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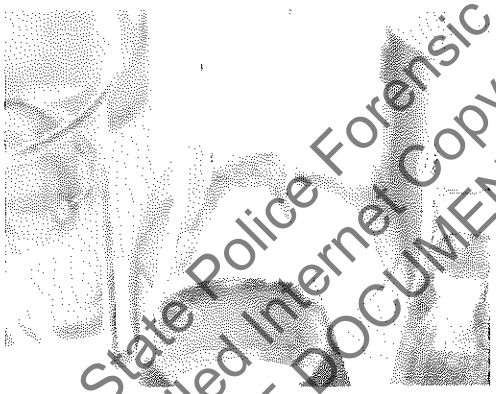

Discipline/Name of Document: Toxicology
2.2.3 Toxi-Lab® Amine Differentiation with Acetaldehyde

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Quality Manager

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Date Signed

Section Two
Urine Toxicology

2.2 ANSYS® Thin Layer Chromatography (TLC) Methods
2.2.3 Toxi-Lab® Amine Differentiation with Acetaldehyde

2.2.3.1 BACKGROUND

Refer to 2.2.1.

2.2.3.2 SCOPE

This procedure describes a modification of the TOXI-LAB A Drug Detection System. The system is optimized for the separation and thus the differentiation of urinary phenylethylamine compounds through the addition of acetaldehyde to the TOXI-A elution solvent. The results serve to support the results of the enzyme immunoassay (EIA) screen or used in lieu of a EIA screen.

2.2.3.3 EQUIPMENT AND SUPPLIES

2.2.3.3.1 Tube rocker
2.2.3.3.2 Laboratory centrifuge
2.2.3.3.3 Solvent concentrator with appropriate concentration cups or tubes
2.2.3.3.4 Electric (plate) warmer
2.2.3.3.5 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
2.2.3.3.6 Chromatography jar with cap
2.2.3.3.7 Forceps
2.2.3.3.8 Disc handling pins
2.2.3.3.9 Index cards for use as disc press cards
2.2.3.3.10 TOXI-GRAMS A
2.2.3.3.11 TOXI-DISCS Blank A
2.2.3.3.12 TOXI-LAB A Worksheets
2.2.3.3.13 TOXI-DIP A-1 Stand-off Jar
2.2.3.3.14 TOXI-LAB A Elution Solvent Bottle

2.2.3.4 REAGENTS

2.2.3.4.1 Acetaldehyde (Certified ACS Grade)
2.2.3.4.2 Ammonium Hydroxide (ACS Grade)
2.2.3.4.3 Ethyl Acetate (Low acetaldehyde)
2.2.3.4.4 Methanol (ACS Grade)
2.2.3.4.5 Formaldehyde, 37% by weight
2.2.3.4.6 Sulfuric Acid (ACS Grade)
2.2.3.4.7 Elution Solvent

Mix 87mL ethyl acetate, 3mL methanol and 1.5mL water. Add 200µL acetaldehyde and mix well. *Store tightly capped at room temperature.*

2.2.3.5 STANDARDS AND CONTROLS

2.2.3.5.1 Sympathomimetic amines standard disc
2.2.3.5.2 Custom amine standard disc
2.2.3.5.3 Toxi-Control No. 19
2.2.3.5.4 Toxi-Control No. 2
2.2.3.5.5 Negative urine control

2.2.3.6 PROCEDURE

2.2.3.6.1 Label TOXI-TUBES A for negative control, positive control (TC-19 and/or TC-2) and for case samples with appropriate laboratory numbers.

2.2.3.6.2 Transfer 5mL each of urine specimen, negative urine and positive control to TOXI-TUBE A. Place on rocker for ≥10 minutes.

2.2.3.6.3 Centrifuge tube at ≥2500 rpm for ≥10 minutes.

2.2.3.6.4 Place appropriate number of concentration cup into Omega-12 extraction solvent concentrator. To each cup add a Toxi-A disc. Allow cup to warm prior to the addition of extract.

2.2.3.6.5 Transfer upper solvent layer from tube into pre-heated concentration cup in Omega-12 extraction solvent concentrator.

2.2.3.6.6 Evaporate solvent on disc on electric warmer. Take care not to over dry disc.

2.2.3.6.7 Insert sympathomimetic amines standard disc and/or custom amine disc into labeled channel on 6-channel TOXI-GRAM A.

2.2.3.6.8 Place specimen disc into labeled channel on 6-channel TOXI-GRAM A.

2.2.3.6.9 Heat the GRAM, with the disc end slightly off the warmer edge.

2.2.3.6.10 Add 3mL of developing solution to chromatography jar.

2.2.3.6.11 To developing solution, add the volume of ammonium hydroxide indicated on TOXI-GRAM A box and swirl vigorously.

2.2.3.6.12 Place GRAM into chromatography jar. Allow dye marker to migrate to ≅ 10 cm.

2.2.3.6.13 Remove GRAM from jar and place face down on warmer for 30-60 seconds.

2.2.3.6.14 Place GRAM into TOXI-DIP A-1 for formaldehyde fuming for ≥5 minutes.

2.2.3.6.15 Slowly dip GRAM into TOXI-DIP A-2 [concentrated sulfuric acid]. Remove and observe the R_f value and color characteristics of compounds versus those exhibited by the compounds in the standard disc for 15 to 60 seconds.

2.2.3.6.16 Place GRAM into a page protector and label samples and controls.

2.2.3.6.17 Photocopy GRAM, with header information, for each case file.

2.2.3.7 DETECTION AND IDENTIFICATION CRITERIA

2.2.3.7.1 The phenylethylamine constituents in the standard disc should exhibit the elution order and color characteristics indicated in the chart below.

2.2.3.7.2 Positive control should establish the presence of appropriate phenylethylamine compounds by exhibiting the proper R_f and color characteristics.

2.2.3.7.3 Negative control should not exhibit characteristics supporting the presence of phenylethylamine compounds or contain interfering substances.

2.2.3.7.4 The method supports the presence of a phenylethylamine class drug compound if there are no significant differences in the R_f value and color characteristics for the sample versus appropriate standard. Consideration should be given to concentration differences and/or interfering/coeluting substances.

2.2.3.7.5 The following table indicates the elution order and color characteristics of commonly encountered phenylethylamines and interfering substances. Absolute R_f is provided only to establish elution order.

Compound	R_f	Stage I Color Characteristics
Ephedrine/pseudoephedrine	0.85	Yellow → green center
Phenylpropanolamine (PPA)	0.70	Yellow → green center
Amphetamine	0.55	Yellow → brown center
3,4-Methylenedioxyamphetamine (MDA)	0.55	Deep purple-blue
β-Phenylethylamine	0.52	Yellow → brown center
Para-Methoxyamphetamine (PMA)	0.50	Deep purple-blue
Labetalol	0.50	Melon yellow
Phentermine	0.39	Yellow → brown center
3,4-Methylenedioxyethylamphetamine (MDE)	0.35	Deep purple-blue
Methamphetamine	0.25	Yellow → brown center
3,4-Methylenedioxymethamphetamine (MDMA)	0.25	Deep purple-blue
Normeperidine	0.18	Yellow → green center

2.2.3.8 REFERENCES

2.2.3.8.1 TOXI-LAB Drug Compendium, Adams, D.J., ed., ANSYS Diagnostics, Inc., 1998.

2.2.3.8.2 Moore, K., *Amphetamines/Sympathomimetic Amines*. pp. 277. *in:* Principles of Forensic Toxicology. Levine, B. ed., AACC, 1999.

2.2.3.8.3 Phenylethylamines, ANSYS Diagnostics, Inc., 2000.

Revision History

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Revision #	Issue Date	Revision
0	10-18-02	SOP format
1	05-07-2007	Reformat