FORENSIC URINE TOXICOLOGY METHODS

TABLE OF CONTENTS

f. Opiates.

g. Phencyclidine (PCP).

h. Antidepressants.

i. Barbiturates.

5. Criteria for Identification

6. Glossary for Section I.

SECTION II.

1. Gas Chromatograph Injection Port Sleeve Cleaning Procedure and Preparation.

2. Principles of Quantitation Using a GC/MSD System.

3. Amphetamine/Methamphetamine in Urine: Confirmation by GC/MS. (4-CB).

4. 11-nor-9-carboxy-Δ^9-THC (Carboxy-THC) in Urine: Qualitative Confirmation by GC/MS (TMS).

5. 11-nor-9-carboxy-Δ^9-THC (Carboxy-THC) in Urine Using c-THC-D9 as Internal Standard: Quantitative Confirmation by GC/MS (TMS).

6. Cocaine/Benzoylecgonine GC/MS Confirmation in Urine: Summary Procedure.

7. Benzodiazepines in Urine Solid Phase Extraction for GC/MS Confirmation: Summary Method.

8. Benzodiazepines in Urine Liquid-Liquid Extraction for GC/MS Confirmation: Summary Method.

9. Opiates in Urine GC/MS Confirmation: Summary Method.

10. Heroin Metabolite (6-Monoacetyl Morphine) in Urine GC/MS Confirmation: Summary Method.

11. Memo to the Record Regarding c-THC-D9 as Internal Standard.

12. Reagent and Solution Formulations for GC/MS Confirmations.

13. Request for Specimen Reanalysis Protocol

FORENSIC URINE TOXICOLOGY METHODS

TABLE OF CONTENTS

SPECIMEN REQUIREMENTS

URINE: Drug screen: Minimum requirement - 1 ml

Confimation: Minumum requirement - 10 ml

Total amount required for complete analysis - 10 to 15 ml *

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

GI
JW 1/5/95

3H124UL.9

Emit® d.a.u.™ Cocaine Metabolite Assay

▶ New A calibrators. See Section 4, Reagents.
▶ Updated specimen storage information. See Section 6, Specimen Collection and Preparation.
▶ Updated crossreactivity, see Section 11, Performance.

1 Intended Use

The Emit® d.a.u.™ Cocaine Metabolite Assay is a homogeneous enzyme immunoassay intended for use in the qualitative and semiquantitative analysis of benzoylecgonine (cocaine metabolite) in human urine. This assay uses a cutoff level of 150 ng/mL or 300 ng/mL to distinguish positive from negative samples.

The Emit® d.a.u.™ Cocaine Metabolite Assay provides only a preliminary analytical test result. A more specific alternative chemical method (1) must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method (1). Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

2 Summary and Explanation of the Test

Cocaine is a central nervous system stimulant that is extracted from the coca plant. As a drug of abuse, it is self-administered in a variety of ways, including inhalation and intravenous injection. Cocaine base can be smoked in a form that is commonly known as "crack." Cocaine is rapidly absorbed, especially when smoked. While all forms are potentially addicting, "crack" is especially likely to lead to dependence because of its more rapid and heightened effect on the abuser (2).

Excretion rate patterns vary with the mode of administration and from individual to individual. Cocaine is almost completely metabolized, primarily in the liver, with only about one percent excreted in the urine unchanged. Most cocaine is eliminated as benzoylecgonine, the major metabolite of cocaine. Cocaine is also excreted in relatively lesser amounts as ecgonine methyl ester and ecgonine. Cocaine metabolites may be detected in urine for up to approximately two days after cocaine is used. Benzoylecgonine can be detected in urine within four hours after cocaine inhalation and remain detectable in concentrations greater than 1000 ng/mL for as long as 48 hours (3-6).

The Emit® d.a.u.™ Cocaine Metabolite Assay tests for benzoylecgonine, the major metabolite of cocaine, in human urine. Positive results for samples containing other compounds structurally unrelated to benzoylecgonine have not been observed. The cutoff levels for distinguishing positive from negative samples are 150 ng/mL and 300 ng/mL.*

***The cutoff level recommended by NIDA is 300 ng/mL.**

Methods historically used for detecting benzoylecgonine in biological fluids include high-performance liquid chromatography, gas-liquid chromatography, and enzyme immunoassay (7-9).

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result.

3 Principle

The Emit® assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine (10). The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6P-DH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 Reagents

Catalog Number	Product Description	Quantity Volume
3H019	**Emit® d.a.u.™ Cocaine Metabolite Assay**	
	Antibody/Substrate Reagent A Sheep antibodies reactive to benzoylecgonine, glucose-6-phosphate, nicotinamide adenine dinucleotide, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL*
	Enzyme Reagent B Benzoylecgonine labeled with glucose-6-phosphate dehydrogenase, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL*
	Emit® Drug Assay Buffer Concentrate When diluted, contains tris buffer, surfactant, and 0.05% sodium azide	13.3 mL
3H119	**Emit® d.a.u.™ Cocaine Metabolite Assay** (Contents of reagents listed above)	
	Antibody/Substrate Reagent A	four 15.0 mL vials*
	Enzyme Reagent B	four 15.0 mL vials*
	Emit® Drug Assay Buffer Concentrate	two 66.7 mL bottles
	For 300 and 150 ng/mL cutoffs use:	
9A049/9A059	**Emit® Calibrator Level Ø****	5 mL/25 mL*
	For 300 ng/mL cutoff use:	
9A169/9A369	**Emit® Calibrator A Level 1 (cutoff)****	5 mL/25 mL*
9A189/9A389	**Emit® Calibrator A Level 2 (high)****	5 mL/25 mL*
	For 150 ng/mL cutoff use:	
9A279/9A479	**Emit® Calibrator B Level 1 (cutoff)****	5 mL/25 mL*
9A299/9A499	**Emit® Calibrator B Level 2 (high)****	5 mL/25 mL*
	(See Table 1 for drug concentrations)	

***Reagents and calibrators are shipped in dry form. The indicated volume is that required for reconstitution. Number of assays will vary depending on the instrument used.**

****Required for use with the Emit® d.a.u.™ Cocaine Metabolite Assay. Sold separately.**

Note: Reagents 1 and 2 are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

The Emit® Calibrators, when reconstituted, contain the concentrations of benzoylecgonine (cocaine metabolite) listed in Table 1.

Table 1 — Benzoylecgonine Concentrations in Emit® Calibrators

Calibrator	Concentration (ng/mL)
Calibrator Level Ø	0
Calibrator A Level 1 (cutoff)*	300
Calibrator A Level 2 (high)*	3000
Calibrator B Level 1 (cutoff)*	150
Calibrator B Level 2 (high)*	3000

***These calibrators also contain additional drugs of abuse, which do not affect the assay.**

Precautions

- The Emit® d.a.u.™ Cocaine Metabolite Assay is for in vitro diagnostic use.
- Reagent A contains nonsterile sheep antiserum.
- Reagents and buffer contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
- On initial reconstitution, each kit contains no more than 0.0025% mercury, wt/vol, as thimerosal (0.005% thimerosal, wt/vol). Please handle and dispose of appropriately.
- Do not use the kit after the expiration date.

Preparation and Storage of Assay Components

Reagents

To reconstitute Reagents A and B:

- Record the date of reconstitution.
- Remove the metal seal and rubber stopper from the vial.
- Mark the stopper to identify it with the vial.
- Add the amount of distilled or deionized water listed in Table 2.
- Put the stopper back on the vial.
- Swirl the vial until the powder is dissolved.

Let the reconstituted reagents remain at a room temperature of 18-25°C (64-77°F) for at least one hour before use; alternatively, reconstitute the reagents the day before use and store refrigerated (2-8°C, 36-46°F) overnight. Allow reconstituted reagents to reach room temperature before use.

Buffer

To prepare the buffer solution from the buffer concentrate:

- Record the date of buffer preparation.
- Remove the lid and the seal from the buffer concentrate bottle.
- Pour all of the buffer concentrate into a clean, graduated, plastic or glass container.
- Rinse the concentrate bottle several times with distilled or deionized water, pouring the water into the container each time.
- Fill the container to the 200 mL mark with distilled or deionized water.
- Put the cap on the container and invert several times to mix thoroughly.

4 Reagents (cont.)

Table 2 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temperature	Recon Volume (mL)	Minimum Recon Time & Temp	Stability* Unopened	Stability* Prepared/ Opened
Reagent A	2-8°C (36-46°F)	6.0/15.0	1 h 18-25°C (64-77°F)	Exp date	12 wk
Reagent B	2-8°C (36-46°F)	6.0/15.0	1 h 18-25°C (64-77°F)	Exp date	12 wk
Buffer					
Unopened	2-8°C (36-46°F)		None	Exp date	—
Diluted	18-25°C (64-77°F)	200		—	12 wk
Calibrators	2-8°C (36-46°F)	5.0/25.0	1 h 18-25°C (64-77°F)	Exp date	12 wk

Stability depends on handling reagents as directed.

****After reconstitution, always store the calibrators upright.***

Allow all refrigerated components to reach a room temperature of 18-25°C (64-77°F) before use. Do not freeze or expose to temperatures above 32°C (90°F).

5 Instruments

Syva provides instructions for using this assay on the Syva ETS® Plus System (11, 12). Contact the Syva Technical Consultation Center in the USA or your local Syva representative for more information.

6 Specimen Collection and Preparation

- Urine samples may be collected in plastic (i.e., polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics adsorb drugs.
- If not analyzed immediately, samples may be stored unrefrigerated for up to seven days following collection (13).
- Samples should be at a room temperature of 18-25°C (64-77°F) for testing.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine samples within the normal urine pH range (4.5-8) do not require prior adjustment of pH.
- Samples outside the normal pH range should be suspected of adulteration.
- Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample.
- Human urine samples should be handled and disposed of as if they were potentially infectious.

7 Procedure

Materials Provided
Emit® d.a.u.™ Cocaine Metabolite Assay
Reagent A
Reagent B
Drug Assay Buffer Concentrate

Materials Required But Not Provided
For a 300 ng/mL cutoff:
Emit® Calibrator Level Ø
Emit® Calibrator A Level 1 (300 ng/mL cutoff)
Emit® Calibrator A Level 2 (3000 ng/mL high)

For a 150 ng/mL cutoff:
Emit® Calibrator Level Ø
Emit® Calibrator B Level 1 (150 ng/mL cutoff)
Emit® Calibrator B Level 2 (3000 ng/mL high)

Other Items:
Class A volumetric pipette
Distilled or deionized water

7 Procedure (cont.)

Assay Sequence
To run the assay, see the ETS® Plus System Operator's Manual.

Calibration
For calibration at the 300 ng/mL level use:
- Emit® Calibrator Level Ø
- Emit® Calibrator A Level 1 (300 ng/mL cutoff)
- Emit® Calibrator A Level 2 (3000 ng/mL high)

For qualitative analysis of your data use the Emit® Calibrator A Level 1. For semiquantitative analysis, use all three calibrators. Recalibrate if you change reagents or as indicated by control results.

For calibration at the 150 ng/mL level use:
- Emit® Calibrator Level Ø (negative)
- Emit® Calibrator B Level 1 (cutoff)
- Emit® Calibrator B Level 2 (high)

For qualitative analysis of your data use Calibrator B Level 1. For semiquantitative analysis, use all three calibrators. Calibrate at the beginning of each workday. Recalibrate if you change reagents or as indicated by control results.

Refer to the Emit® Calibrator A and Emit® Calibrator B package inserts or the ETS® Plus System Operator's Manual.

Quality Control
Validate the calibration by running positive and negative controls. Ensure that control results fall within acceptable limits as defined by your own laboratory. Once the calibration is validated, run samples.

Daily Maintenance
Refer to the instrument operating manual(s) for maintenance instructions.

8 Results

The Emit® Calibrator A Level 1 (cutoff) is used as a reference for distinguishing "positive" from "negative" results at the 300 ng/mL cutoff level. At the 150 ng/mL level, the Emit® Calibrator B Level 1 (cutoff) is used as the reference.

Positive Results
At the 300 ng/mL cutoff, a sample that gives a change in absorbance (ΔA) value equal to or higher than the Emit® Calibrator A Level 1 (cutoff) ΔA value is interpreted as positive. At the 150 ng/mL cutoff, a sample that gives a ΔA value equal to or higher than the Emit® Calibrator B Level 1 (cutoff) ΔA value is interpreted as positive. The sample contains benzoylecgonine.

Negative Results
At the 300 ng/mL cutoff, a sample that gives a ΔA value lower than the Emit® Calibrator A Level 1 (cutoff) ΔA value is interpreted as negative. At the 150 ng/mL cutoff, a sample that gives a ΔA value lower than the Calibrator B Level 1 (cutoff) ΔA value is interpreted as negative. Either the sample does not contain benzoylecgonine or benzoylecgonine is present in concentrations below the cutoff level for this assay.

Semiquantitative Results
Using the Emit® d.a.u.™ Cocaine Metabolite Assay, semiquantitative determinations of cocaine are possible. Where estimates of relative metabolite concentrations are desired, a standard curve should be prepared by plotting the ΔA values of the Emit® Calibrator Level Ø, Emit® Calibrator A Level 1 (cutoff), and Emit® Calibrator A Level 2 (high) (or Emit® Calibrator Level Ø, Calibrator B Level 1, and Calibrator B Level 2) against the calibrator benzoylecgonine concentrations. The ΔA values of positive samples may then be compared to this standard curve.

Immunoassays that produce a single result in the presence of multiple components cannot fully quantitate the concentration of individual components. Interpretation of results must also take into account that urine concentrations can vary extensively with fluid intake and other biological variables. A more specific alternative chemical method must be used to obtain a confirmed analytical result (see section 1, Intended Use).

9 Limitations

- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of cocaine or its metabolites but does not indicate or measure intoxication.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

10 Expected Values

When the Emit® d.a.u.™ Cocaine Metabolite Assay is used as a qualitative assay, the amount of drugs and metabolites detected by the assay in any given sample cannot be estimated. The assay results distinguish positive from negative samples - positive indicating samples that contain cocaine metabolites.

When used semiquantitatively, the assay yields approximate concentrations of the metabolite detected by the assay (see Section 8, Results).

11 Performance

The data appearing in this section were collected on the Syva ETS® Plus System.

Accuracy
Ninety-nine (99) clinical urine specimens were tested using the Emit® d.a.u.™ Cocaine Metabolite Assay at the 300 ng/mL and 150 ng/mL cutoff levels. Forty-seven (47) samples were negative at both cutoff levels, and 44 samples were positive at both cutoff levels. Eight (8) samples were positive at the 150 ng/mL cutoff and negative at the 300 ng/mL cutoff. These 8 samples were confirmed to contain cocaine metabolite at a level between 150 and 300 ng/mL.

In clinical investigations, within-run precision was determined using the Emit® Calibrator Level Ø, Emit® Calibrator A Levels 1 and 2, and Emit® Calibrator B Levels 1 and 2. Results are in Table 3.

Table 3 — Within-Run Precision

	N	Mean (ΔA)	Standard Deviation (ΔA)	Coefficient of Variation (%)
Calibrator Level Ø (0 ng/mL benzoylecgonine)	20	373	2.46	0.66
Calibrator A Level 1 (300 ng/mL benzoylecgonine)	20	466	5.20	1.12
Calibrator A Level 2 (3000 ng/mL benzoylecgonine)	20	609	4.94	0.81
Calibrator B Level 1 (150 ng/mL benzoylecgonine)	20	427	3.37	1.02
Calibrator B Level 2 (3000 ng/mL benzoylecgonine)	20	610	5.68	0.93

Specificity
The Emit® d.a.u.™ Cocaine Metabolite Assay detects benzoylecgonine, the major metabolite of cocaine, in urine. The concentrations of cocaine and ecgonine which are approximately equivalent to the 150 ng/mL cutoff are 40 and 8 μg/mL, respectively. The concentrations of cocaine and ecgonine which are approximately equivalent to the 300 ng/mL cutoff are 100 and 20 μg/mL, respectively. These levels are much higher than those normally found in the urine of people using cocaine (14).

Table 4 lists concentrations of compounds that were tested and found to give a negative response. Positive results for samples containing other compounds structurally unrelated to benzoylecgonine have not been observed.

Table 4 — Concentrations of Compounds Showing a Negative Response (for both cutoff levels)

Compound	Concentration Tested (μg/mL)
Acetaminophen (Paracetamol)	1000
Acetylsalicylic Acid	1000
Amitriptyline	100
Amphetamine	500
Chlorpromazine	12*
Codeine	500
Dextromethorphan	175
Methadone	500
Methaqualone	100
Monoethylglycinexylidide	1000
Morphine	200
Oxazepam	250
p-Aminobenzoic acid	1000
Phencyclidine	750
Procainamide	1000
Propoxyphene	500
Secobarbital	1000

Chlorpromazine has been tested for cross-reactivity at its solubility limit under assay conditions. No cross-reactivity has been observed.

12 Bibliography

1. Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Wyngarrden JB, Smith LH Jr (eds): Cecil Textbook of Medicine. Philadelphia, WB Saunders Co, 1988, pp 54-55.
3. Pearman K: Cocaine: A review. J. Laryngol Otol 1979;93;1191-1199.
4. Stewart DJ et al: Cocaine metabolism: Cocaine and norcocaine hydrolysis by liver and serum esterases. Clin Pharmacol Ther 1978;25:464-468.
5. Hamilton HE et al: Cocaine and benzoylecgonine excretion in humans. J Forensic Sci Sas 1977;22:697-707.
6. Van Dyke C et al: Urinary excretion of immunologically reactive metabolite(s) after intranasal administration of cocaine as followed by enzyme immunoassay. Clin Chem 1977;23:241-244.
7. Eilenhorn MJ, Barceloux DG: Medical Toxicology. New York, Elsevier Science Publishing Company, Inc, 1988, pp 644-659.
8. Logan MJ et al: Quantitative determination of benzoylecgonine and cocaine in human biofluids by gas-liquid chromatography. Anal Chem 1977;49:1965-1969.
9. Lindgren JE: Guide to the analysis of cocaine and its metabolites in biological material. J Ethnopharmacol 1981;3:337-351.
10. Oellerich M: Enzyme immunoassays in clinical chemistry: Present status and trends. J Clin Chem Clin Biochem 1980;18:197-208.
11. Syva ETS® System Operator's Manual. Palo Alto, CA, Syva Co.
12. Syva ETS® System Operating Guide, Palo Alto, CA, Syva Co.
13. Notice of mandatory guidelines for federal workplace drug testing program; Final Guidelines. *Federal Register* 1988;69 (April 11):11983.
14. Baselt RC, Cravey RH: Disposition of Toxic Drugs and Chemicals in Man, ed 3. Chicago, IL, Year Book Medical Publishers Inc, 1989, pp 208-213.

Syva Australia, North Sydney, N.S.W. 2060, Australia
Syva Diagnostica GmbH, A-1110 Vienna, Austria
Syva Belgium, 1140 Bruxelles, Belgium
Syva Canada, Kanata, Ontario, K2M 1P6, Canada
Syva Diagnostika, 2970 Horsholm, Denmark
Syva France, 69570 Dardilly, France
Syva Diagnostica, GmbH, D-64289 Darmstadt, Germany
BRACCO Industria Chimica S.p.A., 20134, Milano, Italy
Nippon Syntex KK, Tokyo 150, Japan
División Diagnósticos Syntex, 11910 Mexico D.F., Mexico
Syva Diagnostica BV, NDL-2288 Rijswijk, Netherlands
Syva New Zealand, Browns Bay, Auckland, New Zealand
Syva Norge A/S, 2000 Lillestrom, Norway
Syntex Latino, División Diagnósticos, 1495 Lisboa, Portugal
Syntex Latino, División Diagnósticos, 08028 Barcelona, Spain
Syva Scandinavia, S-127 23 Skärholmen, Sweden
Syva Diagnostica, CH-8800 Thalwil, Switzerland
Syva U.K., Maidenhead, Berkshire SL6 1RD, United Kingdom
Syva Company, San Jose, CA 95135 USA

For additional assistance call a Syva technical consultant toll-free:
In the USA, (800) 227-8994.
In Canada, (800) 267-6205.

Notice: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in this labeling can affect performance characteristics and stated or implied labeling claims.

The price of reagents includes a royalty for a license under U.S. Patent Nos. 3,975,237 and 3,917,582 for use of this product only.

Syva Company
3403 Yerba Buena Road
P.O. Box 49013
San Jose, CA 95161-9013

Syva a Syntex company

June 1994
Printed in USA
3H124UL.9

3M584UL.6

FZA
01/20/95 SW

Emit® d.a.u.™ Cannabinoid 50 ng Assay

1 Intended Use

The Emit® d.a.u.™ Cannabinoid 50 ng Assay is a homogeneous enzyme immunoassay intended for use in the qualitative and semiquantitative analysis of cannabinoids in human urine. This assay uses a cutoff level of 50 ng/mL to distinguish positive from negative samples.

The Emit® d.a.u.™ Cannabinoid 50 ng Assay provides only a preliminary analytical test result. A more specific alternative chemical method (1) must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method (1). Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

2 Summary and Explanation of the Test

Marijuana is a mixture of dried leaves and flowering tops of the plant *Cannabis sativa L.* The agents that produce the hallucinogenic and other biological effects of marijuana are called cannabinoids.

The cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) is the principal psychoactive ingredient in marijuana and hashish. The compound Δ^9-THC is quickly and effectively absorbed by inhalation or from the gastrointestinal tract (2), and is almost completely metabolized by liver enzymes (3). Peak plasma levels of Δ^9-THC occur within 10 minutes of inhalation, and approximately one hour after ingestion (2). Excretion of urinary metabolites and excretion by way of the feces begins within 72 hours after exposure (2, 3). Concentrations depend on the total amount of THC absorbed, frequency of abuse, rate of release from fatty tissue, and time of sample collection with respect to use. In chronic users, THC may accumulate in fatty tissues faster than it can be eliminated. This accumulation leads to longer detection times in urinalysis for chronic users than for occasional users (4).

The Emit® d.a.u.™ Cannabinoid 50 ng Assay detects the major metabolite of Δ^9-THC, 11-nor-Δ^9-THC-9-carboxylic acid, in human urine. It also detects other Δ^9-THC metabolites. The cutoff level for distinguishing positive from negative samples is 50 ng/mL.* Positive results for samples containing other compounds structurally unrelated to cannabinoids have not been observed.

This assay is not for testing under National Institute on Drug Abuse (NIDA) guidelines. The cutoff level recommended by NIDA is 100 ng/mL.

Methods historically used for detecting cannabinoids in biological fluids include radioimmunoassay, gas chromatography/mass spectrometry, gas chromatography, and enzyme immunoassay (2, 3).

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result (1).

3 Principle

The Emit® assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine (5). The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6P-DH does not interfere because the coenzyme functions only with the bacterial (*Leuconostoc mesenteroides*) enzyme employed in the assay.

4 Reagents

Catalog Number	Product Description	Quan Vo
3M519	**Emit® d.a.u.™ Cannabinoid 50 ng Assay**	Approximately 300
	Antibody/Substrate Reagent A Mouse monoclonal antibodies reactive to Δ^9-THC, glucose-6-phosphate, nicotinamide adenine dinucleotide, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0
	Enzyme Reagent B Δ^9-THC labeled with glucose-6-phosphate dehydrogenase, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0
	Emit® Drug Assay Buffer Concentrate When diluted, contains tris buffer, surfactant, and 0.05% sodium azide	13.
9A049	**Emit® Calibrator Level Ø (negative)****	5.0
9M509	**Emit® Δ^9 Cannabinoid 50 ng/mL Calibrator****	5.0
9M109	**Emit® Δ^9 Cannabinoid 100 ng/mL Calibrator****	5.0
9A059	**Emit® Calibrator Level Ø (negative)**** (See Table 1 for drug concentrations)	25.0

****Reagents and calibrators are shipped in dry form. The indicated volume is that required for reconstitution.***

*****Required for use with the Emit® d.a.u.™ Cannabinoid 50 ng Assay. Sold separately.***

Note: Reagents A and B are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

The Emit® Δ^9 Cannabinoid calibrators, when reconstituted, contain the concentrations of 11-Δ^9-THC-9-COOH listed in Table 1.

Table 1 — 11-nor-Δ^9-THC-9-COOH Concentrations in Emit® Δ^9 Cannabinoid Calibrator

Calibrator	Concentration (ng/mL)
Emit® Calibrator Level Ø (negative)	0
Emit® Δ^9 Cannabinoid 50 ng/mL Calibrator	50
Emit® Δ^9 Cannabinoid 100 ng/mL Calibrator	100

Precautions

- The Emit® d.a.u.™ Cannabinoid 50 ng Assay is for in vitro diagnostic use.
- Reagent A contains nonsterile mouse monoclonal antibodies.
- Reagents and buffer contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it w a large volume of water to prevent azide buildup.
- On initial reconstitution, each kit contains no more than 0.0025% mercury, wt/vol, as thime sal (0.005% thimerosal, wt/vol). Please handle and dispose of appropriately.
- Do not use the kit after the expiration date.

Preparation and Storage of Assay Components

Reagents

To reconstitute Reagents A and B:

- Record the date of reconstitution.
- Remove the metal seal and rubber stopper from the vial.
- Mark the stopper to identify it with the vial.
- Add 6 mL distilled or deionized water.
- Put the stopper back on the vial.
- Swirl the vial until the powder is dissolved.

Let the reconstituted reagents remain at a room temperature of 18-25°C (64-77°F) for at leas one hour before use; alternatively, reconstitute the reagents the day before use and store refr erated (2-8°C, 36-46°F) overnight. Allow reconstituted reagents to reach room temperature before use.

Buffer

To prepare the buffer solution from the buffer concentrate:

- Record the date of buffer preparation.
- Remove the lid and the seal from the buffer concentrate bottle.
- Pour all of the buffer concentrate into a clean, graduated, plastic or glass container.
- Rinse the concentrate bottle several times with distilled or deionized water, pouring the wat into the container each time.
- Fill the container to the 200 mL mark with distilled or deionized water.
- Put the cap on the container and invert several times to mix thoroughly.

4 Reagents (cont.)

Table 2 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temp	Recon Volume (mL)	Minimum Recon Time & Temp	Stability* Unopened	Stability* Prepared/ Opened
Reagent A	2-8°C (36-46°F)	6.0	1 h 18-25°C (64-77°F)	Exp date	9 wk
Reagent B	2-8°C (36-46°F)	6.0	1 h 18-25°C (64-77°F)	Exp date	9 wk
Buffer					
Unopened	2-8°C (36-46°F)		None	Exp date	—
Diluted	18-25°C (64-77°F)	200		—	12 wk
Calibrators**	2-8°C (36-46°F)	5.0 or 25.0 (neg cal)	1 h 18-25°C (64-77°F)	exp date	12 wk

Stability depends on handling reagents as directed.

****After reconstitution, always store the calibrators upright.***

Allow all refrigerated components to reach a room temperature of 18-25°C (64-77°F) before use. Do not freeze or expose to temperatures above 32°C (90°F).

5 Instruments

Syva provides instructions for using this assay on the Syva ETS® Plus system (6,7). Contact the Syva Technical Consultation Center in the USA or your local Syva representative for more information.

6 Specimen Collection and Preparation

- Urine samples may be collected in plastic (i.e., polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics adsorb drugs.
- If not analyzed immediately, samples may be stored unrefrigerated for up to seven days following collection.
- Samples should be at a room temperature of 18-25°C (64-77°F) for testing.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine samples within the normal urine pH range (4.5-8) do not require prior adjustment of pH.
- Samples outside the normal pH range should be suspected of adulteration.
- Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample.
- Human urine samples should be handled and disposed of as if they were potentially infectious.

7 Procedure

Materials Provided
Emit® d.a.u.™ Cannabinoid 50 ng Assay
- Reagent A
- Reagent B
- Drug Assay Buffer Concentrate

Materials Required But Not Provided
Emit® Calibrator Level Ø (negative)
Emit® Δ⁹ Cannabinoid 50 ng/mL Calibrator
Emit® Δ⁹ Cannabinoid 100 ng/mL Calibrator

Other Items:
Class A volumetric pipette
Distilled or deionized water

Assay Sequence
To run the assay, see the ETS® Plus System Operator's Manual.

Calibration
The following three calibrator levels available from Syva are for use with the Emit® d.a.u.™ Cannabinoid 50 ng Assay: the Emit® Calibrator Level Ø (negative), Emit® Δ⁹ Cannabinoid 50 ng/mL Calibrator (cutoff), and Emit® Δ⁹ Cannabinoid 100 ng/mL Calibrator (high). To analyze your data, use the Emit® Δ⁹ Cannabinoid 50 ng/mL Calibrator for qualitative analysis and all three calibrators for semiquantitative analysis. Calibrate at the beginning of each workday. Recalibrate if you change reagents or as indicated by control results.

Refer to the Emit® Δ⁹ Cannabinoid Calibrator package insert or the ETS® Plus System Operator's Manual.

7 Procedure (cont.)

Quality Control
Validate the daily calibration by assaying positive and negative controls. If results fall within acceptable limits as defined by your laboratory and minimum separations are maintained, ru samples. (See base label for minimum separations for each reagent lot.)

Daily Maintenance
Refer to the system shutdown and maintenance procedures in the ETS® Plus System Operatc Manual

8 Results

The Emit® Δ⁹ Cannabinoid 50 ng/mL Calibrator is used as a reference for distinguishing "positive" from "negative" samples.

Positive Results
A sample that gives a change in absorbance (ΔA) value equal to or higher than the ΔA value the Emit® Δ⁹ Cannabinoid 50 ng/mL Calibrator (cutoff) is interpreted as positive. The sample contains cannabinoids.

Negative Results
A sample that gives a change in absorbance (ΔA) value lower than the ΔA value of the Emit® Δ⁹ Cannabinoid 50 ng/mL Calibrator (cutoff) is interpreted as negative. Either the sample doe not contain cannabinoids or cannabinoids are present in concentrations below the cutoff leve for this assay.

Semiquantitative Results
Using the Emit® d.a.u.™ Cannabinoid 50 ng Assay, semiquantitative determinations of cannabinoid concentrations are possible. Where estimates of relative cannabinoid concentrations are desired, a standard curve should be prepared by plotting the ΔA values of the Emit® Calibrat Level Ø and the Emit® Δ⁹ 50 ng/mL and 100 ng/mL calibrators against their respective 11-no Δ⁹-THC-9-carboxylic acid concentrations. The ΔA values of positive samples may then be compared to this standard curve.

Immunoassays that produce a single result in the presence of multiple components cannot fully quantitate the concentration of individual components. Interpretation of results must also take into account that urine concentrations can vary extensively with fluid intake and other biologica variables. A more specific alternative chemical method must be used to obtain a confirmed analytical result (see section 1, Intended Use).

9 Limitations

- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of cannabinoids but does not indicate or measure intoxication.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

10 Expected Values

When the Emit® d.a.u.™ Cannabinoid 50 ng Assay is used as a qualitative assay, the amount o drugs and metabolites detected by the assay in any given sample cannot be estimated. The assay results distinguish positive from negative samples — positive indicating samples that contain cannabinoids.

When used semiquantitatively, the assay yields approximate cumulative concentrations of the drug detected by the assay (see section 8, Results).

11 Performance

The data appearing in this section were collected on the Syva ETS® System.

Accuracy
One hundred one (101) clinical urine specimens were tested by the Emit® d.a.u.™ Cannabinoid 50 ng Assay. These same samples were tested by GC/MS, using a reference level of 15 ng/mL 11-nor-Δ⁹-THC-9-carboxylic acid to distinguish positive from negative results. Fifty-two (52) samples tested positive by both methods and 49 tested negative by both methods.

In clinical investigations, within-run precision was determined using Emit® calibrators containing 0 ng/mL, 50 ng/mL, and 100 ng/mL 11-nor-Δ⁹-THC-9-carboxylic acid. Results are shown in Table 3.

