Procedure for Measurement Assurance

1.0 Purpose - This procedure specifies the required elements for measurement assurance in the Drug Chemistry Sections of the State Crime Laboratory.

2.0 Scope – This procedure applies to Drug Chemistry at the Raleigh, Triad, and Western locations of the State Crime Laboratory.

3.0 Definitions

- Measurement – a process of experimentally obtaining one or more quantity values, typically of physical, chemical, or biological nature. Implies comparison of quantities.
- Metrology – the science of measurement.
- Measurand – the (unknown) quantity subject to measurement.
- Reference standard – measurement standard designated for the calibration of other measurement standards (reference standards or equipment)
- Reference material – material sufficiently homogeneous and stable, with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.
- Measurement Traceability – an unbroken chain of comparisons (using acceptable and documented methods) to national or international standards (SI) with each comparison having stated uncertainties.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Section balances (individual, analytical, and bulk)
- Liquid Handling System
- Headspace GC
- Toxicology GC-MS

4.2 Materials and Reagents

- Class 1 Reference Standard Weights
- Primary Reference materials
- Volumetric flasks
- Class A Pipettes
- Glassware

5.0 Procedure

5.1 Standards and Control

5.1.1 Class 1 Reference Standard Weights shall be used for QC checks and to determine the Uncertainty of Measurement for section balances.

5.1.2 Primary reference standards, primary reference materials, Class A pipettes and Class A volumetric flasks shall be used for Section Blood Alcohol Quantitations, Cannabinoid Quantitations, and GHB Quantitations.

5.2 Section Balances

5.2.1 The process to determine the Uncertainty of Measurement for Section balances shall be conducted on a yearly basis for ten consecutive business days according to the procedure outlined below.

5.2.2 In order to determine uncertainty for balances, several factors must be taken into consideration. These factors include but are not limited to:

5.2.2.1 The uncertainty of the measuring instrument (expressed as C1) shall be obtained from the statement of uncertainty from the approved vendor's current Calibration Report.

- If the expanded uncertainty was reported on the certificate provided by the vendor, divide the expanded uncertainty value by the coverage factor K.

5.2.2.2 The uncertainty of the item being measured (expressed as C2) shall be obtained from the approved vendor's current Calibration Report for the Class 1 Reference Standard Weights.

5.2.2.3 The uncertainty of human/environmental influences (expressed as C3) shall be obtained from the data collection performed by the Forensic Scientists in the Drug Chemistry Sections of the North Carolina State Crime Laboratory on an annual basis.

5.2.2.3.1 These factors include, but are not limited to:

- Position and leveling of the balance
- Position of weight on the balance pan
- Draft
- Ambient temperature changes
- Vibration

5.2.3 All common use balances (analytical and bulk) as well as all individual top loading balances currently being used for case analysis shall be included in the data collection.

5.2.4 A rotation list of Forensic Scientists shall ensure that multiple users contribute to the data collection of common use balances.

5.2.5 If a Forensic Scientist is out of the office for a partial day or partial week during the data collection period, a substitute Forensic Scientist shall collect data on that individual's balance.

5.2.6 The monthly QC check shall be performed at the start of each work day in accordance with the Drug Chemistry Technical Procedure for Balances, but shall be documented with the replicate weight determinations.

5.2.7 In addition to the monthly QC check, each morning and afternoon three replicate weight determinations shall be obtained for two reference standard weights. The Forensic Scientist performing the determination shall record these values on a data collection sheet along with the identifier for each weight used.

5.2.7.1 The specific weights used for each type of balance will depend on certified reference weights at the respective laboratories.

5.2.8 The standard deviation of all occurrences for each weight on each balance shall be used.

5.2.9 Calculations

5.2.9.1 Data collection and data manipulation may be done in an Excel spreadsheet ("Determination of Uncertainty") due to the volume of data collected.

