## 1.0 PURPOSE:

Sympathomimetic Amines (Amphetamines, and its structural analogues; "SMA's") can cause profound behavioral changes and have high potential for abuse due to both chemical and psychological dependence. MDMA and MDA are metabolized to Methamphetamine and Amphetamine respectively. SMAs are water soluble and excreted in the urine. Blood and urine samples, screening positive for SMAs, are extracted using a solid phase extraction column, co-extracting materials are washed from the column and any SMAs that may be present are eluted using a methylene chloride/isopropanol/NH4OH mixture. After evaporation of the solvent, the extracted drug is derivatized with TFAA. Derivatized samples are analyzed by GC/MS.

Qualitative identification of the SMAs is based on retention time and ion ratios for 3 ions compared to the corresponding ion ratios from a calibrator extracted and run in the same batch. Concentrations of SMAs are determined by single point calibration, using d3-analogue of the amphetamine analyte as the internal standard. Each GC/MS batch is separately evaluated using control and blank samples, and is processed by a custom spreadsheet program, on which calibrator, blank and control results are summarized and tabulated, and batch review process by both the analyst and technical reviewer is documented. Matrix-specific (blood and/or urine) positive and negative controls are extracted and analyzed in each analytical batch. The presence of SMAs may be confirmed in urine, blood or other aqueous fluids.



## 2.0 SPECIMEN

Specimens requiring confirmation for SMA's are listed by lab case number on the clip board marked "SMA List" which is maintained in the Toxicology Instrument room. Analysts preparing a batch for analysis should generate their batch sample list (see form 23.4, appended) from this document.

2.1 All evidence transfers, either between individuals or between an individual and a storage location must be documented on the Chain of Custody for the case, either in the LIMS, or on hard-copy COC document maintained in the Case Jacket.

2.2 When not in the sampling or aliquot process, samples in the Toxicology section must be stored in the secure and locked Toxicology evidence storage room.

2.3 Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.

2.4 When analysis of samples in the toxicology section is complete, they must be maintained "Under Proper Seal." This is interpreted to mean that the sample, or a container in which the sample is kept, is sealed with tamper-evident tape, with the initials and date of person placing the seal clearly marked on, or proximate to that seal.

2.5 Samples are maintained in the Toxicology Section for 8 weeks after case is completed, in the absence of notification of any legal action, or other reason to maintain the samples. After this period, samples are discarded in the appropriate medical waste disposal container. Sample from fatalities, or cases with requests for retention are maintained by the laboratory.



## 3.0 EQUIPMENT:

GC/MS and associated data station/computer (HP6890/5973 or equivalent)
General laboratory glassware and equipment
Solid phase extraction manifold and associated vacuum equipment.
Analytical evaporator (Zymark Turbovap or equivalent)
UCT; ZSDAU020 "Clean Screen" extraction columns

## 4.0 Reagents

**4.1** Reagents available as stock items:

Methanol (Baker HPLC grade or equivalent)
Ammonium Hydroxide (Baker reagent grade or equivalent)
Ethyl acetate (Baker HPLC grade or equivalent)
Methylene Chloride (Baker reagent grade or equivalent)
Isopropanol (Baker reagent grade or equivalent)
Triflouroacetic anhydride (TFA) (Pierce or equivailent)
Blank Blood (Received from Blood bank source – Outdated supply)

Drug free urine
Amphetamine Stock: 1 mL vial 1.0 mg/mL (Alltech or equivalent)
Methamphetamine Stock: 1 mL vial 1.0 mg/mL(Alltech or equivalent)
Methylenedioxyamphetamine Stock: 1 mL vial 1.0 mg/mL(Alltech or equivalent)
Methylenedioxymethamphetamine Stock: 1 mL vial 1.0 mg/mL(Alltech or equivalent)
Amphetamine deuterated Stock: 1 mL vial 1.0 mg/mL (Alltech or equivalent)
Deionized water (DIW; Millipore or equivalent In-House supply)
Glacial Acetic acid (Baker reagent grade or equivalent)
Sodium Phosphate Dibasic (Na2HPO4).
Sodium Phosphate Monobasic (NaH2PO4).
Hydrochloric acid (Baker reagent grade or equivalent)

**4.2** Reagents prepared in the Toxicology Laboratory:

1 M Acetic acid; dilute 28.6 mL glacial acetic acid to 500 mL DIW
0.1 M Acetic acid; dilute 50 mL 1 M acetic acid to 500 mL DIW (or equivalent ratio).
0.1 M phosphate buffer pH 6.0; combine 48.6 g Na2HPO4 and 6.8 g NaH2PO4' dilute to 4000 mL using DIW.
1% methanolic HCl; dilute 1 mL concentrated HCl to 100 mL methanol.
Methylene chloride/isopropanol/ammonium hydroxide (39/10/1; prepare volume as appropriate) Note: Must be prepared fresh each day of use.