11 Performance (cont.)

Table 3 — Within-Run Precision

	N	Mean (ΔA)	Standard Deviation (ΔA)	Coefficient of Variation (%)
Negative Calibrator (0 ng/mL 11-nor-Δ⁹-THC-9-carboxylic acid	24	569	5.9	1.0
Δ⁹ 50 ng/mL Calibrator (50 ng/mL 11-nor-Δ⁹-THC-9-carboxylic acid)	24	622	6.4	1.0
Δ⁹ 100 ng/mL Calibrator (100 ng/mL 11-nor-Δ⁹-THC-9-carboxylic acid)	24	743	6.0	0.81

Specificity

The Emit® d.a.u.™ Cannabinoid 50 ng Assay detects the major metabolites of Δ⁹-THC in urine. Table 4 gives the compounds this assay is designed to detect and the levels at which the compounds have been found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Δ⁹ Cannabinoid 50 ng/mL Calibrator). Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. If a sample contains more than one compound detected by the assay, lower concentrations than those listed below may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 4 — Concentration of Compounds that Produce a Result Approximately Equivalent to the Emit® Δ⁹ Cannabinoid 50 ng/mL Calibrator

Compound	Concentration (ng/mL)
11-nor-Δ⁹-THC-9-carboxylic acid	50
8-ß-11-Dihydroxy-Δ⁹-THC	50
8-ß-Hydroxy-Δ⁹-THC	65
11-Hydroxy-Δ⁸-THC	80
11-Hydroxy-Δ⁹-THC	90

Table 5 lists compounds that were tested and found to give a negative response. Positive results for samples containing other compounds structurally unrelated to cannabinoids have not been observed.

Table 5 — Compounds That Produce a Negative Response

Compound	Concentration Tested (µg/mL)
Acetylsalicylic acid	1000
Amitriptyline	1000
Amphetamine	100
Benzoylecgonine	400
Chlorpromazine	12
Meperidine	1000
Methaqualone	500
Morphine	200
Oxazepam	300
Phencyclidine	1000
Promethazine	125
Propoxyphene	100
Secobarbital	1000

12 Bibliography

1. Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Baselt RC: Disposition of Toxic Drugs and Chemicals in Man, ed 3. Chicago, IL, Year Book Medical Publishers Inc., 1990, pp 780-783.
3. Eilenhorn MJ, Barceloux DG: Medical Toxicology. New York, Elsevier Science Publishing Company, Inc, 1988, pp 675-682.
4. Wyngarrden JB, LH Jr (eds): Cecil Textbook of Medicine. Philadelphia, WB Saunders Co, 1986, pp 52-58.
5. Henry JB: Clinical Diagnosis and Management by Laboratory Methods. Philadelphia, WB Saunders Co. 1991, pp 866.
6. Syva ETS® Plus System Operator's Manual, Palo Alto, CA, Syva Co.
7. Syva ETS® Plus System Guide, Palo Alto, CA, Syva Co.

Syva Australia, North Sydney, N.S.W. 2060, Australia
Syva Belgium, Bruxelles, Belgium
Syva Canada, Kanata, Ontario K2K 2A9, Canada
Syva Diagnostika, 2970 Hørsholm, Denmark
Syva U.K., Maidenhead, Berkshire SL6 1RD, England
Syva France, 69570 Dardilly, France
BRACCO Industria Chimica S.p.A., 20134 Milano, Italy
Nippon Syntex KK, Tokyo, Japan
División Diagnósticos Syntex, 11910 Mexico D.F., Mexico
Syva New Zealand, Linden, Wellington, New Zealand
Syva Norge A/S, 2000 Lillestrøm, Norway
Syntex Latino, División Diagnósticos, 08028 Barcelona, Spain
Syva Scandinavia, S-127 23 Skärholmen, Sweden
Syva Diagnostica, D-6100 Darmstadt, West Germany

For additional assistance, call Syva toll-free:
1-800-227-8994 in USA
1-800-267-6205 in Canada

Notice: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in this labeling can affect performance characteristics and stated or implied labeling claims.

Syva Company
3403 Yerba Buena Road
P.O. Box 49013
San Jose, CA 95161-9013

Syva a Syntex company

July 1993
Printed in USA
3M584UL.6

FZ
01/20/95

3G124UL.5

Emit® d.a.u.™ Propoxyphene Assay

1 Intended Use

The Emit® d.a.u.™ Propoxyphene Assay is a homogeneous enzyme immunoassay intended for use in the qualitative and semiquantitative analysis of propoxyphene in human urine. This assay uses a cutoff level of 300 ng/mL to distinguish positive from negative samples.

The Emit® d.a.u.™ Propoxyphene Assay provides only a preliminary analytical test result. A more specific alternative chemical method (1) must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method (1). Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

2 Summary and Explanation of the Test

Propoxyphene and propoxyphene napsylate are used therapeutically as mildly effective narcotic analgesics. They may be toxic and even fatal at levels which exceed the recommended therapeutic dosages, particularly because they are metabolized quickly.

The Emit® d.a.u.™ Propoxyphene Assay uses a cutoff of 300 ng/mL propoxyphene. The assay detects propoxyphene and propoxyphene salts, such as propoxyphene napsylate, in human urine (see Table 4). It also detects norpropoxyphene (N-desmethyldextropropoxyphene), the major urinary metabolite of propoxyphene. Positive results for samples containing other compounds structurally unrelated to propoxyphene have not been observed.

Methods historically used for detecting propoxyphene in biological fluids include ultraviolet spectrophotometry, gas chromatography, and enzyme immunoassay (2).

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result (1).

3 Principle

The Emit® assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine (3). The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6P-DH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 Reagents

Catalog Number	Product Description	Quantity Volum
3G029	**Emit® d.a.u.™ Propoxyphene Assay**	Approximately 300 test
	Antibody/Substrate Reagent A Sheep antibodies reactive to propoxyphene, glucose-6-phosphate, nicotinamide adenine dinucleotide, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL
	Enzyme Reagent B Propoxyphene labeled with glucose-6-phosphate dehydrogenase, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL
	Emit® Drug Assay Buffer Concentrate When diluted, contains tris buffer, surfactant, and 0.05% sodium azide	13.3 m
9A049	**Emit® Calibrator Level Ø (negative)****	5.0 mL
9A279	**Emit® Calibrator B Level 1 (cutoff)****	5.0 mL
9A299	**Emit® Calibrator B Level 2 (high)****	5.0 mL
9A059	**Emit® Calibrator Level Ø (negative)****	25.0 mL
9A479	**Emit® Calibrator B Level 1 (cutoff)****	25.0 mL
9A499	**Emit® Calibrator B Level 2 (high)**** (See Table 1 for drug concentrations)	25.0 mL

****Reagents and calibrators are shipped in dry form. The indicated volume is that required for reconstitution.***

*****Required for use with the Emit® d.a.u.™ Propoxyphene Assay. Sold separately.***

Note: Reagents A and B are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

The Emit® calibrators, when reconstituted, contain the concentrations of propoxyphene listed in Table 1.

Table 1 — Propoxyphene Concentrations in Emit® Calibrators

Calibrator	Concentration (ng/mL)
Emit® Calibrator Level Ø (negative)	0
Emit® Calibrator B Level 1 (cutoff)*	300
Emit® Calibrator B Level 2 (high)*	1000

****These calibrators also contain additional drugs of abuse, which do not affect the assay.***

Precautions

- The Emit® d.a.u.™ Propoxyphene Assay is for in vitro diagnostic use.
- Reagent A contains nonsterile sheep antiserum.
- Reagents and buffer contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
- On initial reconstitution, each kit contains no more than 0.0025% mercury, wt/vol, as thimerosa (0.005% thimerosal, wt/vol). Please handle and dispose of appropriately.
- Do not use the kit after the expiration date.

Preparation and Storage of Assay Components

Reagents

To reconstitute Reagents A and B:

- Record the date of reconstitution.
- Remove the metal seal and rubber stopper from the vial.
- Mark the stopper to identify it with the vial.
- Add 6 mL of distilled or deionized water.
- Put the stopper back on the vial.
- Swirl the vial until the powder is dissolved.

Let the reconstituted reagents remain at a room temperature of 18-25°C (64-77°F) for at least one hour before use; alternatively, reconstitute the reagents the day before use and store refrigerated (2-8°C, 36-46°F) overnight. Allow reconstituted reagents to reach room temperature before use.

Buffer

To prepare the buffer solution from the buffer concentrate:

- Record the date of buffer preparation.
- Remove the lid and the seal from the buffer concentrate bottle.
- Pour all of the buffer concentrate into a clean, graduated, plastic or glass container.
- Rinse the concentrate bottle several times with distilled or deionized water, pouring the water into the container each time.
- Fill the container to the 200 mL mark with distilled or deionized water.
- Put the cap on the container and invert several times to mix thoroughly.

4 Reagents (cont.)

Table 2 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temp	Recon Volume (mL)	Minimum Recon Time & Temp	Stability* Unopened	Stability* Prepared/ Opened
Reagent A	2-8°C (36-46°F)	6.0	1 h 18-25°C (64-77°F)	Exp date	12 wk
Reagent B	2-8°C (36-46°F)	6.0	1 h 18-25°C (64-77°F)	Exp date	12 wk
Buffer					
Unopened	2-8°C (36-46°F)		None	Exp date	—
Diluted	18-25°C (64-77°F)	200		—	12 wk
Calibrators **	2-8°C (36-46°F)	5.0/25.0	1 h 18-25°C (64-77°F)	Exp date	12 wk

Stability depends on handling reagents as directed.

****After reconstitution, always store the calibrators upright.***

Allow all refrigerated components to reach a room temperature of 18-25°C (64-77°F) before use. Do not freeze or expose to temperatures above 32°C (90°F).

5 Instruments

Syva provides instructions for using this assay on the Syva ETS® Plus System (4,5). Contact the Syva Technical Consultation Center in the USA or your local Syva representative for more information.

6 Specimen Collection and Preparation

- Urine samples may be collected in plastic (ie polypropolene, polycarbonate, polyethylene) or glass containers. Some plastics adsorb drugs.
- If not analyzed immediately, samples may be stored unrefrigerated for up to 7 days following collection.
- Samples should be at a room temperature of 18-25°C (64-77°F) for testing.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine samples within the normal urine pH range (4.5-8) do not require prior adjustment of pH.
- Samples outside the normal pH range should be suspected of adulteration.
- Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample.
- Human urine samples should be handled and disposed of as if they were potentially infectious.

7 Procedure

Materials Provided
Emit® d.a.u.™ Propoxyphene Assay
- Reagent A
- Reagent B
- Drug Assay Buffer Concentrate

Materials Required But Not Provided
Emit® Calibrator Level Ø (negative)
Emit® Calibrator B Level 1 (cutoff)
Emit® Calibrator B Level 2 (high)

Other Items:
Class A volumetric pipette
Distilled or deionized water

Assay Sequence
To run the assay, see the ETS® Plus System Operator's Manual.

Calibration
Three calibrator levels are available from Syva: the Emit® Calibrator Level Ø (negative), Emit® Calibrator B Level 1 (cutoff), and Emit® Calibrator B Level 2 (high). To analyze your data, use the Emit® Calibrator B Level 1 for qualitative analysis and all three calibrators for semiquantitative analysis. Calibrate at the beginning of each workday. Recalibrate if you change reagents or as indicated by control results.

Refer to the Emit® Calibrator B package insert or the ETS® Plus System Operator's Manual.

7 Procedure (cont.)

Quality Control
Validate the daily calibration by assaying positive and negative controls. If results fall within acceptable limits as defined by your laboratory and minimum separations are maintained, run samples. (See base label for minimum separations for each reagent lot.)

Daily Maintenance
Refer to the system shutdown and maintenance procedures in the ETS® Plus System Operator's Manual.

8 Results

The Emit® Calibrator B Level 1, which contains a concentration of 300 ng/mL propoxyphene, is used as a reference for distinguishing "positive" from "negative" samples.

Positive Results
A sample that gives a change in absorbance (ΔA) value equal to or higher than the Calibrator B Level 1 ΔA value is interpreted as positive. The sample contains propoxyphene and/or 10 μg/mL or more of the major urinary metabolite, norpropoxyphene.

Negative Results
A sample that gives a change in absorbance (ΔA) value lower than the Calibrator B Level 1 ΔA value is interpreted as negative: Either the sample does not contain propoxyphene or norpropoxyphene or they are present in concentrations below the cutoff level for this assay.

Semiquantitative Results
Using the Emit® d.a.u.™ Propoxyphene Assay, semiquantitative determinations of propoxyphene are possible. Where estimates of relative total drug concentrations are desired, a standard curve should be prepared by plotting the ΔA values of the Emit® Calibrator Level Ø, Calibrator B Level 1, and Calibrator B Level 2 against the calibrator propoxyphene concentrations. The ΔA values of positive samples may then be compared to this standard curve.

Immunoassays that produce a single result in the presence of multiple components cannot fully quantitate the concentration of individual components. Interpretation of results must also take into account that urine concentrations can vary extensively with fluid intake and other biological variables. A more specific alternative chemical method must be used to obtain a confirmed analytical result (see section 1, Intended Use).

9 Limitations

- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of propoxyphene but does not indicate or measure intoxication.
- Methadone can interfere with the assay at a level of greater than 50 μg/mL. Urinary concentrations of methadone and its metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), from 1-50 μg/mL are commonly encountered in methadone maintenance patients (2).
- Imipramine can interfere with the assay concentrations greater than 25 μg/mL. Imipramine levels as high as 54 μg/mL have been reported in fatalities (2).
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

10 Expected Values

When the Emit® d.a.u.™ Propoxyphene Assay is used as a qualitative assay, the amount of drugs and metabolites detected by the assay in any given sample cannot be estimated. The assay results distinguish positive from negative samples — positive indicating samples that contain propoxyphene or norpropoxyphene.

When used semiquantitatively, the assay yields approximate, cumulative concentrations of the drug and metabolite detected by the assay (see section 8, Results).

11 Performance

Data appearing in this section were collected on the Syva® AutoLab Instrument System and on the Syva ETS® analyzer.

Accuracy
One hundred seventy-two (172) clinical urine specimens were tested using the Emit® d.a.u.™ Propoxyphene Assay. Eighty-four (84) were positive by the Emit® d.a.u.™ assay and 88 were negative by the Emit® d.a.u.™ assay. One sample was positive by the Emit® d.a.u.™ assay, but negative by thin layer chromatography (TLC). This sample was confirmed positive for norpropoxyphene by gas-liquid chromatography (GLC). Five samples were negative by the Emit® d.a.u.™ assay, but positive by TLC. On additional analysis by GLC, these samples were all confirmed to contain norpropoxyphene at concentrations below the Emit® d.a.u.™ assay cutoff level.

Propoxyphene-free urine samples and urine samples containing 500 ng/mL propoxyphene were tested with the Emit® d.a.u.™ Propoxyphene Assay. The assay correctly identified more than 99% of these samples.

In clinical investigations, within-run precision was determined using Emit® calibrators containing 0 ng/mL, 300 ng/mL, and 1000 ng/mL propoxyphene. Results are shown in Table 3.

GIA
01/20/95

3D222UL.3E

Emit® d.a.u.™ Barbiturate Assay

Updated Information:
▶ Dual cutoff capability, note changes throughout.

1 Intended Use

The Emit® d.a.u.™ Barbiturate Assay is a homogeneous enzyme immunoassay intended for use in the qualitative and semiquantitative analysis of barbiturates in human urine.

The Emit® d.a.u.™ Barbiturate Assay provides only a preliminary analytical test result. A more specific alternative chemical method (1) must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method (1). Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

2 Summary and Explanation of the Test

Barbiturates, a class of central nervous system depressants, are usually taken orally but are sometimes injected intravenously or intramuscularly. They are absorbed rapidly; 30-40% of the compound is bound to plasma protein, and the rest is distributed to muscle, to fat, and to the liver (where it is ultimately inactivated) (2). Barbiturates are classified based on their duration of action, ranging from very short acting (approximately 15 minutes) to long acting (a day or more). Some of the most commonly abused barbiturates are the short-acting ones, including pentobarbital and secobarbital. An example of a long-acting barbiturate is phenobarbital. The ratio of unchanged drug to metabolites varies depending upon duration of action. Short-acting barbiturates will generally be excreted in urine as metabolites, while the long-acting barbiturates will primarily appear unchanged (3,4).

▶ The Emit® d.a.u.™ Barbiturate Assay tests for both long- and short-acting barbiturates in human urine. Positive results for samples containing other compounds structurally unrelated to barbiturates usually have not been observed. The cutoff levels for distinguishing positive from negative samples are 200 ng/mL or 300 ng/mL.

Methods historically used for detecting barbiturates in biological fluids include thin-layer chromatography, gas chromatography, ultraviolet spectrophotometry, enzyme immunoassay, and radioimmunoassay (5).

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result (1).

3 Principle

The Emit® assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine (6). The assay is based on competition for antibody binding sites between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH). Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6P-DH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 Reagents

Catalog Number	Product Description	Quantity/ Volume
3D229UL	**Emit® d.a.u.™ Barbiturate Assay**	
	Antibody/Substrate Reagent A Sheep antibodies reactive to secobarbital, glucose-6-phosphate, nicotinamide adenine dinucleotide, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL
	Enzyme Reagent B Secobarbital labeled with glucose-6-phosphate-dehydrogenase, bulking agents, stabilizers, tris buffer, and 0.05% sodium azide	6.0 mL
	Emit® Drug Assay Buffer Concentrate When diluted, contains tris buffer, surfactant, and 0.05% sodium azide	13.3 m
	▶For 200 ng/mL cutoff use:	
9A049/9A059	**Emit® Calibrator Level Ø****	5 mL/25 mL
9A169/9A369	**Emit® Calibrator A Level 1 (cutoff)****	5 mL/25 mL
9A189/9A389	**Emit® Calibrator A Level 2 (high)****	5 mL/25 mL
	▶For 300 ng/mL cutoff use:	
9A049/9A059	**Emit® Calibrator Level Ø****	5 mL/25 mL
9A279/9A479	**Emit® Calibrator B Level 1 (cutoff)****	5 mL/25 mL
9A299/9A499	**Emit® Calibrator B Level 2 (high)**** (See Table 1 for drug concentrations)	5 mL/25 mL

***Reagents and calibrators are shipped in dry form. The indicated volume is that required for reconstitution. Number of assays will vary depending on the instrument used.**

****Required for use with the Emit® d.a.u.™ Barbiturate Assay. Sold separately.**

Note: Reagents A and B are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

The Emit® calibrators, when reconstituted, contain the concentrations of secobarbital listed in Table 1.

▶ **Table 1 — Secobarbital Concentrations in Emit® Calibrators**

Calibrator	Concentration (ng/mL)
Emit® Calibrator Level Ø	0
Emit® Calibrator A Level 1 (cutoff)*	200
Emit® Calibrator A Level 2 (high)*	1000
Emit® Calibrator B Level 1 (cutoff)*	300
Emit® Calibrator B Level 2 (high)*	1000

***These calibrators also contain additional drugs of abuse, which do not affect the assay.**

Precautions
- The Emit® d.a.u.™ Barbiturate Assay is for in vitro diagnostic use.
- Reagent A contains nonsterile sheep antiserum.
- Reagents and buffer contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
- On initial reconstitution, each kit contains no more than 0.0025% mercury, wt/vol, as thimerosal (0.005% thimerosal, wt/vol). Please handle and dispose of appropriately.
- Do not use the kit after the expiration date.

Preparation and Storage of Assay Components
Reagents
To reconstitute Reagents A and B:
- Record the date of reconstitution.
- Remove the metal seal and rubber stopper from the vial.
- Mark the stopper to identify it with the vial.
- Add distilled or deionized water to equal 6 mL.
- Put the stopper back on the vial.
- Swirl the vial until the powder is dissolved.

Let the reconstituted reagents remain at a room temperature of 18-25°C (64-77°F) for at least one hour before use; alternatively, reconstitute the reagents the day before use and store refrigerated (2-8°C, 36-46°F) overnight. Allow reconstituted reagents to reach room temperature before use.

Buffer
To prepare the buffer solution from the buffer concentrate:
- Record the date of buffer preparation.
- Remove the lid and the seal from the buffer concentrate bottle.
- Pour all of the buffer concentrate into a clean, graduated, plastic or glass container.
- Rinse the concentrate bottle several times with distilled or deionized water, pouring the water into the container each time.
- Fill the container to the 200 mL mark with distilled or deionized water.
- Put the cap on the container and invert several times to mix thoroughly.

11 Performance (cont.)

Table 3 — Within-Run Precision

	N	Mean (ΔA)	Standard Deviation (ΔA)	Coefficient of Variation (%)
Negative Calibrator (0 ng/mL Propoxyphene)	24	225	3.5	1.5
Cutoff Calibrator (300 ng/mL Propoxyphene)	24	277	4.6	1.7
High Calibrator (1000 ng/mL Propoxyphene)	24	419	5.6	1.3

Specificity

The Emit® d.a.u.™ Propoxyphene Assay detects propoxyphene and the major urinary metabolite, norpropoxyphene, in urine. Imipramine can interfere with the assay at concentrations greater than 25 μg/mL.

Table 4 lists the compounds this assay is designed to detect and the levels at which the compounds have been found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Calibrator B Level 1). These concentrations are within the range of levels found in urine following use of propoxyphene. Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. If a sample contains more than one compound detected by the assay, lower concentrations than those listed below may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 4 — Concentration of Compounds That Produce a Result Approximately Equivalent to Calibrator B Level 1

Compound	Concentration (ng/mL)
Propoxyphene	300
Norpropoxyphene	4500

Table 5 lists concentrations that are not detected by the Emit® d.a.u.™ Propoxyphene Assay. The concentrations tested are all substantially above levels found in urine.

Table 5 — Concentrations of Compounds Showing a Negative Response

Compound	Concentration Tested (μg/mL)
Amphetamine	1000
Benzoylecgonine	1000
Caffeine	1000
Chlorpromazine	12
Codeine	500
Dextromethorphan	100
Methadone	50
Methaqualone	1000
Morphine	1000
Oxazepam	300
Phencyclidine	1000
Secobarbital	1000

12 Bibliography

1. Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Baselt RC: Disposition of Toxic Drugs and Chemicals in Man, ed 2. Davis, CA, Biomedical Publications, 1982, pp 723-726, 512-515, 424.
3. Henry JB: Clinical Diagnosis and Management by Laboratory Methods. Philadelphia, WB Saunders Co. 1991, pp 866.
4. Syva ETS® Plus System Operator's Manual, Palo Alto, CA, Syva Co.
5. Syva ETS® Plus System Guide, Palo Alto, CA, Syva Co.

Syva Australia, North Sydney, N.S.W. 2060, Australia
Syva Diagnostica GmbH, A-1110 Vienna, Austria
Syva Belgium, 1140 Bruxelles, Belgium
Syva Canada, Kanata, Ontario K2M 1P6, Canada
Syva Diagnostika, 2970 Hørsholm, Denmark
Syva France, 69570 Dardilly, France
Syva Diagnostica GmbH, D-64289 Darmstadt, Germany
BRACCO Industria Chimica S.p.A. 20134, Milano, Italy
Nippon Syntex KK, Tokyo 150, Japan
División Diagnósticos Syntex, 11910 Mexico D.F., Mexico
Syva Diagnostica BV, NDL-2288 Rijswijk, Netherlands
Syva New Zealand, Browns Bay, Auckland, New Zealand
Syva Norge A/S, 2000 Lillestrøm, Norway
Syntex Latino, División Diagnósticos, 1495 Lisboa, Portugal
Syntex Latino, División Diagnósticos, 08028 Barcelona, Spain
Syva Scandinavia, S-127 23 Skärholmen, Sweden
Syva Diagnostica, CH-8800 Thalwil, Switzerland
Syva U.K., Maidenhead, Berkshire SL6 1RD, United Kingdom
Syva Company, San Jose, CA 95135 USA

For additional assistance, call Syva toll-free:
1-800-227-8994 in USA
1-800-267-6205 in Canada

Notice: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in this labeling can affect performance characteristics and stated or implied labeling claims.

Syva Company
3403 Yerba Buena Road
P.O. Box 49013
San Jose, CA 95161-9013

Syva a Syntex company

September 1
Printed in U
3G124U

4 Reagents (cont.)

Table 2 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temp	Recon Volume	Minimum Recon Time & Temp	Stability* Unopened	Stability* Prepared/Opened
Reagent A	2-8°C (36-46°F)	6.0 mL	1 h 18-25°C (64-77°F)	Exp date	12 wk
Reagent B	2-8°C (36-46°F)	6.0 mL	1 h 18-25°C (64-77°F)	Exp date	12 wk
Buffer					
Unopened	2-8°C (36-46°F)		None	Exp date	—
Diluted	18-25°C (64-77°F)	200 mL			12 wk
Calibrators**	2-8°C (36-46°F)	5.0/25.0 mL	1 h 18-25°C (64-77°F)	Exp date	12 wk

Stability depends on handling reagents as directed.

****After reconstitution, always store the calibrators upright.***

Allow all refrigerated components to reach a room temperature of 18-25°C (64-77°F) before use. Do not freeze or expose to temperatures above 32°C (90°F).

5 Instruments

Syva provides instructions for using this assay on the Syva ETS® Plus system (7,8). Contact the Syva Technical Consultation Center in the USA or your local Syva representative for more information.

6 Specimen Collection and Preparation

- Urine samples may be collected in plastic (i.e., polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics adsorb drugs.
- If not analyzed immediately, samples may be stored unrefrigerated for up to seven days following collection.
- Samples should be at a room temperature of 18-25°C (64-77°F) for testing.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine samples within the normal urine pH range (4.5-8) do not require prior adjustment of pH.
- Samples outside the normal pH range should be suspected of adulteration.
- Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample.
- Human urine samples should be handled and disposed of as if they were potentially infectious.

7 Procedure

Materials Provided
Emit® d.a.u.™ Barbiturate Assay
- Reagent A
- Reagent B
- Drug Assay Buffer Concentrate

Materials Required But Not Provided
For 200 ng/mL cutoff:
- Emit® Calibrator Level Ø
- Emit® Calibrator A Level 1 (cutoff)
- Emit® Calibrator A Level 2 (high)

▶ For 300 ng/mL cutoff:
- Emit® Calibrator Level Ø
- Emit® Calibrator B Level 1 (cutoff)
- Emit® Calibrator B Level 2 (high)

Other Items:
Class A volumetric pipette
Distilled or deionized water

7 Procedure (cont.)

Assay Sequence
To run the assay, see the ETS® Plus System Operator's Manual.

Calibration
For calibration at the 200 ng/mL level use:
- Emit® Calibrator Level Ø
- Emit® Calibrator A Level 1 (cutoff)
- Emit® Calibrator A Level 2 (high)

▶ For calibration at the 300 ng/mL level use:
- Emit® Calibrator Level Ø
- Emit® Calibrator B Level 1 (cutoff)
- Emit® Calibrator B Level 2 (high)

To analyze your data, use the Emit® Calibrator A Level 1 (or Emit® Calibrator B Level 1) for qualitative analysis and all three calibrators for semiquantitative analysis. Recalibrate if you change reagents or as indicated by control results. Refer to the Emit® Calibrator A (or Emit® Calibrator B) package insert or the ETS® Plus System Operator's Manual.

Quality Control
Validate the daily calibration by assaying positive and negative controls. If results fall within acceptable limits as defined by your laboratory and minimum separations are maintained, run samples. (See base label for minimum separations for each reagent lot.)

Daily Maintenance
Refer to the instrument operating manual for maintenance instructions.

8 Results

▶ The Emit® Calibrator A Level 1 (cutoff) and Emit® Calibrator B Level 1 (cutoff), contain 200 and 300 ng/mL secobarbital, respectively. The cutoff calibrators are used as a reference for distinguishing "positive" from "negative" samples.

Positive Results
A sample that gives a change in absorbance (ΔA) value equal to or higher than the calibrator A Level 1 (or calibrator B Level 1) ΔA value is interpreted as positive. The sample contains barbiturates.

Negative Results
A sample that gives a (ΔA) value lower than the Calibrator A Level 1 (or Calibrator B Level 1) ΔA value is interpreted as negative. Either the sample does not contain barbiturates or they are present in concentrations below the cutoff level for this assay.

Semiquantitative Results
Using the Emit® d.a.u.™ Barbiturate Assay, semiquantitative determinations of barbiturates or barbiturate metabolites are possible. Where estimates of relative total drug concentrations are desired, a standard curve should be prepared by plotting the ΔA values of the Emit® Calibrator Level Ø, Calibrator A Level 1, and Calibrator A Level 2 (or Emit® Calibrator Level Ø, Emit® Calibrator B Level 1, and Calibrator B Level 2) against the calibrator secobarbital concentrations. The ΔA values of positive samples may then be compared to this standard curve.

Immunoassays that produce a single result in the presence of multiple detectable components cannot fully quantitate the concentration of individual components. Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables. A more specific alternative chemical method must be used to obtain a confirmed analytical result (see Section 1, Intended Use).

9 Limitations

- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of barbiturates but does not indicate or measure intoxication.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

10 Expected Values

When the Emit® d.a.u.™ Barbiturate Assay is used as a qualitative assay, the amount of barbiturates detected by the assay in any given sample cannot be estimated. The assay results distinguish positive from negative samples — positive indicating samples that contain barbiturate

When used semiquantitatively, the assay yields approximate cumulative concentrations of th drug detected by the assay (see Section 8, Results).

11 Performance

The data appearing in this section were collected on the Syva ETS® Plus system.

▶ **Accuracy**

A total of 120 urine samples were analyzed by the Emit® d.a.u.™ Barbiturate Assay. The samples were all tested on the ETS® Plus system using both the 200 ng/mL and 300 ng/mL cutoffs. All discrepant results were confirmed by GC/MS.

Sixty-four (64) samples were positive at both cutoffs. Fifty-five (55) samples were negative at both cutoffs. One sample was positive at the 200 ng/mL cutoff and negative at the 300 ng/mL cutoff. The sample was confirmed to contain barbiturates.* The differing result was due to the difference in cutoffs.

The GC/MS method tested for butalbital, butabarbital, amobarbital, pentobarbital, secobarbital, and phenobarbital.

▶ Precision was determined by assaying 4 samples on 5 consecutive days, 1 run per day in replicates of 5. Precision data were calculated in a manner consistent with National Committee of Clinical Laboratory Standards (NCCLS) Guidelines EP-5T2 (March 1992). Results are summarized in Table 3.

▶ **Table 3 — Precision**

	N	Mean (ΔA)	Standard Deviation (ΔA)	Coefficient of Variation (%)
Within-Run Precision				
Negative Calibrator (0 ng/mL secobarbital)	25	206	1.67	0.81
Calibrator A Level 1 (200 ng/mL secobarbital)	25	285	2.42	0.85
Calibrator B Level 1 (300 ng/mL secobarbital)	25	331	3.59	1.08
Calibrator A Level 2 (1000 ng/mL secobarbital)	25	508	6.08	1.20
Total Precision				
Negative Calibrator (0 ng/mL secobarbital)	25	207	1.68	0.81
Calibrator A Level 1 (200 ng/mL secobarbital)	25	285	2.22	0.78
Calibrator B Level 1 (300 ng/mL secobarbital)	25	331	2.89	0.87
Calibrator A Level 2 (1000 ng/mL secobarbital)	25	510	6.53	1.28

Specificity

The Emit® d.a.u.™ Barbiturate Assay is designed to detect and the levels at which the compounds have been found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Calibrator A Level 1, or Emit® Calibrator B Level 1). These compound or metabolite concentrations are within the range of levels found in urine following use of the compound. Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. If a sample contains more than one compound detected by the assay, lower concentrations than those listed below may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 4 lists the compounds this assay detects both long- and short-acting barbiturates in urine.

▶ **Table 4 — Concentrations of Barbiturate Compounds That Produce a Result Approximately Equivalent to Calibrator A Level 1 (200 ng/mL cutoff) and Calibrator B Level 1 (300 ng/mL cutoff).**

Compound	Concentration at 200 ng/mL	Concentration at 300 ng/mL
Alphenal	300	500
Amobarbital	300	700
Aprobarbital	180	200
Barbital	1000	3500
Butabarbital	300	500
Butalbital	150	400
Cyclopentobarbital	200	200
5-Ethyl-5-(4-hydroxyphenyl) barbituric acid	600	3000
Pentobarbital	300	400
Phenobarbital	700	2500
Secobarbital	200	300
Talbutal	200	300
Thiopental	10000	45000

11 Performance (cont.)

Table 5 lists compounds that are not detected by the Emit® d.a.u.™ Barbiturate Assay. The concentrations tested are all substantially above levels found in urine.