5.2.9.2 After completion of the data collection, the uncertainty of human/environmental influences (C3) shall be determined. The following equation shall be used to determine C3:

$$C3 = \frac{s}{\sqrt{n}}$$

Where s = standard deviation
Where n = number of measurements

5.2.9.3 Combined Uncertainties (u)

5.2.9.3.1 In order to accurately reflect the total uncertainty from all of the contributing factors, the following equation shall be used to determine the combined uncertainty (u):

$$u = \sqrt{[(C1)^2+(C2)^2+(C3)^2]}$$

Where C1 = uncertainty of measuring device
C2 = uncertainty of items being measured
C3 = uncertainty of human/environmental influences

5.2.9.4 Expanded Uncertainties at 99.7 % Confidence Level (U)

5.2.9.4.1 In order to determine the expanded uncertainty (U), the combined uncertainty (u) shall be multiplied by a coverage factor (k) of 3, which states the uncertainty at a 99.7 % level of confidence.

$$U=ku$$

Where k = a coverage factor of 3 for a 99.7 % confidence level
u = the combined uncertainty for each type of measurement

5.2.10 The expanded uncertainties for each type of measurement on each type of balance (top loading/analytical/bulk) included in the uncertainty study shall be evaluated. The highest value for each type of balance (top loading/analytical/bulk) shall be used as the section value. These values shall be updated annually and used as directed in the Drug Chemistry technical procedure for each type of measurement.

5.2.10.1 Calculations shall be verified by a second Forensic Scientist and documented on the yearly summary chart for each type of uncertainty.

5.2.11 Reporting of Final Expanded Uncertainty for the Weighing Process

5.2.11.1 The Expanded Uncertainty for each type of balance (see above) shall be used to calculate the Final Expanded Uncertainty for the weighing process. This process is repeated when multiple units are weighed for a combined net weight. The following equation shall be used:

$U_{final} = \sqrt{(U_{balance})^2 \times N}$ which can be simplified to

$U_{final} = \sqrt{N} \times U_{balance}$

Where:

U_{final} = Final expanded uncertainty for the weighing process
$U_{balance}$ = Expanded Uncertainty of the Balance
N = Number of weighings
99.7 % Confidence Level using k=3 coverage factor and normal distribution

5.2.11.2 The expanded uncertainty for the weighing process for the type of balance used shall be reported with the results of reported net weights. The calculations shall be recorded in the case notes.

5.2.11.2.1 Top loading (individual) balances (lower range):
Net weight of material – XX.XX (+/- 0.0X) grams

When the upper range of a top loading balance is triggered, the readout drops to the one tenths place. Yearly uncertainty calculations are based on the lower range; therefore, any evidence which triggers the upper range shall be weighed on a bulk balance.

5.2.11.2.2 Analytical balances:
Net weight of material – XX.XXXX (+/- 0.000X) grams

5.2.11.2.3 Bulk balances:
(Raleigh/Triad Regional Laboratories)
Net weight of material – XX.XX (+/- 0.0X) kilograms
OR
(Western Regional Laboratory)
Net weight of material – XX.XXX (+/- 0.XXX) kilograms

5.2.11.2.3.1 A notice shall be posted near the bulk balance stating the range for which the balance has been calibrated and certified for use, as needed.

5.2.11.2.4 Gross weights shall not require a reported uncertainty and shall be truncated to the 0.1 place or whole number.

5.2.12 Annual Secondary Reference Standard Weight Checks

5.2.12.1 During the month of September, the Section Balances Coordinator shall record the weights of the Section Primary and Secondary Reference Standard Weights. Identifiers for the weights and balances used shall also be documented.

5.2.12.2 The balances used shall be checked with the primary standard reference weights according to the monthly QC Check criteria listed in the Technical Procedure for Balances before the secondary weights are checked.

5.2.12.3 A successful recheck will require the weights recorded for the secondary reference standard weights agree with expected values within the expanded uncertainty measurement as stated on the annual balance calibration certificate of the balance used.

