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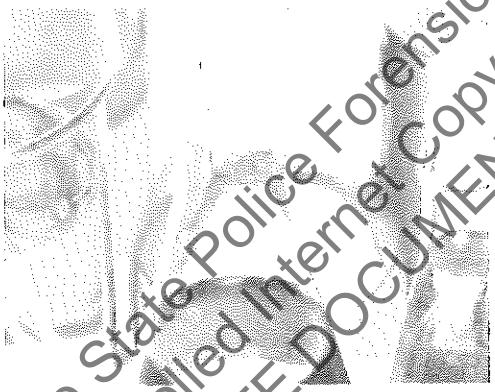

Discipline/Name of Document: Toxicology
3.9.3 – Quantitation of Basic and Neutral Drug Compounds **(FOR QUALITATIVE USE ONLY)**

Revision Number: 0

Issue Date: 11/21/2006

APPROVED BY: Carina C Duskey
Quality Manager

6/26/07
Date Signed

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Forensic Services
Toxicology Discipline

Section Three
Blood Toxicology

3.9 Liquid-Liquid Extraction Methods for Quantitative Gas Chromatography
3.9.3 Quantitation of Basic and Neutral Drug Compounds

3.9.3.1 BACKGROUND

This method is a general blood extraction procedure for a variety of commonly encountered basic and neutral drug compounds along with their metabolites. This method prepares an extract for confirmatory quantitative analysis with a gas chromatograph (GC) equipped with a mass selective (MSD) or nitrogen phosphorus (NPD) detector.

3.9.3.2 PRINCIPLE

The method is based upon the principle of liquid/liquid extraction. The sample pH is adjusted with a pH 9.2 saturated borate buffer and extracted with n-butyl chloride. Following mixing and centrifugation the supernatant is transferred and 1%HCl in MeOH is added to prevent to loss of volatile analytes. This method may be performed with or without a back extraction for sample clean-up. If the back-extraction is used, it must be applied to all calibrators, controls and samples. The final extraction solvent is evaporated to dryness and reconstituted with methanol. Quantitation is accomplished with an appropriate internal standard and a 5 to 6 point calibration curve.

3.9.3.3 EQUIPMENT AND SUPPLIES

3.9.3.3.1 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
3.9.3.3.2 Tube rocker
3.9.3.3.3 Evaporative concentrator equipped with nitrogen tank.
3.9.3.3.4 Vortex mixer
3.9.3.3.5 Laboratory centrifuge capable of 3200rpm
3.9.3.3.6 16 x 100mm round bottom glass screw-top tubes
3.9.3.3.7 Screw Cap for 16mm O.D. tubes
3.9.3.3.8 GC/MS Automated Liquid Sampler (ALS) vials
3.9.3.3.9 GC/MS vial microinsert
3.9.3.3.10 GC equipped with Dual NPDs
3.9.3.3.11 GC equipped with a MSD
3.9.3.3.12 Non-polar Capillary Column (GC-NPD and GC-MSD) 100%-Dimethylsiloxane or a 5%-Diphenyl-95%-Dimethyl-siloxane copolymer, 12.5 to 30M.

3.9.3.3.13 Mid-Polar Capillary Column (GC-NPD)
50% Phenyl, 50% methyl-polysiloxane copolymer, 12.5 to 30M.

3.9.3.4 REAGENTS

Refer to Manual section 5.12 for solution preparation instructions.

3.9.3.4.1 Methanol (Certified ACS Grade)
3.9.3.4.2 pH 9.2 Saturated Borate Buffer
3.9.3.4.3 n-Butyl chloride (Certified ACS Grade)
3.9.3.4.4 1% Hydrochloric Acid in Methanol

3.9.3.5 QUALITY ASSURANCE MATERIAL

3.9.3.5.1 Drug Stock Solutions
1mg/mL (1µg/µL) drugs standards used for calibrator and control preparation must be obtained from different vendors.

3.9.3.5.2 Working Drug Solutions

3.9.3.5.2.1 10ng/µL
Add 100.0µL each 1mg/mL Stock Solution drug(s) of interest to ≅9mL Methanol in a 10mL volumetric class A flask. QS to 10mL. Working drug solutions may be mixed or single compound depending on the compound's retention time.

3.9.2.5.2.2 1ng/µL
Add 1.0mL 10ng/µL working drug solution to ≅8mL Methanol in a 10mL volumetric class A flask. QS to 10mL.

3.9.3.5.2.3 Working solutions are stable for 6 months when stored at 4°C.

3.9.3.5.2.4 Store remaining stock solution in ALS vial in freezer.

3.9.3.5.3 Internal Standard Stock Solutions
Select appropriate internal standard for drug of interest. For GC-MSD use deuterated standard when available. The highest degree of deuteration available should be used when using GC-MS.

3.9.3.5.4 1ng/µL Working Internal Standard Solution

Add 10µL each 1mg/mL or 100.0µL each 100µL/mL Stock Solution to to ≅9mL Methanol in a 10mL volumetric class A flask. QS to 10mL. Store remaining stock solution in ALS vial in freezer. More than one internal standard may be added to this working solution.

Working solution is stable for 6 months when stored at 4 ℃.

3.9.3.5.5 Negative Control
Negative Blood Control

3.9.3.6 PROCEDURE

3.9.3.6.1 Initial set-up
For each calibrator, control and case sample, label two sets of extraction tubes and an ALS vial with microinserts,

3.9.3.6.2 Calibration Standard Preparation

3.9.3.6.2.1 Add 2mL of negative whole blood to five screw-top extraction tubes.

3.9.3.6.2.2 Add the volume of working 1ng/µL working calibration standard as indicated in the chart below.

Level	Desired ng/mL	µL Working Standard
1	25	50
2	50	100

3.9.3.6.2.3 Add the volume of working 10ng/µL working calibration standard as indicated in the chart below.

Level	Desired ng/mL	µL Working Standard
3	100	20
4	250	50
5	500	100
6	1000	200

3.9.3.6.3 Positive Control Sample Preparation

3.9.3.6.3.1 Add indicated amount of working 10ng/µL mixed control solution to 2mL negative whole blood.

Desired ng/mL	µL Working Control
75	15
750	150

3.9.3.6.3.2 Positive controls must be run in duplicate. For every 10 case samples prepare an additional control.

3.9.3.6.4 Negative Control Sample Preparation
Add 2mL of negative whole blood to screw top tube.

3.9.3.6.5 Case Sample Preparation
Add 2mL neat or diluted sample to labeled screw top tube.

3.9.3.6.6 Internal Standard Addition

3.9.3.6.6.1 To calibrators, controls and case samples, add 200µL of internal standard. Vortex.

3.9.3.6.6.2 Allow tubes to stand 15 minutes.

3.9.3.6.7 Extraction

3.9.3.6.7.1 Add 2mL pH 9.2 borate buffer. Vortex.

3.9.3.6.7.2 Add 4mL n-Butyl Chloride into each tube, cap.

3.9.3.6.7.3 Place tube on rocker for a minimum of 10 minutes.

3.9.3.6.7.4 Centrifuge for 10 minutes at 3200 - 3400rpm.

3.9.3.6.7.5 Transfer the upper n-Butyl Chloride layer to second tube.

3.9.3.6.7.6 Add 50µL 1% HCl in MeOH

3.9.3.6.7.7 Evaporate n-Butyl Chloride to dryness under a gentle stream of nitrogen at ≤37°C.

3.9.3.6.8 Back-extraction Clean-up

3.9.3.6.8.1 Reconstitute with 50ul 100mM HCl.

3.9.3.6.8.2 Add 1ml of n-Butylchloride. Vortex.

3.9.3.6.8.3 Centrifuge for 2 minute at ≥3200 rpm.

3.9.3.6.8.4 Draw off and discard the upper n-butyl-chloride layer.

3.9.3.6.8.5 Transfer lower acidic layer in to a 16 x 100mm screw top tube.

3.9.3.6.8.6 Add 2ml of borate (pH 9.2) solution.

3.9.3.6.8.7 Add 4 ml of n-butylchloride and vortex.

3.9.3.6.8.8 Centrifuge for 10 minutes at ≥3200rpm.

3.9.3.6.8.9 Transfer upper solvent layer into a 16 x 100mm culture tube.

3.9.3.6.8.10 Evaporate to dryness under a gentle stream of nitrogen at ≤37°C.

3.9.3.6.9 Reconstitution

3.9.3.6.9.1 Add 50uL methanol to the residue, vortex.

3.9.3.6.9.2 Transfer extract to labeled ALS vial with microinsert.

3.9.3.6.10 Preparation for Analysis Run

3.9.3.6.10.1 Into Sequence log table, enter the sample case numbers, blanks and controls.

3.9.3.6.10.2 Load samples, standards, blank and controls into the quadrant rack as noted in the sequence table.

3.9.3.6.11 Analysis Parameters

3.9.3.6.11.1 Refer to instrument METHOD printouts for analysis parameters.

3.9.3.6.11.2 Appropriate ions for quantitative analysis are selected from full scan analysis of standard. Selected ion monitoring at the corresponding retention time is configured accordingly.

3.9.3.6.11.3 Current analysis method must be stored centrally as a hard or electronic copy.

3.9.3.6.12 GC-MS Calibration Curve

3.9.3.6.12.1 The calibration curve should be established with a minimum of four data points.

3.9.3.6.12.2 All reported results must be bracketed by calibrators.

3.9.3.6.12.3 Calibrators should be analyzed in order of increasing concentration.

3.9.3.6.12.4 The least squares line resulting from the analysis of calibrators must have a coefficient of correlation of ≥0.98.

3.9.3.6.12.5 If calibration standards are run in duplicate, it is not required that duplicate calibration points are included as long as the linearity requirement is met.

3.9.3.7 REPORTING CRITERIA

3.9.3.7.1 Qualitative Chromatographic Criteria

Qualitative results can be accepted when the following two criteria are met.

1. The retention time falls within the ±0.2 minute window established by calibrators.
2. For mass spectral SIM data, ion ratios for the analyte and its corresponding internal standard, established by calibrators for target and qualifier ions, do not differ by more than ±20%.

3.9.3.7.2 Quantitative Criteria

3.9.3.7.2.1 Quantitative results can be accepted if the calculated concentration of all calibration standards and control samples are within ±20% of their respective concentrations.

3.9.3.7.2.2 Quantitation is achieved through the plotting of the compound's response ratio versus the concentration for each calibrator.

3.9.3.7.2.3 Quantitative values for case samples, calibrators and controls will be truncated for reporting purposes.

3.9.3.7.2.4 Limit of quantitation is the lowest calibrator.

3.9.3.7.2.5 If the concentration exceeds the calibration range, the sample can either be appropriately diluted with DI water for reanalysis or reported as greater than 1000ng/mL.

3.9.3.8 REPORTING OF RESULTS

3.9.3.8.1 Quantitative Value

Analysis results should be truncated and reported out without decimal places.

3.9.3.8.2 Uncertainty Value

Based on the current uncertainty assessment, the +/– range should be included on the analysis report. Refer to quality monitoring spreadsheet for current uncertainty figure.

3.9.3.9 QUALITY ASSURANCE REQUIREMENTS

3.9.3.9.1 General

3.9.3.1.1 Blood samples are to be stored under refrigeration after aliquots are removed for analysis.

3.9.3.1.2 Refer to toxicology analytical method 5.1 for pipette calibration options.

3.9.3.1.3 Refer to toxicology analytical method 5.2 for balance calibration requirements.

3.9.3.1.4 Refer to toxicology analytical method 5.3.1 for GC-MSD maintenance guidelines.

3.9.3.1.5 Refer to toxicology analytical methods 5.8 and 5.10 for reference standard authentication and additional GC-MSD quality assurance requirements.

3.9.3.9.2 Monitoring of Control Values

Upon the completion of analysis, input blood control values on spreadsheet used to assess uncertainty for this method.

3.9.3.10 ANALYSIS DOCUMENTATION

3.9.3.10.1 A packet containing original data for controls and standards will be prepared for each analysis run and stored centrally in

the laboratory where the analysis was performed until archiving.

3.9.2.10.2 A copy of controls and standards need not be included in individual case files. When necessary, a copy of the control and standard printouts can be prepared from the centrally stored document.

3.9.3.11 REFERENCES

3.9.3.11.1 Procedure for Basic Drug Analysis, Courtesy of Jim Hutchison, Montana Department of Justice, Forensic Services Division, 2005.

3.9.3.11.2 Strong Bases Extractions - Screening SOP, Courtesy of Dr. Graham Jones, Office of the Chief Medical Examiner, Edmonton, Canada, 2003.

3.9.2.11.3 Jones, G., *Postmortem Toxicology*. pp. 98-102, ***in:*** Clarke's Analysis of Drugs and Poisons, 3rd Edition, Moffat, A.C, Osselton, M.D. and Widdop, B., eds., Pharmaceutical Press, 2004.

3.9.3.11.4 Hearn, W.L. and Walls, H.C. Strategies for Postmortem Toxicology Investigation. pp. 937-939. ***in:*** Drug Abuse Handbook, S.B. Karch, ed., CRC Press, Boca Raton, FL, 1998.

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Discipline Leader: _____ **Date:** _____
Susan C. Williamson

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