▶ **Table 5 — Compounds That Produce a Negative Response**

Compound	Concentration Tested at 200 ng/mL cutoff (μg/mL)	Concentration Tested at 300 ng/mL cutoff (μg/mL)
Aminoglutethimide	25	25
Amphetamine	1000	1000
Barbituric Acid	100	100
Benzoylecgonine	1000	1000
Chlorpromazine	12	12
Fenoprofen	1000	1000
Glutethimide	25	25
Hexobarbital	1000	1000
5-(Hydroxyphenyl)-5-phenylhydantoin	500	500
5-(Hydroxyphenyl)-5-phenylhydantoin glucuronide	500	500
Ibuprofen	1000	1000
Iminostilbene	100	100
Methadone	1000	1000
Metharbital	1000	1000
Morphine	1000	1000
Naproxen	1000	1000
Oxazepam	250	250
Phencyclidine	1000	1000
Phenytoin	1000	1000
Primidone	500	500
Propoxyphene	1000	1000

12 Bibliography

1. Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Hofmann FE: A Handbook on Drug and Alcohol Abuse: The Biomedical Aspects. New York, Oxford University Press, 1983.
3. Eilenhorn MJ, Barceloux DG: Medical Toxicology. New York, Elsevier Science Publishing Company, Inc. 1988, pp 575-580.
4. Wyngaarden JB, Smith LH Jr (eds): Cecil Textbook of Medicine. Philadelphia, WB Saunders Co, 1988, pp 53-54.
5. Goldetzky CW: Detection of drugs of abuse in biological fluids, In Martin WR (ed): Drug Addiction I. New York, Springer-Verlag, 1977, pp 319-409.
6. Henry JB: Clinical Diagnosis and Management by Laboratory Methods. Philadelphia, WB Saunders Co. 1991, pp 866.
7. Syva ETS® System Operator's Manual, Palo Alto, CA, Syva Co.
8. Syva ETS® System Operating Guide, Palo Alto, CA, Syva Co.

Syva Australia, North Sydney, N.S.W. 2060, Australia
Syva Belgium, Bruxelles, Belgium
Syva Canada, Kanata, Ontario K2K 2A9, Canada
Syva Diagnostika, 2970 Hørsholm, Denmark
Syva U.K., Maidenhead, Berkshire SL6 1RD, England
Syva France, 69570 Dardilly, France
BRACCO Industria Chimica S.p.A., 20134 Milano, Italy
Nippon Syntex KK, Tokyo, Japan
División Diagnósticos Syntex, 11910 Mexico D.F., Mexico
Syva New Zealand, Linden, Wellington, New Zealand
Syva Norge A/S, 2000 Lillestrøm, Norway
Syntex Latino, División Diagnósticos, 08028 Barcelona, Spain
Syva Scandinavia, S-127 23 Skärholmen, Sweden
Syva Diagnostica, D-6100 Darmstadt, West Germany

For additional assistance, call Syva toll-free:
1-800-227-8994 in USA
1-800-267-6205 in Canada

Notice: Adulteration of reagents, use of instruments without appropriate capabilities, o other failure to follow instructions as set forth in this labeling can affect performance characteristics and stated or implied labeling claims.

Syva Company
3403 Yerba Buena Road
P.O. Box 49013
San Jose, CA 95161-9013

May
Printed in
3D222(

Syva a Syntex company

G1
01/20/95

3J222UL.2E

Emit® d.a.u.™ Phencyclidine Assay

1 Intended Use

The Emit® d.a.u.™ Phencyclidine Assay is a homogeneous enzyme immunoassay intended for use in the qualitative and semiquantitative analysis of phencyclidine in human urine. This assay uses a cutoff level of 25 ng/mL to distinguish positive from negative samples.

The Emit® d.a.u.™ Phencyclidine Assay provides only a preliminary analytical test result. A more specific alternative chemical method (1) must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method (1). Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

2 Summary and Explanation of the Test

Phencyclidine, also known as PCP and "angel dust," is a synthetic drug that was originally developed for its anesthetic properties but is now a drug of abuse used solely for its potent hallucinogenic effects. It may be self-administered in a variety of ways, including ingestion, inhalation, and intravenous injection. Phencyclidine is absorbed well and quickly and concentrates in the brain and fatty tissues (2). Excretion patterns vary widely, ranging from several hours to a couple of weeks. Phencyclidine is excreted in the urine unchanged, as conjugated metabolites, and primarily as unidentified compounds.

The Emit® d.a.u.™ Phencyclidine Assay, an enzyme immunoassay technique, tests for phencyclidine in human urine. It also detects the analog 1-[1-(2-thienyl)-cyclohexyl]-piperidine (TCP). High concentrations of several phencyclidine metabolites and analogs can also produce positive results in the assay. Positive results for samples containing other compounds structurally unrelated to phencyclidine usually have not been observed. The cutoff level for distinguishing positive from negative samples is 25 ng/mL.

Emit® homogeneous immunoassays are widely used in therapeutic drug monitoring and drugs-of-abuse testing. The assays are nonradioactive and do not require extraction procedures. Designed for use in screening, the Emit® d.a.u.™ assays give qualitative results that are equivalent to other drug detection methods.

Methods historically used for detecting phencyclidine in biological fluids include thin-layer chromatography (3), gas chromatography (4), ultraviolet spectroscopy, enzyme immunoassay, and radioimmunoassay (5,6).

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result (1).

3 Principle

The Emit® assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine (6). The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6P-DH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 Reagents

Catalog Number	Product Description	Quantity/ Volume
3J229UL	**Emit® d.a.u.™ Phencyclidine Assay**	
	Antibody/Substrate Reagent A Sheep antibodies reactive to phencyclidine, glucose-6-phosphate, nicotinamide adenine dinucleotide, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL*
	Enzyme Reagent B Phencyclidine labeled with glucose-6-phosphate dehydrogenase, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL*
	Emit® Drug Assay Buffer Concentrate When diluted, tris buffer, surfactant, and 0.05% sodium azide	13.3 mL
9A049UL	**Emit® Calibrator Level Ø****	5 mL*
9A169UL	**Emit® Calibrator A Level 1 (cutoff)****	5 mL*
9A189UL	**Emit® Calibrator A Level 2 (high)****	5 mL*
9A059UL	**Emit® Calibrator Level Ø****	25 mL*
9A369UL	**Emit® Calibrator A Level 1 (cutoff)****	25 mL*
9A389UL	**Emit® Calibrator A Level 2 (high)****	25 mL*
	(See Table 1 for drug concentrations)	

Reagents and calibrators are shipped in dry form. The indicated volume is that required for reconstitution. Number of assays will vary depending on the instrument used.

****Required for use with the Emit® d.a.u.™ Phencyclidine Assay. Sold separately.***

Note: Reagents 1 and 2 are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

The Emit® calibrators, when reconstituted, contain the concentrations of phencyclidine listed in Table 1.

Table 1 — Phencyclidine Concentrations in Emit® Calibrators

Calibrator	Concentration (ng/mL)
Emit® Calibrator Level Ø	0
Emit® Calibrator A Level 1 (cutoff)*	25
Emit® Calibrator A Level 2 (high)*	75

***These calibrators also contain additional drugs of abuse, which do not affect the assay.**

Precautions

- The Emit® d.a.u.™ Phencyclidine Assay is for in vitro diagnostic use.
- Reagent A contains nonsterile sheep antiserum.
- Reagents and buffer contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
- On initial reconstitution, each kit contains no more than 0.0025% mercury, wt/vol, as thimerosal (0.005% thimerosal, wt/vol). Please handle and dispose of appropriately.
- Do not use the kit after the expiration date.

Preparation and Storage of Assay Components

Reagents

To reconstitute Reagents A and B:

- Record the date of reconstitution.
- Remove the metal seal and rubber stopper from the vial.
- Mark the stopper to identify it with the vial.
- Add the amount of distilled or deionized water listed in Table 2.
- Put the stopper back on the vial.
- Swirl the vial until the powder is dissolved.

Let the reconstituted reagents remain at a room temperature of 18-25°C (64-77°F) for at least one hour before use; alternatively, reconstitute the reagents the day before use and store refrigerated (2-8°C, 36-46°F) overnight. Allow reconstituted reagents to reach room temperature before use.

Buffer

To prepare the buffer solution from the buffer concentrate:

- Record the date of buffer preparation.
- Remove the lid and the seal from the buffer concentrate bottle.
- Pour all of the buffer concentrate into a clean, graduated plastic or glass container.
- Rinse the concentrate bottle several times with distilled or deionized water, pouring the water into the container each time.
- Fill the container to the 200 mL mark with distilled or deionized water.
- Put the cap on the container and invert several times to mix thoroughly.

4 Reagents (cont.)

Table 2 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temp	Recon Volume (mL)	Minimum Recon Time & Temp	Stability* Unopened	Stability* Prepared/Opened
Reagent A	2-8°C (36-46°F)	6.0	1 h 18-25°C (64-77°F)	Exp date	12 wk
Reagent B	2-8°C (36-46°F)	6.0	1 h 18-25°C (64-77°F)	Exp date	12 wk
Buffer					
Unopened	2-8°C (36-46°F)		None	Exp date	—
Diluted	18-25°C (64-77°F)	200		—	12 wk
Calibrators**	2-8°C (36-46°F)	5/25	1 h 18-25°C (64-77°F)	Exp date	12 wk

Stability depends on handling reagents as directed.

****After reconstitution, always store the calibrators upright.***

Allow all refrigerated components to reach a room temperature of 18-25°C (64-77°F) before use. Do not freeze or expose to temperatures above 32°C (90°F).

5 Instruments

Syva provides instructions for using this assay on the Syva ETS® Plus system (7,8). Contact the Syva Technical Consultation Center in the USA or your local Syva representative for more information.

6 Specimen Collection and Preparation

- Urine samples may be collected in plastic (i.e., polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics adsorb drugs.
- If not analyzed immediately, samples may be stored unrefrigerated for up to seven days following collection (9).
- Samples should be at a room temperature of 18-25°C (64-77°F) for testing.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine samples within the normal urine pH range (4.5-8) do not require prior adjustment of pH.
- Samples outside the normal pH range should be suspected of adulteration.
- Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample.
- Human urine samples should be handled and disposed of as if they were potentially infectious.

7 Procedure

Materials Provided
Emit® d.a.u.™ Phencyclidine Assay
Reagent A
Reagent B
Drug Assay Buffer Concentrate

Materials Required But Not Provided
Emit® Calibrator Level Ø
Emit® Calibrator A Level 1 (cutoff)
Emit® Calibrator A Level 2 (high)

Other Items:
Class A volumetric pipette
Distilled or deionized water

7 Procedure (cont.)

Assay Sequence
To run the assay, see the ETS® Plus System Operator's Manual.

Calibration
Three calibrator levels are available from Syva: the Emit® Calibrator Level Ø, Calibrator A Level 1 (cutoff) and Calibrator A Level 2 (high). To analyze your data, use the Emit® Calibrator A Level 1 for qualitative analysis and all three calibrators for semiquantitative analysis. Calibrate at the beginning of each workday. Recalibrate if you change reagents or as indicated by control results.

Refer to the Emit® Calibrator A package insert or the ETS® Plus System Operator's Manual.

Quality Control
Validate the daily calibration by assaying positive and negative controls. If results fall within acceptable limits as defined by your laboratory and minimum separations are maintained, run samples. (See base label for minimum separations for each reagent lot.)

Daily Maintenance
Refer to the instrument operating manual for maintenance instructions.

8 Results

The Emit® Calibrator A Level 1 (cutoff), which contains a concentration of 25 ng/mL phencyclidine, is used as a reference for distinguishing "positive" from "negative" samples.

Positive Results
A sample that gives a change in absorbance (ΔA) value equal to or higher than the Calibrator A Level 1 ΔA value is interpreted as positive. The sample contains phencyclidine or phencyclidine metabolites or analogs.

Negative Results
A sample that gives a change in absorbance (ΔA) value lower than the Calibrator A Level 1 ΔA value is interpreted as negative. Either the sample does not contain phencyclidine or phencyclidine metabolites or analogs or they are present in concentrations below the cutoff level for this assay.

Semiquantitative Results
Using the Emit® d.a.u.™ Phencyclidine Assay, semiquantitative determinations of phencyclidine or phencyclidine metabolites or analogs are possible. Where estimates of relative total drug concentrations are desired, a standard curve should be prepared by plotting the ΔA values of the Emit® Calibrator Level Ø, Calibrator A Level 1, and Calibrator A Level 2 against the calibrator phencyclidine concentrations. The ΔA values of positive samples may then be compared to this standard curve.

Immunoassays that produce a single result in the presence of multiple components cannot fully quantitate the concentration of individual components. Interpretation of results must also take into account that urine concentrations can vary extensively with fluid intake and other biological variables. A more specific alternative chemical method must be used to obtain a confirmed analytical result (see section 1, Intended Use).

9 Limitations

- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of phencyclidine but does not indicate or measure intoxication.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

10 Expected Values

When the Emit® d.a.u.™ Phencyclidine Assay is used as a qualitative assay, the amount of phencyclidine or phencyclidine metabolites or analogs detected by the assay in any given sample cannot be estimated. The assay results distinguish positive from negative samples — positive indicating samples that contain phencyclidine or phencyclidine metabolites or analogs.

When used semiquantitatively, the assay yields approximate concentrations of the metabolite detected by the assay (see section 8, Results).

Revised September 1993

3B124UL.9

G1
01/20/95

Emit® d.a.u.™ Opiate Assay

Updated Information:
▶ Cross-reactivity information. See Section 11.

1 Intended Use

The Emit® d.a.u.™ Opiate Assay is a homogeneous enzyme immunoassay intended for use in the qualitative and semiquantitative analysis of opiates in human urine. This assay uses a cutoff level of 300 ng/mL to distinguish positive from negative samples.

The Emit® d.a.u.™ Opiate Assay provides only a preliminary analytical test result. A more specific alternative chemical method (1) must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method (1). Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

2 Summary and Explanation of the Test

Opiates are a class of compounds that includes morphine, codeine, and heroin. Morphine and codeine are naturally occurring alkaloids that are found in opium, a substance exuded from the unripe seedpod of the opium poppy *Papaver somniferum*. Heroin is a semisynthetic derivative of morphine (2,3).

Morphine is a potent analgesic. Codeine is used in analgesic preparations and as a cough suppressant. Heroin is an even more potent analgesic than morphine. Both morphine and codeine are legitimate drugs. Heroin is a drug of abuse that may be snorted, smoked, or dissolved and injected subcutaneously or intravenously.

Opiates are absorbed rapidly. Heroin is converted almost immediately to morphine, which is excreted in urine both unchanged and as a glucuronidated metabolite. Excretion takes place over a period of a couple of days. Codeine is excreted in urine as a glucuronidated conjugate, as free and conjugated norcodeine, and as morphine. The presence of opiates in the urine indicates the use of heroin, morphine, and/or codeine.

The Emit® d.a.u.™ Opiate Assay tests for morphine, morphine glucuronide, and codeine in human urine and gives a positive result if any of these opiates are present. It also detects synthetic opiates related to morphine, such as hydromorphone, and high concentrations of the analgesic meperidine and the narcotic antagonist nalorphine (see Table 4). Positive results for samples containing other compounds structurally unrelated to opiates have not been observed.

Methods historically used for detecting opiates in biological fluids include thin-layer chromatography, gas chromatography, high-performance liquid chromatography, fluorometry, microcrystallography, enzyme immunoassay, and radioimmunoassay (4).

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result (1).

3 Principle

The Emit® assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine (5). The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6P-DH does not interfere because the coenzyme functions only with the bacterial (*Leuconostoc mesenteroides*) enzyme employed in the assay.

4 Reagents

Catalog Number	Product Description	Quantity/ Volume
3B019	**Emit® d.a.u.™ Opiate Assay**	Approximately 300 tests
	Antibody/Substrate Reagent A Sheep antibodies reactive to morphine, glucose-6-phosphate, nicotinamide adenine dinucleotide, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL*
	Enzyme Reagent B Morphine labeled with glucose-6-phosphate dehydrogenase, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL*
	Emit® Drug Assay Buffer Concentrate When diluted, contains tris buffer, surfactant, and 0.05% sodium azide	13.3 mL
9A049	**Emit® Calibrator Level Ø (negative)****	5.0 mL*
9A169	**Emit® Calibrator A Level 1 (cutoff)****	5.0 mL*
9A189	**Emit® Calibrator A Level 2 (high)****	5.0 mL*
9A059	**Emit® Calibrator Level Ø (negative)****	25.0 mL*
9A369	**Emit® Calibrator A Level 1 (cutoff)****	25.0 mL*
9A389	**Emit® Calibrator A Level 2 (high)****	25.0 mL*
	(See Table 1 for drug concentrations)	

Reagents and calibrators are shipped in dry form. The indicated volume is that required for reconstitution.

****Required for use with the Emit® d.a.u.™ Opiate Assay. Sold separately.***

Note: Reagents A and B are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

The Emit® calibrators, when reconstituted, contain the concentrations of morphine (opiate) listed in Table 1.

Table 1 — Morphine Concentrations in Emit® Calibrators

Calibrator	Concentration (ng/mL)
Emit® Calibrator Level Ø (negative)	0
Emit® Calibrator A Level 1 (cutoff)*	300
Emit® Calibrator A Level 2 (high)*	1000

****These calibrators also contain additional drugs of abuse, which do not affect the assay.***

Precautions
- The Emit® d.a.u.™ Opiate Assay is for in vitro diagnostic use.
- Reagent A contains nonsterile sheep antiserum.
- Reagents and buffer contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
- On initial reconstitution, each kit contains no more than 0.0025% mercury, wt/vol, as thimerosal (0.005% thimerosal, wt/vol). Please handle and dispose of appropriately.
- Do not use the kit after the expiration date.

Preparation and Storage of Assay Components

Reagents
To reconstitute Reagents A and B:
- Record the date of reconstitution.
- Remove the metal seal and rubber stopper from the vial.
- Mark the stopper to identify it with the vial.
- Add 6 mL of distilled or deionized water listed in Table 2.
- Put the stopper back on the vial.
- Swirl the vial until the powder is dissolved.

Let the reconstituted reagents remain at a room temperature of 18-25°C (64-77°F) for at least one hour before use; alternatively, reconstitute the reagents the day before use and store refrigerated (2-8°C, 36-46°F) overnight. Allow reconstituted reagents to reach room temperature before use.

Buffer
To prepare the buffer solution from the buffer concentrate:
- Record the date of buffer preparation.
- Remove the lid and the seal from the buffer concentrate bottle.
- Pour all of the buffer concentrate into a clean, graduated, plastic or glass container.
- Rinse the concentrate bottle several times with distilled or deionized water, pouring the water into the container each time.
- Fill the container to the 200 mL mark with distilled or deionized water.
- Put the cap on the container and invert several times to mix thoroughly.

11 Performance

The data appearing in this section were collected on the Syva ETS® Plus System.

Accuracy

One hundred (100) urine samples were analyzed by both the Emit® d.a.u.™ Phencyclidine Assay (25 ng/mL) and the Emit® d.a.u.™ Phencyclidine Assay (75 ng/mL) on the ETS® Plus analyzer. Thirty-nine (39) were positive and 46 were negative by both methods. All 39 positive samples were confirmed by GC/MS to contain phencyclidine. Fifteen (15) samples were positive by the Emit® d.a.u.™ Phencyclidine Assay (25 ng/mL) and negative by the Emit® d.a.u.™ Phencyclidine Assay (75 ng/mL). The 15 discrepant samples were confirmed by GC/MS to contain phencyclidine. The samples that were negative by the Emit® d.a.u.™ Phencyclidine Assay (75 ng/mL) contained less than the assay cutoff amount of 75 ng/mL phencyclidine.

In clinical investigations, within-run precision was determined using the Emit® Calibrator Level Ø and Calibrator A Levels 1 and 2. Results are in Table 3.

Table 3 — Within-Run Precision

	N	Mean (ΔA)	Standard Deviation (ΔA)	Coefficient of Variation (%)
Calibrator Level Ø (0 ng/mL phencyclidine)	24	382	5.3	1.39
Calibrator A Level 1 (25 ng/mL phencyclidine)	24	480	5.2	1.07
Calibrator A Level 2 (75 ng/mL phencyclidine)	24	528	6.1	1.16

Specificity

The Emit® d.a.u.™ Phencyclidine Assay detects phencyclidine, phencyclidine metabolites, or analogs in human urine.

Table 4 lists the compounds this assay is designed to detect and the levels at which the compounds have been found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Calibrator A Level 1). These concentrations are within the range of levels found in urine following use of the compound or, in the case of metabolites, the parent compound. Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. If a sample contains more than one compound detected by the assay, lower concentrations than those listed below may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 4 — Approximate Concentrations of Phencyclidine and Its Metabolites and Analogs That Produce a Result Approximately Equivalent to the Cutoff Calibrator

Compound	Concentration ng/mL
Phencyclidine	25
N,N-Diethyl-1-phenylcyclohexylamine (PCDE)	350
1-(4-Hydroxypiperidino)phenylcyclohexane	250
1-(1-Phenylcyclohexyl)morpholine (PCM)	90
1-(1-Phenylcyclohexyl)pyrrolidine (PCPy)	60
4-Phenyl-4-piperidinocyclohexanol	35
1-[1-(2-Thienyl)-cyclohexyl]morpholine (TCM)	170
1-[1-(2-Thienyl)-cyclohexyl]piperidine (TCP)	30
1-[1-(2-Thienyl)-cyclohexyl]pyrrolidine (TCPy)	75

Table 5 lists additional compounds that have produced a positive result at levels not typically found in urine (10,11). The concentration found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Calibrator A Level 1) is given for each compound. Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. If a sample contains more than one compound detected by the assay, lower concentrations than those listed below may combine to produce a rate equivalent to or greater than that of the cutoff calibrator.

Table 5 — Concentration of Additional Compounds Producing Results Approximately Equivalent to the Cutoff Calibrator

Compound	Concentration (μg/mL)	Compound	Concentration (μg/mL)
Dextromethorphan	110	Mesoridazine	10
Dextrorphan	470	Promethazine	135
Imipramine	170	Thioridazine	15
Levallorphan	5	Tripelennamine	300
Meperidine	70		

Table 6 lists compounds that are not detected by the Emit® d.a.u.™ Phencyclidine Assay. The concentrations tested are all substantially above levels found in urine.

11 Performance (cont.)

Table 6 — Compounds That Produce a Negative Response

Compound	Concentration Tested (μg/mL)	Compound	Concentration Tested (μg/mL)
1-Phenylcyclohexylamine (PCA)	50	Ibuprofen	1000
1-Piperidinocyclohexane carbonitrile (PCC)	50	Ketamine	100
Acetaminophen	1000	Ketoprofen	1000
Albuterol	1000	Methadone	100
Benzoylecgonine	500	Methaqualone	500
Buspirone	900	Morphine	200
Chlorpromazine	12	Orphenadrine	300
Cimetidine	1000	Oxazepam	100
d-Amphetamine	1000	Phenytoin	30
Diphenhydramine	500	Propoxyphene	1000
Fenoprofen	1000	Salicylamide	65
Haloperidol	1000	Secobarbital	1000
Hydroxyzine	1000	Sodium Salicylate	100

12 Bibliography

1. Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Aniline O, Pitts FN: Phencyclidine (PCP): A review and perspectives. CRC Crit Rev Toxicol 1982;10:145-177.
3. Finkle HI: Phencyclidine identification by thin-layer chromatography. A rapid screening procedure for emergency toxicology. Am J Clin Pathol 1978;70:287-290.
4. Froelich PE, Gross G: Separation and detection of phencyclidine in urine by gas chromatography. J Chromatogr 1977;137:135-143.
5. Guptas RC et al: Determination of phencyclidine in urine and illicit street drug samples. Clin Toxicol 1975;8:611-621.
6. Henry JB: Clinical Diagnosis and Management by Laboratory Methods. Philadelphia, WB Saunders Co. 1991 p 866.
7. Syva ETS® System Operator's Manual. Palo Alto, CA, Syva Co.
8. Syva ETS® System Operating Guide. Palo Alto, CA, Syva Co.
9. Notice of mandatory guidelines for federal workplace drug testing program; Final Guidelines. *Federal Register* 1988;69 (April 11):11983.
10. Baselt RC, Cravey RH: Disposition of Toxic Drugs and Chemicals in Man 3rd edition. Chicago, Year Book Medical Publishers, Inc. 1989.
11. Park Y et al: Quantitative determination of dextromethorphan and three metabolites in urine by reverse-phase high-performance liquid chromatography. J Pharm Sci 1984;73:24-29.

Syva Australia, North Sydney, N.S.W. 2060, Australia
Syva Belgium, Bruxelles, Belgium
Syva Canada, Kanata, Ontario K2K 2A9, Canada
Syva Diagnostika, 2970 Hørsholm, Denmark
Syva U.K., Maidenhead, Berkshire SL6 1RD, England
Syva France, 69570 Dardilly, France
BRACCO Industria Chimica S.p.A., 20134 Milano, Italy
Nippon Syntex KK, Tokyo, Japan
División Diagnósticos Syntex, 11910 Mexico D.F., Mexico
Syva New Zealand, Linden, Wellington, New Zealand
Syva Norge A/S, 2000 Lillestrøm, Norway
Syntex Latino, División Diagnósticos, 08028 Barcelona, Spain
Syva Scandinavia, S-127 23 Skärholmen, Sweden
Syva Diagnostica, D-6100 Darmstadt, West Germany

For additional assistance, call Syva toll-free:
1-800-227-8994 in USA
1-800-267-6205 in Canada

Notice: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in this labeling can affect performance characteristics and stated or implied labeling claims.

Syva Company
3403 Yerba Buena Road
P.O. Box 49013
San Jose, CA 95161-9013

November 1992
Printed in USA
3J222UL.2E

Syva a Syntex company

4 Reagents (cont.)

Table 2 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temp	Recon Volume (mL)	Minimum Recon Time & Temp	Stability* Unopened	Stability* Prepared/ Opened
Reagent A	2-8°C (36-46°F)	6.0	1 h 18-25°C (64-77°F)	Exp date	12 wk
Reagent B	2-8°C (36-46°F)	6.0	1 h 18-25°C (64-77°F)	Exp date	12 wk
Buffer					
Unopened	2-8°C (36-46°F)		None	Exp date	—
Diluted	18-25°C (64-77°F)	200		—	12 wk
Calibrators **	2-8°C (36-46°F)	5.0/25.0	1 h 18-25°C (64-77°F)	Exp date	12 wk

Stability depends on handling reagents as directed.

****After reconstitution, always store the calibrators upright.***

Allow all refrigerated components to reach a room temperature of 18-25°C (64-77°F) before use. Do not freeze or expose to temperatures above 32°C (90°F).

5 Instruments

Syva provides instructions for using this assay on the Syva ETS® Plus System (6,7). Contact the Syva Technical Consultation Center in the USA or your local Syva representative for more information.

6 Specimen Collection and Preparation

- Urine samples may be collected in plastic (i.e., polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics adsorb drugs.
- If not analyzed immediately, samples may be stored unrefrigerated for up to seven days following collection (9).
- Samples should be at a room temperature of 18-25°C (64-77°F) for testing.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine samples within the normal urine pH range (4.5-8) do not require prior adjustment of pH.
- Samples outside the normal pH range should be suspected of adulteration.
- Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample.
- Human urine samples should be handled and disposed of as if they were potentially infectious.

7 Procedure

Materials Provided
Emit® d.a.u.™ Opiate Assay
- Reagent A
- Reagent B
- Drug Assay Buffer Concentrate

Materials Required But Not Provided
Emit® Calibrator Level Ø (negative)
Emit® Calibrator A Level 1 (cutoff)
Emit® Calibrator A Level 2 (high)

Other Items:
Class A volumetric pipette
Distilled or deionized water

Assay Sequence
To run the assay, see the ETS® Plus System Operator's Manual.

Calibration
Three calibrator levels are available from Syva: the Emit® Calibrator Level Ø (negative), Emit® Calibrator A Level 1 (cutoff), and Emit® Calibrator A Level 2 (high). To analyze your data, use the Emit® Calibrator A Level 1 for qualitative analysis and all three calibrators for semiquantitative analysis. Calibrate at the beginning of each workday. Recalibrate if you change reagents or as indicated by control results.

Refer to the Emit® Calibrator A package insert or the ETS® Plus System Operator's Manual.

7 Procedure (cont.)

Quality Control
Validate the daily calibration by assaying positive and negative controls. If results fall within acceptable limits as defined by your laboratory and minimum separations are maintained, run samples. (See base label for minimum separations for each reagent lot.)

Daily Maintenance
Refer to the system shutdown and maintenance procedures in the ETS® Plus System Operator's Manual.

8 Results

The Emit® Calibrator A Level 1, which contains a concentration of 300 ng/mL morphine, is used as a reference for distinguishing "positive" from "negative" samples.

Positive Results
A sample that gives a change in absorbance (ΔA) value equal to or higher than the Calibrator A Level 1 ΔA value is interpreted as positive. The sample contains opiates.

Negative Results
A sample that gives a change in absorbance (ΔA) value lower than the Calibrator A Level 1 ΔA value is interpreted as negative: Either the sample does not contain opiates or opiates are present in concentrations below the cutoff level for this assay.

Semiquantitative Results
Using the Emit® d.a.u.™ Opiate Assay, semiquantitative determinations of opiates are possible. Where estimates of relative total drug concentrations are desired, a standard curve should be prepared by plotting the ΔA values of the Emit® Calibrator Level Ø, Calibrator A Level 1, and Calibrator A Level 2 against the calibrator morphine concentrations. The ΔA values of positive samples may then be compared to this standard curve.

Immunoassays that produce a single result in the presence of multiple components cannot fully quantitate the concentration of individual components. Interpretation of results must also take into account that urine concentrations can vary extensively with fluid intake and other biological variables. A more specific alternative chemical method must be used to obtain a confirmed analytical result (see section 1, Intended Use).

9 Limitations

- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of opiates but does not indicate or measure intoxication.
- The Emit® d.a.u.™ Opiate Assay also detects high concentrations of the analgesic meperidine and the narcotic antagonist nalorphine.
- Floxin (ofloxacin) can interfere with the assay at concentrations greater than 226 μg/mL.
- Poppy seeds can contain opiates, and ingestion of products containing poppy seeds can cause a positive test result (8).
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

10 Expected Values

When the Emit® d.a.u.™ Opiate Assay is used as a qualitative assay, the amount of drugs and metabolites detected by the assay in any given sample cannot be estimated. The assay result distinguish positive from negative samples—positive indicating samples that contain opiates.

When used semiquantitatively, the assay yields approximate, cumulative concentrations of the drug detected by the assay (see section 8, Results).

11 Performance

Data appearing in this section were collected on the Syva® AutoLab Instrument System and on the Syva ETS® analyzer.

Accuracy
Ninety-eight clinical urine specimens were tested using the Emit® d.a.u.™ Opiate Assay. Fifty-eight were positive by the Emit® d.a.u.™ assay and 40 were negative by the Emit® d.a.u.™ assay. The Emit® d.a.u.™ test results from all 98 samples (100%) agreed with results by thin-layer chromatography (TLC), radioimmunoassay (RIA), and/or GC/MS.

Opiate-free urine samples and urine samples containing 500 ng/mL morphine were tested with the Emit® d.a.u.™ Opiate Assay. The assay correctly identified more than 99% of these samples.

In clinical investigations, within-run precision was determined using Emit® calibrators containing 0 ng/mL, 300 ng/mL, and 1000 ng/mL morphine. Results are shown in Table 3.

11 Performance (cont.)

Table 3 — Within-Run Precision

	N	Mean (ΔA)	Standard Deviation (ΔA)	Coefficient of Variation (%)
Negative Calibrator (0 ng/mL Morphine)	24	233	3.2	1.4
Low Calibrator (300 ng/mL Morphine)	24	332	5.0	1.5
High Calibrator (1000 ng/mL Morphine)	24	477	4.9	1.1

Specificity

▶ The Emit® d.a.u.™ Opiate Assay detects morphine and morphine glucuronide, the major metabolites of heroin, in urine.