5.3 Alcohol/Acetone Concentration Determinations

5.3.1 The method to determine alcohol and/or acetone concentration is Headspace Gas Chromatography (HS-GC). This method is documented in the Technical Procedure for Headspace Gas Chromatography to Quantitate and Identify Volatiles in Liquids. The uncertainty budget for this procedure was prepared to establish the uncertainty associated with the determination of the concentration of ethanol, methanol, isopropanol, and acetone in blood, serum, urine, and liquor samples. While other volatiles (i.e., 1,1-difluoroethane) are identified by this process, they are identified qualitatively; therefore, they are outside the scope of this method.

5.3.2 The analyte concentration is calculated, through least squares regression of calibrator solutions, using the manufacturer's software. The software calculates both the slope and the intercept used to generate the equation of the line that will determine analyte concentration. The equations used are:

Analyte Conc. = (Area Ratio * slope + intercept)/ISTD conc.

Area Ratio = Sample peak area/ ISTD peak area

The calibration curve is a historical curve. When a new lot of internal standard is prepared, a new calibration shall be performed. If maintenance is performed on the HS-GC that may significantly affect the calibration (e.g., new column, detector maintenance), a new calibration shall be performed.

5.3.3 The analytes are analyzed via HS-GC using an internal standard calibration method employing a 0.05 gram per 100 mL n-propanol internal standard solution. The calibration curve for all analytes consists of a 5 point curve with calibrators spaced

between 0.010 gram per 100 mL and 0.500 gram per 100 mL. The correlation coefficient of the curve defined as R^2 must be greater than 0.995. Any concentration beyond this range is not within the scope of the current validated method. The Technical Procedure for Headspace Gas Chromatography to Quantitate and Identify Volatiles in Liquids also requires that four quality controls (0.015, 0.050, 0.100, and 0.400 gram per 100 mL) be analyzed with the calibration. The 0.050, 0.100, and 0.400 gram per 100 mL quality controls are certified reference material solutions. The 0.015 gram per 100 mL solution is prepared in-house. The calibration is valid if all quality control samples are within 5 % of the expected concentration for ethanol. All other analytes must be within 10 % of the expected concentration. Each run shall contain two of the certified reference material solutions to serve as positive controls. These shall meet the same acceptance criteria as the controls used to verify the calibration curve.

5.3.4 Analyte and Sampling

5.3.4.1 The analyte and sampling related factors that contribute to the overall process uncertainty considered in this evaluation were homogeneity, temperature, liquid handling system, matrix effects, reactivity, and partitioning.

5.3.5 Instrumental

5.3.5.1 The instrumental related factors that contribute to the overall process uncertainty considered in this evaluation were peak broadening, peak tailing, resolution, and flame ionization detector (FID).

5.3.6 Calibration

5.3.6.1 The calibration factors that contribute to the overall process uncertainty considered in this evaluation were peak area, calibration solutions, and linear regression.

5.3.7 Internal Standard

5.3.7.1 The internal standard concentration is a unique source of uncertainty. The n-propanol used in the preparation of the internal standard is greater than 99.5 % pure. The internal standard solution is prepared using Class A volumetric glassware and a calibrated analytical balance. Because the calibration is tied to the internal standard lot, all case samples will be analyzed using the same lot of internal standard that was used to calibrate the instrument.

5.3.7.2 The internal standard is subject to the same sources of uncertainty as the analytes. These sources of uncertainty are uniformly applied to both internal standard and analyte. As such, the use of an internal standard normalizes and minimizes these effects on uncertainty.

5.3.8 All factors considered were reflected in the overall variance of replicate sample analysis (repeatability-Ethanol only) and quality control data (repeatability-Methanol, Acetone, Isopropanol and reproducibility-all analytes). These are the two major components of the overall process uncertainty.

5.3.9 Repeatability

5.3.9.1 Quadruplicate sample data for cases worked from September 2012 thru May 2013 were evaluated for mean and standard deviation. Only mean sample results between 0.01 and 0.50 were considered for analysis of variance. Based on the mean of the results, sample data was separated into incremental pools of 0.010 g/100 mL. The overall mean, standard deviation, relative standard deviation, and % CV of the pool was obtained. Mean relative standard deviation was obtained by linear regression analysis. The mean relative standard deviation was assumed to approximate the population mean relative standard deviation, and thus a coverage factor (k) of 3.0 was assumed to approximate the 99.73 % confidence interval. Analysis of quadruplicate sample data was assumed to be reflective of the total process repeatability. Sample regression was performed in Microsoft Excel.

5.3.9.2 For analytes other than ethanol, sample repeatability was estimated from analysis of certified reference material control data. Control results from the same period were analyzed for mean, standard deviation, and % CV using Microsoft Access and Microsoft Excel.

5.3.10 Control Chart Data

5.3.10.1 Certified reference material control data was also evaluated for mean recovery/accuracy (Rm) versus the expected value.

5.3.11 Overall process uncertainty was calculated by:

5.3.11.1 Ethanol process uncertainty = $\sqrt{(((\text{repeatability})^2/\sqrt{4}) + (\text{Rm})^2)}$

5.3.11.2 Other analytes process uncertainty = $\sqrt{(((\text{repeatability})^2) + (\text{Rm})^2)}$

5.3.12 The expanded uncertainty was calculated by:

5.3.12.1 Expanded uncertainty (99.73 % CI) = 3 * process uncertainty

5.3.13 These values shall be updated annually by the Drug Chemistry Forensic Scientist Manager or his/her designee and used as directed in the Drug Chemistry Section-Toxicology Unit Technical Procedure for Headspace Gas Chromatography to Quantitate and Identify Volatiles in Liquids.

5.4 Cannabinoid Quantitations

5.4.1 The procedure for which this uncertainty budget is prepared is the determination of the concentration of delta-9-tetrahydrocannabinol (THC), 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC) and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THCA) in blood. The procedure to determine the analyte concentration is a liquid-liquid extraction with subsequent analysis using LC-MS/MS. This procedure is documented in the Technical Procedure for Blood Cannabinoid Liquid-Liquid Extraction for Analysis by LC-MS/MS.

5.4.2 The analyte concentration is calculated using least squares regression of calibrator solutions by the MassLynx software supplied with the instruments. The software calculates both the slope and the intercept used to generate the equation of the line that will determine analyte concentration. The equations used in determining the analyte concentration are:

Linear:

Analyte Conc. (x) = (Response (y) –y-intercept (b))/Slope (m)

Quadratic:

Response (y) = a*(analyte conc. (x)) 2 + b*(analyte conc. (x)) + c

Response = Analyte peak area* (ISTD concentration/ ISTD peak area)

The calibration curve used to determine the concentration in case specimens is not a historical curve. A new curve is prepared by simultaneous extraction of both calibrator samples with case specimens, so that all data is acquired under as close to the same conditions as can be obtained.

5.4.3 Calibration curve acceptance criteria and the calibration levels are contained in the Technical Procedure for Blood Cannabinoid Liquid-Liquid Extraction for Analysis by LC-MS/MS. Each analyte is analyzed and quantitated using its deuterated analog as an internal standard.

5.4.4 Analyte and Sampling

5.4.4.1 The analyte and sampling related factors that contribute to the overall process uncertainty considered in this evaluation were homogeneity, temperature, pipettes, matrix stability, and reactivity.

5.4.5 Instrumental

5.4.5.1 The instrumental related factors that contribute to the overall process uncertainty considered in this evaluation were peak broadening, peak tailing, resolution, and peak area.

5.4.6 Calibration and Control Solution Concentration

5.4.6.1 The calibration and control solution factors that contribute to the overall process uncertainty considered in this evaluation were volume, pipettes and purity.

5.4.6.2 The certified reference materials used to prepare the QC solutions shall be obtained from a second manufacturer or be a different lot number than the ones used to prepare the calibration solution.

5.4.7 Internal Standard

5.4.7.1 The internal standard concentration is a unique source of uncertainty. Deuterated analogs of each analyte are used as the internal standards for

the procedure. The internal standards are certified reference material standards acquired from an approved vendor. The % purity of the internal standards used is greater than 97.5 %. The stock and working internal standard solution are prepared using calibrated mechanical pipettes and Class A volumetric glassware.

5.4.7.2 The internal standard is subject to the same sources of uncertainty as the analytes. These sources of uncertainty are uniformly applied to both internal standard and analyte. As such, the use of an internal standard normalizes and minimizes these effects on uncertainty.

5.4.7.3 All case samples are analyzed using the same lot of internal standard that was used in the calibration and control samples. Therefore, any uncertainty in the preparation of internal standard is reflected in the quality control data.

5.4.8 All factors considered were reflected in the quality control data used to determine the statistical bias and precision, which are the two major components of the overall process uncertainty.

5.4.9 Bias and Precision

5.4.9.1 Within-run and between-run coefficients of variation (% CV) are determined using the Analysis of Variance (ANOVA) function within Microsoft Excel.

5.4.9.2 Bias was determined by evaluating quality control data for mean recovery/accuracy versus the expected value.

5.4.10 Overall process uncertainty for each analyte was calculated by:

5.4.10.1 process uncertainty = $\sqrt{((\text{Bias})^2 + (\text{Precision})^2)}$

5.4.11 The expanded uncertainty was calculated by:

5.4.11.1 Expanded uncertainty (95.45 % CI) = 2.32 * process uncertainty

5.4.11.2 The k factor of 2.32 was due to the limited number of data points available at the time the uncertainty was determined. This value was obtained from a student-t table.

5.4.12 These values shall be updated annually during March by the Drug Chemistry Forensic Scientist Manager or his/her designee and used as directed in the Drug Chemistry Section-Toxicology Unit Technical Procedure for Blood Cannabinoid Liquid-Liquid Extraction for Analysis by LC-MS/MS.

6.0 Limitations – N/A

7.0 Safety – N/A

8.0 References

ASCLD/LAB Level 100A Traceability presentation. Copyright 2011; Heusser Neweigh, LLC & ASCLD/LAB.

ASCLD/LAB Level 100B Measurement Assurance presentation. Copyright 2011; Heusser Neweigh, LLC & ASCLD/LAB.

ASCLD/LAB Level 100C Measurement Uncertainty Concepts presentation. Copyright 2011; Heusser Neweigh, LLC & ASCLD/LAB.

ASCLD/LAB Level 200 Measurement Confidence for the Forensic Laboratory: Measurement Uncertainty in Drug Chemistry presentation. Copyright 2011; Heusser Neweigh, LLC & ASCLD/LAB

Clark, J.P. and Shull, A.H. *Evaluation of Methods for Estimating the Uncertainty of Electronic Balance Measurements*. Westinghouse Savannah River Company, 2002.

EURACHEM/CITAC Guide CG 4: *Quantifying Uncertainty in Analytical Measurement*, Third Edition 2012.

Gariott, James C. Medicolegal Aspects of Alcohol. 5th ed. Lawyers and Judges Publishing Co. 2008.

Gullberg, Rod. "Estimating the Measurement Uncertainty in Forensic Blood Alcohol Analysis." Journal of Analytical Toxicology. 2012. 36: 153-161.

JCGM 100:2008 Evaluation of measurement data - Guide to the Expression of Uncertainty in Measurement, First Edition September 2008.

Jones, Alan. "Determination of Liquid/Air Partition Coefficients for Dilute Solutions of Ethanol in Water, Whole Blood, and Plasma." Journal of Analytical Toxicology. 1983. 7: 193-197.

Kristiansen, Jesper and Petersen, Henning. "An Uncertainty Budget for the Measurement of Ethanol in Blood by Headspace Gas Chromatography." Journal of Analytical Toxicology. 2004. 28: 456-463.

LeBeau, Marc. "Introduction to Measurement Uncertainty." RTI International. 2009.

Measurement Uncertainty for Weight Determinations in Seized Drug Analysis Supplemental Document SD-3 Revision 2. Copyright 2011; SWGDRUG.

Sklerov, Jason and Couper, Fiona. "Calculation and Verification of Blood Ethanol Measurement Uncertainty for Headspace Gas Chromatography." Journal of Analytical Toxicology. 2011. 35: 402-410.

Taylor, B.N, and Kuyatt, C.E. NIST Technical Note 1297 Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results, September 1994 Edition.

Tilson, Chris. "Analysis of Biological Specimens." FTCB. 2010.

Virginia Department of Forensic Sciences. Controlled Substances Procedure Manual. Document 221-D100 Revision 7, February 6, 2012.

9.0 Records

- Determination of Uncertainty - Yearly Report for Balances
- Calibration Reports for section balances
- Traceability Maps
- Calibration Reports for section Class A pipettes
- Reference Standard Weight Calibration Certificates
- Uncertainty Budget for the Quantitation of Ethanol, Methanol, Isopropanol, and Acetone using Headspace Gas Chromatography
- Uncertainty Budget for the Quantitation of Cannabinoids in blood using Liquid-Liquid Extraction and LC-MS/MS
- Toxicology Control Charts

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document
5/10/2013	2	**Original 5.1**- Moved first statement to apply to Section Balances only. References to traceability maps removed, since this data covered by calibration certificates **Original 5.2, 5.3** – N/A sections removed **5.2** – Section renamed and edited to cover Section Balances only **5.2.2.1** – Removed normalization calculation that is not needed **5.2.2.2** – Added reference to Class A Reference Standard Weights **5.2.2.3.1** – Section moved from below **5.2.3 – 5.2.9** – Sections moved from below; Weights used will depend on availability at laboratories **5.2.9.1** – Added name of Excel spreadsheet used to calculate data **5.2.11** – Added clarification on types of balances **5.2.11.1** – Corrected reference line number **5.3, 5.4** - Revised to "Currently being established" **5.5** – Added explanation for Methamphetamine Quantitations via HPLC **Original 5.7** – Removed section for GHB Quantitations **5.5.7.1 -** Added process evaluated annually **References** – Added EURACHEM/CITAC… Taylor…, and JCGM…references **Records** – Removed Measurement Assurance Cause & Effect Diagram, added Uncertainty Budget for the HPLC Quantitation of Methamphetamine and HPLC Control Charts
06/14/2013	3	Removed Technical in title **5.2.10 -** Removed last sentence **5.3 -** Inserted explanation of measurement uncertainty as it relates to Alcohol/Acetone Concentration Determinations **References -** Added Garriott, Gullberg, Jones, Kristiansen, LeBeau, Sklerov, and Tilson **Records** – Added Uncertainty Budget for the Quantitation of Ethanol, Methanol, Isopropanol, and Acetone using Headspace Gas Chromatography and Toxicology Control Charts

10/16/2013	4	**5.2.11.2.1** – Reworded to remove use of upper range on table top balances **5.2.11.2.3** – Clarified nonuse of upper ranges on bulk balances **5.2.11.2.4** – Deleted unneeded reference to range of balance **5.2.12** – Added section to clarify successful annual Secondary Reference Weight check; added issuing authority to header
12/18/2013	5	**4.2, 5.1.1, 5.2.2.2 –** Typo corrected for Class 1 Weights **5.3.2** – Reworded statement reference calibration curve **5.3.3** – Reworded statement reference validity of calibration curve
03/14/2014	6	**4.1** – Removed HPLC from Equipment list **5.1.3** – Removed reference to HPLC Standards and Controls **5.4** – Amended section to include discussion of measurement uncertainty as it relates to Cannabinoid Concentration Determinations. Records - Added Uncertainty Budget for the Quantitation of Cannabinoids in blood using Liquid-Liquid Extraction and LC-MS/MS **5.5** – Removed section for HPLC Quantitations via HPLC **Records** – Removed Uncertainty Budget for HPLC and HPLC control charts
08/29/2014	7	**5.2.11.1 –** replaced incorrect line reference with "(see above)" **5.3.3 –** 6 % changed to 5 % **Records** – added Traceability Maps