FORENSIC SERVICES PROCEDURE MANUAL

BLOOD ALCOHOL ANALYSIS

QUANTITATIVE ALCOHOL ANALYSIS BY
HEADSPACE GAS CHROMATOGRAPHY

I. Equipment:

A. Hewlett Packard 5890 Series II G.C.

B. Hewlett Packard 7694 Headspace Sampler

C. P.C. with Hewlett Packard ChemStation Version A.06.03 [509]

D. Micro Lab 500 Series, Auto Dilutor

E. Crimper, Hewlett Packard Cat. #9301-0720

II. Supplies:

A. Septa - Hewlett Packard - Cat. #9301-0976

B. Crimp Caps - Hewlett Packard - Cat. #9301-0721

C. 10 ml Headspace Vials - Hewlett Packard - Cat. #5182-0838

D. Whole Blood Control – ToxiChem- Cat. # 2930-14

E. Acetonitrile - Fisher Scientific

F. Methanol - Fisher Scientific

G. Acetone - Fisher Scientific

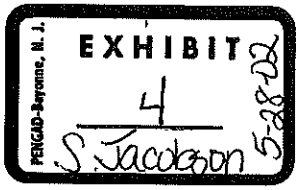
H. Isopropyl Alcohol - Fischer Scientific

I. Acetaldehyde - Fischer Scientific

J. .04, .10, .20, .30, Aqueous Ethanol Controls – College of American Pathologists- Cat. # STO11,17,18,19.

K. Mercuric Chloride - Fischer Scientific

L. Megabore INNOWAX 30 Meter Column - Hewlet Packard - Cat. # 19095N-123



II. Supplies (cont.)

M. Megabore DB-624 30 Meter Column - J & W Scientific - Cat. # 1251334

III. Reagent Preparation:

A. Preparation of Internal Standard Solution

1. Prepare 3% V/V acetonitrile stock solution from acetonitrile and deionized water - 30 ml Acetonitrile / liter of water + a pinch of mercuric chloride.

2. Prepare 0.012% W/V working internal standard solution - 5 ml stock solution / liter of water.

B. Preparation of Mixed standard

1. Acetaldehyde 0.25 ml, methanol 1.00 ml, acetone 0.25 ml, isopropyl alcohol 0.25 ml.

2. Mix with 1 liter of water + a pinch of mercuric chloride.

IV. Dilutor Preparation:

A. Check that there is enough internal standard for the analysis

B. Prime dilutor with internal standard (bubbles can be removed by first flushing the dilutor with acetone).

C. Set syringe volumes
1. Reagent = 2000 ul
2. Sample = 250 ul

V. Sample Preparation:

A. Label each sample vial .

B. Aspirate and dispense sample into vial. Prepare in duplicate.

C. Tightly crimp cap and septa onto vial.

D. Between each sample aspirate water (3x) and dispense into waste to rinse tubing. It is not necessary to rinse between duplicates.

VI. Standard, Blank, and Control Preparation:

A. Prepare .04, .10, .20, and .30 standards with aqueous standards using the same procedures as case samples.

B. Prepare blank with water using the same procedure as case samples.

C. Prepare control with known blood using the same procedures as case samples.

D. Prepare Mixed Standard using the same procedures as case samples.

VII. Calibration:

A. From "Sequence" menu click on "Load Sequence"

B. Highlight "calib.seq" and "OK".

C. From the "Sequence" menu click on "Edit Sequence Parameters".

D. Change the "Data File Subdirectory" to reflect the date of analysis and "OK".

E. Place aqueous calibrators (0.04, 0.100, 0.200, 0.300) in proper location on tray.

F. From the "RunControl" menu click on "Run Sequence".

G. Click on "Method" and "Save Method" and "OK" "Overwrite Method". Enter "Recalibrate" in log

VIII. Run preparation:

A. Place vials in sampler in the following order

1. Aqueous standards (0.04, 0.10, 0.20, 0.30).
2. Mixed standard
3. Blank
4. Blood control in duplicate
5. Case samples in duplicate
6. Blood control (Run a blood control at least every 10 samples).

VIII. Run preparation (cont.)

7. Check standards (0.04, 0.100, 0.200, and 0.300)

IX. Headspace and GC Parameters:

A. Carrier pressure - 0.25 bar

B. Vial pressure - 1.70 bar

C. GC Method - Bldalc1.M

D. Headspace Method - Bloodalc.hsm

BLOOD ALCOHOL
QUALITY ASSURANCE ADDENDUM

I. Proficiency Testing:

The laboratory voluntarily participates on a continuous basis in the following blood alcohol proficiency testing programs administered by independent agencies:

a) U.S. Department of Transportation - NHTSA (National Highway Traffic Safety Administration).

II. Quality Control:

The following rigorous safeguards are employed by each analyst to ensure the validity of their analysis:

a) Blood alcohol analyses are conducted in DUPLICATE. Duplicate values shall be within .01 of each other.

b) Complete calibrations are established at the time of the analysis.

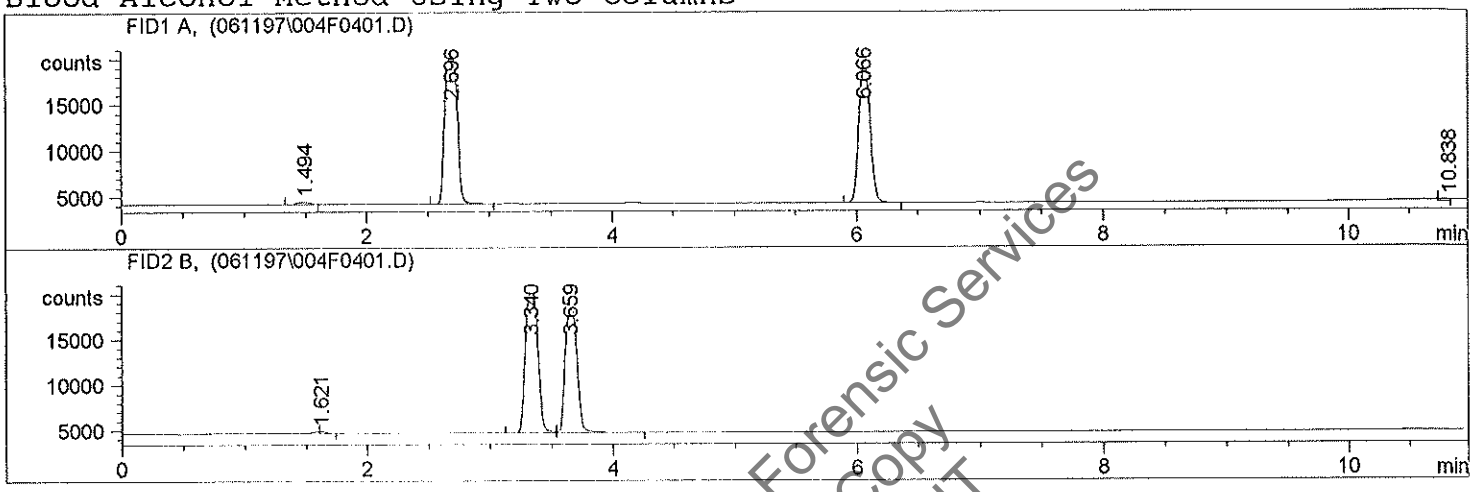
c) Final reports are reviewed by another criminalist.

d) Analytical performance is checked at the time of testing via independently acquired control reference materials. Values for standards and controls shall be within 10% of the known value (GC value on blood control) or .01 whichever is larger.

e) Specimens, while retained in the laboratory, are refrigerated. A chain of custody is maintained on all items while under the control of the Bureau of Forensic Services.

12/28/90 ACS
Revised 11/18/93 SVJ
Revised 06-25-97 SVJ

```
Injection Date  : 6/11/97 9:10:14 AM          Seq. Line :   4
Sample Name     : acetone                        Vial :   4
Acq. Operator   : Stuart V. Jacobson             Inj :   1
                                            Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLOODAL2.M
Last changed    : 6/6/97 11:21:57 AM by Stuart V. Jacobson
Blood Alcohol Method Using Two Columns
```



Internal Standard Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Friday,June 06,1997 11:21:16 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :  2.00000e-1  [g/100ml]   (not used in calc.)
Uncalibrated Peaks    :      not reported

Sample ISTD Information:
ISTD  ISTD Amount  Name
  #    [g/100ml]
----|-------------|-------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE

Signal 1: FID1 A,
 Results obtained with enhanced integrator!

RetTime  Type      Area      Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio    [g/100ml]
-------|------|----------|----------|----------|--|------------------
  4.351           -           -          -          ETHANOL
  6.066 VV    I 9.74633e4     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :                0.00000
```

Signal 2: FID2 B,
Results obtained with enhanced integrator!

RetTime [min]	Type		Area counts*s	Amt/Area ratio	Amount [g/100ml]	Grp	Name
2.957			-	-	-		ETHANOL
3.659	VV	I	9.36619e4	1.00000	1.20000e-2		ACETONITRILE

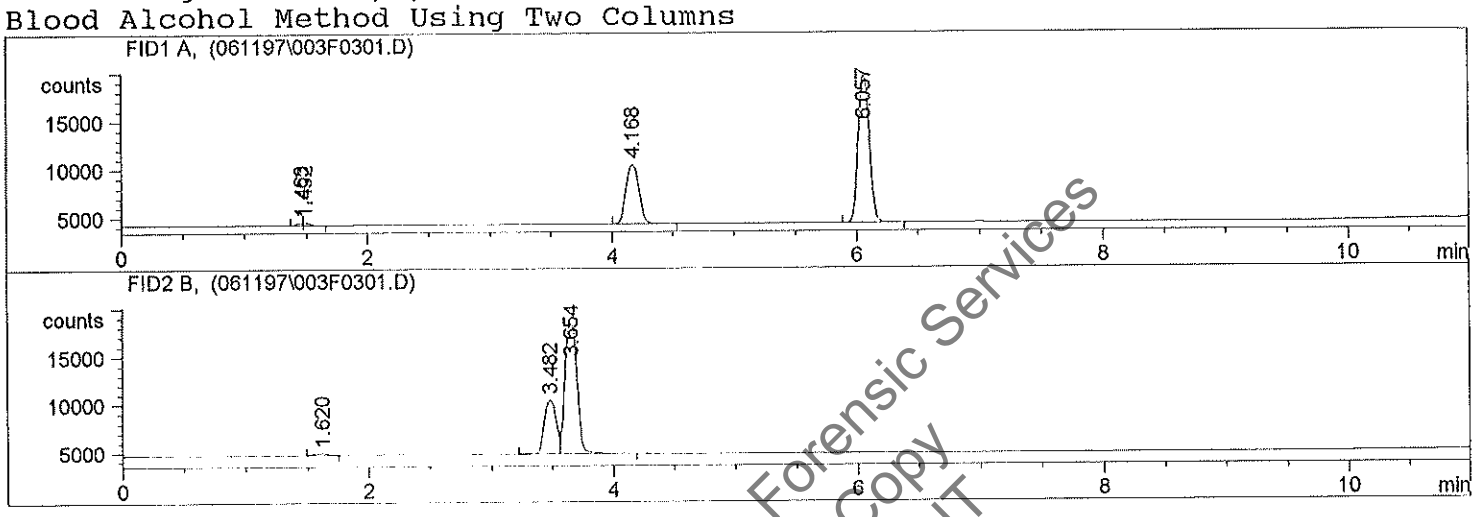
Totals without ISTD(s) : 0.00000

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

```
Injection Date  : 6/11/97 8:53:25 AM          Seq. Line :   3
Sample Name     : isopropanol                     Vial :   3
Acq. Operator   : Stuart V. Jacobson              Inj :   1
                                           Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLOODAL2.M
Last changed    : 6/6/97 11:21:57 AM by Stuart V. Jacobson
Blood Alcohol Method Using Two Columns
```



Internal Standard Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Friday,June 06,1997 11:21:16 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Uncalibrated Peaks    :      not reported

Sample ISTD Information:
```

ISTD #	ISTD Amount [g/100ml]	Name
1	1.20000e-2	ACETONITRILE
2	1.20000e-2	ACETONITRILE

```
Signal 1: FID1 A,
 Results obtained with enhanced integrator!
```

RetTime [min]	Type		Area counts*s	Amt/Area ratio	Amount [g/100ml]	Grp	Name
4.351			-	-	-		ETHANOL
6.057	VV	I	9.63059e4	1.00000	1.20000e-2		ACETONITRILE

```
Totals without ISTD(s) :                    0.00000
```

Signal 2: FID2 B,
Results obtained with enhanced integrator!

RetTime [min]	Type	Area counts*s	Amt/Area ratio	Amount [g/100ml]	Grp	Name
2.957		-	-	-		ETHANOL
3.654	VV	I 9.55764e4	1.00000	1.20000e-2		ACETONITRILE

Totals without ISTD(s) : 0.00000

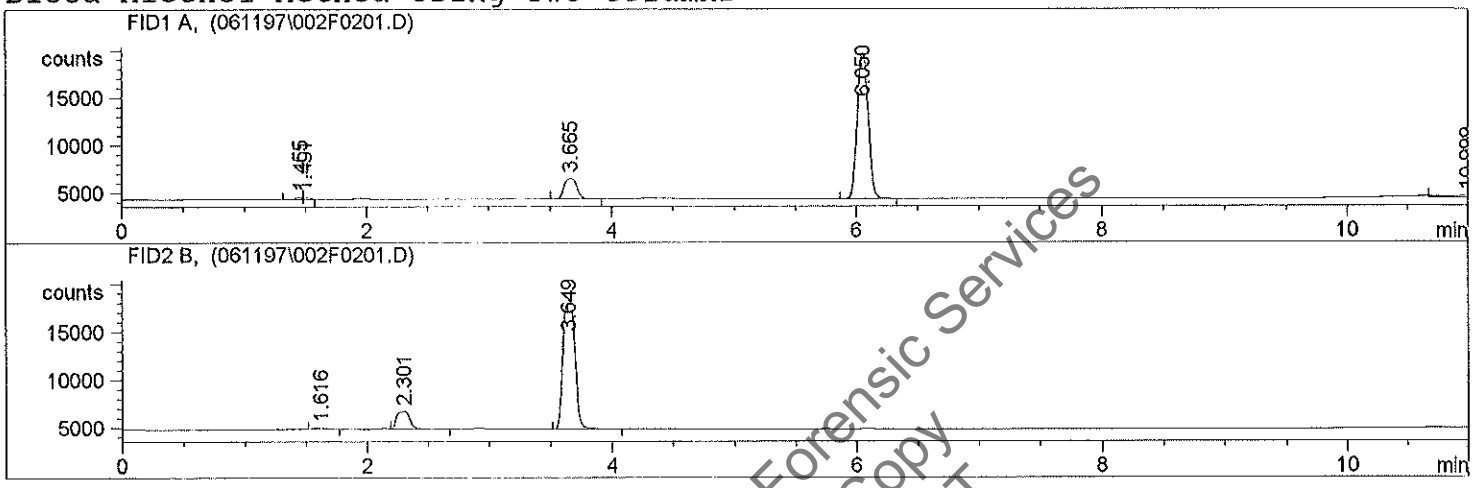
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

```
==========================================================================
Injection Date  : 6/11/97 8:36:40 AM              Seq. Line :   2
Sample Name     : methanol                            Vial :   2
Acq. Operator   : Stuart V. Jacobson                   Inj :   1
                                                  Inj Volume : Manually
Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLOODAL2.M
Last changed    : 6/6/97 11:21:57 AM by Stuart V. Jacobson
Blood Alcohol Method Using Two Columns
```




```
==========================================================================
                        Internal Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday,June 06,1997 11:21:16 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      1.00000e-1  [g/100ml]   (not used in calc.)
Uncalibrated Peaks    :      not reported

Sample ISTD Information:
ISTD  ISTD Amount   Name
  #   [g/100ml]
----|-------------|-------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE

Signal 1: FID1 A,
 Results obtained with enhanced integrator!

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          counts*s     ratio    [g/100ml]
-------|------|----------|----------|----------|--|------------------
  4.351           -           -          -          ETHANOL
  6.050 VV    I 9.72730e4     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :                 0.00000
```

Signal 2: FID2 B,
Results obtained with enhanced integrator!

RetTime [min]	Type	Area counts*s	Amt/Area ratio	Amount [g/100ml]	Grp	Name
2.957		-	-	-		ETHANOL
3.649	VV I	9.28077e4	1.00000	1.20000e-2		ACETONITRILE

Totals without ISTD(s) : 0.00000

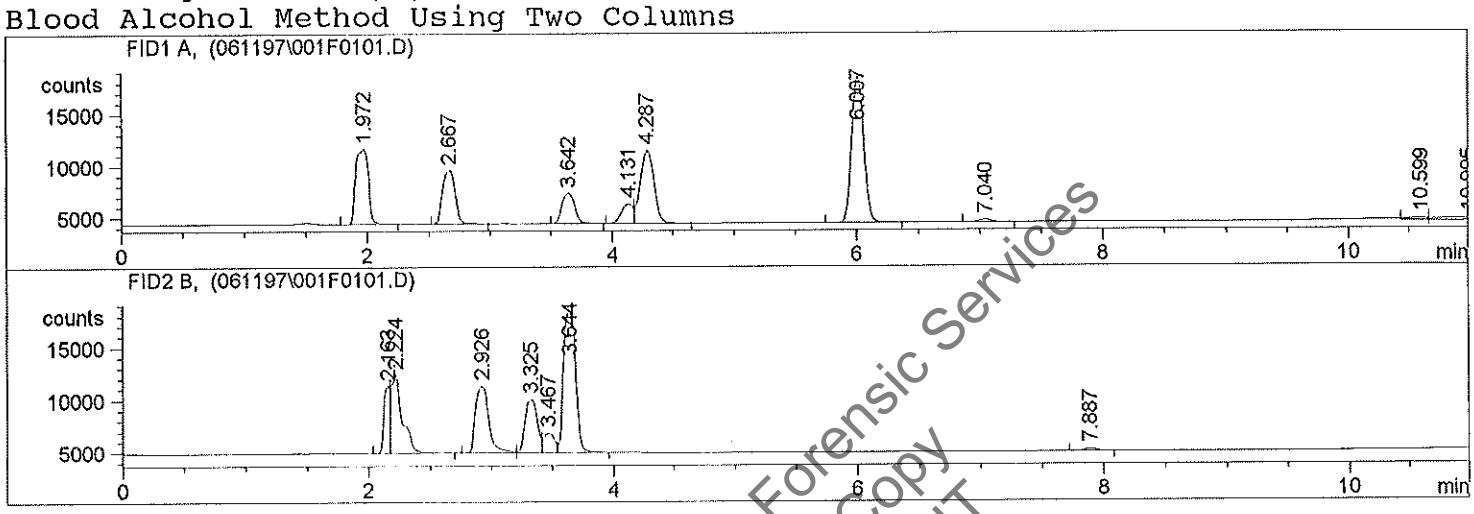
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

```
Injection Date  : 6/11/97 8:19:59 AM          Seq. Line :   1
Sample Name     : MIXED STD                       Vial :   1
Acq. Operator   : Stuart V. Jacobson              Inj :   1
                                           Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLOODAL2.M
Last changed    : 6/6/97 11:21:57 AM by Stuart V. Jacobson
Blood Alcohol Method Using Two Columns
```



Internal Standard Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Friday,June 06,1997 11:21:16 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Uncalibrated Peaks    :      not reported

Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|-------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE

Signal 1: FID1 A,
 Results obtained with enhanced integrator!

RetTime  Type      Area      Amt/Area    Amount    Grp   Name
 [min]           counts*s     ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  4.287 VV      5.63921e4    17.77113 1.24226e-1     ETHANOL
  6.007 VV    I 9.68062e4     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              1.24226e-1
```

Signal 2: FID2 B,
Results obtained with enhanced integrator!

RetTime [min]	Type		Area counts*s	Amt/Area ratio	Amount [g/100ml]	Grp	Name
2.926	VV		4.89165e4	17.96944	1.12832e-1		ETHANOL
3.644	VV	I	9.34840e4	1.00000	1.20000e-2		ACETONITRILE

Totals without ISTD(s) : 1.12832e-1

*** End of Report ***

50 ml 1000 ng/ml → 50 000 ng / 10 ml

5000 ng/ml

5 ng/ml

150 ml .1 mg/ml 15 000 ng / 10 ml

1 500 ng/ml

1.5 ng/ml

20 ng / 1.5 ng/ml

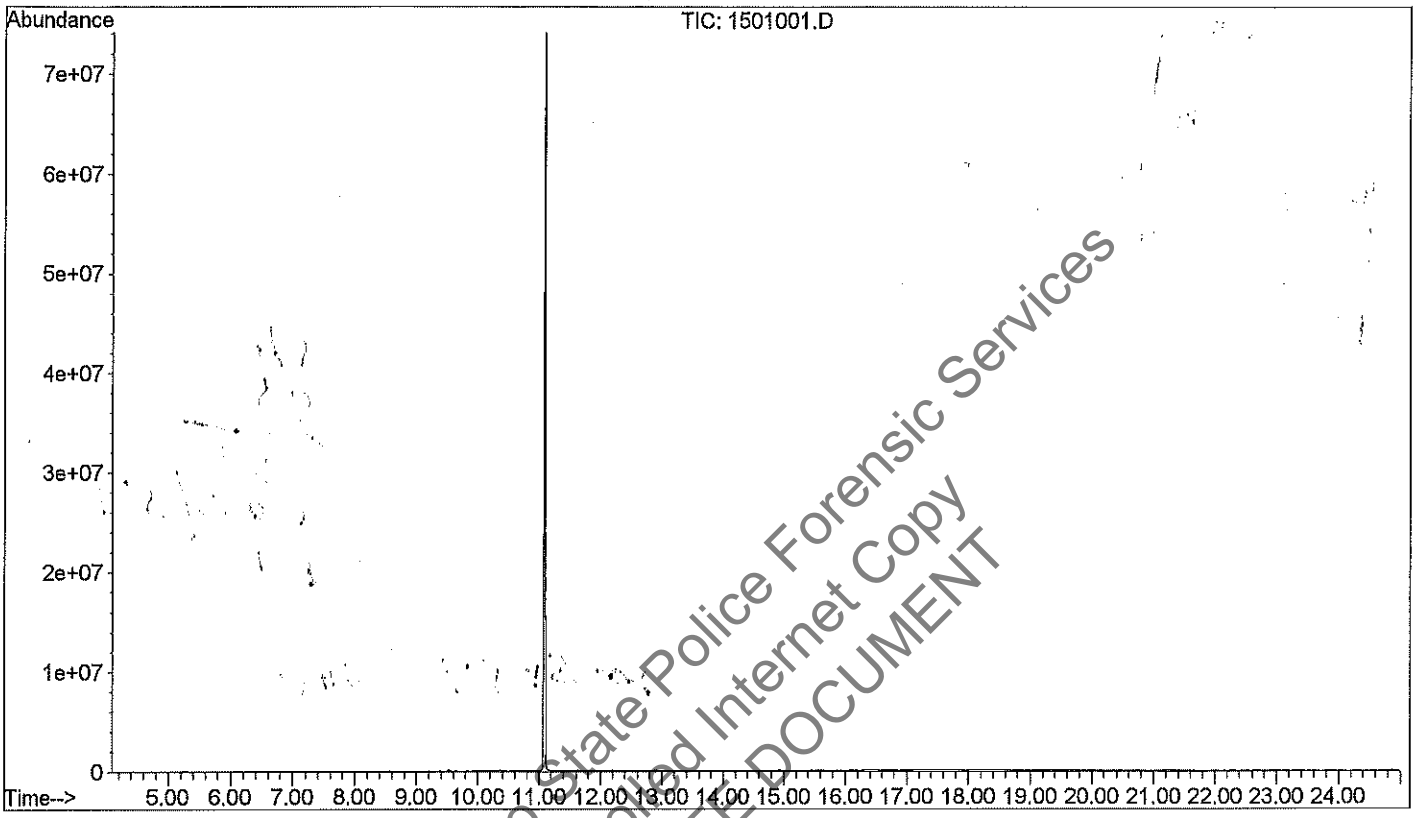
$$1.5 \overline{)\,200} \quad 13$$

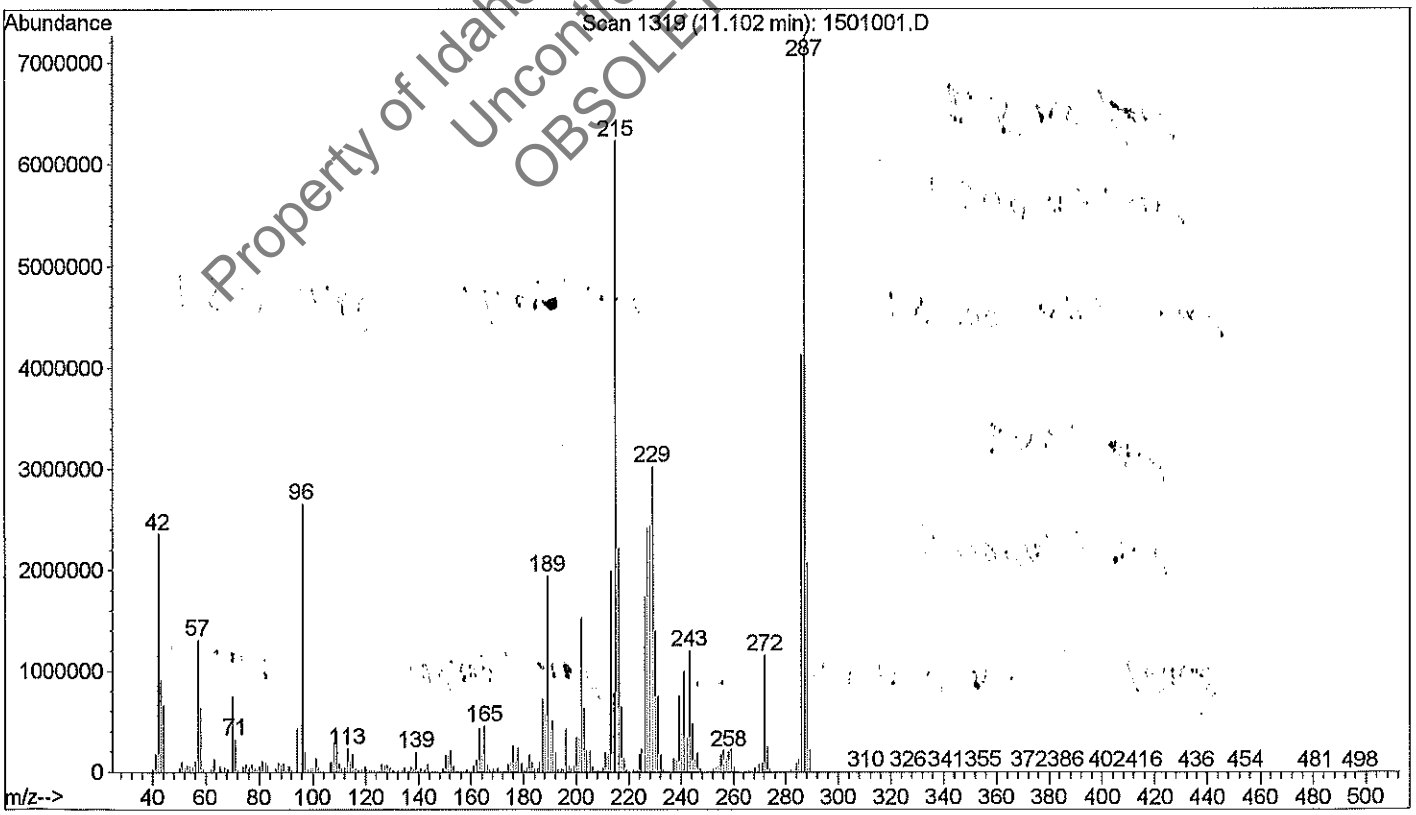
15

50

45

File : D:\HPCHEM\1\DATA\SVJ\111901\1501001.D
Operator : SVJ
Acquired : 19 Nov 2001 14:54 using AcqMethod 100(250)
Instrument : GC/MS Ins
Sample Name: CYPROHEPTADINE STANDARD
Misc Info : TABLET, GOLDLINE LOT # 2929-749
Vial Number: 15





BD

BD Home

VACUTAINER Systems Tube Guide

VACUTAINER BRAND TUBES with HEMOGARD® CLOSURE

Available in a full line, VACUTAINER Brand Tubes with HEMOGARD Closure bring a new feeling of confidence to the laboratory environment. For more information or to order, contact your laboratory supply dealer or your Becton Dickinson VACUTAINER Systems representative.

VACUTAINER® Tubes with HEMOGARD Closure	*VACUTAINER Tubes*	*Additive*	*Number of Inversions at Blood Collection (Invert gently, do not shake)*	*Laboratory Use*
 *Gold*		• Clot activator and gel for serum separation	5	SST Brand Tube for serum determinations in chemistry. Tube inversions ensure mixing of clot activator with blood and clotting within 30 minutes.
 *Light Green*		• Lithium heparin and gel for plasma separation	8	PST Brand Tube for plasma determinations in chemistry. Tube inversions prevent clotting.
 *Red*		• None	0	For serum determinations in chemistry, serology and blood banking.
				For stat serum determinations in

  *Orange*	• Thrombin	8	chemistry. Tube inversions ensure complete clotting, usually in less than 5 minutes.
 *Royal Blue*	• Sodium heparin • Na_2EDTA • None	8 8 0	For trace element, toxicology and nutrient determinations. Special stopper formulation offers low levels of trace elements (see package insert).
 *Green* 	• Sodium heparin • Lithium heparin	8 8	For plasma determinations in chemistry. Tube inversions prevent clotting.
 *Gray* 	• Potassium oxalate/ sodium fluoride • Sodium fluoride • Lithium iodoacetate • Lithium iodoacetate/ lithium heparin	8 8 8 8	For glucose determinations. Tube inversions ensure proper mixing of additive and blood. Oxalate and heparin, anticoagulants, will give plasma samples. Without them, samples are serum.
 *Brown*	• Sodium heparin	8	For lead determinations. This tube is certified to contain less than .01 µg/ml (ppm) lead. Tube inversions prevent clotting.
 *Yellow* 	• Sodium polyanetholesulfonate (SPS) OR • ACD - Acid Citrate Dextrose Additions: Solution A - 22.0g/L trisodium citrate,	8 8	For blood culture specimen collections in microbiology. Tube inversions prevent clotting. For use in blood bank studies, HLA phenotyping, DNA and

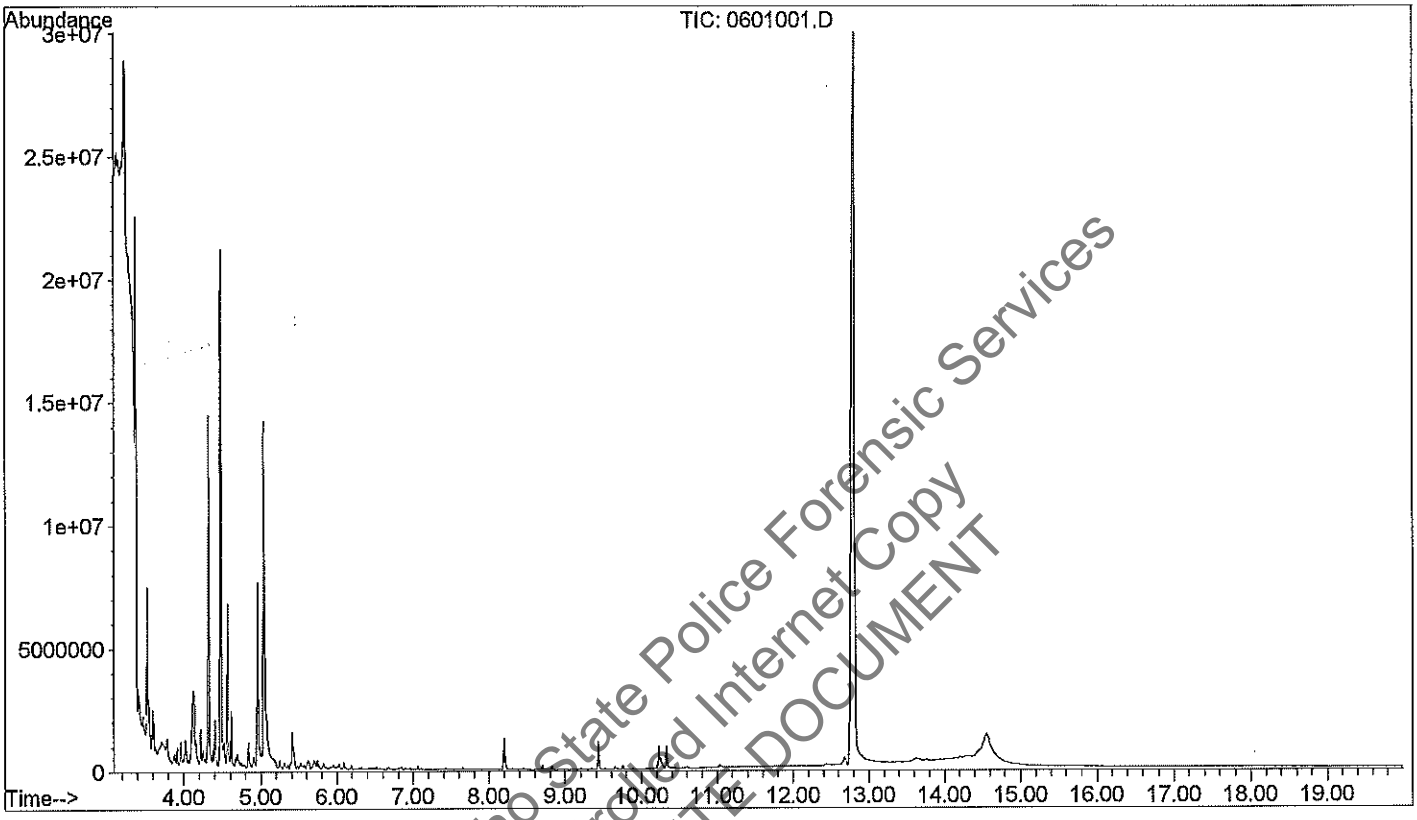
	8.0g/L citric acid, 24.5g/L dextrose Solution B - 13.2g/L trisodium citrate, 4.8g/L citric acid, 14.7g/L dextrose	8	Paternity testing.
  *Lavender*	• Liquid K_3EDTA • Spray-dried K_3EDTA	8 8	For whole blood hematology determinations. Tube inversions prevent clotting.
  *Light Blue*	• .105M Sodium citrate (3.2%) • .129M Sodium citrate (3.8%)	8 8	For coagulation determinations on plasma specimens. Tube inversions prevent clotting. NOTE: Certain tests require chilled specimens. Follow recommended procedures for collection and transport of specimen.

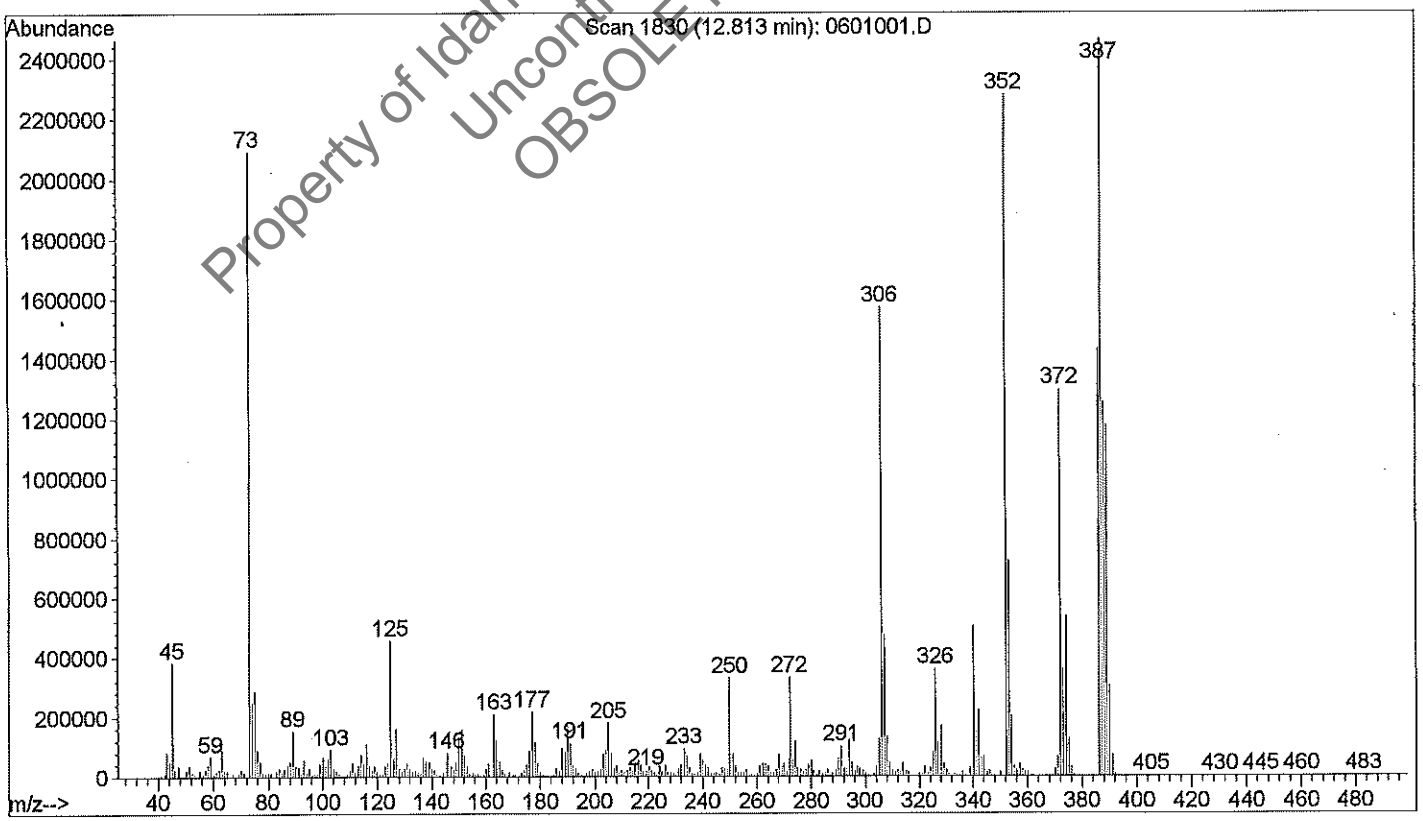
For technical information, call 1-800-631-0174

BD
1 Becton Drive
Franklin Lakes, New Jersey 07417-1883
201-847-6800

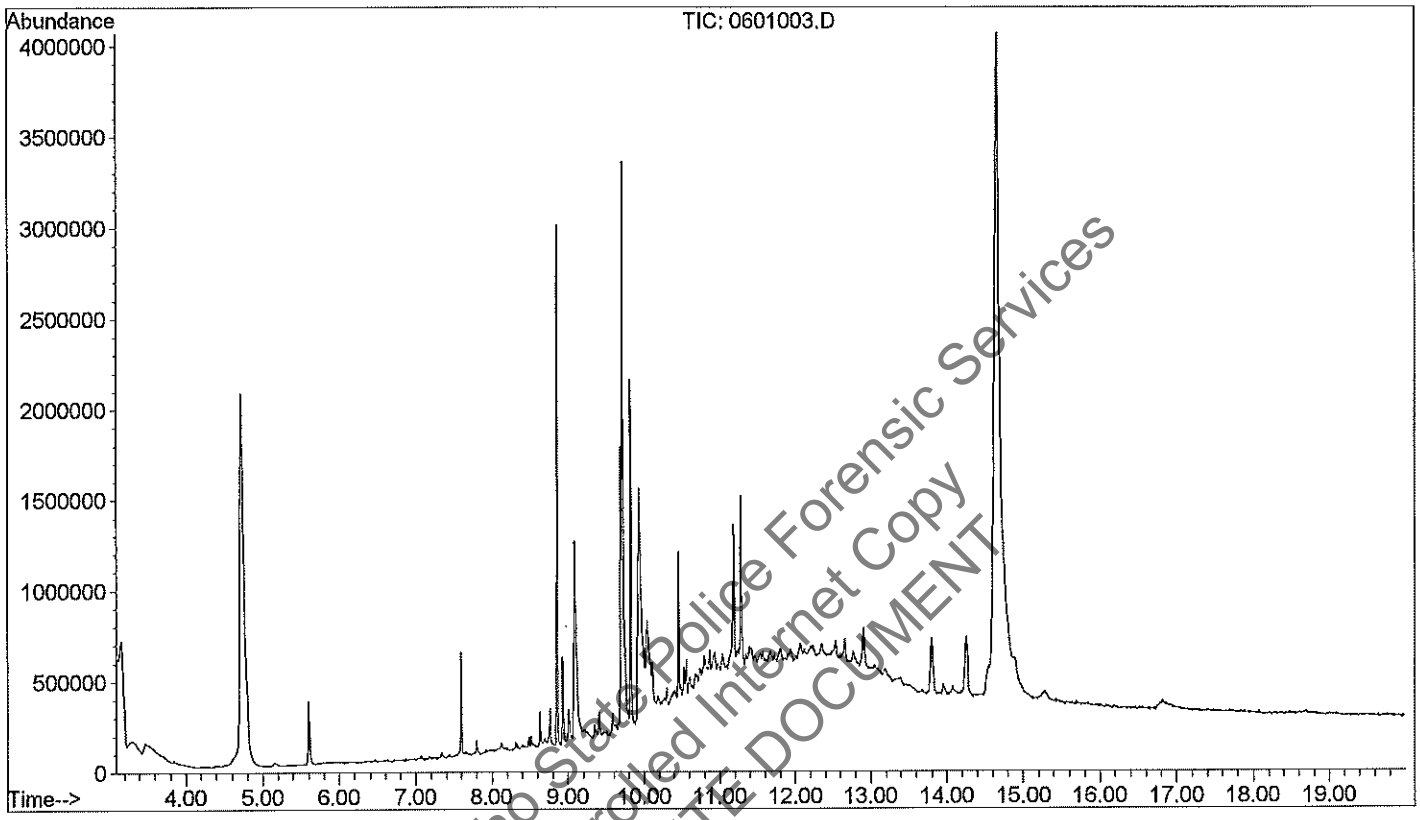
Top of Page

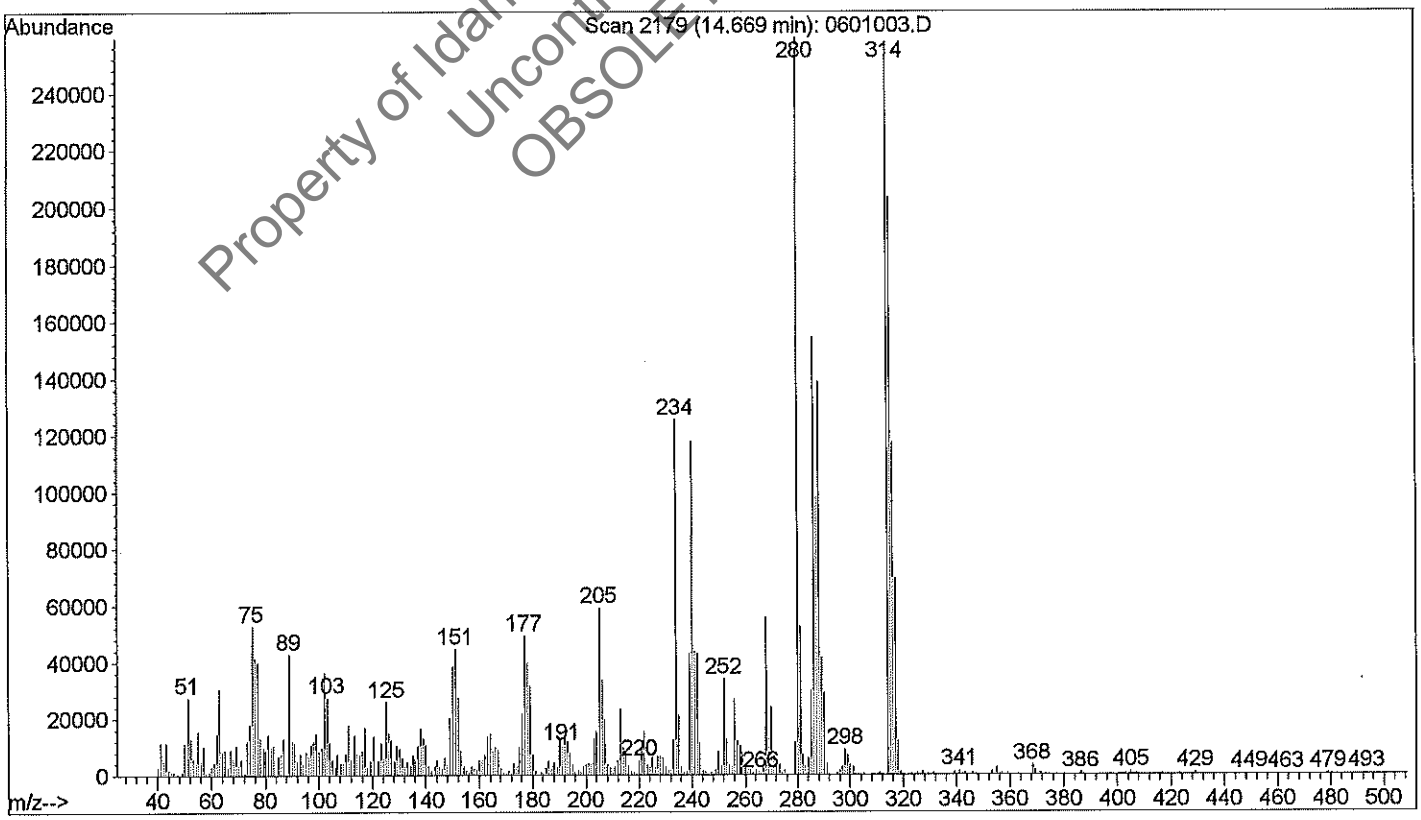
File : D:\HPCHEM\1\DATA\SVJ\021800\0601001.D
Operator : SVJ
Acquired : 18 Feb 2000 9:47 using AcqMethod 80(250)
Instrument : GC/MS Ins
Sample Name: CLONAZEPAM BSTFA DIR.
Misc Info :
Vial Number: 6



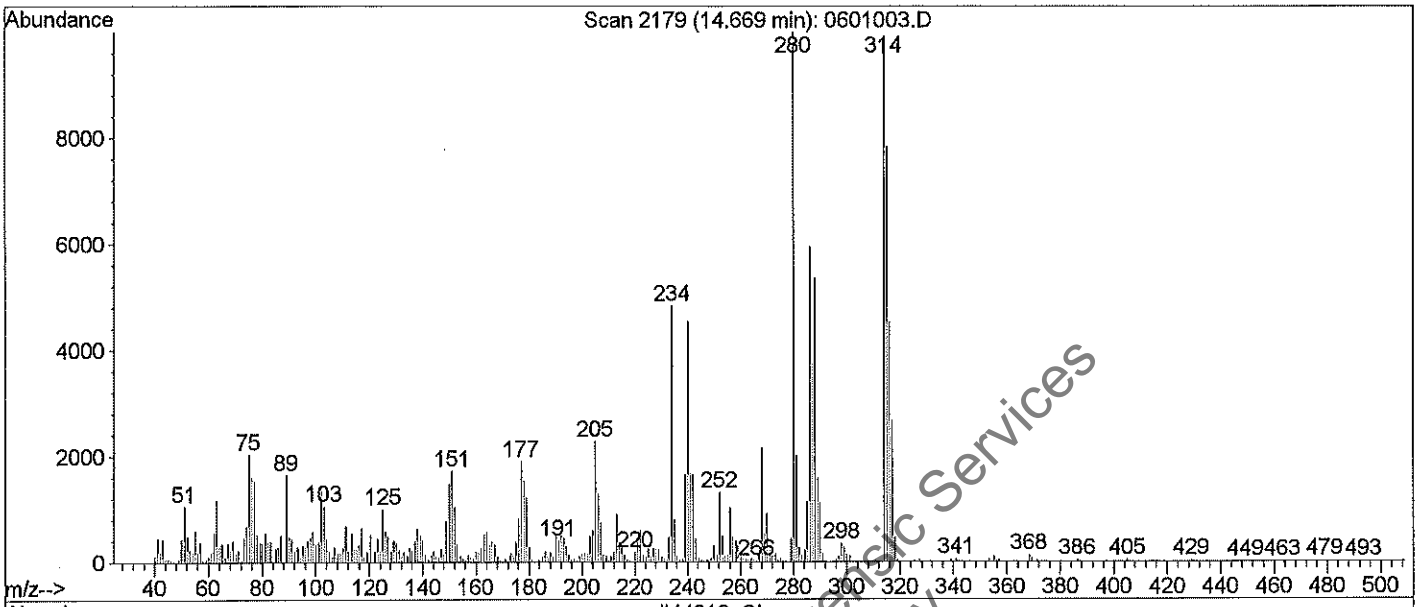


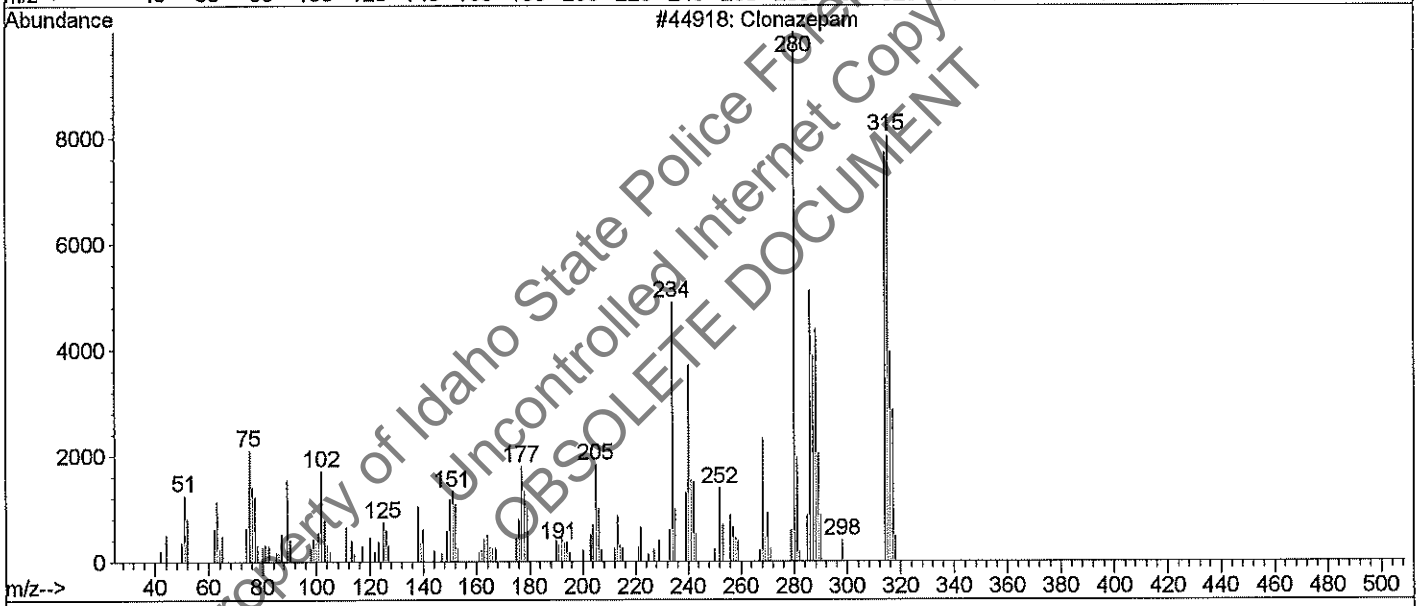
File : D:\HPCHEM\1\DATA\SVJ\021700\0601003.D
Operator : SVJ
Acquired : 17 Feb 2000 10:06 using AcqMethod 80(250)
Instrument : GC/MS Ins
Sample Name: CLONAZEPAM STANDARD
Misc Info : RADIAN LOT # 31567-65B
Vial Number: 6

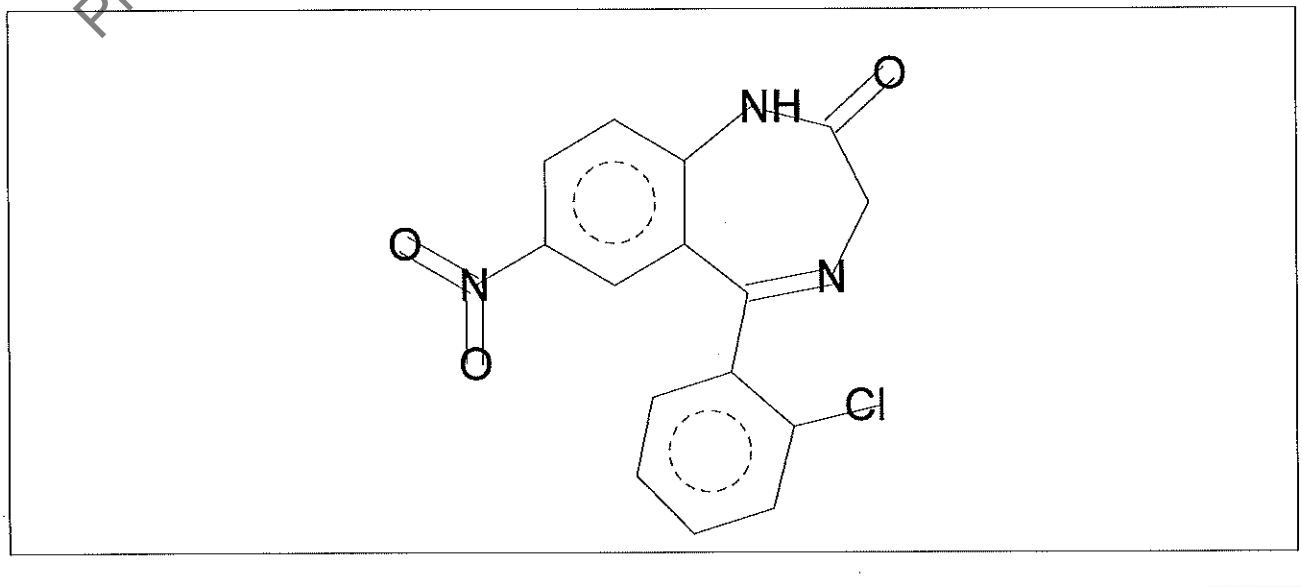




Library Searched : D:\DATABASE\NBS75K.L
Quality : 99
ID : Clonazepam







Method C:\HPCHEM\1\METHODS\BLDALC1.M

Calibration Table

Blood Alcohol Calibration

Calib. Data Modified : Friday, January 28, 2000 5:52:29 PM

Calculate : Internal Standard
Based on : Peak Area

Rel. Reference Window : 10.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 10.000 %
Abs. Non-ref. Window : 0.000 min
Uncalibrated Peaks : not reported
Partial Calibration : No recalibration if peaks missing

Curve Type : Linear
Origin : Included
Weight : Equal

Recalibration Settings:
Average Response : Average all calibrations
Average Retention Time: Floating Average New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Default Sample ISTD Information (if not set in sample table):

ISTD #	ISTD Amount [g/100ml]	Name
1	1.20000e-2	ACETONITRILE
2	1.20000e-2	ACETONITRILE

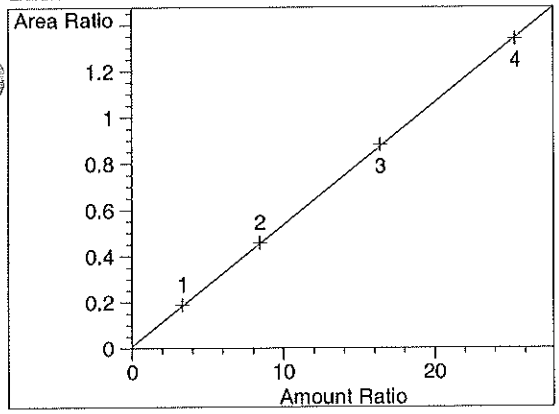
Signal 1: FID1 A,
Signal 2: FID2 B,

RetTime [min]	Sig	Lvl	Amount [g/100ml]	Area	Amt/Area	Ref	Grp	Name
2.515	2	1	4.01800e-2	2.08804e4	1.92429e-6	2		ETHANOL
		2	1.01340e-1	5.00673e4	2.02408e-6			
		3	1.96870e-1	9.85976e4	1.99670e-6			
		4	3.03970e-1	1.47685e5	2.05823e-6			
3.145	2	1	1.20000e-2	1.11591e5	1.07536e-7	I2		ACETONITRILE
		2	1.20000e-2	1.09570e5	1.09519e-7			
		3	1.20000e-2	1.11925e5	1.07215e-7			
		4	1.20000e-2	1.10264e5	1.08830e-7			
3.778	1	1	4.01800e-2	2.17670e4	1.84592e-6	1		ETHANOL
		2	1.01340e-1	5.17576e4	1.95797e-6			
		3	1.96870e-1	1.04792e5	1.87868e-6			
		4	3.03970e-1	1.54753e5	1.96423e-6			
5.352	1	1	1.20000e-2	1.15474e5	1.03920e-7	I1		ACETONITRILE
		2	1.20000e-2	1.12873e5	1.06314e-7			
		3	1.20000e-2	1.17124e5	1.02456e-7			
		4	1.20000e-2	1.13990e5	1.05273e-7			

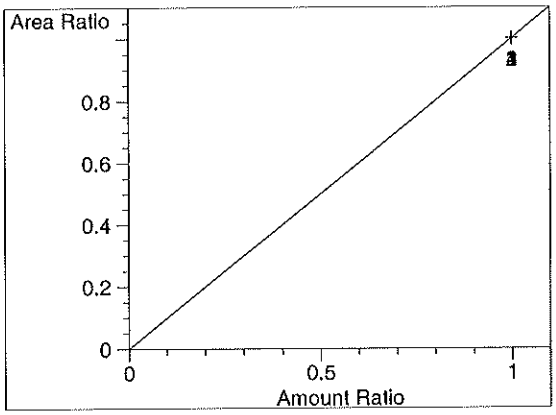
Peak Sum Table

No Entries in table

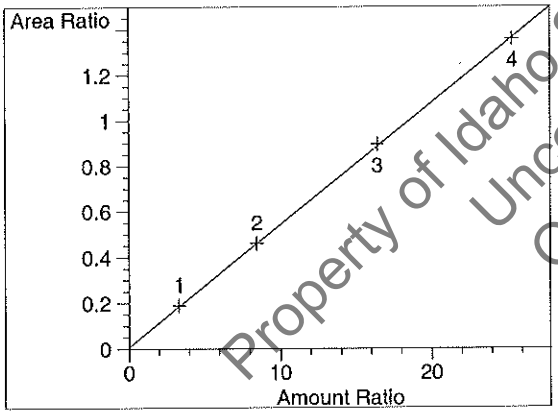
Calibration Curves



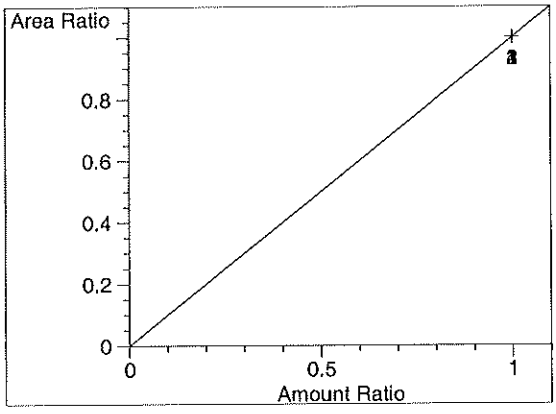
ETHANOL at exp. RT: 2.515
FID2 B,
Correlation: 0.99993
Residual Std. Dev.: 0.00729
Formula: y = mx + b
m: 5.28257e-2
b: 7.32084e-3
x: Amount Ratio
y: Area Ratio



ACETONITRILE at exp. RT: 3.145
FID2 B,
Correlation: 1.00000
Residual Std. Dev.: 0.00000
Formula: y = mx + b
m: 1.00000
b: 0.00000
x: Amount Ratio
y: Area Ratio

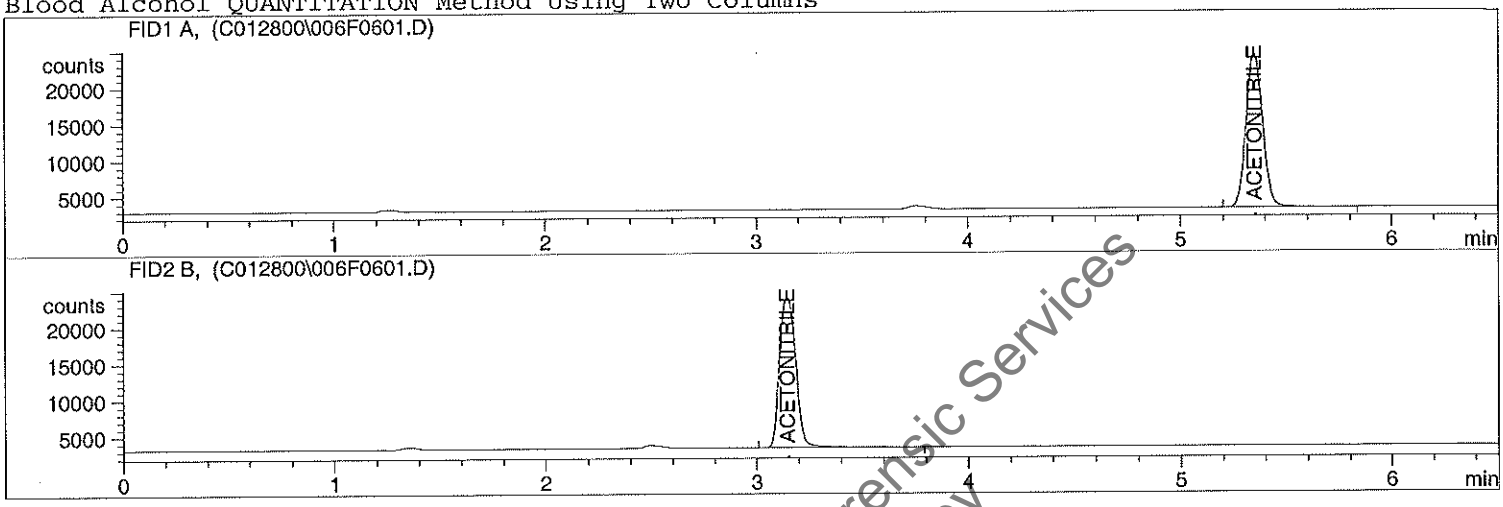


ETHANOL at exp. RT: 3.778
FID1 A,
Correlation: 0.99993
Residual Std. Dev.: 0.00753
Formula: y = mx + b
m: 5.36139e-2
b: 5.88170e-3
x: Amount Ratio
y: Area Ratio



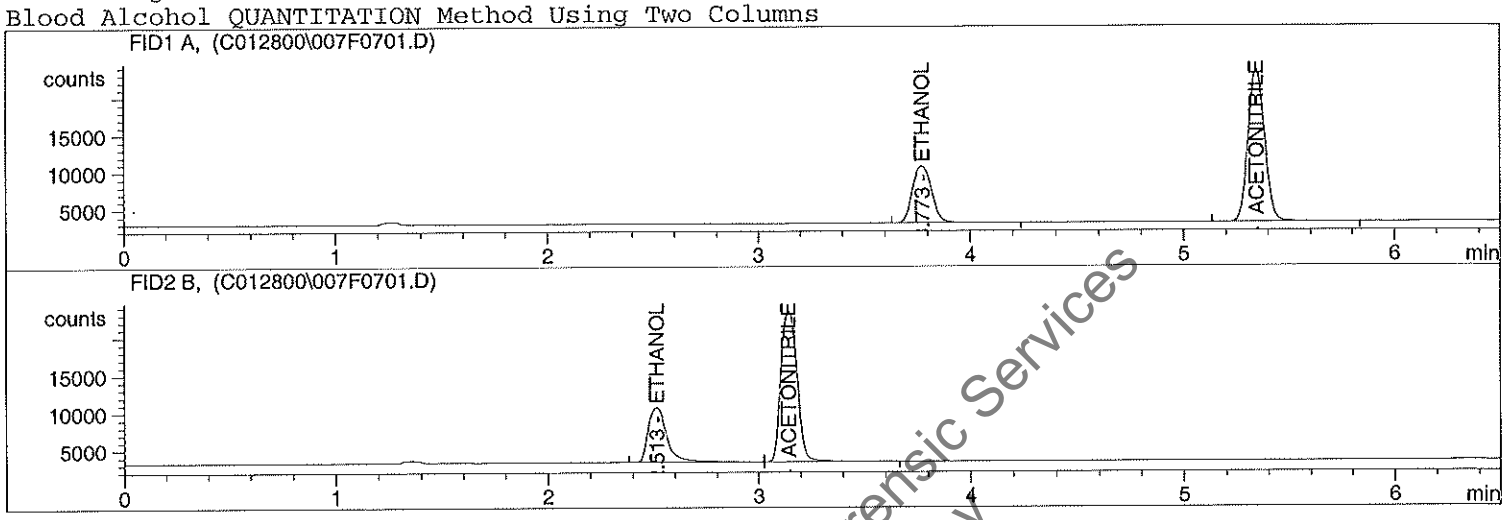
ACETONITRILE at exp. RT: 5.352
FID1 A,
Correlation: 1.00000
Residual Std. Dev.: 0.00000
Formula: y = mx + b
m: 1.00000
b: 0.00000
x: Amount Ratio
y: Area Ratio

```
==========================================================================
Injection Date  : 1/28/00 5:55:44 PM          Seq. Line :   6
Sample Name     : blank                          Vial :   6
Acq. Operator   : Stuart V. Jacobson              Inj :   1
                                            Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed    : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