Table 4 gives the compounds this assay is designed to detect and the levels at which the compounds have been found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Calibrator A Level 1). Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. If a sample contains more than one compound detected by the assay, lower concentrations than those listed below may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Floxin can interfere with the assay at concentrations greater than 226 μg/mL.

Table 4 — Concentration of Opiate Compounds that Produce a Result Approximately Equivalent to Calibrator A Level 1 (300 ng/mL cutoff)

Compound	Concentration (ng/mL)
Morphine	300
Morphine-3-Glucuronide	900
Codeine	200
Hydrocodone	400
Hydromorphone	500
Levallorphan	60000*
Levorphanol	900
Meperidine	50000**
Nalorphine	90000*
Oxycodone	4500

Therapeutic or toxic urinary levels of levallorphan and nalorphine are not reported in the literature.

****Meperidine urinary concentrations of 150000 ng/mL have been measured in cases of fatal meperidine overdosage (10).***

Table 5 lists compounds that are not detected by the Emit® d.a.u.™ Opiate Assay.

Table 5 — Compounds That Produce a Negative Response

Compound	Concentration Tested (μg/mL)
Amphetamine	1000
Benzoylecgonine	1000
Chlorpromazine	12
Dextromethorphan	100
Doxylamine	500
Methadone	500
Naloxone	150
Oxazepam	250
Phencyclidine	1000
Propoxyphene	1000
Secobarbital	1000

12 Bibliography

1. Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Ellenhorn MJ, Barceloux DG: Medical Toxicology. New York, Elsevier Science Publishing Company, Inc, 1988, pp 689-717.
3. Wyngarrden JB, Smith LH Jr (eds): Cecil Textbook of Medicine. Philadelphia, WB Saunders Co, 1988, pp 55-57.
4. Gorodetzky CW: Detection of drugs of abuse in biological fluids, in Martin WR (ed): Drug Addiction I. New York, Springer-Verlag, 1977, pp 319-409.
5. Henry JB: Clinical Diagnosis and Management by Laboratory Methods. Philadelphia, WB Saunders Co. 1991, pp 866.
6. Syva ETS® System Operator's Manual, Palo Alto, CA, Syva Co.
7. Syva ETS® System Guide, Palo Alto, CA, Syva Co.
8. Hayes LW, Krasselt WG, Mueggler PA: Concentrations of morphine and codeine in serum and urine after ingestion of poppy seeds. Clin Chem, 1987, 33/6:806-808.
9. Notice of mandatory guidelines for federal workplace drug testing program; Final Guidelines. *Federal Register* 1988;69 (April 11):11983.
10. Baselt RC,Cravey, RH: Disposition of Toxic Drugs and Chemicals in Man, ed 3. Chicago, Year Book Medical Publishers 1989, p 488.

Syva Australia, North Sydney, N.S.W. 2060, Australia
Syva Diagnostica GmbH, A-1110 Vienna, Austria
Syva Belgium, 1140 Bruxelles, Belgium
Syva Canada, Kanata, Ontario K2M 1P6, Canada
Syva Diagnostika, 2970 Hørsholm, Denmark
Syva France, 69570 Dardilly, France
Syva Diagnostica GmbH, D-64289 Darmstadt, Germany
BRACCO Industria Chimica S.p.A. 20134, Milano, Italy
Nippon Syntex KK, Tokyo 150, Japan
División Diagnósticos Syntex, 11910 Mexico D.F., Mexico
Syva Diagnostica BV, NDL-2288 Rijswijk, Netherlands
Syva New Zealand, Browns Bay, Auckland, New Zealand
Syva Norge A/S, 2000 Lillestrøm, Norway
Syntex Latino, División Diagnósticos, 1495 Lisboa, Portugal
Syntex Latino, División Diagnósticos, 08028 Barcelona, Spain
Syva Scandinavia, S-127 23 Skärholmen, Sweden
Syva Diagnostica, CH-8800 Thalwil, Switzerland
Syva U.K., Maidenhead, Berkshire SL6 1RD, United Kingdom
Syva Company, San Jose, CA 95135 USA

For additional assistance, call Syva toll-free:
1-800-227-8994 in USA
1-800-267-6205 in Canada

Notice: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in this labeling can affect performance characteristics and stated or implied labeling claims.

Syva Company
3403 Yerba Buena Road
P.O. Box 49013
San Jose, CA 95161-9013

Syva a Syntex company

September 1993
Printed in USA
3B124UL.9

G1
01/20/95 (W)

3F222UL.5

Emit® d.a.u.™ Benzodiazepine Assay

Updated Information:
▶ Dual cutoff capability, note changes throughout.

1 Intended Use

The Emit® d.a.u.™ Benzodiazepine Assay is a homogeneous enzyme immunoassay intended for use in the qualitative and semiquantitative analysis of benzodiazepines in human urine.

The Emit® d.a.u.™ Benzodiazepine Assay provides only a preliminary analytical test result. A more specific alternative chemical method (1) must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method (1). Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

2 Summary and Explanation of the Test

Benzodiazepines are sedative-hypnotic drugs that are structurally similar and include widely used drugs such as chlordiazepoxide, diazepam, and oxazepam. The different benzodiazepines are absorbed at different rates, and the timing of their psychoactive effects varies with the absorption rate. Benzodiazepines are usually taken orally and are metabolized in the liver. Some benzodiazepine metabolites are pharmacologically active (2). Benzodiazepines potentiate the effect of other central nervous system depressants, such as ethyl alcohol (3).

▶ The Emit® d.a.u.™ Benzodiazepine Assay, an enzyme immunoassay technique, tests for benzodiazepines and benzodiazepine metabolites in human urine. Positive results for samples containing other compounds structurally unrelated to benzodiazepines have not been observed. The cutoff levels for distinguishing positive from negative samples are 200 ng/mL and 300 ng/mL.

Emit® homogeneous immunoassays are widely used in therapeutic drug monitoring and drugs-of-abuse testing. The assays are nonradioactive and do not require extraction procedures. Designed for use in screening, the Emit® d.a.u.™ assays give qualitative results that are equivalent to other drug detection methods.

Methods historically used for detecting benzodiazepines in biological fluids include gas chromatography with electron-capture (4) or flame-ionization detection (5), high-performance liquid chromatography (6), thin-layer chromatography (7), fluorescence-TLC densitometry (8), enzyme immunoassay (9), and radioimmunoassay (10).

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result (1).

3 Principle

The Emit® assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine (11). The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6P-DH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 Reagents

Catalog Number	Product Description	Quantity Volume
3F229	**Emit® d.a.u.™ Benzodiazepine Assay**	
	Antibody/Substrate Reagent A Sheep antibodies reactive to diazepam, glucose-6-phosphate, nicotinamide adenine dinucleotide, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL*
	Enzyme Reagent B Diazepam labeled with glucose-6-phosphate dehydrogenase, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL*
	Emit® Drug Assay Buffer Concentrate When diluted, contains tris buffer, surfactant, and 0.05% sodium azide	13.3 mL
	▶ For 200 ng/mL cutoff use:	
9A049/9A059	**Emit® Calibrator Level Ø****	5 mL/25 mL*
9A169/9A369	**Emit® Calibrator A Level 1 (cutoff)****	5 mL/25 mL*
9A189/9A389	**Emit® Calibrator A Level 2 (high)****	5 mL/25 mL*
	▶ For 300 ng/mL cutoff use:	
9A049/9A059	**Emit® Calibrator Level Ø****	5 mL/25 mL*
9A279/9A479	**Emit® Calibrator B Level 1 (cutoff)****	5 mL/25 mL*
9A299/9A499	**Emit® Calibrator B Level 2 (high)****	5 mL/25 mL*
	(See Table 1 for drug concentrations)	

Reagents and calibrators are shipped in dry form. The indicated volume is that required for reconstitution. Number of assays will vary depending on the instrument used.

****Required for use with the Emit® d.a.u.™ Benzodiazepine Assay. Sold separately.***

Note: Reagents A and B are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

The Emit® calibrators, when reconstituted, contain the concentrations of oxazepam listed in Table 1.

▶ **Table 1 — Oxazepam Concentrations in Emit® Calibrators**

Calibrator	Concentration (ng/mL)
Emit® Calibrator Level Ø	0
Emit® Calibrator A Level 1 (cutoff)*	200
Emit® Calibrator A Level 2 (high)*	1000
Emit® Calibrator B Level 1 (cutoff)*	300
Emit® Calibrator B Level 2 (high)*	1000

****These calibrators also contain additional drugs of abuse, which do not affect the assay.***

Precautions
- The Emit® d.a.u.™ Benzodiazepine Assay is for in vitro diagnostic use.
- Reagent A contains nonsterile sheep antiserum.
- Reagents and buffer contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
- On initial reconstitution, each kit contains no more than 0.0025% mercury, wt/vol, as thimerosal (0.005% thimerosal, wt/vol). Please handle and dispose of appropriately.
- Do not use the kit after the expiration date.

Preparation and Storage of Assay Components
Reagents
To reconstitute Reagents A and B:
- Record the date of reconstitution.
- Remove the metal seal and rubber stopper from the vial.
- Mark the stopper to identify it with the vial.
- Add distilled or deionized water to equal 6 mL.
- Put the stopper back on the vial.
- Swirl the vial until the powder is dissolved.

Let the reconstituted reagents remain at a room temperature of 18-25°C (64-77°F) for at least one hour before use; alternatively, reconstitute the reagents the day before use and store refrigerated (2-8°C, 36-46°F) overnight. Allow reconstituted reagents to reach room temperature before use.

Buffer
To prepare the buffer solution from the buffer concentrate:
- Record the date of buffer preparation.
- Remove the lid and the seal from the buffer concentrate bottle.
- Pour all of the buffer concentrate into a clean, graduated plastic or glass container.
- Rinse the concentrate bottle several times with distilled or deionized water, pouring the water into the container each time.
- Fill the container to the 200 mL mark with distilled or deionized water.
- Put the cap on the container and invert several times to mix thoroughly.

4 Reagents (cont.)

Table 2 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temp	Recon Volume	Minimum Recon Time & Temp	Stability* Unopened	Stability* Prepared/Opened
Reagent A	2-8°C (36-46°F)	6.0 mL	1 h 18-25°C (64-77°F)	Exp date	12 wk
Reagent B	2-8°C (36-46°F)	6.0 mL	1 h 18-25°C (64-77°F)	Exp date	12 wk
Buffer					
Unopened	2-8°C (36-46°F)		None	Exp date	—
Diluted	18-25°C (64-77°F)	200 mL			12 wk
Calibrators**	2-8°C (36-46°F)	5.0/25.0 mL	1h 18-25°C (64-77°F)	Exp date	12 wk

Stability depends on handling reagents as directed.

****After reconstitution, always store the calibrators upright.***

Allow all refrigerated components to reach a room temperature of 18-25°C (64-77°F) before use. Do not freeze or expose to temperatures above 32°C (90°F).

5 Instruments

Syva provides instructions for using this assay on the Syva ETS® Plus system (12,13). Contact the Syva Technical Consultation Center in the USA or your local Syva representative for more information.

6 Specimen Collection and Preparation

- Urine samples may be collected in plastic (i.e., polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics adsorb drugs.
- If not analyzed immediately, samples may be stored unrefrigerated for up to seven days following collection.
- Samples should be at a room temperature of 18-25°C (64-77°F) for testing.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine samples within the normal urine pH range (4.5-8) do not require prior adjustment of pH.
- Samples outside the normal pH range should be suspected of adulteration.
- Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample.
- Human urine samples should be handled and disposed of as if they were potentially infectious.

7 Procedure

Materials Provided
Emit® d.a.u.™ Benzodiazepine Assay
- Reagent A
- Reagent B
- Drug Assay Buffer Concentrate

Materials Required But Not Provided
For 200 ng/mL cutoff:
- Emit® Calibrator Level Ø
- Emit® Calibrator A Level 1 (cutoff)
- Emit® Calibrator A Level 2 (high)

▶ For 300 ng/mL cutoff:
- Emit® Calibrator Level Ø
- Emit® Calibrator B Level 1 (cutoff)
- Emit® Calibrator B Level 2 (high)

Other Items:
Class A volumetric pipette
Distilled or deionized water

7 Procedure (cont.)

Assay Sequence
To run the assay, see the the ETS® Plus System Operator's Manual.

Calibration
For calibration at the 200 ng/mL level use:
- Emit® Calibrator Level Ø
- Emit® Calibrator A Level 1 (cutoff)
- Emit® Calibrator A Level 2 (high)

▶ For calibration at the 300 ng/mL level use:
- Emit® Calibrator Level Ø
- Emit® Calibrator B Level 1 (cutoff)
- Emit® Calibrator B Level 2 (high)

To analyze your data, use the Emit® Calibrator A Level 1 (or Emit® Calibrator B Level 1) for qualitative analysis and all three calibrators for semiquantitative analysis. Recalibrate if you change reagents or as indicated by control results. Refer to the Emit® Calibrator A (or Emit® Calibrator B) package insert or the ETS® Plus System Operator's Manual.

Quality Control
Validate the daily calibration by assaying positive and negative controls. If results fall within acceptable limits as defined by your laboratory and minimum separations are maintained, run samples. (See base label for minimum separations for each reagent lot.)

Daily Maintenance
Refer to the instrument operating manual for maintenance instructions.

8 Results

▶ The Emit® Calibrator A Level 1 (cutoff) and Emit Calibrator B Level 1 (cutoff), contain 200 and 300 ng/mL oxazepam, respectively. The cutoff calibrators are used as a reference for distinguishing "positive" from "negative" samples.

Positive Results
A sample that gives a change in absorbance (ΔA) value equal to or higher than the Calibrator A Level 1 (or Calibrator B) ΔA value is interpreted as positive. The sample contains benzodiazepines.

Negative Results
A sample that gives a ΔA value lower than the Calibrator A Level 1 (or Calibrator B Level 1) ΔA value is interpreted as negative. Either the sample does not contain benzodiazepines or they are present in concentrations below the cutoff level for this assay.

Semiquantitative Results
Using the Emit® d.a.u.™ Benzodiazepine Assay, semiquantitative determinations of benzodiazepines or benzodiazepine metabolites are possible. Where estimates of relative total drug concentrations are desired, a standard curve should be prepared by plotting the ΔA values of the Emit® Calibrator Level Ø, Calibrator A Level 1, and Calibrator A Level 2 (or Emit® Calibrator Level Ø, Emit® Calibrator B Level 1, and Calibrator B Level 2) against the calibrator oxazepam concentrations. The ΔA values of positive samples may then be compared to this standard curve.

Immunoassays that produce a single result in the presence of multiple detectable components cannot fully quantitate the concentration of individual components. Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables. A more specific alternative chemical method must be used to obtain a confirmed analytical result (see Section 1, Intended Use).

9 Limitations

- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of benzodiazepines but does not indicate or measure intoxication.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

10 Expected Values

When the Emit® d.a.u.™ Benzodiazepine Assay is used as a qualitative assay, the amount of benzodiazepine or benzodiazepine metabolites detected by the assay in any given sample cannot be estimated. The assay results distinguish positive from negative samples — positive indicating samples that contain benzodiazepine or benzodiazepine metabolites.

When used semiquantitatively, the assay yields approximate concentrations of the metabolite detected by the assay (see Section 8, Results).

Revised November 1992

3C534UL.10E

F4 NJ 01/04/94

Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay

1 Intended Use

The Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay is a homogeneous enzyme immunoassay intended for use in the qualitative and semiquantitative analysis of amphetamines in human urine. This assay uses a cutoff level of 1000 ng/mL to distinguish positive from negative samples.

The Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay provides only a preliminary analytical test result. A more specific alternative chemical method (1) must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method (1). Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

2 Summary and Explanation of the Test

Amphetamines are central nervous system stimulants that produce wakefulness, alertness, increased energy, reduced hunger, and an overall feeling of well-being (1,2). The term "amphetamine" includes many drugs, but d-amphetamine, d-methamphetamine (the N-methyl derivative of amphetamine), and d,l-amphetamine are the most common (2). Amphetamines can be taken orally, intravenously, by smoking, or by snorting (1).

Amphetamines are readily absorbed from the gastrointestinal tract and are then either deactivated by the liver or excreted unchanged in the urine. The relative importance of these elimination modes depends on urinary pH. Amphetamine is metabolized to deaminated (hippuric and benzoic acids) and hydroxylated metabolites. Methamphetamine is partially metabolized to amphetamine, its major active metabolite (1).

Amphetamines appear in the urine within 3 hours after any type of administration (3), and can be detected by Emit® assay for as long as 24 to 48 hours after the last dose (1).

The Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay uses a cutoff of 1000 ng/mL d-methamphetamine which is consistent with the level recommended in the National Institute on Drug Abuse (NIDA) guidelines when screening for amphetamines. The assay also detects d-amphetamine, d,l-amphetamine, methylenedioxyamphetamine (MDA), and methylenedioxymethamphetamine (MDMA) in human urine (see Table 4). Because the assay contains monoclonal antibodies, it is less subject to interference by amphetamine-like compounds than assays containing polyclonal antibodies. While interferences are reduced with this assay, like any immunological test, some interfering compounds do exist. For this reason, confirmation of preliminary positive results is always recommended.

Methods historically used for detecting amphetamines in biological fluids include thin-layer chromatography, gas chromatography, fluorometry, microcrystallography, enzyme immunoassay, and radioimmunoassay (4).

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result (1).

3 Principle

The Emit® assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine (5). The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6P-DH does not interfere because the coenzyme functions only with the bacterial (*Leuconostoc mesenteroides*) enzyme employed in the assay.

4 Reagents

Catalog Number	Product Description	Quantity/ Volume
3C549	**Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay**	Approximately 300 Tests
	Antibody/Substrate Reagent A Mouse monoclonal antibodies reactive to d-amphetamine and d-methamphetamine, glucose-6-phosphate, nicotinamide adenine dinucleotide, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL*
	Enzyme Reagent B Amphetamines labeled with glucose-6-phosphate dehydrogenase, tris buffer, bulking agents, stabilizers, and 0.05% sodium azide	6.0 mL*
	Emit® Drug Assay Buffer Concentrate When diluted, contains tris buffer, surfactant, and 0.05% sodium azide	13.3 mL
9A049	**Emit® Calibrator Level Ø (negative)****	5.0 mL*
9A169	**Emit® Calibrator A Level 1 (cutoff)****	5.0 mL*
9A189	**Emit® Calibrator A Level 2 (high)****	5.0 mL*
9A059	**Emit® Calibrator Level Ø (negative)****	25.0 mL*
9A369	**Emit® Calibrator A Level 1 (cutoff)****	25.0 mL*
9A389	**Emit® Calibrator A Level 2 (high)**** (See Table 1 for drug concentrations)	25.0 mL*

Reagents and calibrators are shipped in dry form. The indicated volume is that required for reconstitution.

****Required for use with the Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay. Sold separately. The Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay has been optimized for use with the Emit® set A calibrators which contain d-methamphetamine; it should not be used with Emit® set B calibrators which contain d-amphetamine.***

Note: Reagents A and B are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

The Emit® calibrators, when reconstituted, contain the concentrations of d-methamphetamine listed in Table 1.

Table 1 — d-Methamphetamine Concentrations in Emit® Calibrators

Calibrator	Concentration (ng/mL)
Emit® Calibrator Level Ø (negative)	0
Emit® Calibrator A Level 1 (cutoff)*	1000
Emit® Calibrator A Level 2 (high)*	3000

***These calibrators also contain additional drugs of abuse, which do not affect the assay.**

Precautions

- The Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay is for in vitro diagnostic use.
- Reagent A contains nonsterile mouse monoclonal antibodies.
- Reagents and buffer contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
- On initial reconstitution, each kit contains no more than 0.0025% mercury, wt/vol, as thimerosal (0.005% thimerosal, wt/vol). Please handle and dispose of appropriately.
- Do not use the kit after the expiration date.

Preparation and Storage of Assay Components

Reagents

To reconstitute Reagents A and B:

- Record the date of reconstitution.
- Remove the metal seal and rubber stopper from the vial.
- Mark the stopper to identify it with the vial.
- Add 6 mL of distilled or deionized water.
- Put the stopper back on the vial.
- Swirl the vial until the powder is dissolved.

Let the reconstituted reagents remain at a room temperature of 18-25°C (64-77°F) for at least one hour before use; alternatively, reconstitute the reagents the day before use and store refrigerated (2-8°C, 36-46°F) overnight. Allow reconstituted reagents to reach room temperature before use.

Buffer

To prepare the buffer solution from the buffer concentrate:

- Record the date of buffer preparation.
- Remove the lid and the seal from the buffer concentrate bottle.
- Pour all of the buffer concentrate into a clean, graduated, plastic or glass container.
- Rinse the concentrate bottle several times with distilled or deionized water, pouring the water into the container each time.
- Fill the container to the 200 mL mark with distilled or deionized water.
- Put the cap on the container and invert several times to mix thoroughly.

11 Performances

Les données de cette section ont été obtenues avec le Système Syva ETS® Plus.

Précision

Cent vingt-neuf échantillons cliniques d'urine ont été testés avec le test Emit® d.a.u.™ Benzodiazépine. Les échantillons ont été testés avec le Système ETS® Plus en utilisant les deux seuils : 200 ng/mL et 300 ng/mL. Tous les résultats inconsistants étaient confirmés par CPG/SM. Soixante-trois (63) échantillons étaient positifs et cinquante-sept échantillons étaient négatifs avec les deux seuils. Neuf (9) échantillons étaient positifs avec le seuil de 200 ng/mL et négatifs avec le seuil de 300 ng/mL. Il a été confirmé par CPG/SM que ces neuf (9) échantillons contenaient des benzodiazépines.* La différence de résultats était due à la différence des seuils.

**** La méthode CPG/SM testait la présence des substances suivantes : alprazolam, lorazépam, nordiazépam, oxazépam, triazolam et métabolites α-hydroxy de l'alprazolam et du triazolam.***

La précision a été déterminée en testant 4 échantillons 5 jours consécutifs, à raison d'un test par jour en 5 exemplaires. La précision a été évaluée d'une manière conforme à la directive EP-5T2 (mars 1992) du Comité national d'établissement des normes pour les laboratoires cliniques (National Committee for Clinical Laboratory Standards ou NCCLS). des résultats sont résumés au tableau 3. Les résultats sont résumés au tableau 3.

Tableau 3 — Précision

	N	Moyenne (ΔA)	Ecart-type (ΔA)	Coefficient de variation (%)
Répétabilité				
Calibrateur négatif (oxazépam à 0 ng/mL)	25	296	2,42	0,82
Calibrateur A - Niveau 1 (oxazépam à 200 ng/mL)	25	389	3,21	0,82
Calibrateur B - Niveau 1 (oxazépam à 300 ng/mL)	25	420	3,14	0,75
Calibrateur A - Niveau 2 (oxazépam à 1000 ng/mL)	25	501	3,73	0,74
Précision totale				
Calibrateur négatif (oxazépam à 0 ng/mL)	25	298	2,80	0,94
Calibrateur A - Niveau 1 (oxazépam à 200 ng/mL)	25	387	4,64	1,20
Calibrateur B - Niveau 1 (oxazépam à 300 ng/mL)	25	417	3,77	0,90
Calibrateur A - Niveau 2 (oxazépam à 1000 ng/mL)	25	496	6,39	1,29

Spécificité

Le test Emit® d.a.u.™ Benzodiazépine détecte les benzodiazépines et leurs métabolites dans l'urine humaine. Le tableau 4 indique les composés que ce test est sensé détecter et les concentrations pour lesquelles ces composés ont donné des résultats approximativement équivalents à ceux du calibrateur seuil (calibrateur Emit® A - Niveau 1 ou calibrateur Emit® B - Niveau 1). Ces concentrations tombent dans l'intervalle de celles détectées dans l'urine après la prise du composé. Chaque concentration représente le niveau de réactivité pour le composé indiqué lorsque ce dernier est ajouté à un échantillon d'urine négatif. Lorsqu'un échantillon contient plusieurs composés détectés par ce test, des concentrations inférieures à celles indiquées ci-dessous peuvent s'ajouter pour produire une concentration équivalente ou supérieure à celle du calibrateur seuil.

Tableau 4 — Concentrations de composés produisant un résultat approximativement équivalent au calibrateur A - Niveau 1 (seuil de 200 ng/mL) et au calibrateur B - Niveau 1 (seuil de 300 ng/mL)

Composé	Concentration (ng/mL) avec un seuil de 200 ng/mL	Concentration (ng/mL) avec un seuil de 300 ng/mL
Oxazépam	200	300
Alprazolam	50	100
Bromazépam	400	550
Chlordiazépoxyde	500	1400
Clobazam	100	270
Clonazépam	500	640
Clorazépate	*	*
Clotiazépam	100	250
Démoxépam	900	1000
N-Désalkylflurazépam	100	180
Diazépam	40	80
Flunitrazépam	100	230
Flurazépam	100	130
Halazépam	80	160
α-Hydroxyalprazolam	60	100
α-Hydroxytriazolam	100	140
1-N-Hydroxyéthylflurazépam	60	100
Lorazépam	1000	1300
Lormétazépam	200	280
Médazépam	100	140
Midazolam	120	180
Nitrazépam	200	260
Norchlordiazépoxyde	1800	1800
Nordiazépam	60	100
Prazépam	80	100
Témazépam	70	190
Tétrazépam	100	100
Triazolam	70	110

****Le clorazépate se dégrade rapidement dans l'acide gastrique en nordiazépam. Le nordiazépam s'hydroxyle en oxazépam qui est détecté par le test avec un seuil de 200 ng/mL et de 300 ng/mL.***

11 Performances (suite)

Le tableau 5 indique les composés à structure différente des benzodiazépines qui ne sont pas détectés par le test Emit® d.a.u.™ Benzodiazépine. Les concentrations testées sont toutes substantiellement supérieures aux concentrations urinaires normales.

Tableau 5 — Concentrations de composés produisant un résultat négatif

Composé	Concentration (μg/mL) avec un seuil de 200 ng/mL	Concentration (μg/mL) avec un seuil de 300 ng/mL
Amphétamines	1000	1000
Benzoylecgonine	1000	1000
Chlorpromazine	12	12
Dextrométhorphane	1000	1000
Fénoprofène	1000	1000
Ibuprofène	1000	1000
Méthadone	1000	1000
Méthaqualone	1000	1000
Morphine	1000	1000
Naproxène	1000	1000
Phencyclidine	1000	1000
Propoxyphène	1000	1000
Sécobarbital	1000	1000

12 Bibliographie

1. Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Woolf DS: CNS depressants: Other sedative hypnotics in, Bennett G, Vourakis C, Woolf DS (eds): Substance Abuse: Pharmacologic, Development, and Clinical Perspectives. New York, John Wiley and Sons, 1983, pp 39-56.
3. Ellenhorn MJ, Barceloux DG: Medical Toxicology. New York, Elsevier Science Publishing Company, Inc, 1988, p 561
4. Fergeson J, Couri D: Electron-capture gas chromatography determination of benzodiazepines and metabolites. J Anal Toxicol 1977;1:171-174.
5. Basett RC, Steward CB, Franch SJ: Toxicological determination of benzodiazepines in biological fluids and tissues by flame-ionization gas chromatography. J Anal Toxicol 1977;1:10-13.
6. Bogge A: Quantitative high-performance liquid chromatography of diazepam and N-desmethyldiazepam in blood. J Chromatogr 1976;128:111-116.
7. Wad N, Rosenmund H, Hanlé E: A simplified quantitative method for the simultaneous determination of diazepam and its metabolites in serum by thin-layer chromatography. J Chromatogr 1976;128:231-234.
8. Sun S: Fluorescence—TLC densitometric determination of diazepam and other 1,4-benzodiazepines in serum. J Pharm Sci 1978;67:1413-1415.
9. Rubenstein KE: Homogeneous enzyme immunoassay today. Scand J Immunol 1978;8(suppl 7):57-62.
10. Dixon R: Radioimmunoassay of benzodiazepines. Methods Enzymol 1982;84:490-515.
11. Henry JB: Clinical Diagnosis and Management by Laboratory Methods. Philadelphia, WB Saunders Co. 1991, pp 866.
12. Syva ETS® System Operator's Manual, Palo Alto, CA, Syva Co.
13. Syva ETS® System Operating Guide, Palo Alto, CA, Syva Co.

Syva Australia, North Sydney, N.S.W. 2060, Australia
Syva Diagnostica GmbH, A-1110 Vienna, Austria
Syva Belgium, 1140 Bruxelles, Belgium
Syva Canada, Kanata, Ontario K2M 1P6, Canada
Syva Diagnostika, 2970 Hørsholm, Denmark
Syva France, 69570 Dardilly, France
Syva Diagnostica GmbH, D-64289 Darmstadt, Germany
BRACCO Industria Chimica S.p.A. 20134, Milano, Italy
Nippon Syntex KK, Tokyo 150, Japan
División Diagnósticos Syntex, 11910 Mexico D.F., Mexico
Syva Diagnostica BV, NDL-2288 Rijswijk, Netherlands
Syva New Zealand, Browns Bay, Auckland, New Zealand
Syva Norge A/S, 2000 Lillestrøm, Norway
Syntex Latino, División Diagnósticos, 1495 Lisboa, Portugal
Syntex Latino, División Diagnósticos, 08028 Barcelona, Spain
Syva Scandinavia, S-127 23 Skärholmen, Sweden
Syva Diagnostica, CH-8800 Thalwil, Switzerland
Syva U.K., Maidenhead, Berkshire SL6 1RD, United Kingdom
Syva Company, San Jose, CA 95135 USA

Note : L'utilisation de réactifs modifiés ou endommagés, d'instruments non performants, ainsi que le non respect des instructions contenues dans cette notice, peuvent affecter les résultats et les affirmations explicites ou implicites paraissant sur cette même notice.

Syva Company
3403 Yerba Buena Road
P.O. Box 49013
San Jose, CA 95161-9013

Syva a Syntex company

April 1994
Printed in USA
3F222UL.5

4 Reagents (cont.)

Table 2 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temp	Recon Volume (mL)	Minimum Recon Time & Temp	Stability* Unopened	Stability* Prepared/ Opened
Reagent A	2-8°C (36-46°F)	6.0	1 h 18-25°C (64-77°F)	Exp date	4 wk
Reagent B	2-8°C (36-46°F)	6.0	1 h 18-25°C (64-77°F)	Exp date	4 wk
Buffer					
Unopened	2-8°C (36-46°F)		None	Exp date	—
Diluted	18-25°C (64-77°F)	200		—	12 wk
Calibrators**	2-8°C (36-46°F)	5.0/25.0	1 h 18-25°C (64-77°F)	Exp date	12 wk

Stability depends on handling reagents as directed.

****After reconstitution, always store the calibrators upright.***

Allow all refrigerated components to reach a room temperature of 18-25°C (64-77°F) before use. Do not freeze or expose to temperatures above 32°C (90°F).

5 Instruments

Syva provides instructions for using this assay on the Syva ETS® Plus system (6,7). Contact the Syva Technical Consultation Center in the USA or your local Syva representative for more information.

6 Specimen Collection and Preparation

- Urine samples may be collected in plastic (i.e., polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics adsorb drugs.
- If not analyzed immediately, samples may be stored unrefrigerated for up to seven days following collection (8).
- Samples should be at a room temperature of 18-25°C (64-77°F) for testing.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine samples within the normal urine pH range (4.5-8) do not require prior adjustment of pH.
- Samples outside the normal pH range should be suspected of adulteration.
- Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample.
- Human urine samples should be handled and disposed of as if they were potentially infectious.

7 Procedure

Materials Provided
Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay
- Reagent A
- Reagent B
- Drug Assay Buffer Concentrate

Materials Required But Not Provided
Emit® Calibrator Level Ø (negative)
Emit® Calibrator A Level 1 (cutoff)
Emit® Calibrator A Level 2 (high)

Other Items:
Class A volumetric pipette
Distilled or deionized water

Assay Sequence
To run the assay, see the ETS® Plus System Operator's Manual.

Calibration
Three calibrator levels are available from Syva: the Emit® Calibrator Level Ø (negative), Emit® Calibrator A Level 1 (cutoff), and Emit® Calibrator A Level 2 (high). To analyze your data, use the Emit® Calibrator A Level 1 for qualitative analysis and all three calibrators for semiquantitative analysis. Calibrate at the beginning of each workday. Recalibrate if you change reagents or as indicated by control results.