Note: Reagent Preparation and Validation is documented in the Toxicology "Reagent Preparation/Validation Logbook" maintained in the Toxicology section. Validation of reagents is addressed below (sec. 4.5).

**4.3** Calibrators and Internal Standard:
Note: Preparation of all calibrator and control solutions is documented in the "Calibrator and Control Preparation Log" (maintained in the Toxicology Wet Laboratory)

4.3.1 SMA internal standard solution (Amphetamine $D_5$, or related deuterated SMA; Alltech or equilvalent; 100 ug/mL)

4.3.2 SMA Stock Calibrator solution: 100 ul of each standard solution (Amphetamine, Methamphetamine, Methylenedioxyamphetamine and Methylene dioxyamphetamine; each 1 mg/mL) to a 10 mL volumetric flask, Q.S. with MeOH.

4.4 Controls:

4.4.1 SMA Stock Control solution: SMA Stock Calibrator solution: 100 ul of each standard solution (Amphetamine, Methamphetamine, Methylenedioxyamphetamine and Methylene dioxyamphetamine; each 1mg/mL) to a 10 mL volumetric flask, Q.S. with MeOH.

4.4.2 Each run will incorporate a high and low control for each analyte. Blood matrix calibrators/controls may be used to validate urine results, but not the reverse. Controls are prepared by addition of SMAs from validated stock solutions to blank sample matrix aliquots, prior to extraction, (details in Procedure below). Acceptable control performance is target value +/-20%.

4.5 Validation of Reagents: Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Validated reagents are marked with a green dot, detailing the specific procedure for which the reagent was validated, and the batch on which that process was documented. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample results. Reagents so validated are marked with a green sticker as noted above. Preparation of reagents, and their validation is documented in the Toxicology Section Reagent Preparation Validation Logbook, maintained in the Toxicology laboratory. See SOP #11.



## 5.0 Procedure

Departure from procedures as specified in this SOP is not anticipated. Should an issue arise that may require such a departure, the issue must be raised with Section Supervisor, Quality Manager and/or the Director. If the proposed change will not present a change of such a magnitude so as to require validation, the change may be approved, and the Director will modify and re-issue the SOP accordingly.

Any such procedural changes would be subject to the review process afforded by the quality control measures of the analytical scheme described herein. Hence, any modification or change that produces an unexpected deleterious effect on the analytical procedure would be expected to trigger control or batch failure in the QC review stages.

5.1 Batch Format: Analytical batches for SMA confirmation should follow the format indicated below: Note that samples are analyzed in duplicate.

Calibrator (500 ng/mL each; Amph., Methamp., MDA, MDMA)
Matrix blank
Control High(1000 ng/mL each; Amph., Methamp., MDA, MDMA)
Control Low(200 ng/mL each; Amph., Methamp., MDA, MDMA)
Sample 1 rep 1
Sample 1 rep 2
Samples 2-? Rep 1 and 2
Control High(1000 ng/mL each; Amph., Methamp., MDA, MDMA)
(Final control, in the absence of additional samples)
(Additional Samples)
(Final control (may be high or low))

5.2 Label a 13x100 culture tube for each sample replicate, blank, calibrator and control. Using a validated dispensing pipette, place 1.0 mL aliquot of each sample replicate into each appropriately labeled 13x100 culture tube.

Note: Samples requiring dilution as a function of concentration greater than the high control, should be diluted with 0.1M pH 6.0 Phosphate buffer as appropriate. The initial quantitative values may be used as a guide for the dilution process. The final dilution volume should be greated than the 1.0 ml aliquot that will be taken. The dilution process shall be documented on the batch worksheet, including the pipette(s) used in the process:

Example: Blood sample; 1st run result, ~ 1250 ng/mL amphetamine ~ 2.8 mg/L methamphetamine. Since a 1:1 dilution would be inadequate for the methamphetamine (final result would be expected to be ~1.9 ug/mL, still in excess of the 1.0 ug/mL high control), a 1:4 dilution is selected. Therefore, 300 uL

sample is added to a 13 x 100 test tube using a 0 – 1 mL adjustable volume pipette, along with 3 x 300 uL 0.1 M pH 6.0 Phosphate buffer. (Note; because equal volumes of sample were utilized, pipette precision determines the accuracy of the dilution). The solution is mixed, and aliquotted as per step 5.3. The dilution details, including the dilution factor (Volume sample: Volume Diluent + Volume Sample) are documented on the batch worksheet.