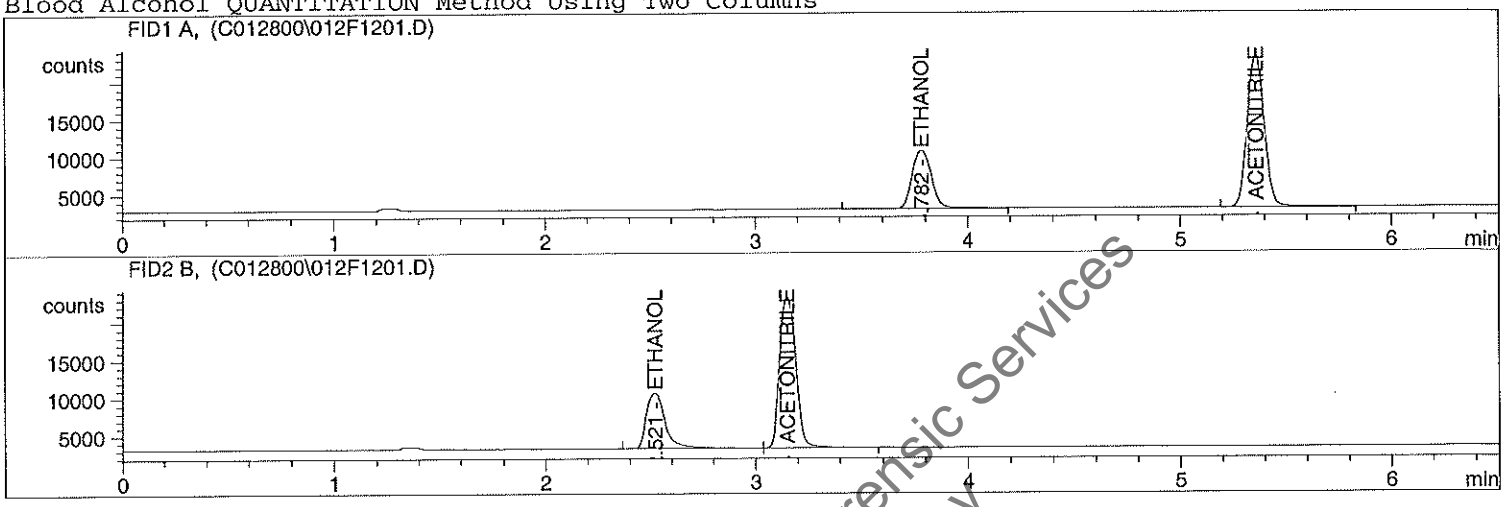

```
==========================================================================
                      Internal Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #   [g/100ml]
----|-------------|-------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          counts*s     ratio    [g/100ml]
-------|------|----------|----------|----------|--|------------------
  3.778                -          -          -       ETHANOL
  5.348 BV    I 1.15292e5    1.00000 1.20000e-2       ACETONITRILE

Totals without ISTD(s) :                 0.00000

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          counts*s     ratio    [g/100ml]
-------|------|----------|----------|----------|--|------------------
  2.515                -          -          -       ETHANOL
  3.144 VV    I 1.11539e5    1.00000 1.20000e-2       ACETONITRILE

Totals without ISTD(s) :                 0.00000

 Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========================================================================
```

```
==========================================================================
Injection Date  : 1/28/00 6:05:29 PM              Seq. Line :   7
Sample Name     : no NaF                             Vial :   7
Acq. Operator   : Stuart V. Jacobson                  Inj :   1
                                                 Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed    : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```




```
==========================================================================
                        Internal Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|----------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|-----------|--|-------------------
  3.773 VV     4.80030e4    18.39593 9.46167e-2     ETHANOL
  5.345 VV   I 1.11996e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.46167e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|-----------|--|-------------------
  2.513 VV     4.62292e4    18.60378 9.47843e-2     ETHANOL
  3.140 VV   I 1.08884e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.47843e-2

 Results obtained with enhanced integrator!
==========================================================================
                          *** End of Report ***
```

```
Injection Date  : 1/28/00 6:54:36 PM          Seq. Line :  12
Sample Name     : no NaF                        Vial :  12
Acq. Operator   : Stuart V. Jacobson             Inj :   1
                                            Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed    : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



Internal Standard Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
```

ISTD #	ISTD Amount [g/100ml]	Name
1	1.20000e-2	ACETONITRILE
2	1.20000e-2	ACETONITRILE

Signal 1: FID1 A,

RetTime [min]	Type	Area counts*s	Amt/Area ratio	Amount [g/100ml]	Grp	Name
3.782	VV	4.97405e4	18.40295	9.73200e-2		ETHANOL
5.358	VV I	1.12870e5	1.00000	1.20000e-2		ACETONITRILE

Totals without ISTD(s) : 9.73200e-2

Results obtained with enhanced integrator!

Signal 2: FID2 B,

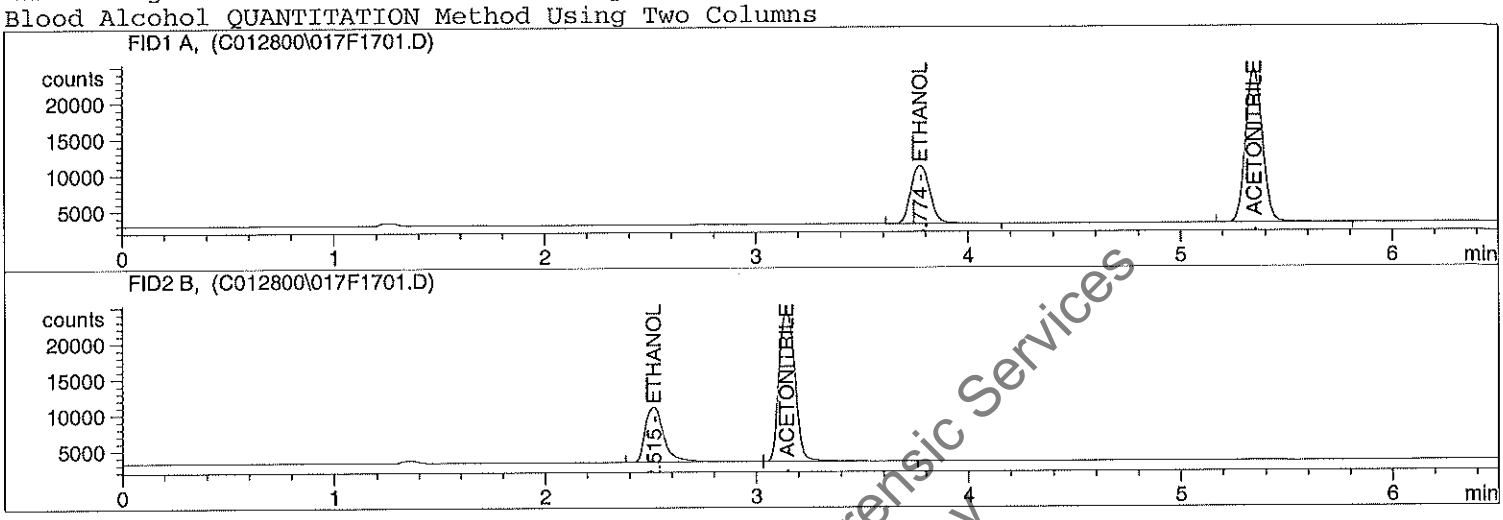
RetTime [min]	Type	Area counts*s	Amt/Area ratio	Amount [g/100ml]	Grp	Name
2.521	VV	4.57232e4	18.60602	9.54521e-2		ETHANOL
3.149	VV I	1.06951e5	1.00000	1.20000e-2		ACETONITRILE

Totals without ISTD(s) : 9.54521e-2

Results obtained with enhanced integrator!

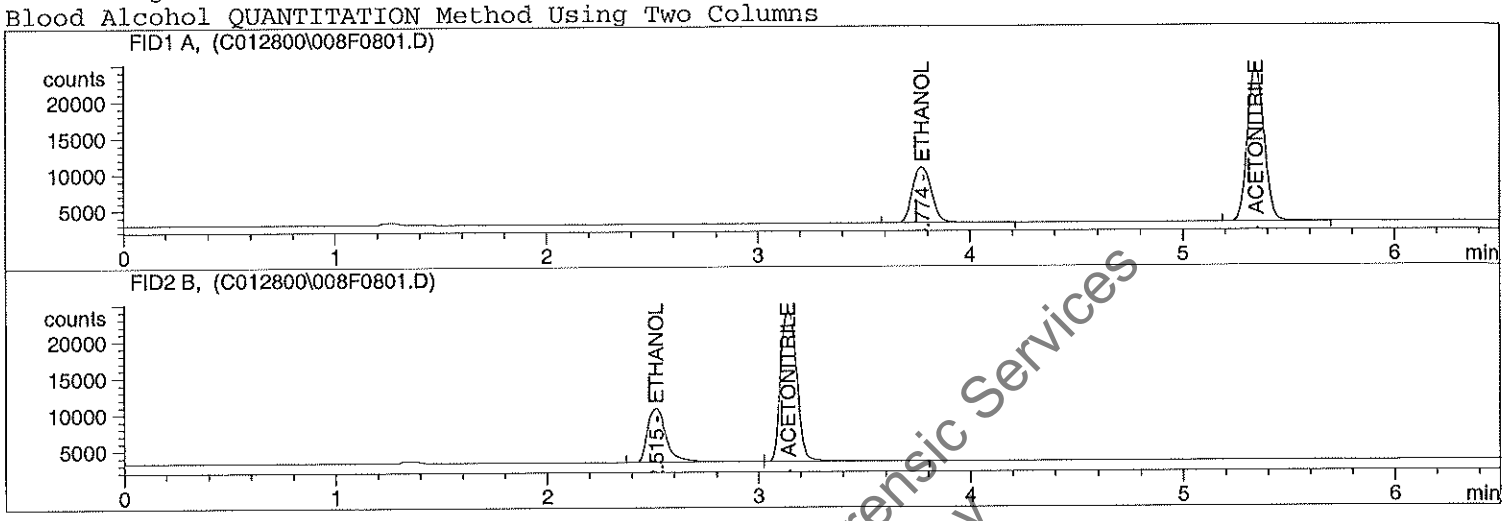
*** End of Report ***

```
==============================================================================
Injection Date  : 1/28/00 7:43:47 PM              Seq. Line :  17
Sample Name     : no NaF                             Vial :  17
Acq. Operator   : Stuart V. Jacobson                  Inj :   1
                                                  Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed    : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



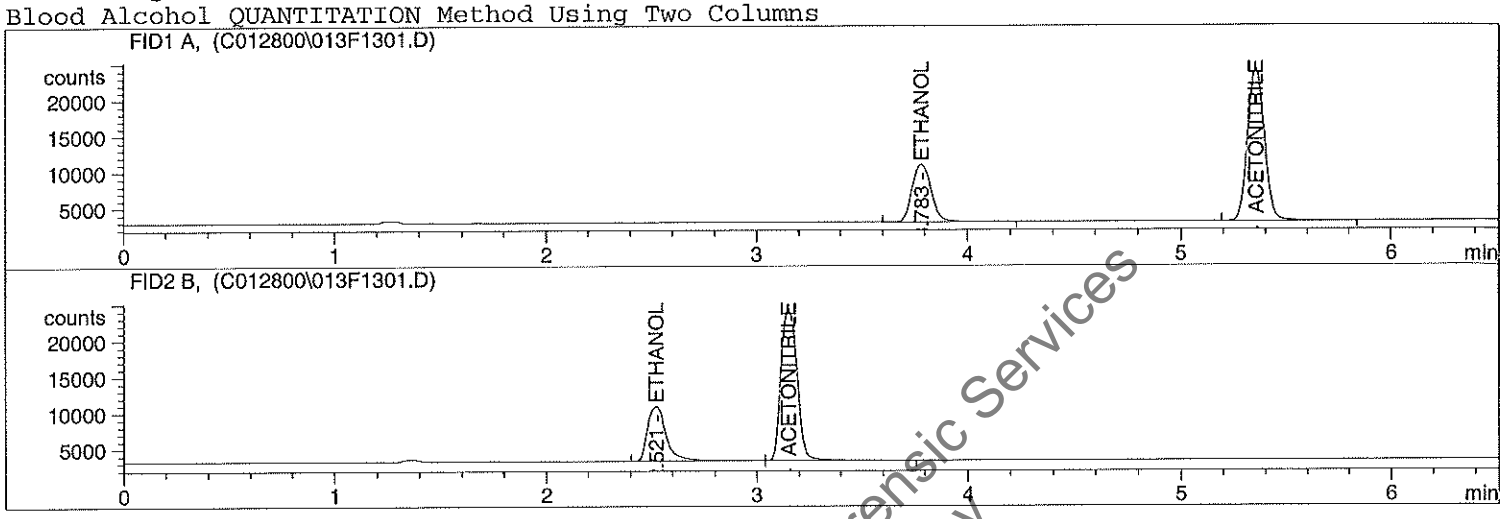

```
==============================================================================
                        Internal Standard Report
==============================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|-------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|-------------------
  3.774 BV     5.09974e4    18.39959 9.60049e-2     ETHANOL
  5.346 VV   I 1.17285e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.60049e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|-------------------
  2.515 VV     4.76586e4    18.60408 9.48729e-2     ETHANOL
  3.142 VV   I 1.12147e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.48729e-2

 Results obtained with enhanced integrator!
==============================================================================
                          *** End of Report ***
```

```
==============================================================================
Injection Date   : 1/28/00 6:15:18 PM              Seq. Line :   8
Sample Name      : 8ml/tube                            Vial :   8
Acq. Operator    : Stuart V. Jacobson                   Inj :   1
                                                 Inj Volume : Manually

Sequence File    : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method           : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed     : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



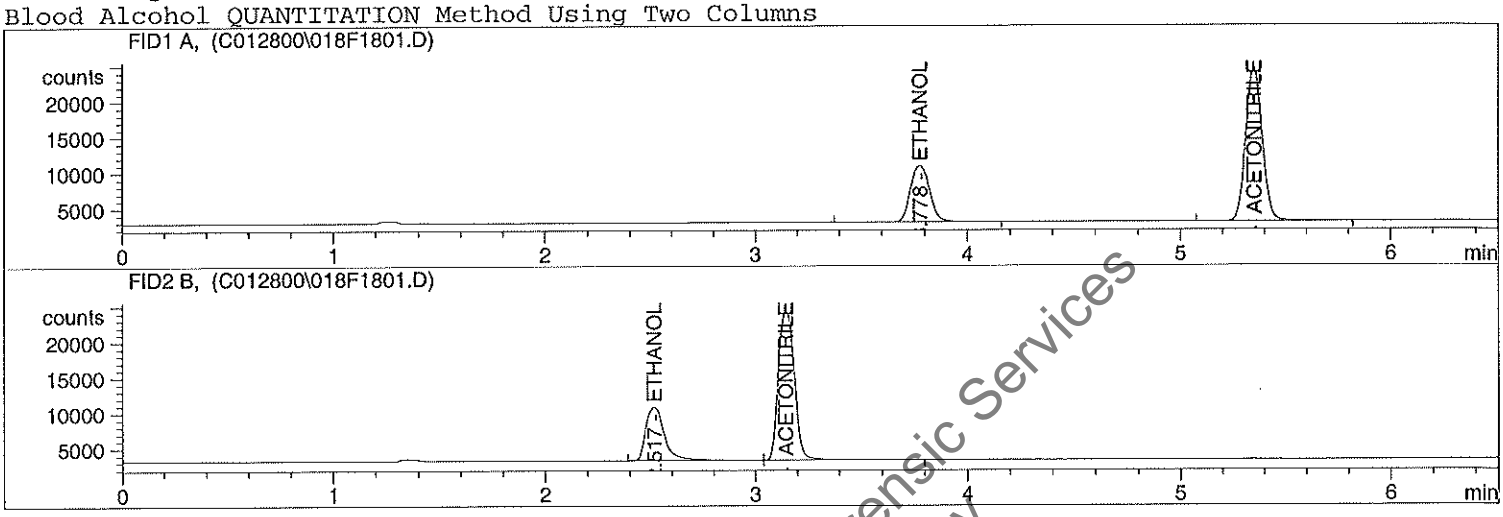

```
==============================================================================
                        Internal Standard Report
==============================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|---------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio      [g/100ml]
-------|------|----------|-----------|-----------|--|------------------
  3.774 VB     4.79604e4    18.38793 9.17070e-2     ETHANOL
  5.345 VV   I 1.15397e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.17070e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio      [g/100ml]
-------|------|----------|-----------|-----------|--|------------------
  2.515 VV     4.60246e4    18.59165 9.13299e-2     ETHANOL
  3.141 VV   I 1.12429e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.13299e-2

 Results obtained with enhanced integrator!
==============================================================================
                          *** End of Report ***
```

```
==============================================================================
Injection Date   : 1/28/00 7:04:30 PM              Seq. Line :  13
Sample Name      : 8 ml/tube                          Vial :  13
Acq. Operator    : Stuart V. Jacobson                  Inj :   1
                                                  Inj Volume : Manually

Sequence File    : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method           : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed     : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



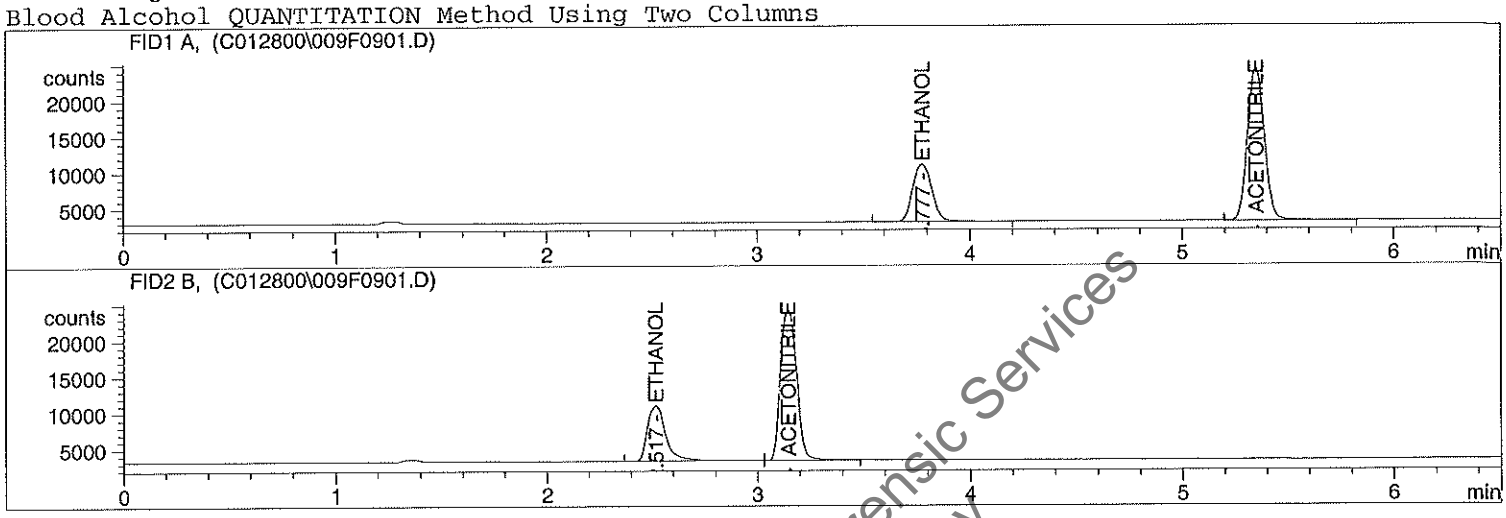

```
==============================================================================
                        Internal Standard Report
==============================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|----------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|--------------------
  3.783 VV     5.11800e4    18.39856 9.56090e-2     ETHANOL
  5.357 BV   I 1.18186e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.56090e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|--------------------
  2.521 VV     4.71152e4    18.59990 9.36514e-2     ETHANOL
  3.150 VV   I 1.12289e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.36514e-2

 Results obtained with enhanced integrator!
==============================================================================
                          *** End of Report ***
```

```
==============================================================================
Injection Date   : 1/28/00 7:53:34 PM              Seq. Line :  18
Sample Name      : 8 ml/tube                          Vial :  18
Acq. Operator    : Stuart V. Jacobson                  Inj :   1
                                                  Inj Volume : Manually

Sequence File    : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method           : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed     : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



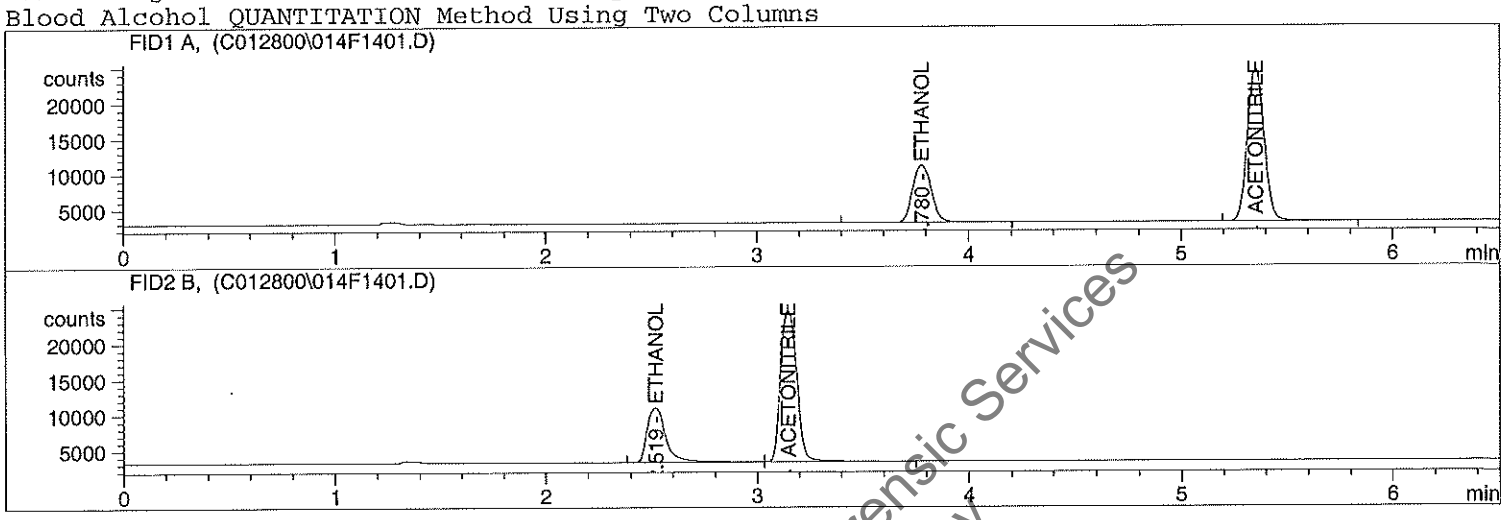

```
==============================================================================
                        Internal Standard Report
==============================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|--------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  3.778 VV     5.09253e4    18.39609 9.46743e-2     ETHANOL
  5.350 VV   I 1.18743e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :               9.46743e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  2.517 VV     4.70117e4    18.59511 9.22880e-2     ETHANOL
  3.144 VB   I 1.13669e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :               9.22880e-2

 Results obtained with enhanced integrator!
==============================================================================
                          *** End of Report ***
```

```
==============================================================================
Injection Date   : 1/28/00 6:25:12 PM              Seq. Line :   9
Sample Name      : 6ml/tube                          Vial :   9
Acq. Operator    : Stuart V. Jacobson                 Inj :   1
                                                  Inj Volume : Manually

Sequence File    : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method           : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed     : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



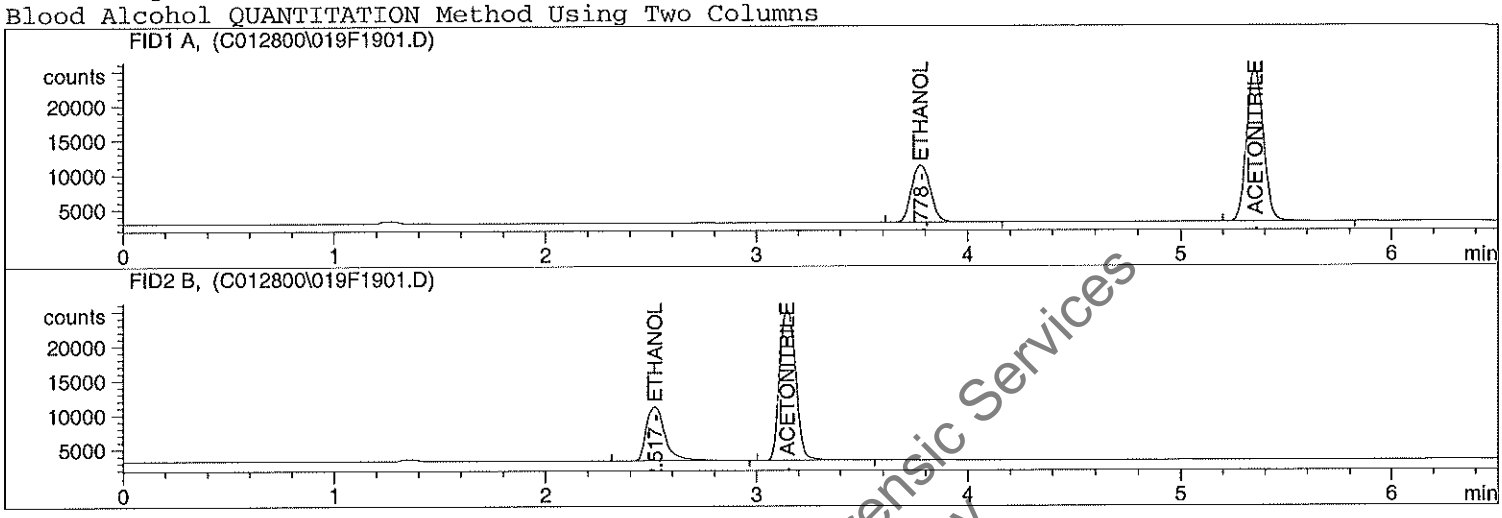

```
==============================================================================
                        Internal Standard Report
==============================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|----------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  3.777 VV     5.10046e4    18.39854 9.56044e-2     ETHANOL
  5.349 VV   I 1.17787e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :               9.56044e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  2.517 VV     4.74738e4    18.60338 9.46660e-2     ETHANOL
  3.145 VV   I 1.11952e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :               9.46660e-2

 Results obtained with enhanced integrator!
==============================================================================
                          *** End of Report ***
```

```
==========================================================================
Injection Date   : 1/28/00 7:14:14 PM              Seq. Line :  14
Sample Name      : 6 ml/tube                          Vial :  14
Acq. Operator    : Stuart V. Jacobson                  Inj :   1
                                                  Inj Volume : Manually

Sequence File    : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method           : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed     : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



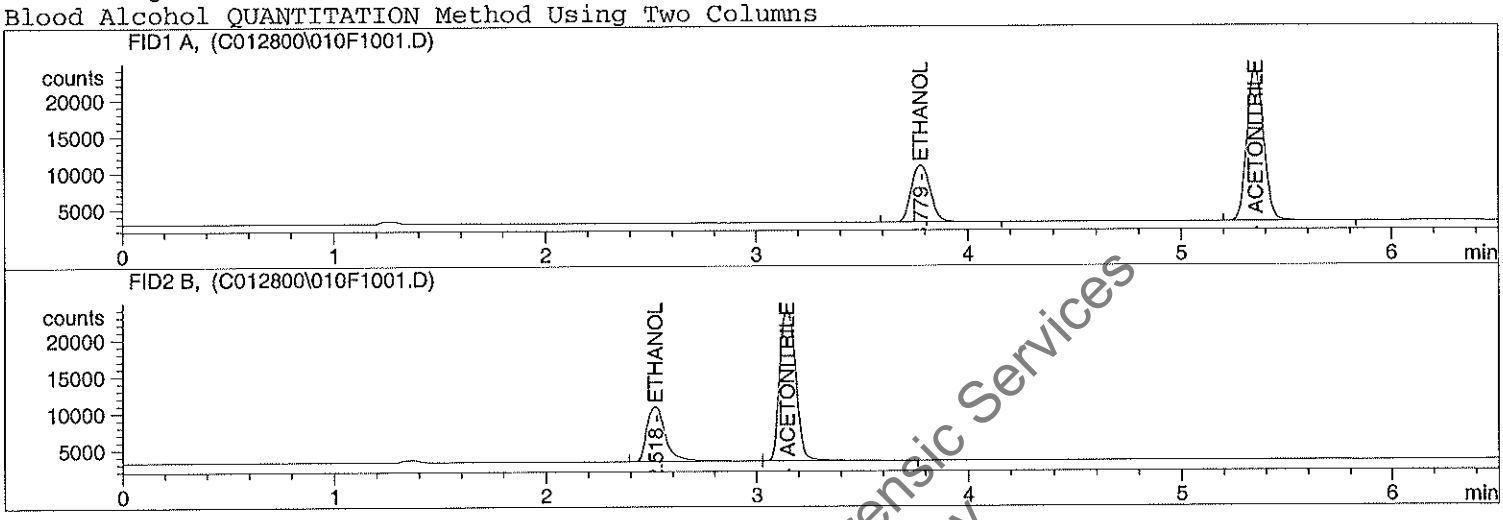

```
==========================================================================
                        Internal Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|-----------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  3.780 VB     5.13114e4    18.39547 9.44423e-2     ETHANOL
  5.353 VV   I 1.19933e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.44423e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  2.519 VV     4.82954e4    18.59867 9.32971e-2     ETHANOL
  3.146 VB   I 1.15532e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.32971e-2

 Results obtained with enhanced integrator!
==========================================================================
                          *** End of Report ***
```

```
==============================================================================
Injection Date  : 1/28/00 8:03:25 PM          Seq. Line :  19
Sample Name     : 6 ml/tube                       Vial :  19
Acq. Operator   : Stuart V. Jacobson               Inj :   1
                                             Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed    : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



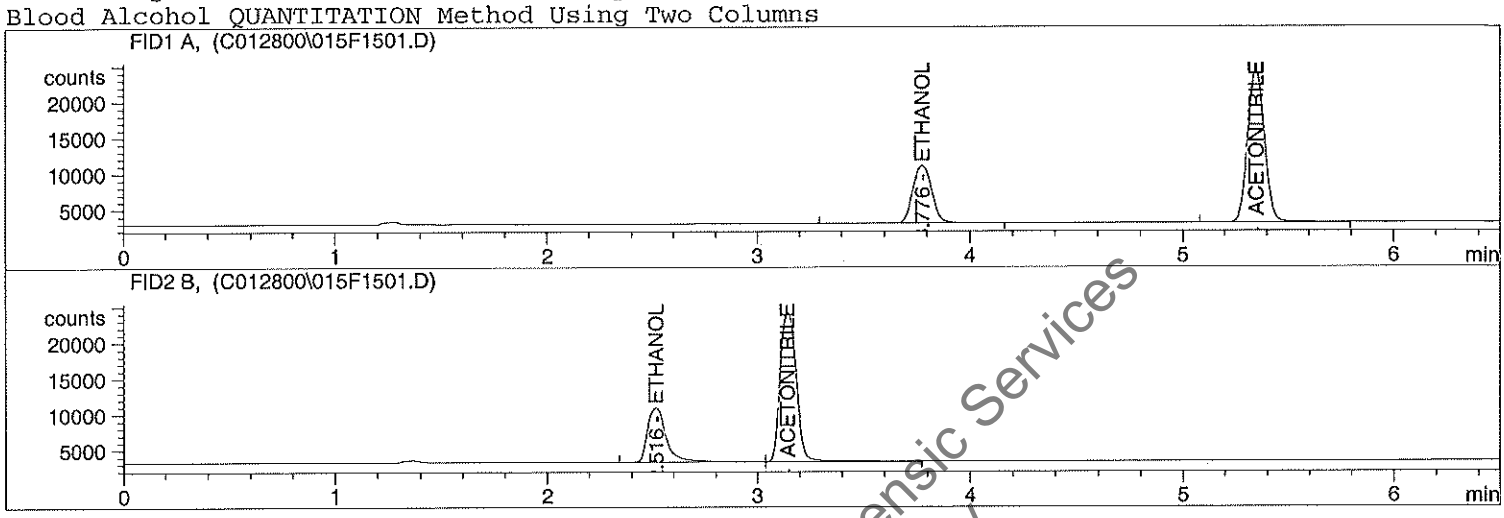

```
==============================================================================
                        Internal Standard Report
==============================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|--------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|---------|----------|----------|--|------------------
  3.778 VV     5.17051e4    18.39216 9.32214e-2     ETHANOL
  5.352 BV   I 1.22414e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :               9.32214e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  2.517 VV     4.83431e4    18.59638 9.26473e-2     ETHANOL
  3.146 VV   I 1.16442e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :               9.26473e-2

 Results obtained with enhanced integrator!
==============================================================================
                          *** End of Report ***
```

```
==========================================================================
Injection Date   : 1/28/00 6:34:57 PM              Seq. Line :  10
Sample Name      : 4ml/tube                          Vial :  10
Acq. Operator    : Stuart V. Jacobson                 Inj :   1
                                                  Inj Volume : Manually

Sequence File    : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method           : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed     : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



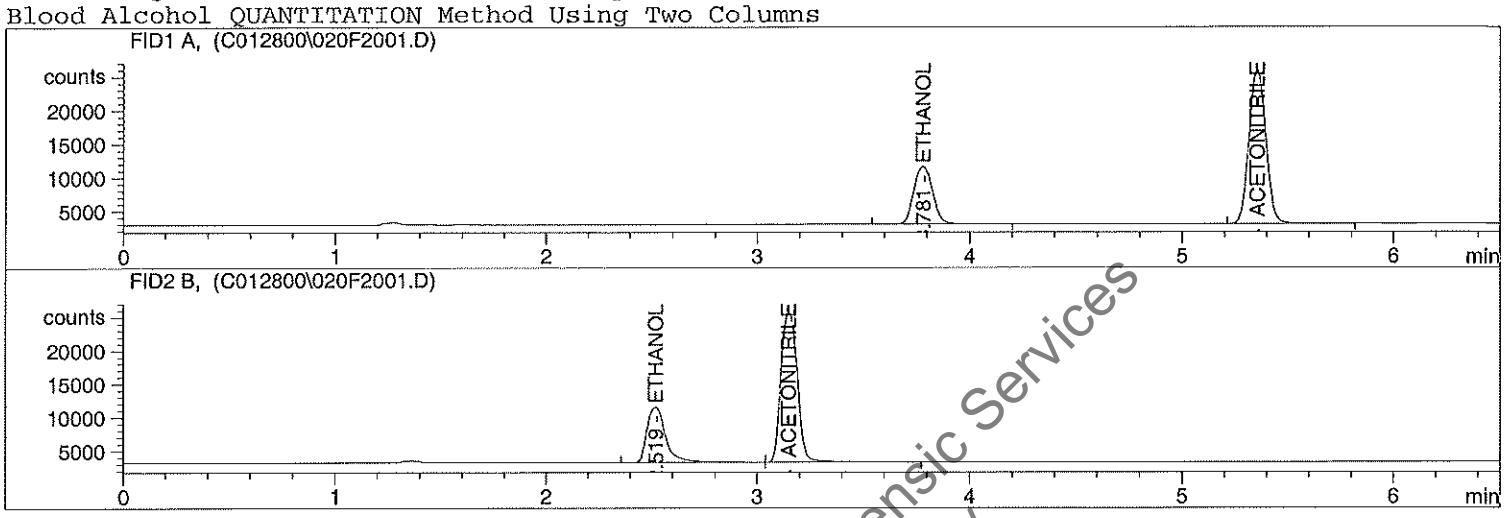

```
==========================================================================
                        Internal Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|----------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  3.779 VV     4.87609e4    18.39392 9.38680e-2     ETHANOL
  5.352 VV   I 1.14659e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :               9.38680e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  2.518 VV     4.69868e4    18.60075 9.38990e-2     ETHANOL
  3.146 VV   I 1.11693e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :               9.38990e-2

 Results obtained with enhanced integrator!
==========================================================================
                          *** End of Report ***
```

```
==========================================================================
Injection Date  : 1/28/00 7:24:09 PM              Seq. Line :  15
Sample Name     : 4ml/tube                            Vial :  15
Acq. Operator   : Stuart V. Jacobson                   Inj :   1
                                                  Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed    : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



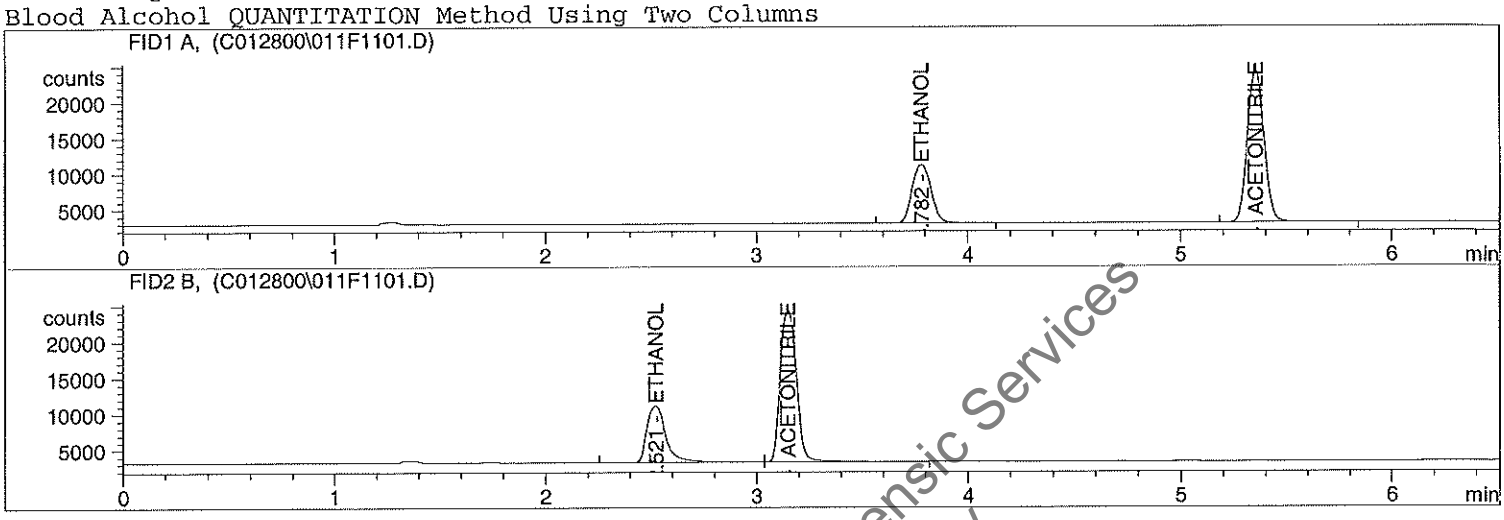

```
==========================================================================
                        Internal Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|-------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  3.776 VV     5.07565e4    18.39817 9.54610e-2     ETHANOL
  5.348 VV   I 1.17387e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.54610e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  2.516 VV     4.82262e4    18.60402 9.48561e-2     ETHANOL
  3.143 VV   I 1.13503e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.48561e-2

 Results obtained with enhanced integrator!
==========================================================================
                          *** End of Report ***
```

```
==========================================================================
Injection Date  : 1/28/00 8:13:09 PM            Seq. Line :  20
Sample Name     : 4ml/tube                          Vial :  20
Acq. Operator   : Stuart V. Jacobson                 Inj :   1
                                                Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed    : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



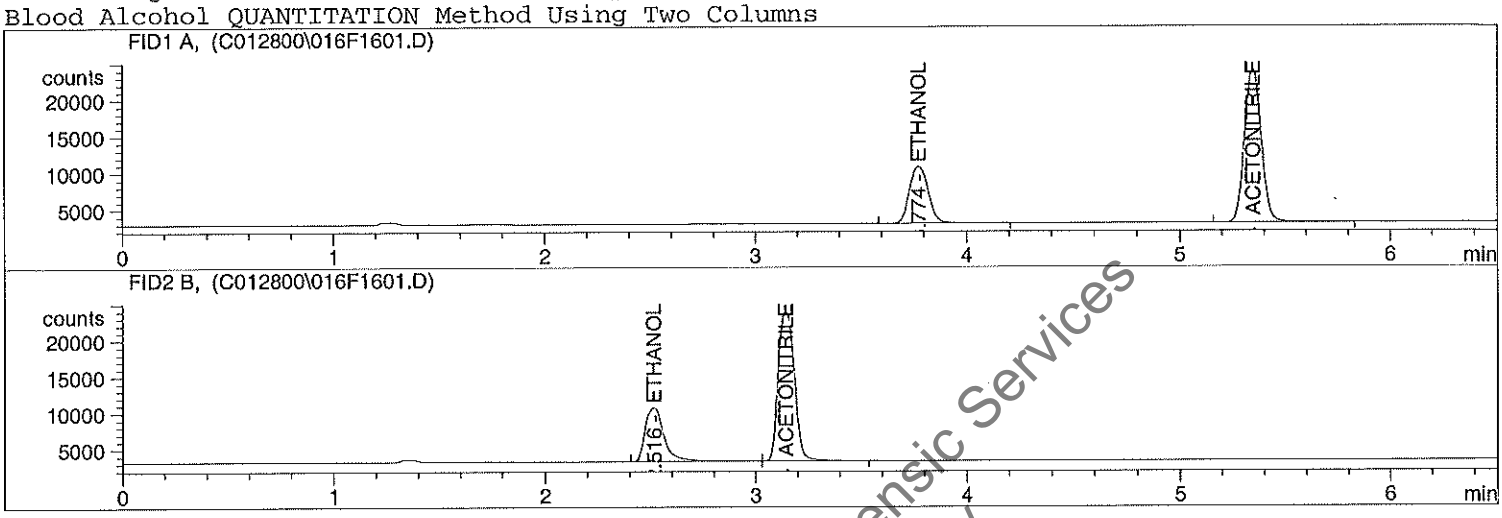

```
==========================================================================
                        Internal Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|-------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type      Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s       ratio     [g/100ml]
-------|------|----------|----------|----------|--|-------------------
  3.781 VV     5.46368e4    18.39817 9.54634e-2     ETHANOL
  5.354 VV   I 1.26359e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.54634e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type      Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s       ratio     [g/100ml]
-------|------|----------|----------|----------|--|-------------------
  2.519 VV     5.16776e4    18.60262 9.44447e-2     ETHANOL
  3.147 VV   I 1.22146e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.44447e-2

 Results obtained with enhanced integrator!
==========================================================================
                          *** End of Report ***
```

```
==========================================================================
Injection Date  : 1/28/00 6:44:48 PM            Seq. Line :  11
Sample Name     : 2ml/tube                          Vial :  11
Acq. Operator   : Stuart V. Jacobson                 Inj :   1
                                                Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed    : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```



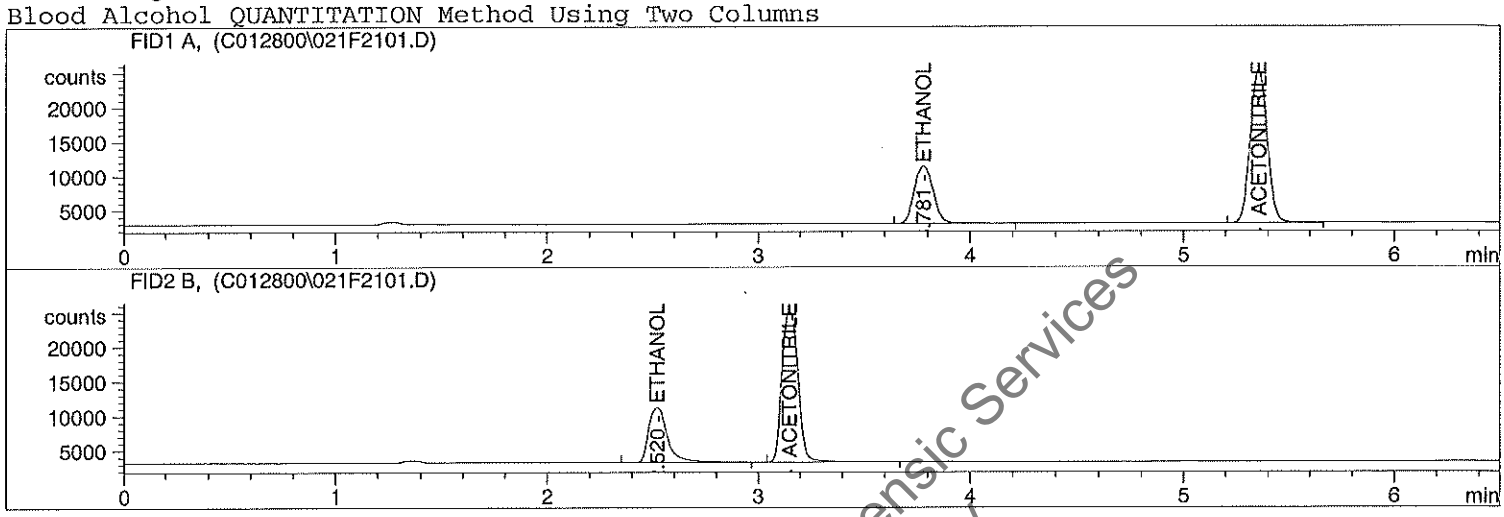

```
==========================================================================
                        Internal Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|-------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type      Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s       ratio     [g/100ml]
-------|------|----------|----------|----------|--|--------------------
  3.782 VV     5.08630e4    18.40057 9.63872e-2     ETHANOL
  5.355 PV   I 1.16519e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.63872e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type      Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s       ratio     [g/100ml]
-------|------|----------|----------|----------|--|--------------------
  2.521 VV     4.94404e4    18.61072 9.68800e-2     ETHANOL
  3.148 VV   I 1.13970e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.68800e-2

 Results obtained with enhanced integrator!
==========================================================================
                          *** End of Report ***
```

```
==========================================================================
Injection Date  : 1/28/00 7:33:54 PM              Seq. Line :  16
Sample Name     : 2 ml/tube                           Vial :  16
Acq. Operator   : Stuart V. Jacobson                   Inj :   1
                                                  Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed    : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```




```
==========================================================================
                        Internal Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|-------------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  3.774 VV     4.83777e4    18.38919 9.21519e-2     ETHANOL
  5.347 VV   I 1.15847e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.21519e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  2.516 VV     4.60546e4    18.59530 9.23413e-2     ETHANOL
  3.142 VV   I 1.11291e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.23413e-2

 Results obtained with enhanced integrator!
==========================================================================
                          *** End of Report ***
```

```
==============================================================================
Injection Date  : 1/28/00 8:23:03 PM              Seq. Line :  21
Sample Name     : 2ml/tube                            Vial :  21
Acq. Operator   : Stuart V. Jacobson                   Inj :   1
                                                  Inj Volume : Manually

Sequence File   : C:\HPCHEM\1\SEQUENCE\CALIB.S
Method          : C:\HPCHEM\1\METHODS\BLDALC1.M
Last changed    : 1/28/00 5:52:29 PM by Stuart V. Jacobson
Blood Alcohol QUANTITATION Method Using Two Columns
```




```
==============================================================================
                        Internal Standard Report
==============================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, January 28, 2000 5:52:29 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
ISTD  ISTD Amount    Name
  #    [g/100ml]
----|-------------|-----------------------
  1   1.20000e-2   ACETONITRILE
  2   1.20000e-2   ACETONITRILE


Signal 1: FID1 A,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  3.781 VV     5.20909e4    18.39435 9.40254e-2     ETHANOL
  5.356 VV   I 1.22288e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.40254e-2

 Results obtained with enhanced integrator!

Signal 2: FID2 B,

RetTime  Type     Area       Amt/Area     Amount    Grp   Name
 [min]          counts*s      ratio     [g/100ml]
-------|------|----------|----------|----------|--|------------------
  2.520 VV     4.99259e4    18.59921 9.34525e-2     ETHANOL
  3.148 VV   I 1.19237e5     1.00000 1.20000e-2     ACETONITRILE