Refer to the Emit® Calibrator A package insert or the ETS® Plus System Operator's Manual.

7 Procedure (cont.)

Quality Control
Validate the daily calibration by assaying positive and negative controls. If results fall within acceptable limits as defined by your laboratory and minimum separations are maintained, run samples. (See base label for minimum separations for each reagent lot.)

Daily Maintenance
Refer to the system shutdown and maintenance procedures in the ETS® Plus System Operator's Manual.

8 Results

The Emit® Calibrator A Level 1, which contains a concentration of 1000 ng/mL d-methamphetamine, is used as a reference for distinguishing "positive" from "negative" samples.

Positive Results
A sample that gives a change in absorbance (ΔA) value equal to or higher than the Calibrator A Level 1 ΔA value is interpreted as positive. The sample contains amphetamines.

Negative Results
A sample that gives a change in absorbance (ΔA) value lower than the Calibrator A Level 1 ΔA value is interpreted as negative: Either the sample does not contain amphetamines or amphetamines are present in concentrations below the cutoff level for this assay.

Semiquantitative Results
Using the Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay, semiquantitative determinations of amphetamines are possible. Where estimates of relative total drug concentrations are desired, a standard curve should be prepared by plotting the ΔA values of the Emit® Calibrator Level Ø, Calibrator A Level 1, and Calibrator A Level 2 against the calibrator d-methamphetamine concentrations. The ΔA values of positive samples may then be compared to this standard curve.

Immunoassays that produce a single result in the presence of multiple components cannot fully quantitate the concentration of individual components. Interpretation of results must also take into account that urine concentrations can vary extensively with fluid intake and other biological variables. A more specific alternative chemical method must be used to obtain a confirmed analytical result (see section 1, Intended Use).

9 Limitations

- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of amphetamines, or possibly amphetamine-like compounds, but does not indicate or measure intoxication.
- Therapeutic doses of the following drugs may produce positive results with this assay: chloroquine (Aralen®), chlorpromazine (Thorazine®), methoxyphenamine, quinacrine, phentermine, ranitidine (Zantac®), procainamide and its metabolite N-acetylprocainamide (NAPA). Because benzphetamine (Didrex®) metabolizes to amphetamine and methamphetamine, therapeutic doses of this drug may also produce a positive result.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

10 Expected Values

When the Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay is used as a qualitative assay, the amount of drugs and metabolites detected by the assay in any given sample cannot be estimated. The assay results distinguish positive from negative samples — positive indicating samples that contain amphetamines or amphetamine-like compounds or both.

When used semiquantitatively, the assay yields approximate, cumulative concentrations of the drug and metabolites detected by the assay (see section 8, Results).

11 Performance

The data appearing in this section were collected on the Syva ETS® System.

Accuracy
Two hundred clinical urine specimens were tested using the Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay. Eighty-nine samples were positive by the Emit® d.a.u.™ assay and 111 samples were negative by the Emit® d.a.u.™ assay. Eighty-eight of the samples found positive by the Emit® d.a.u.™ assay were confirmed by GC/MS to contain amphetamines. The remaining sample was determined by GC/MS to contain a combination of amphetamine-like compounds but did not contain amphetamine or methamphetamine.

The Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay was correct in 99% of the tests.

In clinical investigations, within-run precision was determined using Emit® calibrators containing 0 ng/mL, 1000 ng/mL, and 3000 ng/mL d-methamphetamine. Results are shown in Table 3.

11 Performance (cont.)

Table 3 — Within-Run Precision

	Lab	N	Mean (ΔA)	Standard Deviation (ΔA)	Coefficient of Variation (%)
Negative Calibrator (0 ng/mL d-Methamphetamine)	1	20	424	4.6	1.1
	2	20	421	3.3	0.8
Low Calibrator (1000 ng/mL d-Methamphetamine)	1	20	478	5.2	1.1
	2	20	477	4.9	1.0
High Calibrator (3000 ng/mL d-Methamphetamine)	1	20	544	3.8	0.7
	2	20	545	5.8	1.1

Specificity

Emit® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay detects amphetamine compounds in urine.

Table 4 lists the amphetamine compounds that have been found to produce a positive response at concentrations at or above those listed.

Table 4 — Concentrations of Amphetamine Compounds Showing a Positive Response

Compound	Concentration (ng/mL)
d-Amphetamine	≤400
d,l-Amphetamine	1000
d-Methamphetamine	1000
Methylenedioxyamphetamine (MDA)	1000
Methylenedioxymethamphetamine (MDMA)	3000

Table 5 lists the amphetamine compounds that have been found to produce a negative response at concentrations below those listed. Concentrations above those listed may produce a positive response.

Table 5 — Concentrations of Amphetamine Compounds Showing a Negative Response (Higher Concentrations May Produce a Positive Response)

Compound	Concentration (ng/mL)
l-Amphetamine	<2000*
l-Methamphetamine	<7000*

Urinary concentrations of 1600 ng/mL l-amphetamine (9) and 3160 ng/mL l-methamphetamine (10) (average peak) have been reported in the literature.

Table 6 lists concentrations of amphetamine-like compounds that were tested and found to give a negative response. Concentrations above the stated levels may produce a positive response and may be found in therapeutic situations. Therefore, confirm all positive results by an alternative method.

Table 6 — Concentrations of Amphetamine-Like Compounds Showing a Negative Response

Compound	Concentration Tested (μg/mL)
l-Ephedrine	50
Fenfluramine	10
Mephentermine	10
Phendimetrazine	100
Phenethylamine	10
Phenmetrazine	100
Phenylephrine	200
Phenylpropanolamine	75
Propylhexedrine	2
Pseudoephedrine	100
nor-Pseudoephedrine	250
3-OH-Tyramine	200

Table 7 lists concentrations of compounds structurally unrelated to amphetamines that were tested and found to give a negative response.

Table 7 — Concentrations of Compounds Showing a Negative Response

Compound	Concentration Tested (μg/mL)
Benzoylecgonine	1000
Dextromethorphan	1000
Diethylpropion	750
Labetalol	750
Methadone	1000
Morphine	1000
Oxazepam	1000
Phencyclidine	500
Propoxyphene	1000
Secobarbital	1000

Therapeutic doses of the following drugs may produce positive results with this assay: chloroquine (Aralen®), chlorpromazine (Thorazine®), methoxyphenamine, quinicrine, phentermine, ranitidine (Zantac®), procainamide and its metabolite N-acetylprocainamide (NAPA). Because benzphetamine (Didrex®) metabolizes to amphetamine and methamphetamine, therapeutic doses of this drug may also produce a positive result.

Table 8 lists concentrations of compounds that were tested for at least one lot and were found to give a negative response.

11 Performance (cont.)

Table 8 — Concentrations of Compounds Tested for at Least One Lot Showing a Negative Response.

Compound	Concentration Tested (μg/mL)
Amitriptyline	1000
Desipramine	1000
Imipramine	1000
Isometheptene	100
d,l-Isoproterenol	1000
Isoxsuprine	500
Methylphenidate	1000
Nicotine	250
Nortriptyline	1000
Nylidrin	750
Phenelzine	100
Phthalic Acid	1000
Scopolamine	500
Trifluoperazine	500
Trimethoprim	500
Tyramine	100

12 Bibliography

1. Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Cox TC et al: Drugs and Drug Abuse, Addiction Research Foundation, 1983, pp 153-166.
3. AHFS Drug Information '87, American Society of Hospital Pharmacists, Inc, 1987, pp 1105-1107.
4. Gorodetzky CW: Detection of drugs of abuse in biological fluids, in Martin WR (ed): Drug Addiction I. New York, Springer-Verlag, 1977, pp 319-409.
5. Henry JB: Clinical Diagnosis and Management by Laboratory Methods. Philadelphia, WB Saunders Co: 1991, pp 868.
6. Syva ETS® Plus System Operator's Manual, Palo Alto, CA, Syva Co.
7. Syva ETS® Plus System Guide, Palo Alto, CA, Syva Co.
8. Notice of mandatory guidelines for federal workplace drug testing program; Final Guidelines. Federal Register 1988;69 (April 11):11983.
9. Erfurth SC: Methamphetamine stereoisomer resolution can prevent false positives. Forensic Urine Drug Testing, 1990, pp 6-7.
10. Fitzgerald RL et al: Resolution methamphetamine stereoisomers in urine drug testing: urinary excretion of R(—)-methamphetamine following use of nasal inhalers. J Anal Toxicol 1988;12:225-259.

Syva Australia, North Sydney, N.S.W. 2060, Australia
Syva Belgium, Bruxelles, Belgium
Syva Canada, Kanata, Ontario K2K 2A9, Canada
Syva Diagnostika, 2970 Hørsholm, Denmark
Syva U.K., Maidenhead, Berkshire SL6 1RD, England
Syva France, 69570 Dardilly, France
BRACCO Industria Chimica S.p.A., 20134 Milano, Italy
Nippon Syntex KK, Tokyo, Japan
División Diagnósticos Syntex, 11910 Mexico D.F., Mexico
Syva New Zealand, Linden, Wellington, New Zealand
Syva Norge A/S, 2000 Lillestrøm, Norway
Syntex Latino, División Diagnósticos, 08028 Barcelona, Spain
Syva Scandinavia, S-127 23 Skärholmen, Sweden
Syva Diagnostica, D-6100 Darmstadt, West Germany

For additional assistance, call Syva toll-free:
1-800-227-8994 in USA
1-800-257-6205 in Canada

Notice: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in this labeling can affect performance characteristics and stated or implied labeling claims.

Syva Company
3403 Yerba Buena Road
P.O. Box 49013
San Jose, CA 95161-9013

Syva a Syntex company

November 199
Printed in US
3C534UL.10E

GENERAL GS/MS TOXI-A AND TOXI-B TUBE SAMPLE EXTRACTION PROCEDURE

This procedure describes the use of TOXI-A or TOXI-B extraction tubes for sample extraction in preparation for GS/MS analysis.

Materials

TOXI-A extraction tubes

Extraction of basic and neutral drugs @ pH 9.

TOXI-B extraction tubes

Extraction of acidic and neutral drugs @ pH 4.5.

A. EXTRACTION

1. **Place 5 mL of urine in TOXI-A OR TOXI-B extraction tube.**
2. **Mix tube on mixer for a minimum of 5 minutes.**
3. **Centrifuge @ 2500 rpm for 5 minutes.**

B. CONCENTRATION

1. **Transfer upper organic layer to a centrifuge tube or preheated evaporation cup on hot plate.**
2. **Evaporate to approximately 50 microliters.**
3. **Place sample into a 100 microliter GC/MS vial insert and cap.**

C. ANALYSIS

1. **Inject 1-2 microliters into GC/MS.**
2. **Acquire data in FULL SCAN or monitor selected ions in SIM mode.**

METHAMPHETAMINE AND OTHER SYMPATHOMIMETIC AMINES

I. METHAMPHETAMINE, AMPHETAMINE, PHENTERMINE, AND OTHER SYMPATHOMIMETIC AMINES:

OPTION A] REFER TO GENERAL GS/MS SAMPLE EXTRACTION PROCEDURE WITH TOXI-A EXTRACTION TUBE.

OPTION B] REFER TO SPEC 511801.01: MP1 EXTRACTION OF AMPHETAMINE AND METHAMPHETAMINE FROM URINE.

SPEC®•3ML•MP1 EXTRACTION OF AMPHETAMINE AND METHAMPHETAMINE FROM URINE

-Vacuum Method-

This procedure describes the use of the SPEC®•3ML•MP1 Microcolumn* for the extraction of amphetamine and methamphetamine from urine. Following elution from the SPEC extraction disc and derivatization, amphetamine and methamphetamine may be detected by GC/MS or other analytical techniques.

Materials

SPEC•3ML•MP1 Microcolumns* (15 mg), SPEC Cat. No. 531-11-20
Reacti-Therm® Heating Module, Pierce Cat. No. 18800 or equivalent
Reacti-Vap® Evaporator, Pierce Cat. No. 18790 or equivalent
Flat-bottomed glass vials with screw-top caps, Baxter Cat. No. B7799-21
dl-Amphetamine-D_5 Reference Standard, Radian Cat. No. A-005
dl-Methamphetamine-D_5 Reference Standard, Radian Cat. No. M-004
Volumetric flasks, 10-mL, 100-mL, 500-mL, 1-L
Vacuum manifold/pump
Test tubes, 16- x 100-mm

Reagents

Heptafluorobutyric acid anhydride (HFBA), Pierce Cat. No. 63164 or equivalent
Potassium phosphate monobasic (KH_2PO_4) (ACS)
Potassium hydroxide (KOH) (ACS)
Ammonium hydroxide (NH_4OH), concd (ACS)
Hydrochloric acid (HCl), concd (ACS)
Ethyl acetate (TOXI•LAB Grade), Cat. No. 202
Glacial acetic acid (ACS)
Methanol (chromatography grade)
Water (deionized)

Solutions

1.0 M Potassium hydroxide (1.0 M KOH) (100 mL)

Dissolve 5.6 g of KOH in 80 mL of deionized water in 100-mL volumetric flask. Bring to volume with deionized water and mix.

0.1 M Phosphate buffer, pH 6.0 (100 mL)

Dissolve 13.61 g of KH_2PO_4 in 900 mL of deionized water in 1-L volumetric flask. Adjust pH to 6.0 with 1.0 M KOH. Bring to volume with deionized water and mix.

1.0 M Acetic acid (500 mL)

To 500-mL volumetric flask half filled with deionized water, add 28.6 mL of glacial acetic acid and mix. Bring to volume with deionized water and mix.

*Patent Pending

1% Acidic methanol (10 mL)

Pipet 100 µL of concentrated HCl into 10-mL volumetric flask half filled with methanol. Bring to volume with methanol and mix.

Elution solvent (2% ammonium hydroxide in ethyl acetate) (100 mL)

Pipet 2 mL of NH_4OH (concd) into 98 mL of ethyl acetate. Shake vigorously. Make fresh daily.

A. SAMPLE PRETREATMENT

1. Add 2 mL of urine specimen to a labeled test tube.
2. Add internal standard (e.g., dl-amphetamine-D_5 and dl-methamphetamine-D_5), if required.
3. Add 700 µL of 0.1 M phosphate buffer, pH 6.0. Vortex.

B. EXTRACTION

Note: All vacuum aspiration rates are at <5 in. Hg (17 kPa) unless otherwise noted (1 in. Hg = 3.4 kPa).

1. Insert labeled SPEC•3ML•MP1 Microcolumn in vacuum manifold.
2. Add 100 µL of methanol to microcolumn and aspirate. Stop vacuum. Do not allow disc to dry.
3. Immediately add 200 µL of 0.1 M phosphate buffer, pH 6.0, to microcolumn and aspirate.
4. Pour prepared sample into microcolumn and aspirate.
5. Add 500 µL of 1.0 M acetic acid and aspirate. Increase vacuum to 5-10 in. Hg (17-34 kPa) and dry extraction disc for 5 min.
6. Add 500 µL of methanol to microcolumn and aspirate. Increase vacuum to 10-20 in. Hg (34-68 kPa) and dry disc for 3 min.
7. Open vacuum manifold, wipe collection tips, and insert collection rack holding labeled flat-bottomed vials in vacuum manifold.
8. Add 0.5 mL of elution solvent. Aspirate slowly at <2 in. Hg (6.8 kPa).

C. DERIVATIZATION

1. Remove collection vials with eluates from rack, add 50 µL of 1% acidic methanol, and vortex.
2. Evaporate to dryness under gentle stream of nitrogen at room temperature (approximately 3 min).
3. Add 20 µL of HFBA. Cap immediately and vortex. Heat for 20 min at 70° C.
4. Cool to room temperature and add 700 µL of ethyl acetate. Cap and vortex.
5. Inject 1 µL of extract into GC/MS.
6. If analyzing in SIM mode, monitor ions at mass 91, 118, and 240 for amphetamine and mass 118, 210, and 254 for methamphetamine.

   If using full-scan data acquisition, scan from 50-350 amu.

COCAINE AND COCAINE METABOLITES

I. COCAINE, ECGONINE METHYLESTER, COCAETHYLENE, AND ECGONINE ETHYLESTER:
REFER TO GENERAL GS/MS SAMPLE EXTRACTION PROCEDURE WITH TOXI-A EXTRACTION TUBE.

II. BENZOYLECGONINE
REFER TO SPEC 311202.01: MP1 EXTRACTION OF BENZOYLECGONINE FROM URINE USING ON-DISC DERIVATIZATION.

TOXI·LAB®
SPEC®·VC·MP1 EXTRACTION OF BENZOYLECGONINE FROM URINE USING ON·DISC™ DERIVATIZATION*

-Vacuum Method-

This procedure describes the use of the TOXI·LAB® SPEC®·VC·MP1 Microcolumn* for the extraction of benzoylecgonine (BE), a cocaine metabolite, from urine. Following extraction and ON·DISC™ Derivatization, benzoylecgonine is detected by GC/MS or other analytical techniques.

Materials

SPEC·VC·MP1 Microcolumns (15 mg), TOXI·LAB Cat. No. 500-11-20
Disposable Concentration Cups, TOXI·LAB Cat. No. 152
Omega-12 (cup holder), TOXI·LAB Cat. No. 153
Disc-Handling Pins, TOXI·LAB Cat. No. 186
Custom Electric Warmer, TOXI·LAB Cat. No. 118
Reacti-Therm™ Heating Module, Pierce Cat. No. 18800 or equivalent
Flat-bottomed Sample Vials with caps, 15- x 45-mm, 4-mL, Baxter Cat. No. B7799-21
Benzoylecgonine standard, Sigma® Chemical Co., Cat. No. B8900
Benzoylecgonine-D_3 reference standard, Sigma Chemical Co. Cat. No. B3277
Volumetric flasks, 100-mL, 500-mL
Vacuum manifold/pump
Test tubes, 16- x 100-mm

Reagents

N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), Pierce Cat. No. 48910 or equivalent
Acetone (ACS)
Ethyl acetate (ACS)
Hydrochloric acid (HCl), concd (ACS)
Methanol (MeOH) (chromatography grade)
Water (deionized)

Solutions

Hydrochloric acid, 0.1N

To a 500-mL volumetric flask half-filled with deionized water, add 4.2 mL of concentrated HCl and mix. Bring to volume with deionized water and mix.

20% Acetone/water

To 16 mL of distilled water, add 4.0 mL of acetone. Mix. Make fresh daily.

A. SAMPLE PREPARATION

1. Add 3 mL of sample to labeled test tube.
2. Add internal standard (e.g., BE-D_3), if required.
3. Add 4 mL of 0.1N HCl to tube and mix. pH <2.0.

NOTE:Before start of extraction, place Omega-12 with cups on hot plate to preheat cups.

B. EXTRACTION

1. Insert SPEC·VC·MP1 Microcolumn in vacuum manifold.
2. Add 200 µL of MeOH to sample reservoir.
3. Aspirate through sample reservoir at 5 in. Hg. Stop vacuum.
4. Add 200 µL of 0.1N HCl to sample reservoir and aspirate. Increase to approximately 15 in. Hg until all fluid is taken through microcolumn. Turn off vacuum.
5. Pour sample into sample reservoir. Turn on vacuum and maintain a flow rate of 2 mL/min (approximately 2 in. Hg) until sample has gone through microcolumn. Increase to 15 in. Hg to completely empty column and until no air bubbles are observed at neck of column.
6. Remove sample reservoir and add 500 µL of 0.1N HCl to reagent reservoir. Maintain a flow rate of 2 mL/min (approximately 1 in. Hg.) Increase to 5 in. Hg for 1 min.
7. Add 500 µL of 20% acetone/water to reagent reservoir. Maintain a flow rate of 2 mL/min (approximately 1 in. Hg.) After liquid has gone through, turn off pump, remove reagent reservoir to expose disc completely. Increase vacuum to approximately 10 in. Hg for 1 min.
8. Remove disc from holder and place in concentration cup in Omega-12 cup holder. Place Omega-12 on electric warmer for 10 minutes to dry disc. Temperature inside cup should be 70°–75° C. It is important that the disc be completely dry before starting derivatization procedure.
9. Transfer disc to 15- x 45-mm sample vial.

C. DERIVATIZATION

1. To the sample vial containing disc, add 50 µL of MSTFA, ensuring that disc is completely wetted with liquid.
2. Cap immediately, shake vial gently, and heat at 90° C for 15 min. on heating module.
3. Cool. Add 50 µL of ethyl acetate to solubilize derivatized drug off disc and shake gently by hand.
4. Inject 1–2 µL into GC/MS. If using an autosampler, transfer to appropriate vial.
5. If analyzing samples in SIM mode, monitor ions at mass 361, 346, 256, and 240. If using full-scan data acquisition, scan from 50–400.

*Patent Pending

MARIHUANA METABOLITE

I. 11-NOR-^9-TETRAHYDROCANNABINOL-9-CARBOXYLIC ACID (^9-THC-COOH)

REFER TO SPEC 303201.01: VC-C18 EXTRACTION OF THE THC METABOLITE FROM URINE USING ON-DISC DERIVATIZATION.

TOXI·LAB®
SPEC®·VC·C18 EXTRACTION OF THC METABOLITE FROM URINE USING ON·DISC™ DERIVATIZATION*

-Vacuum Method-

This procedure describes the use of the TOXI·LAB® SPEC®·VC·C18 Microcolumn for the extraction of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (Δ^9-THC-COOH) from urine. Following extraction and ON·DISC™ Derivatization, Δ^9-THC-COOH may be detected by GC/MS or other analytical techniques.

Materials

SPEC·VC·C18 Microcolumns*, TOXI·LAB Cat. No. 500-03-10 (100/pkg)
Hydrolysis tubes with caps, TOXI·LAB Cat. No. 475
Disc-Handling Pins, TOXI·LAB Cat. No. 186
Reacti-Therm™ Heating Module, Pierce Cat. No. 18800 or equivalent
Flat-bottomed Sample Vials with caps, 15- x 45-mm, 4-mL, Baxter Cat. No. B7799-21
11-nor-9-carboxy-Δ^9-THC-D_3 Reference standard, Radian Cat. No. T-004
Vacuum manifold/pump
Volumetric flask, 100-mL

Reagents

TOXI·LAB Hydrolysis Reagent (11.8N potassium hydroxide), Cat. No. 207-4
N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), Pierce Cat. No. 48910 or equivalent
Glacial acetic acid (ACS)
Water (deionized)

Solutions

20% Acetic acid

To a 100-mL volumetric flask half filled with deionized water, add 20 mL of glacial acetic acid and mix. Bring to volume with deionized water and mix.

A. SAMPLE HYDROLYSIS

1. Add 3 mL of urine specimen to labeled hydrolysis tube.
2. Add internal standard (e.g., Δ^9-THC-COOH-D_3), if required.
3. Add 200 µL of TOXI·LAB Hydrolysis Reagent. Vortex.
4. Heat specimen for 15 min at 60° C. Cool to room temperature.
5. Add 700 µL of glacial acetic acid. Vortex.

B. EXTRACTION

1. Insert SPEC·VC·C18 in vacuum manifold.
2. Pour hydrolyzed sample into sample reservoir. Initiate sample flow at 15 in. Hg. Reduce vacuum to 5 in. Hg and continue aspiration at 2 mL/min. Stop vacuum.
3. Remove sample reservoir and discard.
4. Add 1 mL of 20% acetic acid to reagent reservoir and aspirate at 2 mL/min.
5. Increase vacuum (15-20 in. Hg) and dry extraction disc for a minimum of 10 min.

Note: Disc must be completely dry before proceeding.

C. DERIVATIZATION

1. Remove disc assembly (reagent reservoir + disc holder) from vacuum manifold. Remove reagent reservoir from disc holder.
2. Using disc-handling pin, remove extraction disc from disc holder and transfer disc to flat-bottomed vial.
3. Add 100 µL of MSTFA, ensuring that disc is completely submerged in solution.
4. Cap immediately and heat for 10 min at 90° C.
5. Cool and inject 1-2 µL of extract into GC/MS. If using an autosampler, transfer extract to 100-µL insert in autosampler vial.
6. Monitor ions at mass 371, 473, and 488.

*Patent Pending

BENZODIAZEPINES: HYDROLYSIS PROCEDURE
AMINOBENZOPHENONE PRODUCTION

This procedure describes the use of the benzodiazepine hydrolysis method for the extraction of benzodiazepines from urine. Upon hydrolysis with acid and heat, both diazepam and nordiazepam form amino-benzophenones which may be detected by GC/MS or other analytical technique.

Materials/Reagents
TOXI-B extraction tubes
Hydrolysis tubes with caps
TOXI-DISCS impregnated with diazepam and nordiazepam
Water bath (capable of 100C) or autoclave (115C at 25 psi)
Concentrated hydrochloric acid

A. ACID HYDROLYSIS

1. **Place the diazepam/nordiazepam standard disc into a 10 mL screw-top hydrolysis tube containing 4 mL deionized water and 1 mL concentrated HCl. Cap and mix by inversion for 10 seconds. Prepare an additional tube with 4 mL deionized water and 1 mL HCl for the negative control.**

2. **Place 1 mL of specimen into a 10-mL screw-top hydrolysis bue containing 3 mL deionized water and 1 mL concentrated HCl. Cap and mix by inversion for 10 seconds.**

3. **Place tubes in a water bath at 100C for a minimum of 30 minutes. If an autoclave is used for the hydrolysis, the tubes should be heated at 115C @ 25 psi for a minimum of 15 minutes. These conditions should result in complete hydrolysis of the benzodiazepines to amino-benzophenones.**

4. **Remove the tubes from water bath/autoclave after specified time period and allow to cool to room temperature.**

B. EXTRACTION

1. **Extract the hydrolysate in a TOXI-B extraction tube per TOXI-B Instruction Manual. (Neutralization of the hydrolysate is not necessary.)**

C. CONCENTRATION

1. **Evaporate each organic layer of TOXI-B extraction to approximately 50 microliters and transfer to a 100 microliter GC-MS vial insert.**

D. ANALYSIS

1. **Inject 1-2 microliters of extraction into GC/MS.**

2. **Monitor ions at mass 230, 231, 232, 233, 234, 154, 126 and 77 for 2-amino-5-chloro-benzophenone (from nordiazepam) and at 244, 245, 246, 247, 248, 249, 228, 193 and 168 for 2-methylamino-5-chlorobenzophenone (from diazepam), or acquire using full scan mode.**

BENZODIAZEPINES

I. BENZODIAZEPINES INCLUDING BUT NOT LIMITED TO:
DIAZEPAM, LORAZEPAM, PRAZEPAM, OXAZEPAM, FLURAZEPAM, CHLORDIAZEPOXIDE, ALPRAZOLAM, TEMAZEPAM, CHLORAZEPATE, HALAZEPAM, AND TRIAZOLAM.

OPTION A] AMINOBENZOPHENONE PRODUCTION

1. DIRECT EXTRACTION ACID HYDROLYSIS PROCEDURE

2. REFER TO SPEC 320803.01: MP3 DISC ELUTION ACID HYDROLYSIS

BENZODIAZEPINES WHICH WILL FORM AMINOBENZOPHENONES INCLUDE CHLORAZEPOXIDE, CLORAZEPATE, DIAZEPAM, NORDIAZEPAM, TEMAZEPAM, OXAZEPAM, HALAZEPAM, AND PRAZEPAM.

BENZODIAZEPINES WHICH WILL NOT PRODUCE AMINOBENZOPHENONES INCLUDE ALPRAZOLAM, LORAZEPAM AND TRIAZOLAM.

OPTION B] REFER TO SPEC 520803.01: MP3 EXTRACTION OF BENZODIAZEPINES FROM URINE (ENZYME HYDROLYSIS).

SPEC®•3ML•MP3 EXTRACTION OF BENZODIAZEPINES FROM URINE (ENZYME HYDROLYSIS)

-Vacuum Method-

This procedure describes the use of the SPEC®•3ML•MP3 Microcolumn* for the extraction of benzodiazepines from urine. Following elution from the SPEC extraction disc, benzodiazepines may be detected by GC/MS or other analytical techniques.

Materials

SPEC•3ML•MP3 Microcolumns* (15 mg), SPEC Cat. No.531-20-20
Hydrolysis Tubes with Caps, TOXI•LAB Cat. No. 475
Reacti-Therm® Heating Module, Pierce Cat. No. 18800 or equivalent
Reacti-Vap® Evaporator, Pierce Cat. No. 18790 or equivalent
Flat-bottomed glass vials with screw-top caps, Baxter Cat. No. B7799-21
Volumetric flasks, 50-mL, 100-mL
Vacuum manifold/pump
pH paper

Reagents

Ethyl acetate, TOXI•LAB Grade, Cat. No. 202
Potassium hydroxide (KOH) (ACS), 11.8N, TOXI•LAB Cat. No. 207-4
Potassium phosphate (K_2HPO_4), MW 174.18, Fisher Cat. No. P288
β-Glucuronidase (*Patella vulgata*), lyophilized, 2,000,000 units, Sigma Cat. No. CG-8132
N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), Pierce Cat. No. 48910 or equivalent
Ammonium hydroxide (NH_4OH), concd (ACS)
Glacial acetic acid (ACS)
Hydrochloric acid (HCl), concd
Methanol (chromatography grade)
Water (deionized)

Solutions

β-Glucuronidase (*Patella vulgata*), 5000 units/mL (50 mL)

Weigh 100 mg of lyophilized β-glucuronidase powder into 50-mL volumetric flask. Bring to volume with deionized water. Mix well. Store at 2°–8° C. Stable for 1 month. Bring to room temperature before use.

1.0 M Acetate buffer, pH 3.8 (100 mL)

Add 90 mL of deionized water to 250-mL beaker. Pipet 5.7 mL of glacial acetic acid into beaker. Stir. Adjust to pH 3.8 with 11.8N KOH. Transfer to 100-mL volumetric flask and bring to volume with deionized water. Mix well.

0.1 M Potassium phosphate solution (100 mL)

Dissolve 1.74 g of K_2HPO_4 in deionized water. Bring to final volume of 100 mL. Mix well.

Elution solvent (2% ammonium hydroxide in ethyl acetate)

Mix 98 mL of ethyl acetate with 2 mL NH_4OH and shake vigorously. Make fresh daily.

*Patent Pending

A. SAMPLE PRETREATMENT

1. Add 1.0 mL of urine specimen to labeled hydrolysis tube.
2. Add internal standard (e.g., prazepam), if required.
3. Add 100 µL of 1.0 M acetate buffer, pH 3.8. Vortex.

B. ENZYME HYDROLYSIS

1. Add 0.5 mL of *Patella vulgata* solution to sample. Cap and vortex.
2. Heat sample at 60° C for 2 hours.
3. Cool sample to room temperature.
4. Add 1.0 mL of potassium phosphate solution. Vortex.
5. Check the pH of the sample with pH paper. The pH should be between 8.0 and 9.0. Adjust with KOH or HCl if necessary.
6. Centrifuge sample for 5 min at 3000–3500 rpm.

C. EXTRACTION

Note: All vacuum aspiration rates are at <5 in. Hg (17 kPa) unless otherwise noted (1 in. Hg = 3.4 kPa).

1. Insert labeled SPEC•3ML•MP3 Microcolumn in vacuum manifold.
2. Add 200 µL of methanol to reservoir and aspirate. Stop vacuum. Do not allow disc to dry.
3. Immediately add 200 µL of potassium phosphate solution to reservoir and aspirate.
4. Pour sample into reservoir and aspirate.
5. Add 200 µL of water and aspirate.
6. Add 200 µL of potassium phosphate solution and aspirate.
7. Increase vacuum to 10-20 in. Hg (34-68 kPa) and dry extraction disc for a minimum of 5 min.
8. Open vacuum manifold, wipe collection tips, and insert collection rack holding labeled flat-bottomed vials in vacuum manifold.
9. Add 0.5 mL of elution solvent and allow to drip through disc into collection vial. Apply gentle vacuum (≤1 in. Hg, 3.4 kPa) to assist final amount of elution solvent through disc.
10. Repeat step 9 to ensure complete elution.