5.3 Using a validated dispensing pipette, add 20 ul of deuterated amphetamine internal standard solution to each replicate, blank, calibrator and control.

5.4 Using a validated dispensing pipette, add 50 ul of SMA calibrator standard stock solution to the tube labeled Calibrator.

5.5 Using a validated dispensing pipette, add 100 ul of SMA control standard stock solution to the tube labeled High Control.

5.6 Using a validated dispensing pipette, add 20 ul of SMA control standard stock solution to the tube labeled Low Control.

5.7 Add 1 mL of negative urine to each control, calibrator and blank to be run if sample matrix is urine. Add 1 mL of negative blood to each control, calibrator and blank to be run if sample matrix is blood.

5.8 Add 3 mL of 0.1 M pH 6.0 Phosphate buffer to all samples. Label SPE tubes to correspond with each culture tube, place a clean thru tip on each end and load in the manifold.

5.9 Condition the columns:
1 x 3 mL methanol; drain (vacuum assist)
1 x 3 mL DIW; drain (vacuum assist)
1 x 1 mL 100 mM Phosphate buffer; drain (vacuum assist)
DO NOT LET COLUMN GO DRY!

5.10 Transfer contents of each tube to the appropriately labeled SPE tube, and allow gentle drop wise flow until the level reaches the top if the column bed.

5.11 Wash column:
1 x 2 mL DIW; (vacuum assist)
1 x 2 mL 100mM Acetic acid; (vacuum assist)
1 x 3 mL Methanol; (vacuum assist)
Dry column (15 minutes at > 10 inches Hg)

5.12 Position appropriately labeled 13x100 test tubes under each SPE column, with clean thru tip inside collection test tube.

5.13 Elute analytes:
1 x 3 mL CH2Cl2/Isopropanol/NH4OH (78:20:2).
Collect Eluate at 1 to 2 mL/minute.
Add 1 drop of 1% methanolic HCl to each calibrator, control, sample and blank tubes.

5.14 Remove receiver tubes and evaporate to dryness at < 40 C using a gentle flow of Nitrogen (Turbovap).

5.15 Reconstitute residue with 150 ul of ethyl acetate and transfer to gc/ms vial with limited volume insert. Add 10 micro liters TFAA (Trifluoroacetic anhydride).

NOTE: Do not evaporate TFA solution

## 6.0 Chromatography and Mass Spectroscopy

6.1 Instrument and Setup:

GCMS/Autosampler: (Hewlett-Packard 6890/5973, or equivalent)
Column: 30M RTX-5MS (0.25 mm ID; 0.25 micron film)
Inj. Temp. 250 $^{o}$
Det. Temp. 160 $^{o}$
Oven (init.): 80$^{o}$, 25 $^{o}$ /min to 300 $^{o}$, (1.50 min hold).
13.80 min total run time
1 ul inj

6.2 Injection sequence; Samples are injected on the GC/MS in the following sequence:

Calibrator
Matrix blank

Control High
Control Low
Sample 1 rep 1
Sample 1 rep 2
Samples 2-? Rep 1 and 2
Control High
Additional Samples
Final Control (may be high or low)

6.3 Detection and Identification:

Determination of the presence of SMAs in the sample extract is by appearance and ratio of the 3 ions characteristic of each species at the appropriate retention time. Hence, both retention time (a GC characteristic) and fragmentation pattern and ratio (MS characteristics) are used as the basis of qualitative identification. Retention time must be within 5% of the corresponding analyte in the calibrator injection, and the ion ratios for both qualitative ions must be within 20% of the corresponding ratio calibrator sample. Qualitative identification of each analyte is independent.

6.4 Calibration:

6.4.1 Calibration:

Calibration for each batch is done independently. Hence, no sample analysis conducted under CTDPS guidelines is quantitated based on an historical calibration curve. Calibration is accomplished by the incorporation into the sample procedures of a blank sample of the matrix being analyzed that has known quantities (0.5 mg/L) of Amphetamine, Methamphetamine, MDA and MDMA (in addition to the deuterated internal standard). The response of the system to this calibrator, and the assumption of a 0 response to a 0 concentration, defines a run-specific standard curve that is used as the basis for the quantitative calculation in all controls and samples. The system is therefore “single-point calibration, multi-point control”.

