Section Two
Urine Toxicology

2.4 Liquid-Liquid Extraction Methods for Qualitative GC/MSD Confirmation

2.4.1 General Extraction of Urine for Basic and Neutral or Acidic and Neutral Compounds

2.4.1.1 BACKGROUND

These extraction procedures are extensions of the TOXI-LAB® TOXI-A and TOXI-B thin layer chromatography (TLC) drug detection systems. The samples are extracted as with the TLC system, however, instead of concentrating the extract onto a disc, the solvent extract is concentrated and placed into an automated liquid sampler (ALS) vial for analysis by a gas chromatograph equipped with a mass selective detector (GC/MSD). Discussions of TLC and GC/MS theory can be found in most college-level chemistry and/or instrumental texts. In 2013 the TOXI-LAB line was discontinued. An equivalent product, De-Tox Tubes by Dyna-Tek, were evaluated and found to be a suitable replacement.

2.4.1.2 SCOPE

This procedure describes the extraction of drug compounds from urine. Depending upon the pK_a of a drug compound, either De-Tox Tubes A or B (or verified equivalents) are used. Basic and neutral compounds are extracted with an A tube. Addition of urine to the De-Tox A tube results in the sample becoming alkaline and basic and neutral drugs thus extract into a solvent mixture. The B tube is used for acidic and neutral compounds. Urine placed into the De-Tox B tube becomes acidic resulting in acidic and neutral compounds being extracted into a solvent mixture. Either resulting extract is analyzed by full scan GC/MS in EI mode.

2.4.1.3 EQUIPMENT AND SUPPLIES

2.4.1.3.1 Tube Rocker
2.4.1.3.2 Evaporative concentrator and appropriate concentration cups or tubes
2.4.1.3.3 Laboratory Centrifuge capable of 3000 rpm
2.4.1.3.4 Laboratory oven or waterbath
2.4.1.3.5 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
2.4.1.3.6 Automated Liquid Sampler (ALS) vials
2.4.1.3.7 GC/MS Vial Microinsert
2.4.1.3.8 Gas Chromatograph equipped with a mass selective detector and a low bleed (5%-Diphenyl-95%-Dimethylsiloxane copolymer) capillary column.

2.4.1.4 REAGENTS

2.4.1.4.1 De-Tox Tubes A and B (or equivalent Toxi Tubes)

2.4.1.4.2 β-Glucuronidase Solution

2.4.1.4.3 2M Acetate buffer, pH 4.8

2.4.1.5 QUALITATIVE CONTROLS

2.4.1.5.1 Positive control

Tube A positive control may be commercially obtained or prepared in-house. At a minimum, the control must contain at least one phenethylamine at an approximate concentration between 500 and 3000 ng/mL, and one opiate at an approximate concentration between 300 and 3000 ng/mL.

Tube B positive control may also be commercially obtained or prepared in-house. At a minimum, the control must contain two barbiturates at an approximate concentration between 300 and 1000 ng/mL.

2.4.1.5.2 Negative Urine

Negative urine can be commercially obtained or in-house urine verified to be negative for drugs of interest. Refer to AM 5.8.6.1.1.3 for additional details.

2.4.1.5.3 Morphine-Glucuronide Positive and Negative Controls for Optional Enzymatic Hydrolysis Step

Commercially-obtained control or in-house spiked urine containing morphine-glucuronide should be used. The same negative urine must be used to prepare both the positive and negative glucuronide controls for in-house spiking. Morphine-glucuronide should be used for these controls and must be at a minimum concentration of 375ng/mL. The positive and negative glucuronide controls are used to demonstrate the glucuronidase cleavage was effective.

2.4.1.6 QUALITATIVE NON-EXTRACTED REFERENCE MATERIAL

2.4.1.6.1 Run necessary reference material as indicated by examination of GC/MSD data. Reference material mixes may be used.

2.4.1.6.2 Dilute reference material as necessary. A suggested dilution for a 1mg/mL solution is 1 in 3 parts of appropriate solvent.

2.4.1.7 METHOD

2.4.1.7.1 De-Tox Tubes-A Extraction (Basic and Neutral Compounds)

2.4.1.7.1.1 Label DE-TOX TUBES A and ALS vials with microinserts for negative control, positive control and appropriate laboratory numbers.

2.4.1.7.1.2 Agitate the DE-TOX tube to break up salts. This assists in reducing the occurrence of emulsions.

2.4.1.7.1.3 Transfer ≅ 5 mL of casework, negative and positive control urine to appropriate DE-TOX TUBE A (pH=9).

2.4.1.7.1.4 Rock DE-TOX TUBE A for at least 10 minutes.

2.4.1.7.1.5 Centrifuge tube at ≅2500-3000 rpm for ≅10minutes.

2.4.1.7.1.6 Transfer solvent and evaporate to ≅100-300µL.

2.4.1.7.1.7 Transfer solvent to labeled GC/MS ALS vial with microinsert.

2.4.1.7.1.8 OPTIONAL: Analyst may, at their discretion, perform an enzymatic hydrolysis on a sample aliquot prior to the above De-Tox Tube A extraction. If done, this must be done in addition to the regular (non-hydrolyzed) extraction of the sample. Positive and negative morphine glucuronide controls (see section 2.4.1.5.4) should be run in addition to the regular controls required by the method.

2.4.1.7.1.9 Optional Enzyme Hydrolysis: To 4.5mL of urine, add 150µL of 2M acetate buffer and vortex. To all but the glucuronidase negative control, add 75µL of β-glucuronidase solution. Cap and vortex *gently* to mix. Place in a 60°C laboratory oven or waterbath for 2 hours. Allow sample to cool before proceeding with steps 2.4.1.7.1.2 through 2.4.1.7.1.7.

2.4.1.7.2 De-Tox Tubes-B Extraction (Acidic and Neutral Compounds)

2.4.1.7.2.1 Label DE-TOX TUBES B and ALS vials with microinserts for negative control, positive control and appropriate laboratory numbers.

2.4.1.7.2.2 Transfer ≅4.5 mL of casework, negative and positive control urine to appropriate DE-TOX TUBE B (pH=4.5).

2.4.1.7.2.3 Rock DE-TOX TUBE B for at least 10 minutes.

2.4.1.7.2.4 Centrifuge tube at ≅2500-3000 rpm for ≅10 minutes.

2.4.1.7.2.5 Transfer solvent and evaporate to ≅100-300µL.

2.4.1.7.2.6 Transfer solvent to labeled GC/MS ALS vial with microinsert.

2.4.1.7.3 Preparation for Analysis Run

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

2.4.1.7.3.2 Load samples, reference materials, blanks and controls into the quadrant rack as noted in the sequence table.

2.4.1.7.4 GC-MSD Analysis Parameters

2.4.1.7.4.1 Refer to instrument METHOD for current analysis parameters.

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

2.4.1.7.5 Detection and Identification Criteria

The presence of a drug compound is indicated if the retention time for the sample versus applicable reference material does not differ by more than ±0.2 minutes and there are no significant differences in the mass spectral data.

NOTE: early eluting drugs, as well as drugs known to have similar retention times and mass spectral fragmentation patterns (e.g. Phentermine and methamphetamine), may not differ from the retention time of the applicable reference material by more than ±0.1 minutes.

2.4.1.8 QUALITY ASSURANCE REQUIREMENTS

2.4.1.8.1 Refer to toxicology analytical methods 5.8 and 5.10 for additional quality assurance and reference material authentication requirements.

2.4.1.9 ANALYSIS DOCUMENTATION

2.4.1.9.1 Case results are to be recorded in the LIMS system.

2.4.1.9.2 Original data for controls will be prepared for each analysis run and stored centrally in the laboratory where the analysis was performed until archiving.

2.4.1.9.3 A copy of controls may be stored electronically in a central location and need not be included in individual case files. When necessary, a copy of control printouts can be prepared from the centrally stored document.

Revision History

Section Two
Urine Toxicology

2.4 Liquid-Liquid Extraction Methods for Qualitative GC/MSD Confirmation
2.4.1 General Extraction of Urine for Basic and Neutral or Acidic and Neutral Compounds

Revision #	Issue Date	Revision
1	11-27-2001	Original Issue in SOP format
2	10-17-2002	Refinements
3	05-07-2007	Updated QA measures and reformatting.
4	07-28-2008	QA requirements clarified
5	12-16-2011	Added BRC3 as a positive control option, reduced concentration amount of extract from 200-300ul to 100-300ul. Clarified that centrifuge times and speeds are approximated. Changed tube rocking from 15 minutes to at least 10 minutes Changed centrifuge time from 15 minutes to about 10.
6	09-06-2013	Replaced TOXI-Lab tubes with De-Tox tubes and allowed for equivalent tubes to be used, added additional options for positive controls.
7	01-16-2014	Amendment to 2.4.1.9 in accordance with new LIMS system. Minor formatting changes.
8	04-02-2015	Added analysis technique hint (2.4.1.7.1.2). Increased rigor of identification criteria (2.4.1.7.5) for certain compounds. Minor formatting changes. Addition of optional enzymatic hydrolysis in addition to regular extraction of sample – added required controls for this option, added reagents necessary. This change is consistent with AM 6.1.1 and AM 2.4.3 enzymatic hydrolysis procedures.