D. CONCENTRATION AND DERIVATIZATION[1]

1. Evaporate eluate to dryness under gentle stream of nitrogen at 35° C.
2. Add 50 µL of MSTFA. Cap immediately. Vortex.
3. Heat for 10 min at 60° C. Cool to room temperature.
4. Inject 1 µL of extract into GC/MS.
5. Monitor ions at mass 269 for prazepam, mass 256 for diazepam, mass 329 for oxazepam, mass 341 for nordiazepam, mass 86 for flurazepam, and mass 282 for chlordiazepoxide[1].

[1]Chlordiazepoxide will decompose with derivatizing agent/heat. To analyze chlordiazepoxide, in Step 2 substitute 50 µL of ethyl acetate and vortex. Skip Step 3.

SPEC®·VC·MP3 EXTRACTION OF BENZODIAZEPINES FROM URINE USING DISC ELUTION (ACID HYDROLYSIS)

-Vacuum Method-

This procedure describes the use of the SPEC®·VC·MP3 Microcolumn* for the extraction of benzodiazepines as benzophenones from urine. Following elution the benzophenones may be detected by GC/MS or other analytical techniques.

Materials

SPEC·VC·MP3 Microcolumns (15 mg), Cat. No. 500-20-20
Hydrolysis Tubes with Caps, TOXI·LAB Cat. No. 475
2-Amino-5-chlorobenzophenone (ACB), Sigma Cat. No. A4632
2-Methylamino-5-chlorobenzophenone (MACB), Aldrich Cat. No. 19, 135-3
Reacti-Therm® Heating Module, Pierce Cat. No. 18800 or equivalent
Reacti-Vap® Evaporator, Pierce Cat. No. 18790 or equivalent
Flat-bottomed glass vials with screw-top caps, Baxter Cat. No. B7799-21
Volumetric flasks, 1-L, 100-mL, 500-mL
Vacuum manifold/pump
pH paper

Reagents

Ethyl Acetate, TOXI·LAB Grade, Cat. No. 202
Potassium hydroxide (KOH) (ACS), 11.8 N, TOXI·LAB Cat. No. 207-4
Boric acid (M.W. 61.83), Sigma Cat. No. B-0252
Methylene chloride ($MeCl_2$) (chromatography grade)
Isopropyl alcohol (IPA) (chromatography grade)
Potassium hydroxide (KOH) (ACS), M.W. 56.11
Methanol (MeOH) (chromatography grade)
Hydrochloric acid (HCl), concd (ACS)
Ammonium hydroxide (NH_4OH), concd (ACS)
Water (deionized)

Solutions

1.0 M Hydrochloric acid (HCl) (100 mL)

To a 100-mL volumetric flask half-filled with deionized water, add 8.3 mL of concentrated HCl. Bring to volume with deionized water and mix.

1.0 M Potassium hydroxide (KOH) (100 mL)

Dissolve 5.6 g of KOH in approximately 90 mL of deionized water and bring to a final volume of 100 mL. Mix well.

Boric acid solution (1000 mL)

Dissolve 12.37 g of boric acid in approximately 500 mL of deionized water. Add 100 mL of 1.0 M KOH and bring to a final volume of 1 L with deionized water. Mix well.

0.1 M Hydrochloric acid (HCl) (500 mL)

To a 500-mL volumetric flask half-filled with deionized water, add 4.2 mL of concentrated HCl. Bring to volume with deionized water and mix well.

*Patent Pending

Borate buffer, pH 8.5 (100 mL)

Combine 66 mL of boric acid solution and 34 mL of 0.1 M HCl. pH should equal 8.5. If necessary, use HCl or KOH solution to adjust pH.

Elution solvent (100 mL)

Mix 80 mL of $MeCl_2$ with 20 mL of IPA. Add 2.0 mL of NH_4OH and shake vigorously. Make fresh daily.

A. SAMPLE PRETREATMENT

1. Add 0.5 mL of urine specimen to labeled hydrolysis tube.
2. Add internal standard, if required.

B. ACID HYDROLYSIS

1. Add 100 µL of concentrated HCl to sample. Cap and vortex.
2. Hydrolyze for 20 min in steam autoclave at 125° C and 15 psi.
3. Cool sample to room temperature.
4. Add 2.0 mL of boric acid solution. Vortex. Add 100 µL of 11.8 N KOH solution. Vortex.
5. Check the pH of the sample with pH paper. The pH should be between 8.0 and 9.0. Adjust with KOH or HCl solution, if necessary.

C. EXTRACTION

Note: All vacuum aspiration rates are at ≤5 in. Hg unless otherwise noted.

1. Insert labeled SPEC·VC·MP3 Microcolumn in vacuum manifold.
2. Add 200 µL of methanol to sample reservoir and aspirate. Stop vacuum. Do not allow disc to dry.
3. Immediately add 200 µL of borate buffer, pH 8.5, to sample reservoir and aspirate.
4. Pour sample into sample reservoir and aspirate.
5. Remove sample reservoir and discard.
6. Add 200 µL of water and aspirate.
7. Increase vacuum (10–20 in. Hg) and dry extraction disc for a minimum of 5 min.
8. Open vacuum manifold, wipe collection tips, and insert collection rack holding labeled, flat-bottomed vials into vacuum manifold.
9. Add 0.5 mL of elution solvent and allow to drip through disc into collection vial. Apply gentle vacuum (1–2 in. Hg) to assist final amount of elution solvent through disc.

D. CONCENTRATION AND RECONSTITUTION

1. Evaporate eluate to dryness under gentle stream of nitrogen at 35° C.
2. Add 100 µL of ethyl acetate. Vortex.
3. Inject 1 µL of extract into GC/MS.
4. If analyzing sample in SIM mode, monitor ions at mass 230, 231, 232 for ACB and mass 244, 245, 246 for MACB.

   If using full-scan data acquisition, scan from 50–600 amu.

OPIATES

I. OPIATES INCLUDING BUT NOT LIMITED TO:
CODEINE, HYDROCODONE, OXYCODONE, HEROIN, MORPHINE, HYDROCODEINE, AND HYDROMORPHONE.

OPTION A] REFER TO GENERAL GS/MS SAMPLE EXTRACTION PROCEDURE WITH TOXI-A EXTRACTION TUBE.

OPTION B] REFER TO SPEC 320802.01: MP3 EXTRACTION OF CODEINE AND MORPHINE FROM URINE USING DISC ELUTION (ACID HYDROLYSIS).

OPTION C] REFER TO SPEC 320802.01E: MP3 EXTRACTION OF CODEINE AND MORPHINE FROM URINE USING DISC ELUTION (ENZYME HYDROLYSIS).

SPEC®•VC•MP3 EXTRACTION OF CODEINE AND MORPHINE FROM URINE USING DISC ELUTION (ACID HYDROLYSIS)

-Vacuum Method-

This procedure describes the use of the SPEC®•VC•MP3 Microcolumn* for the extraction of codeine and morphine from urine. Following elution and derivatization, codeine and morphine may be detected by GC/MS or other analytical techniques.

Materials

SPEC•VC•MP3 Microcolumns (30 mg), Cat. No. 500-20-30
Hydrolysis Tubes with Caps, TOXI•LAB Cat. No. 475
Flat-bottomed Sample Vials, Screw Cap, Baxter Cat. No. B7799-21
Codeine-D_3 Reference Standard, Radian Cat. No. C-005
Morphine-D_3 Reference Standard, Radian Cat. No. M-003
Reacti-Therm® Heating Module, Pierce Cat. No. 18800 or equivalent
Reacti-Vap® Evaporator, Pierce Cat. No. 18780 or equivalent
Volumetric flask, 500-mL
Vacuum manifold/pump
pH paper

Reagents

Potassium hydroxide (KOH) (ACS), 11.8 N, TOXI•LAB Cat. No. 207-4
Hydrochloric acid (HCl), concd (ACS)
Glacial acetic acid (ACS)
Methanol (MeOH) (chromatography grade)
Methylene chloride ($MeCl_2$) (chromatography grade)
Isopropyl alcohol (IPA) (chromatography grade)
Ammonium hydroxide (NH_4OH), concd (ACS)
N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), Pierce Cat. No. 48910
Water (deionized)

Solutions

0.1 M Acetic acid

To a 500-mL volumetric flask half-filled with deionized water, add 2.9 mL of glacial acetic acid and mix. Bring to volume with deionized water and mix.

Elution solvent

Mix 80 mL of $MeCl_2$ with 20 mL of IPA. Add 2.0 mL of NH_4OH and shake vigorously. Make fresh daily.

*Patent Pending

A. SAMPLE PRETREATMENT

1. Add 0.5 mL of urine specimen to labeled hydrolysis tube.
2. Add internal standard (e.g., codeine-D_3 and morphine-D_3), if required.

B. ACID HYDROLYSIS

1. Add 50 µL of concentrated HCl to sample. Cap and vortex.
2. Hydrolyze for 20 min in steam autoclave at 125° C and 15 psi (100 kPa).
3. Cool sample.
4. Add 2.0 mL of 0.1 M acetic acid. Vortex. Add 50 µL of 11.8 N KOH solution. Vortex.
5. Check the pH of the sample with pH paper. The pH should be between 3.0 and 4.0. Adjust with KOH or HCl if necessary.
6. Centrifuge sample.

C. EXTRACTION

Note: All vacuum aspiration rates are at <2 in. Hg (<7 kPa) unless otherwise noted (1 in. Hg = 3.4 kPa).

1. Insert labeled SPEC•VC•MP3 Microcolumn in vacuum manifold.
2. Add 200 µL of methanol to reservoir and aspirate. Stop vacuum. Do not allow disc to dry.
3. Immediately add 200 µL of 0.1 M acetic acid to reservoir and aspirate.
4. Decant sample into reservoir and aspirate.
5. Remove reservoir and discard.
6. Wash sequentially with 200 µL of water, 200 µL of 0.1 M acetic acid, and 200 µL of methanol.
7. Increase vacuum to 10–20 in. Hg (34–68 kPa) and dry extraction disc for a minimum of 5 min.
8. Open vacuum manifold, wipe collection tips, and insert collection rack holding labeled sample vials in vacuum manifold.
9. Add 0.5 mL of elution solvent and allow to drip through disc into collection vial. Apply gentle vacuum to assist final amount of elution solvent through disc. Repeat one time.

D. DERIVATIZATION

1. Evaporate eluate to dryness under gentle stream of nitrogen at 35° C.
2. Add 100 µL of MSTFA. Cap immediately and vortex.
3. Heat for 10 min at 90° C. Cool.
4. Inject 1 µL of extract into GC/MS.
5. If analyzing sample in SIM mode, monitor ions at mass 429 for morphine, mass 371 for codeine, mass 432 for morphine-D_3, and mass 374 for codeine-D_3.

   If using full-scan data acquisition, scan from 50–600 amu.

TOXI·LAB®
SPEC®·VC·MP3 EXTRACTION OF CODEINE AND MORPHINE FROM URINE USING DISC ELUTION (ENZYME HYDROLYSIS)

-Vacuum Method-

This procedure describes the use of the TOXI·LAB® SPEC®·VC·MP3 Microcolumn* for the extraction of codeine and morphine from urine. Following elution and derivatization, codeine and morphine may be detected by GC/MS or other analytical techniques.

Materials

SPEC·VC·MP3 Microcolumns (30 mg), Cat. No. 500-20-30
Hydrolysis Tubes with Caps, TOXI-LAB Cat. No. 475
Codeine-D_3 Reference Standard, Radian Cat. No. M-003
Morphine-D_3 Reference Standard, Radian Cat. No. C-005
Flat-bottomed glass vials with screw-top caps, Baxter Cat. No. B7799-21
Reacti-Therm® Heating Module, Pierce Cat. No. 18800 or equivalent
Reacti-Vap® Evaporator, Pierce Cat. No. 18780 or equivalent
Volumetric flasks, 50-mL, 100-mL, 500-mL
Vacuum manifold/pump
pH paper

Reagents

Potassium hydroxide (KOH) (ACS), 11.8 N, TOXI-LAB Cat. No. 207-4
β-Glucuronidase (*Patella vulgata*), lyophilized, 2,000,000 units, Sigma Cat. No. G8132
Hydrochloric acid (HCl), concd (ACS)
Glacial acetic acid (ACS)
Methanol (MeOH) (chromatography grade)
Methylene chloride ($MeCl_2$) (chromatography grade)
Isopropyl alcohol (IPA) (chromatography grade)
Ammonium hydroxide (NH_4OH), concd (ACS)
N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), Pierce Cat. No. 48910
Water (deionized)

Solutions

1.0 M Acetate buffer, pH 3.8 (100 mL)

Add 90 mL of deionized water to a 250-mL beaker. Pipet 5.7 mL of glacial acetic acid into beaker. Stir. Adjust pH to 3.8 with 11.8N KOH. Transfer to a 100-mL volumetric flask and bring to volume with deionized water. Mix well.

β-Glucuronidase (*Patella vulgata*) (5000 units/mL) (50 mL)

Weigh 100 mg of lyophilized β-Glucuronidase powder into a 50-mL volumetric flask. Bring to volume with deionized water. Mix well. Store at 2°–8° C. Stable for 1 month. Bring to room temperature before use.

0.1 M Acetic acid

To a 500-mL volumetric flask half-filled with deionized water, add 2.9 mL of glacial acetic acid and mix. Bring to volume with deionized water and mix.

Elution solvent

Mix 80 mL of $MeCl_2$ with 20 mL of IPA. Add 2.0 mL of NH_4OH and shake vigorously. Make fresh daily.

A. SAMPLE PRETREATMENT

1. Add 0.5 mL of urine specimen to labeled hydrolysis tube.
2. Add internal standard (e.g., codeine-D_3 and morphine-D_3), if required.
3. Add 100 µL of 1.0 M acetate buffer, pH 3.8. Vortex. pH should be 3.8-4.4.

B. ENZYME HYDROLYSIS

1. Add 0.5 mL of *Patella vulgata* solution to sample. Cap and vortex.
2. Heat sample at 60° C for 2 hours.
3. Cool sample.
4. Add 1.5 mL of 0.1 M acetic acid.
5. Check the pH of the sample with pH paper. The pH should be between 3.0 and 4.0. Adjust with KOH or HCl if necessary.
6. Centrifuge sample.

C. EXTRACTION

Note: All vacuum aspiration rates are at <5 in. Hg unless otherwise noted.

1. Insert labeled SPEC·VC·MP3 Microcolumn in vacuum manifold.
2. Add 200 µL of methanol to reservoir and aspirate. Stop vacuum. Do not allow disc to dry.
3. Immediately add 200 µL of 0.1 M acetic acid to reservoir and aspirate.
4. Decant sample into reservoir and aspirate.
5. Remove reservoir and discard.
6. Wash sequentially with 200 µL of water, 200 µL of 0.1 M acetic acid, and 200 µL of methanol.
7. Increase vacuum (10–20 in. Hg) and dry extraction disc for a minimum of 5 min.
8. Open vacuum manifold, wipe collection tips, and insert collection rack holding labeled flat-bottomed vials in vacuum manifold.
9. Add 0.5 mL of elution solvent and allow to drip through disc into collection vial. Apply gentle vacuum to assist final amount of elution solvent through disc. Repeat one time.

D. DERIVATIZATION

1. Evaporate eluate to dryness under gentle stream of nitrogen at 35° C.
2. Add 100 µL of MSTFA. Cap immediately and vortex.
3. Heat for 10 min at 90° C. Cool.
4. Inject 1 µL of extract into GC/MS.
5. If analyzing sample in SIM mode, monitor ions at mass 429 for morphine, mass 371 for codeine, mass 432 for morphine-D_3, and mass 374 for codeine-D_3.

   If using full-scan data acquisition, scan from 50–600 amu.

*Patent Pending

PHENCYCLIDINE
{PCP}

I. PHENCYCLIDINE

REFER TO SPEC 311005.01: MP1 EXTRACTION OF PHENCYCLIDINE FROM URINE

TOXI·LAB®
SPEC®·VC·MP1 EXTRACTION OF PHENCYCLIDINE FROM URINE
-Vacuum Method-

This procedure describes the use of the TOXI·LAB® SPEC®·VC·MP1 Microcolumn* for the extraction of phencyclidine (PCP) from urine. Following elution from the SPEC extraction disc, PCP may be detected by GC/MS or other analytical techniques.

Materials

SPEC·VC·MP1 Microcolumns (15 mg), TOXI·LAB Cat. No.500-11-20
Phencyclidine-D_5 Reference Standard, Radian Cat. No. P-003
Reacti-Therm® Heating Module, Pierce Cat. No. 18800 or equivalent
Reacti-Vap® Evaporator, Pierce Cat. No. 18790 or equivalent
Reacti-Vials®, Pierce Cat. No. 13222 or equivalent
Volumetric flasks, 100-mL, 500-mL, 1-L
Vacuum manifold/pump
Test tubes, 16- x 100-mm

Reagents

Ethyl Acetate (TOXI·LAB grade), Cat. No. 202
Potassium phosphate monobasic (KH_2PO_4) (ACS)
Potassium hydroxide (KOH) (ACS)
Ammonium hydroxide, concentrated (ACS)
Methanol (chromatography grade)
Glacial acetic acid (ACS)
Water (deionized)

Solutions

1.0 M Potassium hydroxide (1.0 M KOH)

Dissolve 5.6 g of KOH in 80 mL of deionized water in a 100-mL volumetric flask. Bring to volume with deionized water.

0.1 M Phosphate buffer, pH 6.0

Dissolve 13.61 g of KH_2PO_4 in 900 mL of deionized water in a 1-L volumetric flask. Adjust pH to 6.0 with 1.0 M KOH. Bring to volume with deionized water.

*Patent Pending

1.0 M Acetic acid

To a 500-mL volumetric flask half-filled with deionized water, add 28.6 mL of glacial acetic acid and mix. Bring to volume with deionized water and mix.

Elution solvent (2% ammonium hydroxide in ethyl acetate)

Pipet 2 mL of concentrated ammonium hydroxide into a 100-mL reagent container containing 98 mL of ethyl acetate. Shake vigorously. Prepare fresh daily.

A. SAMPLE PREPARATION

1. Add 2 mL of urine specimen to labeled test tube.
2. Add internal standard (e.g., PCP-D_5), if required.
3. Add 800 µL of 0.1 M phosphate buffer, pH 6.0. Vortex.

B. EXTRACTION

1. Insert labeled SPEC·VC·MP1 Microcolumn in vacuum manifold.
2. Add 500 µL of methanol to sample reservoir and aspirate at 2 mL/min. (<5 in. Hg). Stop vacuum.
3. Immediately add 500 µL of 0.1 M phosphate buffer to sample reservoir and aspirate at 2 mL/min. (<5 in. Hg).
4. Pour sample into sample reservoir and aspirate at 2 mL/min. (5 in. Hg).
5. Remove sample reservoir and discard.
6. Add 500 µL of 1.0 M acetic acid to reagent reservoir and aspirate at 2 mL/min. (<5 in. Hg).
7. Add 500 µL of methanol to reagent reservoir and aspirate at 2 mL/min. (<5 in. Hg).
8. Increase vacuum (5–10 in. Hg) and dry extraction disc for approximately 30 seconds.
9. Open vacuum manifold, wipe collection tips, and insert collection rack holding labeled Reacti-Vials.
10. Add 500 µL of elution solvent to reagent reservoir and aspirate slowly (1 in. Hg).

C. CONCENTRATION AND RECONSTITUTION

1. Remove Reacti-Vials from vacuum manifold and evaporate eluate to dryness under nitrogen at 35° C (approximately 5 min).
2. Reconstitute with 50 µL of ethyl acetate. Vortex.
3. Inject 1 µL of extract into GC/MS.
4. If analyzing samples in SIM mode, monitor ions at mass 200, 243, and 186. If using full-scan data acquisition, scan from 50–400 amu.

*Patent Pending

ANTIDEPRESSANTS

I. **ANTI-DEPRESSANTS AND THEIR METABOLITES INCLUDING BUT NOT LIMITED TO:**

AMITRIPTYLINE, NORTRIPTYLINE, IMIPRAMINE, DESIPRAMINE, DOXEPIN, AND DESMETHYLDOXEPIN.

OPTION A] **REFER TO GENERAL GS/MS SAMPLE EXTRACTION PROCEDURE WITH TOXI-A EXTRACTION TUBE.**

OPTION B] **REFER TO SPEC 201804.01: C18-II EXTRACTION OF ANTIDEPRESSANTS FROM URINE.**

SPEC®·VC·MP3 EXTRACTION OF TRICYCLIC ANTIDEPRESSANTS FROM SERUM OR URINE

-Vacuum Method-

This procedure describes the use of the SPEC®·VC·MP3 Microcolumn* for the extraction of tricyclic antidepressants (TCA) from serum or urine. Following elution from the SPEC extraction disc, TCA may be detected by HPLC with UV detection or other analytical techniques.

Materials

SPEC·VC·MP3 Microcolumns (15 mg), Cat. No.500-20-20
Reacti-Therm® Heating Module, Pierce Cat. No. 18800 or equivalent
Reacti-Vap® Evaporator, Pierce Cat. No. 18790 or equivalent
Flat-bottomed glass vials with screw-top caps, Baxter Cat. No. B7799-21
Volumetric flasks, 100-mL, 500-mL, 1-L
Vacuum manifold/pump
Test tubes, 16- x 100-mm

Reagents

Potassium phosphate monobasic (KH_2PO_4) (ACS)
Potassium hydroxide (KOH) (ACS)
Ammonium hydroxide (NH_4OH), concd (ACS)
Methanol (chromatography grade)
Methylene chloride ($MeCl_2$) (chromatography grade)
Isopropyl alcohol (IPA) (chromatography grade)
Acetonitrile (chromatography grade)
Glacial acetic acid (ACS)
Water (deionized)

Solutions

1.0 M Potassium hydroxide (1.0 M KOH)

Dissolve 5.6 g of KOH in 80 mL of deionized water in a 100-mL volumetric flask. Bring to volume with deionized water.

0.1 M Phosphate buffer, pH 6.0

Dissolve 13.61 g of KH_2PO_4 in 900 mL of deionized water in a 1-L volumetric flask. Adjust pH to 6.0 with 1.0 M KOH. Bring to volume with deionized water.

*Patent Pending

1.0 M Acetic acid

To a 500-mL volumetric flask half-filled with deionized water, add 28.6 mL of glacial acetic acid and mix. Bring to volume with deionized water and mix.

Elution solvent

Mix 80 mL of $MeCl_2$ with 20 mL IPA. Add 2.0 mL of NH_4OH and shake vigorously. Make fresh daily.

Acetonitrile/water (60/40)

Mix 60 mL of acetonitrile with 40 mL water. Mix well.

A. SAMPLE PREPARATION

1. Add 1 mL of serum or urine specimen to labeled test tube.
2. Add internal standard (e.g., trimipramine), if required.
3. Add 1 mL of 0.1 M phosphate buffer, pH 6.0. Vortex.

B. EXTRACTION

Note: All vacuum aspiration rates are at <5 in. Hg unless otherwise noted.

1. Insert labeled SPEC·VC·MP3 Microcolumn in vacuum manifold.
2. Add 200 µL of methanol to sample reservoir and aspirate. Stop vacuum.
3. Immediately add 200 µL of 0.1 M phosphate buffer to sample reservoir and aspirate.
4. Pour sample into sample reservoir and aspirate.
5. Remove sample reservoir and discard.
6. Add 200 µL of 1.0 M acetic acid to reagent reservoir and aspirate.
7. Add 200 µL of methanol to reagent reservoir and aspirate.
8. Increase vacuum (5–10 in. Hg) and dry extraction disc for approximately 2 min.
9. Open vacuum manifold, wipe collection tips, and insert collection rack holding labeled flat-bottomed vials.
10. Add 500 µL of elution solvent to reagent reservoir and aspirate slowly (1 in. Hg).

C. CONCENTRATION AND RECONSTITUTION

1. Remove flat-bottomed vials from vacuum manifold and evaporate eluate to dryness under nitrogen at ≤40° C (approximately 3 min).
2. Reconstitute with 150 µL of acetonitrile/water (60/40). Vortex.
3. Inject 100 µL of extract into HPLC.
4. Monitor detector wavelength at 215 nm.

BARBITURATES

I. **BARBITURATES INCLUDING BUT NOT LIMITED TO: AMOBARBITAL, BUTABARBITAL, BUTALBITAL, MEPHOBARBITAL, PENTOBARBITAL, PHENOBARBITAL AND SECOBARBITAL.**

OPTION A] **REFER TO GENERAL GS/MS SAMPLE EXTRACTION PROCEDURE WITH TOXI-B EXTRACTION TUBE.**

OPTION B] **REFER TO GENERAL GC/MS SAMPLE EXTRACTION PROCEDURE WITH TOXI-A EXTRACTION TUBE.**

CRITERIA FOR IDENTIFICATION

I. METHAMPHETAMINE AND OTHER SYMPATHOMIMETIC AMINES

a. POSITIVE EMIT MONOCLONAL AMPHETAMINE ASSAY.

b. POSITIVE TOXI-LAB ACETALDEHYDE OR ACETONE AMINE SEPARATION. R_f CORRESPONDENCE WITH APPROPRIATE STANDARD(S).

c. GC/MS CONFIRMATION AFTER APPROPRIATE EXTRACTION PROCEDURE. RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH APPROPRIATE AMINE STANDARD(S).

II. COCAINE AND COCAINE METABOLITES

a. POSITIVE EMIT COCAINE METABOLITE ASSAY

b. [OPTIONAL] TOXI-LAB BENZOYLECGONINE TLC PROCEDURE. R_f CORRESPONDENCE WITH BENZOYLECGONINE STANDARD.

c. GC/MS CONFIRMATION AFTER APPROPRIATE EXTRACTION PROCEDURE(S). RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH APPROPRIATE STANDARDS(S).

III. MARIHUANA METABOLITE

a. POSITIVE EMIT CANNABINOID 50 NG ASSAY

AT LEAST ONE OF THE FOLLOWING CONFIRMATION METHODS:

b. POSITIVE TOXI-LAB THCII-PLUS THIN LAYER CHROMATOGRAPHY. R_f CORRESPONDENCE WITH THC-COOH STANDARD.

c. GC/MS CONFIRMATION AFTER APPROPRIATE EXTRACTION PROCEDURE. RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH 11-NOR-^9-THC-9-COOH+TMS STANDARD.

IV. BENZODIAZEPINES

a. POSITIVE EMIT BENZODIAZEPINE ASSAY

b. GC/MS CONFIRMATION AFTER APPROPRIATE EXTRACTION PROCEDURE(S). RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH APPROPRIATE STANDARD(S).

V. OPIATES

a. POSITIVE EMIT OPIATE ASSAY

b. [OPTIONAL] TOXI-LAB TOXI-A GRAM. R_f CORRESPONDENCE WITH APPROPRIATE STANDARD(S).

c. GC/MS CONFIRMATION AFTER APPROPRIATE EXTRACTION PROCEDURE(S). RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH APPROPRIATE STANDARD(S).

CRITERIA FOR IDENTIFICATION

VI. PHENCYCLIDINE (PCP)

a. POSITIVE EMIT PHENCYCLIDINE ASSAY

b. [OPTIONAL] TOXI-LAB TOXI-A GRAM. R_f CORRESPONDENCE WITH PCP STANDARD.

c. GC/MS CONFIRMATION AFTER APPROPRIATE EXTRACTION PROCEDURE(S). RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH APPROPRIATE STANDARD(S).

VII. ANTI-DEPRESSANTS

a. [OPTIONAL] TOXI-LAB TOXI-A GRAM. R_f CORRESPONDENCE WITH APPROPRIATE STANDARD.

b. GC/MS CONFIRMATION AFTER APPROPRIATE EXTRACTION PROCEDURE(S). RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH APPROPRIATE STANDARD(S).

VII. BARBITURATES

a. [OPTIONAL] TOXI-LAB TOXI-B GRAM. R_f CORRESPONDENCE WITH APPROPRIATE STANDARD.

b. GC/MS CONFIRMATION AFTER APPROPRIATE EXTRACTION PROCEDURE(S). RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH APPROPRIATE STANDARD(S).

VIII. PROPOXYPHENE

a. [OPTIONAL] TOXI-LAB TOXI-A GRAM. R_f CORRESPONDENCE WITH PROPOXYPHENE AND/OR PROPOXYPHENE METABOLITE STANDARD.

b. GC/MS CONFIRMATION AFTER TOXI-A EXTRACTION PROCEDURE. RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH PROPOXYPHENE AND/OR PROPOXYPHENE METABOLITE STANDARDS.

IX. CARBAMATES - MEPROBAMATE AND CARISOPRODOL

a. [OPTIONAL] TOXI-LAB TOXI-A GRAM. R_f CORRESPONDENCE WITH MEPROBAMATE AND CARISOPRODOL STANDARDS.

b. GC/MS CONFIRMATION AFTER TOXI-A EXTRACTION PROCEDURE. RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH MEPROBAMATE AND CARISOPRODOL STANDARDS.

CRITERIA FOR IDENTIFICATION

X. OTHER DRUGS

a. APPROPRIATE TOXI-LAB TLC PROCEDURE IF WARRANTED. R_f CORRESPONDENCE WITH APPROPRIATE STANDARD(S).

b. GC/MS CONFIRMATION AFTER APPROPRIATE EXTRACTION PROCEDURE. RETENTION TIME AND MASS SPECTRA CORRESPONDENCE WITH APPROPRIATE STANDARD(S).

TECHNICAL TERMS

Activation	The process of heating a TLC plate or chromatogram to drive off moisture absorbed from the atmosphere
Artifact	A substance detected that is not drug-related
Chromatography	Process of separating constituents of a mixture
Desiccant	A substance having great affinity for water and used as a drying agent
Development	See migration
DPC solution (TOXI-DIP™ B-1)	Diphenylcarbazone + methylene chloride - preliminary reagent for TOXI-GRAMS B detection
Dragendorff's reagent (TOXI-DIP A-3)	Potassium iodide/iodine/bismuth subnitrate. Reagent for detection at Stage IV for TOXI-GRAMS A
Emulsion	Suspension of organic solvent and aqueous phases in the TOXI-TUBES
Inoculate	To introduce a test analyte via a TOXI-DISCS to a chromatographic media (TOXI-GRAMS)
Metabolite	Product of metabolism
Migration	Movement of analyte by a solvent through chromatographic media
Polarity	In the chromatographic sense, the ability of the mobile phase (solvent) to move through the stationary phase (silica gel).
R_f	Calculated by: $\frac{\text{distance migration of analyte}}{\text{distance migration of solvent front}}$
Stand-off jar	A jar to allow exposure of a chromatogram to vapor without contact with fluid
TOXI-DISCS®	Small discs for the concentration of drugs; made of glass microfiber paper impregnated with silica gel
TOXI-GRAMS®	Chromatograms for separation of drugs; made of glass microfiber paper impregnated with silica gel
TOXI-TUBES®	Tubes for extraction of drugs; contain a mixture of solvents, buffering salts, and a phase-marking dye

DRUG TERMS

Alkaloid	Organic base derived from plants
Analgesic	Reduces or eliminates pain (narcotic and nonnarcotic)
Anorectic (Anorexic)	Diminishes appetite
Antiarrhythmic	Prevents or alleviates cardiac arrhythmia
Antiasthmatic	Prevents or alleviates asthma symptoms; bronchodilator
Antibiotic	Inhibits or destroys bacterial and microorganism growth
Anticholinergic	Blocks the passage of impulses through the parasympathetic nerves
Anticonvulsant	Prevents or relieves convulsions
Antidepressant	Prevents or alleviates depression
Antidiarrheal	Prevents and relieves diarrhea
Antiemetic	Alleviates or prevents nausea and vomiting
Antihistamine	Counteracts the action of histamine (side effect: drowsiness; may be used as OTC sleeping aid)
Antimalarial	Prevents or alleviates malaria
Antipyretic	Reduces fever
Antitussive	Relieves or prevents cough
Barbiturate	Acidic hypnotic - induces sleep
Benzodiazepine	Tranquilizer/hypnotic
Carbamate	Tranquilizer/muscle relaxant
Cardiac depressive/ antihypertensive	Slows the heart/counteracts high blood pressure
Diuretic/ antihypertensive	Relieves hypertension and/or edema by increasing excretion of urine
Emetic	Causes vomiting
Expectorant	Promotes elimination of mucous congestion by liquifying bronchial secretions (ingredient in cough medicines)
Gastric antisecretory	Prevents or diminishes secretion of gastric acid
Hallucinogen	Produces hallucinations

CLASSIFICATION OF DRUGS BY PHARMACOLOGICAL GROUP

Analgesics - reduce or eliminate pain

Narcotic

Synthetic

- meperidine (DEMEROL®)
- methadone (DOLOPHINE®)
- pentazocine (TALWIN®)
- propoxyphene (DARVON®)

Semisynthetic - (opiates) - derived from the opium plant

- codeine
- diacetylmorphine (heroin)
- hydrocodone (DICODID®)
- hydromorphone (DILAUDID®)
- morphine
- oxycodone (PERCODAN®)

Non-narcotic

- acetaminophen (TYLENOL®)
- phenacetin
- salicylates (aspirin)
- salicylamide

Antidepressants - relieve depression

Tricyclic

- amitriptyline (ELAVIL®)
- amoxapine (ASENDIN®)
- desipramine (NORPRAMIN®)
- doxepin (SINEQUAN®)
- imipramine (TOFRANIL®)
- nortriptyline (AVENTYL®)
- trimipramine (SURMONTIL®)

Tetracyclic

- maprotiline (LUDIOMIL®)

Other

- trazodone (DESYREL®)

Drug Terms (Cont.)