6.4.2 Quantitation:

Quantitation is accomplished by the comparison of the response ratio of the analyte in a specific sample, to the response ratios of the calibrators expressed as a "standard curve." The concentration of the analyte in the sample is then extrapolated from the standard curve, and corrected for any dilution that may have been performed to facilitate the analysis of relatively concentrated samples.

## 7.0 QUALITY CONTROL AND RUN EVALUATION:

7.1 Verification of Vial Sequence; Vial sequence is checked both before and after the injection of samples. The check after the samples are injected is documented on the run summary sheet.

7.2 Chromatography - Evaluation and Acceptance Criteria; Chromatographic quality is evaluated for each peak. General guidelines are that peaks should be symmetrical, and resolved to baseline on at least one side, with 90% resolution on the other side. Significant departures from those guidelines may be experienced with forensic samples. In many cases, chromatographic quality will warrant rejection of the chromatographic run, or specific samples, by the operator. Any such action should be clearly documented on the batch summary sheet. Questionable peak shape, resolution, or other problems with chromatography can be discussed with the Section Supervisor, Director or the Quality Manager.

7.3 Evaluation of Potential Carryover; Carryover in the chromatographic quality is evaluated by injection of a blank sample immediately following the calibrator. Carryover of greater than 2% (10 ng/mL) requires batch rejection, and remedial action for the instrument (e.g. replacement of injector insert, new septum and perhaps column trim or even replacement). Demonstrated carryover If less than 2% will require operator consideration with regard to the potential for effects on specific samples, and may require re-extraction of specific samples. Carryover is further evaluated on a per sample basis by the requirement that quantitative results between replicates agree within 20%. Any significant carrover effect should cause the first of the two replicate samples to exceed the second by an amount in excess of the 20% differential. If not, any carryover may be considered inconsequential. In practice, when a question of potential carryover exists, the potentially affected sample

replicates may be repeated at the end of the batch.

7.4 Control Results: For the batch to be considered acceptable for any particular analyte, both control results for that analyte must be within 20% of the target value.

7.5 Internal Standard: Minimal acceptable internal standard abundance is 500K. For any individual injection to be acceptable, the internal standard abundance must be at least 20% of the corresponding abundance in the calibrator, and greater than the minimal abundance noted above.

7.6 Linarity: Linearity of the calibration curve is demonstrable in each batch, for each analyte as a function of quantitative results of control materials.

Note; Blood, or body fluid quantitative analysis with quantitative results of

either cocaine or benzoylecgonine concentrations greater than thecorresponding calibrator will be re-analyzed with an appropriate dilution (see section 5.2, above) such that the target analyte concentration will be above the LOQ, but below the calibrator concentration. Because of the lack of correlation between blood and urine concentrations, urine samples will be subject to consideration for such re-analysis on a case-by case basis, as determined by the analyst

7.7 Sensivity- LIMITS OF DETECTION (LOD) and QUANTITATION (LOQ): For the purposes of this procedure, the LOD and LOQ are defined as equal to the lowest concentration of the lowest control. Qualitative Identification and/or Quantitative analysis below the concentration of the low control may be accepted on a case by case basis with the concurrence of the analyst, technical reviewer, Director and/or Quality Manager.

7.8 Accuracy and precision:
Precision of the procedure is evaluated on a batch by batch basis or by repeat analyses of control or PT materials. Accuracy is expressed as a mean (absolute value) percentage difference between mean quantitative value of 10 reps of the specific control, and the target value. Precision is expressed as the CV of that value

*7.9 Specificity:*

Specificity is a function of both the resolution of target analyte during the analytical process, and the mass spectral fragmentation that analyte molecules undergo during the instrumental analysis. There has been no report of any material either than SMAs that elute within 5% if the retention time of known standard materials, and produce the same fragmentation ions and ratios.

**8.0 Reporting of Results**:

SMA runs runs are performed as part of GC/MS batches, containing controls and calibrators. The complete batch packets are in the Toxicology Laboratory. This packet contains all run evaluation documentation. Specific chromatograms for each case are filed in the appropriate case file. Results are documented on the "Batch Summary" sticker on each case file and include a reference number for the batch as a whole. A Batch summary sheet will be produced with each batch. Data on each batch should include fields such as: Sample name, Batch ID ( Date of Batch), analysts who generated data, matrix, analyte found (and concentration if applicable), controls run with the batch and results obtained. If controls do not meet the criteria, the batch can be rejected as a whole or by a case by case basis. The supervisor is notified and proper action is taken to correct any problem. Batches and/or cases shall be repeated as needed.