Totals without ISTD(s) :              9.34525e-2

 Results obtained with enhanced integrator!
==============================================================================
                          *** End of Report ***
```

ANALYSIS OF BLOOD FOR COMMON DRUGS OF ABUSE BY GAS CHROMATOGRAPHY USING NITROGEN PHOSPHORUS DETECTORS

INTRODUCTION:

The presence of nitrogen in the structure of most drugs facilitates the detection of these compounds using a gas chromatograph equipped with nitrogen-phosphorus detectors. The purpose of this method is to screen a blood specimen for a large number of common neutral and basic drugs of abuse (excluding morphine, dilaudid, thc, and benzoylecgonine). The method is based upon the principle of liquid / liquid extraction of the drugs from the blood and then identifying them on two (2) g.c. columns by their relative retention times versus an external standard using nitrogen - phosphorus detectors.

INSTRUMENTATION:

Hewlett Packard 5890 Series II. Gas Chromatograph with dual Nitrogen Phosphorus detectors.
Hewlett Packard 7673, Automatic Sampler
Hewlett Packard 3365 Series II, ChemStation

COLUMNS:

12.5 meter J & W DB-17, catalog # 123-1732; film thickness 0.25 microns, internal diameter 0.32 mm.
12.5 meter HP Ultra 1, catalog # 19091A-112; film thickness 0.52 microns, internal diameter 0.32 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw caps for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro insert, 0.200ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979
Transfer pipets, Fisher Scientific Catalog # 13-711-7

REAGENTS:

Blank whole blood
Methanol
Hexane
N-butyl Chloride
Sodium borate
Sodium hydroxide
Ethanol - 200 proof
Sulfuric acid - concentrated
Drug standards

Prepare the following:

1. 500ml of saturated aqueous sodium borate solution at room temperature
2. 250ml of 1:1 hexane:ethanol solution
3. 500ml of 1 N sulfuric acid
4. Stock solutions of drugs to be tested (2.5mg/ml free drug in meoh)
5. Working solution of drugs to be tested (5.0 ng/ul free drug in 1:1 hexane:ethanol).
    a. Place 5.0ml hexane:ethanol in screw cap tube.
    b. Add 10ul of stock solution
6. 250ml 10 N NaOH
7. Reference standard (5.0 ng/ul of methamphetamine, pcp, iprindole, alprazolam, and strychnine in 1:1 hexane:ethanol).
    a. Pipet 20ul of each stock solution into 10ml volumetric flask.
    b. Fill to mark with 1:1 hexane:ethanol.

CALIBRATION:

1. From the "Sequence" menu load "calib.seq"and "OK"..
2. From the "Sequence" menu click on "Edit Sequence Parameters"change the subdirectory to reflect the date and "OK.
3. Place vial of Reference standard in space #1.
4. From the "RunControl" menu click on "Run Sequence".
5. After the run is completed; from the "Method" menu click on "Edit Run Time Checklist". *
6. Clear "Parameter" box and add "/r" and "OK"..
7. From the "Method" menu click on "Save".
8. From the "Sequence" menu click on "Edit Sequence Parameters".
9. In the "Part of methods to run" box click on the "Reprocessing only" button and "OK".
10. From the "RunControl" menu click on "Run Sequence".
11. After the reprocessing is over, from the "Method" menu click on "Edit Run Time Checklist".

CALIBRATION (cont.):

12. Clear the "Parameter" box , add "/a c:\hpchem\front2.txt c:\hpchem\rear2.txt" and "OK".
13. From the "Method" menu click on "Save" and "Save".

* The chromatograph should have five integrated peaks. If more peaks are present, small extraneous peaks can be removed by adjusting the "Initial Area Reject" value in the "Integration Events" menu and reprocessing the data.

PROCEDURE:

1. Pipet 2.0ml sample, blank blood and control blood into tubes. The control blood is made by taking 2.0ml of blank blood and adding drugs of interest.
2. Pipet 500ng iprindole internal standard (100ul of 5 ng/ul).
3. Pipet 2.0ml pH 9.5 saturated borate buffer to each sample and vortex.
4. Pipet 10ml N-butyl chloride into each tube, cap and extract for 10 minutes.
5. Centrifuge for approx. 5 minutes. **
6. Transfer the butyl chloride (top) layer to a second tube.
7. Pipet 2.0ml of 1N sulfuric acid, cap and extract for 5 minutes.
8. Centrifuge for approx. 5 minutes and discard butyl chloride (top) layer.
9. Pipet 5.0ml hexane into each tube, cap and extract for 5 minutes.
10. Centrifuge for approx. 5 minutes and discard hexane (top) layer.
11. Check the pH of the aqueous phase (it should be acidic).
12. Add 10 N NaOH (approx. 6-8 drops) until the pH is basic (greater than 9).
13. Pipet 10ml butyl chloride into each tube, cap and extract for 5 minutes.
14. Centrifuge for approx. 5 minutes.
15. Transfer butyl chloride (top) layer into centrifuge tube.
16. Evaporate under a gentle stream of nitrogen at 37 C to near dryness.
17. Finish drying under nitrogen at room temperature. As each sample dries, immediately add 50ul of 1:1 hexane;ethanol to the residue and vortex.
18. Transfer the extract to an insert in an auto sampler vial and crimp.
19. Run on NP g.c. using NPBLOOD method.
20. Run hexane:ethanol wash between each case sample.

** For clean samples proceed to step 15.

INTERPRETATION OF RESULTS:

1. The relative retention times of the peaks are compared to the relative retention times from the list of standards.
2. The control blood should be positive for the drugs spiked in it.
3. The blank blood should be negative (but positive for the internal standard).
4. Any standards run should have relative retention times comparable to the list.
5. Run positive samples on GCMS for confirmation.

METHOD REFERENCE:

"A Rapid, Comprehensive Screening Procedure for Basic Drugs in Blood of Tissues by Gas Chromatography" by Foerster, Hatchett and Garriott. Journal of Analytical Toxicology, Vol. 2, pgs. 50-55.

0.820,amphetamine
0.929,phentermine
0.996,methamphetamine
100,methamphetamine
117.743,phenylpropanolamine
123.902,chlorphentermine
125.385,ephedrine
128.915,nicotine?
139.836,phendimetrazine
150.183,carisprodol
186.001,caffeine
196.002,diphenhydramine
196.005,fluoxetine
196.807,lidocane
200,phencyclidine
200.688,theophyline
206.165,carbamazepine
222.696,procane
251.251,methaqualone
251.540,dextromethorphan
254.609,methadone
264.233,cocaine
265.400,amitriptyline
265.518,dextropropoxyphene
268.560,nortriptyline
271.940,imipramine
273.293,doxepin
275.124,desipramine
293.734,oxazepam*
295.564,oxazepam*
300,iprindole
306.078,codeine
311.487,lorazepam
317.004,desalkuflurazepam
318.090,diazepam
318.402,hydrocodone
327.822,desmethyldiazepam
331.919,chlordiazepoxide
333.683,oxycodone
359.363,prazepam
371.600,fentanyl
380.298,flurazepam
381.481,quinine?
400,alprazolam
407.940,haliperidol
500,strychnine

0.821,amphetamine
0.956,phentermine
100,methamphetamine
125.266,chlorphentermine
126.675,nicotine
130.032,phenylpropanolamine
133.057,ephedrine
144.633,phendimetrazine
153.212,carisprodol*
193.383,fluoxetine
200,phencyclidine
204.998,diphenhydramine
205.757,lidocane
210.500,carisprodol*
220.733,caffeine
240.369,carbamazepine*
246.686,procane
254.671,methadone
257.778,dextromethorphan
258.979,theophyline
261.620,dextropropoxyphene
271.400,amitriptyline
279.804,imipramine
283.012,nortriptyline
284.247,doxepin
289.238,cocaine
289.715,methaqualone
292.874,desipramine
300,iprindole
315.714,oxazepam
323.655,codeine
328.271,lorazepam
333.066,diazepam
336.283,hydrocodone
338.543,desalkulflurazepam
344.738,desmethyldiazepam
344.822,oxycodone
347.137,chlordiazepoxide
353.292,fentanyl
354.297,flurazepam
357.116,prazepam
365.021,quinine?
375.951,haliperidol
400,alprazolam
500,strychnine

TOXICOLOGY UNIT

Standard Operating Procedure

SCREENING BY ENZYME IMMUNOASSAY OF WHOLE BLOOD

PRINCIPLE

The micro-plate enzyme immunoassay is a competitive immunoassay for the qualitative determination of drugs in biological specimens. Sample or calibrator/control is added to each well along with enzyme-labeled hapten derivative. There is a competition to bind the antibody fixed onto the well. The wells are washed, substrate is added, and color is produced. The absorbance produced (450nm) is inversely proportional to the amount of drug present in the sample or control/calibrator.

SPECIMEN REQUIREMENT

1 milliliter (mL) of sample is used for the analysis

EQUIPMENT

This procedure uses the following laboratory equipment and supplies:

- 5 mL disposable plastic culture tubes
- 75 mL plastic containers from Bio-Chem
- 35 mL plastic containers from Bio-Chem
- 5 mL plastic cups from Bio-Chem
- 3.5 mL plastic transfer pipettes
- disposable plastic pipette tips

Screening by Enzyme Immunoassay, continued

STC Kit Reagents

This procedure uses the following reagents provided in the kits from STC:

- micro-plates coated with anti-drug antibodies
- enzyme conjugate
- substrate reagent
- stopping reagent

Prepared Reagents and Controls

Package insert for the proper preparation of the following reagents and standards:

- STC negative calibrator
- STC negative control
- STC cutoff calibrator
- STC positive control

Quality Control

The following quality control (QC) samples are analyzed with every batch of unknowns:

- 15/50 ng/ml QC in human blood
- 30/100 ng/ml QC in human blood
- 60/200 ng/ml QC in human blood

Sample Preparations

The samples are prepared as follows:

1. Label 5 mL disposable culture tubes for each sample and QC.
2. Aliquot 1 mL of sample of QC into the corresponding labeled tube.
3. Place samples in carousel on the Labo-Tech instrument.

Screening by Enzyme Immunoassay, continued

Instrument Preparation	The instrument is prepared daily by: 1. Filling wash bottle 1, 2 and 3 with distilled water. 2. Fill pipette tip tray with disposable tips. 3. Check printer paper—you need at least 25 sheets.
Instrument	1. Turn on the computer, monitor and printer by pressing power switches. 2. Computer will initialize and a Labo-Tech logo will fill the screen. At this point hit **ENTER.** 3. The computer will prompt you to turn the instrument on. Turn the power switch located on the back right hand side of the instrument to on. 4. The instrument will prompt you for doing a self-test. Hit **ENTER** for yes and perform a self-test.
Instrument Daily Maintenance	The instrument will have the following daily maintenance performed before samples are analyzed: • Drawer loading • Syringe filling • Wash head filling
Drawer Loading	To perform the drawer loading perform the following steps: 1. When the "Drawer Loading" text is highlighted in red, hit **ENTER.** 2. The instrument will then prompt you to open the drawer. Hit **C** to continue. 3. The instrument will then prompt you to empty the tips waste. Empty the tips and hit **C** to continue. 4. You are now done with drawer loading.

Syringe Filling

To fill the syringes and the lung the following steps must be done:

1. Using the arrow key, scroll down until "Syringe Filling" is highlighted in red. Hit **ENTER**.
2. At the filling screen there will be two choices, "syringes" and "lung". By default syringes will be highlighted. Hit **ENTER** to fill syringes.
3. The instrument will begin pumping water through the syringes. When no air bubbles are detected in either syringe, hit any key on the keyboard to stop the process.
4. At the filling menu, use the arrow key to scroll down to the "Lung" choice and highlight it in black. Hit **ENTER** to fill the lung.
5. You will be asked if you need to fill the lung. Press **Y** if it needs filling and **N** if it doesn't.
6. If **Y** is pressed, the syringe will fill and then a prompt will tell you to press **ENTER** to fill. It will then prompt you with "are you sure". Hit **Y** for yes.
7. Fill the lung using step 6 repeatedly until the level falls between the two lines marked on the outside of the lung.
8. When through filling, hit **ESC** to leave the lung menu.
9. Hit **ESC** again to return to main menu.

Wash Head Filling

To fill the wash head, the following steps must be performed:

1. Using the arrow key, scroll down until "Wash Head Filling" is highlighted in red. Hit **ENTER.**
2. A pop-up screen for washing solution will appear. The default will be "Washing solution tank 1". Hit **ENTER** to use this tank.
3. After 3 cycles the instrument will have flushed the wash head and stop on its own.
4. Hit **ESC** to return to the main menu.

Profile Selection

To begin the analysis a profile is selected by:

1. Using the arrow key, scroll down to "Select a Profile". Hit **ENTER.**
2. Use the arrow key to highlight the profile to be performed. Hit **ENTER.**
3. The instrument will now indicate the position of the reagents on the instrument rack.

Reagent Cup and Containers

To insure proper placement of reagents the following steps are used:

1. All cups are marked for either blood or urine, drug name and numbered according to their positions on the instrument rack.
2. Using the computer screen as a template, place control and calibrator cups into their designated location by number on the instrument rack.
3. All 35 mL and 75 mL containers are marked for either blood or urine, drug name and numbered according to their designated location on the instrument rack.
4. Using the computer screen as a template, place containers into their designated location by number on the instrument rack.
5. When finished placing cups and containers into the rack, hit **ESC**.
6. A microplate view will appear on the screen. Hit **ESC** to return to main menu.

Sample Loading The instrument is told how many samples to run by performing the following steps:

1. From the main menu use the arrow key to scroll down to "Operating menu". Hit **ENTER**.
2. The screen will give you three choices: "Assay Processing", "Sample Loading" and "Printouts". Use the arrow key to scroll down to "Sample Loading". Hit **ENTER**.
3. The screen will give three choices: "Analysis", "Samples" and "Quality Control". The default is "Analysis" and it will be highlighted. Hit **ENTER.**
4. The screen will have a table on it with the assay name on top and sample number along the side. There will be D's in all the boxes, which means the last run is done for that sample and that particular protocol. Hit the **F1** to clear the table.
5. Hit **ENTER** for each protocol to be run on each sample. An **X** will be placed in the table boxes designating which protocol will be run. Do not include the STC controls in the sample list, only patient and in-house QC's.
6. When the proper number of samples is entered into the table, save the table by pressing **F10**.
7. You will get another micro-plates view that shows how many wells or strips are needed to do the analysis including all controls and calibrators and which drugs are positioned where on the plates. Before continuing, load the strips in the carriages.

Microplate Strip Loading

Before leaving the sample loading menu, the microplate strips must be loaded using the following steps:

1. Following the guidelines on the screen, load as many wells or strips that are needed for a particular protocol onto an empty strip tray.
2. Load one drug at a time only, so as not to mix up the strips.
3. Press down on the wells/strips to insure they are seated firmly into the tray, otherwise during the mixing step they will pop up and jam the instrument (strips may be taped into place).
4. The strips are loaded into the tray with the tray positioned with A1 at the top right hand corner.
5. After loading the first tray, place it into the 1st carriage on the instrument.
6. Load the second tray and place it into the 2nd carriage on the instrument.
7. After the trays are loaded into the carriages, hit **C** to continue.

Reagent Loading

The proper reagents will be loaded into their respective wells using the following steps:

1. After hitting **C** in the above section, a diagram of the instrument reagents will be provided, listing where reagents are located and how much of each is needed for the analysis.
2. All STC calibrator, cutoff, conjugate and control bottles are labeled with type of specimen, drug and instrument position by number.
3. Using a plastic transfer pipette, transfer the proper reagents to the proper cups located on the instrument rack.
4. Use a separate pipette for each reagent to avoid contamination.
5. Each drug has it's own unique cutoff, low control, high control, and conjugate. The diluent, negative calibrator, substrate and stop reagent are common for all drug protocols.
6. After all reagents have been dispensed, hit **ESC**.
7. At the next menu, "Introduction For" hit **ESC** to return to "Operating Menu".

ASSAY PROCESSING

After the samples and reagents have been loaded, the assay process starts by:

1. At the "Operating Menu" use the arrow key to scroll up to "Assay processing". Hit **ENTER.**
2. A screen with two choices will pop up: "Automatic" and "Semi-Automatic".
3. Using the arrow key scroll up to "Automatic". Hit **ENTER.**
4. A screen will appear that states reading in process. At this point the sample ID numbers will be entered. They can be entered either manually or by scanning the barcode of the sample.
5. To enter the ID manually, first hit the **NUM LOCK** button.
6. The screen cursor will be positioned at sample number 1. Enter the sample ID (i.e. C9800001). Hit **ENTER** to accept the number. Proceed with the rest of the samples manually.
7. The system will know how many samples you are supposed to be running from the number of samples you loaded during sample loading. When all the IDs of these samples have been entered, the computer will beep and refresh the screen. Now present on the screen will be a complete list of sample IDs and their position.
8. If there is an error in the ID number, it can be changed. Hit **C** for correct. You will be prompted for a sample number. Enter the position number of the incorrect sample. Hit **ENTER**. It will display the old sample number. Enter the new sample number and hit **ENTER** to accept.
9. When all samples have been correctly entered, hit **F10** to save the list.
10. When F10 is hit at this step, the analysis will begin. The instrument needs to be fully loaded with all samples and reagents. If the microstrips are missing the instrument will prompt for them to be added. **It will not prompt you for missing samples.**

Screening by Enzyme Immunoassay, continued

Time Delay

After looking for any missing microstrips, the instrument will ask you if you would like to insert a time delay between plates. Hit **N** for no. The instrument will now begin the analysis.

Post-Run

To prepare for data analysis use the following steps:

1. After the assay is complete, the screen will have all "end incubation" on the counters for both plates. The highlighted text will be at the bottom for all four protocol lists. Hit **ESC** to return to operating menu.

Data Analysis

The data is analyzed using the following steps:

1. At the "Operating menu", use the arrow key to scroll down to "Printouts". Hit **ENTER.**
2. The next screen will have five choices listed at the top: "Protocol Report", "Patient Report", "Archives", "Utility", "Exit". To move between these choices use the arrow key.
3. In the "Printouts" menu (which you are currently in) use the arrow keys to scroll to "Protocol Report". Hit **ENTER.**
4. The default will be "Single Protocol Report". Highlight this selection and hit **ENTER.**
5. A screen will appear with the list of protocols listed separately and one choice with entire. Use the arrow key to scroll down to "Entire". Hit **ENTER**. The reports will be printed out for each assay with the controls and samples listed. Flip printer paper towards front to prevent paper jam.
6. After printing is complete the same screen will be present. Using the **TAB** key move the cursor to EXIT and it will have white parentheses surrounding it (two hits on the TAB key). Hit **ENTER** to exit from this.
7. To print a table of results, use the arrow key to move to "Patient Report" and hit **ENTER.**
8. Use the arrow key to scroll down to "Results Table" and hit **ENTER.**

Archiving Data

The following steps will save the data:

1. At the "Printouts" screen, use the arrow key to move to "Archives". Hit **ENTER.**
2. Use the arrow key to scroll down to "Store in the Archive". Hit **ENTER.**
3. The following message will appear: "The archive will be updated with data of current profile: xxxxx)" where xxxxx stands for the profile that was just used for this analysis. Hit **ENTER** to continue.

Additional Runs Same Profile

To perform another run with different samples using the same profile, follow these steps:

1. Remove tubes containing the analyzed blood samples. Dispose of them in a biohazard cntainer.
2. Place new set of samples into the carousel on the instrument.
3. Remove microplate trays from the carriages. Pop out the strips from behind into the sink. Wash the strips out with plenty of water and dispose.
4. Follow above procedure from "Sample Loading" step (page 6).

Screening by Enzyme Immunoassay, continued

New Profile Same Day

To run a different profile on the same day on the same samples follow these steps:

1. After analysis is complete from profile just ran, remove microstrip plates from the carriages. Pop out the microstrips from behind into the sink. Wash the strips out with plenty of water and dispose.
2. Remove the calibrator, control and conjugate cups/containers one drug at a time and using a clean plastic pipette, transfer each into its respective container.
3. Wash out all plastic containers with distilled water and set aside to dry.
4. From the "Operating Menu" hit **ESC** to return to "Main menu".
5. Follow above procedure from "Profile Selection" (page 5).

Cleanup

The following steps need to be performed at the end of the day:

1. All reagents, diluent, start and stop must be returned to their original containers by transferring them with plastic disposable pipettes.
2. All plastic cups and containers must be rinsed out and set out to dry.
3. All microstrips must be rinsed and disposed of.
4. All blood and urine samples must be disposed in biohazard containers.

Instrument Shutdown

The following must be done to shut down the instrument:

1. Make sure all data has been transferred for the day.
2. From the Main Menu, use the arrow key to scroll down to "End of Work". Hit **ENTER.**
3. The instrument will pump water through the syringes and prompt you with "Drawer unlocked to work-end". Hit **C** to continue.
4. You will now be prompted with "Hydraulic Circuit Cleaning". Use the arrow key to scroll down to "Washing Solution Tank 2". Hit **ENTER.**
5. After it cycles 2 times, hit any key to stop it.
6. Hit **ESC** to end the "Hydraulic Circuit Cleaning".
7. You will be prompted to empty the waste tank now. Hold in the button located on the left side of the Lab-Tech instrument. This is a gravity flow waste system and the button must be held in for it to fully empty. After waste has emptied, hit **C** to continue.
8. You will now be prompted to turn off the instrument. Turn off the powder switch located on the right hand side of the Labo-Tech instrument. Hit **C** to continue.
9. You will now be at the DOS prompt. Turn off the computer and monitor at this time.
10. Empty biohazard waste receptacle located on floor under instrument.

Important Stuff The following things **cannot** be done:

- Mix different lot numbers of controls and microstrips. When you have used all the microstrips for a particular kit, the reagents must be disposed of also.
- Mixing of any controls or calibrators with any other lot numbers.
- Mixing of any controls or calibrators of one drug with a different drug (one negative serum calibrator may be used for all samples).

References The following references were used for this procedure:

1. Labo-Tech Automated Microplate Analyzer, Operation Guidelines, Rev. 4.
2. STC package forensic application notes for enzyme immunoassay kits.
3. Sacramento County Laboratory of Forensic Services - Standard Operating Procedures.

Washing Volume	: 300
Number of Cycles	: 6
Soak Time	: 1
Test Procedure	: DISP. DILUENT
	DISP. STANDARDS
	DISP. SAMPLES
	DISP. CONJUGATE
	0h 30m @ 0°°C
	WASHING
	DISP. SUBSTRATE
	0h 30m @ 0°°C
	DISP. STOP
	READING
Calculation Method	: CUTOFF
Reading Filter	: 450 - double beam measure -
Cutoff Formula	: ((c5+c6)/2)
Controls Validation :	
- NEG	:)1.2
- NEG	: (2.7
- QC1	: (((n1+n2)/2)
- QC1	:)((c5+c6)/2)
- POS	: (((c5+c6)/2)
Test Validation :	
- Positives mean value	:
- Negatives mean value	:
- Pos./Neg. Difference	:
- Cutoff	:

:\1

Date 19-08-1997
Time 13,54
Pag. 1

Protocol ID : SERUMDOA
Number of Standards or Controls : 4
Name of Standards or Controls : NEG
QC1
CUTOFF
POS

Number of Blanks : 0
Immunocontrol Strip : NO
Number of Reagents : 4
Name of Reagents : DILUENT
CONJUGATE
SUBSTRATE
STOP

Mother Rack : MUD-100
Daughter Rack : MICRO-8
Predilutions : NO
Volumes to dispense (ul.):

	NEG	QC1	CUTOFF	POS	SAMPLES	TIP
NEG	25	0	0	0	0	NEEDLE
QC1	0	25	0	0	0	NEEDLE
CUTOFF	0	0	25	0	0	NEEDLE
POS	0	0	0	25	0	NEEDLE
SAMPLES	0	0	0	0	25	NEEDLE
DILUENT	50	50	50	50	50	PLAST.
CONJUGATE	100	100	100	100	100	PLAST.
SUBSTRATE	100	100	100	100	100	PLAST.
STOP	100	100	100	100	100	PLAST.
REPLICATES	2	2	2	2	2	

To: Bob Martin, Laboratory Manager

From: Stuart V. Jacobson

Subject: STC Printouts

Date: June 19, 1998

In response to the ASCLD deficiency regarding STC drug screen printouts in blood toxicology cases (section 1.4.2.14) I now make a copy of the relevant printouts and include them in the case file. I believe that this action corrects the deficiency. A copy of this memo will be placed in the Blood Toxicology manual.

Respectfully submitted;

Stuart V. Jacobson
Senior Criminalist

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Date 09-07-1999
Time 09,30
Pag. 1

Protocol ID	: S-METH
Number of Standards or Controls	: 4
Name of Standards or Controls	: NEG
	QC1
	CUTOFF
	POS
Number of Blanks	: 0
Immunocontrol Strip	: NO
Number of Reagents	: 4
Name of Reagents	: DILUENT
	CONJUGATE
	SUBSTRATE
	STOP
Mother Rack	: MUD-100
Daughter Rack	: MICRO-8
Predilutions	: NO

Volumes to dispense (ul.):

	NEG	QC1	CUTOFF	POS	SAMPLES	TIP
NEG	25	0	0	0	0	NEEDLE
QC1	0	25	0	0	0	NEEDLE
CUTOFF	0	0	25	0	0	NEEDLE
POS	0	0	0	25	0	NEEDLE
SAMPLES	0	0	0	0	25	NEEDLE
DILUENT	50	50	50	50	50	PLAST.
CONJUGATE	100	100	100	100	100	PLAST.
SUBSTRATE	100	100	100	100	100	PLAST.
STOP	100	100	100	100	100	PLAST.
REPLICATES	2	2	2	2	2	

Washing Volume	: 300
Number of Cycles	: 6
Soak Time	: 1
Test Procedure	: DISP. DILUENT DISP. STANDARDS DISP. SAMPLES DISP. CONJUGATE 0h 30m a 0°°C WASHING DISP. SUBSTRATE 0h 30m a 0°°C DISP. STOP READING
Calculation Method	: CUTOFF
Reading Filter	: 450 - double beam measure -
Cutoff Formula	: ((c5+c6)/2)
Controls Validation :	
- NEG	:).7
- NEG	: (2.7
- QC1	: (((n1+n2)/2)
- QC1	:)((c5+c6)/2)
- CUTOFF	: (0.5*((n1+n2)/2)
- POS	: (0.85*((c5+c6)/2)
Test Validation :	
- Positives mean value	:
- Negatives mean value	:
- Pos./Neg. Difference	:
- Cutoff	:

Date 09-07-1999
Time 09:30
Pag. 1

Protocol ID : S-OPIATE
Number of Standards or Controls : 4
Name of Standards or Controls : NEG
QC1
CUTOFF
POS
Number of Blanks : 0
Immunocontrol Strip : NO
Number of Reagents : 4
Name of Reagents : DILUENT
CONJUGATE
SUBSTRATE
STOP
Mother Rack : MOD-100
Daughter Rack : MICRO-8
Predilutions : NO
Volumes to dispense (ul.):

	NEG	QC1	CUTOFF	POS	SAMPLES	TIP
NEG	25	0	0	0	0	NEEDLE
QC1	0	25	0	0	0	NEEDLE
CUTOFF	0	0	25	0	0	NEEDLE
POS	0	0	0	25	0	NEEDLE
SAMPLES	0	0	0	0	25	NEEDLE
DILUENT	50	50	50	50	50	PLAST.
CONJUGATE	100	100	100	100	100	PLAST.
SUBSTRATE	100	100	100	100	100	PLAST.
STOP	100	100	100	100	100	PLAST.
REPLICATES	2	2	2	2	2	

Washing Volume	: 300
Number of Cycles	: 6
Soak Time	: 1
Test Procedure	: DISP. DILUENT DISP. STANDARDS DISP. SAMPLES DISP. CONJUGATE 0h 30m a 00°C WASHING DISP. SUBSTRATE 0h 30m a 00°C DISP. STOP READING
Calculation Method	: CUTOFF
Reading Filter	: 450 - double beam measure -
Cutoff Formula	: ((c5+c6)/2)
Controls Validation :	
- NEG	:)0.7
- NEG	: (2.7
- QC1	: ((n1+n2)/2)
- QC1	:)((c5+c6)/2)
- CUTOFF	: (0.5*((n1+n2)/2)
- POS	: (0.85*((c5+c6)/2)
Test Validation :	
- Positives mean value	:
- Negatives mean value	:
- Pos./Neg. Difference	:
- Cutoff	:

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Date 09-07-1995
Time 09:13
Pag. 1

Protocol ID : S-AMPH
Number of Standards or Controls : 4
Name of Standards or Controls : NEG
QC1
CUTOFF
POS

Number of Blanks : 0
Immunocontrol Strip : NO
Number of Reagents : 4
Name of Reagents : DILUENT
CONJUGATE
SUBSTRATE
STOP

Mother Rack : MOD-100
Daughter Rack : MICRO-8
Predilutions : NO
Volumes to dispense (ul.):

	NEG	QC1	CUTOFF	POS	SAMPLES	TIP
NEG	25	0	0	0	0	NEEDLE
QC1	0	25	0	0	0	NEEDLE
CUTOFF	0	0	25	0	0	NEEDLE
POS	0	0	0	25	0	NEEDLE
SAMPLES	0	0	0	0	25	NEEDLE
DILUENT	50	50	50	50	50	PLAST.
CONJUGATE	100	100	100	100	100	PLAST.
SUBSTRATE	100	100	100	100	100	PLAST.
STOP	100	100	100	100	100	PLAST.
REPLICATES	2	2	2	2	2	

Washing Volume	: 300
Number of Cycles	: 6
Soak Time	: 1
Test Procedure	: DISP. DILUENT
	DISP. STANDARDS
	DISP. SAMPLES
	DISP. CONJUGATE
	0h 30m a 0°°C
	WASHING
	DISP. SUBSTRATE
	0h 30m a 0°°C
	DISP. STOP
	READING
Calculation Method	: CUTOFF
Reading Filter	: 450 - double beam measure -
Cutoff Formula	: ((c5+c6)/2)
Controls Validation :	
- NEG	:).7
- NEG	: (2.7
- QC1	: (((n1+n2)/2)
- QC1	:)((c5+c6)/2)
- CUTOFF	: (0.5*((n1+n2)/2)
- POS	: (0.85*((c5+c6)/2)
Test Validation :	
- Positives mean value	:
- Negatives mean value	:
- Pos./Neg. Difference	:
- Cutoff	:

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Date 04-07-1994
Time 09:13
Pag. 1

Protocol ID : S-BARBS
Number of Standards or Controls : 4
Name of Standards or Controls : NEG
QC1
CUTOFF
POS

Number of Blanks : 0
Immunocontrol Strip : NO
Number of Reagents : 4
Name of Reagents : DILUENT
CONJUGATE
SUBSTRATE
STOP

Mother Rack : MOD-100
Daughter Rack : MICRO-8
Predilutions : NO
Volumes to dispense (ul.):

	NEG	QC1	CUTOFF	POS	SAMPLES	TIP
NEG	25	0	0	0	0	NEEDLE
QC1	0	25	0	0	0	NEEDLE
CUTOFF	0	0	25	0	0	NEEDLE
POS	0	0	0	25	0	NEEDLE
SAMPLES	0	0	0	0	25	NEEDLE
DILUENT	50	50	50	50	50	PLAST.
CONJUGATE	100	100	100	100	100	PLAST.
SUBSTRATE	100	100	100	100	100	PLAST.
STOP	100	100	100	100	100	PLAST.
REPLICATES	2	2	2	2	2	

Washing Volume	: 300
Number of Cycles	: 6
Soak Time	: 1
Test Procedure	: DISP. DILUENT
	DISP. STANDARDS
	DISP. SAMPLES
	DISP. CONJUGATE
	0h 30m a 0°°C
	WASHING
	DISP. SUBSTRATE
	0h 30m a 0°°C
	DISP. STOP
	READING
Calculation Method	: CUTOFF
Reading Filter	: 450 - double beam measure -
Cutoff Formula	: ((c5+c6)/2)
Controls Validation :	
- NEG	:).7
- NEG	: (2.7
- QC1	: (((n1+n2)/2)
- QC1	:)((c5+c6)/2)
- CUTOFF	: (0.5*((n1+n2)/2)
- POS	: (0.85*((c5+c6)/2)
Test Validation :	
- Positives mean value	:
- Negatives mean value	:
- Pos./Neg. Difference	:
- Cutoff	:

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Date 09-07-1999
Time 09, 13
Pag. 1

Protocol ID : S-BENZO
Number of Standards or Controls : 4
Name of Standards or Controls : NEG
QC1
CUTOFF
POS

Number of Blanks : 0
Immunocontrol Strip : NO
Number of Reagents : 4
Name of Reagents : DILUENT
CONJUGATE
SUBSTRATE
STOP

Mother Rack : MOD-100
Daughter Rack : MICRO-8
Predilutions : NO
Volumes to dispense (ul.):

	NEG	QC1	CUTOFF	POS	SAMPLES	TIP
NEG	25	0	0	0	0	NEEDLE
QC1	0	25	0	0	0	NEEDLE
CUTOFF	0	0	25	0	0	NEEDLE
POS	0	0	0	25	0	NEEDLE
SAMPLES	0	0	0	0	25	NEEDLE
DILUENT	50	50	50	50	50	PLAST.
CONJUGATE	100	100	100	100	100	PLAST.
SUBSTRATE	100	100	100	100	100	PLAST.
STOP	100	100	100	100	100	PLAST.
REPLICATES	2	2	2	2	2	

Washing Volume	: 300
Number of Cycles	: 6
Soak Time	: 1
Test Procedure	: DISP. DILUENT
	DISP. STANDARDS
	DISP. SAMPLES
	DISP. CONJUGATE
	0h 30m a 0°°C
	WASHING
	DISP. SUBSTRATE
	0h 30m a 0°°C
	DISP. STOP
	READING
Calculation Method	: CUTOFF
Reading Filter	: 450 - double beam measure -
Cutoff Formula	: ((c5+c6)/2)
Controls Validation :	
- NEG	:).7
- NEG	: (2.7
- QC1	: (((n1+n2)/2)
- QC1	:)((c5+c6)/2)
- CUTOFF	: (0.5*((n1+n2)/2)
- POS	: (0.85*((c5+c6)/2)
Test Validation :	
- Positives mean value	:
- Negatives mean value	:
- Pos./Neg. Difference	:
- Cutoff	:

Date 09-07-1999
Time 09:29
Pag. 1

Protocol ID	: S-CANNAB
Number of Standards or Controls	: 4
Name of Standards or Controls	: NEG
	QC1
	CUTOFF
	POS
Number of Blanks	: 0
Immunocontrol Strip	: NO
Number of Reagents	: 4
Name of Reagents	: DILUENT
	CONJUGATE
	SUBSTRATE
	STOP
Mother Rack	: MOD-100
Daughter Rack	: MICRO-8
Predilutions	: NO

Volumes to dispense (ul.):

	NEG	QC1	CUTOFF	POS	SAMPLES	TIP
NEG	25	0	0	0	0	NEEDLE
QC1	0	25	0	0	0	NEEDLE
CUTOFF	0	0	25	0	0	NEEDLE
POS	0	0	0	25	0	NEEDLE
SAMPLES	0	0	0	0	25	NEEDLE
DILUENT	50	50	50	50	50	PLAST.
CONJUGATE	100	100	100	100	100	PLAST.
SUBSTRATE	100	100	100	100	100	PLAST.
STOP	100	100	100	100	100	PLAST.
REPLICATES	2	2	2	2	2	

Washing Volume	: 300
Number of Cycles	: 6
Soak Time	: 1
Test Procedure	: DISP. DILUENT DISP. STANDARDS DISP. SAMPLES DISP. CONJUGATE 0h 30m a 0°°C WASHING DISP. SUBSTRATE 0h 30m a 0°°C DISP. STOP READING
Calculation Method	: CUTOFF
Reading Filter	: 450 - double beam measure -
Cutoff Formula	: ((c5+c6)/2)
Controls Validation :	
- NEG	:).7
- NEG	: (2)
- QC1	:)((m1+m2)/2)
- QC1	:)((c5+c6)/2)
- CUTOFF	: (0.5*((m1+m2)/2)
- POS	: (0.85*((c5+c6)/2)
Test Validation :	
- Positives mean value	:
- Negatives mean value	:
- Pos./Neg. Difference	:
- Cutoff	:

Date 09-07-1999
Time 09.29
Pag. 1

Protocol ID	: S-COC
Number of Standards or Controls	: 4
Name of Standards or Controls	: NEG
	QC1
	CUTOFF
	POS
Number of Blanks	: 0
Immunocontrol Strip	: NO
Number of Reagents	: 4
Name of Reagents	: DILUENT
	CONJUGATE
	SUBSTRATE
	STOP
Mother Rack	: MOD-180
Daughter Rack	: MICRO-8
Predilutions	: NO

Volumes to dispense (ul.):

	NEG	QC1	CUTOFF	POS	SAMPLES	TIP
NEG	25	0	0	0	0	NEEDLE
QC1	0	25	0	0	0	NEEDLE
CUTOFF	0	0	25	0	0	NEEDLE
POS	0	0	0	25	0	NEEDLE
SAMPLES	0	0	0	0	25	NEEDLE
DILUENT	50	50	50	50	50	PLAST.
CONJUGATE	100	100	100	100	100	PLAST.
SUBSTRATE	100	100	100	100	100	PLAST.
STOP	100	100	100	100	100	PLAST.
REPLICATES	2	2	2	2	2	

Washing Volume	: 300
Number of Cycles	: 6
Soak Time	: 1
Test Procedure	: DISP. DILUENT DISP. STANDARDS DISP. SAMPLES DISP. CONJUGATE 0h 30m a 0°°C WASHING DISP. SUBSTRATE 0h 30m a 0°°C DISP. STOP READING
Calculation Method	: CUTOFF
Reading Filter	: 450 - double beam measure -
Cutoff Formula	: ((c5+c6)/2)
Controls Validation :	
- NEG	: 0.7
- NEG	: 2.7
- QC1	: (((n1+n2)/2)
- QC1	:)((c5+c6)/2)
- CUTOFF	: (0.5*((n1+n2)/2)
- PUS	: (0.85*((c5+c6)/2)
Test Validation :	
- Positives mean value	:
- Negatives mean value	:
- Pos./Neg. Difference	:
- Cutoff	:

FORENSIC SERVICES PROCEDURE MANUAL

BLOOD ALCOHOL ANALYSIS

QUANTITATIVE ALCOHOL ANALYSIS BY
HEADSPACE GAS CHROMATOGRAPHY

I. Equipment:

A. Hewlett Packard 5890 G.C.

B. Hewlett Packard 7694 Headspace Sampler

C. P.C. with Hewlett Packard ChemStation Version A.04.01

D. Micro Lab 500 Series, Auto Dilutor

E. Crimper, Hewlett Packard Cat. #9301-0720

II. Supplies:

A. Septa - Hewlett Packard - Cat. #9301-0976

B. Crimp Caps - Hewlett Packard - Cat. #9301-0721

C. 10 ml Headspace Vials - Hewlett Packard - Cat. #5182-0838

D. Whole Blood Control – ToxiChem- Cat. # 2930-14

E. Acetonitrile - Fisher Scientific

F. Methanol - Fisher Scientific

G. Acetone - Fisher Scientific

H. Isopropyl Alcohol - Fischer Scientific

I. Acetaldehyde - Fischer Scientific

J. .04, .10, .20, .30, Aqueous Ethanol Controls – College of American Pathologists- Cat. # STO11,17,18,19.

K. Mercuric Chloride - Fischer Scientific

L. Megabore INNOWAX 30 Meter Column - Hewlet Packard - Cat. # 19095N-123

II. Supplies (cont.)

M. Megabore DB-624 30 Meter Column - J & W Scientific - Cat. # 1251334

III. Reagent Preparation:

A. Preparation of Internal Standard Solution

1. Prepare 3% V/V acetonitrile stock solution from acetonitrile and deionized water - 30 ml Acetonitrile / liter of water + a pinch of mercuric chloride.

2. Prepare 0.012% W/V working internal standard solution - 5 ml stock solution / liter of water.

B. Preparation of Mixed standard

1. Acetaldehyde 0.25 ml, methanol 1.00 ml, acetone 0.25 ml, isopropyl alcohol 0.25 ml.

2. Mix with 1 liter of water + a pinch of mercuric chloride.

IV. Dilutor Preparation:

A. Check that there is enough internal standard for the analysis

B. Prime dilutor with internal standard (bubbles can be removed by first flushing the dilutor with acetone).

C. Set syringe volumes
1. Reagent = 2000 ul
2. Sample = 250 ul

V. Sample Preparation:

A. Label each sample vial .

B. Aspirate and dispense sample into vial. Prepare in duplicate.

C. Tightly crimp cap and septa onto vial.

D. Between each sample aspirate water (3x) and dispense into waste to rinse tubing. It is not necessary to rinse between duplicates.

VI. Standard, Blank, and Control Preparation:

A. Prepare .04, .10, .20, and .30 standards with aqueous standards using the same procedures as case samples.

B. Prepare blank with water using the same procedure as case samples.

C. Prepare control with known blood using the same procedures as case samples.

D. Prepare Mixed Standard using the same procedures as case samples.

VII. Calibration:

A. From "Sequence" menu click on "Load Sequence"

B. Highlight "calib.seq" and "OK".

C. From the "Sequence" menu click on "Edit Sequence Parameters".

D. Change the "Data File Subdirectory" to reflect the date of analysis and "OK".

E. Place aqueous calibrators (0.04, 0.100, 0.200, 0.300) in proper location on tray.

F. From the "RunControl" menu click on "Run Sequence".

G. From the "View" menu click on "Data Analysis".

H. From the "File" menu click on "Load Signal".

I. Highlight the first file (0.04 g/100 ml) and "OK".

J. From the "Calibration" menu click on "New Calibration Table" and click "OK" on the "Level One" box.

K. Click "yes" on the "Overwrite Existing Calibration Table" box.

L. Complete table by writing in "Name" (Ethanol or acetonitrile), "g/100 ml" (concentration) ,"ISTD" (ethanol-no, acetonitrile-yes), and "#" (be sure acetonitrile # matches ethanol # for each chromatogram.

VII. Calibration (cont.)

M. From the "File" menu click on "Load Signal", highlight the second file (0.100 g/100 ml) and "OK".

N. From the "Calibration" menu click on "Add Level" and "OK" the "Add Level 2" box. Fill in "Name" and "g/100 ml".

O. Repeat until all four levels are complete.

P. From the "View" menu click on "Method and Run Control". Click on "Method" and "Save Method" and "OK" "Overwrite Method". Enter "Recalibrate" in log.

VIII. Run preparation:

A. Place vials in sampler in the following order

1. Aqueous standards (0.04, 0.10, 0.20, 0.30).
2. Mixed standard
3. Blank
4. Blood control in duplicate
5. Case samples in duplicate
6. Blood control (Run a blood control at least every 10 samples).
7. Check standards (0.04, 0.10, 0.20, 0.30)

IX. Headspace and GC Parameters:

A. Carrier pressure - 0.25 bar

B. Vial pressure - 1.70 bar

C. GC Method - Bldalc1.M

D. Headspace Method - Bloodalc.hsm

BLOOD ALCOHOL
QUALITY ASSURANCE ADDENDUM

I. Proficiency Testing:

The laboratory voluntarily participates on a continuous basis, in the following blood alcohol proficiency testing programs administered by independent agencies:

a) U.S. DEPARTMENT OF TRANSPORTATION - NHTSA (National Highway Traffic Safety Administration).

II. Quality Control:

The following rigorous safeguards are employed by each analyst to ensure the validity of their analysis:

a) Blood alcohol analyses are conducted in DUPLICATE. Duplicate values shall be within 0.01 of each other.
b) Complete calibrations are established at the time of the analysis.
c) Final reports are reviewed by another criminalist.
d) Analytical performance is checked at the time of testing via independently acquired control reference materials. Values for standards and controls shall be within 10% of the known value (GC value on blood control).
e) Specimens, while retained in the laboratory, are refrigerated. A chain of custody is maintained on all items while under the control of the Bureau of Forensic Services.

12/28/90 ACS
Revised 11/18/93 SVJ

FORENSIC SECTION PROCEDURE MANUAL

FORENSIC ALCOHOL ANALYSIS

QUANTITATIVE ALCOHOL ANALYSIS BY
HEADSPACE GAS CHROMATOGRAPHY

I. Equipment Used:

1. Hewlett Packard 5890 G.C.

2. Hewlett Packard 19395A Autosampler

3. Hewlett Packard 3396A Integrator

4. Micro Lab 400 Series. Auto Dilutor

II. Preparing Dilutor:

1. Check that there is enough internal standard for the analysis.

2. Place dilutor in "Run" mode.
   A. Set Reagent = 2000 µl.
   B. Set Sample = 250 µl.

III. Preparing Samples:

1. Label each sample vial with Blood Alcohol Number (i.e. CB92-222).

2. Pour approximately 0.5 ml of case sample into a disposable tube.

3. Aspirate and dispense sample into vial. Prepare a separate duplicate sample.

4. Aspirate and dispense water into waste flask.

5. Continue with next sample.

IV. Preparing the Blank and Controls:

1. Prepare the blank (a vial containing room air, internal standard and water).

2. Prepare the controls. The control blood is an independently acquired blood sample containing known

amount of ethyl alcohol and is prepared exactly like a case sample.

V. Preparing Standards:

1. Prepare the standards using the same procedure as the case samples.

2. Use a .10, .20, .30, w/v ethyl alcohol standards and a mixed standard.

VI. Preparing for a Run:

1. Place the vials in the headspace sampler in the following order:

.10, .10, .20, .20, .30, .30, mix, blank, blood control, cases (in duplicate), check standards (.10, .20, .30).

VII. Headspace and GC Parameters:

Headspace analyzer:		GC parameters:	
Headspace method	1	Inlet temp	250 C
Equilibration time	20min	Oven temp	40 C
Bath temp.	40 C	Temp prog	2C/min
Valve/loop temp.	65 C	Detect. temp	250 C
Sampling interval	remote	Final temp	50 C
Last vial number	?		
Method sequence	1		
Vial	1		
Injections/vial	1		
Valve timing	min:sec		
Probe	"01"		
Pressurize	"03"		
Pressurize	"13"		
Vent/fill loop	"14"		

Vent/fill loop	"19"
Inject	"20"
Inject	"30"
Probe	"31"
Carrier flow (He)	1.6 bar
Aux pressure (He)	1.7 bar

VIII. Post Run Analysis:

1. Check analytical results including linearity, accuracy and precision.

2. Record duplicate results of each case sample and report the average of the values.

I. Equipment:

A. Hewlett Packard Instruments:

1. 5890 Gas Chromatograph
2. 19395A Autosampler
3. 3396A Integrator

B. Micro Lab 400 Series. Auto Dilutor

C. Crimper - Hewlett Packard - Cat. #9301-0720

II. Supplies:

A. Septa - Hewlett Packard - Cat. #9301-0976

B. Crimp Caps - Hewlett Packard - Cat. #9301-0721

C. 20 ml Headspace Vials - Hewlett Packard - Cat. #9301-0717

D. Whole Blood Control - Behring Diagnostics - Cat. #860161

E. Acetonitrile - Fisher Scientific

F. Methanol - Fisher Scientific

G. Acetone - Fisher Scientific

H. Isopropyl Alcohol - Fisher Scientific

I. Acetaldehyde - Fisher Scientific

J. .10, .20, .30, Ethanol Controls - High Purity Chemical, Inc. - Cat. #750-053 through 750-057

K. Mercuric Chloride - Fisher Scientific

L. MEGABORE D-B-WAX 30 meter Column - J & W Scientific Cat. #125-7032

III. Reagent Preparation:

A. Preparation of Internal Standard Solution

1. Prepare 3% V/V acetonitrile stock solution from acetonitrile and deionized water

$$\frac{3\text{ml acetonitrile}}{100\text{ ml soln}} \times 1000\text{ml soln} = 30\text{ml acetonitrile/liter of soln}$$

Add a pinch of mercuric chloride

2. Prepare 0.012% W/V working internal standard solution

by diluting stock

$$\frac{0.012\text{g acetone}}{100\text{ ml soln}} \times 2000\text{ml soln} \times \frac{100\text{ml stock}}{3\text{ml acetone}} \times \frac{1\text{ml acetone}}{.8\text{g acetone}}$$

= 10 ml stock for 2 liters working internal standard solution. Add a pinch of mercuric chloride

B. Preparation of Mixed Standard

1. Acetaldehyde 0.02% W/V

$$\frac{.02\text{g acetone}}{100\text{ ml soln}} \times 1000\text{ml soln} \times \frac{1\text{ml acetone}}{.8\text{g acetone}} = 0.25\text{ml acetaldehyde}$$

NOTE: Acetaldehyde and pipette must be cold. Boiling point is 20.8 degrees

2. Methanol 0.08% W/V

$$\frac{0.08\text{g MeOH}}{100\text{ml soln}} \times 1000\text{ ml soln} \times \frac{1\text{ml MeOH}}{.8\text{g MeOH}} = 1.00\text{ ml methanol}$$

3. Acetone 0.02% W/V

$$\frac{.02\text{g acetone}}{100\text{ ml soln}} \times 1000\text{ml soln} \times \frac{1\text{ml acetone}}{.8\text{g acetone}} = 0.25\text{ml acetone}$$

4. Isopropanol 0.02% W/V

$$\frac{.02\text{g IPA}}{100\text{ml soln}} \times 1000\text{ml soln} \times \frac{1\text{ml IPA}}{.8\text{g IPA}} = 0.25\text{ml isopropanol}$$

Add deionized water to 1 liter mark
Add a pinch of mercuric chloride

IV. Vial Preparation:

A. Check internal standard solution to be sure there is enough to complete run

B. Aspirate sample. Dispense sample into headspace vial. Prepare in duplicate

C. Tightly crimp cap and septa onto vial

D. Between each blood sample aspirate water 3 times (3x) and dispense into waste to rinse tubing. It is not necessary to rinse tubing between duplicates.

E. When finished with pump, rinse tubing.

F. Specimen vials are loaded in the sample chamber in the following order:

1. Ethanol standards (0.10%, 0.20%, 0.30% w/v)

2. Mixed standard

3. Blank containing deionized water and internal standard solution.

4. Behring blood control in duplicate

5. Cases in duplicate

6. Check standards

BLOOD ALCOHOL
QUALITY ASSURANCE ADDENDUM

I. Proficiency Testing:

The laboratory voluntarily participates on a continuous basis, in the following blood alcohol proficiency testing programs administered by independent agencies:

a) U.S. DEPARTMENT OF TRANSPORTATION - NHTSA (National Highway Traffic Safety Administration).

II. Quality Control:

The following rigorous safeguards are employed by each analyst to ensure the validity of their analysis:

a) Blood alcohol analyses are conducted in DUPLICATE.
b) Complete calibrations are established at the time of the analysis.
c) Final reports are reviewed by another Criminalist.
d) Analytical performance is checked at the time of testing via independently acquired control reference materials.
e) Specimens, while retained in the laboratory, are refrigerated. A chain of custody is maintained on all items while under the control of the Bureau of Forensic Services.

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0.840,amphetamine
0.931,phentermine
0.996,methamphetamine
100,methamphetamine
110.173,phenylpropanolamine
115.210,chlorphentermine
118.588,ephedrine
128.915,nicotine
128.947,phendimetrazine
138.184,carisprodol*
180.489,caffeine
191.758,carisprodol*
193.510,diphenhydramine
194.461,fluoxetine
194.907,lidocane
200,phencyclidine
200.688,theophyline
206.165,carbamazepine*
221.278,procane
250.337,methaqualone
250.540,dextromethorphan
253.759,methadone
263.698,cocaine
265.205,amitriptyline
265.518,dextropropoxyphene
267.724,nortriptyline
271.235,imipramine
272.515,doxepin
275.124,desipramine
284.431,carbamazepine*
292.925,oxazepam*
295.564,oxazepam*
300,iprindole
305.929,codeine
312.952,lorazepam
316.367,desalkulflurazepam
317.499,diazepam
318.026,hydrocodone
327.760,desmethyldiazepam
331.919,chlordiazepoxide
334.814,oxycodone
359.363,prazepam
371.600,fentanyl
380.298,flurazepam
381.481,quinine
400,alprazolam
500,strychnine

0.903,amphetamine
0.912,methamphetamine
0.956,phentermine
100,methamphetamine
115.400,chlorphentermine
117.641,nicotine
119.113,phenylpropanolamine
119.447,ephedrine
132.996,phendimetrazine
140.346,carisprodol*
191.061,fluoxetine
200,phencyclidine
204.037,diphenhydramine
204.904,lidocane
210.500,carisprodol*
220.092,caffeine
240.369,carbamazepine*
245.932,procane
254.671,methadone
257.778,dextromethorphan
258.979,theophyline
261.620,dextropropoxyphene
270.983,amitriptyline
279.109,imipramine
283.012,nortriptyline
283.875,doxepin
289.238,cocaine
289.715,methaqualone
292.874,desipramine
300,iprindole
314.181,oxazepam
322.382,codeine
322.717,lorazepam
327.025,carbamazepine*
329.962,diazepam
333.493,hydrocodone
335.299,desalkulflurazepam
341.230,desmethyldiazepam
342.215,oxycodone
347.137,chlordiazepoxide
353.292,fentanyl
354.297,flurazepam
359.363,prazepam
365.021,quinine
400,alprazolam
500,strychnine

Blank whole blood
Methanol
Hexane
N-butyl Chloride
Sodium borate
Sodium hydroxide
Ethanol - 200 proof
Sulfuric acid - concentrated
Drug standards

Prepare the following:

1. 500ml of saturated aqueous sodium borate solution at room temperature
2. 250ml of 1:1 hexane:ethanol solution
3. 500ml of 1 N sulfuric acid
4. Stock solutions of drugs to be tested (2.5mg/ml free drug in meoh)
5. Working solution of drugs to be tested (5.0 ng/ul free drug in 1:1 hexane:ethanol).
    a. Place 5.0ml hexane:ethanol in screw cap tube.
    b. Add 10ul of stock solution
6. 250ml 10 N NaOH
7. Reference standard (5.0 ng/ul of methamphetamine, pcp, iprindole, alprazolam, and strychnine in 1:1 hexane:ethanol).
    a. Pipet 20ul of each stock solution into 10ml volumetric flask.
    b. Fill to mark with 1:1 hexane:ethanol.

PROCEDURE:

1. Pipet 2.0ml sample, blank blood and control blood into tubes. The control blood is made by taking 2.0ml of blank blood and adding drugs of interest.
2. Pipet 500ng iprindole internal standard (100ul of 5 ng/ul).
3. Pipet 2.0ml pH 9.5 saturated borate buffer to each sample and vortex.
4. Pipet 10ml N-butyl chloride into each tube, cap and extract for 10 minutes.
5. Centrifuge for approx. 5 minutes.
6. Transfer the butyl chloride (top) layer to a second tube.
7. Pipet 2.0ml of 1N sulfuric acid, cap and extract for 5 minutes.
8. Centrifuge for approx. 5 minutes and discard butyl chloride (top) layer.
9. Pipet 5.0ml hexane into each tube, cap and extract for 5 minutes.
10. Centrifuge for approx. 5 minutes and discard hexane (top) layer.
11. Check the pH of the aqueous phase (it should be acidic).
12. Add 10 N NaOH (approx. 6-8 drops) until the pH is basic (greater than 9).
13. Pipet 10ml butyl chloride into each tube, cap and extract for 5 minutes.
14. Centrifuge for approx. 5 minutes.
15. Transfer butyl chloride (top) layer into centrifuge tube.

16. Evaporate under a gentle stream of nitrogen at 37 C to near dryness.
17. Finish drying under nitrogen at room temperature. As each sample dries, immediately add 50ul of 1:1 hexane;ethanol to the residue and vortex.
18. Transfer the extract to an insert in an auto sampler vial and crimp.
19. Run on NP g.c. using NPBLOOD method.
20. Run hexane:ethanol blanks between each case sample.

INTERPRETATION OF RESULTS:

1. The relative retention times of the peaks are compared to the relative retention times from the list of standards.
2. The control blood should be positive for the drugs spiked in it.
3. The blank blood should be negative (but positive for the internal standard).
4. Any standards run should have relative retention times comparable to the list.
5. The hexane:ethanol blanks between case samples should be negative.
6. Run positive samples on GCMS for confirmation.

METHOD REFERENCE:

"A Rapid, Comprehensive Screening Procedure for Basic Drugs in Blood of Tissues by Gas Chromatography" by Foerster, Hatchett and Garriott. Journal of Analytical Toxicology, Vol. 2, pgs. 50-55.

ANALYSIS OF BLOOD FOR COMMON DRUGS OF ABUSE BY GAS CHROMATOGRAPHY USING NITROGEN PHOSPHORUS DETECTORS

INTRODUCTION:

The presence of nitrogen in the structure of most drugs facilitates the detection of these compounds using a gas chromatograph equipped with nitrogen-phosphorus detectors. The purpose of this method is to screen a blood specimen for a large number of common neutral and basic drugs of abuse (excluding morphine, dilaudid, thc, and benzoylecgonine). The method is based upon the principle of liquid / liquid extraction of the drugs from the blood and then identifying them on two (2) g.c. columns by their relative retention times versus an external standard using nitrogen - phosphorus detectors.

INSTRUMENTATION:

Hewlett Packard 5890 Series II. Gas Chromatograph with dual Nitrogen Phosphorus detectors.
Hewlett Packard 7673, Automatic Sampler
Hewlett Packard 3365 Series II, ChemStation

COLUMNS:

12.5 meter J & W DB-17, catalog # 123-1732; film thickness 0.25 microns, internal diameter 0.32 mm.
12.5 meter HP Ultra 1, catalog # 19091A-112; film thickness 0.52 microns, internal diameter 0.32 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw caps for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro insert, 0.200ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979
Transfer pipets, Fisher Scientific Catalog # 13-711-7

REAGENTS:

AMPHETAMINE/METHAMPHETAMINE BLOOD EXTRACTION AND DERIVATIZATION PROCEDURE

INTRODUCTION:

Sympathomimetic amines are central nervous system stimulants. They produce increased alertness, euphoria, excitement, wakefulness, a reduced sense of fatigue, loss of appetite and an increased feeling of power. They may enhance performance in athletic competition. The drugs may be introduced into the system by smoking, snorting or injection. Sympathomimetic amines may be extracted from biological samples with organic solvents under basic conditions.

INSTRUMENTATION:

Hewlett Packard 5890 Gas Chromatograph
Hewlett Packard 7673A Auto Sampler
Hewlett Packard 5970 Mass Select Detector (MSD)

COLUMN:

15 meter HP Ultra 1, catalog # 19091A-102; film thickness 0.33 microns, internal diameter 0.20 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.

REAGENTS:

Blank whole blood
N-butyl chloride
Trifluoroacetic Anhydride

REAGENTS (cont):

Sodium borate
Ethyl acetate
Drug standards

Prepare the following:

1. 500ml of saturated aqueous sodium borate solution at room temperature

PROCEDURE:

1. Pipet 2.0ml sample (case samples, blank and control) into screw cap tubes.
2. Pipet 2.0ml saturated sodium borate buffer to each sample and vortex.
3. Pipet 10ml of N-butyl chloride into each tube, cap and extract for 10 minutes.
4. Centrifuge for approx. 5 minutes.
5. Transfer N-butyl chloride layer to centrifuge tube.
6. Evaporate under nitrogen at 37°C to near dryness.
7. Finish drying under nitrogen at room temperature. As each sample dries, immediately add 50ul trifluoroacetic anhydride to the residue and cap.
8. Heat samples at 70°C for 20 minutes.
9. Evaporate samples to dryness with nitrogen at room temperature.
10. Pipet 20ul of ethyl acetate to each sample and vortex.
11. Transfer ethyl acetate to vials with micro inserts and crimp
12. Run on GC/MS using "drugscreen" full scan method or
13. Run on GC/MS using "drugscreen" method set for SIM and monitor the following ions:
    a. Amphetamine tfa derivative - 63, 65, 66, 69, 70, 89, 91, 92, 93, 113, 115, 117, 118, 119, 140, 141.
    b. Methamphetamine tfa derivative - 65, 69, 89, 91, 92, 110, 115, 117, 118, 119, 154, 155, 176.

PROCEDURE:

1. Pipet 1.5 ml blood (case sample, blank, or control) into 16 x 100 culture tube.
2. While vortexing add 4 ml 1:1 N-butyl chloride:isopropanol.
3. Vortex for 30 seconds.
4. Allow tubes to stand for 15 - 30 minutes.
5. Vortex for 10 seconds
6. Centrifuge tubes at high (3000+) speed for 10 - 20 minutes.
7. Transfer supernate to centrifuge tube being careful not to transfer red particles.
8. Add 2 drops of 1% methanolic HCl.
9. Evaporate to dryness at 37°C under nitrogen.
10. Reconstitute residue with 375 ul of 1:1 buffer:methanol
11. Vortex for 15 seconds and allow to sit for 10 minutes.
12. Centrifuge tubes on high speed for 5 minutes.
13. Transfer supernate to EMIT immunoassay sample cup without disturbing pellet.
14. Analyze using standard EMIT - ETS procedures.

REFERENCES:

1. Lewellen, L.J. and McCurdy, H.H., (1988). "A novel Procedure for the Analysis of Drugs in Whole Blood by Homogenous Enzyme Immunoassay (EMIT)." J. Anal. Toxicol. 12: 260 - 264.
2. Peel, H.W. and Perrigo, B.J. (1981). "Detection of Cannabinoids in Whole Blood Using EMIT." J. Anal. Toxicol. 6: 88 - 90.
3. Perrigo, B.J. and Joynt, B.P. (1989). "Optimization of the EMIT Immunoassay Procedure for the Analysis of Cannabinoids in Methanolic Blood Extracts." J. Anal. Toxicol. 13: 235 - 237.
4. Asselin, W.M., Leslie, J.M., and McKinley, B. (1988). "Direct Detection of Drugs of Abuse in Whole Hemolyzed Blood using the EMIT d.a.u. Urine Assays." J. Anal. Toxicol. 12: 207 - 215.

SCREENING FOR DRUGS ON ABUSE IN WHOLE BLOOD BY HOMOGENOUS ENZYME IMMUNOASSAY

INTRODUCTION:

Homogenous enzyme immunoassays (EMIT) are commonly used for the detection of drugs of abuse in urine. This method describes a procedure by which whole blood can be analyzed using nine (9) Emit assays: Amphetamine monoclonal, benzodiazepine class, cocaine metabolite, opiate class, barbiturate class,phencyclidine, cannabinoids, propoxyphene and tricyclic antidepressants.

INSTRUMENTATION:

Syva"s Emit ETS Plus analyzer

SUPPLIES:

Culture tubes, 16 x 100mm. Fisher Scientific Catalog # 14-958F
Centrifuge tubes, 16 x 144, Fisher Scientific Catalog # 05-538-41C

REAGENTS:

EMIT calibrators from Syva:

1. Calibrator Level 0.
2. Calibrator A, Level 1
3. Calibrator A, Level 2
4. Calibrator B, Level 1
5. Calibrator B, Level 2
6. Cannabinoid 20 ng/ml Calibrator
7. Cannabinoid 50 ng/ml Calibrator
8. Serum Tricyclic Antidepressants, Negative Control
9. Serum Tricyclic Antidepressants, Calibrator
10. Serum Tricyclic Antidepressants, Positive Control

EMIT assays from Syva

1. Amphetamines/methamphetamine, Monoclonal
2. Cocaine metabolite
3. Opiates
4. Benzodiazepines
5. Phencyclidine

REAGENTS (cont):

6. Cannabinoids (20 ng)
7. Propoxyphene
8. Trycyclic antidepressants

Blank whole blood
0.825M EMIT Tris-HCl buffer
Methanol
Isopropanol
N - butyl chloride
Hcl (concentrated)
Drug standards

a. Benzoylecgonine
b. Amphetamine
c. Nordiazepam
d. Phencyclidine
e. Morphine
f. Phenobarbital
g. THC-COOH
h. Propoxyphene
i. Nortriptyline

Prepare the following:

1. Calibrators and Assays according to directions.
2. 0.825M EMIT Tris-HCl buffer according to directions.
3. Tris-HCl buffer:Methanol 1:1.
4. Isopropanol:N-butyl chloride 1:1.
5. 1 % methanolic HCl.
6. Stock solution of drugs (2.5mg/ml free drug in meoh).
7. Whole blood standard containing:
   a. Phenobarbital - 300 ng/ml
   b. Nordiazepam - 300 ng/ml
   c. Benzoylecgonine - 300 ng/ml
   d. Morphine - 300 ng/ml
   e. amphetamine - 300 ng/ml
   f. THC-COOH - 25 ng/ml
   g. Nortriptyline - 300 ng/ml
   h. Phencyclidine - 25 ng/ml
   I. Propoxyphene - 300 ng/ml

BENZODIAZEPINE BLOOD EXTRACTION AND DERIVATRZATION PROCEDURE

INTRODUCTION:

Benzodiazepines are antianxiety agents. They are classified as long-acting: diazepam, intermediate-acting: lorazepam, or short-acting: triazolam. Effects can include sedation, drowsiness, light-headedness and lethargy. Benzodiazepines are often used in conjunction with other drugs such as cocaine and alcohol.

INSTRUMENTATION:

Hewlett Packard 5890 Gas Chromatograph
Hewlett packard 7673A Auto Sampler
Hewlett Packard 5970 Mass Select Detector (MSD)

COLUMN:

15 meter HP Ultra 1, catalog # 19091A-102; film thickness 0.33 microns, internal diameter 0.20 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol

REAGENTS (cons):

Hydrochloric acid - concentrated
Methylene chloride
Isopropanol
Ammonium hydroxide
BSTFA

Prepare the following:

1. 100 mM, pH 6.0 Phosphate buffer
2. 100 mM HCl
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).

PROCEDURE:

1. Pipet 2ml of sample (case sample, blank, control) into screw top tube
2. Add 8ml DI water, vortex, let stand for 5 minutes.
3. Centrifuge for 10 minutes
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
   a. 1 x 3ml methanol
   b. 1 x 3ml DI water
   c. 1 x 1ml 100 mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column
   a. 1 x 2ml DI H_2O
   b. 1 x 2ml 100mM HCl
   c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum ≥10 inches Hg.
9. Elute with elution solvent into centrifuge tube
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul BSTFA, cap, vortex heat at 90°C for 15 minutes.
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using "Drugscreen" method set to SIM using the following ions:
   a. desalkylflurazepam - 245, 247, 341, 342, 343, 344, 345, 346, 347, 348, 359, 360, 361, 362, 363.
   b. desalkyldiazepam - 227, 327, 328, 329, 341, 342, 343, 344, 345.
   c. lorazepam - 313, 324, 327, 329, 340, 341, 401, 415, 429, 430, 431, 432.
   d. diazepam - 165, 177, 221, 255, 256, 257, 258, 283, 284, 285, 286.

e. oxazepam - 347, 349, 429, 430, 431, 432, 449, 451.
f. prazepam - 241, 242, 243, 267, 268, 269, 270, 271, 295, 296, 297, 298, 323, 324, 326, 327.
g. flurazepam - 245, 315, 318, 387, 388, 389, 390.
h. triazolam - 238, 239, 279, 313, 314, 315, 343, 344, 345.
I. alparzolam - 204, 273, 279, 280, 281, 307, 308, 309, 310.
j. chlordiazepoxide -

PROPOXYPHENE/NORPROPOXYPHENE CONFIRMATION IN BLOOD BY GC/MS

INTRODUCTION:

Propoxyphene is an analgesic compound that is structurally similar to methadone. It's potency is about half that of codeine. Taken in large doses it can have opiate-like effects.

INSTRUMENTATION:

Hewlett Packard 5890 Gas Chromatograph
Hewlett packard 7673A Auto Sampler
Hewlett Packard 5970 Mass Select Detector (MSD)

COLUMN:

15 meter HP Ultra 1, catalog # 19091A-102; film thickness 0.33 microns, internal diameter 0.20 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Blank whole blood
Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol
Sodium acetate trihydrate

REAGENTS (cons):

Glacial acetic acid
Hydrochloric acid - concentrated
Methylene chloride
Isopropanol
Ammonium hydroxide
Ethyl acetate

Prepare the following:

1. 100 mM, pH 6.0 phosphate buffer
2. 100 mM, pH 4.5 acetate buffer
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).

PROCEDURE:

1. Pipet 2ml of sample (case sample, blank and control) into a screw top tube.
2. Add 8ml DI water, vortex and let stand for 5 minutes.
3. Centrifuge for 10 minutes.
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
    a. 1 x 3ml methanol
    b. 1 x 3ml DI water
    c. 1 x 2ml 100mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column.
    a. 1 x 2ml DI water
    b. 1 x 2ml 100mM acetate buffer
    c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum $\geq$ 10 inches Hg.
9. Elute with elution solvent into centrifuge tube.
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul ethyl acetate and vortex for 15 sec.
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using "Drugscreen" method set to SIM monitoring the following ions:
    a. Propoxyphene/nor propoxyphene - 44, 58, 59, 91, 100, 115, 117, 129, 130, 178, 193, 205, 208, 220, 265, 325.

OPIATE CONFIRMATION IN BLOOD USING GC/MS

INTRODUCTION:

The term opiate refers to those compounds which are natural or semisynthetic alkaloidal derivatives of the opium poppy. Opiates are used widely as pain relievers. The compounds of interest in this method are morphine, codeine, hydrocodone, oxycodone, heroin and hydromorphone.

INSTRUMENTATION:

Hewlett Packard 5890 Gas Chromatograph
Hewlett packard 7673A Auto Sampler
Hewlett Packard 5970 Mass Select Detector (MSD)

COLUMN:

15 meter HP Ultra 1, catalog # 19091A-102; film thickness 0.33 microns, internal diameter 0.20 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Blank whole blood
Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol
Sodium acetate trihydrate

REAGENTS (cons):

Glacial acetic acid
Hydrochloric acid - concentrated
Methylene chloride
Isopropanol
Ammonium hydroxide
BSTFA

Prepare the following:

1. 100 mM, pH 6.0 phosphate buffer
2. 100 mM, pH 4.5 acetate buffer
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).

PROCEDURE:

1. Pipet 2ml of sample (case sample, blank and control) into a screw top tube.
2. Add 8ml DI water, vortex and let stand for 5 minutes.
3. Centrifuge for 10 minutes.
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
    a. 1 x 3ml methanol
    b. 1 x 3ml DI water
    c. 1 x 2ml 100mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column.
    a. 1 x 2ml DI water
    b. 1 x 2ml 100mM acetate buffer
    c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum $\geq$ 10 inches Hg.
9. Elute with elution solvent into centrifuge tube.
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul BSTFA, cap, vortex and heat at 90°C for 15 minutes.
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using "Drugscreen" method set to SIM monitoring the following ions:
    a. Morphine - 429, 414, 401, 236, 196, 371, 287.
    b. Codeine - 371, 178, 73, 196, 234, 229.
    c. Hydrocodone - 371, 73, 234, 313, 314, 356, 242, 243, 299, 185, 214.
    d. Oxycodone - 387, 73, 179, 315, 330, 388, 459, 242, 312, 446, 460.
    e. Hydromorphone - 357, 300, 73, 59, 342, 243, 272, 301, 358.

COCAINE/BENZOYLECGONINE BLOOD EXTRACTION AND DERIVITIZATION PROCEDURE

INTRODUCTION:

Cocaine is a naturally occurring alkaloid. It is a powerful central nervous system stimulant. It increases mental awareness and alertness and gives a feeling of well-being and euphoria. Cocaine may be snorted, injected and in the case of the free base smoked. Cocaine converts to benzoylecgonine over time in blood tubes containing sodium floride.

INSTRUMENTATION:

Hewlett Packard 5890 Gas Chromatograph
Hewlett packard 7673A Auto Sampler
Hewlett Packard 5970 Mass Select Detector (MSD)

COLUMN:

15 meter HP Ultra 1, catalog # 19091A-102; film thickness 0.33 microns, internal diameter 0.20 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol
Hydrochloric acic - concentrated

REAGENTS (cont):

Methylene chloride
Isopropanol
Ammonium hydroxide
BSTFA

Prepare the following:

1. 100 mM, pH 6.0 Phosphate buffer
2. 100 mM HCl
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).

PROCEDURE:

1. Pipet 2ml of sample (case sample, blank, control) into screw top tube
2. Add 8ml DI water, vortex, let stand for 5 minutes.
3. Centrifuge for 10 minutes
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
   a. 1 x 3ml methanol
   b. 1 x 3ml DI water
   c. 1 x 1ml 100 mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column
   a. 1 x 2ml DI H_2O
   b. 1 x 2ml 100mM HCl
   c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum ≥10 inches Hg.
9. Elute with elution solvent into centrifuge tube
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul BSTFA, cap, vortex heat at 90°C for 15 minutes.
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using "Drugscreen" method set to SIM using the following ions: 82, 83, 94, 96, 105, 182, 198, 240, 241, 256, 303, 346, 361.

GENERAL SOLID PHASE BLOOD EXTRACTION AND CONFIRMATION PROCEDURE FOR NEUTRAL AND BASIC DRUGS USING GC/MS

INTRODUCTION:

A large number of basic and neutral drugs can be extracted from blood using a general solid phase extraction procedure. The extract may be derivitized or analyzed without derivitization.

INSTRUMENTATION:

Hewlett Packard 5890 Gas Chromatograph
Hewlett packard 7673A Auto Sampler
Hewlett Packard 5970 Mass Select Detector (MSD)

COLUMN:

15 meter HP Ultra 1, catalog # 19091A-102; film thickness 0.33 microns, internal diameter 0.20 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol
Drug standards

REAGENTS (cont):

Hydrochloric acid - concentrated
Methylene chloride
Isopropanol
Ammonium hydroxide
Ethyl acetate

Prepare the following:

1. 100 mM, pH 6.0 Phosphate buffer
2. 100 mM HCl
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).

PROCEDURE:

1. Pipet 2ml of sample (case sample, blank, control) into screw top tube
2. Add 8ml DI water, vortex, let stand for 5 minutes.
3. Centrifuge for 10 minutes
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
   a. 1 x 3ml methanol
   b. 1 x 3ml DI water
   c. 1 x 1ml 100 mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column
   a. 1 x 2ml DI H_2O
   b. 1 x 2ml 100mM HCl
   c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum $\geq$10 inches Hg.
9. Elute with elution solvent into centrifuge tube
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul ethyl acetate and vortex.
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using "Drugscreen" method set to SCAN or SIM monitoring the appropriate ions.

FORENSIC SERVICES PROCEDURE MANUAL

BLOOD ALCOHOL ANALYSIS

QUANTITATIVE ALCOHOL ANALYSIS BY
HEADSPACE GAS CHROMATOGRAPHY

I. Equipment:

A. Hewlett Packard 5890 G.C.

B. Hewlett Packard 7694 Headspace Sampler

C. P.C. with Hewlett Packard ChemStation Version A.04.01

D. Micro Lab 500 Series, Auto Dilutor

E. Crimper, Hewlett Packard Cat. #9301-0720

II. Supplies:

A. Septa - Hewlett Packard - Cat. #9301-0976

B. Crimp Caps - Hewlett Packard - Cat. #9301-0721

C. 10 ml Headspace Vials - Hewlett Packard - Cat. #5182-0838

D. Whole Blood Control - Behring Diagnostics - Cat. #860161

E. Acetonitrile - Fisher Scientific

F. Methanol - Fisher Scientific

G. Acetone - Fisher Scientific

H. Isopropyl Alcohol - Fischer Scientific

I. Acetaldehyde - Fischer Scientific

J. .10, .20, .30, Aqueous Ethanol Controls - Stephens Scientific - Cat. #4462-10 through 4462-30

K. Mercuric Chloride - Fischer Scientific

L. Megabore INNOWAX 30 Meter Column - Hewlet Packard - Cat. #

III. Reagent Preparation:

A. Preparation of Internal Standard Solution

1. Prepare 3% V/V acetonitrile stock solution from acetonitrile and deionized water - 30 ml Acetonitrile / liter of water + a pinch of mercuric chloride.

2. Prepare 0.012% W/V working internal standard solution - 5 ml stock solution / liter of water.

B. Preparation of Mixed standard

1. Acetaldehyde 0.25 ml, methanol 1.00 ml, acetone 0.25 ml, isopropyl alcohol 0.25 ml.

2. Mix with 1 liter of water + a pinch of mercuric chloride.

IV. Dilutor Preparation:

A. Check that there is enough internal standard for the analysis

B. Prime dilutor with internal standard.

C. Set syringe volumes
1. Reagent = 2000 ul
2. Sample = 250 ul

V. Sample Preparation:

A. Label each sample vial .

B. Aspirate and dispense sample into vial. Prepare in duplicate.

C. Tightly crimp cap and septa onto vial.

D. Between each sample aspirate water (3x) and dispense into waste to rinse tubing. It is not necessary to rinse between duplicates.

VI. Standard, Blank, and Control Preparation:

A. Prepare .10, .20, and .30 standards with aqueous standards using the same procedures as case samples.

B. Prepare blank with water using the same procedure as case samples.

C. Prepare control with known blood using the same procedures as case samples.

VII. Run preparation:

A. Place vials in sampler in the following order

1. Aqueous standards (.10, .20, .30) in duplicate.
2. Mixed standard
3. Blank
4. Blood control in duplicate
5. Case samples in duplicate
6. Check standards (.10, .20, .30)

VII. Headspace and GC Parameters:

A. Carrier pressure - 0.25 bar

B. Vial pressure - 1.70 bar

C. GC Method - Bloodalc.m

D. Headspace Method - Bloodalc.hsm

BLOOD TOXICOLOGY WORKSHEET

DATE:_____ **LAB. NUMBER:**_____

STC SCREEN:	**POS**	**NEG**
Amphetamine	[ ]	[ ]
Barbituates	[ ]	[ ]
Benzodiazepines	[ ]	[ ]
Cannabinoids	[ ]	[ ]
Cocaine Metabolite	[ ]	[ ]
Methamphetamine	[ ]	[ ]
Opiates	[ ]	[ ]

NP SCREEN:

RTT: FRONT	REAR	IDENTIFICATION
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

CONFIRMATION:

GC/MS: Column:_____ Temp:_____°C Rate:_____°C/Min.
Extraction Procedure:

GC/MS: Column:_____ Temp:_____°C Rate:_____°C/Min.
Extraction Procedure:

RESULTS:

CRIMINALIST:_____

BLOOD TOXICOLOGY WORKSHEET

DATE:_____ **LAB. NUMBER:**_____

ETS SCREEN:	**POS**	**NEG**
Cocaine metabolite	[ ]	[ ]
Amphetamine/methamphetamine	[ ]	[ ]
Opiates	[ ]	[ ]
Benzodiazepines	[ ]	[ ]
Phencyclidine	[ ]	[ ]
Barbituates	[ ]	[ ]
Cannabinoids	[ ]	[ ]
Propoxyphene	[ ]	[ ]
Tricyclic Antidepressants	[ ]	[ ]

NP SCREEN:

RTT: FRONT	REAR	IDENTIFICATION
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

CONFIRMATION:

GC/MS: Column:_____ Temp:_____°C Rate:_____°C/Min.
Extraction Procedure:

GC/MS: Column:_____ Temp:_____°C Rate:_____°C/Min
Extraction Procedure:

RESULTS:

_____
_____
_____
_____
_____

CRIMINALIST: _____

SPECIMEN REQUIREMENTS

BLOOD SCREEN MINIMUM REQUIREMENTS:

1. STC screen - 1.0 ml
2. NP screen - 2.0 ml

BLOOD CONFIRMATION MINIMUM REQUIREMENTS:

1. 2 ml per constituent *

* complete analysis may be completed on a lesser amount depending on concentration and class of drug present.

CONFIRMATIONS

The following drugs are routinely confirmed at or above the following levels:

1. Carboxy-THC - 15 ng/ml
2. Amphetamine - 50 ng/ml
3. Methamphetamine - 50 ng/ml
4. Morphine - 50 ng/ml
5. Benzoylecgonine - 50 ng/ml
6. Secobarbital - 50 ng/ml
7. Nordiazepam - 50 ng/ml

Whole Blood
Alcohol Control

thyl Alcohol Control

at. No. 860161: 10 x 2 mL

ended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

mmary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, wever, be measured and controlled by proper use of stable control materials. Most reliable results achieved when the quality control material matrix is similar to that of the unknown samples. ole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the .R.™ Ethyl Alcohol Test.

nciples of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The varia- in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of analytical system. The measured values can also be compared with the acceptable range presented ethyl alcohol.

gents

nposition

Whole Blood Alcohol Control is a liquid trol prepared from a stabilized human blood e to which ethyl alcohol has been added. ues established with selected analytical tems are given in the Table of Assay Values.

cautions

For *In Vitro* Diagnostic Use. Individual blood nations used to manufacture this control re examined for hepatitis B surface antigen BsAg) and for antibodies to HTLV III/LAV FDA-required test and only those found to nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

ce no test method can insure that infectious nts are absent, handle following the practices ommended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

ehring Diagnostics Inc.

BEHRING®

Whole Blood
Alcohol Control

Ethyl Alcohol Control

Cat. No. 860161: 10 x 2 mL

Intended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

Summary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, however, be measured and controlled by proper use of stable control materials. Most reliable results are achieved when the quality control material matrix is similar to that of the unknown samples. Whole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation of a protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the E.R.™ Ethyl Alcohol Test.

Principles of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The variation in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of the analytical system. The measured values can also be compared with the acceptable range presented for ethyl alcohol.

Reagents

Composition

Whole Blood Alcohol Control is a liquid control prepared from a stabilized human blood base to which ethyl alcohol has been added. Values established with selected analytical systems are given in the Table of Assay Values.

Precautions

For *In Vitro* Diagnostic Use. Individual blood donations used to manufacture this control were examined for hepatitis B surface antigen (HBsAg) and for antibodies to HTLV III/LAV by FDA-required test and only those found to be nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

Since no test method can insure that infectious agents are absent, handle following the practices recommended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

Procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

Behring Diagnostics Inc.

BEHRING® E. Behring

ssay

Swirl the Whole Blood Alcohol Control ntly to insure homogeneity. Assay as an un- own test sample following instructions for e method used. The control is assayed after eparation of a protein-free filtrate when tested h the Behring Diagnostics Stat-Pack™ Ethyl cohol Test or the S.V.R.™ Ethyl Alcohol Test.

uality Control

Inability to obtain values within an estab- hed acceptable range or a shift in measured ues suggests a change in the analytical system s occurred.

sults

Assay values for ethyl alcohol are calculated specified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

al Lot No.: 7180

	ETHYL ALCOHOL CONCENTRATION (g/dL) Mean	Acceptable Range
at-Pack™ Ethyl Alcohol Test- Protein free filtrate:	0.144	0.130 - 0.158
V.R.™ Ethyl Alcohol Test- Protein free filtrate:	0.147	0.132 - 0.162
s Chromatography[1]:	0.144	0.130 - 0.158

HIGH

ferences

Jain NC: Clin Chem 17:82, 1971.

Centers for Disease Control/National Institutes of Health, 1984 (HHS Pub. No. [CDC] 84-8395).

ehring Diagnostics Inc.
1 University Avenue
estwood, Massachusetts 02090

blished September 1995

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BEHRING®

Doc. No. N00116

Whole Blood Alcohol Control

Ethyl Alcohol Control

Cat. No. 860161: 10 x 2 mL

Intended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

Summary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, however, be measured and controlled by proper use of stable control materials. Most reliable results are achieved when the quality control material matrix is similar to that of the unknown samples. Whole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation of a protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the V.R.™ Ethyl Alcohol Test.

Principles of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The variation in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of the analytical system. The measured values can also be compared with the acceptable range presented for ethyl alcohol.

Reagents

Composition

Whole Blood Alcohol Control is a liquid control prepared from a stabilized human blood base to which ethyl alcohol has been added. Values established with selected analytical systems are given in the Table of Assay Values.

Precautions

For *In Vitro* Diagnostic Use. Individual blood donations used to manufacture this control were examined for hepatitis B surface antigen (HBsAg) and for antibodies to HTLV III/LAV by FDA-required test and only those found to be nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

Since no test method can insure that infectious agents are absent, handle following the practices recommended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

Procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

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say

Swirl the Whole Blood Alcohol Control tly to insure homogeneity. Assay as an un-own test sample following instructions for method used. The control is assayed after paration of a protein-free filtrate when tested n the Behring Diagnostics Stat-Pack™ Ethyl ohol Test or the S.V.R.™ Ethyl Alcohol Test.

ality Control

Inability to obtain values within an estab-ed acceptable range or a shift in measured ues suggests a change in the analytical system occurred.

sults

Assay values for ethyl alcohol are calculated pecified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

al Lot No.: 5280

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
t-Pack™ Ethyl Alcohol Test-Protein free filtrate:	0.141	0.127 - 0.155
V.R.™ Ethyl Alcohol Test-Protein free filtrate:	0.138	0.124 - 0.152
s Chromatography[1]:	0.139	0.125 - 0.153

ferences

Jain NC: Clin Chem 17:82, 1971.
Centers for Disease Control/National Institutes of Health, 1984 (HHS Pub. No. [CDC] 84-8395).

ehring Diagnostics Inc.
1 University Avenue
estwood, Massachusetts 02090

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olished September 1995

Doc. No. N00116

Whole Blood
Alcohol Control

thyl Alcohol Control

at. No. 860161: 10 x 2 mL

tended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

mmary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, wever, be measured and controlled by proper use of stable control materials. Most reliable results achieved when the quality control material matrix is similar to that of the unknown samples. ole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation a protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the V.R.™ Ethyl Alcohol Test.

inciples of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The varia- n in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of analytical system. The measured values can also be compared with the acceptable range presented ethyl alcohol.

agents

mposition

Whole Blood Alcohol Control is a liquid trol prepared from a stabilized human blood e to which ethyl alcohol has been added. ues established with selected analytical tems are given in the Table of Assay Values.

cautions

For *In Vitro* Diagnostic Use. Individual blood nations used to manufacture this control re examined for hepatitis B surface antigen BsAg) and for antibodies to HTLV III/LAV FDA-required test and only those found to nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

ce no test method can insure that infectious nts are absent, handle following the practices ommended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

ehring Diagnostics Inc.

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y

Swirl the Whole Blood Alcohol Control ly to insure homogeneity. Assay as an un-wn test sample following instructions for method used. The control is assayed after aration of a protein-free filtrate when tested the Behring Diagnostics Stat-Pack™ Ethyl hol Test or the S.V.R.™ Ethyl Alcohol Test.

lity Control

nability to obtain values within an estab-ed acceptable range or a shift in measured es suggests a change in the analytical system occurred.

ults

Assay values for ethyl alcohol are calculated pecified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

al Lot No.: 3170

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
t-Pack™ Ethyl Alcohol Test-Protein free filtrate:	0.159	0.143 - 0.175
V.R.™ Ethyl Alcohol Test-Protein free filtrate:	0.157	0.141 - 0.173
s Chromatography[1]:	0.159	0.143 - 0.175

ferences

Jain NC: Clin Chem 17:82, 1971.
Centers for Disease Control/National Institutes of Health, 1984 (HHS Pub. No. [CDC] 84-8395).

rranty:

s product is warranted to perform as described in the labeling and in Behring Diagnostic's literature when using the pro-dure indicated herein. Any changes or modifications in the procedure may affect the results. In such event, Behring gnostics disclaims all warranties, expressed, implied or statutory, including any implied warranty of merchantability or ess for use. In no event shall Behring Diagnostics be liable for any indirect or consequential damages arising out of the ove mentioned express warranty.

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blished June 1, 1987

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Doc. No. N00116

Whole Blood
Alcohol Control

thyl Alcohol Control

at. No. 860161: 10 x 2 mL

tended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

mmary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, wever, be measured and controlled by proper use of stable control materials. Most reliable results e achieved when the quality control material matrix is similar to that of the unknown samples. hole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation a protein-free filtrate when using the Behring Diagnostics Dat-Pack™ Ethyl Alcohol Test or the V.R.™ Ethyl Alcohol Test.

rinciples of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The varia- n in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of e analytical system. The measured values can also be compared with the acceptable range presented r ethyl alcohol.

agents

mposition

Whole Blood Alcohol Control is a liquid ntrol prepared from a stabilized human blood se to which ethyl alcohol has been added. lues established with selected analytical stems are given in the Table of Assay Values.

acautions

For *In Vitro* Diagnostic Use. Individual blood nations used to manufacture this control ere examined for hepatitis B surface antigen BsAg) and for antibodies to HTLV III/LAV FDA-required test and only those found to nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

ace no test method can insure that infectious ents are absent, handle following the practices commended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

ehring Diagnostics Inc.

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Assay

Swirl the Whole Blood Alcohol Control gently to insure homogeneity. Assay as an unknown test sample following instructions for the method used. The control is assayed after preparation of a protein-free filtrate when tested with the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the S.V.R.™ Ethyl Alcohol Test.

Quality Control

Inability to obtain values within an established acceptable range or a shift in measured values suggests a change in the analytical system has occurred.

Results

Assay values for ethyl alcohol are calculated as specified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

Vial Lot No.: 1264

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
Stat-Pack™ Ethyl Alcohol Test-Protein free filtrate:	0.163	0.147 - 0.179
S.V.R.™ Ethyl Alcohol Test-Protein free filtrate:	0.166	0.150 - 0.182
Gas Chromatography[1]:	0.162	0.146 - 0.178

References

Jain NC: Clin Chem 17:82, 1971.
Centers for Disease Control/National Institutes of Health 1984 (HHS Pub. No. [CDC] 84-8395).

Warranty

This product is warranted to perform as described in the labeling and in Behring Diagnostic's literature when using the procedure indicated herein. Any changes or modifications in the procedure may affect the results. In such event, Behring Diagnostics disclaims all warranties, expressed, implied or statutory, including any implied warranty of merchantability or fitness for use. In no event shall Behring Diagnostics be liable for any indirect or consequential damages arising out of the above mentioned express warranty.

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Doc. No. N00116

Whole Blood
Alcohol Control

thyl Alcohol Control

at. No. 860161: 10 x 2 mL

tended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

mmary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, wever, be measured and controlled by proper use of stable control materials. Most reliable results achieved when the quality control material matrix is similar to that of the unknown samples. ole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation a protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the V.R.™ Ethyl Alcohol Test.

inciples of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The varia- n in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of analytical system. The measured values can also be compared with the acceptable range presented ethyl alcohol.

agents

mposition

Whole Blood Alcohol Control is a liquid trol prepared from a stabilized human blood e to which ethyl alcohol has been added. ues established with selected analytical tems are given in the Table of Assay Values.

cautions

For *In Vitro* Diagnostic Use. Individual blood nations used to manufacture this control re examined for hepatitis B surface antigen BsAg) and for antibodies to HTLV III/LAV FDA-required test and only those found to nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

ce no test method can insure that infectious nts are absent, handle following the practices ommended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

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say

Swirl the Whole Blood Alcohol Control ntly to insure homogeneity. Assay as an un-own test sample following instructions for method used. The control is assayed after paration of a protein-free filtrate when tested h the Behring Diagnostics Stat-Pack™ Ethyl ohol Test or the S.V.R.™ Ethyl Alcohol Test.

ality Control

Inability to obtain values within an estab-hed acceptable range or a shift in measured ues suggests a change in the analytical system occurred.

sults

Assay values for ethyl alcohol are calculated pecified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

al Lot No.: 2042

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
t-Pack™ Ethyl Alcohol Test-Protein free filtrate:	0.151	0.136 - 0.166
V.R.™ Ethyl Alcohol Test-Protein free filtrate:	0.150	0.135 - 0.165
s Chromatography[1]:	0.150	0.135 - 0.165

ferences

Jain NC: Clin Chem 17:82, 1971.
Centers for Disease Control/National Institutes of Health. 1984 (HHS Pub. No. [CDC] 84-8395).

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Doc. No. N00116

Whole Blood
Alcohol Control

thyl Alcohol Control

at. No. 860161: 10 x 2 mL

ended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

mmary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, wever, be measured and controlled by proper use of stable control materials. Most reliable results achieved when the quality control material matrix is similar to that of the unknown samples. ole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the .R.™ Ethyl Alcohol Test.

nciples of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The varia- in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of analytical system. The measured values can also be compared with the acceptable range presented ethyl alcohol.

gents

nposition

Whole Blood Alcohol Control is a liquid trol prepared from a stabilized human blood e to which ethyl alcohol has been added. ues established with selected analytical ems are given in the Table of Assay Values.

cautions

For *In Vitro* Diagnostic Use. Individual blood ations used to manufacture this control e examined for hepatitis B surface antigen sAg) and for antibodies to HTLV III/LAV FDA-required test and only those found to nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

e no test method can insure that infectious nts are absent, handle following the practices ommended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

ehring Diagnostics Inc.

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ay

Swirl the Whole Blood Alcohol Control tly to insure homogeneity. Assay as an unown test sample following instructions for method used. The control is assayed after paration of a protein-free filtrate when tested the Behring Diagnostics Stat-Pack™ Ethyl ohol Test or the S.V.R.™ Ethyl Alcohol Test.

ality Control

Inability to obtain values within an established acceptable range or a shift in measured ues suggests a change in the analytical system occurred.

ults

Assay values for ethyl alcohol are calculated pecified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

al Lot No.: 4069

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
t-Pack™ Ethyl Alcohol Test- Protein free filtrate:	0.164	0.139 - 0.169
.R.™ Ethyl Alcohol Test- Protein free filtrate:	0.155	0.140 - 0.170
Chromatography[1]:	0.151	0.136 - 0.166

erences

Jain NC: Clin Chem 17:82, 1971.
Centers for Disease Control/National Institutes of Health 1984 (HHS Pub. No. [CDC] 84-8395).

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blished June 1, 1987

Doc. No. N00116

Whole Blood Alcohol Control

thyl Alcohol Control

at. No. 860161: 10 x 2 mL

tended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

mmary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, wever, be measured and controlled by proper use of stable control materials. Most reliable results achieved when the quality control material matrix is similar to that of the unknown samples. ole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation a protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the V.R.™ Ethyl Alcohol Test.

rinciples of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The varia-n in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of analytical system. The measured values can also be compared with the acceptable range presented ethyl alcohol.

agents

mposition

Whole Blood Alcohol Control is a liquid ntrol prepared from a stabilized human blood se to which ethyl alcohol has been added. lues established with selected analytical tems are given in the Table of Assay Values.

ecautions

For *In Vitro* Diagnostic Use. Individual blood nations used to manufacture this control re examined for hepatitis B surface antigen BsAg) and for antibodies to HTLV III/LAV FDA-required test and only those found to nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

ce no test method can insure that infectious nts are absent, handle following the practices ommended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

ehring Diagnostics Inc.

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say

Swirl the Whole Blood Alcohol Control tly to insure homogeneity. Assay as an unown test sample following instructions for method used. The control is assayed after paration of a protein-free filtrate when tested the Behring Diagnostics Stat-Pack™ Ethyl hol Test or the S.V.R.™ Ethyl Alcohol Test.

ality Control

Inability to obtain values within an estabed acceptable range or a shift in measured ues suggests a change in the analytical system occurred.

ults

Assay values for ethyl alcohol are calculated pecified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

al Lot No.: 5280

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
t-Pack™ Ethyl Alcohol Test- Protein free filtrate:	0.141	0.127 - 0.155
.R.™ Ethyl Alcohol Test- Protein free filtrate:	0.138	0.124 - 0.152
s Chromatography[1]:	0.139	0.125 - 0.153

erences

Jain NC: Clin Chem 17:82, 1971.

Centers for Disease Control/National Institutes of Health. 1984 (HHS Pub. No. [CDC] 84-8395).

ranty:

product is warranted to perform as described in the labeling and in Behring Diagnostic's literature when using the prore indicated herein. Any changes or modifications in the procedure may affect the results. In such event, Behring gnostics disclaims all warranties, expressed, implied or statutory, including any implied warranty of merchantability or ss for use. In no event shall Behring Diagnostics be liable for any indirect or consequential damages arising out of the ve mentioned express warranty.

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Doc. No. N00116

blished September 1995

Whole Blood Alcohol Control

thyl Alcohol Control

at. No. 860161: 10 x 2 mL

tended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

mmary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, wever, be measured and controlled by proper use of stable control materials. Most reliable results achieved when the quality control material matrix is similar to that of the unknown samples. hole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation a protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the V.R.™ Ethyl Alcohol Test.

rinciples of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The varia- n in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of e analytical system. The measured values can also be compared with the acceptable range presented r ethyl alcohol.

agents

mposition

Whole Blood Alcohol Control is a liquid ntrol prepared from a stabilized human blood se to which ethyl alcohol has been added. lues established with selected analytical stems are given in the Table of Assay Values.

ecautions

For *In Vitro* Diagnostic Use. Individual blood nations used to manufacture this control ere examined for hepatitis B surface antigen HBsAg) and for antibodies to HTLV III/LAV FDA-required test and only those found to nonreactive were used.

WARNING--POTENTIAL BIOHAZARD

nce no test method can insure that infectious ents are absent, handle following the practices commended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

ehring Diagnostics Inc.

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say

Swirl the Whole Blood Alcohol Control ntly to insure homogeneity. Assay as an un-own test sample following instructions for method used. The control is assayed after paration of a protein-free filtrate when tested n the Behring Diagnostics Stat-Pack™ Ethyl ohol Test or the S.V.R.™ Ethyl Alcohol Test.

ality Control

Inability to obtain values within an estab-ed acceptable range or a shift in measured ues suggests a change in the analytical system occurred.

sults

Assay values for ethyl alcohol are calculated pecified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

al Lot No.: 6120

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
at-Pack™ Ethyl Alcohol Test- Protein free filtrate:	0.157	0.141 - 0.173
V.R.™ Ethyl Alcohol Test- Protein free filtrate:	0.161	0.145 - 0.177
s Chromatography[1]:	0.159	0.143 - 0.175

ferences

Jain NC: Clin Chem 17:82, 1971.
Centers for Disease Control/National Institutes of Health, 1984 (HHS Pub. No. [CDC] 84-8395).

rranty:

s product is warranted to perform as described in the labeling and in Behring Diagnostic's literature when using the pro-ure indicated herein. Any changes or modifications in the procedure may affect the results. In such event, Behring gnostics disclaims all warranties, expressed, implied or statutory, including any implied warranty of merchantability or ess for use. In no event shall Behring Diagnostics be liable for any indirect or consequential damages arising out of the ve mentioned express warranty.

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Doc. No. N00116

blished September 1995

Whole Blood
Alcohol Control

thyl Alcohol Control

at. No. 860161: 10 x 2 mL

tended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

mmary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, wever, be measured and controlled by proper use of stable control materials. Most reliable results achieved when the quality control material matrix is similar to that of the unknown samples. ole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation a protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the .R.™ Ethyl Alcohol Test.

inciples of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The varia- n in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of analytical system. The measured values can also be compared with the acceptable range presented ethyl alcohol.

agents

nposition

Whole Blood Alcohol Control is a liquid trol prepared from a stabilized human blood e to which ethyl alcohol has been added. ues established with selected analytical tems are given in the Table of Assay Values.

cautions

For *In Vitro* Diagnostic Use. Individual blood nations used to manufacture this control re examined for hepatitis B surface antigen BsAg) and for antibodies to HTLV III/LAV FDA-required test and only those found to nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

ce no test method can insure that infectious nts are absent, handle following the practices ommended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

ehring Diagnostics Inc.

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ay

Swirl the Whole Blood Alcohol Control tly to insure homogeneity. Assay as an unown test sample following instructions for method used. The control is assayed after paration of a protein-free filtrate when tested the Behring Diagnostics Stat-Pack™ Ethyl ohol Test or the S.V.R.™ Ethyl Alcohol Test.

ality Control

Inability to obtain values within an established acceptable range or a shift in measured ues suggests a change in the analytical system occurred.

ults

Assay values for ethyl alcohol are calculated pecified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean values derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

al Lot No.: 3315

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
t-Pack™ Ethyl Alcohol Test- Protein free filtrate:	0.169	0.152 - 0.186
.R.™ Ethyl Alcohol Test- Protein free filtrate:	0.174 ~~.185~~ ~~0.162~~	0.157 - 0.191 .152 - .213 ~~0.146 - 0.178~~
s Chromatography[1]:		

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erences

Jain NC: Clin Chem 17:82, 1971.

Centers for Disease Control/National Institutes of Health 1984 (HHS Pub. No. [CDC] 84-8395).

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blished June 1, 1987

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Doc. No. N00116

Whole Blood
Alcohol Control

Ethyl Alcohol Control

Cat. No. 860161: 10 x 2 mL

Intended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

Summary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, however, be measured and controlled by proper use of stable control materials. Most reliable results are achieved when the quality control material matrix is similar to that of the unknown samples. Whole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation of a protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the E.R.™ Ethyl Alcohol Test.

Principles of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The variation in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of the analytical system. The measured values can also be compared with the acceptable range presented for ethyl alcohol.

Reagents

Composition

Whole Blood Alcohol Control is a liquid control prepared from a stabilized human blood base to which ethyl alcohol has been added. Values established with selected analytical systems are given in the Table of Assay Values.

Precautions

For *In Vitro* Diagnostic Use. Individual blood donations used to manufacture this control were examined for hepatitis B surface antigen (HBsAg) and for antibodies to HTLV III/LAV by FDA-required test and only those found to be nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

Since no test method can insure that infectious agents are absent, handle following the practices recommended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

Procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

Behring Diagnostics Inc.

BEHRING® S. Behring

ssay

Swirl the Whole Blood Alcohol Control ntly to insure homogeneity. Assay as an un- own test sample following instructions for e method used. The control is assayed after eparation of a protein-free filtrate when tested h the Behring Diagnostics Stat-Pack™ Ethyl cohol Test or the S.V.R.™ Ethyl Alcohol Test.

uality Control

Inability to obtain values within an estab- hed acceptable range or a shift in measured lues suggests a change in the analytical system s occurred.

sults

Assay values for ethyl alcohol are calculated specified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

ial Lot No.: 2042

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
at-Pack™ Ethyl Alcohol Test- Protein free filtrate:	0.151	0.136 - 0.166
V.R.™ Ethyl Alcohol Test- Protein free filtrate:	0.150	0.135 - 0.165
as Chromatography[1]:	0.150	0.135 - 0.165

ferences

Jain NC: Clin Chem 17:82, 1971.
Centers for Disease Control/National Institutes of Health, 1984 (HHS Pub. No. [CDC] 84-8395).

rranty:

s product is warranted to perform as described in the labeling and in Behring Diagnostic's literature when using the pro- dure indicated herein. Any changes or modifications in the procedure may affect the results. In such event, Behring gnostics disclaims all warranties, expressed, implied or statutory, including any implied warranty of merchantability or ess for use. In no event shall Behring Diagnostics be liable for any indirect or consequential damages arising out of the ve mentioned express warranty.

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Chubb Way
merville, NJ 08876

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blished June 1, 1987

Doc. No. N00116

Whole Blood
Alcohol Control

thyl Alcohol Control

at. No. 860161: 10 x 2 mL

ended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

mmary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, wever, be measured and controlled by proper use of stable control materials. Most reliable results achieved when the quality control material matrix is similar to that of the unknown samples. ole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation protein-free filtrate when using the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the .R.™ Ethyl Alcohol Test.

nciples of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The varia- in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of analytical system. The measured values can also be compared with the acceptable range presented ethyl alcohol.

gents

nposition

Whole Blood Alcohol Control is a liquid trol prepared from a stabilized human blood e to which ethyl alcohol has been added. ues established with selected analytical ems are given in the Table of Assay Values.

cautions

For *In Vitro* Diagnostic Use. Individual blood ations used to manufacture this control e examined for hepatitis B surface antigen BsAg) and for antibodies to HTLV III/LAV FDA-required test and only those found to nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

e no test method can insure that infectious nts are absent, handle following the practices mmended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

ehring Diagnostics Inc.

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ay

Swirl the Whole Blood Alcohol Control tly to insure homogeneity. Assay as an un-wn test sample following instructions for method used. The control is assayed after paration of a protein-free filtrate when tested the Behring Diagnostics Stat-Pack™ Ethyl hol Test or the S.V.R.™ Ethyl Alcohol Test.

lity Control

inability to obtain values within an estab-ed acceptable range or a shift in measured es suggests a change in the analytical system occurred.

ults

Assay values for ethyl alcohol are calculated pecified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

l Lot No.: 4069

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
t-Pack™ Ethyl Alcohol Test-Protein free filtrate:	0.154	0.139 - 0.169
.R.™ Ethyl Alcohol Test-Protein free filtrate:	0.155	0.140 - 0.170
Chromatography[1]:	0.151	0.136 - 0.166

erences

lain NC: Clin Chem 17:82, 1971.
Centers for Disease Control/National Institutes of Health 1984 (HHS Pub. No. [CDC] 84-8395).

ehring Diagnostics Inc.
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olished June 1, 1987

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Doc. No. N00116

Whole Blood Alcohol Control

Ethyl Alcohol Control

Cat. No. 860161: 10 x 2 mL

Intended use

For use in measuring the precision of analytical systems for the determination of ethyl alcohol.

Summary and explanation

All analytical systems are subject to inherent measurement error. This unavoidable error can, however, be measured and controlled by proper use of stable control materials. Most reliable results are achieved when the quality control material matrix is similar to that of the unknown samples. Whole Blood Alcohol Control is prepared from a human blood base which is assayed after preparation of a protein-free filtrate when using the Behring Diagnostics StatPack™ Ethyl Alcohol Test or the A.V.R.™ Ethyl Alcohol Test.

Principles of the procedure

Whole Blood Alcohol Control is assayed as an unknown with each batch of specimens. The variation in values observed for ethyl alcohol upon repetitive assay serves as a measure of the precision of the analytical system. The measured values can also be compared with the acceptable range presented for ethyl alcohol.

Reagents

Composition

Whole Blood Alcohol Control is a liquid control prepared from a stabilized human blood base to which ethyl alcohol has been added. Values established with selected analytical systems are given in the Table of Assay Values.

Precautions

For *In Vitro* Diagnostic Use. Individual blood donations used to manufacture this control were examined for hepatitis B surface antigen (HBsAg) and for antibodies to HTLV III/LAV by FDA-required test and only those found to be nonreactive were used.

WARNING—POTENTIAL BIOHAZARD

Since no test method can insure that infectious agents are absent, handle following the practices recommended for Biosafety Level 2.[2]

Preparation

Each vial contains 2 mL of liquid control requiring no further preparation. Swirl gently to insure homogeneity prior to use.

Storage and Stability

Store at 2° to 8°C. When opened, the control is stable for at least 5 days at 2° to 8°C if the vial is tightly stoppered immediately after use. If visible evidence of microbial contamination appears, consider the control unsuitable and discard.

Procedure

Materials Provided

Whole Blood Alcohol Control

Materials Required But Not Provided

Reagents and equipment for assay of ethyl alcohol.

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Assay

Swirl the Whole Blood Alcohol Control gently to insure homogeneity. Assay as an unknown test sample following instructions for the method used. The control is assayed after preparation of a protein-free filtrate when tested with the Behring Diagnostics Stat-Pack™ Ethyl Alcohol Test or the S.V.R.™ Ethyl Alcohol Test.

Quality Control

Inability to obtain values within an established acceptable range or a shift in measured values suggests a change in the analytical system has occurred.

Results

Assay values for ethyl alcohol are calculated as specified in the instructions for the method.

limitations

Each laboratory should verify or determine the acceptable range of performance under its own assay conditions.

performance

The procedures listed were used to generate the mean values and acceptable ranges given in the Table of Assay Values. The mean value is derived from replicate assays. The acceptable range is for the assay conditions and procedure given in the instructions for the method used. Different methods or assay conditions may yield different results.

Results may be expressed in SI units as follows:

1 g/dL = 217 mmol/L

TABLE OF ASSAY VALUES

Vial Lot No.: 7270

	ETHYL ALCOHOL CONCENTRATION (g/dL)	
	Mean	Acceptable Range
Stat-Pack™ Ethyl Alcohol Test- Protein free filtrate:	0.169	0.152 - 0.186
S.V.R.™ Ethyl Alcohol Test- Protein free filtrate:	0.164	0.148 - 0.180
Gas Chromatography[1]:	0.166	0.149 - 0.183

References

1. Jain NC: Clin Chem 17:82, 1971.
2. Centers for Disease Control/National Institutes of Health, 1984 (HHS Pub. No. [CDC] 84-8395).

Warranty:

This product is warranted to perform as described in the labeling and in Behring Diagnostic's literature when using the procedure indicated herein. Any changes or modifications in the procedure may affect the results. In such event, Behring Diagnostics disclaims all warranties, expressed, implied or statutory, including any implied warranty of merchantability or fitness for use. In no event shall Behring Diagnostics be liable for any indirect or consequential damages arising out of the above mentioned express warranty.

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Published September 1995
Doc. No. N00116

SOLUTIONS

Saturated Sodium Borate Buffer:

To 250 ml DI H2O add sodium borate until solution is saturated.
Storage: Plastic or glass.
Stability: 6 months.

1 N SULFURIC ACID:

To 200 ml DI H2O add 6.7 ml concentrated H2SO4. Dilute to 250 ml.
Storage: Plastic or glass.
Stability: 6 months.

1 N HYDROCHLORIC ACID:

To 200 ml DI H2O add 21 ml concentrated HCl. Dilute of 250 ml.
Storage: Plastic or glass.
Stability: 6 months.

10 N SODIUM HYDROXIDE:

In 200 ml DI H2O disolve 100 g NaOH. Dilute to 250 ml.
Storage: Plastic or glass.
Stability: 6 months.

1% METHANOLIC HCl:

To 30 ml MeOH add 0.5 ml concentrated HCl. Dilute to 50 ml.
Storage: Glass at 5°C.
Stability: 6 months.

100 mM PHOSPHATE BUFFER, pH 6.0:

Dissolve 0.42 g Na2HPO4 and 3.03 g NaH2PO4-H2O in 200 ml DI H2O. Dilute to 250 ml. Adjust to pH 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).
Storage: 5°C in glass.
Stability: 1 month.

100 mM HCl:

To 200 ml DI H2O add 2.1 ml concentrated HCl. Dilute to 250 ml.
Storage: Plastic or glass.
Stability: 6 months.

SOLUTIONS:

0.45 N SODIUM HYDROXIDE:

In 200 ml DI H2O dissolve 4.5 g NaOH. Dilute to 250 ml.
Storage: Plastic or glass.
Stability: 6 months.

100 mM ACETATE BUFFER, pH 4.5:

Dissolve 1.47 g sodium acetate trihydrate in 200 ml DI H2O. Add 0.81 ml glacial acetic acid. Dilute to 250 ml. Adjust pH to 4.5 ± 0.1 with 100 mM sodium acetate or 100 mM acetic acid.
Storage: Plastic or glass.
Stability: 6 months.

.2 N NaOH:

Dissolve 2 g NaOH in 200 ml DI H2O. Dilute to 250 ml.
Storage: Plastic or glass.
Stability: 6 months.

STC DRUG STANDARD WORKING SOLUTION:

Fill 10 ml volumetric flask 1/2 full with methanol. Add 50 ul each of 1.0 mg/ml nordiazepam, methamphetamine, amphetamine, morphine, benzoylecgonine, and secobarbital. Add 150 ul of 100 ug/ml (-)-11-nor-9-carboxy-delta9-THC. Fill flask to 10 ml with methanol.
Storage: Glass at < 0°C.
Stability: 6 months.

STC BLOOD QUALITY CONTROLS:

To 1 ml of blank blood add the following:
1: 10 ul working solution for 15/50 ng/ml QC.
2: 20 ul working solution for 30/100 ng/ml QC.
3: 40 ul working solution for 60/100 ng/ml QC.
Storage: Glass at < 0°C.
Stability: 6 months.

GENERAL CONFIRMATION PROCEDURE FOR NEUTRAL AND BASIC DRUGS OF ABUSE USING GC/MS

INTRODUCTION:

The purpose of this method is to provide a general confirmation procedure for a large number of common neutral and basic drugs of abuse (excluding morphine, dilaudid, thc, and benzoylecgonine) in blood samples. The method is based upon the principle of liquid / liquid extraction of the drugs from the blood and then identifying them on a GC/MS using S.I.M. or scan monitoring.

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph.
Hewlett Packard 6890, Automatic Sampler.
Hewlett Packard 5973 Mass Select Detector (MSD).

COLUMN:

30 meter HP5-MS, catalog # 19091S-433; film thickness 0.25 microns, internal diameter 0.25mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw caps for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro insert, 0.200ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979
Transfer pipets, Fisher Scientific Catalog # 13-711-7

REAGENTS:

Blank whole blood
Methanol
Hexane
N-butyl Chloride
Sodium borate

REAGENTS (cont.):

Sodium hydroxide
Ethanol - 200 proof
Sulfuric acid - concentrated
Drug standards
Hydrochloric acid - concentrated
Methanol

Prepare the following:

1. 500 ml of saturated aqueous sodium borate solution at room temperature
2. 250 ml of 1:1 hexane:ethanol solution
3. 500 ml of 1 N sulfuric acid
4. Stock solutions of drugs to be tested (2.5mg/ml free drug in meoh)
5. Working solution of drugs to be tested (5.0 ng/ul free drug in 1:1 hexane:ethanol).
    a. Place 5.0ml hexane:ethanol in screw cap tube.
    b. Add 10ul of stock solution
6. 250 ml 10 N NaOH
7. 100 ml 1 % HCl in methanol.

PROCEDURE:

1. Pipet 2.0ml sample, blank blood and control blood into tubes. The control blood is made by taking 2.0ml of blank blood and adding drugs of interest.
2. Pipet 2.0ml pH 9.5 saturated borate buffer to each sample and vortex.
3. Pipet 10ml N-butyl chloride into each tube, cap and extract for 10 minutes.
4. Centrifuge for approx. 5 minutes.***
5. Transfer the butyl chloride (top) layer to a second tube.
6. Pipet 2.0ml of 1N sulfuric acid, cap and extract for 5 minutes.
7. Centrifuge for approx. 5 minutes and discard butyl chloride (top) layer.
8. Pipet 5.0ml hexane into each tube, cap and extract for 5 minutes.
9. Centrifuge for approx. 5 minutes and discard hexane (top) layer.
10. Check the pH of the aqueous phase (it should be acidic).
11. Add 10 N NaOH (approx. 6-8 drops) until the pH is basic (greater than 9).
12. Pipet 10ml butyl chloride into each tube, cap and extract for 5 minutes.
13. Centrifuge for approx. 5 minutes.
14. Transfer butyl chloride (top) layer into centrifuge tube.
15. Add 2-5 drops of 1 % HCl in methanol.
16. Evaporate under a gentle stream of nitrogen at 37 C to near dryness.
17. Finish drying under nitrogen at room temperature. As each sample dries, immediately add 50ul of 1:1 hexane;ethanol to the residue and vortex (samples may be derivitized if appropriate).

PROCEDURE (cont.)

18. Transfer the extract to an insert in an auto sampler vial and crimp.
    Run on GC/MS using scan mode or S.I.M. monitoring appropriate ions

*** If sample is clean proceed to step 14.

REFERENCES:

"A Rapid, Comprehensive Screening Procedure for Basic Drugs in Blood of Tissues by Gas Chromatography" by Foerster, Hatchett and Garriott. Journal of Analytical Toxicology, Vol. 2, pgs. 50-55.

GENERAL SOLID PHASE BLOOD EXTRACTION AND CONFIRMATION PROCEDURE FOR NEUTRAL AND BASIC DRUGS USING GC/MS

INTRODUCTION:

A large number of basic and neutral drugs can be extracted from blood using a general solid phase extraction procedure. The extract may be derivitized or analyzed without derivitization.

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph
Hewlett Packard 6890 Auto Sampler
Hewlett Packard 5973 Mass Select Detector (MSD)

COLUMN:

30 meter HP5-MS, catalog # 19091S-433; film thickness 0.25 microns, internal diameter 0.25 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol

REAGENTS (cons):

Hydrochloric acid - concentrated
Methylene chloride
Isopropanol
Ammonium hydroxide
Ethyl acetate

Prepare the following:

1. 100 mM, pH 6.0 Phosphate buffer
2. 100 mM HCl
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).

PROCEDURE:

1. Pipet 2ml of sample (case sample, blank, control) into screw top tube
2. Add 8ml DI water, vortex, let stand for 5 minutes.
3. Centrifuge for 10 minutes
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
    a. 1 x 3ml methanol
    b. 1 x 3ml DI water
    c. 1 x 1ml 100 mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column
    a. 1 x 2ml DI H_2O
    b. 1 x 2ml 100mM HCl
    c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum of10 inches Hg.
9. Elute with 6 ml of elution solvent into centrifuge tube
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul ethyl acetate and vortex.*
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using scan method or SIM monitoring the appropriate ions.

*Sample may be derivitized if appropriate.

Region 1 Idaho State Police Lab Role

The role of the region one forensic lab is to provide accurate facts, interpretations, and expert opinions based on scientific testing to the criminal justice system with out bias, in the areas of controlled substance analysis, toxicology, blood alcohol determination, firearm and toolmark comparison, serial # restoration, and crime scene processing.

Training: In order to support the role of the laboratory, a continuing education program will be encouraged. Although training in each staff member's field of expertise should be the primary focus, cross-training and study in fields of general interest should also be encouraged. Providing information as to the procedures and capabilities of the laboratory to our clients will promote a solid working relationship.

The region one lab should strive to use their time and resources to the highest level of efficiency possible. It is important to keep an open mind that the tax paying citizens of the state of Idaho expect maximum service at a reasonable cost.

We will accomplish this forensic science mission with fairness, impartiality, integrity, professionalism and commitment to the truth wherever it leads.

Just the Facts

Region 1 Idaho State Police Lab Role

Provide accurate facts, interpretations and expert opinions based on scientific evidence to the criminal justice system without bias.

The lab will strive to use their time and resources to the highest level of efficiency possible.

The role is better achieved through continued education, cross training, and consulting with user agencies on how to better serve and train them.

We will strive to accomplish out role with fairness, impartiality, integrity, professionalism and commitment to the truth wherever it leads.

HYDROCODONE CONFIRMATION IN BLOOD USING GC/MS

INTRODUCTION:

Hydrocodone is a narcotic analgisic and an antitussive with actions similar to codeine. It may produce drowsiness, changes in mood and mental clouding .

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph
Hewlett packard 6890 Series Injector
Hewlett Packard 5973 Mass Select Detector (MSD)

COLUMN:

30 meter HP-5MS, catalog # 19091S-433 or its' equivilent.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Blank whole blood
Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol
Sodium acetate trihydrate

REAGENTS (cons):

Glacial acetic acid
Methylene chloride
Isopropanol
Ammonium hydroxide
Hexane
Ethyl alcohol

Prepare the following:

1. 100 mM, pH 6.0 phosphate buffer
2. 100 mM, pH 4.5 acetate buffer
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).
4. 1:1 hexane/ethyl achohol

PROCEDURE:

1. Pipet 2ml of sample (case sample, blank and control) into a screw top tube.
2. Add 8ml DI water, vortex and let stand for 5 minutes.
3. Centrifuge for 10 minutes.
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
    a. 1 x 3ml methanol
    b. 1 x 3ml DI water
    c. 1 x 2ml 100mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column.
    a. 1 x 2ml DI water
    b. 1 x 2ml 100mM acetate buffer
    c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum $\geq$ 10 inches Hg.
9. Elute with 6ml of elution solvent into centrifuge tube.
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul 1:1 hexane/ethyl alcohol and vortex.
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using "HYCOUDIR" method set to SIM monitoring the following ions: 185, 199, 214, 228, 242, 299.

GENERAL ACIDIC DRUG BLOOD EXTRACTION AND GC/MS CONFIRMATION PROCEDURE

INTRODUCTION:

Acidic drugs may be extracted from biological samples using n-butyl chloride in a liquid/liquid extraction under acidic conditions.

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph
Hewlett Packard 6890 Auto Sampler
Hewlett Packard 5973 Mass Select Detector (MSD)

COLUMN:

30 meter HP5-MS, catalog # 19091S-433; film thickness 0.25 microns, internal diameter 0.25 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.

REAGENTS:

Blank whole blood
N-butyl chloride
Sodium hydroxide
Hydrochloric acid -concentrated
Hexane
Ethanol - 200 proof
Drug standards

REAGENTS (cont):

Prepare the following:

1. 250ml of 1:1 hexane:ethanol solution
2. 250 ml of 0.45 N sodium hydroxide

PROCEDURE:

1. Pipet 1 ml of blood (sample, blank or control) into a screw top tube.
2. Extract with 10 ml N-butyl chloride for three minutes.
3. Centrifuge for five minutes.***
4. Transfer N-butyl chloride to another screw cap tube.
5. Add 2 ml of 0.45 N sodium hydroxide and mix for three minutes.
6. Centrifuge for five minutes
7. Discard N-butyl chloride
8. Adjust the pH to acid with concentrated HCl.
9. Extract with 10 ml N-butyl chloride for five minutes.
10. Centrifuge for five minutes.
11. Transfer the N-butyl chloride layer to a centrifuge tube and evaporate at 37°C under nitrogen to dryness.
12. Reconstitute the residue in 100 ul 1:1 hexane:ethanol.
13. Run on GC/MS using scan method or
14. Run on GC/MS using SIM method and monitor the appropriate ions.

***for clean samples proceed to step 11.

CARBOXY-THC BLOOD EXTRACTION AND DERIVATIZATION PROCEDURE

INTRODUCTION:

The plant Cannabis sativa L. produces compounds, grouped as CANNABINOIDS, responsible for the hallucinogenic and other physiological effects of marijuana. The primary cannabinoid responsible for these effects is delta-9-tetrahydrocannabinol (THC). THC undergoes extensive metabolism in the body. The primary metabolite of THC is 11-nor-9-carboxy-delta-9-THC (carboxy-THC or c-THC). This method uses a protein precipitation and liquid/liquid extraction to separate and identify c-THC in blood.

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph
Hewlett Packard 6890 Auto Sampler
Hewlett Packard 5973 Mass Select Detector (MSD)

COLUMN:

30 meter HP5-MS, catalog # 19091S-433; film thickness 0.25 microns, internal diameter 0.25 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Culture tubes, 16 x 100 mm, Fisher Scientific Catalog # 14-958F
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Pasteur pipets, Fisher Scientific Catalog # 22-230-482

REAGENTS:

Blank whole blood
Acetonitrile
C-THC standard
Hydrochloric acid - concentrated
Hexane
Ethyl acetate
MSTFA

REAGENTS (cont.)

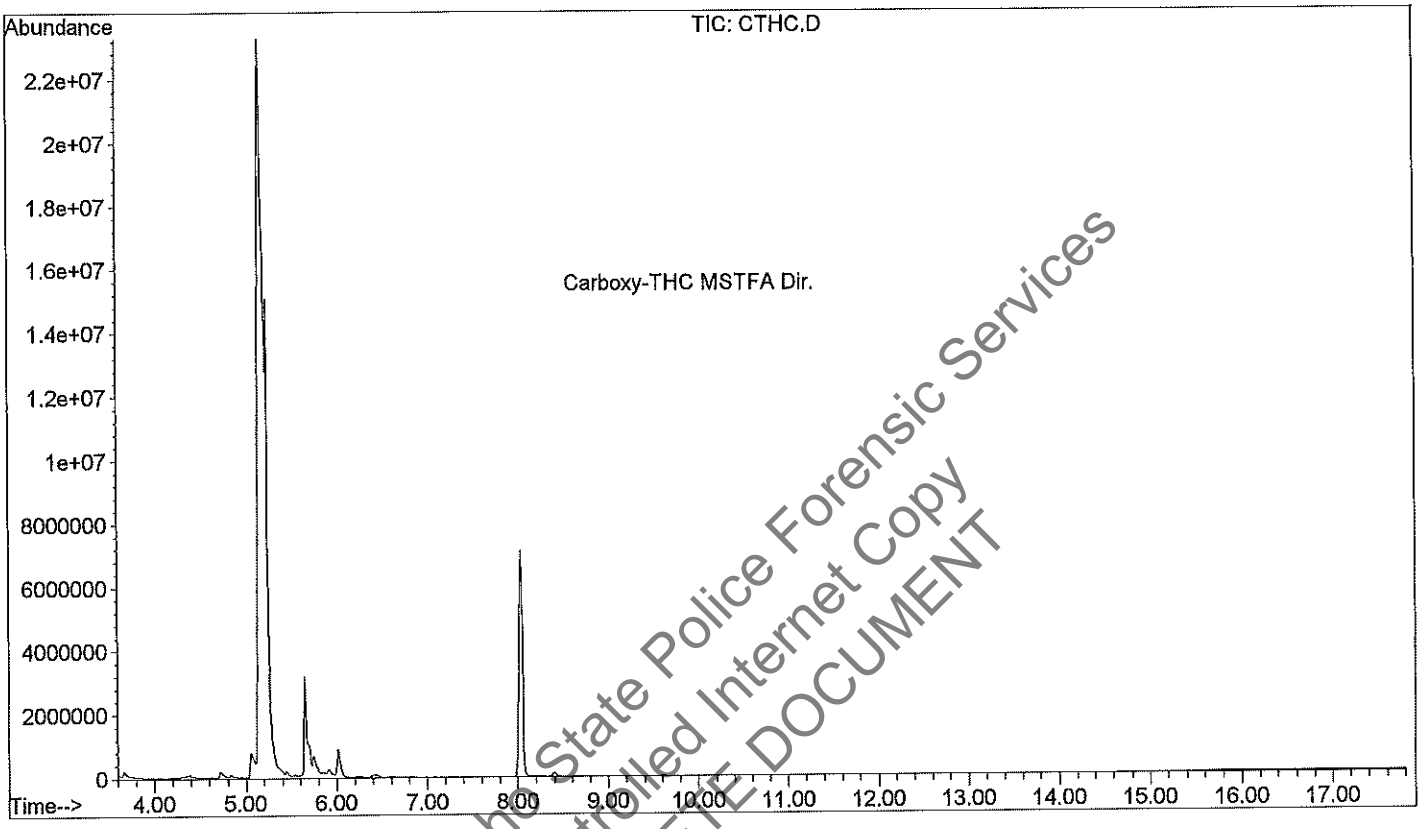
Prepare the following:

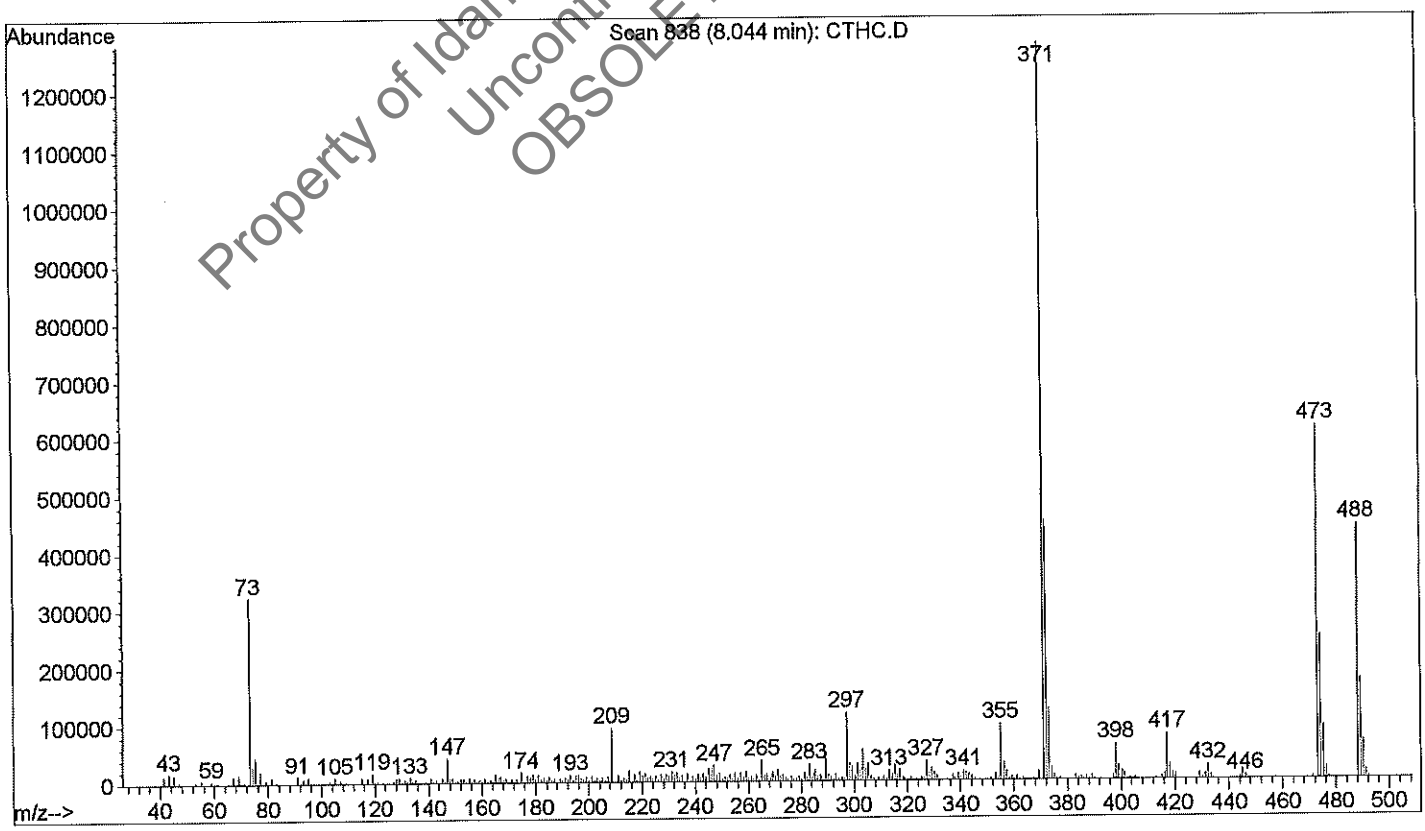
1. 1 N HCl
2. Hexane:ethyl acetate (9:1)

PROCEDURE:

1. Pipet 2 ml of sample (case sample, blank and control) into culture tube.
2. Add standard to control blood and allow to equilibrate for 60 minutes.
3. While vortexing add 4 ml acetonitrile. Continue vortexing for 30 seconds.
4. Centrifuge for 10 minutes and decant supernate into screw top tube.
5. Reduce the solvent under nitrogen at 37°C to approx. 1 ml remains (do not take to dryness).
6. Add 1 ml 1 N HCl and 6 ml hexane:ethyl acetate (9:1). Cap and extract for 30 minutes.
7. Centrifuge for 10 minutes and transfer top layer to centrifuge tube with glass, pasteur pipet.
8. Evaporate under nitrogen at 37°C.
9. Add 50 ul MSTFA and derivatize at 90°C for 15 minutes.
10. Run on GC/MS using SIM method and monitoring the following ions: 371, 473 and 488.

File : D:\HPCHEM\1\DATA\CTHC.D
Operator : SVJ
Acquired : 18 Feb 1998 17:17 using AcqMethod 3
Instrument : GC/MS Ins
Sample Name: THC/CTHC BSTFA DIR
Misc Info : BSTFA DIR
Vial Number: 2





AMPHETAMINE/METHAMPHETAMINE BLOOD EXTRACTION AND DERIVATIZATION PROCEDURE

INTRODUCTION:

Sympathomimetic amines are central nervous system stimulants. They produce increased alertness, euphoria, excitement, wakefulness, a reduced sense of fatigue, loss of appetite and an increased feeling of power. They may enhance performance in athletic competition. The drugs may be introduced into the system by smoking, snorting or injection. Sympathomimetic amines may be extracted from biological samples with organic solvents under basic conditions.

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph
Hewlett Packard 6890 Auto Sampler
Hewlett Packard 5973 Mass Select Detector (MSD)

COLUMN:

30 meter HP5-MS, catalog # 19091S-433, film thickness 0.25 microns, internal diameter 0.25 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.

REAGENTS:

Blank whole blood
N-butyl chloride
Trifluoroacetic Anhydride

REAGENTS (cont):

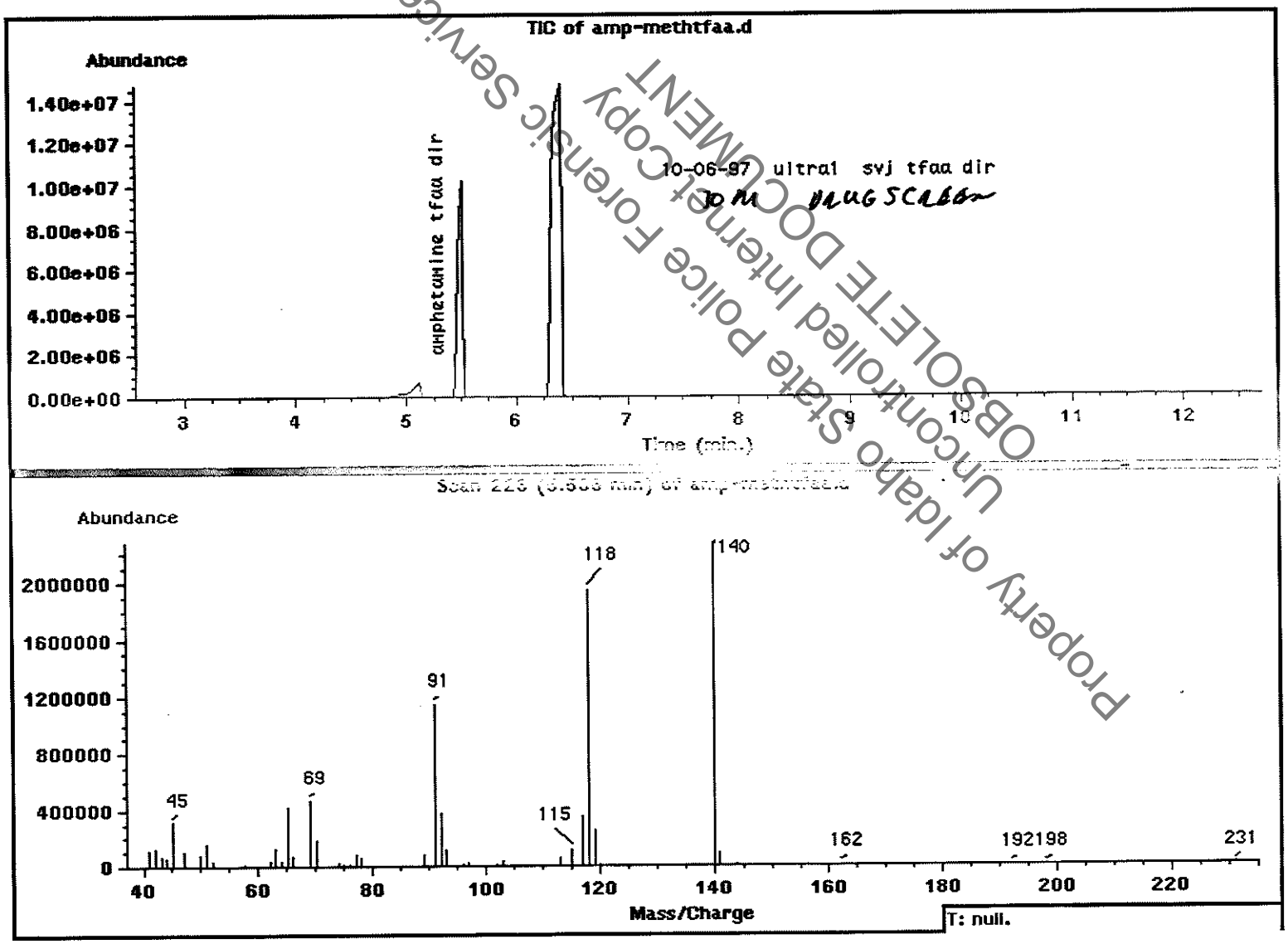
Sodium borate
Ethyl acetate
Drug standards
Concentrated Hcl
Methanol

Prepare the following:

1. 500ml of saturated aqueous sodium borate solution at room temperature
2. 1 % HCl solution in methanol

PROCEDURE:

1. Pipet 2.0ml sample (case samples, blank and control) into screw cap tubes.
2. Pipet 2.0ml saturated sodium borate buffer to each sample and vortex.
3. Pipet 10ml of N-butyl chloride into each tube, cap and extract for 10 minutes.
4. Centrifuge for approx. 5 minutes.
5. Transfer N-butyl chloride layer to centrifuge tube.
6. Add 2-5 drops 1% HCl in methanol.
7. Evaporate under nitrogen at 37°C to near dryness.
8. Finish drying under nitrogen at room temperature. As each sample dries, immediately add 50ul trifluoroacetic anhydride to the residue and cap.
9. Heat samples at 70°C for 20 minutes.
10. Evaporate samples to dryness with nitrogen at room temperature.
11. Pipet 50ul of ethyl acetate to each sample and vortex.
12. Transfer ethyl acetate to vials with micro inserts and crimp
13. Run on GC/MS using full scan method or
14. Run on GC/MS using SIM method and monitor the following ions:
    a. Amphetamine tfa derivative - 65, 91, 92, 117, 118, 140.
    b. Methamphetamine tfa derivative - 65, 91, 110, 118, 154.

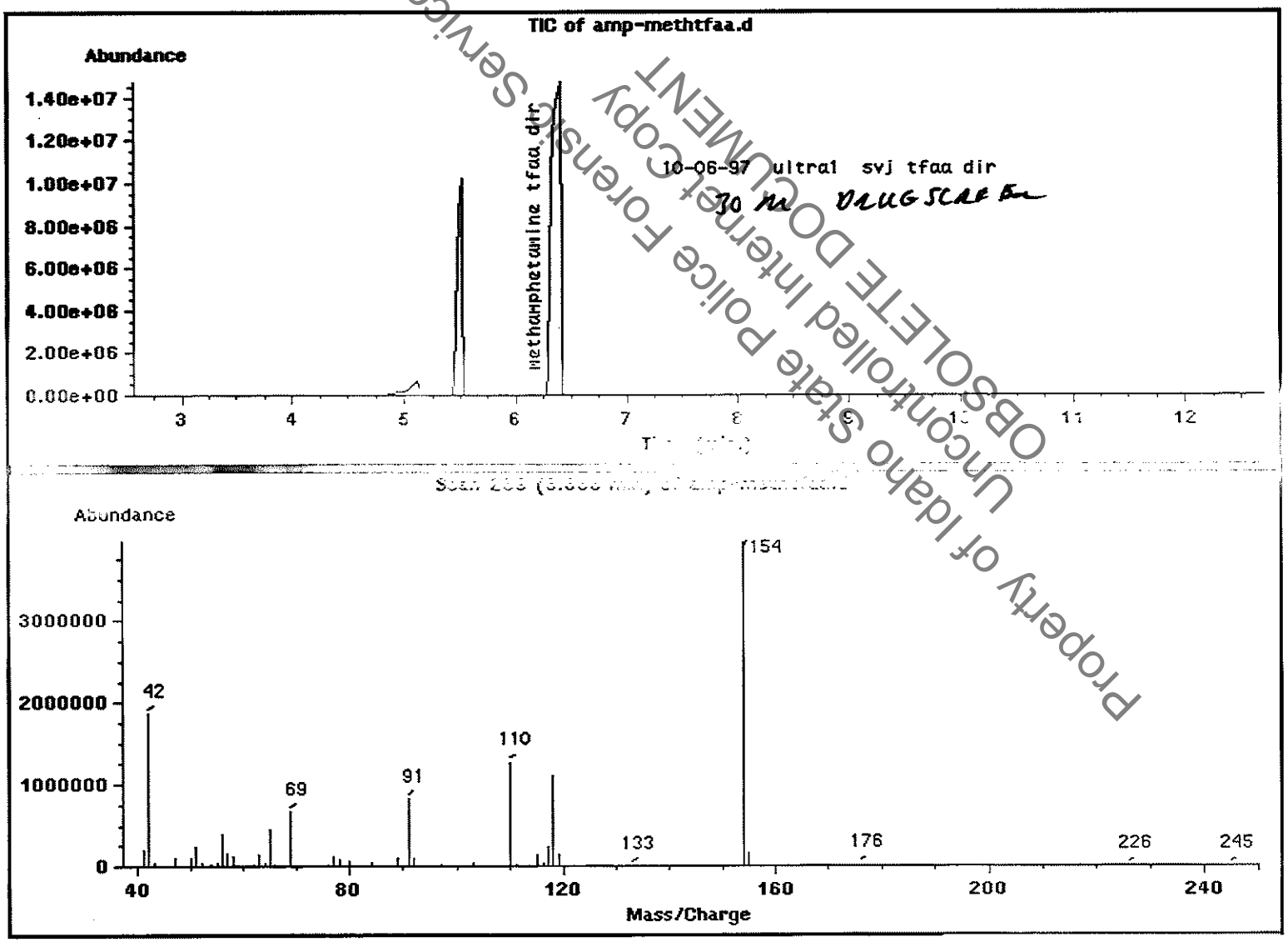


Scan 223 (5.506 min) of amp-methtfaa.d

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
41.05	121672	65.05	429952	89.05	91288	119.10	261888
42.05	137408	66.05	81464	91.05	1158144	120.10	23368
43.05	76752	67.15	4677	92.05	382656	121.00	7891
44.05	66112	68.95	476544	92.95	121344	122.00	3390
45.05	327936	70.05	191808	93.95	7090	126.00	2354
46.15	14164	71.05	6565	95.95	23760	127.00	1787
47.05	118736	72.05	3144	96.95	33552	128.10	5414
48.05	3554	73.05	5209	97.95	1617	129.10	3716
49.95	90552	73.95	31176	100.95	4377	130.10	4141
51.05	172992	75.05	20344	102.05	24408	132.00	4487
52.05	42368	76.05	20232	103.05	48664	133.10	5444
53.05	15753	77.05	94304	104.05	13559	134.10	2650
53.95	4862	78.05	64520	105.05	4305	136.30	2144
55.05	3437	79.05	11927	106.05	2548	140.00	2285568
55.95	4500	80.05	1866	109.10	1964	141.00	103976
57.55	26056	81.05	2219	109.90	4222	142.00	6180
61.05	7784	82.05	1777	113.00	70944	144.10	18392
62.05	42144	83.05	2187	115.10	125696	145.00	3048
63.05	137792	84.05	8245	117.10	356608	146.00	3961
64.15	50544	85.05	9050	118.10	1958400	147.10	2555

Scan 223 (5.506 min) of [illegible]methtfaa.d

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
149.10	5152	160.10	4742	178.00	4164	214.15	1727
152.00	1669	160.90	2187	192.05	4835	216.05	5459
162.10	16856	170.10	1566	198.05	5444	231.05	6936
163.10	2842	171.10	1264				



Scan 289 (6.383 min) of amp-methtfaa.d

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
41.15	224448	63.05	159488	89.05	109464	117.10	256960
42.05	1880576	64.15	56336	91.05	839296	118.10	1124352
43.05	61280	65.05	471424	92.05	115880	119.10	151104
44.05	11417	66.05	37192	93.05	5238	120.10	9905
45.05	3066	67.05	4152	96.05	8066	124.10	1787
47.05	118416	68.05	679488	96.95	30256	126.00	2408
48.05	3352	69.05	22440	98.05	10376	127.00	3544
50.05	121016	71.05	11830	100.15	1594	128.00	5326
51.05	248768	71.95	2108	101.05	4588	129.10	4729
52.05	67016	72.95	22744	102.05	24144	130.00	12058
53.05	22376	73.95	22496	103.05	52648	131.10	12124
54.05	38744	76.05	33008	104.05	29048	132.10	17048
55.15	49192	77.05	134848	105.05	15246	133.10	18952
56.05	408384	77.95	94824	106.05	5881	134.10	3485
57.05	171648	79.05	25080	110.00	1287168	135.00	5138
58.05	131264	89.05	71720	111.10	46088	139.20	1363
59.05	6440	89.95	56648	112.00	1732	140.10	3939
60.05	10548	91.05	5551	113.00	3614	144.10	3287
61.05	8709	91.95	6785	115.10	152768	145.00	1930
62.05	42832	92.95	10751	116.10	52360	146.10	8177

Scan 289 (6.383 min) of amp-methtfaa.d

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
147.10	9174	159.05	10751	175.10	2891	230.05	2998
148.10	11417	159.95	30138	176.10	28656	244.15	1757
149.10	6547	161.05	2704	177.10	4016	245.05	8110
154.00	3971072	161.95	8554	178.10	4957	246.15	2126
155.00	185152	163.05	5015	226.05	1631		

COCAINE/BENZOYLECGONINE BLOOD EXTRACTION AND DERIVATRZATION PROCEDURE

INTRODUCTION:

Cocaine is a naturally occurring alkaloid. It is a powerful central nervous system stimulant. It increases mental awareness and alertness and gives a feeling of well-being and euphoria. Cocaine may be snorted, injected and in the case of the free base smoked. Cocaine converts to benzoylecgonine over time in blood tubes containing sodium floride.

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph
Hewlett packard 6890 Auto Sampler
Hewlett Packard 5973 Mass Select Detector (MSD)

COLUMN:

30 meter HP5-MS, catalog # 19091S-433, film thickness 0.25 microns, internal diameter 0.25 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol

REAGENTS (cont):

Hydrochloric acid - concentrated
Methylene chloride
Isopropanol
Ammonium hydroxide
BSTFA

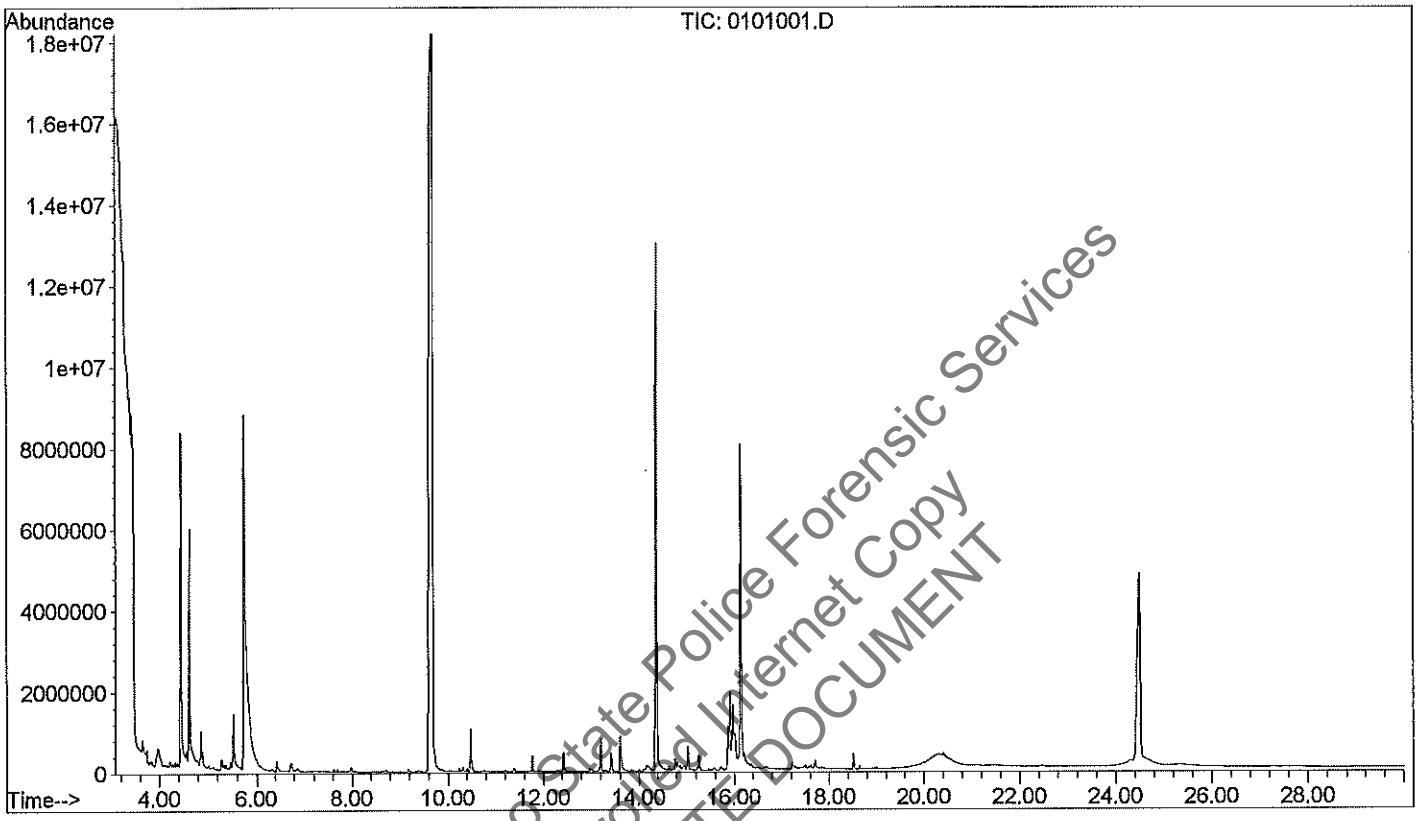
Prepare the following:

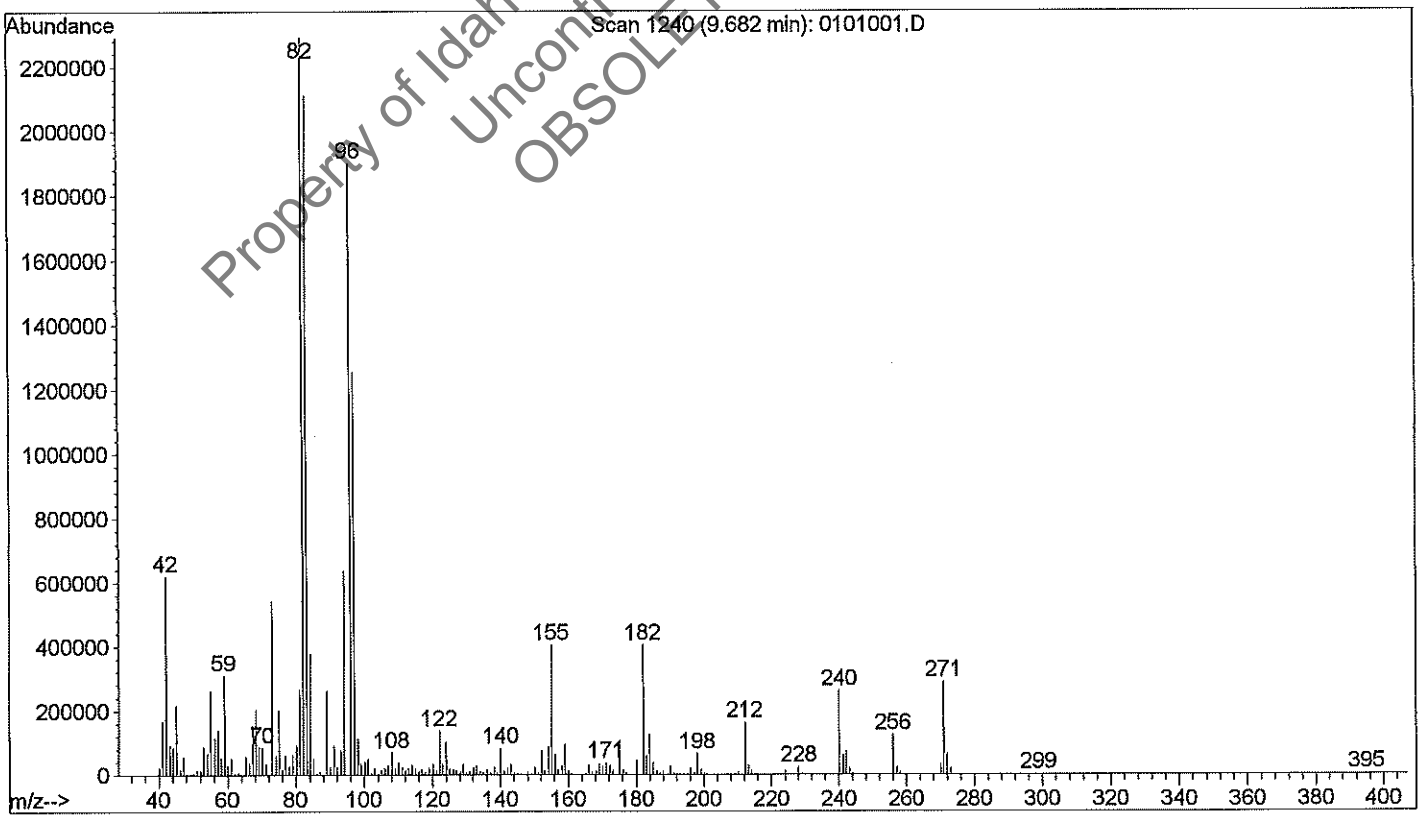
1. 100 mM, pH 6.0 Phosphate buffer
2. 100 mM HCl
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).

PROCEDURE:

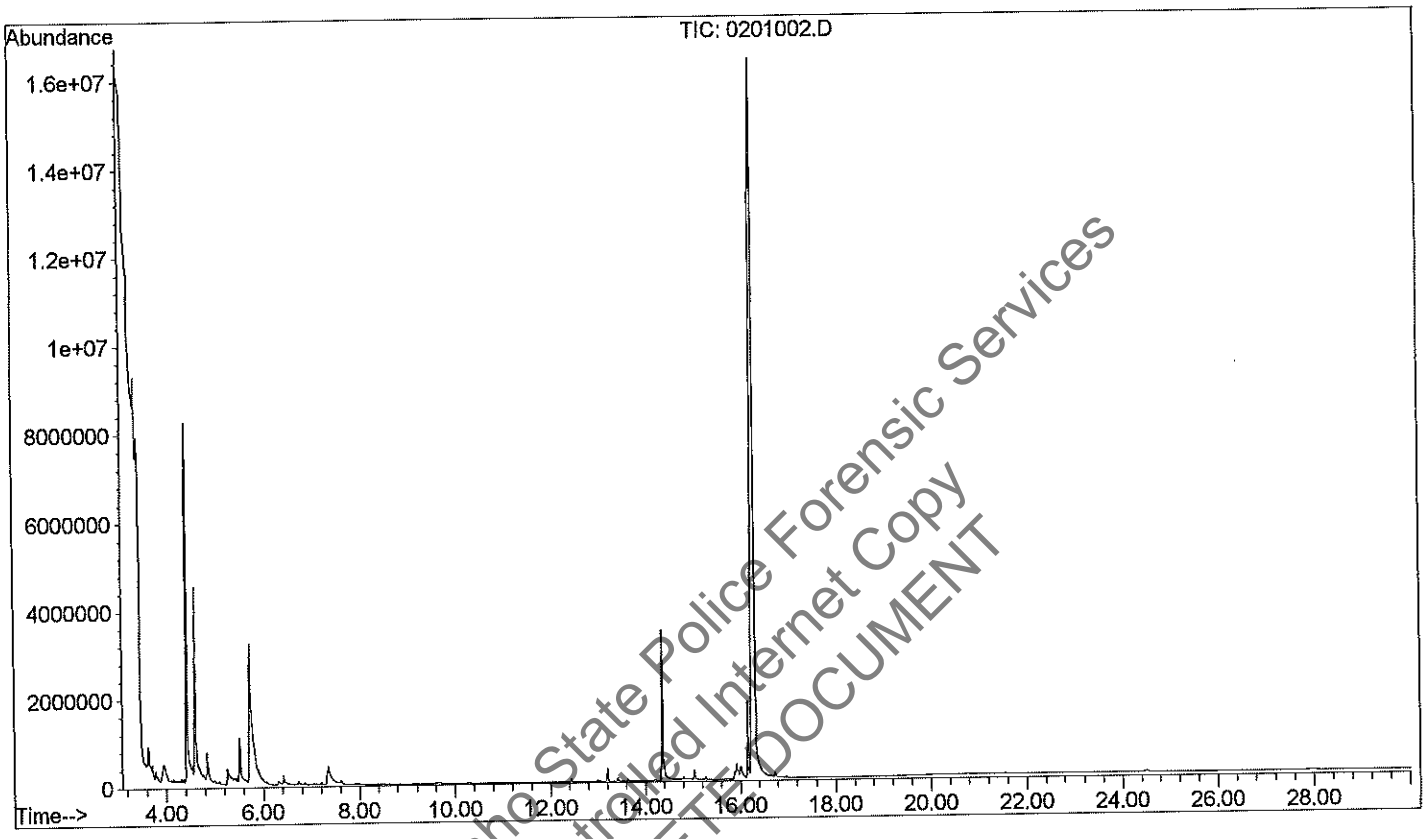
1. Pipet 2ml of sample (case sample, blank, control) into screw top tube
2. Add 8ml DI water, vortex, let stand for 5 minutes.
3. Centrifuge for 10 minutes
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
    a. 1 x 3ml methanol
    b. 1 x 3ml DI water
    c. 1 x 1ml 100 mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column
    a. 1 x 2ml DI H_2O
    b. 1 x 2ml 100mM HCl
    c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum of10 inches Hg.
9. Elute with 6ml of elution solvent into centrifuge tube
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul BSTFA, cap, vortex heat at 90°C for 15 minutes.
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using SIM method monitoring the following ions: 82, 83, 94, 96, 105, 182, 198, 240, 241, 256, 303, 346, 361.

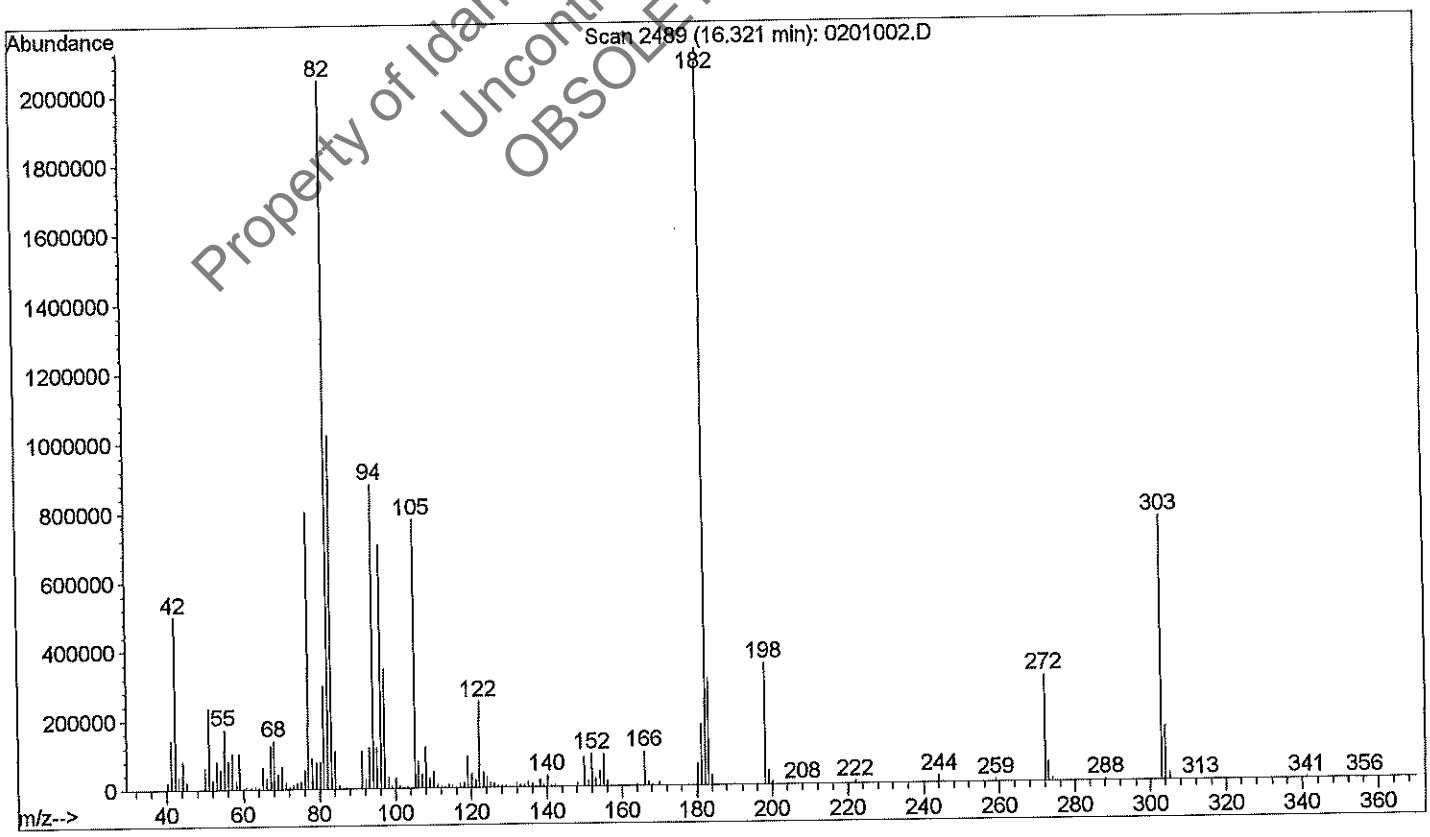
File : D:\HPCHEM\1\DATA\SVJ\050100\0101001.D
Operator : SVJ
Acquired : 1 May 2000 13:53 using AcqMethod COCSCAN
Instrument : GC/MS Ins
Sample Name: ECGONINE METHYL ESTER
Misc Info :
Vial Number: 1



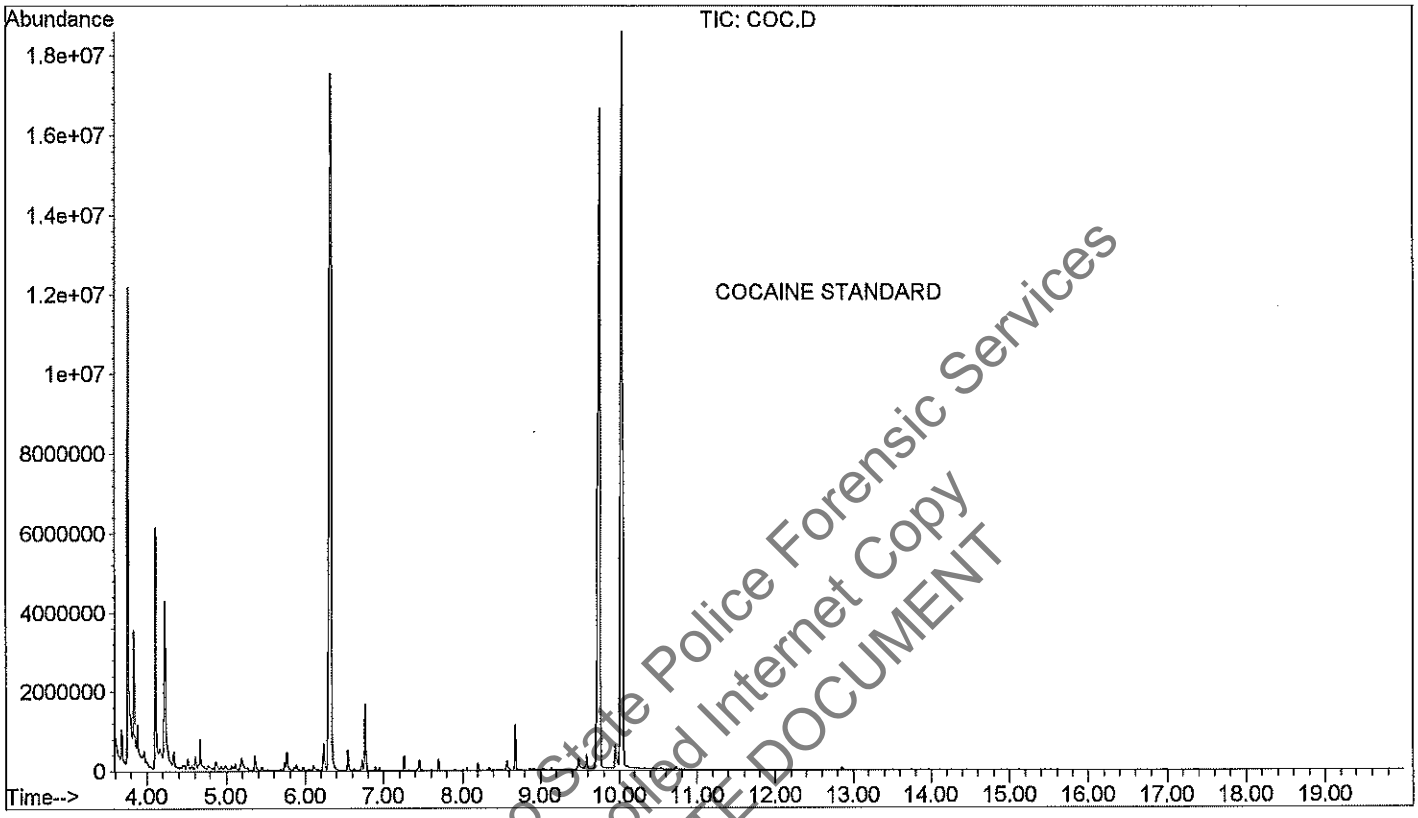


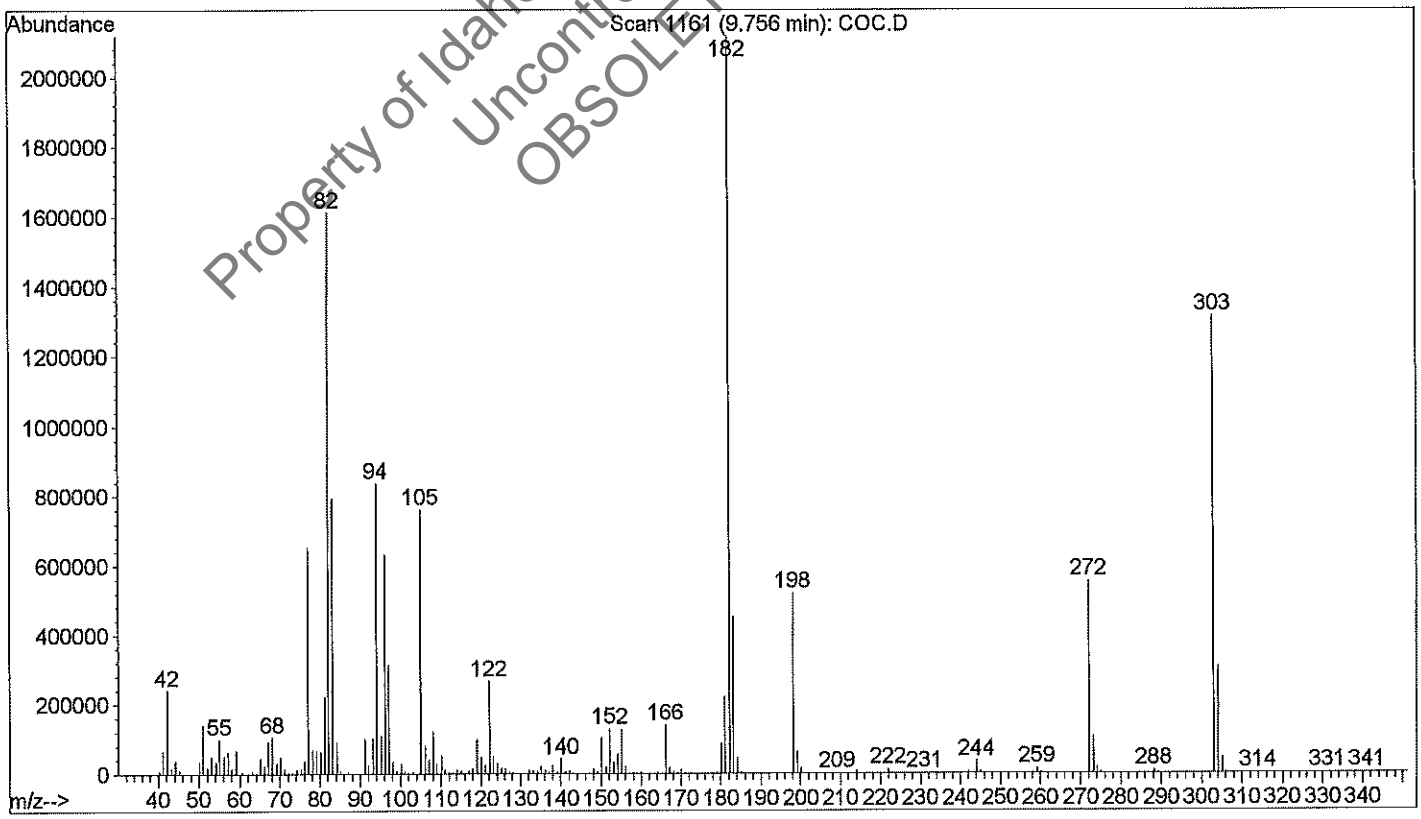
File : D:\HPCHEM\1\DATA\SVJ\050100\0201002.D
Operator : SVJ
Acquired : 1 May 2000 14:42 using AcqMethod COCSCAN
Instrument : GC/MS Ins
Sample Name: COCAINE
Misc Info : BSTFA DIR
Vial Number: 2





File : D:\HPCHEM\1\DATA\COC.D
Operator : SVJ
Acquired : 18 Feb 1998 11:35 using AcqMethod 2
Instrument : GC/MS Ins
Sample Name: COCAINE/ECGONINE METHYLESTER/BENZOYL
Misc Info : BSTFA DIR
Vial Number: 1





Scan 1161 (9.756 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
40.15	7846	52.10	16159	64.15	2439	75.20	12964
41.15	64160	53.10	47528	65.15	45048	76.10	35976
42.15	240896	54.10	33232	66.15	23256	77.05	651136
43.15	16680	55.10	97752	67.10	91992	78.05	68320
44.15	37720	56.15	49424	68.10	104096	79.15	64128
45.15	9443	57.15	62600	69.10	30248	80.15	61992
46.00	277	58.15	13910	70.10	48704	81.15	221120
47.00	207	59.05	66432	71.10	15249	82.15	1612288
47.60	216	60.45	3137	72.10	3872	83.15	794304
50.10	33272	62.05	1493	73.10	3523	84.15	89488
51.10	139456	63.05	4610	74.10	11521	85.15	8195

Scan 1161 (9.756 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
86.15	1852	98.15	32400	110.10	50808	121.15	23080
87.10	3073	99.05	6479	111.10	10978	122.15	266240
88.10	303	100.15	27288	112.10	4042	123.15	49800
89.10	1587	101.05	5688	113.10	3724	124.15	32104
91.10	100288	102.05	1379	114.10	10993	125.15	14385
92.10	23680	103.15	4365	115.10	6864	126.15	13175
93.10	100776	105.05	760640	116.10	1768	127.05	3464
94.10	835840	106.15	80240	117.10	6928	128.10	4465
95.20	109016	107.15	39664	118.05	15334	129.00	1493
96.20	631488	108.10	121776	119.05	97752	130.10	1640
97.15	312640	109.10	27528	120.05	48144	131.10	1995

Scan 1161 (9.756 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
132.10	10110	143.05	1325	154.10	57864	166.15	138240
133.10	9138	144.15	621	155.10	124832	167.15	16608
134.10	7091	145.05	858	156.10	20200	168.15	6834
135.10	19880	146.05	732	157.10	2752	169.10	1263
136.10	11824	147.15	1698	158.10	1220	170.10	11315
137.10	3189	148.15	12065	159.05	1881	171.10	1338