Hypnotic	Calms or induces sleep
Laxative	Promotes evacuation of the bowel
Local anesthetic	Reduces or eliminates pain in a limited area
Narcotic	Produces insensibility or stupor
Opiate	Substance derived from opium poppy
Phenothiazine	Tranquilizer
Smooth-muscle relaxant/antispasmodic	Relaxes smooth muscle (primarily of the blood vessels)/prevents smooth muscle spasm
Steroid	Prevents or alleviates inflammation
Sympathomimetic amine	Mimics the effects of the sympathetic nervous system; stimulates the central nervous system, producing mood elevation or agitation; constricts blood vessels, hence may be used as a decongestant
Tranquilizer	Acts on emotional state, quieting or calming
Volatile	May be vaporized easily with low heat (ie, amphetamines, ethchlorvynol)
Xanthines	Naturally occurring class of chemicals that possess diuretic action; affect smooth muscle and the myocardium; stimulate central nervous system (ie, caffeine, theophylline, theomomin)

Anticonvulsants - prevent or relieve convulsions

carbamazepine (TEGRETOL®)
phenytoin (DILANTIN®)
phenobarbital (LUMINAL®)

Antihistamines - counteract action of histamine; produce drowsiness; antiemetic

chlorpheniramine (CHLOR-TRIMETON®)
cyclizine (MAREZINE®)
dexchlorpheniramine (POLARAMINE®)
dimenhydrinate (DRAMAMINE®)
diphenhydramine (BENADRYL®)
doxylamine (BENDECTIN®)
pyrilamine (TRIAMINIC®)

Hallucinogens - produce hallucinations

marijuana
lysergic acid diethylamide (LSD)
phencyclidine (PCP)
psilocybin (magic mushroom)
mescaline (peyote)

Hypnotics - induce sleep

Barbiturates

amobarbital (AMYTAL®)
aprobarbital (ALURATE®)
barbital
butabarbital (BUTISOL®)
butalbital (FIORINAL®)
pentobarbital (NEMBUTAL®)
phenobarbital (LUMINAL®)
secobarbital (SECONAL®)

Non-barbiturates

ethchlorvynol (PLACIDYL®)
ethinamate (VALMID®)
flurazepam (DALMANE®)
glutethimide (DORIDEN®)
methaqualone (QUAALUDE®)

Antibiotics Erythromycin Ampicillin Clindamycin

Stimulants

Sympathomimetic amines - mimic sympathetic nervous system

amphetamine (BENZEDRINE®)
ephedrine (PRIMATENE®)
methamphetamine (DESOXYN®)
methylphenidate (RITALIN®) - rarely seen in urine. (STage 4 - Braun)
phenmetrazine (PRELUDIN®)
phentermine (FASTIN®)
phenylpropanolamine (PPA)
pseudoephedrine (SUDAFED®)

Others

caffeine - Theophylline
cocaine -
nicotine -
strychnine -

Tranquilizers - induce a quieting or calming effect

Benzodiazepines

alprazolam (XANAX®)
chlordiazepoxide (LIBRIUM®)
clorazepate (TRANXENE®)
diazepam (VALIUM®)
halazepam (PAXIPAM®)
oxazepam (SERAX®)
temazepam (RESTORIL®)

Phenothiazines

chlorpromazine (THORAZINE®)
prochlorperazine (COMPAZINE®)
thioridazine (MELLARIL®)
trifluoperazine (STELAZINE®)
} only metabolites detected

Carbamates

carisoprodol (SOMA®)
meprobamate (MILTOWN®)
ethinamate (VALMID®)

DEA CLASSIFICATION (Drug Enforcement Agency)

Classification for control purposes

ELUOTROPIC SERIES FOR CHROMATOGRAPHIC SOLVENTS

This is a partial list of chromatographic solvents taken from "The Chemist's Companion: A Handbook of Practical Data, Techniques, and References" by Arnold J. Gordon and Richard A. Ford (1972, John Wiley & Sons, Inc.). Solvents are listed in order of increasing polarity. Corresponding boiling points are from The Merck Index, 10th Ed. (1983).

Solvent	B.P. (°C)
1. n-Heptane	98.4
2. n-Hexane	69.0
3. n-Pentane	36.1
4. Cyclohexane	83.0
5. Carbon disulfide	46.5
6. Carbon tetrachloride	76.7
7. Toluene	110.6
8. Benzene	80.1
9. Chloroform	61.0
10. Dichloromethane	39.7
11. Isopropyl ether	68.0
12. Ethyl ether	34.6
13. 2-Butanol	99.5
14. Acetonitrile	81.6
15. Ethyl acetate	77.0
16. Acetone	56.5
17. Ethanol	78.5
18. Tetrahydrofuran	66.0
19. Methanol	64.7
20. Pyridine	115.0

TOXICOLOGY MANUAL ADDENDUM

TABLE OF CONTENTS

IDAHO DEPARTMENT OF LAW ENFORCEMENT
BUREAU OF FORENSIC SERVICES

GAS CHROMATOGRAPH INJECTION PORT SLEEVE CLEANING PROCEDURE AND PREPARATION

OVERVIEW

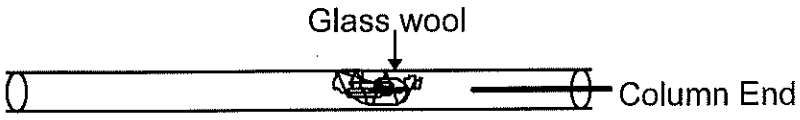
With use, injection port sleeves develop residual active sites along inner liner surfaces. For this reason, the old sleeve must by replaced regularly with a newly conditioned sleeve. This procedure details the process of sleeve conditioning, which cuts down considerably on the expense of purchasing new sleeves.

1. SLEEVE CLEANING AND CONDITIONING

a.) Remove glass wool from used liners and discard wool.
b.) Soak empty liners in concentrated nitric acid for at least one hour. Sonicate in sonicating water bath for the last 10 minutes. **Note: Do NOT use chromic acid.**
c.) Rinse the liners in the following order: water, distilled water, methanol, and hexane.
d.) Soak the liners in dichlorodimethylsiloxane: toluene for a minimum of 30 minutes. Recycle silanizing solution. **NOTE:** Silanize liners under a hood to prevent exposure to toluene fumes.
e.) Rinse the silanized liners in the following order: hexane, methanol. Allow to air dry.
f.) Wear lint-free gloves when handling the silanized liners. Do not touch liners with bare hands as this will lead to the deposition of finger oils and contaminants.

2. SLEEVE PREPARATION FOR USE

a.) Pack the liners prior to use using either silanized glass wool or deactivated borosilicate glass wool, using the wooden end of a cotton-tipped applicator stick to compress it. Packing should be of medium density. Note the distance from needle insertion and column insertion ends of the liner as shown below:



b.) Condition the packed liners at 275°C injection port temperature for at least 30 minutes prior to use.

PRINCIPLES OF QUANTITATION USING A GC/MSD SYSTEM

1. INTRODUCTION

1.1 A quantitation procedure for urine toxicology should ideally result in reliable, reproducible results. Utilizing a gas chromatograph/mass selective detector (GC/MS) system set in selected ion monitoring mode (SIM) provides a means by which quantitation may be achieved.

1.2 Furthermore, use of an internal standard is facilitated by GC/MS in that deuterated internal standards may be used. Deuterated internal standards, when utilized as analogs of the target analytes, function well in urine toxicology in that they usually extract and derivatize in the same fashion as the target analyte, allowing them to be added at the start of the extraction procedure.

1.3 SIM provides a highly accurate and sensitive means for data acquisition on the GC/MS system. Ion selection is important in SIM, however, and care should be taken to select ions which are unique for each target analyte.

1.3.1 In the case of using deuterated internal standards, care should be taken to select ions on which the deuterium masses will be located to ensure no cross-reactivity between the target compound and the internal standard.

1.3.2 In method development, ions are frequently provided by the author(s) of a method. Verify that these ions are present and are the ones desired by running full scan acquisitions of both the deuterated and non-deuterated compounds, and that the ions fulfill the requirements of 1.3.1. Mistakes have been known to be made.

1.3.3 Additionally, the selection of quantitation ion may vary according to instrument, and the selected ion in the method may not be the ion which works best for the particular detector used in the laboratory.

1.4 Using SIM acquisition, the ion selected as the quantitation ion is typically the most abundant ion, although it does not have to be. In typical quantitation, the target analyte is identified by the quantitation ion and two qualifier ions, and the internal standard is identified by one quantitation ion and one qualifier ion.

2. CALCULATIONS FOR AN INTERNAL STANDARD REPORT

2.1 The quantitation software is generally capable of performing calculations of the corrected amount ratio of a particular compound based on the area of the quantitation ion. This typically occurs in four steps.

2.1.1 The calibration points are constructed by calculating an amount ratio and a response ratio for each level of a particular peak in the calibration table. The *amt ratio* is the amount of the compound divided by the amount of the internal standard for a given level. The *response ratio* is the response of the compound divided by the response of the internal standard at this level.

2.1.2 An equation for the curve through the calibration points is calculated using the instructions provided in the calibration table (typically, use of linear regression with force through origin).

2.1.3 The response of the compound in the unknown sample is divided by the response of the internal standard in the unknown sample to give a *response ratio* for the unknown.

2.1.4 A *corrected amt ratio* for the unknown is calculated using the curve fit equation determined in 2.1.2.

2.2 The quantitation software will also perform a calculation of the compound *q* using an internal standard calculation using the following formulas:

Amount of q = Corrected Amt Ratio X Amt [ISTD]

Absolute Amt of q = Amount of q X M

Where *Corrected Amt Ratio* is obtained in step 2.1.4 above, *M* is a multiplier entered into the data system (*optional*), and *Amt [ISTD]* is the amount of the internal standard specified in the calibration table.

AMPHETAMINE/METHAMPHETAMINE IN URINE: CONFIRMATION by GC/MS

1. INTRODUCTION

1.1 Sympathomimetic amines are central nervous system stimulants, and result in anorexia, alertness, excitability, and provide a feeling of overall well-being. Amphetamine and methamphetamine are sympathomimetic amines which may be prescribed for legitimate medical purposes in weight loss, although their use has declined considerably for this purpose due to the high potential for abuse of these drugs. The only other legitimate source of methamphetamine is the availability of *l*-methamphetamine in the Vick's inhaler. Amphetamine and methamphetamine are readily absorbed by smoking, intravenous injection, inhalation, or ingestion. Their elimination rates depend on urinary pH. Metabolites as well as parent compounds may be detected in urine anywhere from 3 to 48 hours after dosage.

1.2 This method is intended for the confirmation of amphetamine and methamphetamine in urine. It is unable to differentiate the isomeric forms of these drugs, and it cannot rule out legal use. Also, a positive result does not indicate of degree of intoxication or length of time since use.

2. PRINCIPLE

2.1 Subject samples, along with all standards and controls, are subjected to oxidation using sodium metaperiodate, a strong oxidizing agent, to destroy any ephedrine or pseudoephedrine present. Samples are further alkalinized and extracted into n-butyl chloride. The n-butyl-chloride extract is derivatized with 4-carbethoxyhexaflurobutyrl chloride (4-CB), and the derivatized product is analyzed on a gas chromatograph/mass selective detector system for quantitative analysis.

2.2 Specimen requirements: 2 mL urine for standard assay. Minimum amount depends on sample concentration.

3. ANALYTICAL SUPPLIES

3.1 MATERIALS

Screw-cap vials w/ caps, solvent resistant. Min Vol: 8

mL
13 X 100 mm disposable culture tubes w/ snap caps or similar
50 deg C heat block
ALS vials w/ caps
Precision pipettes at appropriate volumes
Tube Mixer/Rocker

3.2 REAGENTS

n-Butyl-Chloride, reagent grade or better
Sodium Carbonate Anhydrous
Saturated Sodium Carbonate (Saturate D.I. water w/ Sodium Carbonate Anhydrous. Stable 1 year at room temp)
0.35M Sodium Metaperiodate
40% Sodium Phosphate Dibasic Buffer
4-Carbethoxyhexaflurobutyrl chloride (4CB)
D.I. Water
Methanol, reagent grade or better
Derivatization Reagent: To 1 mL n-butyl-chloride, add 10 uL 4CB. Mix well. **Prepare fresh daily.** Use appropriate precautions.

4. STANDARDS/CONTROLS PROTOCOL

4.1 STOCK SOLUTIONS

4.1.1 STOCK STANDARDS:

(±)-AMPHETAMINE 1.0 mg/mL in Methanol, Radian Cat # A-007.
(±)-METHAMPHETAMINE 1.0 mg/mL in Methanol, Radian Cat #M-009.

4.1.2 STOCK INTERNAL STANDARDS

(±)-AMPHETAMINE-D5: 100 ug/mL in Methanol, Radian Cat # A-005.

(±)-METHAMPHETAMINE-D5: 100 ug/mL in Methanol, Radian Cat #M-004.

4.1.3 STOCK CONTROLS:

d,l-AMPHETAMINE SULFATE: 1 mg/mL in Methanol, SIGMA Cat # A-2262.

+-METHAMPHETAMINE HCL 1 mg/mL in Methanol, SIGMA Cat# M-5260.

4.2 **WORKING SOLUTIONS:** [**Note:** To avoid excessive waste of stock material, adjust volume of working solutions prepared according to usage rates.]

4.2.1 WORKING STANDARD: To 48.0 mL methanol, add 1.0 mL each of stock (±)-amphetamine and (±)-methamphetamine standards. This yields a 20,000 ng/mL working standard solution stable 4 months at -4 deg C. Cap tightly.

4.2.2 WORKING INTERNAL STANDARD: To 6.0 mL methanol add 2.0 mL each of stock internal standards. This yields a 20,000 ng/mL working internal standard solution. Cap tightly. Stable 4 months at -4 deg C.

4.2.3 WORKING CONTROL: To 19.2 mL methanol, add 400 uL of each stock control solution. This yields a 20,000 ng/mL working control solution. Cap tightly. Stable 4 months at -4 deg C.

5. **PROCEDURE**

5.1 **CALIBRATOR/CONTROL PREPARATION**

5.1.1 STANDARDS: To appropriately labeled extraction tubes add 2 mL negative urine. For each calibrator pipet the indicated volume into the appropriate tube. **500 ng/mL CAL:** 50 uL working standard **1000 ng/mL CAL:** 100 uL working standard.

5.1.2 CONTROLS: 750 ng/mL ABOVE CUT-OFF CONTROL: To appropriately labeled extraction tube add 2 mL negative urine. Pipet 75 uL working control solution to yield a 750 ng/mL control. **NEGATIVE CONTROL:** To appropriately labeled extraction tube add 2 mL negative urine.

5.1.3 NON-EXTRACTED STANDARD: The inclusion of a non-extracted standard can provide a gauge of extraction efficiency. To an appropriately labeled 13 x 100 mm tube, or equivalent, add 100 uL working standard solution and 50 uL working internal standard solution. Vortex. Take ***just*** to dryness under N_2 at 37 °C. Add 1.5 mL n-

Butyl chloride, vortex mix and insert at step 5.5.2.

5.2 SUBJECT SAMPLES

5.2.1 Set up subject samples at full-strength or at dilution factors of 4, 10, 20, or more, depending on EMIT result protocol or according to previous extraction results.

5.2.2 Pipet 2 mL subject urine into appropriately labeled tube. **OR** Pipet 0.5 mL subject urine into 1.5 mL blank matrix for a 4X dilution. **OR** Pipet 200 uL subject urine into 1.8 mL blank matrix for a 10X dilution. **OR** Pipet 100 uL subject urine into 1.9 mL blank matrix for a 20X dilution. **Notate dilutions on worksheet.**

5.3 PREPARATION FOR OXIDATION

5.3.1 To each sample tube, add 50 uL working internal standard solution, 0.5 mL 0.35M sodium metaperiodate, and 0.5 mL 40% sodium phosphate dibasic solution. Mix gently.

5.3.2 Allow tubes to sit at room temperature for at least 5 minutes. **OR** Cap tubes and mix for 5-10 minutes.

5.4 SAMPLE EXTRACTION

5.4.1 To each sample tube add the following: approx. 100 mg sodium carbonate, 1 mL saturated sodium carbonate, and 1.5 mL n-butyl-chloride. Cap and extract for 10 minutes.

5.4.2 Centrifuge 5 minutes at 3500 rpm. Transfer upper organic layer to appropriately labeled 13 X 100 mm culture tube, conical centrifuge tube or similar. Take caution not to transfer any aqueous layer.

5.5 DERIVATIZATION

5.5.1 Prepare derivatization reagent by adding 10 uL of 4-CB to 1 mL n-Butyl-Chloride. Vortex.

5.5.2 To each tube add 50 uL derivatization reagent (MAKE FRESH DAILY). Vortex, cap, and incubate 20 minutes

at 50 deg C.

5.5.3 Remove tubes from heat, allow to equilibrate to room temperature, and transfer contents of tubes to appropriately labeled ALS vials. Cap and inject 1-2 uL on GC/MS.

6. GC/MS PARAMETERS

6.1 ANALYTICAL EQUIPMENT

Hewlett-Packard Ultra I 30 m/12 m column
Hewlett-Packard 5890 Series Gas Chromatograph
Hewlett-Packard 5970 Series Mass Selective Detector

6.2 RAMPS:

INITIAL TEMP: 110 deg C **INITIAL TIME:** 1.00 min
RAMP: 20 deg C/min to 270 deg C **HOLD TIME:** 10.00 min
DETECTOR TEMP: 280 deg C **INJECTOR B TEMP:** 175 deg C

6.3 IONS MONITORED, SIM MODE

GROUP 1	IONS	DWELL
AMPHETAMINE-D5	270*, 298	80
AMPHETAMINE	266*, 294, 248	80

GROUP 2	IONS	DWELL
METHAMPHETAMINE-D5	312*, 284	80
METHAMPHETAMINE	308*, 262, 280	80

*[NOTE: * indicates quantitation ion]*

7. CALIBRATION PROTOCOL

3-level calibration: Calibrators at 500 and 1000 ng/mL will be set up. Calibration will be based on linear regression with force through the origin.

8. RUN AND SAMPLE CRITERIA

8.1 RUN CRITERIA: The following are evaluated in order to assess whether a run is considered acceptable. Failure of the negative control and/or the 750 positive control to fit criteria results in rejection of the run. Failure of the non-extracted standard to quantitate within ± 20% of 1000 ng/mL

does not affect run acceptability.

8.1.1 All standards must display acceptable chromatography and peak shapes. All ions must be present for all compounds and internal standards.

8.1.2 Calibration curve must be linear, with r > 0.98.

8.1.3 Negative control must not display peaks for amphetamine or methamphetamine, or peak areas must be less than 10 % of respective areas for the cutoff calibrator (500 CAL). Internal standards must be present with acceptable ion ratios and chromatography.

8.1.4 The 750 ng/mL above-cut-off control must quantitate within ± 20% of its target value [between 600 and 900 ng/mL].

8.2 POSITIVE SAMPLE CRITERIA

8.2.1 All criteria for an acceptable run must be met.

8.2.2 Subject sample must display acceptable chromatography for either amphetamine or methamphetamine, or both. The quantitative value for either amphetamine or methamphetamine must be greater than the cutoff of 500 ng/mL. Calibrated peaks **MUST** correlate to retention times of standards and be within ± 20% of target ion ratio values. **Strict adherence to retention time and ion ratio criteria must be met to ensure accurate reporting of amphetamine and methamphetamine due to the possibility of other sympathomimetic amines being present. Under this policy of strict adherence, there are no interfering compounds using this methodology.**

8.2.3 Samples which are positive according to the above criteria may be reported out as positive for either amphetamine or methamphetamine, or both.

8.3 NEGATIVE SAMPLE CRITERIA

8.3.1 Subject samples with quantitative results for both amphetamine and methamphetamine which are below 500 ng/mL are negative and are subject to additional

confirmatory procedures as necessary.

8.3.2 Absence of amphetamine and/or methamphetamine peaks combined with the presence of internal standard peaks at the appropriate times with the appropriate ratios are reported as negative.

8.4 ION RATIO PROBLEMS IN AMPH/METH CONFIRMATIONS

8.4.1 An overloaded sample may give ion ratios which fall outside of the acceptable range. A sample in which this occurs may be diluted by removing a portion of the derivatized sample and adding an equal amount of n-butyl-chloride. Mix the sample, and reinject.

8.4.2 Phentermine may be picked up in the methamphetamine window if parameters for the windows have been set too wide. This may result in ion ratio problems. Verify that the retention time is consistent with standards and consider narrowing peak window times.

8.5 DATA MANAGEMENT

8.5.1 Each subject result will contain copies of the following:

a copy of the subject results.
a copy of the data sheet for the run.

8.5.2 Data pack results containing all of the original data for the entire run will be kept on file in accordance with the laboratory filing procedures.

8.6 PROCEDURE LIMITATIONS

8.6.1 The following have been experimentally determined in the past to reflect the limitations of this procedure:

	AMPH/METH
Upper range of linearity:	10,000 ng/mL
Carryover limit:	5000 ng/mL
Limit of detection:	50 ng/mL
Limit of quantitation:	50 ng/mL
Interference:	None of the compounds below

ephedrine/pseudoephedrine
phenylpropanolamine
MDA
MDMA
phentermine

11-nor-9-carboxy-Delta 9-THC (carboxy-THC) IN URINE: CONFIRMATION by GC/MS

1. INTRODUCTION

1.1 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 hepatic metabolism in the body, and blood, urinary, and fecal metabolite concentrations depend on a number of factors: 1) The total amount of THC absorbed, 2) The number of exposures, 3) The amount of fatty tissue present, and 4) The time of exposure in relation to the time of sample collection.

1.2 The primary urinary metabolite of THC is 11-nor-9-carboxy-delta-9-THC (carboxy-THC or c-THC). Confirmation of c-THC in urine offers no reflection of length of time since exposure nor does it measure degree of intoxication due to marijuana use. Confirmation of c-THC at or above the federally mandated cut-off of 15 ng/mL indicates past use of marijuana and reflects a level not possible due to passive inhalation.

2. PRINCIPLE

2.1 Subject samples, along with all standards and controls, are subjected to alkaline hydrolysis to free the c-THC from its glucuronide conjugate. Hydrolyzed samples are then made acidic and extracted. The extract is dried under nitrogen then derivatized to form the TMS derivative. The derivatized end-product is analyzed on a gas chromatograph/mass selective detector system for quantitative analysis.

2.2 Specimen requirements: 3 mL urine for standard assay. Minimum depends on sample concentration.

3. ANALYTICAL SUPPLIES

3.1 MATERIALS

16 X 125 mm or 16 x 100 mm screw top tubes with caps
10 mL conical centrifuge tubes with plastic caps
(alternative: 13 X 100 mm culture tubes with snap caps)
40 deg C incubator or water bath
95 deg C heat block

ALS vials with inserts and caps
MLA pipettes at 1-5 mL volumes, and appropriate tips
Precision pipettes at appropriate volumes
Evaporating block with N_2 at 37 deg C

3.2 REAGENTS

1.0N KOH (11.8 g KOH + 200 mL D.I. water)
Saturated potassium phosphate monobasic (Saturate D.I. water w/ KH_2PO_4; adjust to pH ~1.8 with phosphoric acid)
12.5% ethyl acetate/Hexane (v:v). Make as needed.
MSTFA (n-Methyl-n-trimethylsilyl-trifluoro-acetimide): Pierce Chemicals Cat # 48910 **OR** BSTFA
Ethyl acetate, reagent grade or better
Hexane, reagent grade or better

4. CALIBRATOR/STANDARD PROTOCOL:

4.1 STOCK SOLUTIONS

4.1.1 STOCK STANDARD: 100 ug/mL (±)11-nor-9-carboxy-delta 9-THC (Radian cat # T-006)

4.1.2 STOCK INTERNAL STANDARD: 100 ug/mL (±)11-nor-9-carboxy-delta 9-THC-D3 (Radian cat# T-004)

4.1.3 STOCK CONTROL: 50 ug/mL 11-nor-delta-9-THC-delta 9 carboxylic acid (Sigma cat # N6893)

4.2 WORKING SOLUTIONS: [**NOTE:** Prepare working solutions in volumes which are appropriate to usage in order to prevent waste of stock solutions.]

4.2.1 WORKING STANDARD: To 49.1 mL methanol add 900 uL stock standard to yield an 1800 ng/mL working standard. Stable 6 months at -4 deg C.

4.2.2 WORKING INTERNAL STANDARD: To 47.0 mL methanol add 3 mL stock internal standard to yield a 6000 ng/mL working internal standard. 50 uL of this solution, when added to 3 mL sample, will yield 100 ng/mL c-THC-D3 as internal standard. Stable 6 months at -4 deg C.

4.2.3 WORKING CONTROL: To 9.7 mL methanol add 300 uL stock control to yield a 1500 ng/mL working control solution. Stable 6 months at -4 deg C.

5. PROCEDURE:

5.1 CALIBRATOR/CONTROL PREPARATION

5.1.1 STANDARDS: To appropriately labeled screw top tubes add 3 mL negative urine. For each calibrator pipet the indicated volume into the appropriate tube. **15 ng/mL CAL:** 25 uL working standard. **60 ng/mL CAL:** 100 uL working standard. **120 ng/mL CAL:** 200 uL working standard.

5.1.2 CONTROLS: 25 NG/ML POSITIVE CONTROL: To appropriately labeled screw top tube add 3 mL negative urine. Add 50 uL working control solution to yield a 25 ng/mL control. **NEGATIVE CONTROL:** 3 mL blank urine in appropriately labeled screw top tube.

5.1.3 NON-EXTRACTED STANDARD: The addition of a non-extracted standard to a run can provide a gauge of extraction efficiency. It is an optional part of the run. To an appropriately labeled centrifuge tube, add 100 uL of working standard and 50 uL working internal standard. Vortex. Take to dryness and insert at step # 5.5.1.

5.2 SUBJECT SAMPLES:

5.2.1 Set up subject samples at full strength or at a dilution factor of 3, depending on EMIT result protocol OR previous sample results.

5.2.2 Pipet 3 mL subject sample into appropriately labeled tube. **OR** Pipet 1 mL subject sample into 2 mL blank urine. Notate dilutions on worksheet.

5.3 PREPARATION FOR HYDROLYSIS

5.3.1 To each tube add 50 uL working internal standard solution, and 0.5 mL 1.0N KOH. Vortex **GENTLY** and heat 15 minutes in 40 deg C water bath.

5.3.2 Remove samples and equilibrate to room temperature.

5.4 SAMPLE EXTRACTION

5.4.1 To each tube add 1.5 mL pH 1.8 phosphate buffer and 3 mL ethyl acetate/hexane 12.5 % v:v extraction solvent. Extract for 10 minutes.

5.4.2 Centrifuge for 5 minutes at 3500 rpm, then transfer organic layer to appropriately labeled centrifuge tube or equivalent. Take caution not to transfer any aqueous layer. Dry tubes under gentle nitrogen. **DO NOT OVERDRY.**

5.5 DERIVATIZATION

5.5.1 To each dried extract add 50 uL MSTFA. Vortex, cap and incubate 15 minutes at 95 deg C.

5.5.2 Remove from heat, equilibrate to room temperature, add 50 uL ethyl acetate to each tube, vortex thoroughly and transfer contents to appropriately labeled ALS vials with inserts. Cap and inject 1-2 uL sample using method **cthc.m.**

6. GC/MS PARAMETERS:

6.1 ANALYTICAL EQUIPMENT
Hewlett-Packard Ultra I 30 m/12 m column
Hewlett-Packard 5890 series Gas Chromatograph
Hewlett Packard 5970 series Mass Selective Detector

6.2 RAMPS:

INITIAL TEMP: 190 deg C **INITIAL TIME:** 1.00 min
RAMP: 30 deg C/min to 270 deg C **HOLD TIME:** 10.00 min
SECOND RAMP: 35 deg C/min to 315 deg C **HOLD TIME:** 3.00 min
DETECTOR TEMP: 280 deg C **INJECTOR B TEMP:** 250 deg C

6.3 IONS MONITORED, SIM MODE
C-THC: 371*, 473, 488 DWELL = 80
C-THC-D3: 374*, 476 DWELL = 80
*[**NOTE:** * indicates quantitation ion]*

7. CALIBRATION PROTOCOL:
4-Level calibration: Calibrators (standards) will be set up at 15 ng/mL, 60 ng/mL and 120 ng/mL. Calibration curves will be established using linear regression and force through

origin.

8. RUN AND SAMPLE CRITERIA:

8.1 RUN CRITERIA: The following are evaluated in order to assess whether a run is considered acceptable. Failure of the negative control and/or of the 25 ng/mL positive control to fit criteria results in rejection of the run. Failure of the non-extracted standard to quantitate within ± 20% of 60 ng/mL does not affect run acceptability.

8.1.1 All standards must display acceptable chromatography and peak shapes. All ions must be present for both c-THC and c-THC-D3.

8.1.2 Calibration curve must be linear, with $r > 0.98$.

8.1.3 Negative control must display no peaks for c-THC, or peak areas must be less than the respective areas for the cutoff (15 ng/mL) cal. Internal standard peaks must be present with acceptable chromatography.

8.1.4 The above-cut-off control (25 ng/mL) must quantitate between ± 20% of its target value, i.e. between 20.0 and 30.0 ng/mL.

8.2 POSITIVE SAMPLE CRITERIA

8.2.1 All criteria for acceptable run must be met.

8.2.2 Subject sample must display acceptable chromatography, quantitate greater than 15 ng/mL c-THC after correction for dilution factor, and have ratios within ± 20% of target value. Calibrated peaks must correlate to retention times of standards.

8.2.3 Report samples as positive for marijuana metabolite carboxy-THC.

8.3 NEGATIVE SAMPLE CRITERIA

8.3.1 Subject samples with acceptable chromatography but with quantitative results below 15 ng/mL after correction for dilution factor are negative.

8.3.2 Absence of c-THC peaks combined with presence of internal standard are reported as negative for carboxy-THC.

8.4 ION RATIO PROBLEMS IN c-THC CONFIRMATIONS

8.4.1 The GC/MS confirmation of c-THC is confounded on occasion by the use of various techniques by the user to avoid detection. This may result in ion ratios which are inconsistent with those of the standards, but in which the chromatography suggests the presence of c-THC at levels above the cutoff. In the event that a sample screens positive on the ETS system but displays ion ratios out of range for the standards, the sample may be confirmed by the TOXI-LAB THC II Plus system. Results for this type of confirmation are reported out accordingly.

8.4.2 Overloaded samples may result in ion ratios for the internal standard being out of the ± 20 % range, but the c-THC ratios are acceptable. At the analyst's discretion, these may be reported as a positive qualitative result with invalid quantitative results. The use of the TOXI-LAB THC II Plus system as an additional confirmatory step is also at the analyst's discretion.

8.5 DATA MANAGEMENT

8.5.1 Each subject result will contain copies of the following:

a copy of the subject results
a copy of the data sheet for the entire run

8.5.2 Data pack results containing all of the original data for the entire run will be kept on file in accordance with laboratory filing procedures.

