

## 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  
May be either in-house verified or commercially obtained.

### 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  $\geq 10$  minutes.
- 2.2.3.6.3 Centrifuge tube at  $\approx 2500$  rpm for  $\geq 10$  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  $\cong$  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  $\geq$ 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
$\beta$ -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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#### Urine Toxicology

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#### 2.2 ANSYS<sup>®</sup> Thin Layer Chromatography (TLC) Methods

##### 2.2.3 Toxi-Lab<sup>®</sup> Amine Differentiation with Acetaldehyde

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<b>Revision #</b>	<b>Issue Date</b>	<b>Revision</b>
0	10-18-2002	SOP format
1	05-07-2007	Reformat
2	07-28-2008	QA requirements clarified

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