Procedural Uncertainty is reported with all quantitative results, and is calculated and tabulated annually for each analytical method, (See SOP TX-19 section 6.3).



**9.0 Quality Assurance**: Quality Assurance is provided by the multiple layers of checks that are performed both during and after analysis. Specifically:

9.1 The GC/MS run is thoroughly checked by the operator, including vial position on the autosampler, both prior to and following the injection of samples.

9.2 The GC/MS run is reviewed and signed off by a reviewer distinct from the operator, with this review including an evaluation of qualitative and quantitative (where applicable) results, including:

a. Control Results
b. Chromatographic Characteristics
c. Transcription errors

9.3 The results, as transcribed in the Case Summary Form are checked against the original run summary sheet during the process of report preparation, and during the administrative review of case results.

9.4 The original run is compared to the Final Report during the Final Director's review, prior to case sign-off.

## 10.0 SOURCES OF ERROR

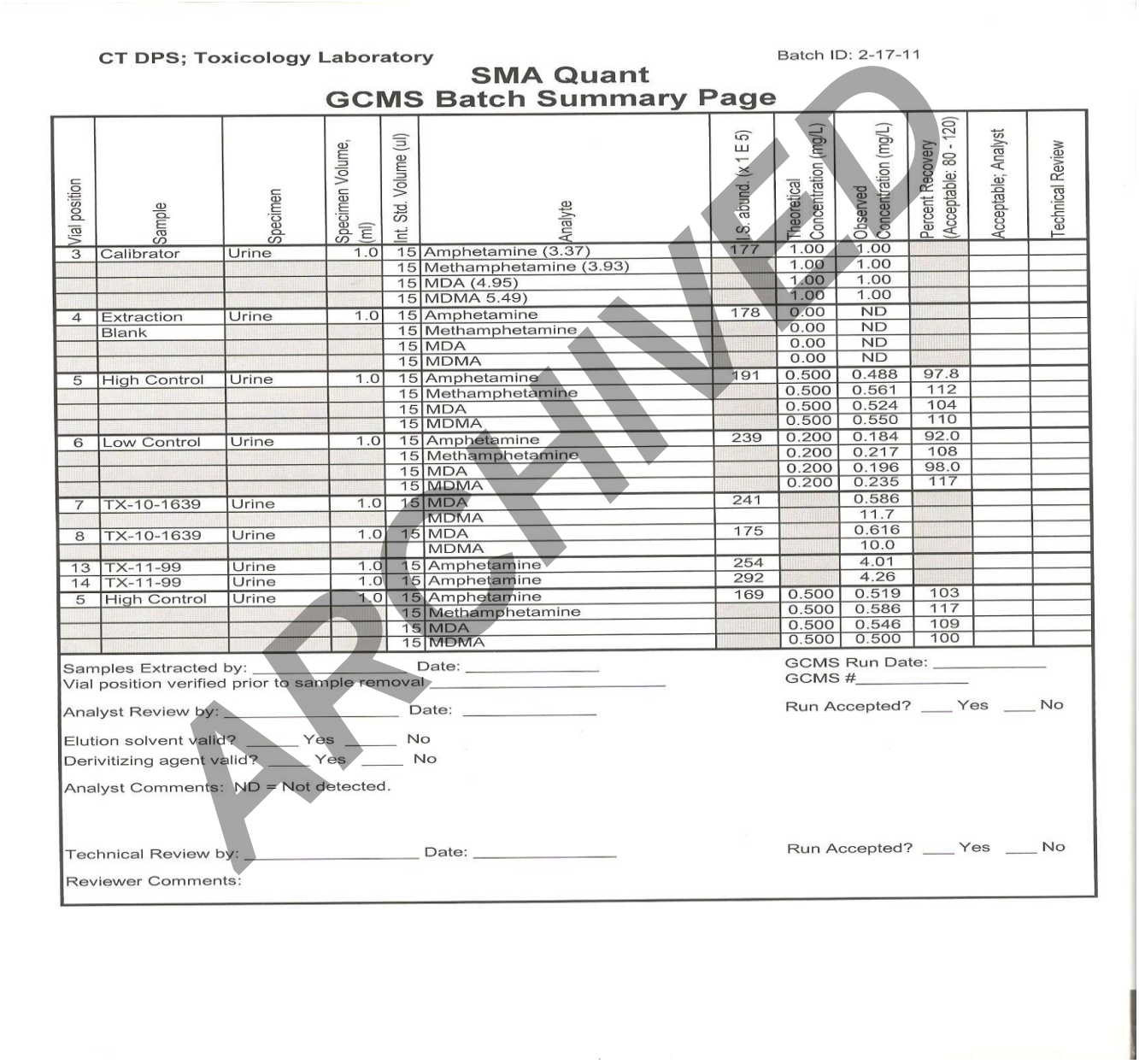
The utilization of 3-ion SIM methodology, with reference to procedural, controls and calibrators yields qualitative drug identification with essentially no uncertainty. Urine drug analyses are reported only as qualitative results.