8.6 PROCEDURE LIMITATIONS

8.6.1 The following have been experimentally determined in the past to reflect the limitations of this procedure:

Upper range of linearity:	600 ng/mL
Carryover Limit:	300 ng/mL
Limit of Detection:	3 ng/mL
Limit of Quantitation:	3 ng/mL
Interference:	None of the compounds below

ibuprofen
Delta-8-carboxy-THC

11-nor-9-carboxy-Delta 9-THC (carboxy-THC) IN URINE USING c-THC-D9 AS INTERNAL STANDARD: CONFIRMATION by GC/MS

1. INTRODUCTION

1.1 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 hepatic metabolism in the body, and blood, urinary, and fecal metabolite concentrations depend on a number of factors: 1) The total amount of THC absorbed, 2) The number of exposures, 3) The amount of fatty tissue present, and 4) The time of exposure in relation to the time of sample collection.

1.2 The primary urinary metabolite of THC is 11-nor-9-carboxy-delta-9-THC (carboxy-THC or c-THC). Confirmation of c-THC in urine offers no reflection of length of time since exposure nor does it measure degree of intoxication due to marijuana use. Confirmation of c-THC at or above the federally mandated cut-off of 15 ng/mL indicates past use of marijuana and reflects a level not possible due to passive inhalation.

2. PRINCIPLE

2.1 Subject samples, along with all standards and controls, are subjected to alkaline hydrolysis to free the c-THC from its glucuronide conjugate. Hydrolyzed samples are then made acidic and extracted. The extract is dried under nitrogen then derivatized to form the TMS derivative. The derivatized end-product is analyzed on a gas chromatograph/mass selective detector system for quantitative analysis. The use of c-THC-D9 provides ion ratio stability over a wide range of concentrations.

2.2 Specimen requirements: 3 mL urine for standard assay. Minimum depends on sample concentration.

3. ANALYTICAL SUPPLIES

3.1 MATERIALS

16 X 125 mm or 16 x 100 mm screw top tubes with caps
10 mL conical centrifuge tubes with plastic caps

(alternative: 13 X 100 mm culture tubes with snap caps)
40 deg C incubator or water bath
95 deg C heat block
ALS vials with inserts and caps
MLA pipettes at 1-5 mL volumes, and appropriate tips
Precision pipettes at appropriate volumes
Evaporating block with N_2 at 37 deg C

3.2 REAGENTS

1.0N KOH (11.8 g KOH + 200 mL D.I. water)
Saturated potassium phosphate monobasic (Saturate D.I. water w/ KH_2PO_4; adjust to pH ~1.8 with phosphoric acid)
12.5% ethyl acetate/Hexane (v:v). Make as needed.
MSTFA(n-Methyl-n-trimethylsilyl-trifluoro-acetimide): Pierce Chemicals Cat # 48910 **OR** BSTFA Pierce Chemicals Cat # 38831
Ethyl acetate, reagent grade or better
Hexane, reagent grade or better

4. CALIBRATOR/STANDARD PROTOCOL:

4.1 STOCK SOLUTIONS

4.1.1 STOCK STANDARD: 100 ug/mL (±)11-nor-9-carboxy-delta 9-THC (Radian cat # T-006)

4.1.2 STOCK INTERNAL STANDARD: 100 ug/mL (±)11-nor-9-carboxy-delta 9-THC-D9 (Radian cat# T-007)

4.1.3 STOCK CONTROL: 50 ug/mL 11-nor-delta-9-THC-delta 9 carboxylic acid (Sigma cat # N6893)

4.2 WORKING SOLUTIONS: [NOTE: Prepare working solutions in volumes which are appropriate to usage in order to prevent waste of stock solutions.]

4.2.1 WORKING STANDARD: To 49.1 mL methanol add 900 uL stock standard to yield an 1800 ng/mL working standard. Stable 6 months at -4 deg C.

4.2.2 WORKING INTERNAL STANDARD: To 47.0 mL methanol add 3 mL stock internal standard to yield a 6000 ng/mL working internal standard. 50 uL of this solution, when added to 3 mL sample, will yield 100 ng/mL c-THC-D9 as

internal standard. Stable 6 months at -4 deg C.

4.2.3 WORKING CONTROL: To 9.7 mL methanol add 300 uL stock control to yield a 1500 ng/mL working control solution. Stable 6 months at -4 deg C.

5. PROCEDURE:

5.1 CALIBRATOR/CONTROL PREPARATION

5.1.1 STANDARDS: To appropriately labeled screw top tubes add 3 mL negative urine. For each calibrator pipet the indicated volume into the appropriate tube. **15 ng/mL CAL:** 25 uL working standard. **60 ng/mL CAL:** 100 uL working standard. **120 ng/mL CAL:** 200 uL working standard.

5.1.2 CONTROLS: 25 NG/ML POSITIVE CONTROL: To appropriately labeled screw top tube add 3 mL negative urine. Add 50 uL working control solution to yield a 25 ng/mL control. **NEGATIVE CONTROL:** 3 mL blank urine in appropriately labeled screw top tube.

5.1.3 NON-EXTRACTED STANDARD: The addition of a non-extracted standard to a run can provide a gauge of extraction efficiency. It is an optional part of the run. To an appropriately labeled centrifuge tube, add 100 uL of working standard and 50 uL working internal standard. Vortex. Take to dryness and insert at step # 5.5.1.

5.2 SUBJECT SAMPLES:

5.2.1 Set up subject samples at full strength or at a dilution factor of 3, depending on EMIT result protocol OR previous sample results.

5.2.2 Pipet 3 mL subject sample into appropriately labeled tube. **OR** Pipet 1 mL subject sample into 2 mL blank urine. Notate dilutions on worksheet.

5.3 PREPARATION FOR HYDROLYSIS

5.3.1 To each tube add 50 uL working internal standard solution, and 0.5 mL 1.0N KOH. Vortex **GENTLY** and heat 15

minutes in 40 deg C water bath.

5.3.2 Remove samples and equilibrate to room temperature.

5.4 SAMPLE EXTRACTION

5.4.1 To each tube add 1.5 mL pH 1.8 phosphate buffer and 3 mL ethyl acetate/hexane 12.5 % v:v extraction solvent. Extract for 10 minutes.

5.4.2 Centrifuge for 5 minutes at 3500 rpm, then transfer organic layer to appropriately labeled centrifuge tube or equivalent. Take caution not to transfer any aqueous layer. Dry tubes under gentle nitrogen. **DO NOT OVERDRY.**

5.5 DERIVATIZATION

5.5.1 To each dried extract add 50 uL MSTFA. Vortex, cap and incubate 15 minutes at 95 deg C.

5.5.2 Remove from heat, equilibrate to room temperature, add 50 uL ethyl acetate to each tube, vortex thoroughly and transfer contents to appropriately labeled ALS vials with inserts. Cap and inject 1-2 uL sample using method **cthc.m.**

6. GC/MS PARAMETERS:

6.1 ANALYTICAL EQUIPMENT

Hewlett-Packard Ultra I 30 m/12 m column
Hewlett-Packard 5890 series Gas Chromatograph
Hewlett Packard 5970 series Mass Selective Detector

6.2 RAMPS:

INITIAL TEMP: 190 deg C **INITIAL TIME:** 1.00 min
RAMP: 30 deg C/min to 270 deg C **HOLD TIME:** 10.00 min
SECOND RAMP: 35 deg C/min to 315 deg C **HOLD TIME:** 3.00 min
DETECTOR TEMP: 280 deg C **INJECTOR B TEMP:** 250 deg C

6.3 IONS MONITORED, SIM MODE

C-THC: 371*, 473, 488 DWELL = 80
C-THC-D9: 380*, 497 DWELL = 80

*[NOTE: * indicates quantitation ion]*

7. CALIBRATION PROTOCOL:

4-Level calibration: Calibrators (standards) will be set up at 15 ng/mL, 60 ng/mL and 120 ng/mL. Calibration curves will be established using linear regression and force through origin.

8. RUN AND SAMPLE CRITERIA:

8.1 RUN CRITERIA: The following are evaluated in order to assess whether a run is considered acceptable. Failure of the negative control and/or of the 25 ng/mL positive control to fit criteria results in rejection of the run. Failure of the non-extracted standard to quantitate within ± 20% of 60 ng/mL does not affect run acceptability.

8.1.1 All standards must display acceptable chromatography and peak shapes. All ions must be present for both c-THC and c-THC-D9.

8.1.2 Calibration curve must be linear, with r > 0.98.

8.1.3 Negative control must display no peaks for c-THC, or peak areas must be less than the respective areas for the cutoff (15 ng/mL) cal. Internal standard peaks must be present with acceptable chromatography.

8.1.4 The above-cut-off control (25 ng/mL) must quantitate between ± 20% of its target value, i.e. between 20.0 and 30.0 ng/mL.

8.2 POSITIVE SAMPLE CRITERIA

8.2.1 All criteria for acceptable run must be met.

8.2.2 Subject sample must display acceptable chromatography, quantitate greater than 15 ng/mL c-THC after correction for dilution factor, and have ratios within ± 20% of target value. Calibrated peaks must correlate to retention times of standards.

8.2.3 Report samples as positive for marijuana metabolite carboxy-THC.

8.3 NEGATIVE SAMPLE CRITERIA

8.3.1 Subject samples with acceptable chromatography but with quantitative results below 15 ng/mL after correction for dilution factor are negative.

8.3.2 Absence of c-THC peaks combined with presence of internal standard are reported as negative for carboxy-THC.

8.4 ION RATIO PROBLEMS IN c-THC CONFIRMATIONS

8.4.1 The GC/MS confirmation of c-THC is confounded on occasion by the use of various techniques by the user to avoid detection. This may result in ion ratios which are inconsistent with those of the standards, but in which the chromatography suggests the presence of c-THC at levels above the cutoff. In the event that a sample screens positive on the ETS system but displays ion ratios out of range for the standards, the sample may be confirmed by the TOXI-LAB THC II Plus system. Results for this type of confirmation are reported out accordingly.

8.4.2 Overloaded samples may result in ion ratios for the internal standard being out of the ± 20 % range, but the c-THC ratios are acceptable. At the analyst's discretion, these may be reported as a positive qualitative result with invalid quantitative results. The use of the TOXI-LAB THC II Plus system as an additional confirmatory step is also at the analyst's discretion.

8.5 DATA MANAGEMENT

8.5.1 Each subject result will contain copies of the following:

a copy of the subject results
a copy of the data sheet for the entire run

8.5.2 Data pack results containing all of the original data for the entire run will be kept on file in accordance with laboratory filing procedures.

8.6 PROCEDURE LIMITATIONS

8.6.1 The following have been experimentally determined in the past to reflect the limitations of this procedure:

Upper range of linearity:	600 ng/mL
Carryover Limit:	300 ng/mL
Limit of Detection:	3 ng/mL
Limit of Quantitation:	3 ng/mL
Interference:	None of the compounds below
	ibuprofen
	Delta-8-carboxy-THC

COCAINE/BENZOYLECGONINE GC/MS CONFIRMATION IN URINE: SUMMARY PROCEDURE

1. SAMPLE PREPARATION:

1.1 STANDARDS/CONTROLS: To appropriately labeled 16 x 100 mm tubes, add 2 mL negative urine. For cocaine extracted standard, add 10 uL stock solution to yield approximately 12,500 ng/mL cocaine. For benzoylecgonine extracted standard, add 10 uL stock solution to yield approximately 12,500 ng/mL benzoylecgonine. For negative control, use 2 mL negative urine.

1.2 SUBJECT SAMPLES: To appropriately labeled 16 x 100 mm tubes, add 2 mL of subject urine.

2. PREPARATION FOR EXTRACTION:

2.1 To each sample tube, add 2 mL 0.1M phosphate buffer, pH 6.0. Vortex gently.

2.2 Centrifuge turbid specimens to avoid column blockage.

3. EXTRACTION:

3.1 COLUMN PREPARATION

3.1.1 Label Worldwide Monitoring Clean Screen ZSDAU020 columns and prepare vacuum extraction manifold accordingly.

3.1.2 Condition the columns by rinsing sequentially with the following: ***[NOTE: Do not allow column drying between applications]***

3 mL Methanol
2 mL 0.1M Phosphate Buffer, pH 6.0

3.2 LOAD SAMPLE

3.2.1 Carefully transfer samples to appropriate column and extract at 1-2 mL/minute (low vacuum)

3.3 WASH COLUMN

3.3.1 After the samples have passed through the columns, rinse sequentially with the following: ***[NOTE:***

Columns may dry after each application after samples have been loaded.]

2 mL D.I. water
2 mL 0.1N HCL
6 mL Methanol

3.3.2 After methanol has passed through, increase vacuum to ≥ 10" Hg, and dry columns for at least 5 minutes.

3.4 ELUTE DRUGS

3.4.1 Prepare elution solvent daily in volumes appropriate for extraction at the following ratios:

80 parts methylene chloride
20 parts isopropanol

Mix the above, remove 2 parts and discard. Add 2 parts concentrated ammonium hydroxide. Mix well. Use appropriate precautions when preparing the above elution solvent. After vigorous mixing, it may need to be vented carefully to avoid spatter.

3.4.2 Add 3 mL elution solvent to each column and collect eluent in appropriately labeled tubes.

3.4.3 Dry eluent under gentle N_2 in 37 deg C heat block until just to dryness.

4. DERIVATIZATION

4.1 To each sample tube, add 50 uL ethyl acetate and 50 uL of BSTFA or MSTFA. Cap and vortex gently. Take appropriate precautions using BSTFA/MSTFA. Derivatize 20 minutes at 70-72 deg C. Remove from heat and allow to cool.

4.2 Transfer samples to appropriately labeled vials with inserts for GC/MS analysis.

5. ANALYSIS

5.1 Inject 1-2 uL on the GC/MS system drugscreen.m, drugscreen2u.m, or drugscreen2.m methods, which have the following parameters:

	drugscreen.m	drugscreen2u.m	drugscreen2.m
Inj Port Temp (deg C)	175	175	250
Inj Volume (uL)	1	2	1
Temp Range (deg C)	100-280	100-280	100-280
Ramp (deg C/min)	20	20	20

5.2 Analyze samples accordingly. Report out finding as cocaine, and/or cocaine metabolite (benzoylecgonine).

BENZODIAZEPINES IN URINE SOLID PHASE EXTRACTION FOR GC/MS CONFIRMATION: SUMMARY METHOD

[NOTE: This method is intended for the qualitative identification of benzodiazepine compounds.]

1. SAMPLE PREPARATION

1.1 STANDARDS/CONTROLS: To appropriately labeled 16 x 125 mm screw cap tubes, add 5 mL negative urine. For each standard desired (ie. oxycodone, alprazolam, etc.), add 20 uL of stock solution. For negative control, use 5 mL negative urine.

1.2 SUBJECT SAMPLES: To appropriately labeled 16 x 125 mm screw cap tubes, add 5 mL of subject urine.

2. SAMPLE HYDROLYSIS

2.1 To each sample tube, add 2 mL 2.0M acetate buffer, pH 5 and 100 uL β-glucuronidase. Cap and vortex mix gently. Hydrolyze for 3 hours at 65 deg C. Allow samples to cool.

3. PREPARATION FOR EXTRACTION

3.1 To each sample tube, add 2mL saturated sodium borate. Vortex mix gently.

3.2 Adjust sample pH for each tube to approximately 9 ± 0.2 by adding 1.0N KOH, 11.8N KOH, 1N HCL, or concentrated HCL.

3.3 Centrifuge samples 5 minutes at 3500 rpm.

4. EXTRACTION:

4.1 COLUMN PREPARATION

4.1.1 Label Worldwide Monitoring Clean Screen ZSDAU020 columns and prepare vacuum extraction manifold accordingly.

4.1.2 Condition the columns by rinsing sequentially with the following: ***[NOTE: Do not allow column drying between applications]***

3 mL methanol

3 mL D.I. water
2 mL 0.1M acetate buffer, pH 4.5

4.2 LOAD SAMPLE

4.2.1 Carefully transfer centrifuged samples to appropriate column and extract at 1-2 mL/minute.

4.3 WASH COLUMN

4.3.1 After the samples have passed through columns, rinse sequentially with the following: ***[NOTE: Columns may dry after applications of the wash solutions.]***

3 mL D.I. water
3 mL 0.1M acetate buffer, pH 4.5
Dry columns under high vacuum for 5-10 minutes
6 mL methanol: D.I. water (85:15)

4.3.2 After methanol:water solution has passed through, increase vacuum to ≥ 10" Hg. Dry columns for at least 15 minutes under high vacuum.

4.4 ELUTE DRUGS

4.4.1 Prepare elution solvent fresh in volumes appropriate for extraction at the following ratios:

80 parts methylene chloride
20 parts isopropanol

Mix the above, remove 2 parts and discard, then add 2 parts concentrated ammonium hydroxide. Mix well. Use appropriate precautions when preparing the above elution solvent. After vigorous mixing, it may need to be vented carefully to avoid spatter.

4.4.2 Add 3 mL elution solvent to each column and collect eluent in appropriately labeled tubes.

4.4.3 Dry eluent under gentle steam of nitrogen in 37 deg C heat block.

5. DERIVATIZATION

5.1 To each sample tube add 50 uL ethyl acetate and 50 uL BSTFA or MSTFA. Cap, vortex mix, and heat for 15-30 minutes in 90-95 deg C heat block. Use appropriate precautions using BSTFA/MSTFA. Remove from heat and allow to cool.

5.2 Transfer samples to appropriately labeled vials with inserts for GC/MS analysis.

6. ANALYSIS

6.1 Inject 1-2 uL on the GC/MS system drugscreen.m, drugscreen2u.m, or drugscreen2.m methods, which have the following parameters:

	drugscreen.m	drugscreen2u.m	drugscreen2.m
Inj Volume (uL)	1	2	1-2
Inj Temp (deg C)	175	175	250
Temp Range (deg C)	100-280	100-280	100-280
Ramp (deg C/min)	20	20	20

6.2 Analyze samples accordingly. Report out findings based on benzodiazepine(s) found.

BENZODIAZEPINES IN URINE LIQUID/LIQUID EXTRACTION FOR GC/MS CONFIRMATION: SUMMARY METHOD

1. SAMPLE PREPARATION

1.1 STANDARDS/CONTROLS: To appropriately labeled 16 x 125 mm screw cap tubes, add 6 mL negative urine. For each standard desired (ie. oxazepam, alprazolam, etc.), add 10-20 uL of stock solution. For negative control, use 6 mL negative urine.

1.2 SUBJECT SAMPLES: To appropriately labeled 16 x 125 mm screw cap tubes, add 6 mL of subject urine.

2. SAMPLE HYDROLYSIS

2.1 To each sample tube, add 200 uL 2M acetate buffer (pH ~4.8) and 100 uL β-glucuronidase. Cap and vortex mix gently. Incubate at 55-65 deg C for 2 hours. Allow samples to cool.

3. PREPARATION FOR EXTRACTION

3.1 To each sample tube, add 2 mL of 50 mM sodium bicarbonate solution (pH 11).

4. EXTRACTION:

4.1 To each sample tube, add 6 mL of chloroform:isopropanol (9:1). Extract for 10 minutes.

4.2 Centrifuge at 3500 rpm for 5-10 minutes.

4.3 Carefully remove lower organic layer and transfer equal portions to two appropriately labeled centrifuge tubes or equivalent.

4.4 Dry extracts under gentle steam of nitrogen in 37 deg C heat block.

5. DERIVATIZATION

5.1 To one set of centrifuge tubes, add 20 uL ethyl acetate and 30 uL BSTFA or MSTFA. Cap, vortex mix, and heat for 15 minutes in 75 deg C heat block. Use appropriate precautions using BSTFA/MSTFA. Remove from heat and allow to cool.

5.2 To the remaining set of centrifuge tubes, add 50 uL ethyl acetate. Vortex mix.

5.3 Transfer samples to appropriately labeled vials with inserts for GC/MS analysis.

6. ANALYSIS

6.1 Inject 1-2 uL of each sample (derivatized and underivatized) on the GC/MS system drugscreen.m, drugscreen2u.m, or drugscreen2.m methods, which have the following parameters:

	drugscreen.m	drugscreen2u.m	drugscreen2.m
Inj Volume (uL)	1	2	1-2
Inj Temp (deg C)	175	175	250
Temp Range (deg C)	100-280	100-280	100-280
Ramp (deg C/min)	20	20	20

6.2 Analyze samples accordingly. Report out findings based on benzodiazepine(s) found.

OPIATES IN URINE GC/MS CONFIRMATION: SUMMARY METHOD

1. SAMPLE PREPARATION

1.1 STANDARDS/CONTROLS: To appropriately labeled 16 x 125 mm screw cap tubes, add 4 mL negative urine. For each standard desired (ie. codeine, morphine, hydrocodone, etc.), add 10 uL of stock solution to yield approximately 5000 ng/mL of standard. For negative control, use 4 mL negative urine.

1.2 SUBJECT SAMPLES: To appropriately labeled 16 x 125 mm screw cap tubes, add 4 mL of subject urine.

2. SAMPLE HYDROLYSIS

2.1 To each sample tube, add 500 uL concentrated HCL. Cap and vortex mix gently. Autoclave for 15 minutes or alternatively, boil sample in boiling water bath for 15-20 minutes. Allow samples to cool.

3. PREPARATION FOR EXTRACTION

3.1 To each sample tube, add 550 uL of 11.8N KOH. Vortex mix gently and allow to return to room temperature.

3.2 Adjust sample pH for each tube to approximately 8 ± 0.2 by adding 1.0N KOH, 11.8N KOH, 1N HCL, or concentrated HCL.

3.3 Centrifuge samples 5 minutes at 3500 rpm.

4. EXTRACTION:

4.1 COLUMN PREPARATION

4.1.1 Label Worldwide Monitoring Clean Screen ZSDAU020 columns and prepare vacuum extraction manifold accordingly.

4.1.2 Condition the columns by rinsing sequentially with the following: ***[NOTE: Do not allow column drying between applications]***

3 mL methanol
3 mL D.I. water

4.2 LOAD SAMPLE

4.2.1 Carefully transfer centrifuged samples to appropriate column and extract at 1-2 mL/minute.

4.3 WASH COLUMN

4.3.1 After the samples have passed through columns, rinse sequentially with the following: ***[NOTE: Columns may dry after applications of the wash solutions.]***

3 mL D.I. water
3 mL 0.1M acetate buffer, pH 4.5
6 mL methanol

4.3.2 After methanol has passed through, increase vacuum to ≥ 10" Hg. Dry columns for at least 5 minutes under high vacuum.

4.4 ELUTE DRUGS

4.4.1 Prepare elution solvent fresh in volumes appropriate for extraction at the following ratios:

80 parts methylene chloride
20 parts isopropanol

Mix the above, remove 2 parts and discard, then add 2 parts concentrated ammonium hydroxide. Mix well. Use appropriate precautions when preparing the above elution solvent. After vigorous mixing, it may need to be vented carefully to avoid spatter.

4.4.2 Add 3 mL elution solvent to each column and collect eluent in appropriately labeled tubes.

4.4.3 Dry eluent under gentle steam of nitrogen in 37 deg C heat block.

5. DERIVATIZATION

5.1 To each sample tube add 50 uL ethyl acetate and 50 uL BSTFA or MSTFA. Cap, vortex mix, and heat for 15 minutes in 90-95 deg C heat block. Use appropriate precautions using BSTFA/MSTFA. Remove from heat and allow to cool.

5.2 Transfer samples to appropriately labeled vials with inserts for GC/MS analysis.

6. ANALYSIS

6.1 Inject 1-2 uL on the GC/MS system drugscreen.m, drugscreen2u.m, or drugscreen2.m methods, which have the following parameters:

	drugscreen.m	drugscreen2u.m	drugscreen2.m
Inj Volume (uL)	1	2	1-2
Inj Temp (deg C)	175	175	250
Temp Range (deg C)	100-280	100-280	100-280
Ramp (deg C/min)	20	20	20

6.2 Analyze samples accordingly. Report out findings based on opiate(s) found.

HEROIN METABOLITE (6-MONOACETYL MORPHINE) IN URINE GC/MS CONFIRMATION: SUMMARY METHOD

1. SAMPLE PREPARATION

1.1 STANDARD/CONTROLS: To appropriately labeled 16 x 100 mm tubes, add 5 mL negative urine plus 10 uL of 6-monoacetyl morphine (6-MAM) stock solution (Radian Cat #A-026 at 100 ug/mL in acetonitrile or Radian Cat #A-027 at 1.0 mg/mL in acetonitrile) to yield approximately 400/4000 ng/mL of 6-MAM. For negative control, pipette 5 mL negative urine.

1.2 SUBJECT SAMPLES: To appropriately labeled 16 X 100 mm tubes, add 5 mL subject sample in which heroin is suspected.

2. PREPARATION FOR EXTRACTION:

2.1 To each sample, add 2 mL 0.1M phosphate buffer, pH 6.0. Vortex gently.

2.2 Centrifuge turbid specimens to avoid column blockage.

3. EXTRACTION:

3.1 COLUMN PREPARATION

3.1.1 Label Worldwide Monitoring Clean Screen ZSDAU020 columns, and prepare vacuum extraction manifold accordingly.

3.1.2 Condition the columns by rinsing sequentially with the following: ***[NOTE: Do not allow column drying between applications.]***

3 mL methanol
3 mL D.I. water
2 mL 0.1M phosphate buffer, pH 6.0

3.2 LOAD SAMPLE

3.2.1 Carefully transfer samples to appropriate column and extract at 1-2 mL/minute (low vacuum).

3.3 WASH COLUMN

3.3.1 After the samples have passed through the

columns, rinse sequentially with the following: ***[NOTE: Columns may dry after these applications following sample loading.]***

2 mL D.I. water
2 mL 0.1M acetate buffer, pH 4.5
6 mL methanol

3.3.2 After methanol has passed through, increase vacuum to ≥ 10" Hg, and dry columns for at least 5 minutes.

3.4 ELUTE DRUGS

3.4.1 Prepare elution solvent daily in volumes appropriate for extraction at the following ratios:

80 parts methylene chloride
20 parts isopropanol

Mix the above thoroughly, remove 2 parts and discard. Add 2 parts concentrated ammonium hydroxide. Mix well. Use appropriate precautions when preparing this elution solvent. After vigorous mixing, it may need to be vented carefully to avoid spatter.

3.4.2 Add 3 mL elution solvent to each column, and collect eluent in appropriately labeled tubes.

3.4.3 Dry eluent under gentle nitrogen stream in 37 deg C heat block just to dryness.

4. DERIVATIZATION

4.1 To each sample tube, add 50 uL ethyl acetate and 50 uL of BSTFA or MSTFA. Cap and vortex gently. Take appropriate precautions using BSTFA/MSTFA. Derivatize 20 minutes at 70-72 deg C. Remove from heat and allow to cool.

4.2 Transfer samples to appropriately labeled vials with inserts for GC/MS analysis.

5. ANALYSIS

5.1 Inject 1-2 uL on the GC/MS system drugscreen.m, drugscreen2u.m, or drugscreen2.m methods, which have the following parameters:

	drugscreen.m	drugscreen2u.m	drugscreen2.m
Inj Volume (uL)	1	2	1-2
Inj Temp (deg C)	175	175	250
Temp Range (deg C)	100-280	100-280	100-280
Ramp (deg C/min)	20	20	20

5.2 Analyze samples accordingly. Report out the presence of 6-MAM as containing heroin metabolite, 6-monoacetyl morphine. ***[NOTE: Sample should also contain morphine, with codeine possibly present as well.]***

MEMO TO THE RECORD

FROM: Trisa Robarge, Senior Criminalist
Coeur d'Alene BFS

DATE: January 29, 1997

RE: Use of c-THC-D9 as Internal Standard

Per a conversation with Jim Kay, formerly of Worldwide Monitoring, he suggested the use of the 9-deuterated carboxy-THC as an internal standard rather than the 3-deuterated carboxy-THC. He suggested this based on the c-THC-D9 exhibiting greater ion ratio stability over a broader concentration range than did the c-THC-D3.

At this suggestion, (±)-11-nor-9-Carboxy-Delta 9-THC-D9 (Cat # T-007) was ordered from Radian Corporation (Concentration: 100 ug/mL in methanol). A scan was performed on the derivatized stock solution to determine ions for SIM analysis. The 380 ion was selected as the quantifier ion for c-THC-D9, and 497 was selected as the qualifier.

Cannabinoid confirmations by GC/MS will utilize the D9 c-THC as internal standard from this time on. The procedure reflecting this change takes the place of the original method.

REAGENT AND SOLUTION FORMULATIONS FOR GC/MS CONFIRMATIONS

NOTE: All reagents should be labeled with identity of contents, preparation date, expiration date/stability, and initials of preparer.

CARBOXY-THC CONFIRMATION:

1.0N KOH: Add 11.8 g KOH pellets to 200 mL D.I. water. Use caution as this releases heat. KOH is caustic and can cause burns. Use appropriate precautions. Store in plastic bottle. Stable indefinitely at room temperature.

SATURATED PHOSPHATE BUFFER: Saturate approximately 500 mL D.I. water with KH_2PO_4. Saturation may be better achieved by the application of moderate heat while using a magnetic stirrer. Adjust pH to ~1.8 with concentrated phosphoric acid. Stable indefinitely at room temperature.

COCAINE/BENZOYLECGONINE/6-MAM/OPIATE CONFIRMATIONS:

0.1M PHOSPHATE BUFFER, pH 6.0: Weight 13.61 g KH2PO4 (MW=136.09) and add to approximately 900 mL D.I. water in a 1000 mL volumetric flask. Adjust buffer pH to approximately 6.0 with 1.0N KOH while stirring. Bring to volume with D.I. water. Store at 4 deg C. Stable 30-60 days when refrigerated, 1 week at room temperature.

0.1N HCL: Into a 250 mL volumetric flask, add 150 mL D.I. water. Add 2.1 mL concentrated hydrochloric acid. Hydrochloric acid may cause burns. Use appropriate precautions. Bring to volume and store at room temperature. Stable indefinitely at room temperature.

0.1M ACETATE BUFFER, pH 4.5: Dissolve 2.93g sodium acetate trihydrate in 400 mL D.I. water. Add 1.62 mL glacial acetic acid. Adjust pH to ~4.5. Bring to 500 mL volume. Stable 6 months at room temperature.

BENZODIAZEPINE CONFIRMATION, SOLID PHASE EXTRACTION:

2.0M POTASSIUM ACETATE BUFFER, pH 5.0: Prepare a 2M concentration solution of potassium acetate. Adjust pH to ~5.0. Stable 6 months at room temperature.

SATURATED SODIUM BORATE BUFFER: Saturate approximately 250 mL D.I. water with sodium borate. The application of heat is

not necessary to achieve saturation. Stable indefinitely at room temperature.

BENZODIAZEPINE CONFIRMATION, LIQUID/LIQUID EXTRACTION:

2M SODIUM ACETATE BUFFER, pH 4.8: Dissolve 68.05g sodium acetate trihydrate in 250 mL D.I. water. Adjust pH to 4.8 as necessary. Stability unknown.

50mM SODIUM BICARBONATE SOLUTION, pH 11: Dissolve 2.1g $NaHCO_3$ in 500 mL D.I. water. Stability unknown.

AMPHETAMINE/METHAMPHETAMINE CONFIRMATION:

0.35M SODIUM METAPERIODATE: ***CAUTION: Sodium metaperiodate is a strong oxidizing agent. Avoid contact with skin.*** Weigh 18.72 g sodium metaperiodate (MW = 213.98)into a volumetric flask containing approximately 150 mL D.I. water. Stir until dissolved. Bring to volume with D.I. water. Stable indefinitely at room temperature.

40% SODIUM PHOSPHATE DIBASIC: To 200 mL water, add 80 g sodium phosphate dibasic (Na_2HPO_4, MW = 142.0). Mix to dissolve. Add additional D.I. water to avoid saturation. Stable indefinitely at room temperature.

SATURATED SODIUM CARBONATE: Place a 500 mL beaker on a magnetic stirrer. Add approximately 350-400 mL D.I. water. Add sodium carbonate anhydrous powder to water while stirring until solution is saturated. The application of heat is not necessary to achieve saturation. Stable indefinitely at room temperature.