138.05	23824	149.10	4754	160.15	3295	172.10	904
139.15	5840	150.10	104016	161.25	887	173.10	442
140.05	41680	151.10	19024	162.15	1935	174.10	526
141.05	5459	152.10	127208	163.15	541	175.10	317
142.15	7016	153.10	31160	164.05	3836	176.10	414

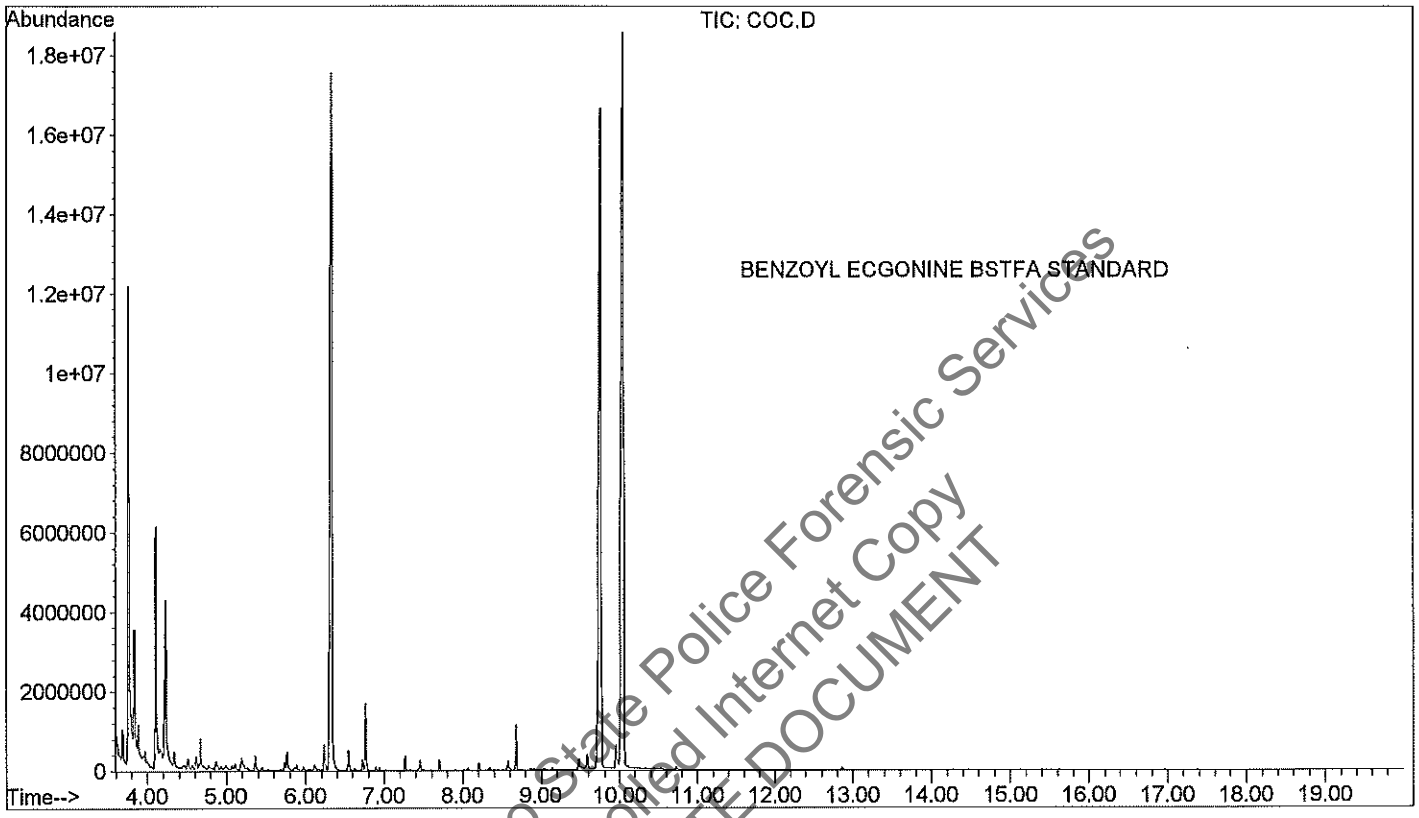
Scan 1161 (9.756 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

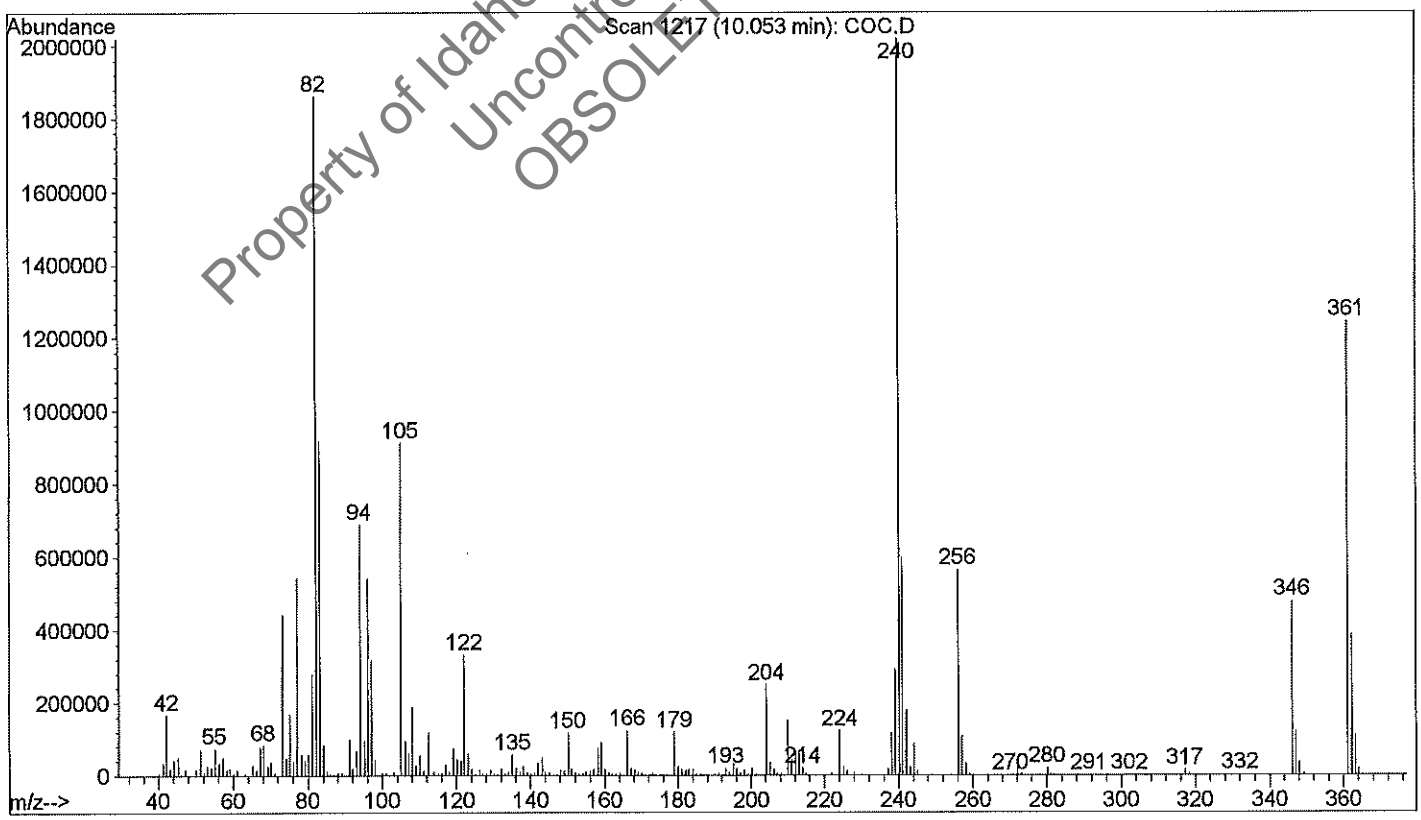
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
177.00	231	188.15	635	201.05	1638	215.10	945
178.10	878	189.05	350	202.05	510	216.10	683
179.15	4464	190.10	1794	203.15	271	217.20	238
180.15	86720	190.90	538	204.05	2471	220.05	280
181.15	221504	193.10	430	204.95	750	222.05	11710
182.15	2117120	194.00	240	206.05	201	223.05	1726
183.15	453632	195.20	387	207.05	758	224.15	391
184.15	43776	196.10	1380	208.15	253	225.05	255
185.15	3062	198.20	519232	209.15	292	226.05	310
186.05	714	199.10	63416	210.00	204	228.05	410
187.15	381	200.15	14788	214.10	4715	230.10	984

Scan 1161 (9.756 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
231.10	428	257.10	282	276.00	551	306.15	4513
237.00	228	258.10	1092	277.10	218	307.15	463
239.20	472	259.20	13400	281.05	438	309.15	203
240.95	230	260.20	4098	281.95	208	312.90	271
242.05	4377	261.05	715	288.15	7809	313.90	272
243.15	1235	262.15	260	289.15	1421	316.40	243
244.15	34696	270.15	522	290.15	286	331.15	212
245.15	6020	272.10	554560	299.20	311	341.10	224
246.15	819	273.10	105544	303.15	1313792		
249.05	202	274.10	15899	304.15	308416		
253.10	329	275.10	3316	305.15	44416		

File : D:\HPCHEM\1\DATA\COC.D
Operator : SVJ
Acquired : 18 Feb 1998 11:35 using AcqMethod 2
Instrument : GC/MS Ins
Sample Name: COCAINE/ECGONINE METHYLESTER/BENZOYL
Misc Info : BSTFA DIR
Vial Number: 1





Scan 1217 (10.053 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
40.15	4538	51.10	70536	62.15	1941	74.10	47840
41.15	34288	52.10	8428	63.15	3767	75.10	168256
42.15	167616	53.10	26304	64.15	1341	76.10	37816
43.15	19280	54.10	21504	65.15	28224	77.05	542336
44.15	41560	55.10	74312	66.15	16063	78.15	57656
45.15	50296	56.15	34104	67.10	79272	79.15	39896
46.10	3614	57.15	49400	68.10	82672	80.15	55424
47.10	16680	58.15	17224	69.10	27256	81.15	279680
48.10	984	59.05	19104	70.10	37352	82.15	1861120
49.10	2407	60.15	4519	71.10	7570	83.15	917376
50.10	15015	61.05	13616	73.10	442880	84.15	84392

Scan 1217 (10.053 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
85.15	10698	97.15	317120	110.10	54776	122.15	331840
86.05	3450	98.15	41648	111.10	11663	123.15	59800
87.10	2118	100.05	6139	112.60	117104	124.15	16904
88.10	6806	101.05	6012	114.00	10949	125.05	4287
89.10	5422	102.05	2174	115.10	4553	126.15	13824
91.10	98136	103.15	7454	116.10	6319	127.05	4435
92.10	19072	105.15	912000	117.10	29032	128.00	3089
93.10	66872	106.15	94736	118.05	10385	129.10	15532
94.10	688640	107.15	62056	119.05	74368	130.10	4888
95.20	96112	108.10	187136	120.15	43432	131.10	4199
96.20	541312	109.10	27816	121.15	40768	132.10	18368

Scan 1217 (10.053 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
133.10	6008	143.95	10116	155.10	9479	167.15	19728
134.10	10309	145.05	6741	156.10	12612	168.15	13913
135.10	56296	146.15	2162	157.10	15382	169.10	7958
136.10	20768	147.15	3702	158.10	76320	170.10	4829
137.10	11026	148.15	14445	159.05	90192	171.10	2342
138.05	25048	149.10	13076	160.05	16776	172.30	4121
139.05	6230	150.10	116496	161.15	7333	173.00	5156
140.05	5803	151.10	18720	162.05	5142	173.90	1116
141.05	4750	152.10	7423	163.05	4078	175.10	2374
142.05	32608	153.10	3127	164.15	4704	176.10	968
143.05	47096	154.10	5609	166.15	122984	177.10	1373

Scan 1217 (10.053 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
179.05	119184	190.00	2935	201.15	3479	214.10	21000
180.05	24344	191.10	4671	202.15	987	215.10	4432
181.05	15468	192.10	2011	204.15	248768	216.10	2211
182.05	13665	193.10	20088	205.15	34624	217.10	689
183.05	15268	194.10	9448	206.15	15053	218.20	491
184.05	15042	195.10	33712	207.15	5007	219.00	460
185.05	3744	196.10	18616	208.15	4767	220.05	941
186.05	2927	197.10	7786	210.10	150144	221.15	2036
187.15	904	198.10	14718	211.10	43304	222.05	6033
188.15	1001	199.10	3313	212.10	66368	224.15	122088
189.10	683	200.15	18840	213.10	58832	225.15	22520

Scan 1217 (10.053 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
226.15	12843	238.20	116264	250.05	354	262.05	696
227.15	2395	239.20	291840	251.20	247	263.05	697
228.15	6625	240.15	2018816	252.00	717	264.05	361
229.05	1410	241.15	596224	253.00	405	265.05	455
230.10	1402	242.15	178624	254.10	1646	267.15	324
231.10	526	243.15	22216	256.10	563520	269.05	272
232.10	375	244.15	85296	257.10	108304	270.15	555
233.00	262	245.15	13135	258.10	31856	271.10	1077
234.00	327	246.15	1458	259.10	4138	272.10	18856
235.10	535	247.05	823	260.20	2307	273.10	3875
237.20	18344	249.05	1238	261.05	434	274.10	607

Scan 1217 (10.053 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
275.10	221	291.25	213	305.05	228	327.05	367
278.10	1390	295.10	374	311.25	247	328.15	825
280.10	19632	296.20	233	312.00	329	330.25	373
281.05	4315	297.10	294	313.10	759	331.15	275
282.05	1350	298.30	209	314.20	497	332.15	2346
283.15	430	299.20	501	315.10	254	333.10	1327
285.05	350	300.10	322	316.10	2013	334.10	524
286.05	250	301.00	301	317.20	15681	335.10	236
288.15	1992	302.25	1377	318.20	4818	339.20	294
289.15	610	303.05	381	319.20	1299	341.30	542
290.15	210	304.05	216	320.20	377	342.20	353

Scan 1217 (10.053 min): COC.D
COCAINE/ECGONINE METHYLESTER/BENZOYL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
343.15	260	362.20	386048				
344.15	355	363.15	110960				
346.15	477824	364.15	17240				
347.15	121768	365.15	2127				
348.15	35328	366.25	305				
349.15	5124						
350.15	624						
355.40	587						
356.50	245						
357.40	224						
361.20	1243136						

THC and CARBOXY THC
IN WHOLE BLOOD
FOR GC/MS CONFIRMATIONS USING:
200 MG CLEAN SCREEN® EXTRACTION COLUMN
ZSTHC020

1. PREPARE SAMPLE

To ~~1~~ 2 ml of whole blood sample add internal standard(s)* and ~~1~~ 2 ml of acetonitrile.
Mix/vortex. Let stand 5 minutes. Vortex.
Centrifuge for 10 minutes at maximum rpm.
Decant and add ~~5~~ 6 ml of 100 mM acetate buffer (pH 4.5) to supernatant.
Mix/vortex, centrifuge 5 minutes to remove blood fragments or foam.

2. PRECONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 ml hexane/ethyl acetate (75/25); aspirate.

3. CONDITION CLEAN SCREEN® EXTRACTION COLUMN

1 x 3 ml CH_3OH; aspirate.
1 x 3 ml DI H_2O; aspirate. NOTE: Use gravity flow or minimal vacuum.
1 x 1 ml 100 mM HCl; aspirate.

4. APPLY SAMPLE

Load at 1 ml/minute. **NOTE**: Use gravity flow or minimal vacuum.

5. WASH COLUMN

1 x 2 ml DI H_2O; aspirate.
1 x 2 ml 100 mM HCl/acetonitrile (70/30); aspirate.
Dry columns (5 minutes at ≥ 10 inches Hg).
1 x 200 µl hexane. **NOTE:** Use gravity flow or minimal vacuum.

6. ELUTE THC AND CARBOXY THC

1 x 3 ml hexane/ethyl acetate (75/25). NOTE: Use gravity flow or minimal vacuum.

7. DRY ELUATE

Evaporate slowly to dryness at ≤ 40° C.

8. DERIVATIZE

Add 50 µl BSTFA (with 1% TMCS) ~~and 50 µl of ethyl/acetate~~.
~~Overlayer with N_2 and~~ cap. Mix/vortex.
React ~~30~~ 15 minutes at ~~70°~~ 160° C. Remove from heat source to cool.
NOTE: Do not evaporate BSTFA solution.

9. QUANTITATE

Inject ~~2~~ 1 µl sample onto chromatograph.
Monitor the following ions (GC/MS):

THC-303**, ~~315~~, 386	D3THC-306**, 318, 389
Carboxy Δ^9 THC-371**, 473, 488	D3Carboxy Δ^9 THC-3744*, 476, 491

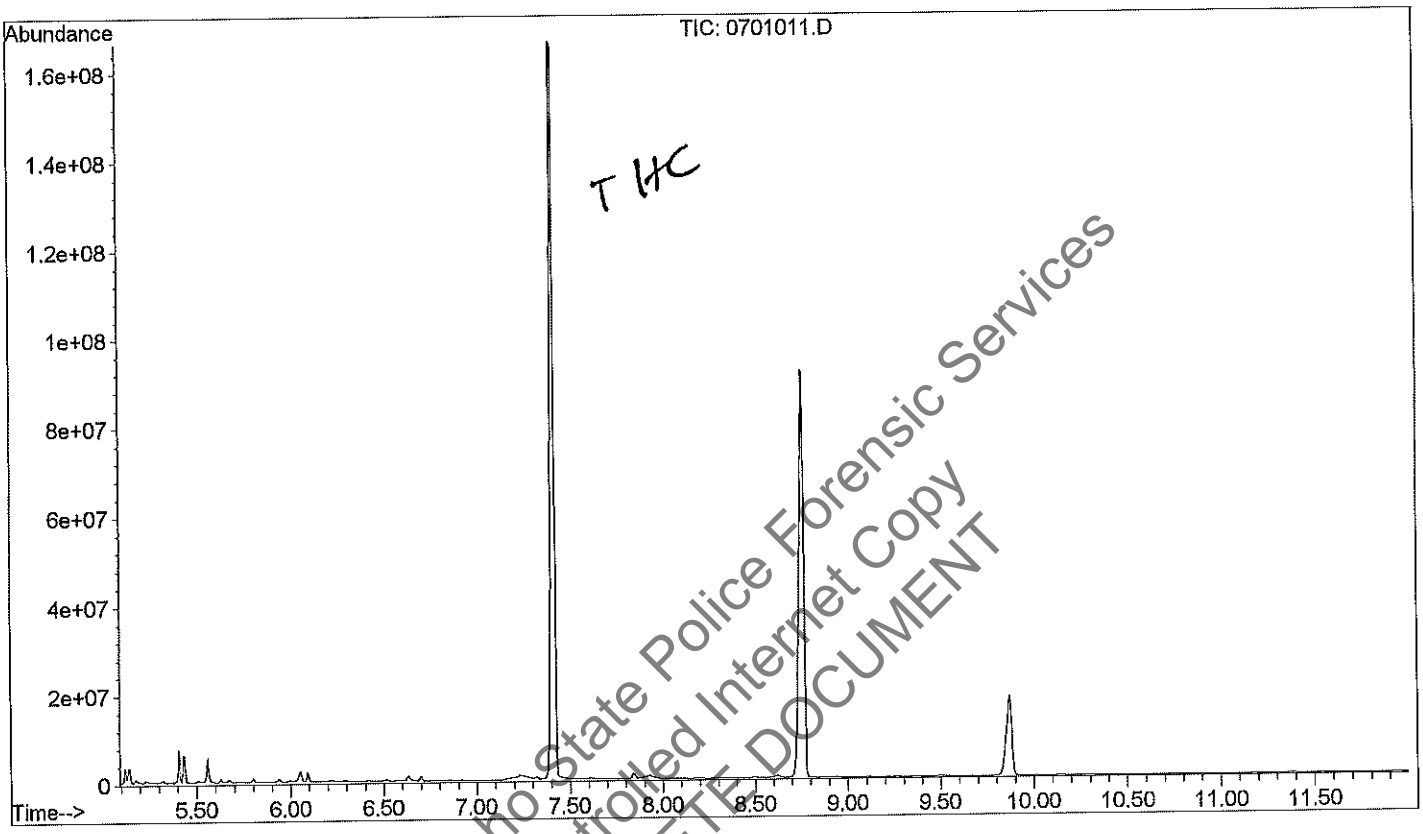
GC/MS Parameters:
HP 5972 MSD, HP-5, using electronic pressure control. MSD set 400V above autotune for THC, 600V abov autotune for THC-COOH. SIM mode with peak area quantitation.

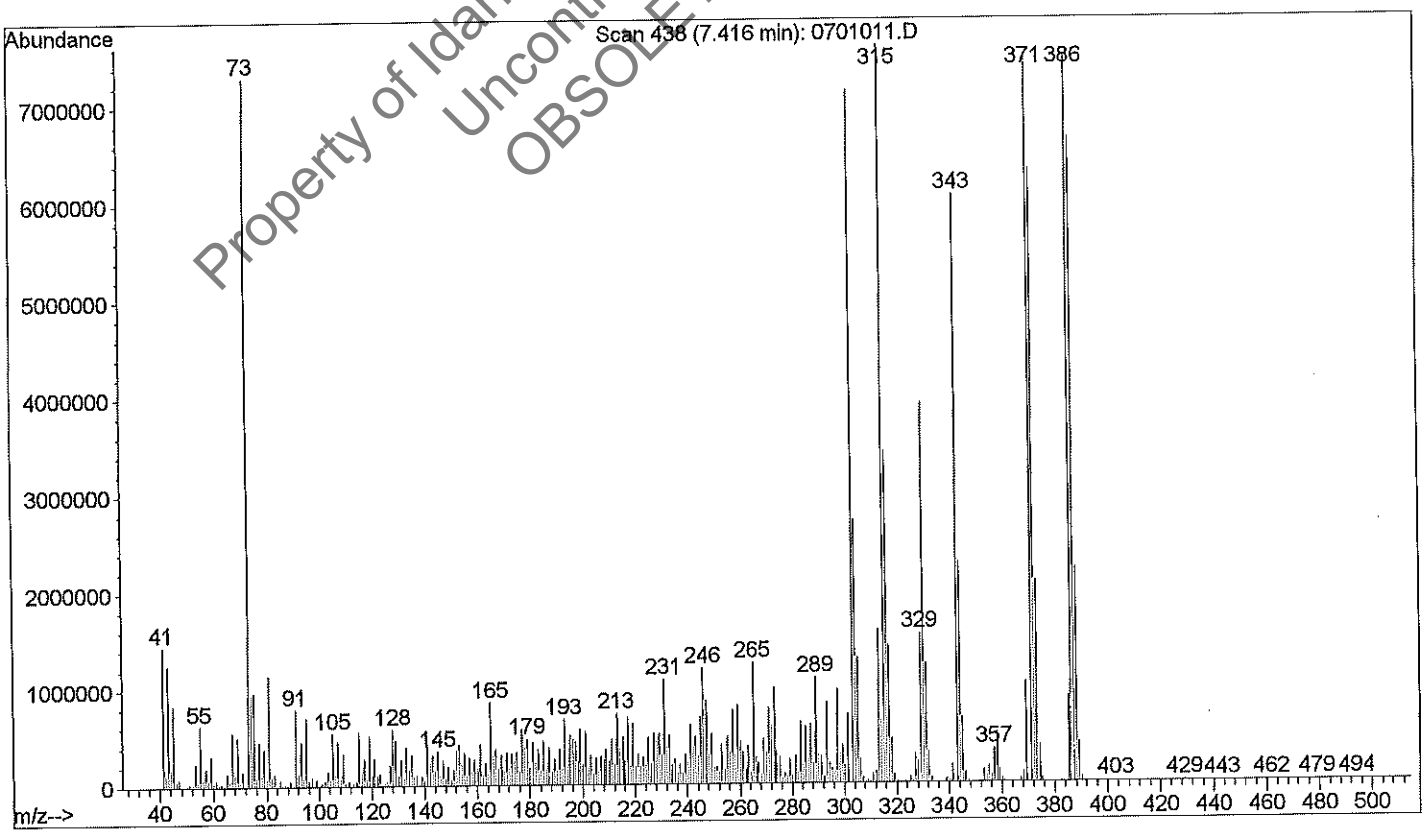
*Suggested internal standards for GC/MS: D_3THC and D_3Carboxy Δ^9 THC
** Quantitation ion

Preliminary Method

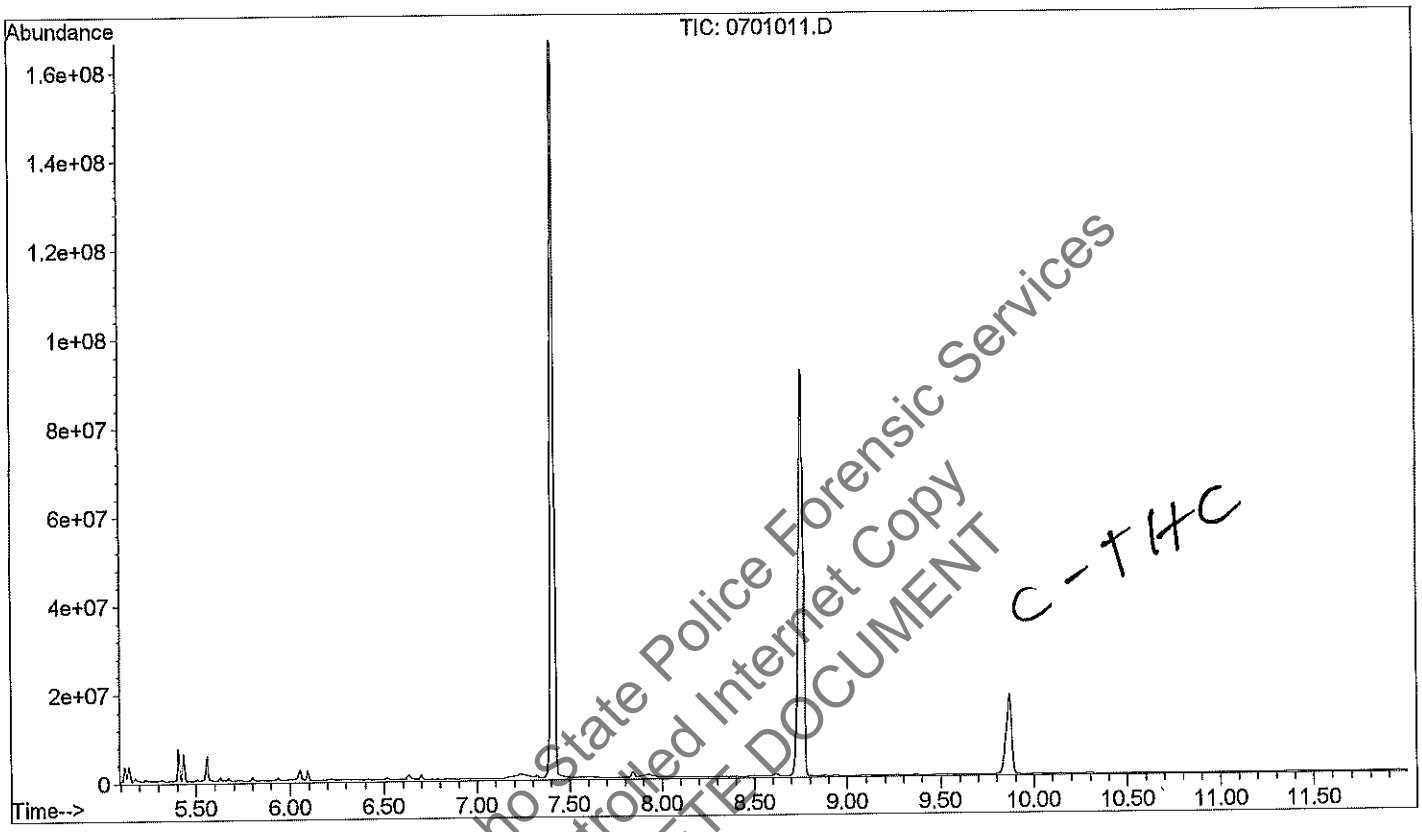
Courtesy of the Las Vegas Metro Police Crime Lab
Procedure Code: THCB200Z081195PRELIM

File : D:\HPCHEM\1\DATA\SVJ\110501\0701011.D
Operator : SVJ
Acquired : 6 Nov 2001 7:44 using AcqMethod BTHCSCN
Instrument : GC/MS Ins
Sample Name: THC STANDARD
Misc Info :
Vial Number: 7

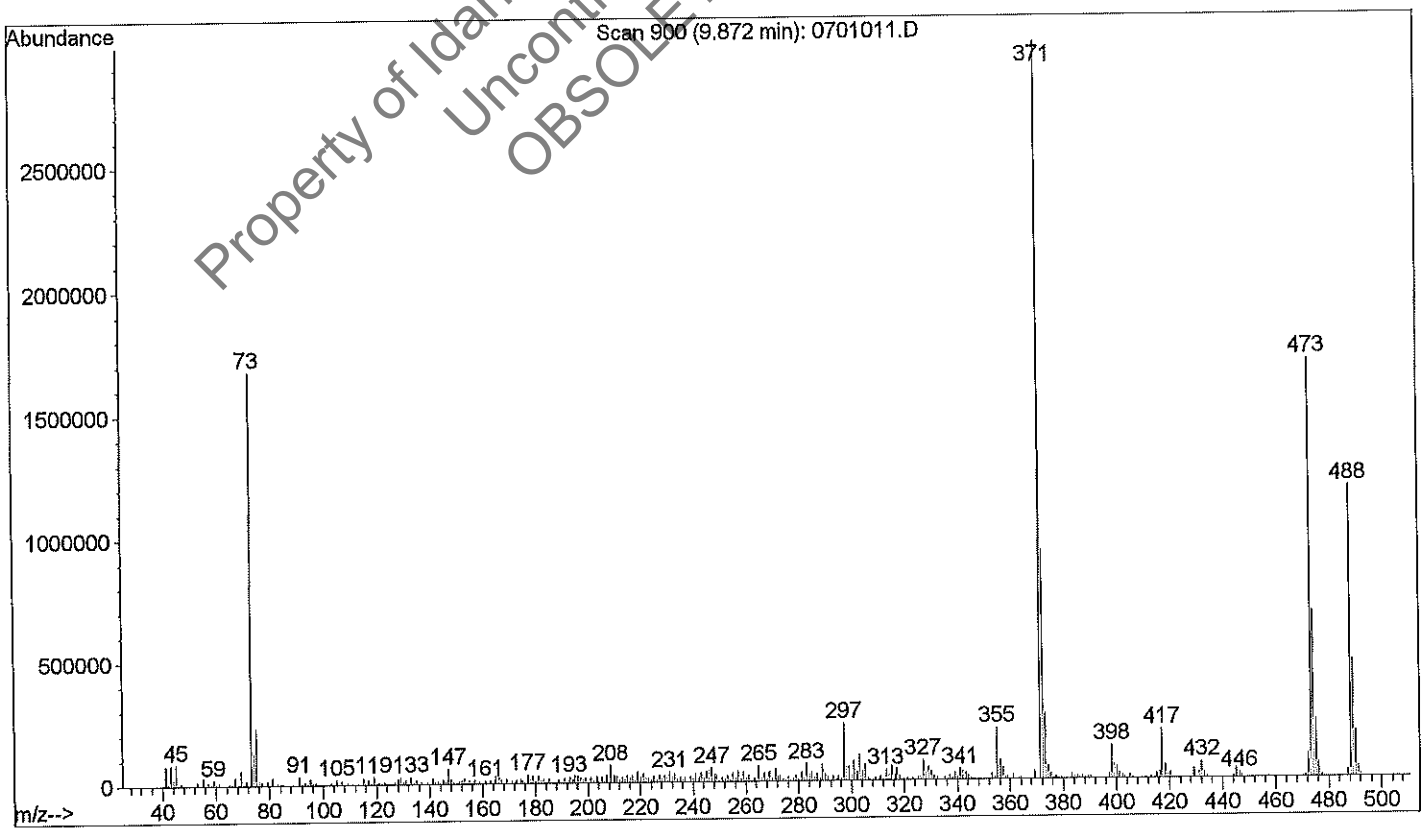




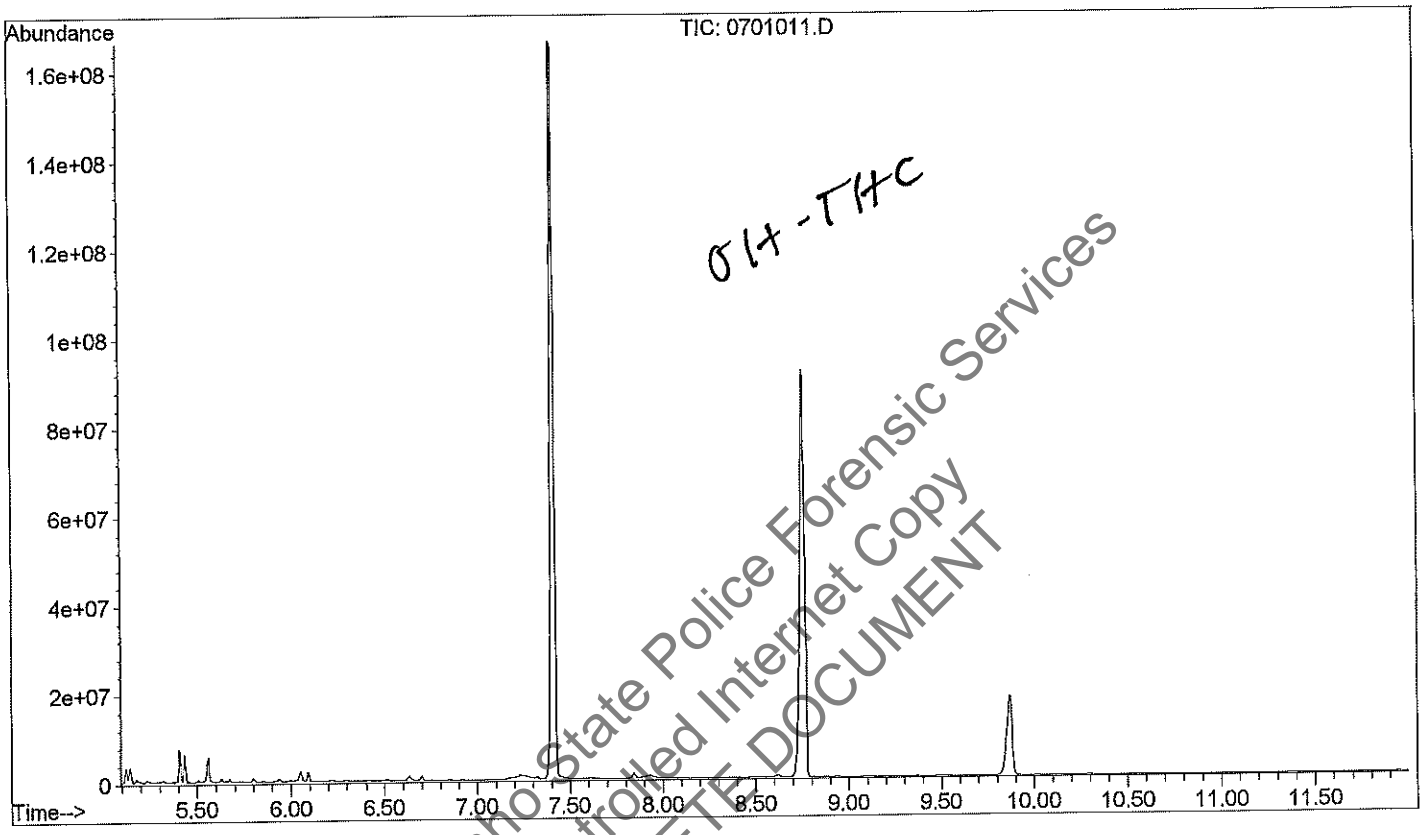
File : D:\HPCHEM\1\DATA\SVJ\110501\0701011.D
Operator : SVJ
Acquired : 6 Nov 2001 7:44 using AcqMethod BTHCSCN
Instrument : GC/MS Ins
Sample Name: THC STANDARD
Misc Info :
Vial Number: 7



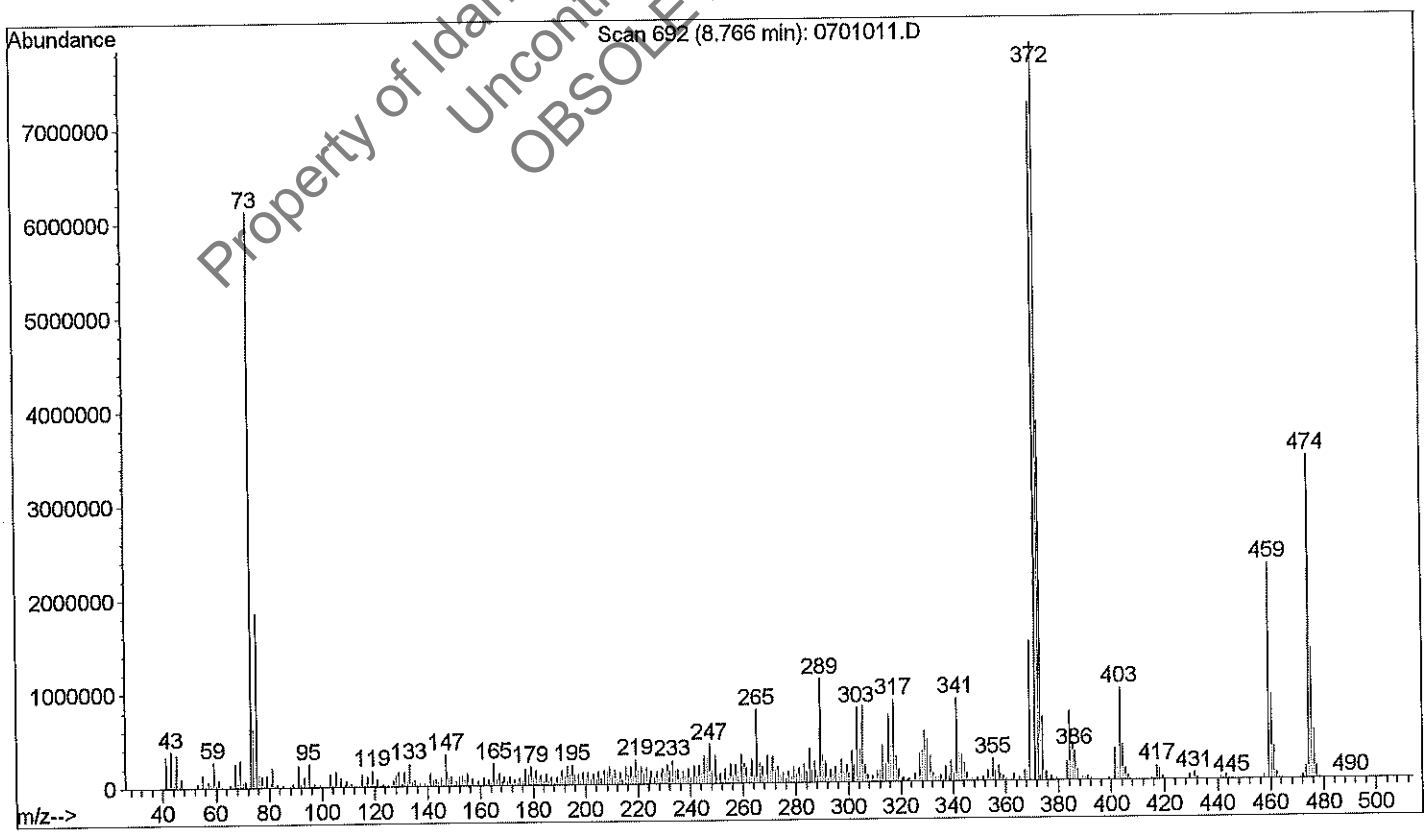
C-THC



File : D:\HPCHEM\1\DATA\SVJ\110501\0701011.D
Operator : SVJ
Acquired : 6 Nov 2001 7:44 using AcqMethod BTHCSCN
Instrument : GC/MS Ins
Sample Name: THC STANDARD
Misc Info :
Vial Number: 7



Δ14-THC



BENZODIAZEPINE BLOOD EXTRACTION AND DERIVATRZATION PROCEDURE

INTRODUCTION:

Benzodiazepines are antianxiety agents. They are classified as long-acting: diazepam, intermediate-acting: lorazepam, or short-acting: triazolam. Effects can include sedation, drowsiness, light-headedness and lethargy. Benzodiazepines are often used in conjunction with other drugs such as cocaine and alcohol.

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph
Hewlett packard 6890 Auto Sampler
Hewlett Packard 5973Mass Select Detector (MSD)

COLUMN:

30 meter HP5-MS, catalog # 19091S-433; film thickness 0.25 microns, internal diameter 0.25 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol

REAGENTS (cont):

Hydrochloric acid - concentrated
Methylene chloride
Isopropanol
Ammonium hydroxide
BSTFA

Prepare the following:

1. 100 mM, pH 6.0 Phosphate buffer
2. 100 mM HCl
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).

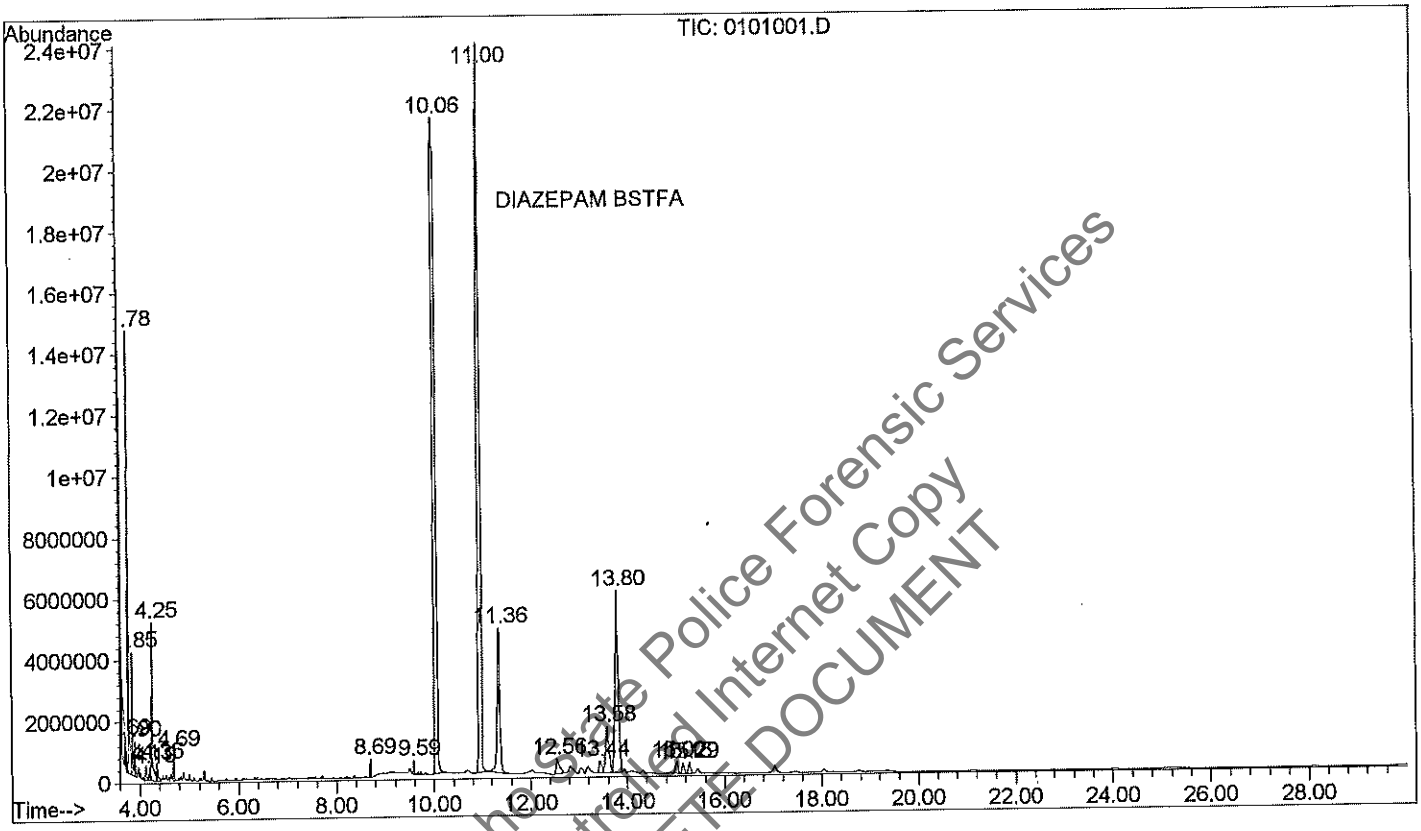
PROCEDURE:

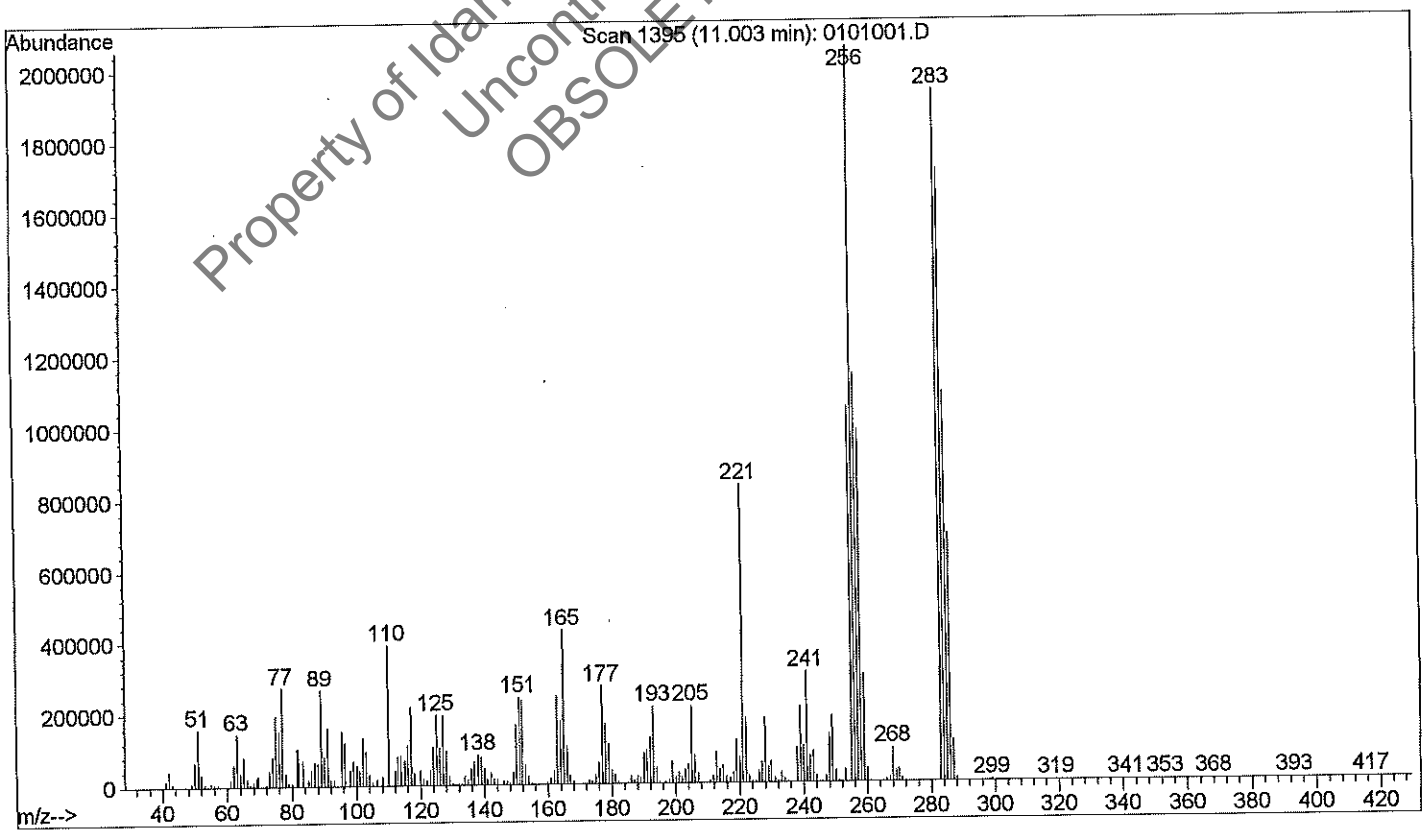
1. Pipet 2ml of sample (case sample, blank, control) into screw top tube
2. Add 8ml DI water, vortex, let stand for 5 minutes.
3. Centrifuge for 10 minutes
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
    a. 1 x 3ml methanol
    b. 1 x 3ml DI water
    c. 1 x 1ml 100 mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column
    a. 1 x 2ml DI H_2O
    b. 1 x 2ml 100mM HCl
    c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum ≈10 inches Hg.
9. Elute with 6ml of elution solvent into centrifuge tube
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul BSTFA, cap, vortex heat at 90°C for 15 minutes.
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using SIM method monitoring the following ions:
    a. desalkylflurazepam - 245, 247, 341, 342, 343, 344, 345, 346, 347, 348, 359, 360, 361, 362, 363.
    b. desmethyldiazepam - 227, 327, 328, 329, 341, 342, 343, 344, 345.
    c. lorazepam -347, 349, 429, 430, 431, 432.
    d. diazepam - 165, 177, 221, 255, 256, 257, 258, 283, 284, 285, 286.
    e. oxazepam - 347, 349, 429, 430, 431, 432, 449, 451.

PROCEDURE (cont):

f. prazepam - 241, 242, 243, 267, 268, 269, 270, 271, 295, 296, 297, 298, 323, 324, 326, 327.
g. flurazepam - 245, 315, 318, 387, 388, 389, 390.
h. triazolam - 238, 239, 279, 313, 314, 315, 343, 344, 345.
I. alparzolam - 204, 273, 279, 307, 308,.
j. chlordiazepoxide -

File : D:\HPCHEM\1\DATA\SVJ\0812\0101001.D
Operator : svj
Acquired : 12 Aug 1998 10:26 using AcqMethod 2-30
Instrument : GC/MS Ins
Sample Name: DIAZEPAM/NORDIA
Misc Info : FULL SCAN DIAZEPAM/NORDIAZEPAM BSTFA
Vial Number: 1





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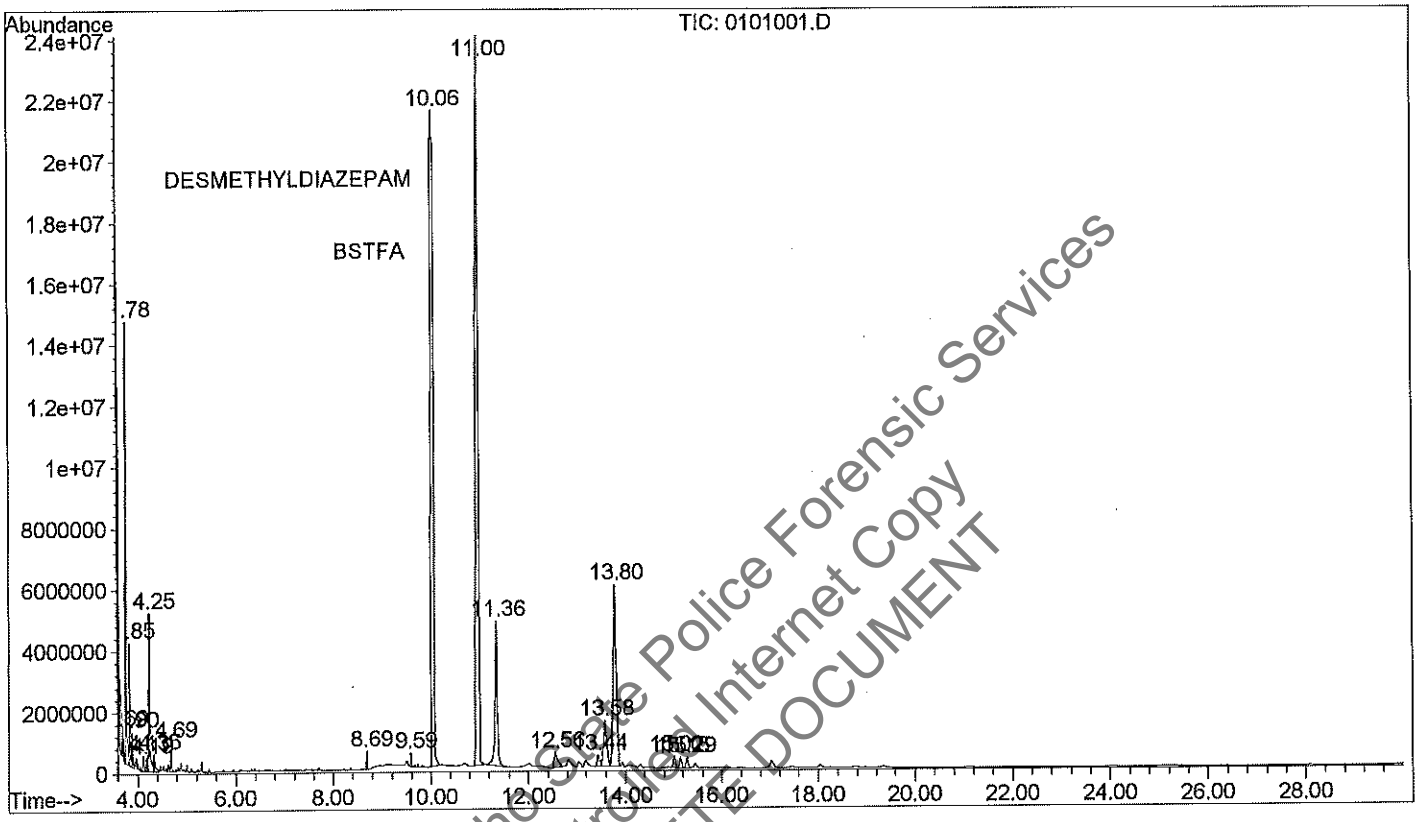
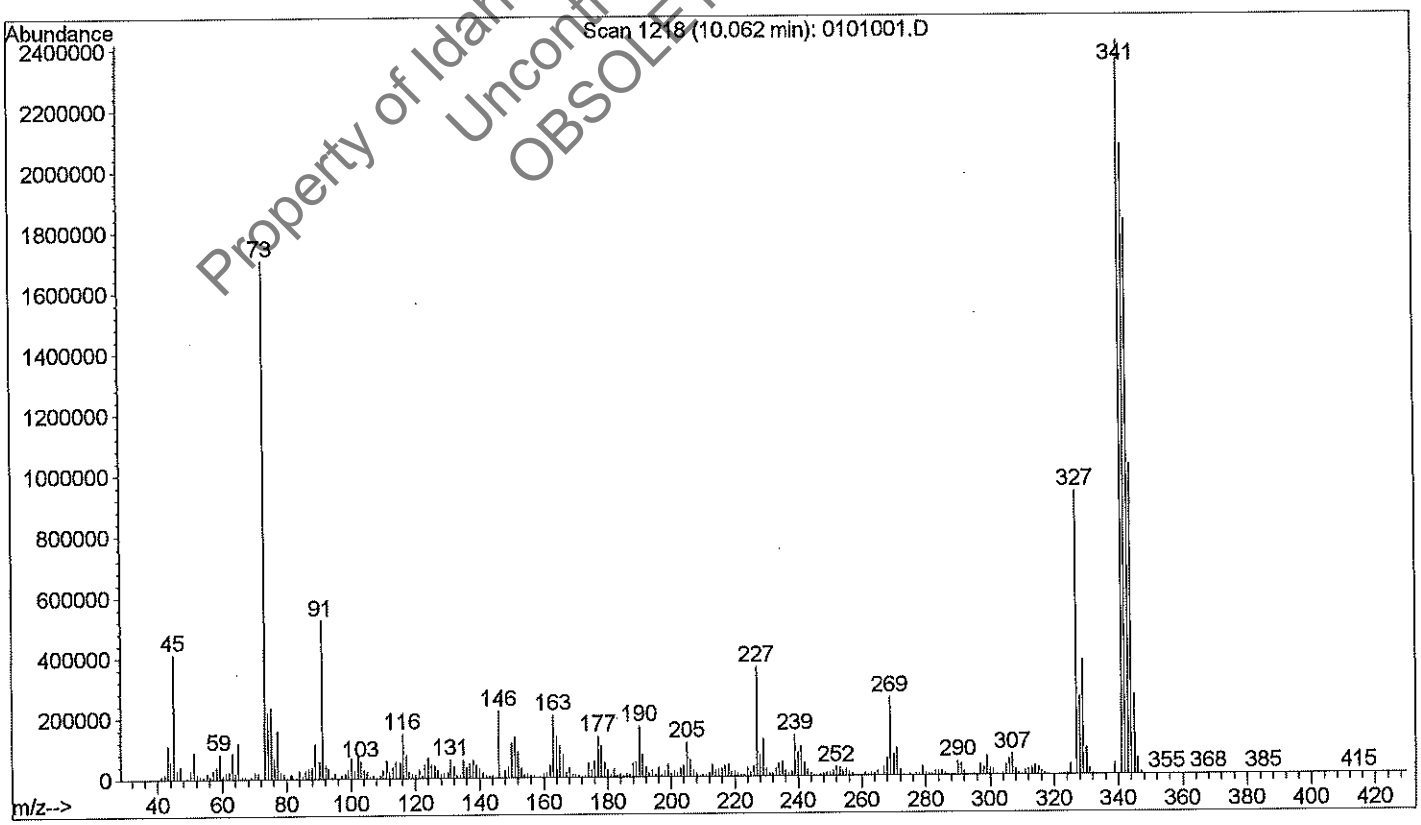
Operator : svj
Acquired : 12 Aug 1998 10:26 using AcqMethod 2-30
Instrument : GC/MS Ins
Sample Name: DIAZEPAM/NORDIA
Misc Info : FULL SCAN DIAZEPAM/NORDIAZEPAM BSTFA
Vial Number: 1





Scan 1218 (10.062 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
41.05	10578	52.10	15206	63.05	84304	75.05	232896
42.05	14934	53.00	10413	64.00	19968	76.05	67816
43.05	109000	54.05	5959	65.00	116880	77.05	157696
44.00	61448	55.05	17184	66.00	9243	78.05	21744
45.00	410688	56.05	11398	67.00	5757	79.05	13934
46.00	29416	57.05	26864	68.00	3423	80.05	4451
47.00	40152	58.05	40968	69.00	5689	81.05	12757
48.00	1787	59.05	80208	70.00	21160	81.45	11774
49.00	4138	60.05	12099	71.10	16856	82.05	10060
50.10	27928	61.05	19928	73.10	1707008	83.00	6504
51.10	87744	62.05	22392	74.05	218560	84.00	26152

Scan 1218 (10.062 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
85.00	11567	97.05	9908	108.00	4806	118.95	13273
86.00	27816	98.05	14548	109.10	10743	119.95	9759
87.00	31760	99.05	30560	110.00	27368	121.05	29192
88.00	35016	100.05	66688	111.00	59768	122.05	10366
89.00	114592	101.05	26056	112.00	21528	123.00	43600
90.10	57104	102.05	72504	112.95	35168	124.00	68264
91.10	522880	103.00	56824	113.95	54248	125.00	43952
92.10	45120	104.00	28320	115.05	49952	126.00	39168
92.95	33704	105.00	22016	116.05	147072	127.00	28280
94.05	6302	106.00	7189	117.05	77280	128.00	15370
94.95	17816	107.00	8307	117.95	22824	129.00	15069

Scan 1218 (10.062 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
130.10	18680	141.05	16400	153.95	11606	165.00	104856
131.00	60952	141.90	8880	154.95	11724	166.00	74408
131.95	38760	142.90	6297	155.95	8819	167.00	17144
132.95	11060	144.00	6080	157.05	5390	168.00	31000
134.05	9948	146.00	220608	158.05	2107	169.00	10379
135.05	58784	148.00	24896	159.05	4651	170.00	8000
135.95	37856	149.00	36464	160.05	11742	171.00	5582
137.05	46760	150.00	115680	161.05	17760	171.95	3357
138.05	58832	151.00	132992	162.00	40200	173.05	6943
139.05	42824	151.95	85912	163.00	204224	174.05	47032
140.05	28536	152.95	32456	164.00	136384	175.05	25024

Scan 1218 (10.062 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
176.05	55544	187.00	10248	197.95	19640	209.00	5813
177.05	134720	188.00	45224	199.05	40272	210.00	5465
178.05	103280	189.00	49600	199.95	12271	210.95	6031
179.05	48136	190.00	166720	201.00	17384	211.95	12076
180.05	25400	191.05	73976	202.00	13778	212.95	37432
181.00	9823	192.05	34040	203.00	26952	213.95	21072
182.00	25448	193.05	14580	204.00	38904	215.05	24384
183.00	8325	193.95	20544	205.00	113304	216.05	26512
184.00	10245	194.95	8574	206.00	57200	217.05	34872
185.00	6320	195.95	28992	207.00	20408	218.05	40448
186.00	11393	196.95	8942	208.00	10970	219.05	16105

Scan 1218 (10.062 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
220.05	16440	231.05	10587	242.00	43184	252.95	27640
221.00	9627	232.05	9483	243.00	21480	253.95	19816
222.00	7204	233.05	25184	244.00	7585	254.95	20368
223.00	6942	234.05	41648	245.00	3915	255.95	15149
224.00	28032	235.05	48032	246.00	2525	256.95	8087
225.00	14307	236.05	19096	247.00	5510	257.95	5253
226.00	32672	237.05	9818	248.00	7888	259.05	2394
227.00	360960	238.05	14579	249.00	11177	260.00	2584
228.00	72096	239.05	135424	250.05	10688	261.00	7734
229.00	122040	240.00	80544	251.05	17216	262.00	5770
229.95	24208	241.00	99400	251.95	29032	263.00	8826

Scan 1218 (10.062 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
264.00	8890	275.05	4255	286.00	8037	297.05	33520
265.00	15018	276.05	3173	287.00	4603	298.05	26400
266.00	7405	277.05	6905	288.10	2066	299.00	62536
267.00	27984	278.05	6924	289.05	3078	300.00	23184
268.00	59904	279.00	29512	290.05	45656	301.00	19104
269.00	258496	280.00	8899	291.05	39352	302.00	5088
269.95	72072	281.00	5786	292.05	14210	303.00	1795
270.95	90736	282.00	7359	293.05	4869	304.10	6448
271.95	18192	283.00	14078	294.05	1764	305.10	33000
273.05	3802	284.00	15811	295.05	4193	306.10	53136
273.95	2005	285.00	14565	296.05	4309	307.10	68728

Scan 1218 (10.062 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
308.10	18184	318.90	468	331.05	21544	345.10	262272
309.05	6025	319.80	263	332.05	3264	346.10	55168
309.95	3519	321.00	346	333.05	658	347.10	7697
310.95	15081	322.00	346	335.05	254	348.05	872
311.95	19720	323.00	1727	335.45	275	355.15	443
313.05	23712	324.00	5660	337.15	1170	356.15	223
314.05	29072	325.00	34424	339.10	38608	368.25	477
315.05	25192	327.10	934976	341.10	2417664	369.15	295
316.05	12095	328.05	258624	342.10	2077696	370.95	284
317.05	7104	329.05	377984	343.10	1831936	385.20	452
318.00	1442	330.05	91448	344.10	1023040	387.05	252

Scan 1218 (10.062 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
405.00	214						
415.05	377						
416.10	293						
417.20	305						

Scan 1395 (11.003 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
41.05	17672	53.10	7315	64.00	38288	76.05	153920
42.05	42784	54.05	4062	65.00	81112	77.05	273024
43.05	9612	55.05	8268	66.00	19664	78.05	36016
44.00	1223	56.05	7640	67.10	6328	79.05	8529
45.00	579	57.05	5179	68.10	11370	80.05	7398
47.00	1401	58.05	2605	69.10	22256	81.65	104208
48.10	674	59.05	1766	69.50	26056	82.05	104416
49.00	9178	60.05	2060	72.10	4754	83.50	69232
50.00	69416	61.05	18264	73.00	43816	85.00	18592
51.10	159168	62.05	60184	74.05	80536	86.00	43888
52.10	33200	63.05	145344	75.05	194624	87.00	66592

Scan 1395 (11.003 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
88.10	63616	101.05	45328	115.05	68960	127.00	192768
89.00	265728	102.05	131776	116.05	112352	128.00	96384
90.00	82136	103.00	95320	117.05	218688	129.00	25488
91.10	160768	104.00	30688	118.05	34184	130.00	4482
92.10	17856	105.00	11101	120.05	41568	131.10	2377
93.05	16800	106.50	16258	120.95	19440	131.95	4086
95.55	151232	108.00	25840	122.05	13529	133.05	5525
96.55	120600	110.00	390208	123.00	41136	133.95	24344
98.05	42496	112.00	42736	124.00	107128	134.95	15952
99.05	68720	112.95	79920	125.00	194880	135.95	47168
100.05	56960	113.95	82368	126.00	105360	136.95	64208

Scan 1395 (11.003 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
138.05	82352	149.00	34736	159.95	4934	171.00	2684
139.05	76720	150.00	166144	161.05	15484	172.05	1593
140.05	46880	151.00	242112	162.00	37768	172.95	8076
141.05	18088	151.95	234880	163.00	245568	173.95	8960
142.00	32480	152.95	54160	164.00	176512	174.95	22816
143.00	16079	153.95	23840	165.00	429184	176.05	61248
144.00	14965	154.95	4583	166.00	106952	177.05	271296
145.00	4421	155.95	1086	167.00	25872	178.05	165632
146.00	11176	157.05	1807	168.00	7491	179.05	110160
147.00	9215	158.05	1401	169.10	1839	180.05	36896
148.00	5433	159.05	1690	170.00	321	181.00	25432

Scan 1395 (11.003 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
182.00	5100	193.05	212992	204.00	51240	214.95	48104
183.00	4158	194.05	44608	205.00	213376	215.95	18272
184.00	1824	195.05	4896	206.00	79400	217.05	14534
185.00	4100	196.05	857	207.00	26312	218.05	30728
186.00	20384	196.95	3198	208.00	4121	219.05	119664
187.00	11586	198.05	8696	209.00	1954	220.10	72472
188.00	21112	199.05	60424	210.10	901	221.00	832832
189.00	17080	200.05	17560	210.95	7464	222.00	180032
190.10	83528	201.00	29040	211.95	20440	223.00	19536
191.05	94720	202.00	20296	212.95	85648	224.00	5006
192.05	128104	203.00	38344	213.95	40048	225.00	5879

Scan 1395 (11.003 min): 0101001.D
DIAZEPAM/NORDIA

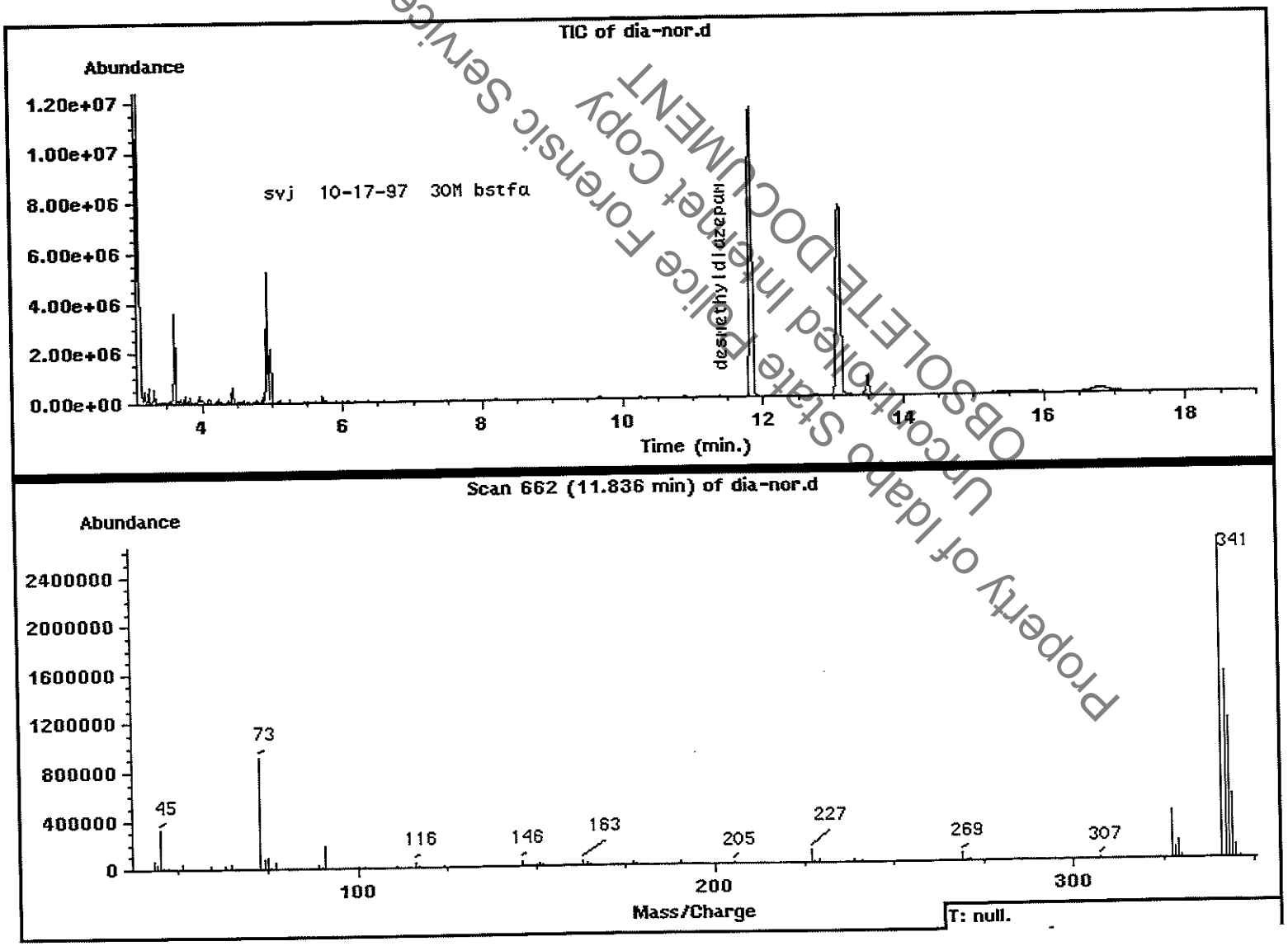
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
226.00	31512	238.05	98376	249.10	184960	261.00	3049
227.00	57472	239.05	208704	250.05	32096	261.90	232
228.00	181312	240.00	103536	251.05	4790	263.00	201
229.00	46552	241.00	306752	252.05	4455	265.00	3190
229.95	58680	242.00	77224	253.05	36360	266.00	7371
231.05	13066	243.00	85144	255.05	1049600	267.00	12508
232.05	5669	244.00	17216	256.05	2056704	268.00	93584
233.05	29488	245.00	3430	257.05	1140736	269.00	32160
234.05	11600	246.00	1833	258.05	984000	269.95	35984
235.05	3056	247.10	17160	259.05	298880	270.95	10423
236.05	785	248.10	135808	260.00	37136	272.05	2431

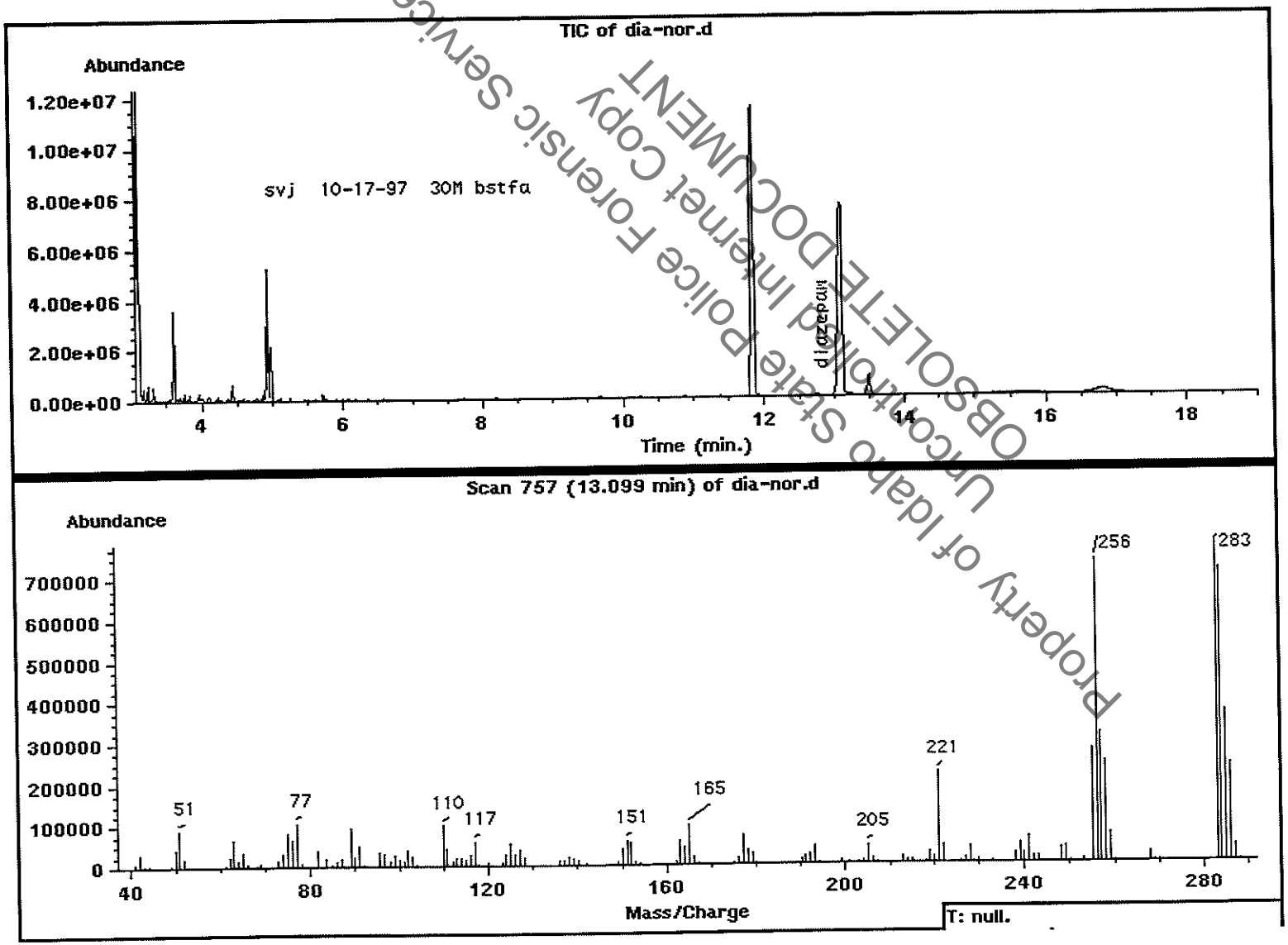
Scan 1395 (11.003 min): 0101001.D
DIAZEPAM/NORDIA

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
273.05	474	295.05	502	320.10	364	344.10	450
274.15	209	296.05	301	325.00	278	349.25	227
279.00	389	297.05	251	326.10	391	353.25	730
283.00	1932800	298.05	313	327.00	326	354.05	308
284.00	1709056	299.00	2736	328.05	228	355.05	701
285.00	1090048	300.00	871	329.05	457	355.85	207
286.00	690240	301.00	404	339.10	494	357.05	247
287.00	115984	313.05	562	340.00	210	359.30	225
288.00	10531	314.25	554	340.90	860	368.25	1737
288.95	901	315.15	254	342.10	606	369.25	375
294.25	261	319.20	965	343.10	490	370.95	230

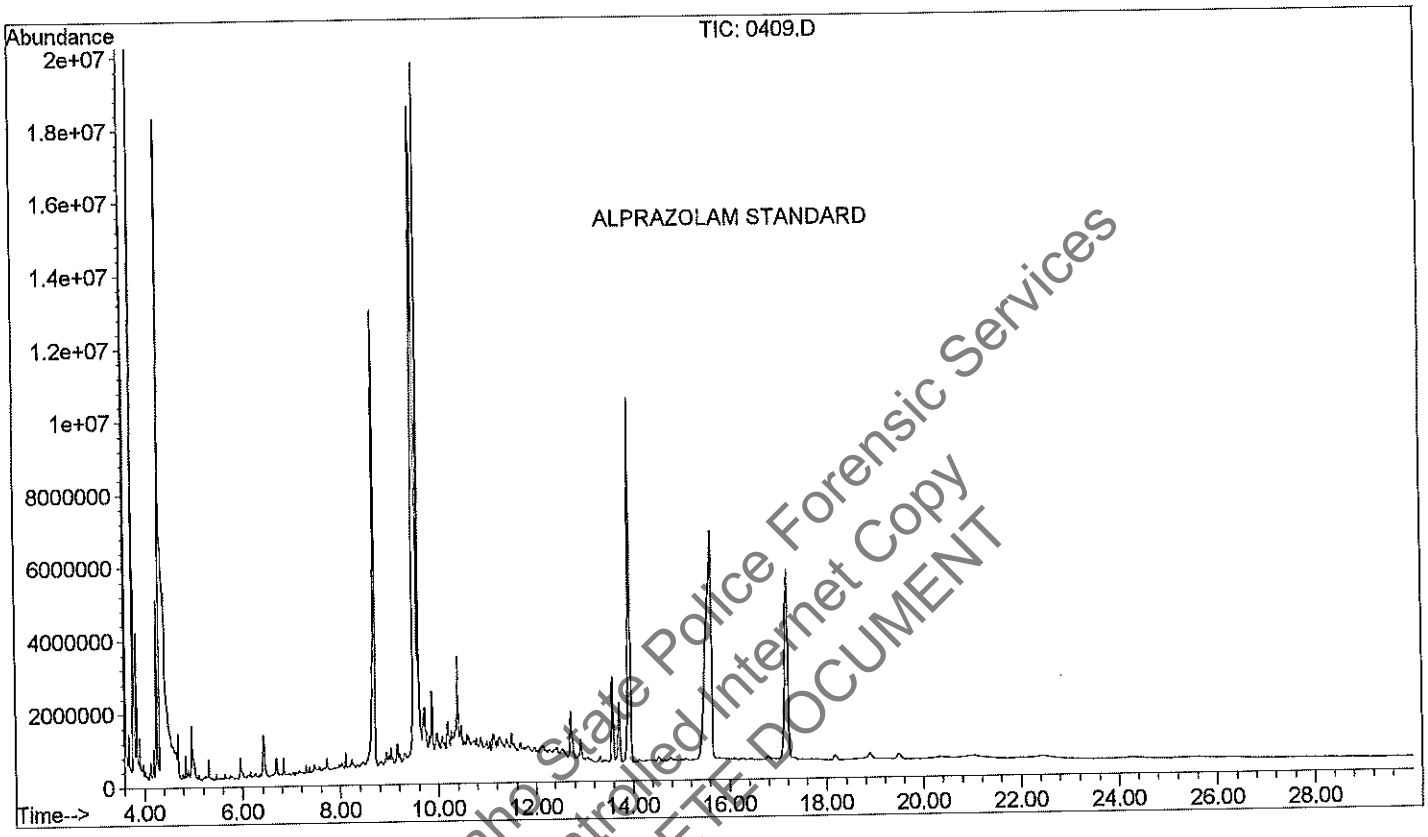
Scan 1395 (11.003 min): 0101001.D
DIAZEPAM/NORDIA

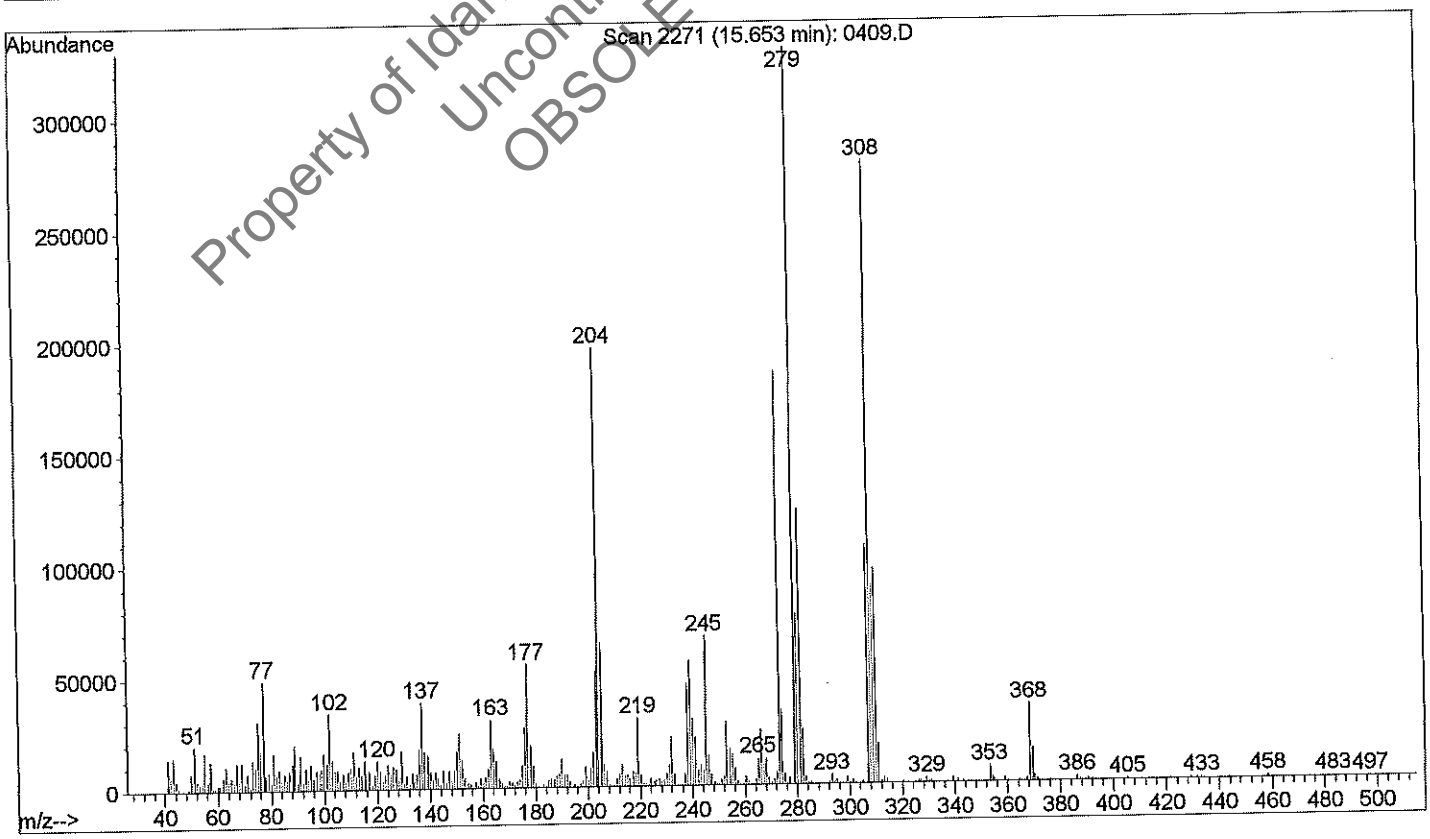
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
379.00	240						
387.05	211						
393.15	286						
402.60	213						
417.00	231						





File : D:\HPCHEM\1\DATA\SVJ\0409.D
Operator : svj
Acquired : 9 Apr 1998 14:06 using AcqMethod 2-30
Instrument : GC/MS Ins
Sample Name: ALPRAZOLAM BSTFA STD
Misc Info : FULL SCAN
Vial Number: 1





Scan 2271 (15.653 min): 0409.D
ALPRAZOLAM BSTFA STD

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
41.05	14142	53.00	3415	64.05	3611	75.00	30840
42.05	6091	54.10	3597	65.05	5381	76.00	17320
43.05	14776	55.10	16928	66.05	3443	77.10	48824
44.05	4248	56.10	4069	67.05	12181	78.00	6545
45.05	1432	57.10	12854	68.00	3835	79.05	7367
46.05	222	58.05	1121	69.00	12221	80.05	3148
46.90	395	58.95	1015	70.00	3073	81.05	16432
49.00	961	60.05	2251	71.10	7340	82.05	6214
50.00	7661	60.95	2186	72.00	1150	83.05	8832
51.00	19880	61.95	5700	73.00	13088	84.05	4037
52.00	4334	63.05	10485	74.00	10146	85.05	7151

Scan 2271 (15.653 min): 0409.D
ALPRAZOLAM BSTFA STD

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
86.05	4318	97.10	8735	108.05	3723	119.10	6729
87.05	8321	98.10	7777	109.05	8079	120.00	12729
88.15	11716	99.00	9395	110.00	9909	121.05	8346
89.00	20192	99.95	16113	111.00	16888	121.95	3557
90.00	3739	101.05	11934	112.00	6333	123.05	6704
91.00	15083	102.05	34008	113.00	9786	123.95	10952
92.10	3222	102.95	13455	114.00	6890	125.05	6588
93.00	9346	104.05	8105	115.20	12738	126.05	10140
94.00	4194	105.05	8571	115.50	12967	127.05	8937
95.10	11226	106.05	3758	117.00	7953	128.05	4640
96.00	5225	107.05	7235	118.10	1979	129.05	17016

Scan 2271 (15.653 min): 0409.D
ALPRAZOLAM BSTFA STD

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
130.05	3199	141.05	3812	152.00	12873	163.05	30480
131.00	5793	142.05	7291	153.00	4499	164.05	18064
132.20	2470	143.05	4930	154.10	2346	165.05	12108
133.20	6867	144.05	1789	155.10	2129	166.05	4111
134.10	3035	145.05	8172	156.00	818	167.05	2115
135.00	6500	146.05	2717	157.10	2579	168.15	854
136.00	17960	147.05	7719	158.10	1459	169.15	1116
137.00	38632	148.05	3196	159.10	4167	170.05	2492
138.00	16616	149.05	8410	160.10	2751	171.05	2254
139.00	15338	150.05	16592	161.10	4855	172.05	847
140.00	7368	151.05	24744	161.95	9036	173.10	2995

Scan 2271 (15.653 min): 0409.D
ALPRAZOLAM BSTFA STD

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
174.00	3712	185.05	3136	196.00	526	207.05	6764
175.00	10050	185.95	3654	197.00	1416	208.05	1577
176.00	27216	187.05	3881	198.00	2963	209.05	1091
177.00	55232	188.05	5028	199.10	8805	210.05	721
178.10	18896	189.05	6004	200.10	3292	211.05	3430
179.00	9865	190.05	12605	201.10	8405	212.05	5867
180.10	1946	191.05	5850	202.10	15507	213.05	9810
181.10	928	192.05	5537	203.10	51832	214.05	5577
182.10	773	193.05	2537	204.05	196800	215.00	5051
183.05	1160	194.20	586	205.05	64440	216.00	3800
184.05	515	195.00	1267	206.05	9919	217.10	6501

Scan 2271 (15.653 min): 0409.D
ALPRAZOLAM BSTFA STD

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
218.10	5690	229.15	3003	240.00	30448	251.05	2547
219.10	30688	230.05	5641	241.00	21712	252.05	3974
220.10	5249	231.05	9392	242.10	7212	253.05	28392
221.10	1315	232.05	21856	243.10	8955	254.05	16464
222.10	849	233.05	5237	244.10	6478	255.15	14107
223.00	1107	234.15	789	245.10	67024	256.00	7875
224.00	3358	235.10	614	246.05	13485	257.00	1624
225.05	1929	236.00	785	247.15	5064	258.10	641
225.95	2459	237.00	5463	248.25	1337	259.20	815
227.05	2998	238.00	45760	249.05	639	260.20	3650
228.05	2450	239.00	55840	250.05	738	261.20	1600

Scan 2271 (15.653 min): 0409.D
ALPRAZOLAM BSTFA STD

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
262.30	856	273.15	185408	285.20	1047	297.25	493
263.20	1230	274.15	33448	285.90	328	298.30	545
264.10	2726	275.15	4918	287.20	384	299.10	3007
265.10	11794	276.05	963	289.05	321	300.00	889
266.10	24664	277.00	3016	290.25	430	301.20	1456
267.05	9513	279.00	329920	291.05	635	302.10	537
268.05	11478	280.10	77368	292.15	1040	305.20	749
269.05	3197	281.10	123400	293.05	4028	307.10	107008
270.05	1472	282.10	25080	294.05	799	308.10	278720
271.15	2236	283.10	3321	295.05	1623	309.05	89368
272.15	5777	284.10	1137	296.05	467	310.05	96512

Scan 2271 (15.653 min): 0409.D
ALPRAZOLAM BSTFA STD

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
311.05	18208	322.20	242	338.25	236	351.15	328
312.15	1566	325.20	707	339.25	1992	352.25	256
313.25	2474	326.30	1023	340.30	584	353.35	7101
314.15	1691	327.10	1198	341.20	1091	354.25	2338
315.15	540	328.20	568	342.20	404	355.15	1534
316.25	356	329.25	2069	343.10	644	355.95	469
317.25	399	330.25	636	345.00	435	357.15	367
318.25	416	331.15	839	346.10	525	358.35	390
319.10	242	333.15	421	347.30	255	359.05	1708
320.20	547	336.35	216	348.30	237	360.15	584
321.20	220	337.25	718	349.30	300	360.90	333

Scan 2271 (15.653 min): 0409.D
ALPRAZOLAM BSTFA STD

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
361.30	327	372.25	343	385.10	250	397.25	281
362.10	317	373.15	307	386.40	1785	398.35	274
363.30	218	373.85	249	387.20	808	399.35	234
364.40	527	374.25	280	388.30	265	400.35	349
365.30	303	375.25	248	389.40	379	401.15	252
366.20	664	376.25	483	390.40	766	401.45	250
367.30	1402	377.15	338	391.40	279	403.00	354
368.40	34664	378.35	255	392.25	539	405.30	438
369.40	15290	379.45	269	393.35	354	406.50	321
370.30	3125	380.15	209	395.35	274	407.30	425
371.25	1030	383.40	205	396.25	260	408.20	284

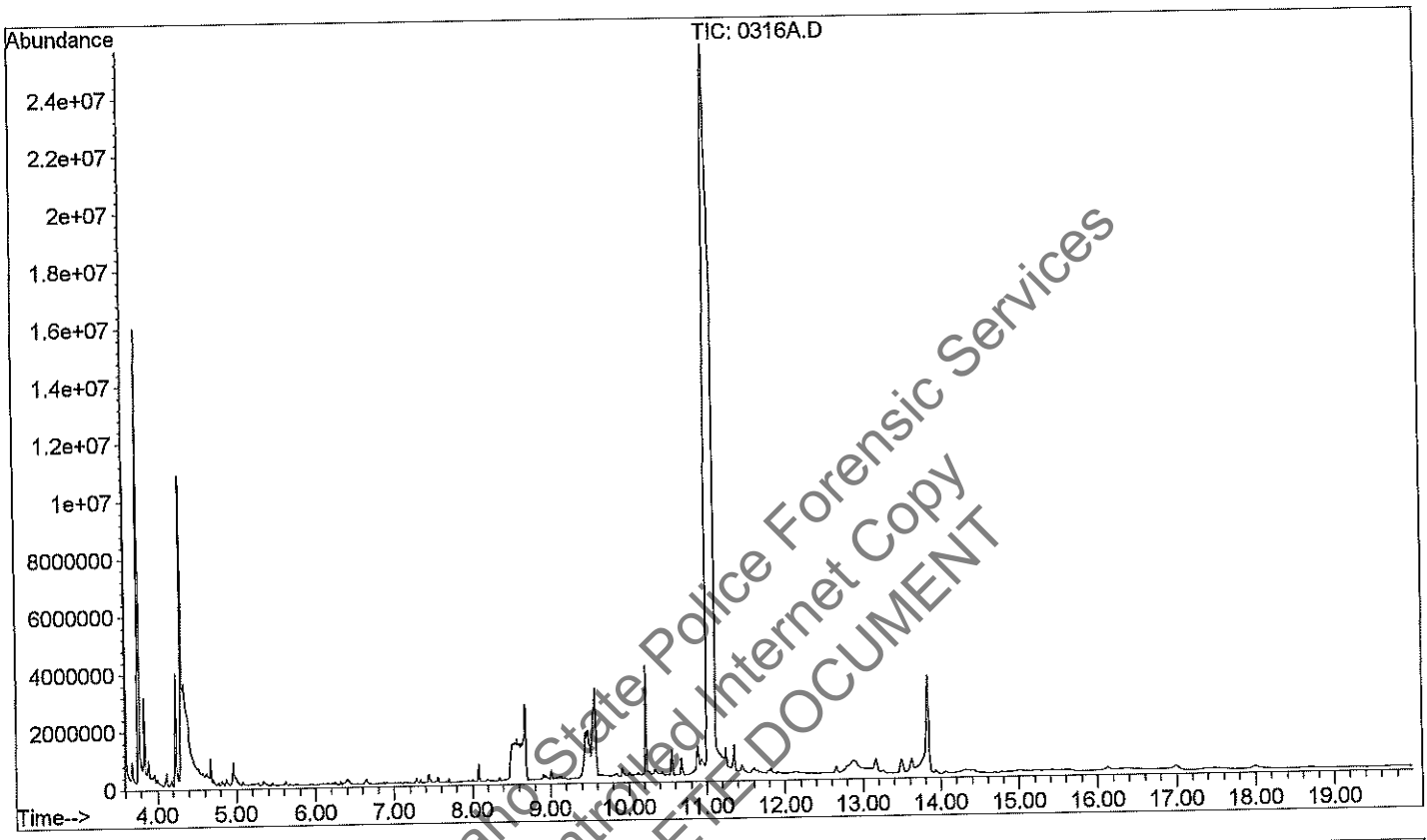
Scan 2271 (15.653 min): 0409.D
ALPRAZOLAM BSTFA STD

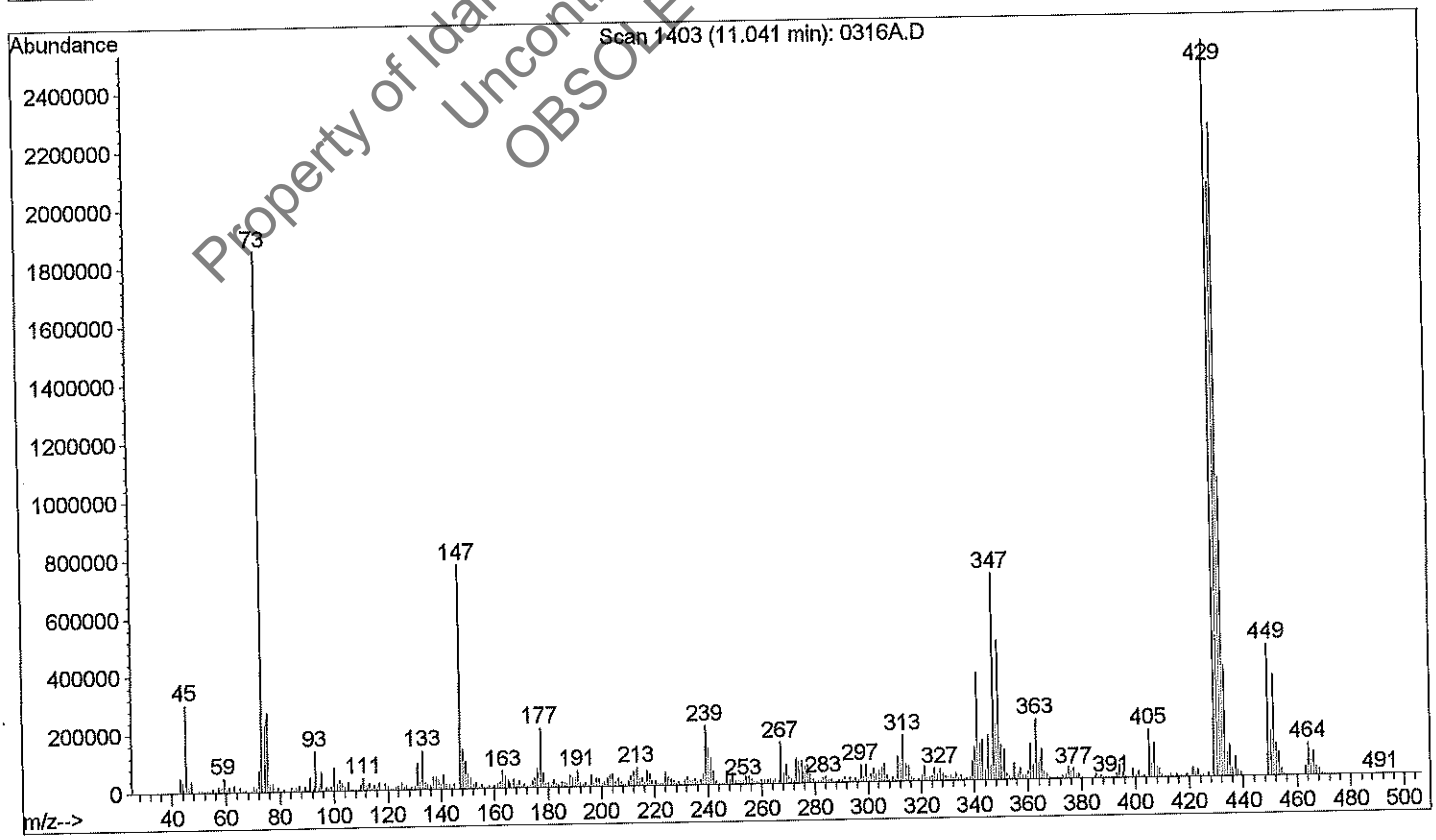
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
410.40	271	423.15	413	444.40	421	471.50	201
413.35	290	426.20	285	446.30	248	474.30	248
414.35	276	429.10	504	449.30	321	476.25	268
415.15	284	429.70	215	456.35	226	479.15	242
416.35	397	430.40	375	457.35	266	481.25	348
417.25	351	431.30	541	458.35	1087	483.15	413
418.55	238	433.20	719	459.35	364	484.25	274
419.15	327	433.85	235	460.25	201	486.40	301
420.15	319	435.15	306	461.25	282	491.40	248
421.35	554	440.35	326	463.25	256	492.10	202
422.05	259	443.45	599	470.40	218	494.50	214

Scan 2271 (15.653 min): 0409.D
ALPRAZOLAM BSTFA STD

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
495.10	322						
496.35	304						
497.25	258						
499.25	201						

File : D:\HPCHEM\1\DATA\SVJ\0316A.D
Operator : svj
Acquired : 16 Mar 1998 13:18 using AcqMethod 2
Instrument : GC/MS Ins
Sample Name: lorazepam
Misc Info : bstfa dir full scan
Vial Number: 6





Scan 1403 (11.041 min): 0316A.D
lorazepam

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
40.15	574	51.10	6842	62.15	4456	73.20	1858048
41.15	3233	52.20	3007	63.05	21304	74.20	228864
42.15	3719	53.10	3366	64.15	2937	75.20	271040
43.15	47696	54.10	2287	65.05	14617	76.20	26008
44.15	39544	55.10	12066	66.15	5014	77.15	25576
45.15	301824	56.05	4788	67.10	6608	78.15	4319
46.10	20680	57.15	19064	68.10	1666	79.05	13252
47.10	36568	58.15	22696	69.20	3992	80.15	3331
48.10	1662	59.15	48408	70.10	17096	81.15	6185
49.10	2268	60.15	8376	71.10	13070	82.15	1822
50.10	6085	61.15	17776	72.20	73480	83.15	5603

Scan 1403 (11.041 min): 0316A.D
lorazepam

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
84.15	13501	95.10	62920	106.15	6053	117.10	26240
85.15	9083	96.10	5787	107.10	5624	118.05	5864
86.15	12281	97.05	10546	108.00	1938	119.15	21144
87.10	18104	98.15	6718	109.20	5231	120.05	14082
88.10	6846	99.05	14786	110.10	20768	121.05	7346
89.10	12950	100.05	78784	111.10	42968	122.05	5636
90.10	4227	101.15	26960	112.10	13965	123.05	11493
91.10	46256	102.15	35472	113.10	24480	124.05	13545
92.20	8816	103.15	25376	114.10	9799	125.15	22856
93.10	137280	104.15	13352	115.10	17832	126.15	12135
94.10	13616	105.15	28040	116.10	9098	127.15	13597

Scan 1403 (11.041 min): 0316A.D
lorazepam

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
128.10	7780	139.05	32416	151.10	39984	162.05	21912
129.10	9176	140.05	15866	152.10	17336	163.15	59352
130.10	12916	141.05	47784	153.10	20736	164.05	42232
131.10	90472	142.05	17944	154.10	6728	165.15	25504
132.10	23784	143.05	12855	155.10	13749	166.05	16840
133.10	132288	144.15	6704	156.10	7464	167.05	29256
134.10	23200	145.15	17368	157.10	7714	168.05	13868
135.10	20496	147.15	771072	158.05	9090	169.00	23840
136.10	15606	148.10	140544	159.15	6351	170.00	9388
137.10	41032	149.10	97744	160.05	11941	171.00	11268
138.05	42832	150.10	49760	161.05	15326	172.10	7369

Scan 1403 (11.041 min): 0316A.D
lorazepam

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
173.10	8392	184.05	11605	195.10	6898	206.15	26312
174.10	24400	185.05	17136	196.10	40088	207.15	14390
175.10	33664	186.05	18704	197.10	16279	208.05	9569
176.10	65800	187.15	15631	198.10	29168	209.00	6453
177.10	205248	188.15	40344	199.05	26960	210.00	16728
178.10	49848	189.10	30048	200.05	14079	211.10	37264
179.05	16234	190.10	33080	201.05	15084	212.10	51528
180.05	14696	191.10	55240	202.05	29248	213.10	64912
181.05	8980	192.10	18144	203.15	38768	214.10	28112
182.05	23512	193.10	14016	204.15	42840	215.10	29048
183.05	16171	194.10	13977	205.15	20568	216.10	12700

Scan 1403 (11.041 min): 0316A.D
lorazepam

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
217.10	49736	228.05	9504	239.10	203776	250.10	13563
218.10	42416	229.15	10902	240.05	128864	251.10	13696
219.05	20688	230.10	6594	241.05	91912	252.10	10319
220.05	15119	231.10	16744	242.05	48480	253.10	14017
221.05	9456	232.10	28128	243.05	12514	254.10	27424
222.05	9023	233.10	20512	244.05	7038	255.10	26752
223.05	8058	234.10	18072	245.05	6218	256.10	18272
224.05	43784	235.10	19000	246.15	6669	257.10	12139
225.05	28624	236.10	12789	247.05	47880	258.10	8930
226.05	23080	237.10	20808	248.05	18032	259.10	6125
227.05	16784	238.10	36936	249.05	34056	260.05	14811

Scan 1403 (11.041 min): 0316A.D
lorazepam

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
261.05	15765	272.10	9302	283.05	18136	294.10	8957
262.05	15763	273.00	85600	284.05	19048	295.10	12399
263.05	16608	274.00	76336	285.05	12442	296.10	8607
264.05	9698	275.00	77120	286.05	10248	297.10	59008
265.05	13546	276.10	60368	287.05	5264	298.10	25040
266.15	9741	277.10	62616	288.05	5031	299.10	58560
267.05	142720	278.10	30016	289.05	9467	300.10	18408
268.05	39568	279.10	13331	290.15	10401	301.05	25816
269.05	64896	280.10	5824	291.10	16896	302.05	45344
270.10	26968	281.05	9956	292.10	9657	303.05	28416
271.00	14702	282.05	11683	293.10	16672	304.05	40048

Scan 1403 (11.041 min): 0316A.D
lorazepam

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
305.05	50360	316.10	15397	327.15	42792	338.10	12771
306.15	62120	317.10	8314	328.15	23752	339.10	65608
307.05	21944	318.10	4077	329.15	17424	340.10	116064
308.05	8638	319.10	9095	330.05	11573	341.10	369664
309.15	13123	320.10	21912	331.05	15258	342.05	124880
310.15	9760	321.15	50928	332.00	8138	343.05	139200
311.10	86312	322.05	16102	333.00	25888	344.05	34168
312.10	61328	323.05	14287	334.00	11546	345.05	153216
313.10	159424	324.05	18672	335.10	16920	346.15	47960
314.10	60192	325.05	43488	336.10	7882	347.05	711936
315.10	55416	326.05	23104	337.10	9187	348.05	191872

Scan 1403 (11.041 min): 0316A.D
lorazepam

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
349.05	479168	360.10	29272	371.15	4504	382.05	771
350.05	122496	361.10	123000	372.10	2305	383.05	3058
351.05	106344	362.05	52272	373.00	12011	384.05	1811
352.00	23792	363.05	207296	374.10	15543	385.15	15212
353.10	10820	364.05	60688	375.10	41312	386.15	6589
354.10	4632	365.05	104664	376.10	21776	387.15	8879
355.10	54264	366.05	26344	377.10	34744	388.05	3306
356.10	20016	367.05	20776	378.10	15209	389.15	3076
357.10	38144	368.15	7546	379.10	15986	390.15	1610
358.10	18112	369.15	5205	380.10	4197	391.15	3464
359.10	17616	370.15	2348	381.20	2404	392.15	3054

Scan 1403 (11.041 min): 0316A.D
lorazepam

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
393.20	17544	405.05	165696	416.10	2702	427.25	12363
394.20	44152	406.05	51616	417.10	5488	429.25	2531840
395.20	18672	407.05	119424	418.00	1857	430.25	2046976
396.20	6845	408.05	37064	419.10	10700	431.25	2248192
397.10	3564	409.05	31136	420.20	3915	432.25	1029632
398.10	2544	410.05	8181	421.10	33968	433.20	383040
399.20	31264	411.15	3489	422.20	12253	434.20	82440
400.20	11331	412.15	1678	423.15	23432	435.10	109912
401.20	22904	413.10	11059	424.15	7090	436.20	35336
402.20	7657	414.10	4850	425.05	5806	437.20	67208
403.15	5801	415.10	8614	426.15	2350	438.10	22128

Scan 1403 (11.041 min): 0316A.D
lorazepam

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
439.10	15686	455.10	7604	468.15	21000		
440.10	5247	456.10	1412	469.15	5881		
441.20	1476	457.20	381	470.25	1773		
442.10	487	458.40	216	471.15	594		
447.15	1624	460.10	214	491.15	244		
449.15	454528	461.20	617				
450.15	156736	463.20	31200				
451.15	348224	464.15	111032				
452.15	111304	465.15	58384				
453.15	83896	466.15	82664				
454.10	24016	467.15	31720				

OPIATE CONFIRMATION IN BLOOD USING GC/MS

INTRODUCTION:

The term opiate refers to those compounds which are natural or semisynthetic alkaloidal derivatives of the opium poppy. Opiates are used widely as pain relievers. The compounds of interest in this method are morphine, codeine, hydrocodone, oxycodone, heroin and hydromorphone.

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph
Hewlett packard 6890 Auto Sampler
Hewlett Packard 5973 Mass Select Detector (MSD)

COLUMN:

30 meter HP5-MS, catalog # 19091S-433; film thickness 0.25 microns, internal diameter 0.25 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Blank whole blood
Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol
Sodium acetate trihydrate

REAGENTS (cont):

Glacial acetic acid
Methylene chloride
Isopropanol
Ammonium hydroxide
BSTFA

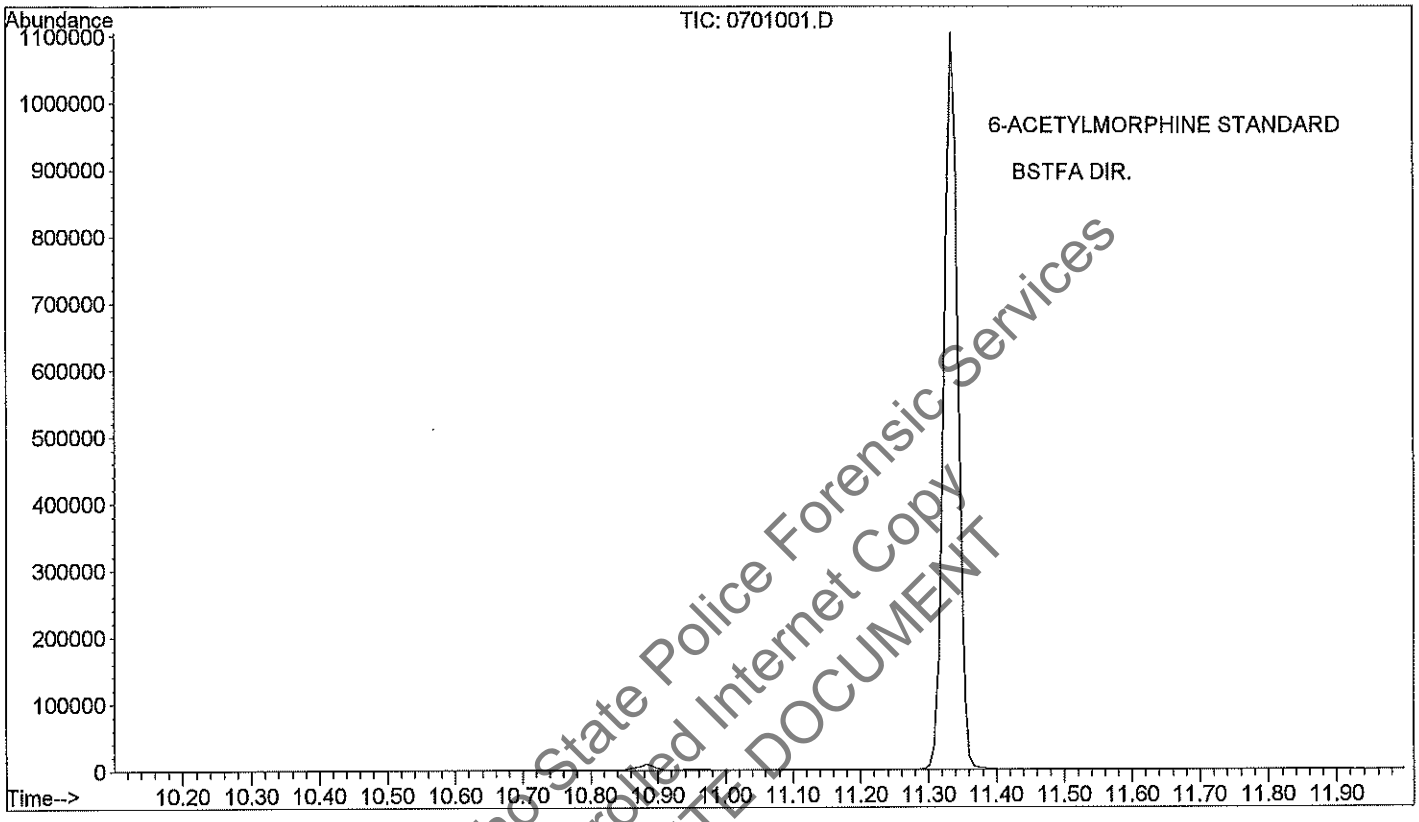
Prepare the following:

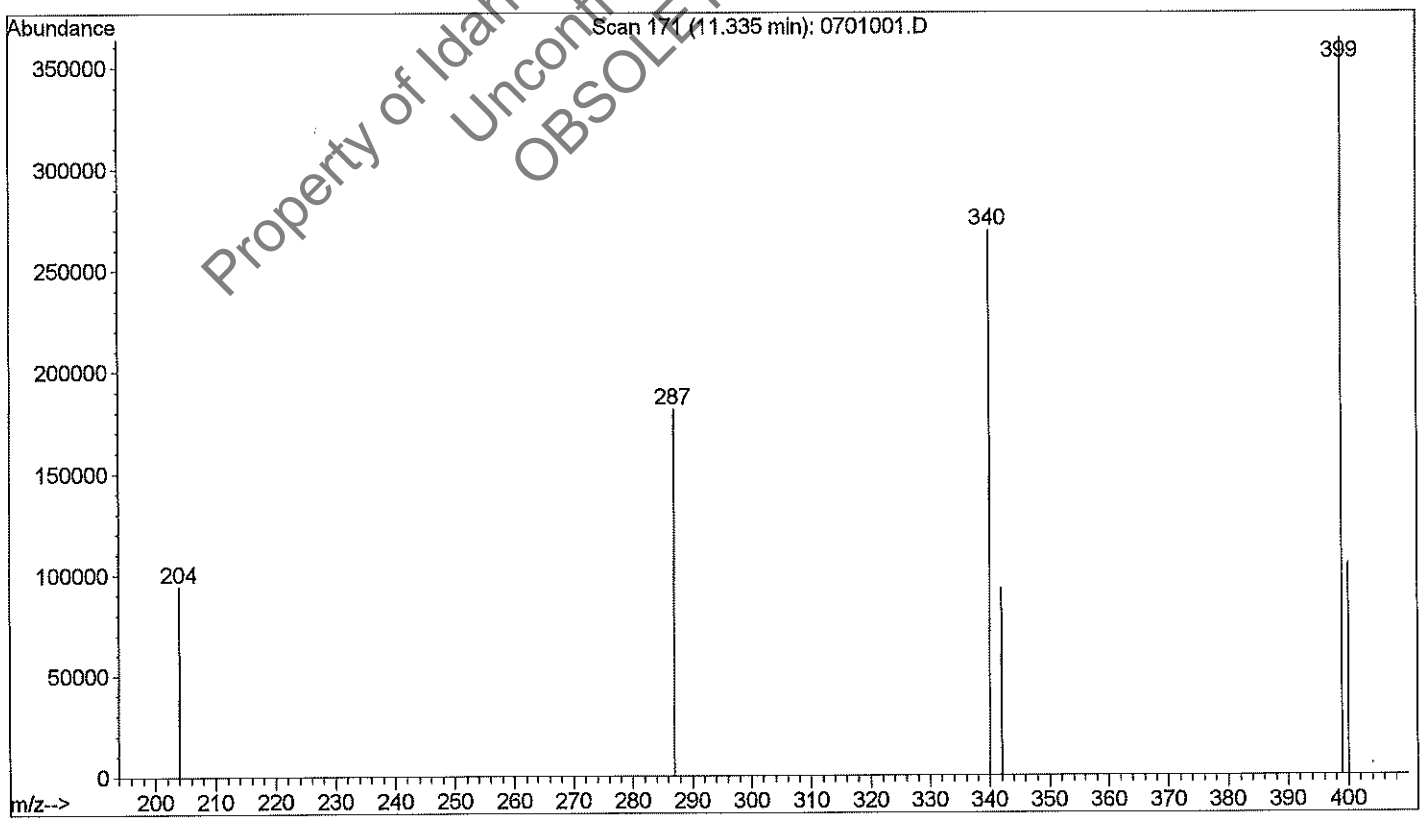
1. 100 mM, pH 6.0 phosphate buffer
2. 100 mM, pH 4.5 acetate buffer
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).

PROCEDURE:

1. Pipet 2ml of sample (case sample, blank and control) into a screw top tube.
2. Add 8ml DI water, vortex and let stand for 5 minutes.
3. Centrifuge for 10 minutes.
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
    a. 1 x 3ml methanol
    b. 1 x 3ml DI water
    c. 1 x 2ml 100mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column.
    a. 1 x 2ml DI water
    b. 1 x 2ml 100mM acetate buffer
    c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum $\geq$ 10 inches Hg.
9. Elute with 6ml of elution solvent into centrifuge tube.
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul BSTFA, cap, vortex and heat at 90°C for 15 minutes.
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using SIM method monitoring the following ions:
    a. Morphine - 196, 234, 236, 287, 371, 401, 414, 429.
    b. Codeine - 178, 196, 229, 234, 371, 372.
    c. Hydrocodone - 371, 73, 234, 313, 314, 356, 242, 243, 299, 185, 214.
    d. Oxycodone - 387, 73, 179, 315, 330, 388, 459, 242, 312, 446, 460.
    e. Hydromorphone - 357, 300, 73, 59, 342, 243, 272, 301, 358..

File : D:\HPCHEM\1\DATA\SVJ\012199\0701001.D
Operator : SVJ
Acquired : 21 Jan 1999 11:27 using AcqMethod OPIASIM
Instrument : GC/MS Ins
Sample Name: 6-ACETYLMORPHINE STANDARD
Misc Info :
Vial Number: 7

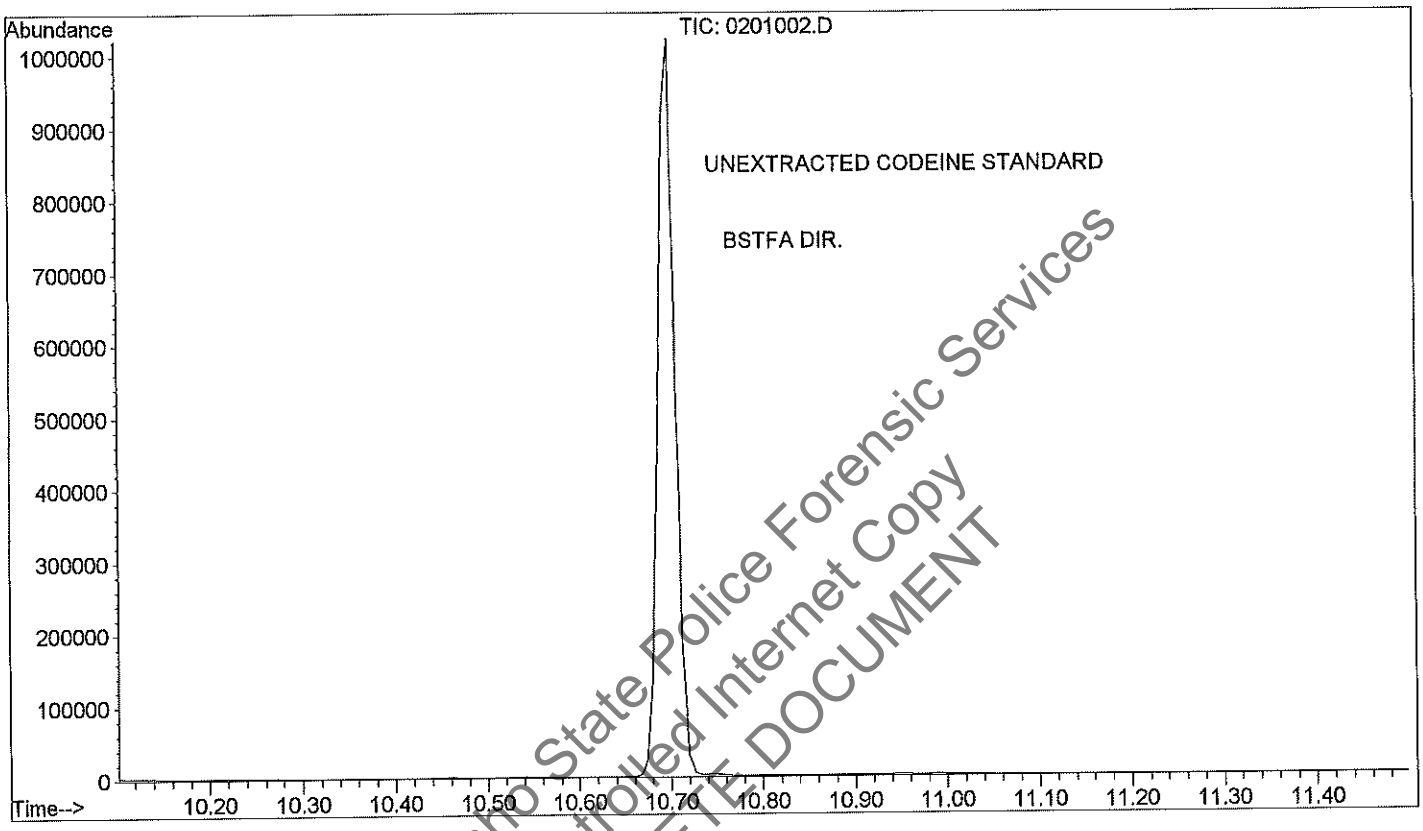


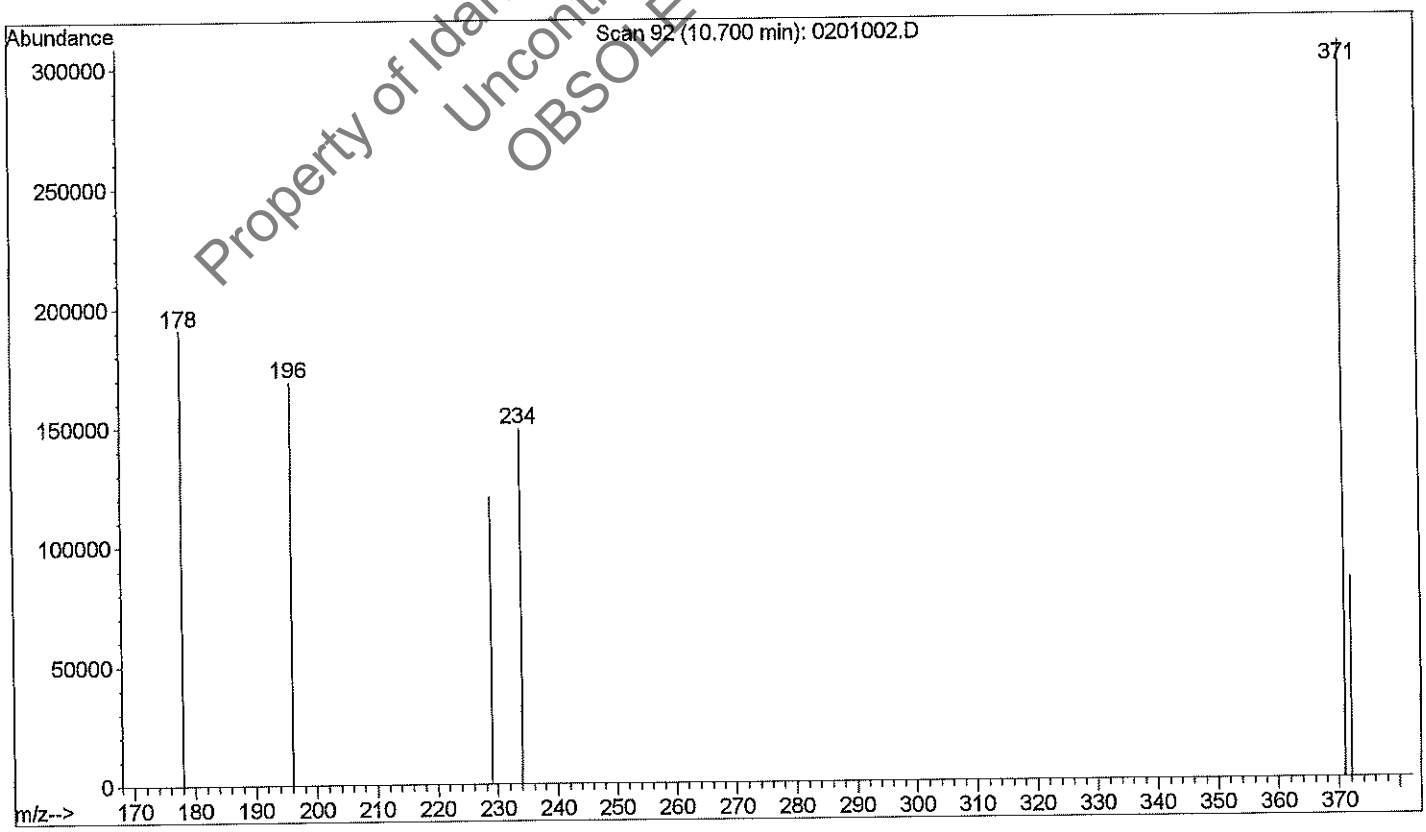


Scan 171 (11.335 min): 0701001.D
6-ACETYLMORPHINE STANDARD

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
204.00	94128						
287.00	181056						
340.00	269184						
342.00	92432						
399.00	363584						
400.00	104792						

File : D:\HPCHEM\1\DATA\SVJ\012099\0201002.D
Operator : SVJ
Acquired : 20 Jan 1999 15:45 using AcqMethod OPIASIM
Instrument : GC/MS Ins
Sample Name: codeine std
Misc Info :
Vial Number: 2

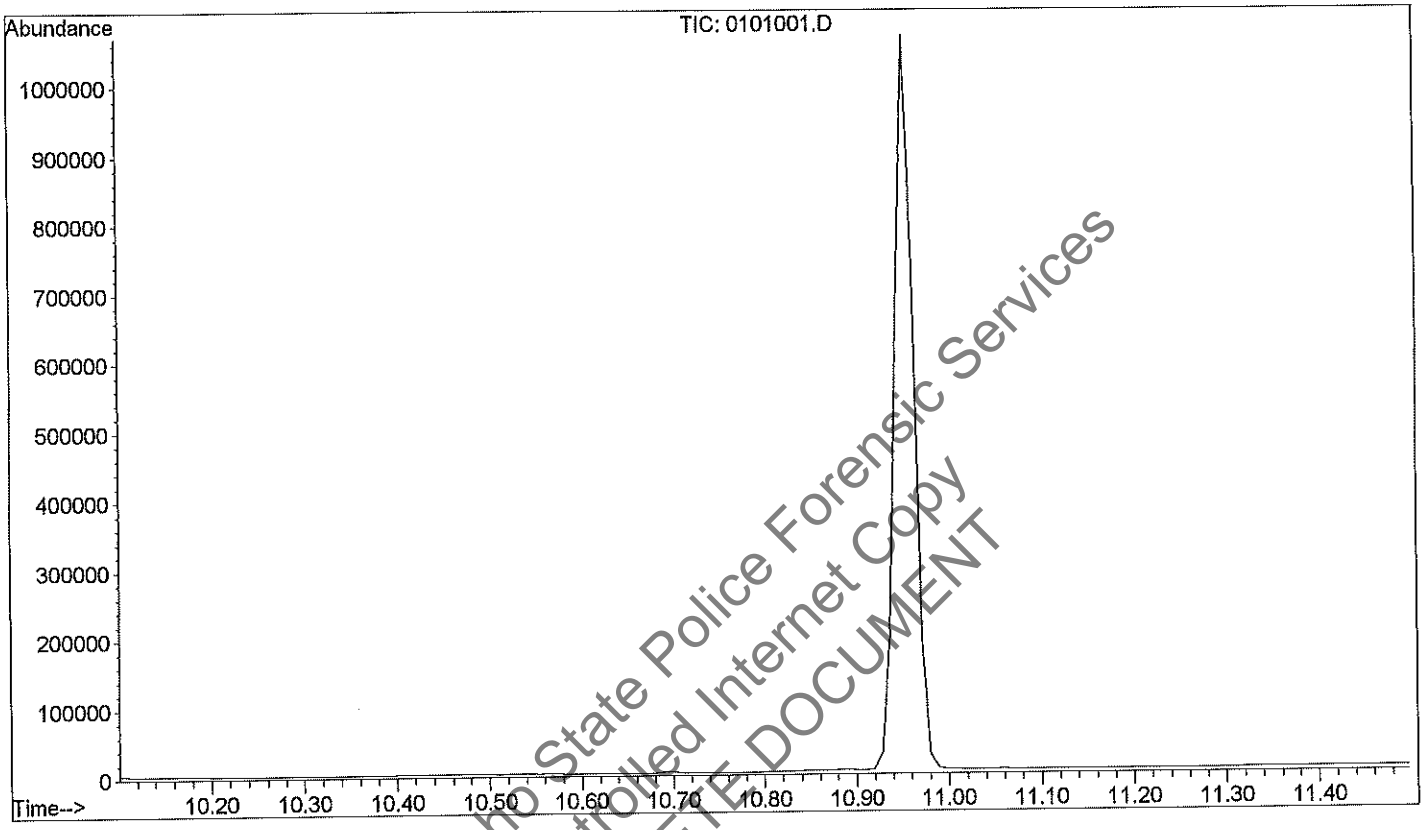


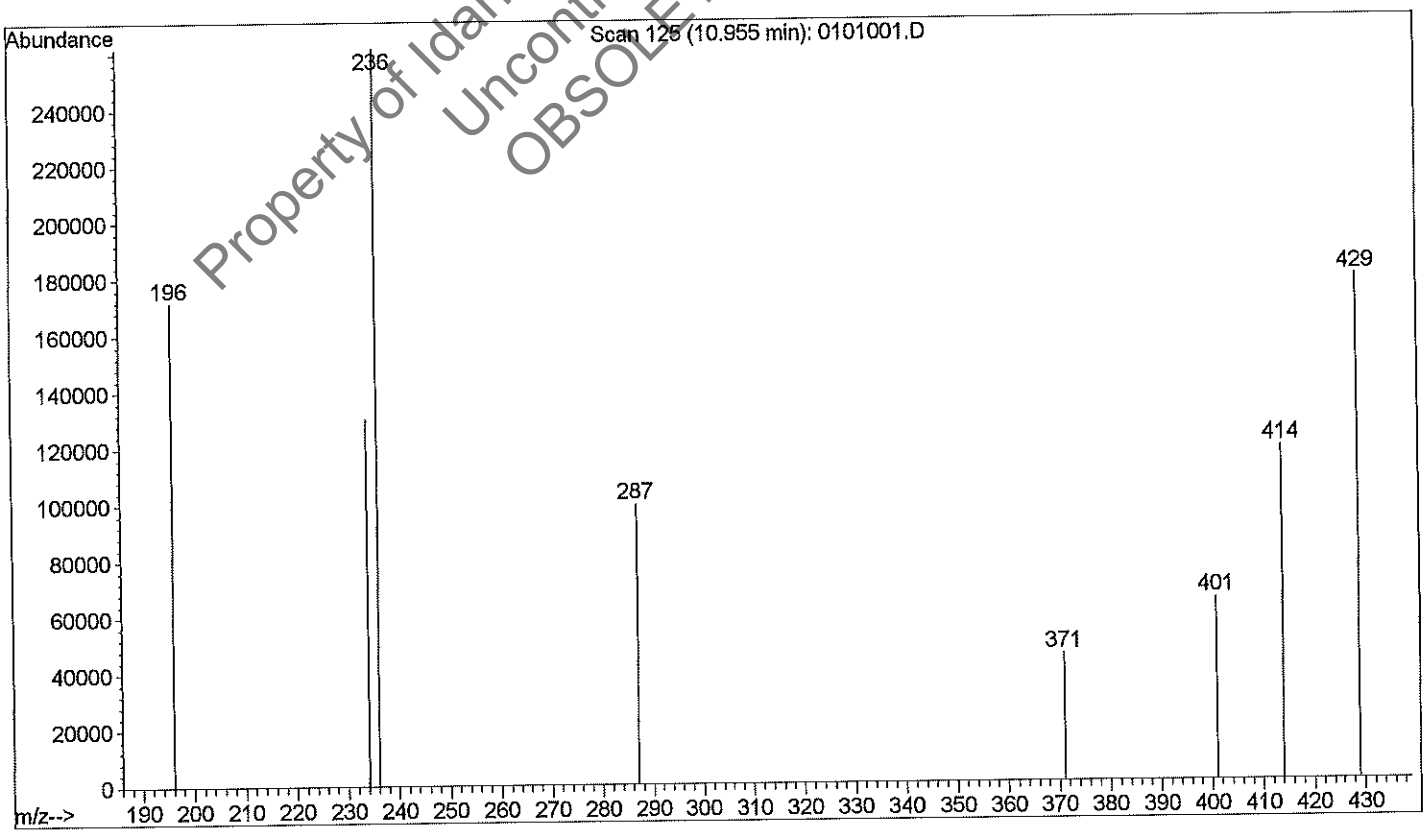


Scan 92 (10.700 min): 0201002.D
codeine std

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
178.00	191104						
196.00	169216						
229.00	120648						
234.00	148992						
371.00	308672						
372.00	83832						

File : D:\HPCHEM\1\DATA\SVJ\012099\0101001.D
Operator : SVJ
Acquired : 20 Jan 1999 15:17 using AcqMethod OPIASIM
Instrument : GC/MS Ins
Sample Name: morphine std
Misc Info :
Vial Number: 1

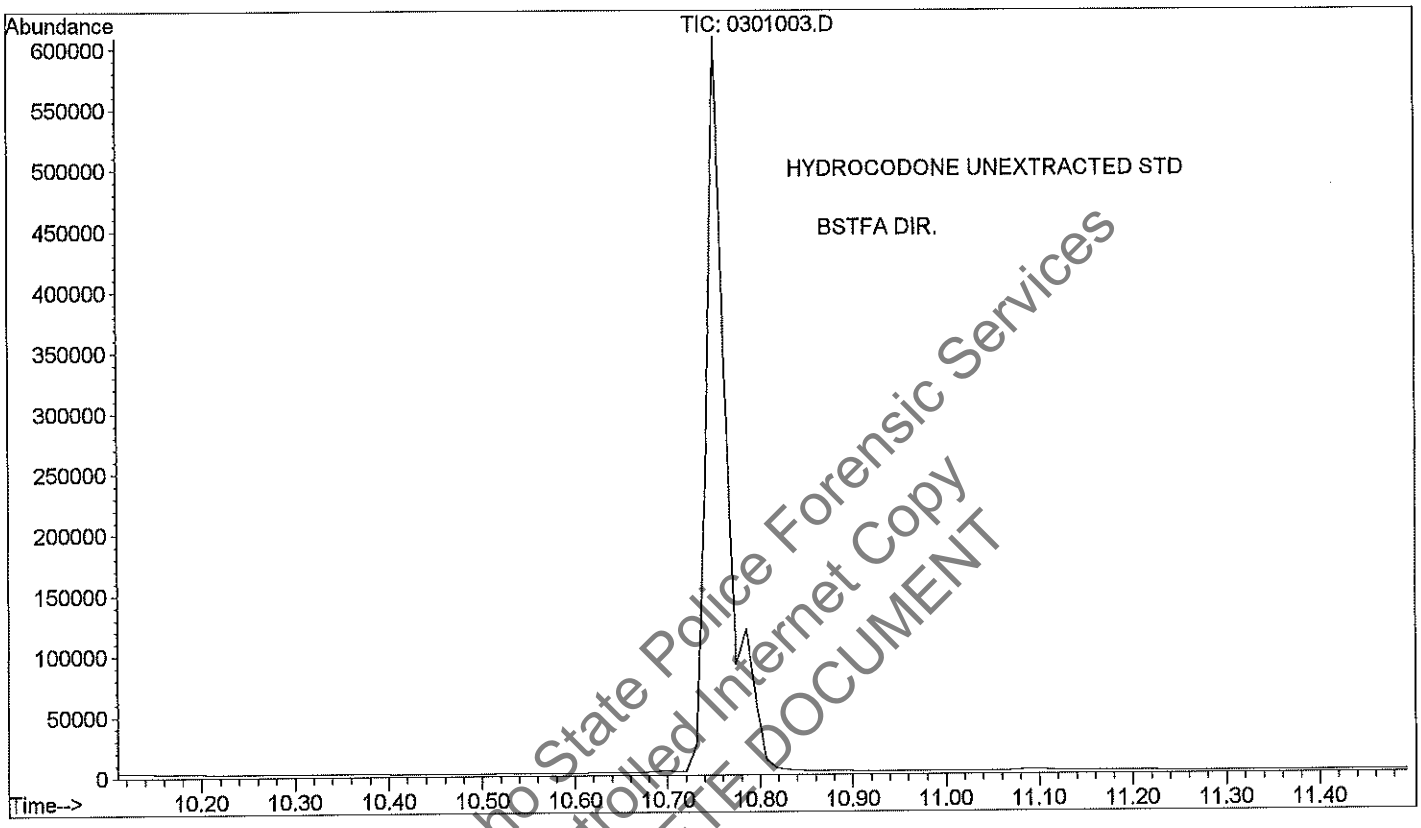


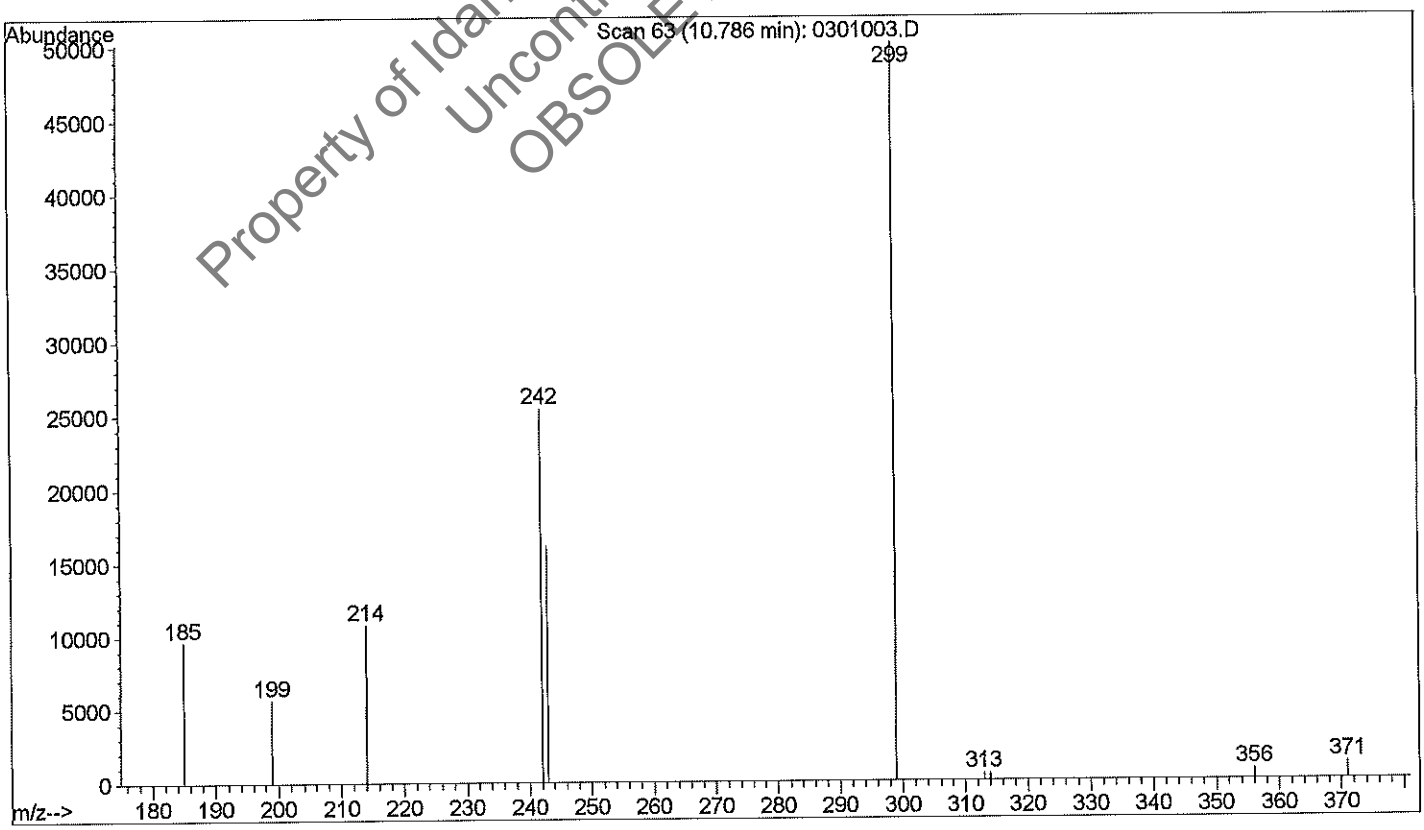


Scan 125 (10.955 min): 0101001.D
morphine std

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
196.00	171712						
234.00	130208						
236.00	261504						
287.00	99464						
371.00	45168						
401.00	64688						
414.00	118464						
429.00	178944						

File : D:\HPCHEM\1\DATA\SVJ\012099\0301003.D
Operator : SVJ
Acquired : 20 Jan 1999 16:14 using AcqMethod HYCODSIM
Instrument : GC/MS Ins
Sample Name: hydrocodone std
Misc Info :
Vial Number: 3

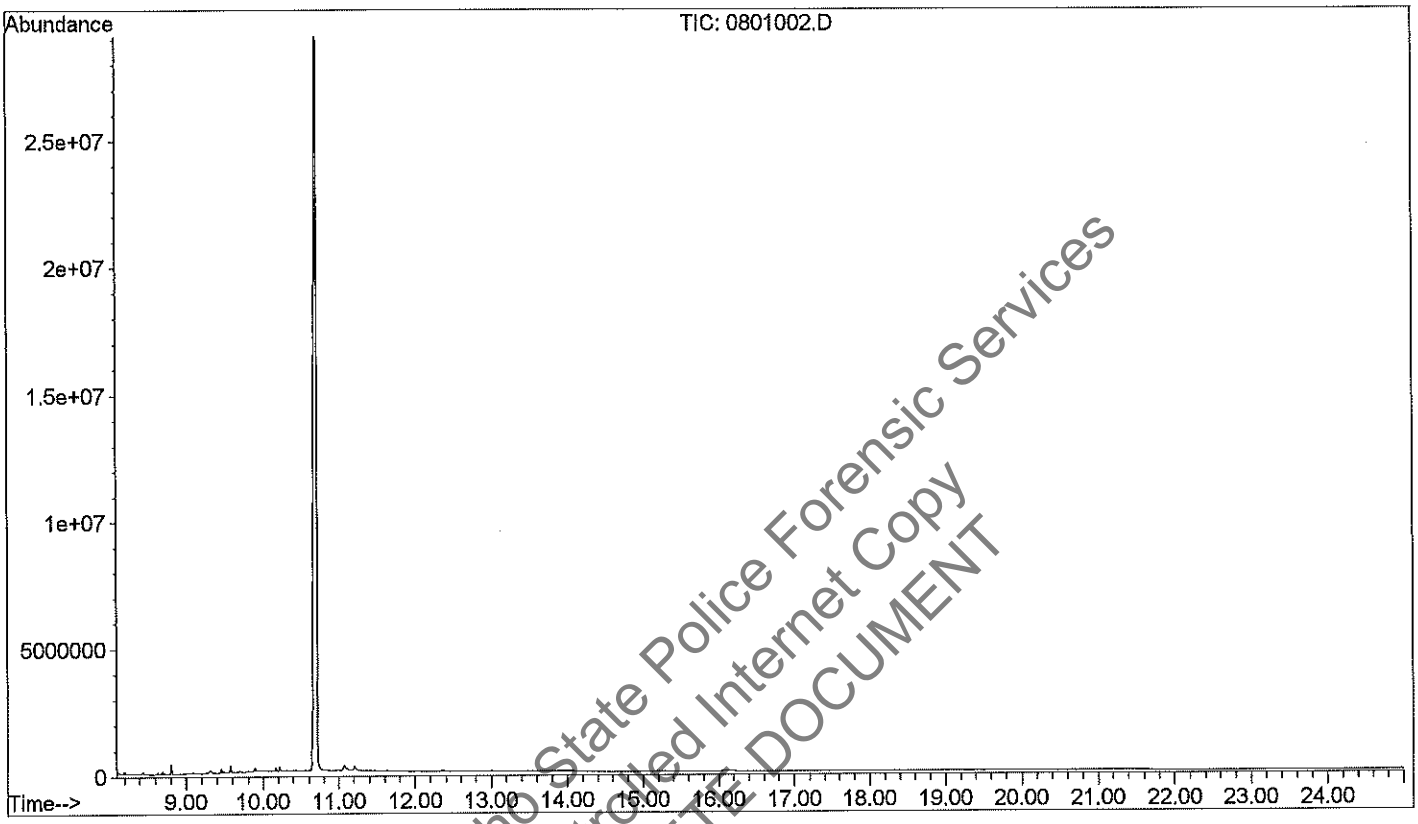


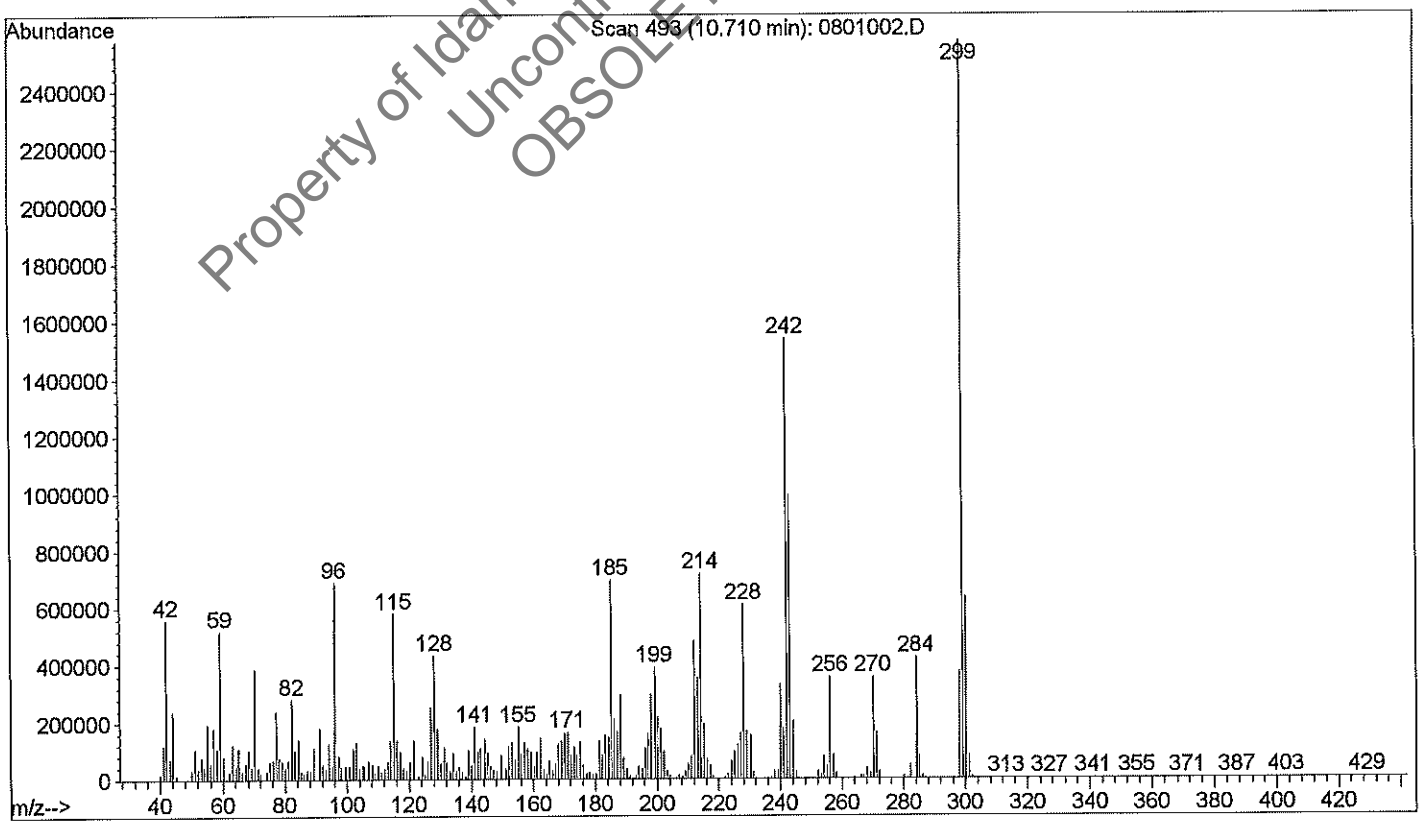


Scan 63 (10.786 min): 0301003.D
hydrocodone std

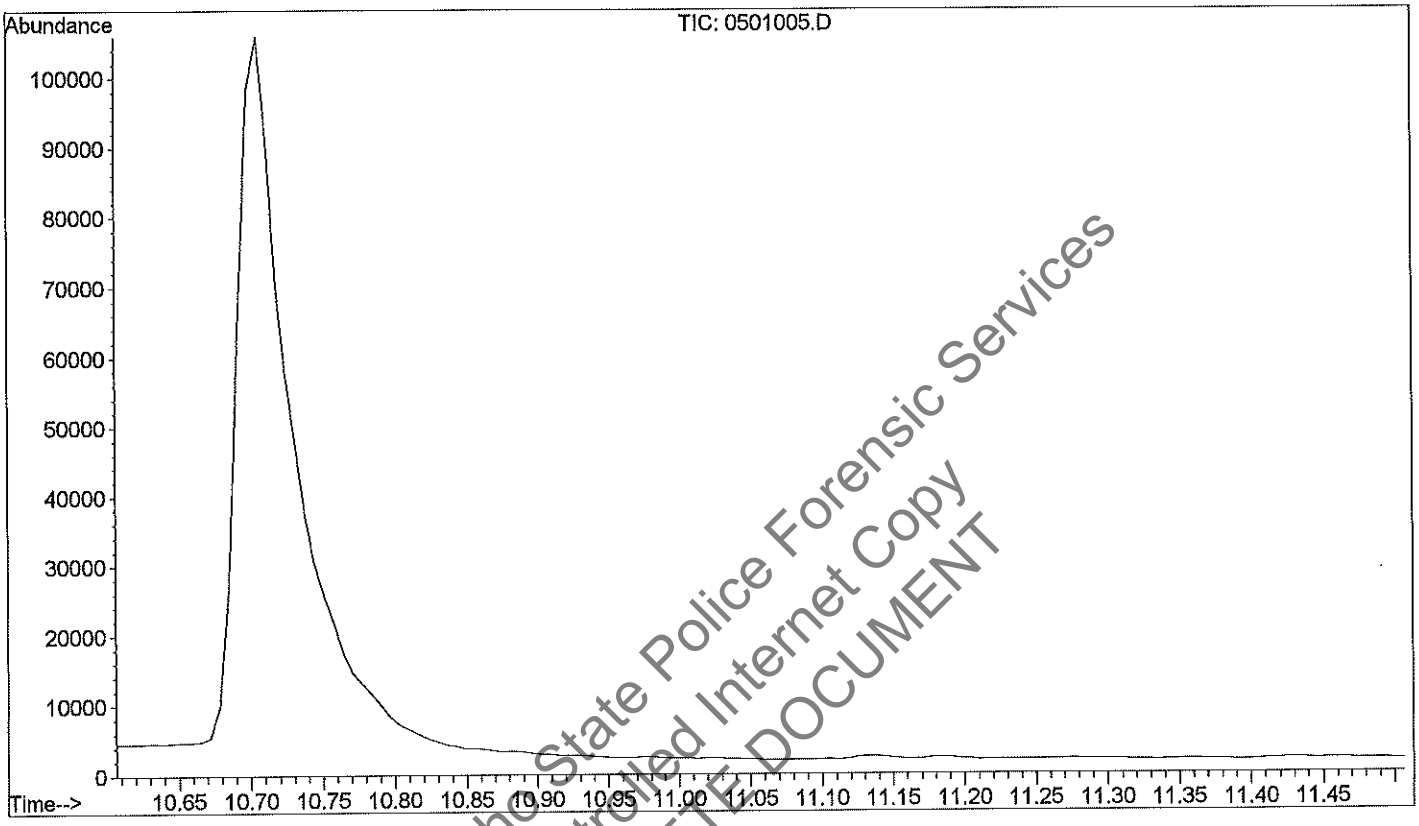
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
185.00	9613						
199.00	5641						
214.00	10774						
242.00	25424						
243.00	16160						
299.00	50128						
313.00	472						
314.00	449						
356.00	690						
371.00	1128						

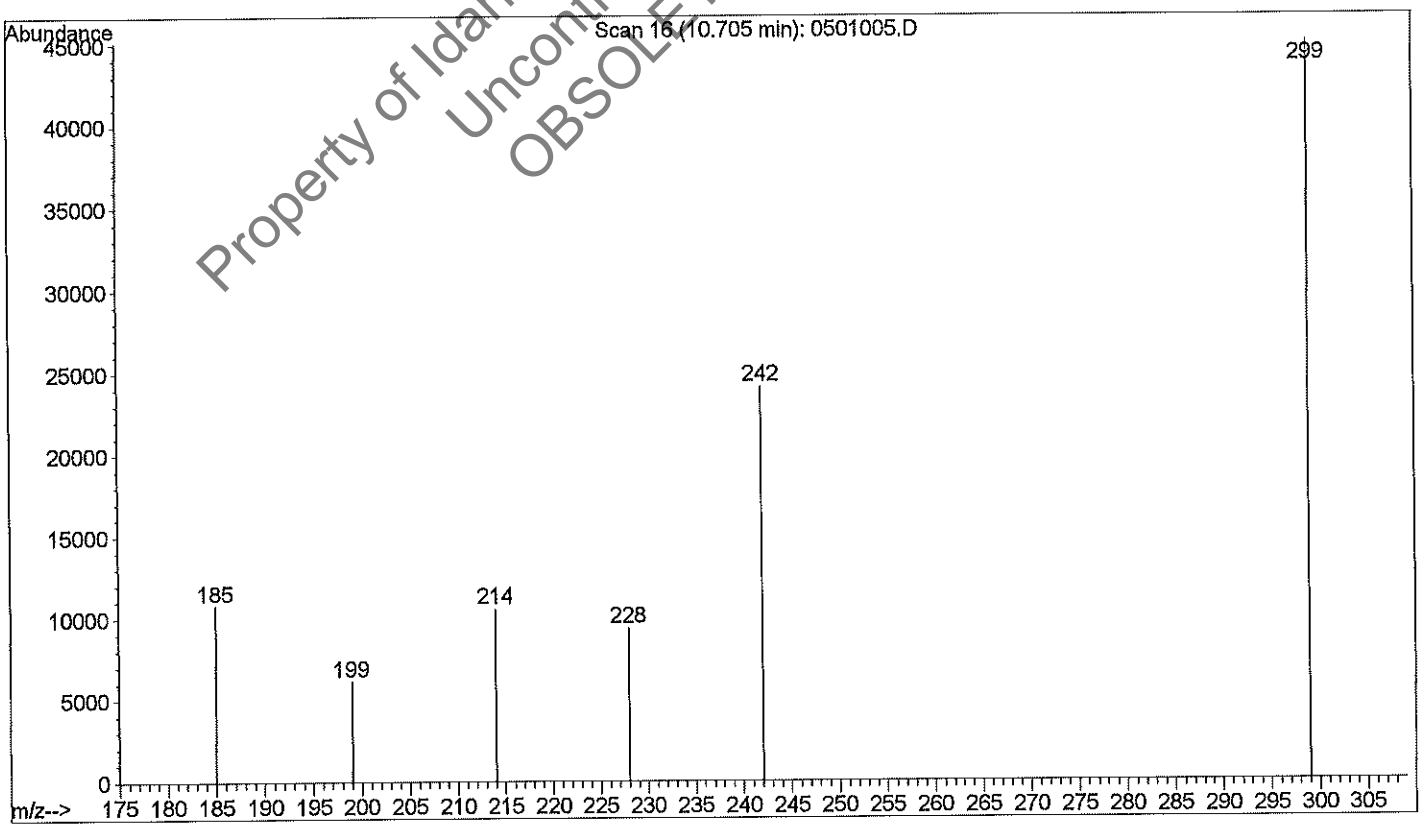
File : D:\HPCHEM\1\DATA\SVJ\083199\0801002.D
Operator : SVJ
Acquired : 31 Aug 1999 10:45 using AcqMethod OPIASCAN
Instrument : GC/MS Ins
Sample Name: HYDROCODONE
Misc Info : MEOH
Vial Number: 8





File : D:\HPCHEM\1\DATA\SVJ\083199A\0501005.D
Operator : SVJ
Acquired : 31 Aug 1999 18:27 using AcqMethod HYCOUDIR
Instrument : GC/MS Ins
Sample Name: 100 HYDROCODONE
Misc Info : UNDERIVITIZED 100 NG/ML HYDROCODONE
Vial Number: 5





BARBITURATE BLOOD EXTRACTION AND GC/MS CONFIRMATION PROCEDURE

INTRODUCTION:

Barbiturates comprise a group of compounds which produce varying degrees of behavioral depression ranging from mild sedation, through anesthesia, to coma and death. Barbiturates are classified as nonselective central nervous system depressants. They are extracted from biological samples using an organic solvent under acidic conditions.

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph
Hewlett Packard 6890 Auto Sampler
Hewlett Packard 5973 Mass Select Detector (MSD)

COLUMN:

30 meter HP5-MS, catalog # 19091S-433; film thickness 0.25 microns, internal diameter 0.25 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.

REAGENTS:

Blank whole blood
N-butyl chloride
Sodium hydroxide
Hydrochloric acid -concentrated
Hexane

REAGENTS (cont):

Ethanol - 200 proof
Drug standards

Prepare the following:

1. 250ml of 1:1 hexane:ethanol solution
2. 250 ml of 0.45 N sodium hydroxide

PROCEDURE:

1. Pipet 1 ml of blood (sample, blank or control) into a screw top tube.
2. Extract with 10 ml N-butyl chloride for three minutes.
3. Centrifuge for five minutes.*
4. Transfer N-butyl chloride to another screw cap tube.
5. Add 2 ml of 0.45 N sodium hydroxide and mix for three minutes.
6. Centrifuge for five minutes
7. Discard N-butyl chloride
8. Adjust the pH to acid with concentrated HCl.
9. Extract with 10 ml N-butyl chloride for five minutes.
10. Centrifuge for five minutes.
11. Transfer the N-buty chloride layer to a centrifuge tube and evaporate at 37°C under nitrogen to dryness.
12. Reconstitute the residue in 100 ul 1:1 hexane:ethanol.
13. Run on GC/MS using full scan method or
14. Run on GC/MS using SIM method and monitor the following ions:
    a. Amobarbital -141, 142, 156, 157, 183, 197, 198.
    b. Secobarbital - 124, 153, 168, 169, 170, 195, 209.
    c. Phenobarbital - 115, 117, 146, 161, 204, 205, 232.
    d. Butalbital -

*For clean samples go to step 11.

PROPOXYPHENE/NORPROPOXYPHENE CONFIRMATION IN BLOOD BY GC/MS

INTRODUCTION:

Propoxyphene is an analgesic compound that is structurally similar to methadone. It's potency is about half that of codeine. Taken in large doses it can have opiate-like effects.

INSTRUMENTATION:

Hewlett Packard 6890 Gas Chromatograph
Hewlett packard 6890 Auto Sampler
Hewlett Packard 5973 Mass Select Detector (MSD)

COLUMN:

30 meter HP5-MS, catalog # 19091S-433; film thickness 0.25 microns, internal diameter 0.25 mm.

SUPPLIES:

Screw cap tubes, 13 x 100mm, Fisher Scientific Catalog # 14-959-35C
Screw cap for tubes, Fisher Scientific Catalog # 14-930-15E
Centrifuge tubes, 16 x 144 mm, Fisher Scientific Catalog # 05-538-41C
Auto sampler vials, 12 x 32mm, Fisher Scientific Catalog # 03-395C
Crimp caps, 11mm, Fisher Scientific Catalog # 06-406-19B
Micro inserts, 0.200 ml, Fisher Scientific Catalog # 03-375-3A
Crimper for 11mm crimp caps, Hewlett Packard Catalog # 8710-0979.
Transfer pipets, Fisher Scientific Catalog # 13-711-7.
Clean Screen extraction column, Worldwide Monitoring # ZSDAU020, 200mg

REAGENTS:

Blank whole blood
Deionized water
Monobasic sodium phosphate
Dibasic sodium phosphate
Methanol
Sodium acetate trihydrate

REAGENTS (cont):

Glacial acetic acid
Hydrochloric acid - concentrated
Methylene chloride
Isopropanol
Ammonium hydroxide
Ethyl acetate

Prepare the following:

1. 100 mM, pH 6.0 phosphate buffer
2. 100 mM, pH 4.5 acetate buffer
3. 78:20:2 methylene chloride:isopropanol:ammonium hydroxide elution solvent (prepare fresh daily).

PROCEDURE:

1. Pipet 2ml of sample (case sample, blank and control) into a screw top tube.
2. Add 8ml DI water, vortex and let stand for 5 minutes.
3. Centrifuge for 10 minutes.
4. Transfer liquid to second tube and add 4ml 100mM phosphate buffer.
5. Condition Clean Screen column.
    a. 1 x 3ml methanol
    b. 1 x 3ml DI water
    c. 1 x 2ml 100mM phosphate buffer
6. Apply sample at 1 to 2ml per minute
7. Wash column.
    a. 1 x 2ml DI water
    b. 1 x 2ml 100mM acetate buffer
    c. 1 x 3ml methanol
8. Dry column for 5 minutes at a vacuum $\geq$ 10 inches Hg.
9. Elute with 6ml of elution solvent into centrifuge tube.
10. Evaporate to dryness at 37°C under nitrogen.
11. Add 50ul ethyl acetate and vortex for 15 sec.
12. Transfer liquid to auto sampler vial with micro insert and cap.
13. Run sample on GC/MS using SIM method monitoring the following ions:
    a. Propoxyphene/nor propoxyphene - 44, 58, 59, 91, 100, 115, 117, 129, 130, 178, 193, 205, 208, 220, 265, 325.

STC Technologies, Inc.

1745 Eaton Avenue, Bethlehem, PA 18018-1799
Phone: (610) 882-1820 ▪ Fax: (610) 882-1830

COCAINE METABOLITE MICRO-PLATE EIA
FORENSIC APPLICATION

14030 (12/96)

INTENDED USE

The STC Cocaine Metabolite Micro-Plate EIA is intended for use in the qualitative determination of cocaine and cocaine metabolites (benzoylecgonine, ecgonine methyl ester) in serum. ***THIS TEST IS INTENDED FOR FORENSIC USE ONLY.***

The STC Cocaine Metabolite Micro-Plate EIA provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result.[1] Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

PRINCIPLE OF THE TEST

The STC Cocaine Metabolite Micro-Plate EIA is a competitive immunoassay for the qualitative determination of cocaine and cocaine metabolites in serum. Sample or calibrator/control is added to each well along with enzyme-labeled hapten derivative. There is a competition to bind the antibody fixed onto the well. The wells are washed, substrate is added, and color is produced. The absorbance produced (450 nm) is inversely proportional to the amount of cocaine or cocaine metabolite present in the sample or calibrator/control.

KIT COMPONENTS	Catalog No. 1122EA 96 Test Kit Min. Qty.	Catalog No. 1122EB 480 Test Kit Min. Qty.	Catalog No. 1122EC 9600 Test Kit Min. Qty.
Micro-Plate Sheep anti-benzoylecgonine antibody immobilized on a polystyrene plate.	1	5	100
Lyophilized Conjugate - Horseradish peroxidase labeled with a benzoylecgonine hapten and diluted in a protein matrix with stabilizers.	1 vial	1 vial	1 vial
Conjugate Diluent - Buffer containing protein stabilizers for reconstituting and diluting lyophilized conjugate.	20 mL	60 mL	1 L
Substrate Reagent - Contains 3,3', 5,5' tetramethylbenzidine.	20 mL	60 mL	1 L
Stopping Reagent - Contains 2 N sulfuric acid.	20 mL	60 mL	1 L
STC Negative Calibrator – Protein matrix tested by GC/MS and found to be negative for benzoylecgonine.	4 mL	4 mL	16 mL
STC Cocaine Metabolite Negative Control – Protein matrix containing 20 ng/mL (± 3 ng/mL) of benzoylecgonine and tested by GC/MS.	4 mL	4 mL	16 mL
STC Cocaine Metabolite Cutoff Calibrator – Protein matrix containing 100 ng/mL (± 10%) of benzoylecgonine and tested by GC/MS.	4 mL	4 mL	16 mL
STC Cocaine Metabolite Positive Control – Protein matrix containing 300 ng/mL (± 10%) of benzoylecgonine and tested by GC/MS.	4 mL	4 mL	16 mL

WARNINGS AND PRECAUTIONS

1. The handling of food or drink near the kit reagents is NOT recommended.
2. Proper handling of all reagents is strongly advised. It is suggested that disposable materials are used to avoid contamination of Substrate Reagent. Discard Substrate Reagent if obvious blue color develops.
3. Do NOT mouth pipet reagents. Handle all specimens and reagents as if potentially infectious.
4. Do NOT add sodium azide to samples as a preservative!
5. Keep all containers closed when not in use to avoid microbial contamination.
6. Do NOT use reagents past the expiration date.
7. Do NOT mix reagents from different kits or manufacturers.
8. Do NOT freeze reagents.
9. It is suggested that all STC reagents be kept out of direct sunlight whenever possible.
10. Stopping Reagent is corrosive; handle with care.

STORAGE/STABILITY

Store all reagents at 2-8°C until the expiration date indicated on the kit label.

SPECIMEN HANDLING

STC Technologies has not tested all possible applications of the assay. Therefore, laboratories must establish their own performance characteristics with fluids other than serum.

Viscous samples may require a predilution into distilled water or PBS. Once diluted, these samples may be run directly.[2]

MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated adjustable pipet (0.1-2.0 mL) for reconstitution and dilution of conjugate.
2. Semi-automated pipets (25 and 100 microliters) with tips.
3. Plate reader capable of reading at a dual wavelength of 450 and 630 nm.
4. Plate washer.

REAGENT PREPARATION

1. Using a calibrated pipet, add 2 mL of Conjugate Diluent to the vial of Lyophilized Stock Conjugate.
2. Replace the stopper and gently mix the contents of the vial by inversion for 10 minutes.
3. Using a calibrated pipet, add the volume of reconstituted Stock Conjugate specified on the Conjugate Dilution Instructions for this lot to the Conjugate Diluent bottle.
4. Replace the lid of the bottle and gently mix the contents by inversion for 1 minute. Allow the reagent to equilibrate for 30 minutes at room temperature or overnight at 2-8°C.
5. This conjugate dilution is stable for 8 weeks and may be used in the STC Cocaine Metabolite Micro-Plate assay as needed.

ASSAY PROCEDURE - *Note: Allow all reagents and samples to come to room temperature (20-27°C) before use.*

1. **At the discretion of the operator, all samples and calibrators/controls may be tested in duplicate. The insertion of calibrators/controls is recommended in each run.**
2. **Add 25 microliters of sample or calibrator/control to each well. Label wells appropriately.**
3. **Add 100 microliters of Enzyme Conjugate to each test well.**
4. **Start the clock with the addition of Enzyme Conjugate to the first well. Incubate for 30 minutes at room temperature (20-27°C) in the dark.**
5. **Using a suitable washer, wash each well 6 times with 300 microliters of distilled water.**
6. **Add 100 microliters of Substrate Reagent to each well and incubate 30 minutes at room temperature (20-27°C) in the dark.**

7. **Add 100 microliters of Stopping Reagent to each well.**
8. **Measure the absorbance at a dual wavelength of 450 and 630 nm. Wells should be read within 15 minutes.**

INTERPRETATION

Positive Result: Any sample with an absorbance less than or equal to the STC Cocaine Metabolite Cutoff Calibrator is considered a positive.

Negative Result: Any sample with an absorbance greater than the STC Cocaine Metabolite Cutoff Calibrator is considered a negative.

When interpreting duplicate results, the operator must be aware of several factors which may influence assay results. These include precise pipetting of specimens and reagents, effective washing of plates, and properly calibrated and maintained instrumentation. At the discretion of the operator, duplicate sample results with a variation greater than 10% may be retested.

A positive EIA result indicates only the presence of cocaine metabolite equal to or greater than the STC Cocaine Metabolite Cutoff Calibrator. It is possible that a negative result may indicate either the absence of cocaine metabolite or a concentration of cocaine metabolite in a specimen less than the STC Cocaine Metabolite Cutoff Calibrator.

QUALITY CONTROL

STC supplies positive and negative controls for monitoring the daily performance of the STC Cocaine Metabolite Micro-Plate EIA. The Negative Control contains 20 ng/mL benzoylecgonine, and the Positive Control contains 300 ng/mL benzoylecgonine. The Negative Control must have an absorbance greater than the STC Cocaine Metabolite Cutoff Calibrator, while the Positive Control must always have an absorbance less than the Cutoff Calibrator.

The testing laboratory should also monitor the percent displacement to cutoff between the STC Cocaine Metabolite Cutoff Calibrator and STC Negative Calibrator (formula listed below). Refer to the Lot Specification Sheet included in each kit for the performance characteristics and recommended limits of acceptance from STC for percent displacement. If the kit is not meeting these criteria, contact STC Technical Service for assistance.

$$\%\text{Displacement to Cutoff} = \frac{A_{450}\text{ Value (Negative Calibrator)} - A_{450}\text{ Value (Cutoff Calibrator)}}{A_{450}\text{ Value (Negative Calibrator)}} \times 100$$

Failure to follow these QC criteria in the STC Cocaine Metabolite Micro-Plate EIA may cause poor results or otherwise compromise the integrity of the assay.

If possible and commercially available, independent controls should be used with the STC Cocaine Metabolite Micro-Plate EIA. These controls should be above and below the STC Cocaine Metabolite Cutoff Calibrator. If commercial controls are used, they should not contain sodium azide.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Sensitivity/Limit of Detection - The limit of detection (LOD) for the STC Cocaine Metabolite Micro-Plate EIA is defined from the signal to noise ratio (S/N) at the zero drug concentration as the mean zero signal (S_o) (absorbance) minus the noise (N) times three (LOD = S_o - 3 N, or LOD = A_o - 3 SD). The limit of detection was determined by obtaining the absorbance values for twenty-four (24) negative samples and determining the standard deviation of the absorbance at zero drug concentration that was considered an estimate of the assay noise. The value for the standard deviation was then multiplied by three and subtracted from the man absorbance value to obtain the absorbance at the limit of detection (A_o - 3 SD). The apparent concentration of the resulting absorbance is the limit of detection of the assay.

The Cocaine Metabolite limit of detection (LOD) extrapolated from the standard curve is less than 1 ng/mL.

Precision - Precision was evaluated for the STC EIA by analyzing four levels of calibrators. Intra-assay was determined by analyzing the data from 24 replicates for each calibrator. The calibration concentration levels were 0, 50, 100 and 300 ng/mL.

The precision results are shown in the following table:

Calibrator	Intra-Assay % CV (n = 24)
0	3.7
50	4.2
100	6.4
300	7.2

Specificity/Cross-Reactivity

The following compounds were spiked in a serum diluent at a concentration of 10,000 ng/mL and tested for cross-reactivity. None were found to cross-react.

Amitriptyline	Gemfibrozil	Nortriptyline
Amobarbital	Gentisic Acid	Penicillin
Amphetamine	Hydrocodone	Pentobarbital
Butabarbital	Hydromorphone	Phenobarbital
Butalbital	Hydroxyalprazolam	Phenylephrine
Chlordiazepoxide	Ibuprofen	Phenylpropanolamine
Chlorpromazine	Imipramine	Primidone
Clonazepam	Lidocaine	Procaine
Clorazepate	Medazepam	Pseudoephedrine
Cotinine	Methadone	Quinine
Dextromethorphan	Methamphetamine	Quinidine
Doxepin	Morphine-3-b Glucuronide	THC
Ephedrine	Nalorphine	Theophylline
Erythromycin	Naproxyn	Trimipramine
Fenoprofen	Norchlordiazepoxide	

There is the possibility that other substances and/or factors not listed above may interfere with the test and cause false results.

The table below shows the concentration of compounds which produce a positive result.

Compound	Cross-Reactivity Level
Cocaethylene	5,000 ng/mL
Cocaine	10,000 ng/mL
Ecgonine	10,000 ng/mL
Ecgonine Methyl Ester	100,000 ng/mL

BIBLIOGRAPHY

1. "Urine Testing for Drugs of Abuse," *National Institute on Drug Abuse (NIDA) Research Monograph 73,* 1986.
2. Perrigo, B.J. and Joynt, B.P., "Use of ELISA for the Detection of Common Drugs of Abuse in Forensic Whole Blood Samples," *Can-Soc. Forens. Sci. J.*, **28** (4): 261-269, 1995.

Note: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions set forth in the labeling can affect performance characteristics and stated or implied label claims.

For additional assistance in the USA, call STC Technical Service toll free (800) 869-3538.

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OPIATES MICRO-PLATE EIA

SERUM APPLICATION

14050 (1/97)

INTENDED USE

The STC Opiates Micro-Plate EIA is intended for use in the qualitative determination of opiates in serum. ***THIS TEST IS INTENDED FOR IN VITRO DIAGNOSTIC USE.***

BACKGROUND

Heroin is metabolized extensively and excreted in the urine as Morphine (4.2% of dose), Conjugated Morphine (38.3% of dose), 6-acetyl Morphine (1.3% of dose), and unchanged Heroin (0.1% of dose). Serum will contain predominately Morphine with an expected half life of 60 to 90 minutes.

Heroin is metabolized extensively and excreted in the urine as Morphine (4.2% of dose), Conjugated Morphine (38.3% of dose), 6-acetyl Morphine (1.3% of dose), and unchanged Heroin (0.1% of dose). Serum will contain predominately Morphine with an expected half life of 60 to 90 minutes.

A detection method for opiates must be able to detect both free morphine as well as other metabolites. Each of the three detection methods have different cross-reactivities for opiates.

Opiates in serum can be detected at levels as low as 5.0 ng/mL.[1] However, patients or chronic users who receive a single large dose of opiates will have a serum opiate concentration well above this level.[2-6] For example, large doses of morphine (55-65 mg) have produced plasma morphine concentrations between 800 and 2,600 ng/mL. Upon administration of opiates, the opiate concentration of the serum will peak rapidly and then decline over time (t 1/2 is 1.3-7.0 hours). The STC Opiates Micro-Plate EIA uses a cutoff concentration of 100 ng/mL or 200 ng/mL for the qualitative detection of opiates including morphine and codeine in serum. The STC Opiates Micro-Plate EIA may be used as a screen for qualitative identification of chronic or single-large-dose users of opiates.

The STC Opiates Micro-Plate EIA provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[6] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

PRINCIPLE OF THE TEST

The STC Opiates Micro-Plate EIA is a competitive enzyme immunoassay for the qualitative determination of opiates in serum. Sample or calibrator/control is added to each well along with enzyme-labeled hapten derivative. There is a competition to bind the antibody fixed onto the well. The wells are washed, substrate is added, and color is produced. The absorbance produced (450 nm) is inversely proportional to the amount of opiates present in the sample or calibrator/control.

KIT COMPONENTS	Catalog No. 1150EA 96 Test Kit Min. Qty.	Catalog No. 1150EB 480 Test Kit Min. Qty.	Catalog No. 1150EC 9600 Test Kit Min. Qty.
Micro-Plate - Rabbit anti-morphine polyclonal antibody immobilized on a polystyrene plate.	1	5	100
Enzyme Conjugate - Morphine labeled with horseradish peroxidase and diluted in a protein matrix with protein stabilizers.	20 mL	60 mL	1 L
Substrate Reagent - Contains 3,3', 5,5' tetramethylbenzidine.	20 mL	60 mL	1 L
Stopping Reagent - Contains 2 N sulfuric acid.	20 mL	60 mL	1 L
STC Negative Calibrator -- Preserved buffer tested by GC/MS and found to be negative for morphine.	4 mL	4 mL	16 mL
STC Opiates Serum Negative Control -- Preserved buffer containing 10 ng/mL (± 3 ng/mL) of morphine.	4 mL	4 mL	16 mL
STC Opiates Serum Cutoff Calibrator -- Preserved buffer containing 100 ng/mL (± 10%) of morphine.	4 mL	4 mL	16 mL
STC Opiates Serum Cutoff Calibrator* -- Preserved buffer containing 200 ng/mL (± 10%) of morphine.	4 mL	4 mL	16 mL
STC Opiates Serum Positive Control -- Preserved buffer containing 500 ng/mL (± 10%) of morphine.	4 mL	4 mL	16 mL

* Provided Separately.

WARNINGS AND PRECAUTIONS

1. The handling of food or drink near the kit reagents is NOT recommended.
2. Proper handling of all reagents is strongly advised. It is suggested that disposable materials are used to avoid contamination of Substrate Reagent. Discard Substrate Reagent if obvious blue color develops.
3. Do NOT mouth pipet reagents. Handle all specimens and reagents as if potentially infectious.
4. Do NOT add sodium azide to samples as a preservative!
5. Keep all containers closed when not in use to avoid microbial contamination.
6. Do NOT use reagents past the expiration date.
7. Do NOT mix reagents from different kits or manufacturers.
8. Do NOT freeze reagents.
9. It is suggested that all STC reagents be kept out of direct sunlight whenever possible.
10. Stopping Reagent is corrosive; handle with care.

STORAGE/STABILITY

Store all reagents at 2-8°C until the expiration date indicated on the kit label.

SPECIMENS

The assay was developed for use with serum. An effort should be made to use fresh samples. If immediate testing is not possible, samples may be stored at 2-8°C for 30 days.

SPECIMEN HANDLING/INSTRUMENTATION

The use of sample handling equipment, such as a Hamilton or Tecan, may require the addition of 50 µL of distilled water along with 25 µL of sample. The addition of 50 µL of distilled water does not affect the overall results of the assay. Contact STC Technical Service for further information.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Semi-automated pipets (25 and 100 microliters) with tips.
2. Plate reader capable of reading at a dual wavelength of 450 and 630 nm.
3. Micro-plate washer.

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AMPHETAMINE-SPECIFIC MICRO-PLATE EIA

FORENSIC APPLICATION

14000 (1/97)

INTENDED USE

The STC Amphetamine-Specific Micro-Plate EIA is intended for use in the qualitative determination of Amphetamine in serum. ***THIS TEST IS INTENDED FOR FORENSIC USE ONLY.***

The STC Amphetamine-Specific Micro-Plate EIA provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

PRINCIPLE

The STC Amphetamine-Specific Micro-Plate EIA is a competitive immunoassay for the qualitative determination of amphetamine in serum. Sample or calibrator/control is added to each well along with enzyme-labeled hapten derivative. There is a competition to bind the antibody fixed onto the well. The wells are washed, substrate is added, and color is produced. The absorbance produced (450 nm) is inversely proportional to the amount of amphetamine present in the sample or calibrator/control.

REAGENTS PROVIDED		
KIT COMPONENTS	Catalog No. 1103EA 96 Test Kit Min. Qty.	Catalog No. 1103EB 480 Test Kit Min. Qty.
Micro-Plate - Sheep anti-amphetamine antibody immobilized on a polystyrene plate.	1	5
Enzyme Conjugate - Horseradish peroxidase labeled with an amphetamine hapten and diluted in a protein matrix with stabilizers.	20 mL	60 mL
Substrate Reagent - Contains 3,3', 5,5' tetramethylbenzidine.	20 mL	60 mL
Stopping Reagent - Contains 2 N sulfuric acid.	20 mL	60 mL
STC Negative Calibrator -- Protein matrix negative for D-amphetamine.	4 mL	4 mL
STC Amphetamine-Specific Serum Negative Control - Protein matrix containing 25 ng/mL (± 3 ng/mL) of D-amphetamine.	4 mL	4 mL
STC Amphetamine-Specific Serum Cutoff Calibrator - Protein matrix containing 100 ng/mL (± 10%) of D-amphetamine.	4 mL	4 mL
STC Amphetamine-Specific Serum Positive Control - Protein matrix containing 500 ng/mL (± 10%) of D-amphetamine.	4 mL	4 mL

WARNINGS AND PRECAUTIONS

1. The handling of food or drink near the kit reagents is NOT recommended.
2. Proper handling of all reagents is strongly advised. It is suggested that disposable materials are used to avoid contamination of Substrate Reagent. Discard Substrate Reagent if obvious blue color develops.
3. Do NOT mouth pipet reagents. Handle all specimens and reagents as if potentially infectious.
4. Keep all containers closed when not in use to avoid microbial contamination.

5. Do NOT add sodium azide to samples as a preservative!
6. Do NOT use reagents past the expiration date.
7. Do NOT mix reagents from different kits or manufacturers.
8. Do NOT freeze reagents.
9. It is suggested that all STC reagents be kept out of direct sunlight whenever possible.
10. Stopping Reagent is corrosive; handle with care.

STORAGE/STABILITY

Store all reagents at 2-8°C until the expiration date indicated on the kit label.

SPECIMEN HANDLING

STC Technologies has not tested all possible applications of the assay. Therefore, laboratories must establish their own performance characteristics with fluids other than serum.

Viscous samples may require a predilution into distilled water or PBS. Once diluted, these samples may be run directly.[2]

MATERIALS REQUIRED BUT NOT PROVIDED

1. Semi-automated pipets (25 and 100 microliters) with tips.
2. Plate reader capable of reading at a dual wavelength of 450 and 630 nm.
3. Micro-plate washer.

ASSAY PROCEDURE

Note: Allow all reagents and samples to come to room temperature (20-27°C) before use.

1. **At the discretion of the operator, it is recommended that all samples, calibrators and controls be tested in duplicate. The insertion of calibrators and controls is recommended in each run or on each new plate.**
2. **Add 25 microliters of sample, calibrator or control to each well. Label wells appropriately.**
3. **Add 100 microliters of Enzyme Conjugate to each test well.**
4. **Start the clock with the addition of Enzyme Conjugate to the first well. Incubate for 30 minutes at room temperature (20-27°C) in the dark.**
5. **Using a suitable plate washer, wash each well 6 times with 300 microliters of distilled water.**
6. **Add 100 microliters of Substrate Reagent to each well and incubate 30 minutes at room temperature (20-27°C) in the dark.**
7. **Add 100 microliters of Stopping Reagent to each well.**
8. **Measure the absorbance at a dual wavelength of 450 and 630 nm. Wells should be read within 15 minutes of stopping the reaction.**

INTERPRETATION

Positive Result: *Any sample with an absorbance less than or equal to the STC Amphetamine-Specific Cutoff Calibrator is considered a positive.*

Negative Result: *Any sample with an absorbance greater than the STC Amphetamine-Specific Cutoff Calibrator is considered a negative.*

When interpreting duplicate results, the operator must be aware of several factors which may influence assay results. These include precise pipetting of specimens and reagents, effective washing of plates, and properly calibrated and maintained instrumentation. At the discretion of the operator, duplicate sample results with a variation greater than 10% may be retested.

ASSAY PROCEDURE

Note: Allow all reagents and samples to come to room temperature (20-27°C) before use.

1. **At the discretion of the operator, samples and calibrators and controls may be tested in duplicate. The insertion of calibrators and controls is recommended in each run.**
2. **Add 25 microliters of sample, calibrator or control to each well. Label wells appropriately.**
3. **Add 100 microliters of Enzyme Conjugate to each test well.**
4. **Start the clock with the addition of Enzyme Conjugate to the first well. Incubate for 30 minutes at room temperature (20-27°C) in the dark.**
5. **Using a suitable plate washer, wash each well 6 times with 300 microliters of distilled water.**
6. **Add 100 microliters of Substrate Reagent to each well and incubate 30 minutes at room temperature (20-27°C) in the dark.**
7. **Add 100 microliters of Stopping Reagent to each well.**
8. **Measure the absorbance at a dual wavelength of 450 and 630 nm. Wells should be read within 15 minutes of stopping the reaction.**

INTERPRETATION

Positive result: Any sample with an absorbance less than or equal to the STC Opiates Cutoff Calibrator is considered a positive.

Negative result: Any sample with an absorbance greater than the STC Opiates Cutoff Calibrator is considered a negative.

When interpreting duplicate results, the operator must be aware of several factors which may influence assay results. These include precise pipetting of specimens and reagents, effective washing of plates, and properly calibrated and maintained instrumentation. At the discretion of the operator, duplicate sample results with a variation greater than 10% may be retested.

A positive EIA result indicates only the presence of opiates above the STC Opiates Cutoff Calibrator. It is possible that a negative result may indicate either the absence of opiates or a concentration of opiates in a specimen less than the STC Opiates Cutoff Calibrator.

QUALITY CONTROL

The Negative Control must have an absorbance greater than the Cutoff Calibrator, while the Positive Control must always have an absorbance less than the Cutoff Calibrator. An additional QC measure to be monitored by the testing laboratory includes the percent displacement between the Cutoff and Negative Calibrator (formula listed below). Refer to the Lot Specification Sheet included in each kit for the performance characteristics and recommended limits of acceptance from STC for percent displacement. If the kit is not meeting these criteria, contact STC Technical Service for assistance.

$$\%\,\text{Displacement to Cutoff} = \frac{A_{450}\ \text{Value (Negative Calibrator)} - A_{450}\ \text{Value (Cutoff Calibrator)}}{A_{450}\ \text{Value (Negative Calibrator)}} \times 100$$

If possible and commercially available, independent controls should be used with the STC Opiates Micro-Plate EIA. These controls should be above and below the STC Opiates Cutoff Calibrator. If commercial controls are used, they should not contain sodium azide.

PERFORMANCE CHARACTERISTICS

Precision – The precision of the STC Opiates Micro-Plate EIA is shown below:

CALIBRATOR	INTRA-ASSAY % CV (n=8)	INTER-ASSAY % CV (n=24, 3 DAYS)
0	5.9	6.8
10	5.8	7.2
100	6.2	7.1
500	6.4	7.5

Accuracy – The accuracy of the STC Opiates Micro-Plate EIA was evaluated in comparison to RIA and GC/MS. All samples evaluated by RIA and the STC EIA were run according to the manufacturer's instructions. Results are presented in the following table.

STC OPIATES MICRO-PLATE EIA (Serum) VERSUS RIA AND GC/MS			
Specimen No.	EIA Result	RIA Result (ng/mL)	GC/MS (ng/mL) TOTAL MORPHINE
1	+	312.83	>500
2	+	140.53	200
3	+	171.65	200
4	+	219.58	350
5	+	103.80	161
6	+	93.66	110
7	+	198.35	247
8	+	217.76	232
9	+	175.55	192
10	+	139.21	160
11	+	181.49	209
12	+	286.59	310
13	+	208.05	232
14	+	181.92	211
15	+	91.85	120
16	+	99.79	127
17	+	405.59	>500
18	+	97.50	115
19	+	109.60	170
20	+	650.06	>500
21	+	136.64	172
22	+	109.34	180
23	+	680.61	>500
24	+	368.85	425
25	+	160.08	193
26	+	399.93	>500
27	+	268.00	310
28	+	98.53	140
29	+	350.72	420
30	+	127.11	156
31	+	222.92	216
32	+	77.25	110
33	+	399.32	>500
34	+	75.73	105
35	+	105.84	109
36	+	121.16	151
37	+	145.46	162
38	+	193.72	220
39	+	118.35	131
40	+	314.23	401

n = 40

A total of 76 specimens were tested by both the RIA and STC EIA. A total of 36 specimens were negative in both immunoassays. Out of the 40 specimens that were positive in the STC EIA, all but seven had values greater than 100 ng/mL in the RIA and GC/MS. Those discrepancies may result from the differences in crossreactivities of the two immunoassays. In such situations, it may be prudent to use GC/MS to differentiate the opiates present in a given specimen.

Sensitivity – The sensitivity of the assay was determined by spiking normal human serum with decreasing concentrations of morphine. These samples were then tested in duplicate in the assay as described. The minimum detectable concentration of morphine was 5.0 ng/mL.

Interfering Compounds -- Ten volunteers negative for opiates donated serum which was collected into Vacutainer™ tubes. All specimens were then tested in the STC Opiates Micro-Plate EIA. None of the specimens gave readings greater than 5.0 ng/mL in the assay. In another experiment whole blood specimens were spiked with 20 mg/dL bilirubin and sonicated to produce hemolysis. None of these specimens interfered with the assay.

Specificity – The minimum concentration at which selected compounds produced a positive response when tested by this assay (100 ng/mL morphine cutoff) is listed in the following table.

Compound Tested	Concentration
Codeine	75 ng/mL
6-Monoacetylmorphine	500 ng/mL
Diacetylmorphine	600 ng/mL
Hydromorphone	600 ng/mL
Hydrocodone	300 ng/mL
Levorphanol	4500 ng/mL
Nalorphine	125,000 ng/mL
Normorphone	125,000 ng/mL
Morphine 3-b-d Glucuronide	125,000 ng/mL
Oxycodone	125,000 ng/mL
Oxymorphone	125,000 ng/mL

There is the possibility that other substances and/or factors not listed above may interfere with the test and cause false results, e.g., technical or procedural errors.

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6. Urine Testing for Drugs of Abuse, National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.

Note: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions set forth in the labeling can affect performance characteristics and stated or implied label claims.

For additional assistance in the USA, call STC Technical Service toll free (800) 869-3538.

A positive EIA result indicates only the presence of amphetamine equal to or greater than the STC Amphetamine-Specific Cutoff Calibrator. It is possible that a negative result may indicate either the absence of amphetamine or a concentration of amphetamine in a specimen less than the STC Amphetamine-Specific Cutoff Calibrator.

QUALITY CONTROL

STC supplies positive and negative controls for monitoring the daily performance of the STC Amphetamine-Specific Micro-Plate EIA. The Negative Control contains 25 ng/mL D-amphetamine, and the Positive Control contains 500 ng/mL D-amphetamine. The Negative Control must have an absorbance greater than the STC Amphetamine-Specific Cutoff Calibrator, while the Positive Control must always have an absorbance less than the Cutoff Calibrator.

The testing laboratory should also monitor the percent displacement to cutoff between the STC Amphetamine Cutoff Calibrator and STC Negative Calibrator (formula listed below). Refer to the Lot Specification Sheet included in each kit for the expected results and acceptable displacement criteria. If the kit is not meeting these criteria, contact STC Technical Service for assistance.

$$\%\,\text{Displacement to Cutoff} = \frac{A_{450}\ \text{Value (Negative Calibrator)} - A_{450}\ \text{Value (Cutoff Calibrator)}}{A_{450}\ \text{Value (Negative Calibrator)}} \times 100$$

Failure to follow these QC criteria in the STC Amphetamine-Specific Micro-Plate EIA may cause poor results or otherwise compromise the integrity of the assay.

If possible and commercially available, independent controls should be used with the STC Amphetamine-Specific Micro-Plate EIA. These controls should be above and below the STC Amphetamine-Specific Cutoff Calibrator. If commercial controls are used, they should not contain sodium azide.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Sensitivity/Limit of Detection - The limit of detection (LOD) for the STC Amphetamine-Specific Micro-Plate EIA is defined from the signal to noise ratio (S/N) at the zero drug concentration as the mean zero signal (S_o) (absorbance) minus the noise (N) times three (LOD = S_o - 3 N, or LOD = A_o - 3 SD). The limit of detection was determined by obtaining the absorbance values for twenty-four (24) negative samples and determining the standard deviation of the absorbance at zero drug concentration that was considered an estimate of the assay noise. The value for the standard deviation was then multiplied by three and subtracted from the mean absorbance value to obtain the absorbance at the limit of detection (A_o - 3 SD). The apparent concentration of the resulting absorbance is the limit of detection of the assay. The limit of detection (LOD) extrapolated from the standard curve for three separate runs is less than 10 ng/mL.

Precision - Precision was evaluated for the STC Amphetamine-Specific Micro-Plate EIA by analyzing four levels of calibrators. Inter-assay precision was determined over a three-day period with twenty-four (24) samples run at each calibrator level per day. Intra-assay precision was determined by analyzing the data from the first 24 replicates for each calibrator.

The precision results are shown in the following table:

D-amphetamine (ng/mL)	Intra-Assay % CV (n = 24)	Inter-Assay % CV (n = 24/day, 3 days)
0	5.6	6.8
25	4.7	7.4
100	3.9	7.1
500	5.6	7.9

Analytical Specificity/Cross-Reactivity

The analytical specificity of an immunoassay is the cross-reactivity characteristics in the assay of substances which are structurally related to the target compound. The percent cross-reactivity of a compound in the STC Amphetamine-Specific Micro-Plate EIA is defined as the apparent D-amphetamine concentration divided by the spiked concentration times 100 give a percentage.

The cross-reactivity of structurally related compounds was calculated at several spiked concentrations in a protein diluent. The following table indicates the apparent concentration of D-amphetamine for the substance tested at the concentrations shown.

Compound	Tested Concentration (ng/mL)	D-amphetamine Equivalents (ng/mL)	% Cross-Reactivity
L-Phenylalanine	100,000	8.8	0
L-Ephedrine	100,000	10.4	0
L-Methamphetamine	100,000	20.2	0
Pseudoephedrine	100,000	16.5	0
Phenylpropanolamine	100,000	20.3	0
Fenfluramine	100,000	13.0	0
Phentermine	1,000	27.7	2.7
	10,000	134.2	1.3
	50,000	311.3	0.62
	100,000	442.1	0.44
MDMA	100,000	77.0	0.07
MDA	10	21.3	213.0
	25	47.7	190.8
	50	76.8	1536
	75	121.2	161.6
	100	146.0	146.0

The following compounds were spiked in a protein diluent at a concentration of 10,000 ng/mL and tested for cross-reactivity. None were found to produce an absorbance less than or equal to the STC Amphetamine-Specific Cutoff Calibrator.

Alprazolam	Fenoprofen	Nordiazepam
Amitriptyline	Gemfibrozil	Nortriptyline
Amobarbital	Gentisic acid	Phencyclidine
Benzoylecgonine	Glipizide	Penicillin
Butabarbital	Hydrocodone	Pentobarbital
Butalbital	Hydromorphone	Phenobarbital
Chlordiazepoxide	Hydroxyalprazolam	Phenylephrine
Chlorpromazine	Ibuprofen	Primidone
Clonazepam	Imipramine	Procaine
Clorazepate	Lidocaine	Procainamide
Cocaethylene	Medazepam	Quinidine
Cocaine	Meperidine	Quinine
Codeine	Methadone	Temazepam
Cotinine	Morphine-3-β-D-glucuronide	Δ^9-THC
Dextromethorphan	Morphine	Theophylline
Diacetylmorphine	Nalorphine	Trimipramine
Doxepin	Naproxen	
Erythromycin	Norchlordiazepoxide	

There is the possibility that other substances and/or factors not listed above may interfere with the test and cause false results.

Effect of Sample pH - Samples in concentrations of 0 and 1000 ng/mL D-amphetamine in a protein diluent were adjusted to various pH levels to determine if sample pH could cause false positive/false negative results. The following table shows the results obtained from this testing.

	pH Level					
D-amphetamine (ng/mL)	4	5	6	7	8	9
0 ng/mL	Negative	Negative	Negative	Negative	Negative	Negative
1000 ng/mL	Positive	Positive	Positive	Positive	Positive	Positive

Note: A sample pH of 4 depressed the negative absorbance value.

Anti-Coagulants - Potassium oxalate/NaF, EDTA (K_3), and sodium heparin do not affect the assay.

BIBLIOGRAPHY

1. "Urine Testing for Drugs of Abuse," *National Institute on Drug Abuse (NIDA) Research Monograph 73*, 1986.
2. Perrigo, B.J. and Joynt, B.P., "Use of ELISA for the Detection of Common Drugs of Abuse in Forensic Whole Blood Samples," *Can. Soc. Forens. Sci. J.*, 28 (4): 261-269, 1995.

Note: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in the labeling can affect performance characteristics and stated or implied label claims.

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METHAMPHETAMINE MICRO-PLATE EIA
FORENSIC APPLICATION

14010 (12/96)

INTENDED USE

The STC Methamphetamine Micro-Plate EIA is intended for use in the qualitative determination of methamphetamine in serum. ***THIS TEST IS INTENDED FOR FORENSIC USE ONLY.***

The STC Methamphetamine Micro-Plate EIA provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result.[1] Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

PRINCIPLE OF THE TEST

The STC Methamphetamine Micro-Plate EIA is a competitive immunoassay for the qualitative determination of methamphetamine in serum. Sample or calibrator/control is added to each well along with enzyme-labeled hapten derivative. There is a competition to bind the antibody fixed onto the well. The wells are washed, substrate is added, and color is produced. The absorbance produced (450 nm) is inversely proportional to the amount of methamphetamine present in the sample or calibrator/control.

KIT COMPONENTS	Catalog No. 1104EA 96 Test Kit Min. Qty.	Catalog No. 1104EB 480 Test Kit Min. Qty.	Catalog No. 1104EC 9600 Test Kit Min. Qty.
Micro-Plate - Rabbit anti-methamphetamine polyclonal antibody immobilized on a polystyrene plate.	1	5	100
Enzyme Conjugate - Horseradish peroxidase labeled with a methamphetamine hapten and diluted in a protein matrix with stabilizers.	20 mL	60 mL	1 L
Substrate Reagent - Contains 3,3', 5,5' tetramethylbenzidine.	20 mL	60 mL	1 L
Stopping Reagent - Contains 2 N sulfuric acid.	20 mL	60 mL	1 L
STC Negative Calibrator -- Protein matrix tested by GC/MS to be negative for methamphetamine.	4 mL	4 mL	16 mL
STC Methamphetamine Negative Control -- Protein matrix containing 50 ng/mL (± 10%) methamphetamine and tested by GC/MS.	4 mL	4 mL	16 mL
STC Methamphetamine Cutoff Calibrator -- Protein matrix containing 100 ng/mL (± 10%) methamphetamine and tested by GC/MS.	4 mL	4 mL	16 mL
STC Methamphetamine Positive Control -- Protein matrix containing 500 ng/mL (± 10%) methamphetamine and tested by GC/MS.	4 mL	4 mL	16 mL

WARNINGS AND PRECAUTIONS

1. The handling of food or drink near the kit reagents is NOT recommended.
2. Proper handling of all reagents is strongly advised. It is suggested that disposable materials are used to avoid contamination of Substrate Reagent. Discard Substrate Reagent if obvious blue color develops.
3. Do NOT mouth pipet reagents. Handle all specimens and reagents as if potentially infectious.
4. Do NOT add sodium azide to samples as a preservative!
5. Keep all containers closed when not in use to avoid microbial contamination.
6. Do NOT use reagents past the expiration date.
7. Do NOT mix reagents from different kits or manufacturers.
8. Do NOT freeze reagents.
9. It is suggested that all STC reagents be kept out of direct sunlight whenever possible.
10. Stopping Reagent is corrosive; handle with care.

STORAGE/STABILITY

Store all reagents at 2-8°C until the expiration date indicated on the kit label.

SPECIMEN HANDLING

STC Technologies has not tested all possible applications of the assay. Therefore, laboratories must establish their own performance characteristics with fluids other than serum.

Viscous samples may require a predilution into distilled water or PBS. Once diluted, these samples may be run directly.[2]

MATERIALS REQUIRED BUT NOT PROVIDED

1. Semi-automated pipets (25 and 100 microliters) with tips.
2. Plate reader capable of reading at a dual wavelength of 450 and 630 nm.
3. Micro-plate washer.

ASSAY PROCEDURE

Note: Allow all reagents and samples to come to room temperature (20-27°C) before use.

1. **At the discretion of the operator, all samples, calibrators and controls may be tested in duplicate. The insertion of calibrators and controls is recommended in each run.**
2. **Add 25 microliters of sample, calibrator or control to each well. Label wells appropriately.**
3. **Add 100 microliters of Enzyme Conjugate to each test well.**
4. **Start the clock with the addition of Enzyme Conjugate to the first well. Incubate for 30 minutes at room temperature (20-27°C) in the dark.**
5. **Using a suitable washer, wash each well 6 times with 300 microliters of distilled water.**
6. **Add 100 microliters of Substrate Reagent to each well and incubate 30 minutes at room temperature (20-27°C) in the dark.**
7. **Add 100 microliters of Stopping Reagent to each well.**
8. **Measure the absorbance at a dual wavelength of 450 and 630 nm. Wells should be read within 15 minutes.**

INTERPRETATION

Positive Result: Any sample with an absorbance less than or equal to the STC Methamphetamine Cutoff Calibrator is considered a positive.

Negative Result: Any sample with an absorbance greater than the STC Methamphetamine Cutoff Calibrator is considered a negative.

When interpreting duplicate results, the operator must be aware of several factors which may influence assay results. These include precise pipetting of specimens and reagents, effective washing of plates, and properly calibrated and maintained instrumentation. At the discretion of the operator, duplicate sample results with a variation greater than 10% may be retested.

A positive EIA result indicates only the presence of methamphetamine equal to or greater than the STC Methamphetamine Cutoff Calibrator. It is possible that a negative result may indicate either the absence of methamphetamine or a concentration of methamphetamine in a specimen less than the STC Methamphetamine Cutoff Calibrator.

QUALITY CONTROL

STC supplies positive and negative controls for monitoring the daily performance of the STC Methamphetamine Micro-Plate EIA. The Negative Control contains 50 ng/mL methamphetamine, and the Positive Control contains 500 ng/mL methamphetamine. The Negative Control must have an absorbance greater than the STC Methamphetamine Cutoff Calibrator, while the Positive Control must always have an absorbance less than the Cutoff Calibrator.

The testing laboratory should also monitor the percent displacement to cutoff between the STC Methamphetamine Cutoff Calibrator and STC Negative Calibrator (formula listed below). Refer to the Lot Specification Sheet included in each kit for the performance characteristics and recommended limits of acceptance from STC for percent displacement. If the kit is not meeting these criteria, contact STC Technical Service for assistance.

$$\%\,\text{Displacement to Cutoff} = \frac{A_{450}\ \text{Value (Negative Calibrator)} - A_{450}\ \text{Value (Cutoff Calibrator)}}{A_{450}\ \text{Value (Negative Calibrator)}} \times 100$$

Failure to follow these QC criteria in the STC Methamphetamine Micro-Plate EIA may cause poor results or otherwise compromise the integrity of the assay.

If possible and commercially available, independent controls should be used with the STC Methamphetamine Micro-Plate EIA. These controls should be above and below the STC Methamphetamine Cutoff Calibrator. If commercial controls are used, they should not contain sodium azide.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Sensitivity/Limit of Detection - The limit of detection (LOD) for the STC Methamphetamine Micro-Plate EIA is defined from the signal to noise ratio (S/N) at the zero drug concentration as the mean zero signal (S_o) (absorbance) minus the noise (N) times three (LOD = S_o - 3N, or LOD = A_o - 3 SD). The limit of detection was determined by obtaining the absorbance values for twenty-four (24) negative samples and determining the standard deviation of the absorbance at zero drug concentration that was considered an estimate of the assay noise. The value for the standard deviation was then multiplied by three and subtracted from the mean absorbance value to obtain the absorbance at the limit of detection (A_o - 3 SD). The apparent methamphetamine concentration at the resulting absorbance is the limit of detection of the assay. The Methamphetamine limit of detection (LOD) extrapolated from the standard curve is less than 20 ng/mL.

Precision – Precision was evaluated for the STC EIA by analyzing four levels of calibrators/controls. Intra-assay precision was determined by analyzing the data from 24 replicates of each sample.

Methamphetamine (ng/mL)	Intra-Assay % CV (n=24)
0	7.3
50	12.2
100	10.7
500	14.6

Specificity/Cross Reactivity

The following compounds were spiked in a serum diluent at a concentration of 10,000 ng/mL and tested for cross-reactivity. None were found to cross-react.

Amitriptyline	Gentisic Acid	Penicillin
Amobarbital	Hydrocodone	Pentobarbital
Butabarbital	Hydromorphone	Phenobarbital
Butalbital	Hydroxyalprazolam	Phenylephrine
Chlordiazepoxide	Ibuprofen	Phenylpropanolamine
Chlorpromazine	Imipramine	Primidone
Clonazepam	Lidocaine	Procaine
Clorazepate	Medazepam	Quinine
Cotinine	Methadone	Quinidine
Dextromethorphan	Morphine-3-b Glucuronide	THC
Doxepin	Nalorphine	Theophylline
Erythromycin	Naproxyn	Trimipramine
Fenoprofen	Norchlordiazepoxide	
Gemfibrozil	Nortriptyline	

There is the possibility that other substances and/or factors not listed above may interfere with the test and cause false results.

The table below shows the concentration of compounds which produce a positive result.

Compound	Cross-Reactivity Level
d-Amphetamine	5,000 ng/mL
Ephedrine	7,000 ng/mL
L-Amphetamine	5,000 ng/mL
L-Methamphetamine	30 ng/mL
MDA	3,000 ng/mL
MDMA	10 ng/mL
Phenylethylamine	50,000 ng/mL
Pseudoephedrine	5,000 ng/mL

BIBLIOGRAPHY

1. "Urine Testing for Drugs of Abuse," *National Institute on Drug Abuse (NIDA) Research Monograph 73,* 1986.
2. Perrigo, B.J. and Joynt, B.P., "Use of ELISA for the Detection of Common Drugs of Abuse in Forensic Whole Blood Samples," *Can-Soc. Forens. Sci. J.*, **28** (4): 261-269, 1995.

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BARBITURATES MICRO-PLATE EIA

11003 (8/97)

INTENDED USE

The STC Barbiturates Micro-Plate EIA is intended for use in the qualitative determination of Barbiturates in urine. *THIS TEST IS INTENDED FOR FORENSIC USE ONLY.*

The STC Barbiturates Micro-Plate EIA provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

PRINCIPLE

The STC Barbiturates Micro-Plate EIA is a competitive immunoassay for the qualitative determination of barbiturates in urine. Sample or calibrator/control is added to each well along with enzyme-labeled hapten derivative. There is a competition to bind the antibody fixed onto the well. The wells are washed, substrate is added, and color is produced. The absorbance produced (450 nm) is inversely proportional to the amount of barbiturates present in the sample or calibrator/control.

KIT COMPONENTS	Catalog No. 1108UA 96 Test Kit Min. Qty.	Catalog No. 1108UB 480 Test Kit Min. Qty.
Micro-Plate - Sheep anti-barbiturate antibody immobilized on a polystyrene plate.	1	5
Enzyme Conjugate - Horseradish peroxidase labeled with a barbiturate hapten and diluted in a protein matrix with stabilizers. This conjugate is supplied as a lyophilized stock solution.	1 vial	1 vial
Conjugate Diluent - Buffer containing protein stabilizers for reconstituting and diluting the stock conjugate.	20 mL	60 mL
Substrate Reagent - Contains 3,3′, 5,5′ tetramethylbenzidine.	20 mL	60 mL
Stopping Reagent - Contains 2 N sulfuric acid.	20 mL	60 mL
STC Negative Calibrator -- Normal human urine negative for secobarbital.	4 mL	4 mL
STC Barbiturates Urine Negative Control -- Normal human urine containing 100 ng/mL (± 10%) of secobarbital.	4 mL	4 mL
STC Barbiturates Urine Cutoff Calibrator -- Normal human urine containing 200 ng/mL (± 10%) of secobarbital.	4 mL	4 mL
STC Barbiturates Urine Positive Control -- Normal human urine containing 1000 ng/mL (± 10%) of secobarbital.	4 mL	4 mL

WARNINGS AND PRECAUTIONS

1. The handling of food or drink near the kit is NOT recommended.
2. Proper handling of all reagents is strongly advised. It is suggested that disposable materials are used to avoid contamination of Substrate Reagent. Discard Substrate Reagent if obvious blue color develops.
3. Do NOT mouth pipet reagents. Handle all specimens and reagents as if potentially infectious.

4. Do NOT add sodium azide to samples as a preservative!
5. Keep all containers closed when not in use to avoid microbial contamination.
6. Do NOT use reagents past the expiration date.
7. Do NOT mix reagents from different kits or manufacturers.
8. Do NOT freeze reagents.
9. It is suggested that all STC reagents be kept out of direct sunlight whenever possible.
10. Stopping Reagent is corrosive; handle with care.
11. Standards are prepared in normal human urine. This material is a potential biohazard and should be treated as such.

STORAGE/STABILITY

Store all reagents at 2-8°C until the expiration date indicated on the kit label.

SPECIMEN HANDLING

Fresh urine samples should be collected in plastic or glass containers. If not analyzed immediately, samples may be stored refrigerated for 3 days. To store samples longer than 3 days, keep them frozen (< 0°C) and thaw before use. Samples should be at room temperature (20-27°C) for testing. Samples should be within the pH range of 5-8. Fresh or properly stored urine samples will generally fall within this range. Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample. Specimens may be encountered that display unusually high turbidity. It is recommended that these be centrifuged before analysis. The effect of urine preservatives on this assay has not been established.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated adjustable pipet (0.1-2.0 mL) for reconstitution and dilution of conjugate.
2. Semi-automated pipets (10 and 100 microliters) with tips.
3. Plate reader capable of reading at a dual wavelength of 450 and 630 nm.
4. Micro-plate washer.

REAGENT PREPARATION

1. Using a calibrated pipet, add 1 mL of Conjugate Diluent to the vial of lyophilized Stock Conjugate.
2. Replace the stopper and gently mix the contents of the vial by inversion for 10 minutes.
3. Using a calibrated pipet, add the volume of reconstituted Stock Conjugate specified on the Conjugate Dilution Instructions for this lot to the Conjugate Diluent bottle.
4. Replace the lid on the bottle and gently mix the contents by inversion for 1 minute. Allow the reagent to equilibrate for 30 minutes at room temperature or overnight at 2-8°C.
5. This conjugate dilution is stable for 8 weeks when stored at 2-8°C and may be used in the STC Barbiturates Micro-Plate assay as needed.

ASSAY PROCEDURE

Note: Allow all reagents and samples to come to room temperature (20-27°C) before use.

1. **At the discretion of the operator, samples, calibrators/controls be tested in duplicate. The insertion of calibrators/controls is recommended in each run.**
2. **Add 10 microliters of sample, calibrator/control to each well. Label wells appropriately.**
3. **Add 100 microliters of Enzyme Conjugate to each test well.**
4. **Start the clock with the addition of Enzyme Conjugate to the first well. Incubate for 30 minutes at room temperature (20-27°C) in the dark.**
5. **Using a suitable plate washer, wash each well 6 times with 300 microliters of distilled water.**
6. **Add 100 microliters of Substrate Reagent to each well and incubate 30 minutes at room temperature (20-27°C) in the dark.**
7. **Add 100 microliters of Stopping Reagent to each well.**
8. **Measure the absorbance at a dual wavelength of 450 and 630 nm. Wells should be read within 15 minutes of stopping the reaction.**

INTERPRETATION

Positive Result: Any sample with an absorbance less than or equal to the STC Barbiturates Cutoff Calibrator is considered a positive.

Negative Result: Any sample with an absorbance greater than the STC Barbiturates Cutoff Calibrator is considered a negative.

When interpreting duplicate results, the operator must be aware of several factors which may influence assay results. These include precise pipetting of specimens and reagents, effective washing of plates, and properly calibrated and maintained instrumentation. At the discretion of the operator, duplicate sample results with a variation greater than 10% may be retested.

A positive EIA result indicates only the presence of barbiturates equal to or greater than the STC Barbiturates Cutoff Calibrator. It is possible that a negative result may indicate either the absence of barbiturates or a concentration of barbiturates in a specimen less than the STC Barbiturates Cutoff Calibrator.

QUALITY CONTROL

STC supplies positive and negative controls for monitoring the daily performance of the STC Barbiturates Micro-Plate EIA. The Negative Control contains 100 ng/mL secobarbital, and the Positive Control contains 1000 ng/mL secobarbital. The STC Barbiturates Negative Control must have an absorbance greater than the STC Barbiturates Cutoff Calibrator, while the Positive Control must always have an absorbance less than the Cutoff Calibrator.

The testing laboratory should also monitor the percent displacement to cutoff between the STC Barbiturates Cutoff Calibrator and STC Negative Calibrator (formula listed below). Refer to the Lot Specification Sheet included in each kit for the performance characteristics and recommended limits of acceptance from STC for percent displacement. If the kit is not meeting these criteria, contact STC Technical Service for assistance.

$$\%\,\text{Displacement to Cutoff} = \frac{A_{450}\ \text{Value (Negative Calibrator)} - A_{450}\ \text{Value (Cutoff Calibrator)}}{A_{450}\ \text{Value (Negative Calibrator)}} \times 100$$

Failure to follow these QC criteria in the STC Barbiturates Micro-Plate EIA may cause poor results or otherwise compromise the integrity of the assay.

If possible and commercially available, independent controls should be used with the STC Barbiturates Micro-Plate EIA. These controls should be above and below the STC Barbiturates Cutoff Calibrator. If commercial controls are used, they should not contain sodium azide.

LIMITATIONS OF PROCEDURE

The assay is designed for use with urine samples. Other samples may produce variable results and their use is not recommended.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Sensitivity/Limit of Detection - The limit of detection (LOD) for the STC Barbiturates Micro-Plate EIA is defined from the signal to noise ratio (S/N) at the zero drug concentration as the mean zero signal (S_o) (absorbance) minus the noise (N) times three (LOD = S_o - 3 N, or LOD = A_o - 3 SD). The limit of detection was determined by obtaining the absorbance values for twenty-four (24) negative samples and determining the standard deviation of the absorbance at zero drug concentration that was considered an estimate of the assay noise. The value for the standard deviation was then multiplied by three and subtracted from the mean absorbance value to obtain the absorbance at the limit of detection (A_o - 3 SD). The apparent concentration of the resulting absorbance is the limit of detection of the assay. The Barbiturates limit of detection (LOD) extrapolated from the standard curve for three separate runs is 5 ng/mL.

Precision - Precision was evaluated for the STC Barbiturates Micro-Plate EIA by analyzing four levels of calibrators. Inter-assay precision was determined from three runs with twenty-four (24) samples tested at each calibrator level per run. Intra-assay precision was determined by analyzing the data from the first 24 replicates for each calibrator (i.e., data from the first test run). The calibrator concentration levels were 0, 100, 200 and 1,000 ng/mL.

The precision results are shown in the following table:

Secobarbital (ng/mL)	Intra-Assay % CV (n = 24)	Inter-Assay % CV (n = 24/Run, 3 Runs)
0	5.3	6.9
100	8.7	11.0
200	8.8	10.5
1,000	8.0	9.5

Analytical Specificity/Cross-Reactivity - The following compounds were spiked in preserved human urine at a concentration of 10,000 ng/mL and tested for cross-reactivity. None were found to produce an absorbance less than or equal to the STC Barbiturates Cutoff Calibrator.

Alprazolam	Dextromethorphan	Lidocaine	Phenylephrine
Amitriptyline	Diacetylmorphine	Medazepam	Phenylpropanolamine
Anabarbital	Doxepin	Meperidine	Primidone
β-Phenethylamine	Erythromycin	Methadone	Procainamide
Benzoylecgonine	Fenoprofen	Methamphetamine	Procaine
Chlordiazepoxide	Gemfibrozil	Methohexital	Pseudoephedrine
Chlorpromazine	Gentisic acid	Morphine-3-β-D-glucuronide	Quinidine
Clonazepam	Glipizide	Morphine	Quinine
Clorazepate	Hydrocodone	Nalorphine	Temazepam
Cocaethylene	Hydromorphone	Naproxen	Δ^9-THC
Cocaine	Hydroxyalprazolam	Norchlordiazepoxide	Theophylline
Codeine	Ibuprofen	Nordiazepam	Trimipramine
Cotinine	Imipramine	Nortriptyline	
D-Amphetamine	L-Ephedrine	Penicillin	
D-Methamphetamine	L-Methamphetamine	Phencyclidine	

The cross-reactivity of structurally-related compounds was calculated at several spiked concentrations in normal human urine. The cross-reactivity data selected for inclusion in this insert was based on Secobarbital equivalents at or near the cutoff.

Compound	Spiked Concentration (ng/mL)	Secobarbital Equivalents (ng/mL)	% Cross-Reactivity
Butabarbital	10,000	713	7
Butalbital	1,000	240	24
Pentobarbital	10,000	810	8
Phenobarbital	10,000	<100	n.d.

BIBLIOGRAPHY

1. "Urine Testing for Drugs of Abuse," *National Institute on Drug Abuse (NIDA) Research Monograph 73,* 1986.

Note: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in the labeling can affect performance characteristics and stated or implied label claims.

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CANNABINOIDS MICRO-PLATE EIA
FORENSIC APPLICATION

14020 (7/97)

INTENDED USE

The STC Cannabinoids Micro-Plate EIA is intended for use in the qualitative determination of Cannabinoids in serum. ***THIS TEST IS INTENDED FOR FORENSIC USE ONLY.***

The STC Cannabinoids Micro-Plate EIA provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

PRINCIPLE OF THE TEST

The STC Cannabinoids Micro-Plate EIA is a competitive immunoassay for the qualitative determination of cannabinoids (marijuana) in serum. Sample or calibrator/control is added to each well along with enzyme-labeled hapten derivative. There is a competition to bind the antibody fixed onto the well. The wells are washed, substrate is added, and color is produced. The absorbance produced (450 nm) is inversely proportional to the amount of drug present in the sample or calibrator/control.

KIT COMPONENTS	Catalog No. 1118EA 96 Test Kit Min. Qty.	Catalog No. 1118EB 480 Test Kit Min. Qty.
Micro-Plate - Rabbit anti-cannabinoids immobilized on a polystyrene plate.	1	5
Enzyme Conjugate - Horseradish peroxidase labeled with Δ^9-THC and diluted in a protein matrix with stabilizers.	20 mL	60 mL
Substrate Reagent - Contains 3,3',5,5'- tetramethylbenzidine.	20 mL	60 mL
Stopping Reagent - Contains 2 N sulfuric acid.	20 mL	60 mL
STC Negative Calibrator – Protein matrix tested by GC/MS to be negative for THC.	4 mL	4 mL
STC Cannabinoids Negative Control – Protein matrix containing 10 ng/mL (±) 11-nor-9-carboxy THC (± 3 ng/mL) and tested by GC/MS.	4 mL	4 mL
STC Cannabinoids Cutoff Calibrator – Protein matrix containing 30 ng/mL (±) 11-nor-9-carboxy THC (± 3 ng/mL) and tested by GC/MS.	4 mL	4 mL
STC Cannabinoids Positive Control – Protein matrix containing 50 ng/mL (±) 11-nor-9-carboxy THC (± 10%) and tested by GC/MS.	4 mL	4 mL

WARNINGS AND PRECAUTIONS

1. The handling of food or drink near the kit is NOT recommended.
2. Proper handling of all reagents is strongly advised. It is suggested that disposable materials are used to avoid contamination of Substrate Reagent. Discard Substrate Reagent if obvious blue color develops.
3. Do NOT mouth pipet reagents. Handle all specimens and reagents as if potentially infectious.
4. Do NOT add sodium azide to samples as a preservative!

5. Keep all containers closed when not in use to avoid microbial contamination.
6. Do NOT use reagents past the expiration date.
7. Do NOT mix reagents from different kits or manufacturers.
8. Do NOT freeze reagents.
9. It is suggested that all STC reagents be kept out of direct sunlight whenever possible.
10. Stopping Reagent is corrosive; handle with care.

STORAGE/STABILITY

Store all reagents at 2-8°C until the expiration date indicated on the kit label.

SPECIMEN HANDLING

STC Technologies has not tested all possible applications of the assay. Therefore, laboratories must establish their own performance characteristics with fluids other than serum.

Viscous samples may require a predilution into distilled water or PBS. Once diluted, these samples may be run directly.[2]

MATERIALS REQUIRED BUT NOT PROVIDED

1. Semi-automated pipets (25 and 100 microliters) with tips.
2. Plate reader capable of reading at a dual wavelength of 450 and 630 nm.
3. Plate washer.

ASSAY PROCEDURE

Note: Allow all reagents and samples to come to room temperature (20-27°C) before use.

1. **At the discretion of the operator, samples and calibrators and controls may be tested in duplicate. The insertion of calibrators and controls is recommended in each run.**
2. **Add 25 microliters of sample, calibrator or control to each well. Label wells appropriately.**
3. **Add 100 microliters of Enzyme Conjugate to each test well.**
4. **Start the clock with the addition of Enzyme Conjugate to the first well. Incubate for 30 minutes at room temperature (20-27°C) in the dark.**
5. **Using a suitable plate washer, wash each well 6 times with 300 microliters of distilled water.**
6. **Add 100 microliters of Substrate Reagent to each well and incubate 30 minutes at room temperature (20-27°C) in the dark.**
7. **Add 100 microliters of Stopping Reagent to each well.**
8. **Measure the absorbance at a dual wavelength of 450 and 630 nm. Wells should be read within 15 minutes.**

INTERPRETATION

Positive Result — Any sample with an absorbance less than or equal to the STC Cannabinoids Serum Cutoff Calibrator is considered a positive.

Negative Result — Any sample with an absorbance greater than the STC Cannabinoids Serum Cutoff Calibrator is considered a negative.

When interpreting duplicate results, the operator must be aware of several factors which may influence assay results. These include precise pipetting of specimens and reagents, effective washing of plates, and properly calibrated and maintained instrumentation. At the discretion of the operator, duplicate sample results with a variation greater than 10% may be retested.

A positive EIA result indicates only the presence of THC equal to or greater than the STC Cannabinoids Cutoff Calibrator. It is possible that a negative result may indicate either the absence of THC or a concentration of THC in a specimen less than the STC Cannabinoids Cutoff Calibrator.

QUALITY CONTROL

STC supplies positive and negative controls for monitoring the daily performance of the STC Cannabinoids Micro-Plate EIA. The Negative Control contains 10 ng/mL (±) 11-nor-9-carboxy THC, and the Positive Control contains 50 ng/mL (±) 11-nor-9-carboxy THC. The Negative Control must have an absorbance greater than the STC Cannabinoids Cutoff Calibrator, while the Positive Control must always have an absorbance less than the Cutoff Calibrator.

The testing laboratory should also monitor the percent displacement to cutoff between the STC Cannabinoids Cutoff Calibrator and STC Negative Calibrator (formula listed below). Refer to the Lot Specification Sheet included in each kit for the performance characteristics and recommended limits of acceptance from STC for percent displacement. If the kit is not meeting these criteria, contact STC Technical Service for assistance.

$$\%\text{Displacement to Cutoff} = \frac{A_{450}\text{ Value (Negative Calibrator) - }A_{450}\text{ Value (Cutoff Calibrator)}}{A_{450}\text{ Value (Negative Calibrator)}} \times 100$$

Failure to follow these QC criteria in the STC Cannabinoids Micro-Plate EIA may cause poor results or otherwise compromise the integrity of the assay.

If possible and commercially available, independent controls should be used with the STC Cannabinoids Micro-Plate EIA. These controls should be above and below the STC Cannabinoids Cutoff Calibrator. If commercial controls are used, they should not contain sodium azide.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Sensitivity/Limit of Detection - The limit of detection (LOD) for the STC Cannabinoids Micro-Plate EIA is defined from the signal to noise ratio (S/N) at the zero drug concentration as the mean zero signal (S_o) (absorbance) minus the noise (N) times three (LOD = S_o - 3 N, or LOD = A_o - 3 SD). The limit of detection was determined by obtaining the absorbance values for twenty-four (24) negative samples and determining the standard deviation of the absorbance at zero drug concentration that was considered an estimate of the assay noise. The value for the standard deviation was then multiplied by three and subtracted from the mean absorbance value to obtain the absorbance at the limit of detection (A_o - 3 SD). The apparent concentration of the resulting absorbance is the limit of detection of the assay. The THC limit of detection (LOD) extrapolated from the standard curve is less than 3 ng/mL.

Precision - Precision was evaluated for the STC EIA by analyzing four levels of calibrators. Intra-assay was determined by analyzing the data from 24 replicates of each calibrator. The calibration concentration levels were 0, 10, 30 and 50 ng/mL.

The precision results are shown in the following table:

Calibrator	Intra-Assay % CV (n = 24)
0	14.0
10	10.5
30	14.2
50	11.5

Specificity/Cross-Reactivity

The following compounds were spiked in a serum diluent at a concentration of 10,000 ng/mL and tested for cross-reactivity. None were found to cross-react.

Amitriptyline	Gemfibrozil	Penicillin
Amobarbital	Gentisic Acid	Pentobarbital
Amphetamine	Hydrocodone	Phenobarbital
Butabarbital	Hydromorphone	Phenylephrine
Butalbital	Ibuprofen	Phenylpropanolamine
Chlorpromazine	Imipramine	Primidone
Clonazepam	Lidocaine	Procaine
Cotinine	Methadone	Pseudoephedrine
Dextromethorphan	Methamphetamine	Quinine
Doxepin	Morphine-3-b Glucuronide	Quinidine
Ephedrine	Nalorphine	Theophylline
Erythromycin	Naproxen	Trimipramine
Fenoprofen	Nortriptyline	

There is the possibility that other substances and/or factors not listed above may interfere with the test and cause false results.

The cross-reactivity of structurally-related compounds was calculated at several spiked concentrations in a protein diluent. The cross-reactivity data selected for inclusion in this insert was based on the concentration of each compound which produced a positive result.

Compound	Tested Concentration (ng/mL)	11-nor-9-carboxy-THC Equivalents (ng/mL)	(%) Cross-Reactivity
Cannabidiol	100,000	26.8	0.03
Cannabinol	500	27.8	5.6
Δ^8-THC	500	50.6	10.1
Δ^9-THC	300	72.7	24.2

BIBLIOGRAPHY

1. "Urine Testing for Drugs of Abuse," *National Institute on Drug Abuse (NIDA) Research Monograph 73*, 1986.
2. Perrigo, B.J. and Joynt, B.P., "Use of ELISA for the Detection of Common Drugs of Abuse in Forensic Whole Blood Samples," *Can-Soc. Forens. Sci. J.*, 28 (4): 261-269, 1995.

Note: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in the labeling can affect performance characteristics and stated or implied label claims.

For additional assistance in the USA, call STC Technical Service toll free (800) 869-3538.

Instrumentation Laboratory ToxiChem® Whole Blood Alcohol Control (Human)

I. INTENDED USE

The Instrumentation Laboratory ToxiChem Whole Blood Alcohol Control is for use as a control for procedures involving qualitative and quantitative assay of ethyl alcohol in whole blood.

II. SUMMARY AND PRINCIPLES

The use of quality control material which resembles specimens being assayed provides the laboratory with reliable means of monitoring day to day performance. Detection of random and systematic errors resulting from a variety of sources such as errors in technique, defects in reagents and instrumentation, or inherent bias of a particular methodology can be accomplished by use of a control with a known constituent value. These steps are necessary to assure the reliability of results reported on patient's specimens.

The Whole Blood Alcohol Control is designed to be used exactly as if it were a patient's specimen and should be subjected to all steps of an analytical procedure. Values obtained in this manner may be compared with the assigned values given in the variable data portion of this circular and a determination made as to whether the given procedure is within control limits.

III. PRODUCT DESCRIPTION

The Whole Blood Alcohol Control is prepared from human blood. Pooled red blood cells are washed in a buffer containing sodium fluoride. Ethanol is added in a concentration of 0.15 g per dL. Sodium azide is used as a preservative.

The constituent value is established by a variety of procedures on the basis of multiple determinations performed by selected referee laboratories in accordance with protocols specified by Instrumentation Laboratory.

IV. PRECAUTIONS

1. The material from which this product has been produced was tested and found nonreactive for Hepatitis B Surface Antigen (HBsAg) and HIV antibodies by immunoassay. No known test method can offer assurance that products derived from human blood will not transmit Hepatitis, AIDS, or other infections. Therefore, all human serum products and patient specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.

2. This product is for *in vitro* diagnostic use.

3. **WARNING: This product contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.**

V. STORAGE AND STABILITY

The Whole Blood Alcohol Control is stable until the date indicated if stored as directed.

Store at refrigerated temperatures, 2-8ºC (35-46ºF). This product may be used for two days after the vial is opened provided it is kept tightly stoppered and refrigerated at all times other than when sampling.

VI. PROCEDURE

1. Mix thoroughly prior to use; avoid excess shaking.

2. Analyze in the same manner as patient specimens including all steps of the assay procedure as specified in the directions for use of the kit or instrument being used.

3. Check to insure that the lot number on this circular matches the lot number on the vial.

VII. LIMITATIONS

1. The listed assay values apply only to this particular lot of product.

2. Assay values given are the result of multiple determinations done in a number of different laboratories. Individual laboratories may not always expect to obtain the mean value listed. Variations in technique and equipment or random errors will produce slightly different results.

   The "expected range" given in this insert encompasses these variations. Additional information should be obtained from the Limitations section of the package insert for the procedure being utilized.

VIII. EXPECTED RESULTS

See listed values on the reverse side.

Product Number 2930-14 6 x 3 mL

Issued January 1998

Instrumentation Laboratory Company
Lexington, MA 02173-3190
300314R3 1/98

Instrumentation Laboratory

TOXICHEM®
WHOLE BLOOD ALCOHOL CONTROL (HUMAN)

Product Number 2930-14
(Values Apply Only To This Lot)

Lot N1289928 Pool 019 Exp 06/00

METHOD	CONVENTIONAL UNITS ETHANOL CONCENTRATION mg/dL	CONVENTIONAL UNITS EXPECTED RANGE mg/dL	S.I. UNITS ETHANOL CONCENTRATION mmol/L	S.I. UNITS EXPECTED RANGE mmol/L
ENZYMATIC (Alcohol Dehydrogenase)				
Abbott TDX[1]	144	116-172	31.3	25.2-37.3
Abbott AxSYM[1]	146	118-174	31.7	25.6-37.8
Dade ACA[2, 5]	154	128-180	33.4	27.8-39.1
Dade DIMENSION[2]	154	126-182	33.4	27.4-39.5
SIGMA[3,6]	148	120-176	32.1	26.1-38.2
GAS CHROMATOGRAPHIC[4]	156	128-184	33.9	27.8-39.9

1. Abbott Laboratories, Abbott Park, Illinois.
2. Dade International, Newark, Delaware.
3. Sigma-Aldrich Chemical Company, St. Louis, Missouri.
4. Includes most available GC and GLC methods, direct and with head space injectors.
5. Performed after deproteinization as per ACA manual.
6. Concensus values obtained using assays done manually and on automated instruments.

TOXICHEM®
WHOLE BLOOD ALCOHOL CONTROL (HUMAN)

Product Number 2930-14
(Values Apply Only To This Lot)

Lot N0375270 **Pool 007** **Exp 09/98**

METHOD	CONVENTIONAL UNITS		S.I. UNITS	
	ETHANOL CONCENTRATION mg/dL	EXPECTED RANGE	ETHANOL CONCENTRATION mmol/L	EXPECTED RANGE
ENZYMATIC (Alcohol Dehydrogenase)				
Abbott TDX[1]	145	120-170	31.5	26.1-36.9
Abbott AxSYM[1]	155	130-180	33.7	28.2-39.1
Dade ACA[2, 5]	154	129-179	33.4	28.0-38.9
Dade DIMENSION[2]	158	133-183	34.3	28.9-39.7
SIGMA[3,6]	149	124-174	32.3	26.9-37.8
GAS CHROMATOGRAPHIC[4]	154	129-179	33.4	28.0-38.9

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6. Concensus values obtained using assays done manually and on automated instruments.

Instrumentation Laboratory ToxiChem® Whole Blood Alcohol Control (Human)

I. INTENDED USE

The Instrumentation Laboratory ToxiChem Whole Blood Alcohol Control is for use as a control for procedures involving qualitative and quantitative assay of ethyl alcohol in whole blood.

II. SUMMARY AND PRINCIPLES

The use of quality control material which resembles specimens being assayed provides the laboratory with reliable means of monitoring day to day performance. Detection of random and systematic errors resulting from a variety of sources such as errors in technique, defects in reagents and instrumentation, or inherent bias of a particular methodology can be accomplished by use of a control with a known constituent value. These steps are necessary to assure the reliability of results reported on patient's specimens.

The Whole Blood Alcohol Control is designed to be used exactly as if it were a patient's specimen and should be subjected to all steps of an analytical procedure. Values obtained in this manner may be compared with the assigned values given in the variable data portion of this circular and a determination made as to whether the given procedure is within control limits.

III. PRODUCT DESCRIPTION

The Whole Blood Alcohol Control is prepared from human blood. Pooled red blood cells are washed in a buffer containing sodium fluoride. Ethanol is added in a concentration of 0.15 g per dL. Sodium azide is used as a preservative.

The constituent value is established by a variety of procedures on the basis of multiple determinations performed by selected referee laboratories in accordance with protocols specified by Instrumentation Laboratory.

IV. PRECAUTIONS

1. The material from which this product has been produced was tested and found nonreactive for Hepatitis B Surface Antigen (HBsAg) and HIV antibodies by immunoassay. No known test method can offer assurance that products derived from human blood will not transmit Hepatitis, AIDS, or other infections. Therefore, all human serum products and patient specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.

2. This product is for *in vitro* diagnostic use.

3. **WARNING: This product contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.**

V. STORAGE AND STABILITY

The Whole Blood Alcohol Control is stable until the date indicated if stored as directed.

Store at refrigerated temperatures, 2-8ºC (35-46ºF). This product may be used for two days after the vial is opened provided it is kept tightly stoppered and refrigerated at all times other than when sampling.

VI. PROCEDURE

1. Mix thoroughly prior to use; avoid excess shaking.

2. Analyze in the same manner as patient specimens including all steps of the assay procedure as specified in the directions for use of the kit or instrument being used.

3. Check to insure that the lot number on this circular matches the lot number on the vial.

VII. LIMITATIONS

1. The listed assay values apply only to this particular lot of product.

2. Assay values given are the result of multiple determinations done in a number of different laboratories. Individual laboratories may not always expect to obtain the mean value listed. Variations in technique and equipment or random errors will produce slightly different results.

   The "expected range" given in this insert encompasses these variations. Additional information should be obtained from the Limitations section of the package insert for the procedure being utilized.

VIII. EXPECTED RESULTS

See listed values on the reverse side.

Product Number 2930-14 6 x 3 mL

Issued January 1992

Instrumentation Laboratory Company
Lexington, MA 02173-3190
300314R2

Instrumentation Laboratory

Instrumentation Laboratory ToxiChem® Whole Blood Alcohol Control (Human)

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See listed values on the reverse side.

Product Number 2930-14 6 x 3 mL

Issued January 1998

Instrumentation Laboratory Company
Lexington, MA 02173-3190
300314R3 1/98

Instrumentation Laboratory

TOXICHEM®
WHOLE BLOOD ALCOHOL CONTROL (HUMAN)

Product Number 2930-14
(Values Apply Only To This Lot)

Lot N1175595 **Pool 012** **Exp 05/99**

METHOD	CONVENTIONAL UNITS		S.I. UNITS	
	ETHANOL CONCENTRATION mg/dL	EXPECTED RANGE mg/dL	ETHANOL CONCENTRATION mmol/L	EXPECTED RANGE mmol/L
ENZYMATIC (Alcohol Dehydrogenase)				
Abbott TDX[1]	145	120-170	31.5	26.1-36.9
Abbott AxSYM[1]	158	133-183	34.3	28.9-39.7
Dade ACA[2, 5]	161	136-186	35.0	29.5-40.4
Dade DIMENSION[2]	154	129-179	33.4	28.0-38.9
SIGMA[3,6]	139	109-169	30.2	23.7-36.7
GAS CHROMATOGRAPHIC[4]	151	126-176	32.8	27.4-38.2

1. Abbott Laboratories, Abbott Park, Illinois.
2. Dade International, Newark, Delaware.
3. Sigma-Aldrich Chemical Company, St. Louis, Missouri.
4. Includes most available GC and GLC methods, direct and with head space injectors.
5. Performed after deproteinization as per ACA manual.
6. Concensus values obtained using assays done manually and on automated instruments.

BEHRING

February 7, 1994

Behring Diagnostics Inc.

151 University Avenue
Westwood, MA 02090
Tel/617.320.3000

Dear Customer,

Recently we have received questions regarding lot no. 3315 of the Whole Blood Alcohol Control. As a result of additional testing using Gas Chromatography, the range for lot 3315 is being re-assigned as follows:

Mean: 0.185 gm% **Range: 0.157-0.213 gm%**

As always, it is recommended that each laboratory establish its own range based on the patient population and conditions common to that local.

If there are additional questions regarding this topic, please feel free to call at (800) 854-5089.

TOXICHEM®
WHOLE BLOOD ALCOHOL CONTROL (HUMAN)

Product Number 2930-14
(Values Apply Only To This Lot)

Lot N0964747 **Pool 002** **Exp 03/98**

METHOD	CONVENTIONAL UNITS ETHANOL CONCENTRATION mg/dL	CONVENTIONAL UNITS EXPECTED RANGE	S.I. UNITS ETHANOL CONCENTRATION mmol/L	S.I. UNITS EXPECTED RANGE
ENZYMATIC (Alcohol Dehydrogenase)				
Abbott TDX[1]	142	117-167	30.8	25.4-36.3
Abbott AxSYM[1]	150	125-175	32.6	27.1-38.0
Dade ACA[2, 5]	153	128-178	33.2	27.8-38.6
SIGMA[3,6]	148	123-173	32.1	26.7-37.6
GAS CHROMATOGRAPHIC[4]	153	128-178	33.2	27.8-38.6

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800 955 9525
FAX 617 861-0175

Instrumentation Laboratory ToxiChem® Whole Blood Alcohol Control (Human)

I. INTENDED USE

The Instrumentation Laboratory ToxiChem Whole Blood Alcohol Control is for use as a control for procedures involving qualitative and quantitative assay of ethyl alcohol in whole blood.

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3. **WARNING: This product contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.**

V. STORAGE AND STABILITY

The Whole Blood Alcohol Control is stable until the date indicated if stored as directed.

Store at refrigerated temperatures, 2-8ºC (35-46ºF). This product may be used for two days after the vial is opened provided it is kept tightly stoppered and refrigerated at all times other than when sampling.

VI. PROCEDURE

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   The "expected range" given in this insert encompasses these variations. Additional information should be obtained from the Limitations section of the package insert for the procedure being utilized.

VIII. EXPECTED RESULTS

See listed values on the reverse side.

Product Number 2930-14 6 x 3 mL

Issued January 1992

Instrumentation Laboratory Company
Lexington, MA 02173-3190
300314R2

Instrumentation Laboratory

Instrumentation Laboratory ToxiChem® Whole Blood Alcohol Control (Human)

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VIII. EXPECTED RESULTS

See listed values on the reverse side.

Product Number 2930-14 6 x 3 mL

Issued January 1992

Instrumentation Laboratory Company
Lexington, MA 02173-3190
300314R2

Instrumentation Laboratory

TOXICHEM®
WHOLE BLOOD ALCOHOL CONTROL (HUMAN)

Product Number 2930-14
(Values Apply Only To This Lot)

Lot N0375270 **Pool 007** **Exp 09/98**

METHOD	CONVENTIONAL UNITS ETHANOL CONCENTRATION mg/dL	CONVENTIONAL UNITS EXPECTED RANGE	S.I. UNITS ETHANOL CONCENTRATION mmol/L	S.I. UNITS EXPECTED RANGE
ENZYMATIC (Alcohol Dehydrogenase)				
Abbott TDX[1]	145	120-170	31.5	26.1-36.9
Abbott AxSYM[1]	155	130-180	33.7	28.2-39.1
Dade ACA[2, 5]	154	129-179	33.4	28.0-38.9
Dade DIMENSION[2]	158	133-183	34.3	28.9-39.7
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4. Includes most available GC and GLC methods, direct and with head space injectors.
5. Performed after deproteinization as per ACA manual.
6. Concensus values obtained using assays done manually and on automated instruments.

Instrumentation Laboratory ToxiChem® Whole Blood Alcohol Control (Human)

I. INTENDED USE

The Instrumentation Laboratory ToxiChem Whole Blood Alcohol Control is for use as a control for procedures involving qualitative and quantitative assay of ethyl alcohol in whole blood.

II. SUMMARY AND PRINCIPLES

The use of quality control material which resembles specimens being assayed provides the laboratory with reliable means of monitoring day to day performance. Detection of random and systematic errors resulting from a variety of sources such as errors in technique, defects in reagents and instrumentation, or inherent bias of a particular methodology can be accomplished by use of a control with a known constituent value. These steps are necessary to assure the reliability of results reported on patient's specimens.

The Whole Blood Alcohol Control is designed to be used exactly as if it were a patient's specimen and should be subjected to all steps of an analytical procedure. Values obtained in this manner may be compared with the assigned values given in the variable data portion of this circular and a determination made as to whether the given procedure is within control limits.

III. PRODUCT DESCRIPTION

The Whole Blood Alcohol Control is prepared from human blood. Pooled red blood cells are washed in a buffer containing sodium fluoride. Ethanol is added in a concentration of 0.15 g per dL. Sodium azide is used as a preservative.

The constituent value is established by a variety of procedures on the basis of multiple determinations performed by selected referee laboratories in accordance with protocols specified by Instrumentation Laboratory.

IV. PRECAUTIONS

1. The material from which this product has been produced was tested and found nonreactive for Hepatitis B Surface Antigen (HBsAg) and HIV antibodies by immunoassay. No known test method can offer assurance that products derived from human blood will not transmit Hepatitis, AIDS, or other infections. Therefore, all human serum products and patient specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.

2. This product is for *in vitro* diagnostic use.

3. **WARNING: This product contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.**

V. STORAGE AND STABILITY

The Whole Blood Alcohol Control is stable until the date indicated if stored as directed.

Store at refrigerated temperatures, 2-8ºC (35-46ºF). This product may be used for two days after the vial is opened provided it is kept tightly stoppered and refrigerated at all times other than when sampling.

VI. PROCEDURE

1. Mix thoroughly prior to use; avoid excess shaking.

2. Analyze in the same manner as patient specimens including all steps of the assay procedure as specified in the directions for use of the kit or instrument being used.

3. Check to insure that the lot number on this circular matches the lot number on the vial.

VII. LIMITATIONS

1. The listed assay values apply only to this particular lot of product.

2. Assay values given are the result of multiple determinations done in a number of different laboratories. Individual laboratories may not always expect to obtain the mean value listed. Variations in technique and equipment or random errors will produce slightly different results.

   The "expected range" given in this insert encompasses these variations. Additional information should be obtained from the Limitations section of the package insert for the procedure being utilized.

VIII. EXPECTED RESULTS

See listed values on the reverse side.

Product Number 2930-14 6 x 3 mL

Issued January 1992

Instrumentation Laboratory Company
Lexington, MA 02173-3190
300314R2

Instrumentation Laboratory

TOXICHEM®
WHOLE BLOOD ALCOHOL CONTROL (HUMAN)

Product Number 2930-14
(Values Apply Only To This Lot)

Lot N0375270 **Pool 007** **Exp 09/98**

METHOD	CONVENTIONAL UNITS		S.I. UNITS	
	ETHANOL CONCENTRATION mg/dL	EXPECTED RANGE	ETHANOL CONCENTRATION mmol/L	EXPECTED RANGE
ENZYMATIC (Alcohol Dehydrogenase)				
Abbott TDX[1]	145	120-170	31.5	26.1-36.9
Abbott AxSYM[1]	155	130-180	33.7	28.2-39.1
Dade ACA[2, 5]	154	129-179	33.4	28.0-38.9
Dade DIMENSION[2]	158	133-183	34.3	28.9-39.7
SIGMA[3,6]	149	124-174	32.3	26.9-37.8
GAS CHROMATOGRAPHIC[4]	154	129-179	33.4	28.0-38.9

1. Abbott Laboratories, Abbott Park, Illinois.
2. Dade International, Newark, Delaware.
3. Sigma-Aldrich Chemical Company, St. Louis, Missouri.
4. Includes most available GC and GLC methods, direct and with head space injectors.
5. Performed after deproteinization as per ACA manual.
6. Concensus values obtained using assays done manually and on automated instruments.

Instrumentation Laboratory ToxiChem® Whole Blood Alcohol Control (Human)

I. INTENDED USE

The Instrumentation Laboratory ToxiChem Whole Blood Alcohol Control is for use as a control for procedures involving qualitative and quantitative assay of ethyl alcohol in whole blood.

II. SUMMARY AND PRINCIPLES

The use of quality control material which resembles specimens being assayed provides the laboratory with reliable means of monitoring day to day performance. Detection of random and systematic errors resulting from a variety of sources such as errors in technique, defects in reagents and instrumentation, or inherent bias of a particular methodology can be accomplished by use of a control with a known constituent value. These steps are necessary to assure the reliability of results reported on patient's specimens.

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2. This product is for *in vitro* diagnostic use.

3. **WARNING: This product contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.**

V. STORAGE AND STABILITY

The Whole Blood Alcohol Control is stable until the date indicated if stored as directed.

Store at refrigerated temperatures, 2-8°C (35-46°F). This product may be used for two days after the vial is opened provided it is kept tightly stoppered and refrigerated at all times other than when sampling.

VI. PROCEDURE

1. Mix thoroughly prior to use; avoid excess shaking.

2. Analyze in the same manner as patient specimens including all steps of the assay procedure as specified in the directions for use of the kit or instrument being used.

3. Check to insure that the lot number on this circular matches the lot number on the vial.

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VIII. EXPECTED RESULTS

See listed values on the reverse side.

Product Number 2930-14 6 x 3 mL

Issued January 1992

Instrumentation Laboratory Company
Lexington, MA 02173-3190
300314R2

Instrumentation Laboratory

TOXICHEM®
WHOLE BLOOD ALCOHOL CONTROL (HUMAN)

Product Number 2930-14
(Values Apply Only To This Lot)

Lot N0375270 **Pool 007** **Exp 09/98**

METHOD	CONVENTIONAL UNITS ETHANOL CONCENTRATION mg/dL	CONVENTIONAL UNITS EXPECTED RANGE	S.I. UNITS ETHANOL CONCENTRATION mmol/L	S.I. UNITS EXPECTED RANGE
ENZYMATIC (Alcohol Dehydrogenase)				
Abbott TDX[1]	145	120-170	31.5	26.1-36.9
Abbott AxSYM[1]	155	130-180	33.7	28.2-39.1
Dade ACA[2, 5]	154	129-179	33.4	28.0-38.9
Dade DIMENSION[2]	158	133-183	34.3	28.9-39.7
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4. Includes most available GC and GLC methods, direct and with head space injectors.
5. Performed after deproteinization as per ACA manual.
6. Concensus values obtained using assays done manually and on automated instruments.

Instrumentation Laboratory ToxiChem® Whole Blood Alcohol Control (Human)

I. INTENDED USE

The Instrumentation Laboratory ToxiChem Whole Blood Alcohol Control is for use as a control for procedures involving qualitative and quantitative assay of ethyl alcohol in whole blood.

II. SUMMARY AND PRINCIPLES

The use of quality control material which resembles specimens being assayed provides the laboratory with reliable means of monitoring day to day performance. Detection of random and systematic errors resulting from a variety of sources such as errors in technique, defects in reagents and instrumentation, or inherent bias of a particular methodology can be accomplished by use of a control with a known constituent value. These steps are necessary to assure the reliability of results reported on patient's specimens.

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2. This product is for *in vitro* diagnostic use.

3. **WARNING: This product contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.**

V. STORAGE AND STABILITY

The Whole Blood Alcohol Control is stable until the date indicated if stored as directed.

Store at refrigerated temperatures, 2-8ºC (35-46ºF). This product may be used for two days after the vial is opened provided it is kept tightly stoppered and refrigerated at all times other than when sampling.

VI. PROCEDURE

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2. Analyze in the same manner as patient specimens including all steps of the assay procedure as specified in the directions for use of the kit or instrument being used.

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Product Number 2930-14 6 x 3 mL

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Instrumentation Laboratory Company
Lexington, MA 02173-3190
300314R2

Instrumentation Laboratory

TOXICHEM®
WHOLE BLOOD ALCOHOL CONTROL (HUMAN)

Product Number 2930-14
(Values Apply Only To This Lot)

Lot N0964747 **Pool 002** **Exp 03/98**

METHOD	CONVENTIONAL UNITS		S.I. UNITS	
	ETHANOL CONCENTRATION mg/dL	EXPECTED RANGE	ETHANOL CONCENTRATION mmol/L	EXPECTED RANGE
ENZYMATIC (Alcohol Dehydrogenase)				
Abbott TDX[1]	142	117-167	30.8	25.4-36.3
Abbott AxSYM[1]	150	125-175	32.6	27.1-38.0
Dade ACA[2, 5]	153	128-178	33.2	27.8-38.6
SIGMA[3,6]	148	123-173	32.1	26.7-37.6
GAS CHROMATOGRAPHIC[4]	153	128-178	33.2	27.8-38.6

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Instrumentation Laboratory ToxiChem® Whole Blood Alcohol Control (Human)

I. INTENDED USE

The Instrumentation Laboratory ToxiChem Whole Blood Alcohol Control is for use as a control for procedures involving qualitative and quantitative assay of ethyl alcohol in whole blood.

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Product Number 2930-14 6 x 3 mL

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Instrumentation Laboratory

TOXICHEM®
WHOLE BLOOD ALCOHOL CONTROL (HUMAN)

Product Number 2930-14
(Values Apply Only To This Lot)

Lot N0964747 **Pool 002** **Exp 03/98**

METHOD	CONVENTIONAL UNITS ETHANOL CONCENTRATION mg/dL	CONVENTIONAL UNITS EXPECTED RANGE	S.I. UNITS ETHANOL CONCENTRATION mmol/L	S.I. UNITS EXPECTED RANGE
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6. Concensus values obtained using assays done manually and on automated instruments.

BEHRING

Behring Diagnostics Inc.

P.O. Box 49013
San Jose, CA 95161-9013

3403 Yerba Buena Road
San Jose, CA 95135
Telephone 408-239-2000

March 28, 1997

Stewart Jacobson
Dept. of Law Enforcement
1000 Hubbard
Coeur D'Alene, ID 83814

Dear Mr. Jacobson:

This letter is in response to your request for documentation regarding the ethyl alcohol concentration in the Whole Blood Alcohol Control lot number 6120.

A retained sample was tested and the Gas Chromatography analysis indicated an average alcohol concentration of 0.149 g/dl. The specification are from 0.143 g/dl to 0.177 g/dl alcohol, therefore the retained sample is within specified values.

I hope this information is useful. In the future, please do not hesitate to contact me with technical issues that you may have. I can be reached at the Behring Technical Support Center at (800) 227-8994.

Sincerely,

Rosemarie Dittrich

Rosemarie Dittrich MT(ASCP)
Technical Support Specialist

Instrumentation Laboratory ToxiChem® Whole Blood Alcohol Control (Human)

I. INTENDED USE

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Instrumentation Laboratory

TOXICHEM®
WHOLE BLOOD ALCOHOL CONTROL (HUMAN)

Product Number 2930-14
(Values Apply Only To This Lot)

Lot N0375270 **Pool 007** **Exp 09/98**

METHOD	CONVENTIONAL UNITS		S.I. UNITS	
	ETHANOL CONCENTRATION mg/dL	EXPECTED RANGE	ETHANOL CONCENTRATION mmol/L	EXPECTED RANGE
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Abbott TDX[1]	145	120-170	31.5	26.1-36.9
Abbott AxSYM[1]	155	130-180	33.7	28.2-39.1
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