## 11.0 References:

Clarke's Isolation and Identification of Drugs. 2nd Edition

UCT United Chemical Technologies "Solid phase extraction methods"

Example of controlled SMA batch document TX SMA-1. Batch documents can vary based on nature of batch.



CT DPS; Toxicology Laboratory

Batch ID: 2-17-11

## SMA Quant
## GCMS Batch Summary Page

| Vial position | Sample | Specimen | Specimen Volume, (ml) | Int. Std. Volume (ul) | Analyte | I.S. abund. (x 1 E 5) | Theoretical Concentration (mg/L) | Observed Concentration (mg/L) | Percent Recovery (Acceptable: 80 - 120) | Acceptable; Analyst | Technical Review |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | Calibrator | Urine | 1.0 | 15 | Amphetamine (3.37) | 177 | 1.00 | 1.00 | | | |
| | | | | 15 | Methamphetamine (3.93) | | 1.00 | 1.00 | | | |
| | | | | 15 | MDA (4.95) | | 1.00 | 1.00 | | | |
| | | | | 15 | MDMA 5.49) | | 1.00 | 1.00 | | | |
| 4 | Extraction | Urine | 1.0 | 15 | Amphetamine | 178 | 0.00 | ND | | | |
| | Blank | | | 15 | Methamphetamine | | 0.00 | ND | | | |
| | | | | 15 | MDA | | 0.00 | ND | | | |
| | | | | 15 | MDMA | | 0.00 | ND | | | |
| 5 | High Control | Urine | 1.0 | 15 | Amphetamine | 191 | 0.500 | 0.488 | 97.8 | | |
| | | | | 15 | Methamphetamine | | 0.500 | 0.561 | 112 | | |
| | | | | 15 | MDA | | 0.500 | 0.524 | 104 | | |
| | | | | 15 | MDMA | | 0.500 | 0.550 | 110 | | |
| 6 | Low Control | Urine | 1.0 | 15 | Amphetamine | 239 | 0.200 | 0.184 | 92.0 | | |
| | | | | 15 | Methamphetamine | | 0.200 | 0.217 | 108 | | |
| | | | | 15 | MDA | | 0.200 | 0.196 | 98.0 | | |
| | | | | 15 | MDMA | | 0.200 | 0.235 | 117 | | |
| 7 | TX-10-1639 | Urine | 1.0 | 15 | MDA | 241 | | 0.586 | | | |
| | | | | | MDMA | | | 11.7 | | | |
| 8 | TX-10-1639 | Urine | 1.0 | 15 | MDA | 175 | | 0.616 | | | |
| | | | | | MDMA | | | 10.0 | | | |
| 13 | TX-11-99 | Urine | 1.0 | 15 | Amphetamine | 254 | | 4.01 | | | |
| 14 | TX-11-99 | Urine | 1.0 | 15 | Amphetamine | 292 | | 4.26 | | | |
| 5 | High Control | Urine | 1.0 | 15 | Amphetamine | 169 | 0.500 | 0.519 | 103 | | |
| | | | | 15 | Methamphetamine | | 0.500 | 0.586 | 117 | | |
| | | | | 15 | MDA | | 0.500 | 0.546 | 109 | | |
| | | | | 15 | MDMA | | 0.500 | 0.500 | 100 | | |

Samples Extracted by: _____ Date: _____
Vial position verified prior to sample removal _____

GCMS Run Date: _____
GCMS #_____

Analyst Review by: _____ Date: _____

Run Accepted? ___ Yes ___ No

Elution solvent valid? _____ Yes _____ No
Derivitizing agent valid? _____ Yes _____ No

Analyst Comments: ND = Not detected.

Technical Review by: _____ Date: _____

Run Accepted? ___ Yes ___ No

Reviewer Comments: