

# **Laboratory Procedure Manual**

**Analyte:** Caffeine and Caffeine Metabolites

Matrix: Urine

Method: UHPLC-ESI-MS/MS

Method No: 4063.08

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as performed by: Nutritional Biomarkers Branch (NBB)

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#### **Important Information for Users**

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

This document details the Lab Protocol for testing the items listed in the following table.

This method file describes measurements of U2CF\_H\_R.

File Name	Variable Name	Analyte Description (and SI units)				
	UR2AMU	5-acetylamino-6-amino-3-methyluracil (AAMU), Urine 2nd collection (umol/L)				
	UR2MU1	1-methyluric acid, Urine 2nd Collection (umol/L)				
	UR2MU2	3-methyluric acid, Urine 2nd Collection (umol/L)				
	UR2MU3	7-methyluric acid, Urine 2nd Collection (umol/L)				
	UR2MU4	1,3-Dimethyluric acid, Urine 2nd Collection (umol/L)				
	UR2MU5	1,7-Dimethyluric acid, Urine 2nd Collection (umol/L)				
	UR2MU6	3,7-Dimethyluric acid, Urine 2nd Collection (umol/L)				
U2CF_H_R	UR2MU7	1,3,7-Trimethyluric acid, Urine 2nd Collection (umol/L)				
	UR2MX1	1-methylxanthine, Urine 2nd Collection (umol/L)				
	UR2MX2	3-methylxanthine, Urine 2nd Collection (umol/L)				
	UR2MX3	7-methylxanthine, Urine 2nd Collection (umol/L)				
	UR2MX4	1,3-dimethylxanthine (theophylline), Urine 2nd Collection (umol/L)				
	UR2MX5	1,7-dimethylxanthine (paraxanthine), Urine 2nd Collection (umol/L)				
	UR2MX6	3,7-dimethylxanthine (theobromine), Urine 2nd Collection (umol/L)				
	UR2MX7	1,3,7-trimethylxanthine (caffeine), Urine 2nd Collection (umol/L)				

#### 1. Overview

#### A. Clinical Relevance

Caffeine is an alkaloid that is known to have psychoactive stimulatory effects. Caffeine naturally occurs in plants (e.g., coffee beans, tea leaves, cocoa beans, cola nuts), and the dietary consumption of caffeine originates mainly from derivative beverages (e.g., coffee, tea, cola drinks) and foods (e.g., chocolate) [1-2]. Caffeine is also used as a food additive in beverages (e.g., caffeinated soft drinks, "energy" drinks) and as a drug either on its own or as an adjuvant in certain medications (e.g., analgesics) [3-5]. Given caffeine's high prevalence in the worldwide diet at behaviorally active doses, significant scientific interest in the health effects of caffeine has developed. As a psychoactive stimulant, the behavioral effects of caffeine, such as its effect on mental alertness, have been studied extensively, and topics such as caffeine tolerance, addiction, and withdrawal have also been examined [2; 5-7]. Caffeine consumption has been studied as a risk factor for many diseases and conditions, including hypertension, bone health, cardiovascular diseases, various cancers, reproduction and developmental abnormalities, and mental and behavioral disorders [6;8-21]. In addition to assessing dietary exposure, the quantitation of caffeine and its urine metabolites provides a potential means of assessing differences in metabolic activity [22-23]. The liver serves as the primary site of caffeine metabolism. Caffeine undergoes an intricate series of reactions via several enzyme systems, primarily Ndemethylations and C-8-hydroxylation, to yield a mixture of N-methylated xanthines, uric acids, and an acetylated uracil [24-26]. Cytochrome P450 1A2 (CYP1A2), CYP2A6, N-acetyltransferase 2 (NAT2) and xanthine oxidase (XO), are involved in caffeine metabolism [24-26]. Caffeine is a preferred metabolic probe for assessing CYP 1A2, CYP2A6, NAT2, and XO enzyme activities, all of these enzymes are involved in the activation or detoxification of various xenobiotic compounds [26-29]. The enzyme activity can be assessed by calculate the ratio of products and precursors (metabolic ratio). We can use our validated method to study these enzyme activities based on dietary caffeine intake with NHANES subjects [30-32].

#### B. Test Principle

Urine caffeine and its 14 metabolites are quantified with ultra-high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS) with stable isotope labeled internal standards. A 50- $\mu$ L aliquot of urine is first diluted with 450  $\mu$ L of water. 100  $\mu$ L of the diluted urine is then combined with 120  $\mu$ L of a 0.2 N NaOH solution containing stable isotope labeled internal standards. The mixture is allowed to incubate for at least 30 min at room temperature, facilitating the conversion of an unstable uracil metabolite (AFMU) into a more stable form (AAMU). Samples are then acidified 30  $\mu$ L of 2.0 N HCl and 250  $\mu$ L of a 10% methanol containing 0.1% formic acid such that the matrix of the sample is similar to the starting mobile phase composition of the initial analysis step. Samples are then filtered and analyzed by use of UHPLC-ESI-MS/MS with polarity switching. Quantitation is based on peak area ratios interpolated against an 11-point calibration curve derived from calibrators in synthetic urine. The following compounds are quantified:

	Abbi	reviation
Compound	Scientific literature (including this document	NHANES t) analyte code
1-methylxanthine	1X	MX1
3-methylxanthine	3X	MX2
7-methylxanthine	7X	MX3
1,3-dimethylxanthine (theophylline)	13X	MX4
1,7-dimethylxanthine (paraxanthine)	17X	MX5
3,7-dimethylxanthine (theobromine)	37X	MX6
1,3,7-trimethylxanthine (caffeine)	137X	MX7
1-methyluric acid	1U	MU1
3-methyluric acid	3U	MU2
7-methyluric acid	7U	MU3
1,3-dimethyluric acid	13U	MU4
1,7-dimethyluric acid	17U	MU5
3,7-dimethyluric acid	37U	MU6
1,3,7-trimethyluric acid	137U	MU7
5-acetylamino-6-amino-3-methyluracil	AAMU	AMU

The preparation of 60 patient samples, 11 calibrators, and quality control materials (QCs) generally takes 1.5 hours with an automated liquid processor (including 30 min for the alkaline conversion step). UHPLC-ESI-MS/MS analysis of each sample requires 9 min (6.5 min to run method) per sample.

# 2. Safety Precautions

Consider all urine specimens as potentially positive for infectious agents including HIV, hepatitis B and hepatitis C. We recommend the hepatitis B vaccination series for all analysts working with urine. Observe universal precautions; wear protective gloves, lab coat, and safety glasses during all steps of this method. Discard any residual sample material by autoclaving after analysis is completed. Place all disposable plastic, glassware, and paper (pipet tips, auto sampler vials, gloves etc.) that contact urine in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Use disposable bench diapers during sample preparation and urine handling and discard after use. Also, wipe down all contaminated work surfaces with a 10% bleach solution when work is finished.

Handle acids and bases used in sample and reagent preparation with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study include those listed in Section 6. Safety data sheets (SDSs) for all chemicals are readily available in the SDS section as hard copies in the laboratory. SDSs for other chemicals can be viewed at http://www.ilpi.com/msds/index.html or at <a href="http://intranet.cdc.gov/ossam/workplace-safety/safety-practices/chemical-safety/index.html">http://intranet.cdc.gov/ossam/workplace-safety/safety-practices/chemical-safety/index.html</a>.

### 3. Computerization and Data System Management

During sample preparation and analysis, samples are identified by their <u>sample ID</u>. The sample ID is a number that is unique to each sample that links the laboratory information to demographic data recorded by those who collected the sample.

The raw data file and respective batch file from the tandem mass spectrometer are collected using the instrument software and stored on the instrument workstation. The data file and batch file are copied to the network where the data file is processed into a results file that is saved on the CDC network. Results are typically generated by auto-integration, but may require manual integration in some cases. The results file (including analyte and internal standard names, peak areas, retention times, sample dilution factor, data file name, acquisition time, etc.) is imported into STARLIMS database for review of the data, statistical evaluation of QC/QA data, and approval of the results. See "4063.08 SOP Computerization and Data System Management" for a step-by-step description of data transfer, review, and approval.

For NHANES, data is transmitted electronically. Abnormal values are confirmed by the analyst, and codes for missing data are entered by the analyst and are transmitted as part of the data file. NCHS makes arrangements for the abnormal report notifications to the NCHS Survey Physician.

Data files from the instrument workstation are typically copied to the CDC network on a run-by-run basis. This is the responsibility of the analyst under the guidance of the team lead and/or supervisor. Further data processing is typically conducted on a networked computer and saved directly to the CDC network. Files stored on the CDC network are automatically backed up nightly by ITSO support staff.

# 4. Specimen Collection, Storage, and Handling Procedures

We recommend that specimen donors fast prior to specimen collection, but fasting is not required. Specimens for caffeine and caffeine metabolite analysis are performed on fresh or frozen urine. One mL of urine is preferable to allow for repeat analyses. A volume of  $50-\mu L$  is required for each analysis. The appropriate amount of urine is dispensed into a Nalgene 2.0 mL cryovial or other plastic screw-capped vial labeled with the participants ID. Specimens collected in the field are frozen, and then shipped on dry ice by overnight carrier. Frozen samples are stored at  $\leq$  -20°C for short-term storage, and  $\leq$  -70°C for long-term storage. Caffeine and its metabolites in urine appear to be stable over the course of at least 3 freeze/thaw cycles at ambient temperature. One of the caffeine metabolites (5-acetylamino-6-amino-3-methyluracil, AAMU) is light sensitive; excessive ambient light exposure (more than 2 hours) should be avoided, and preparation of AAMU standards should be performed under low-UV lighting.

Specimen handling conditions are outlined in the DLS Policies and Procedures Manual. The electronic copy of the file is located at \\cdc.gov\project\CCEHIP NCEH DLS NBB LABS\CLIA\DLS Policies and Procedures Manual). The protocol discusses collection and transport of specimens and the special equipment required. In general, urine should be transported and stored at -20°C. If there is more than one analyte of interest in the specimen and it needs to be divided, the appropriate amount of urine should be transferred into a sterile Nalgene cryovial labeled with the participant's ID.

# 5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Not applicable for this method.

# 6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

#### A. Reagent Preparation

Prepare all solutions, samples and standards with 0.45  $\mu$ m filtered deionized water with a resistance of at least 18 M $\Omega$ /cm, and HPLC-grade solvents and reagents. Use Class A volumetric glassware in all cases. Perform all steps involving concentrated acids, bases, and organic solvents in a chemical fume hood. Though each reagent preparation specifies a total volume of reagent prepared, these directions may be scaled up or down to prepare larger or smaller quantities if desired.

#### (1) 1.2 N NaOH solution

For 100 mL of solution, add approximately 50 mL of deionized water to a 100-mL volumetric flask. Quantitatively transfer 12 mL of 10M NaOH into the flask and mix the contents. Bring the solution up to volume with deionized water. Seal the volumetric flask and mix the contents by inversion. Transfer to a storage vessel. Prepare monthly and store at room temperature.

#### (2) HPLC mobile phase A (aqueous) -5% methanol/0.05% formic acid

For 2 L of solution, quantitatively transfer 1900 mL of deionized water, 100 mL of methanol and 1 mL of formic acid to a 2-L HPLC reservoir bottle. Cap the bottle and mix thoroughly, venting the bottle several times during mixing. Prepare every 7 days and store at room temperature.

#### (3) HPLC mobile phase B (organic) – 90% methanol/0.05% formic acid

For 500 mL of solution, quantitatively transfer 450 mL of methanol, 50 mL of deionized water and 250  $\mu$ L of formic acid to a 1-L HPLC reservoir bottle. Cap the bottle and mix thoroughly, venting the bottle several times during mixing. Prepare every 7 days and store at room temperature.

#### (4) 2X HPLC mobile phase A (aqueous)

For 100 mL of solution, quantitatively transfer 90 mL of deionized water, 10 mL of methanol and 100  $\mu$ L of concentrated formic acid to a storage vessel. Cap the vessel and mix thoroughly, venting the bottle several times during mixing. Prepare every 7 days and store at room temperature.

#### (5) 1X Synthetic Urine

For 1000 mL, quantitatively transfer 500 mL of deionized water to a 2 L beaker. Using a magnetic stir bar to mix the solution, add the following chemicals in the quantities and order specified:

- 3.8 g Potassium Chloride (KCl)
- 8.5 g Sodium Chloride (NaCl)
- 24.5 g Urea
- 1.03 g Magnesium Sulfate (MgSO<sub>4</sub>.7H<sub>2</sub>O)
- 1.03 g Citric Acid
- 0.34 g Ascorbic Acid
- 1.18 g Potassium Phosphate Dibasic (K<sub>2</sub>HPO<sub>4</sub>)
- 1.4 g Creatinine
- 0.64 g Sodium Hydroxide (add slowly)
- 0.47 g Sodium Bicarbonate (NaHCO<sub>3</sub>)
- 0.28 mL Sulfuric Acid (conc.)

Once all compounds have dissolved in solution, transfer the mixture to a 1000 mL volumetric flask. Bring the solution up to volume with deionized water. Seal the volumetric flask and mix the contents by inversion, and transfer to a storage vessel. This solution can be stored at 4°C for up to

one year. The solution should be discarded and re-prepared if there is any visible evidence of precipitates, bacterial growth, or other changes in appearance.

### B. Standards Preparation

A total of 11 calibrators (S1–S11), spanning the reportable range for each analyte, are prepared for this method. Target concentrations for the calibrators appear below (Table I), and the reportable ranges can be found in Section 9 (Table III).

**Table I** Final concentrations (μM) of analytes in calibrators (S1-S11)

Analyte	<b>S1</b>	<b>S2</b>	<b>S3</b>	<b>S4</b>	<b>S</b> 5	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	S10	S11
1X	10.0	25.0	100.0	200.0	5.0	400.0	1.0	300.0	50.0	0.1	0.03
3X	10.0	100.0	50.0	25.0	200.0	1.0	5.0	75.0	300.0	0.1	0.04
7X	600.0	10.0	5.0	400.0	50.0	1.0	100.0	200.0	2.5	0.1	0.02
10	1.00	500.0	200.0	100.0	400.0	50.0	10.0	300.0	20.0	0.2	0.05
3U	6.0	10.0	0.8	0.6	8.0	2.00	1.00	4.00	15.00	0.4	0.1
7U	20.0	5.0	1.0	50.0	200.0	100.0	2.5	40.0	10.0	0.25	0.04
13X	20.0	10.0	0.4	0.25	5.00	15.0	1.0	0.6	2.5	0.05	0.01
17X	200.0	25.0	3.0	1.0	100.0	150.0	10.0	5.0	50.0	0.05	0.006
37X	25.0	250.0	3.0	50.0	10.0	1.0	150.0	5.0	100.0	0.05	0.004
13U	50.0	2.5	0.4	1.0	0.25	10.0	0.8	5.0	25.0	0.15	0.02
17U	5.0	50.0	10.0	100.0	3.0	1.0	250.0	20.0	300.0	0.25	0.02
37U	1.0	8.0	20.0	10.0	0.3	0.8	0.6	4.0	2.0	0.1	0.03
137X	2.5	0.6	0.3	40.0	20.0	1.0	5.0	10.0	50.0	0.05	0.003
137U	5.0	0.3	20.0	40.0	10.0	0.5	2.5	1.0	30.0	0.05	0.005
AAMU	100.0	1.0	500.0	3.0	5.0	200.0	400.0	30.0	50.0	0.4	0.1

**Note:** Special attention should be paid to the preparation of calibrators S10 and S11; they are low-level standards that are prepared in a different manner from calibrators S1–S9.

#### (1) Single-Analyte and Single-Internal Standard Stock Solutions

Separate stock solutions should be prepared for each analyte and stable isotope-labeled internal standard by dissolving an accurately known mass (±0.1 mg or less) of the pure solid compound in aqueous solution, targeting a final concentration of 1 mM based on the formula weight of the compound. The volume of solution prepared should be sufficiently large such that the determined mass of starting material has an imprecision of less than 1%. For example, to prepare 200 mL of a 1-mM stock solution of 1,3-dimethyluric acid (13U), weigh an accurately known mass (target 39.2 mg based on MW of 195.16 g/mol) of solid 1,3-dimethyluric acid into a glass weighing funnel. Carefully transfer the material to a 200-mL volumetric flask, rinsing the contents of the weighing funnel into the flask with deionized water. Partially fill the volumetric flask with deionized water and mix the contents by sonication until dissolved. Bring the solution up to volume with deionized water and mix by inversion. Aliquot the solution into 2-mL polypropylene cryovials (1 mL/vial), and store at -70°C.

**Note:** the following analytes require the addition of sodium hydroxide (final concentration of 1 mM NaOH in solution) for complete dissolution: 1X, 3X, 7X, 1U, 3U, 7U, 17U, 37U, and AAMU. The same is true for the stable isotope-labeled analogues of these analytes. All other analytes and internal standards can be prepared in deionized water.

**Note: AAMU** (5-acetylamino-6-amino-3-methyluracil) **is light sensitive**. The preparation of primary and intermediate stock solutions and the addition of this analyte to the standard mixture needs to be performed under low-UV yellow light.

Assignment of single-analyte stock solution concentration by use of UV-visible absorbance measurements and molar extinction coefficients is preferred. A list of recommended extinction coefficients appears in **Appendix C**. In the absence of reliable extinction coefficients, assignment of stock solution concentration by gravimetric measurement is acceptable.

(2) Intermediate Mixed-Analyte Stock Solutions – Preparation of Calibrators

The intermediate mixed-analyte stock solutions for S1–S9 are prepared by combining the single-analyte stock solutions according to the amounts specified in Table II. For example, to prepare 10 mL of intermediate mixed-analyte standard solution "S1", use an air displacement pipette to transfer the defined amount single-analyte stock solution into a 10-mL polypropylene tube, the appropriate amount is confirmed by weight. Bring the solution up to volume with deionized water and mix thoroughly by inversion. Aliquot each calibrator (1 mL/vial) into 2-mL cryovials and store the calibrators at -70°C.

Table II: Volume of single-analyte stock solution (μL) required to prepare S1–S9

Analyte	<b>S1</b>	S2	<b>S3</b>	<b>S4</b>	<b>S</b> 5	<b>S6</b>	<b>S7</b>	S8	<b>S9</b>
1X	93	234	935	1869	47	3738	28*	2804	467
3X	93	935	467	234	1869	28*	47	701	2804
7X	5505	92	46	3670	459	28*	917	1835	23
1U	29*	4854	1942	971	3883	485	97	2913	194
3U	57	94	23*	29*	75	38*	28*	38	142
7U	206	52	21*	515	2062	1031	26	412	103
13X	202	101	20*	25*	51	152	20*	31*	25
17X	2000	250	30	20*	1000	1500	100	50	500
37X	250	2500	30	500	100	20*	1500	50	1000
13U	481	24	38*	29*	24*	96	23*	48	240
17U	52	515	103	1031	31	21*	2577	206	3093
37U	29*	78	196	98	29	23*	30*	39	20
137X	23	27	27*	360	180	27*	45	90	450
137U	50	30*	198	396	99	25*	25	30*	297
AAMU	877	26*	4386	26	44	1754	3509	263	439
Water	54	187	1539	227	47	1034	1028	491	203

**Note:** Volumes denoted with \* indicate that a 10x dilution of the single-analyte stock solution was used. The 10× dilution of the stock solution is necessary so that the volume of solutions being pipetted is >20  $\mu$ L.

**Note:** The volumes provided in Table II assume all stock solution concentrations are exactly 1 mM. Actual stock solution concentrations should be used and pipetting volumes may be adjusted in order to obtain the target concentrations in Table I.

The intermediate mixed-analyte stock solutions for S10 and S11 are prepared by combining the single-analyte stock solutions according to the amounts specified in Table III. Because S10 and S11 are low-level calibrators, they are prepared using diluted stock solutions so that the volume of solution being pipetted is  $>20~\mu L$ .

Table III: Volume of single-analyte stock solution (μL) required to prepare S10 and S11

Analyte	S10 (using a 50 μM stock solution)	S11 (using a 20 μM stock solution)*†		
1X	100	75		
3X	100	100		
7X	100	50		
1U	200	125		
3U	400	250		
7U	250	100		
13X	50	25		
17X	50	30*		
37X	50	20*		
13U	150	50		
17U	250	50		
37U	100	75		
137X	50	<b>30</b> <sup>†</sup>		
137U	50	25*		
AAMU	400	250		
Water	2700	3745		

**Note:** Volumes denoted with \* indicate that a 10  $\mu$ M stock solution was used. Volumes denoted with a † indicate that a 20  $\mu$ M stock solution was used.

#### (3) Preparation of Working Solution for Calibration Standards

Calibration standards are prepared by combining the appropriate intermediate mixed-analyte stock solution with 1× synthetic urine and deionized water in a relative proportion of 1:1:8 for S1 through S9. The same proportion applies to intermediate mixed-analyte stock solutions S10 and S11; however, these stock solutions need to be diluted 10× prior to use. All calibration standards are prepared in batches, and aliquoted as 125.0  $\mu$ L/vial. An aliquot of 100  $\mu$ L from each standard (S1 through S11) is required to set up a run. For example, to prepare enough calibration standards for approximately 1000  $\mu$ L of diluted calibration standard "S1": combine 100  $\mu$ L of "S1" stock solution, 100  $\mu$ L of synthetic urine, and 800  $\mu$ L of deionized water and mix thoroughly. Accurately aliquot the mixture into 1.5-mL micro-centrifuge vials (125.0  $\mu$ L/vial) and store at -70 °C. The final concentrations ( $\mu$ M) of each analyte in S1–S11 are shown in Table I.

#### (4) Intermediate Mixed-Internal Standard Stock Solutions

Intermediate mixed-internal standard stock solutions are prepared by combining single internal standard stock solutions into a mixture containing 5  $\mu$ M of each compound except for AAMU, which will have a concentration of 15  $\mu$ M. Aliquot the solution into 2-mL polypropylene cryovials (0.5mL/vial or 0.2 ml/vial) and store at -70°C.

# (5) Working Mixed-Internal Standard Solutions

Working mixed-internal standard stock solutions are prepared by diluting the intermediate mixed-internal standard stock solution by 5× with water.

#### C. Preparation of Quality Control Materials

Low, medium, and high quality control (QC) pools are prepared by selecting and pooling urine from anonymous volunteers. Urine samples from anonymous volunteers are first screened for their caffeine and metabolite concentrations and pooled to meet target concentrations for 1X, 17X, 137X, 1U, 17U and AAMU based on currently available reference data. A best-effort is made to meet target concentrations for the remaining analytes but this may not always be possible due to the total number of compounds being analyzed. For the low QC pool, urine samples are selected such that a pool can be generated with analyte concentrations approximating the  $25^{th}$  percentile population estimate. Similarly, the medium QC pool is prepared to approximate the  $50^{th}$  percentile and the high QC pool is prepared to approximate the  $75^{th}$  percentile. Each pool is stored in 500-µL aliquots in 2.0-mL Nalgene cryovials at -70 °C.

#### D. Other Materials

With some exceptions, a material listed herein may be substituted with equivalent product from a different manufacturer provided that it meets or exceeds the specifications of the product listed. In the case of standards, internal standards, chemicals and reagents, the chemical and/or isotopic purity of the substituted must meet or exceed that of the listed product. In the case of the HPLC column and guard cartridge, equivalent performance must be demonstrated experimentally in accordance with DLS policies and procedures.

#### (1) General consumables

- Kinetex 1.7 μ XB-C18 column 100 x 3.0 mm, 100 Å pore (Phenomenex, Torrance, CA)
- Krudkatcher Ultra HPLC In-Line Filter 0.5 μ Depth Filter x 0.004 in ID (Phenomenex)
- 9" Disposable glass Pasteur pipettes (Kimble Glass, Vineland, NJ)
- HPLC autosampler vials (2.0mL/12x32mm, National Scientific, Duluth, GA)
- 1-mL, 96-well plate, 31 mm (Nalgene, Rochester, NY)
- Pre-slit, silicone 96-well plate seal (Fisher Scientific, Suwanee, GA)
- Fisher brand nitrile examination gloves (Fisher Scientific, Suwanee, GA)
- Pipette tips, blue, 50-1000 μL, for Eppendorf pipette (Eppendorf, Hauppauge, NY)
- Pipette tips, yellow, 2-200 μL, for Eppendorf pipettes (Eppendorf)
- Positive displacement pipette tip, Combitip plus, 500 μL, 1mL, 2.5 mL, and 5 mL, for Eppendorf repeater pipette (Eppendorf)
- Hamilton high volume (1mL) tips without filter (Hamilton, Reno, NV)
- Hamilton standard volume (300µL) tips without filter (Hamilton)

- Costar Spin-X Centrifuge Tube filter (0.22 μm Nylon), polypropylene tune, non-sterile (Corning Incorporated, Corning, NY)
- AcroPrep 0.2-μm nylon, 96-well filter plate (Pall Life Sciences, Ann Arbor, MI)
- 2.0 mL Polypropylene cryovials (Nalgene)
- 10 mL Polypropylene T310-10A Cryovial with silicone washer seal (Simport, Beloeil, QC, Canada)
- 15 mL Falcon Tubes (Fisher Scientific, Suwanee, GA)
- 1.5mL micro centrifuge tubes (VWR, Suwanee, GA)
- Various glass beakers, volumetric flasks (Class A), graduated cylinders (Class A), and bottles (various suppliers)

#### (2) Chemicals and solvents

- Methanol, HPLC grade (Burdick & Jackson Laboratories, Muskegon)
- Water, 0.45 µm filtered, ≥ 18.0 MΩ resistance (in-house source, Aqua Solutions, Jasper, GA)
- Sodium hydroxide, 10N (Fisher Scientific Co., Fairlawn, NJ)
- Hydrochloric acid, 2N (Fisher Scientific)
- Formic acid (Sigma, St. Louis, MO)
- Potassium chloride (Sigma)
- Sodium chloride (Sigma)
- Urea (Sigma)
- Magnesium sulfate (MgSO4.7H2O) (Sigma)
- Citric acid (Sigma)
- Ascorbic acid (Sigma)
- Potassium phosphate (Sigma)
- Creatinine (Sigma)
- Sodium hydroxide (Sigma)
- Sodium bicarbonate (Sigma)
- Sulfuric acid, concentrated (Sigma)
- 1,3,7-trimethylxanthine (Sigma)
- 1,3 dimethylxanthine (Sigma)
- 1,7 dimethylxanthine (Sigma)
- 3,7 dimethylxanthine (Sigma)
- 1-methylxanthine (Sigma)
- 3-methylxanthine (Sigma)
- 7-methylxanthine (Sigma)
- 1,3,7-trimethyluric acid (Sigma)
- 1,3-dimethyluric acid (Sigma)
- 1,7-dimethyluric acid (Sigma)
- 3,7-dimethyluric acid (Sigma)
- 1-methyluric acid (Santa Cruz, Dallas, Texas)
- 3-methyluric acid (Toronto Research Chemicals, Toronto, ON, Canada)
- 7-methyluric acid (Toronto Research Chemicals)
- 5-acetylamino-6-amino-3-methyluracil (Toronto Research Chemicals)
- 1,3-dimethyl xanthine -13-(methyl-(<sup>2</sup>H<sub>3</sub>)<sub>2</sub>) (CDN Isotopes, Point Claire. QC, Canada)
- 1,3,7-trimethylxanthine -(1,3,7, -(methyl-(<sup>2</sup>H<sub>3</sub>)<sub>3</sub>) (CDN Isotopes, Point Claire. QC, Canada)

- 1,3,7-trimethyl xanthine -2H9 (CDN Isotopes)
- 1,3-dimethyl xanthine -13C<sub>4</sub>15N<sub>3</sub> (IsoSciences, LLC, King of Prussia, PA)
- 1,7-dimethyl xanthine -13C<sub>4</sub>15N<sub>3</sub> (Iso Sciences)
- 3,7-dimethyl xanthine -13C<sub>4</sub>15N<sub>3</sub> (Iso Sciences)
- 1-methylxanthine -<sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>3</sub> (Iso Sciences)
- 3-methylxanthine -<sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>3</sub> (Iso Sciences)
- 7-methylxanthine <sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>2</sub> (Iso Sciences)
- 1,3,7-trimethyluric acid -13C<sub>4</sub>15N<sub>3</sub> (Iso Sciences)
- 1,3-dimethyluric acid -13C<sub>4</sub>15N<sub>3</sub> (Iso Sciences)
- 1,7-dimethyluric acid -13C<sub>4</sub>15N<sub>3</sub> (Iso Sciences)
- 3,7-dimethyluric acid <sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>1</sub> (Iso Sciences)
- 1-methyluric acid -<sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>3</sub> (Iso Sciences)
- 3-methyluric acid -<sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>3</sub> (Iso Sciences)
- 7-methyluric acid -13C<sub>4</sub>15N<sub>3</sub> (Iso Sciences)
- 5-acetylamino-6-amino-3-methyluracil-<sup>13</sup>C<sub>4</sub><sup>15</sup>N<sub>3</sub> (Iso Sciences)

#### E. Instrumentation

In the case of simple laboratory instrumentation (e.g., pipettes, vortex mixer, analytical balance, etc.) a product listed herein may be substituted with equivalent product from a different manufacturer provided that it meets or exceeds the specifications of the product listed. In the case of analysis instrumentation (e.g., UHPLC components, tandem quadrupole mass spectrometer) equivalent performance must be demonstrated experimentally in accordance with DLS policies and procedures if a product substitution is made. Equivalent performance must also be demonstrated in accordance with DLS policies and procedures when multiple analysis systems are used in parallel, even if they are of the exact same type.

- (1) Agilent 1290 UHPLC system (Agilent Technologies, Palo Alta, CA), including:
  - Model 4208A-Control Module
  - Model G4220A-Binary pump
  - Model G4226A-High Performance Autosampler
  - Model G1330B-Autosampler Thermostat
  - Model G1316C-Thermostatted Column Compartment
- (2) AB Sciex 6500 triple quad mass spectrometer (AB Sciex, Foster City, CA), including:
  - Turbo V Ion source, operated in ESI mode (AB Sciex)
  - Analyst 1.6.2 software (AB Sciex)
- (3) Hamilton Starlet 8-channel with auto-load arm (Hamilton), including:
  - Two pipette tip carriers, TIP CAR 480 A00
  - Three sample vial carriers, SMP CAR-32 A00
  - One reagent carrier, RGT\_CAR\_5X50\_G
  - One plate carrier, PLT\_CAR\_L5AC\_A00
- (4) Other laboratory instrumentation:
  - Harvard syringe pump (Harvard Apparatus, Inc., Holliston Massachusetts)
  - Eppendorf pipette,100-1000μL (Eppendorf)
  - Eppendorf pipette, 1-10 mL (Eppendorf)

- Eppendorf pipette, 20-200 μL (Eppendorf)
- Eppendorf pipette, 100 μL (Eppendorf)
- Eppendorf pipette, 10-100μL (Eppendorf)
- Eppendorf pipette, 2-20µL (Eppendorf)
- Eppendorf Repeater Plus pipette (Eppendorf)
- Vortexer (VWR)
- Accumet pH/mV meter (XL150, Fisher Scientific)
- Magnetic stirrer (Fisher Scientific)
- Eppendorf Centrifuge (5810R, Eppendorf)
- Analytical balance (AG104, Mettler Instrument Corp., Hightstown, NJ)

### 7. Calibration and Calibration Verification Procedures

#### A. Method Calibration

Eleven calibrators (S1-S11) prepared in 0.1× synthetic urine are added to the reaction plate and processed as regular samples. These 11 calibrators are analyzed at the beginning of each run. The calibrators are re-analyzed as unknown samples at the end of each run. A quadratic calibration equation with 1/x weighting is used. Samples with the concentrations exceed the highest concentration of the calibrators are re-prepared with appropriate dilution. The measured concentrations of these calibrators should generally agree within 15% of their set values, although >15% agreement will be observed at concentrations approaching the LOD.

Reference materials are not available for urine caffeine and caffeine metabolites. Calibration verification is conducted as outlined in "4063.08 SOP for Calibration and Calibration Verification."

External proficiency testing programs currently do not exist for urine caffeine metabolites. An in-house proficiency testing program has been developed and is conducted at least twice a year, details of which can be found in "4063.08 SOP for In-House Proficiency Testing." For general information on the handling, analysis, review, and reporting of proficiency testing materials see "NBB\_SOP Proficiency Testing Procedure."

Results from a series of in-house ruggedness testing experiments designed to assess how much method accuracy changes when certain experimental parameters are varied are presented in **Appendix B.** 

#### B. Instrument Calibration

#### (1) API 6500 Mass Spectrometer

The calibration of the mass spectrometer is scheduled on a semi-annual basis as part of a preventive maintenance program and is performed by the service engineer from Applied Biosystems. If necessary, the analyst can recalibrate using the calibration standards described below and by following the instructions contained in the operator's manual.

The tuning and mass calibration of the first (Q1) and third (Q3) quadrupoles of the API 6500 is performed using a solution of polypropylene glycol (PPG) by infusion and running the instrument in either Manual Tuning mode or using Automatic Mass Calibration. Please refer to the API 6500 User's Manual for additional details.

## (2) Hamilton Microlab Starlet

Once a year, a qualified service engineer performs preventative maintenance, including volume verification at 10  $\mu$ L and 1000  $\mu$ L.

A volume verification of the various steps of the method can also be performed gravimetrically (e.g., using online gravimetric kit, Hamilton) by the user. Imprecision should be commensurate or exceed that obtained using manual pipettes.

# 8. Procedure Operating Instructions; Calculations; Interpretation of Results

A typical run (in the order in which they are injected into the LC-MS/MS) consists of a blank (with IS), a double blank (buffer only, without IS), 11 calibrators, 3 levels of bench QCs (low, medium, and high), patient samples (up to a maximum of 77), re-inject of 3 bench QCs (low, medium, and high), blanks, and calibrators.

## A. Sample Preparation

- (1) Manual Sample Preparation
  - (a) Sample dilution:
  - Label one set of 1.5-mL micro-centrifuge tubes for all urine samples and two sets of bench QCs (dilution tubes).
  - Quantitatively transfer 450  $\mu$ L of water to each dilution tube. Quantitatively transfer 50  $\mu$ L of each sample and QC to a dilution tube.
  - Cap and mix all dilution tubes thoroughly by vortexing. Transfer 100  $\mu$ L of the diluted urine to the second set of ependorf tubes (reaction tubes).
    - (b) Alkaline treatment:
  - Label one set of 1.5-mL microcentrifuge tubes for all diluted samples and bench QCs from the previous step, plus additional tubes for a blank and calibrators which are pre-diluted (reaction tubes).
  - Quantitatively transfer 80  $\mu$ L of water, 20  $\mu$ L of 1x internal standard, and 20  $\mu$ L of 1.2 N NaOH to each reaction tube (alternatively, prepare a 4:1:1 mixture of these solutions and quantitatively transfer 120  $\mu$ L of the mixture to each reaction tube).
  - Quantitatively transfer 100  $\mu$ L of each diluted sample, bench QC, blank and calibrator to each reaction tube. Cap and mix all reaction tubes and incubate at room temperature for at least 30 minutes.
    - (c) Acidification:
  - Quantitatively transfer 30  $\mu$ L of 2N HCl and 250  $\mu$ L of 2× HPLC mobile phase A to each reaction tube (alternatively, prepare a 3:25 mixture of these solutions and quantitatively transfer 280  $\mu$ L of the mixture to each reaction tube). Cap and mix all reaction tubes thoroughly by vortexing.
    - (d) Filtration:
  - Label one set of 0.2-μm nylon microcentrifuge filter tubes for all samples, QCs, blanks and calibrators.
  - Transfer the contents of each reaction tube to a microcentrifuge filter tube and centrifuge at 10,000 g for 5 min.
    - (e) HPLC Analysis:

- Label one set of HPLC vials for all samples, QC, blanks and calibrators.
- Transfer the filtered contents of each microcentrifuge filter to an HPLC vial with an insert.
- Cap all vials and gently tap each vial to ensure that there are no bubbles in the vial
  contents. The filtrate is ready for the analysis on HPLC (alternatively, transfer the filtered
  contents of each microcentrifuge filter, or use a 96-well filter plate to filter the samples
  directly into a 96-deep well plate and seal the plate with a pre-slit 96-well silicone sealing
  mat).

#### (2) Automated Sample Preparation

"4063.08 SOP Automated Sample Preparation" describes automated sample preparation using the Hamilton Starlet system. These steps directly mimic those described above for manual sample preparation with most pipetting actions being performed by the Hamilton Starlet. In brief: sample dilution steps (a) are performed in a 96-deep well plate; alkaline treatment (b) and acidification steps (c) are performed in a second 96-deep well plate; filtration steps (d) are performed using a 96-well 0.2 μm nylon centrifuge filter plate collecting into a 96-deep well plate; and HPLC analysis (e) is performed on the 96-well collection plate sealed with a pre-slit 96-well silicone sealing mat. All precautions observed in manual sample preparation should be observed when performing automated sample preparation.

The instructions given in the SOP reflect the custom program developed for performing sample preparation that is currently being used. Certain non-critical elements of this program (e.g., positions of samples, wording of user messages) may be modified and differ from the exact instructions given in the SOP. The user is strongly encouraged to be familiar with the exact program being used.

A liquid handling system other than the Hamilton Starlet may be used for this purpose provided that it is able to perform these steps with accuracy and precision that meets or exceeds that of the Hamilton Starlet.

#### B. Instrument Preparation

#### (1) UHPLC (Agilent 1290)

Fill all solvent bottles as follows. UHPLC Mobile Phase A (aqueous) (line A1) and Mobile Phase B (organic) (line B1) should be refilled with freshly prepared solvent before each run (see section 6.a. for preparation instructions). HPLC-grade water (line A2) and a solution of 50% HPLC-grade acetonitrile in water- (line B2) should be checked daily and refilled as needed. The solution of 60% methanol in water (needle wash) should be checked daily and refilled as needed. Clean or replace any solvent bottles, inlet filters or lines as needed.

Check the waste bottle to ensure that it will not overflow during the run. Dispose of all chemical waste according to procedures described in the CDC Hazardous Chemical Waste Management procedures.

Replace the guard column every 5 runs, or when the chromatographic performance has become poor, whichever occurs first.

Replace the HPLC column if chromatographic performance has become poor. Monitor chromatographic performance closely in terms of background noise and accuracy of S10 and S11if the column has been used for >1000 sample injections. Inspect all UHPLC tubing and tubing connections. Ensure that all connections are in place and tightened appropriately.

Using the hand-held control module, purge all solvent lines by running solvent through each line at 5 ml/min for at least 5 min. Purging is necessary if the instrument has been idle for one day or longer, or if air bubbles are visible in any of the solvent lines. Close the waste valve when done.

#### (2) Mass Spectrometer

Check the interface and turbo ion spray probe before each run to make sure that the needle height, probe height/width settings are correct. The probe position is optimized, and usually reset after preventative maintenance. In general, a test run containing standards and QCs is performed after maintenance to ensure that instrument performance (e.g., sensitivity, signal-to-noise ratio) is comparable with previous runs.

Clean the source, probe, and curtain plate interface every 2 full runs (caution: the interface may be very hot if the instrument was recently run). See the API 6500 User's Manual for specific guidance.

# C. Sample Analysis

The UHPLC-MS/MS system is used to quantitate caffeine and caffeine metabolite levels in urine. See "4063.08 SOP Sample Analysis" for a detailed description of the sample analysis steps. UHPLC-MS/MS parameters are given in **Appendix D**. The following is an overview of the sample analysis process.

#### (1) Preliminaries

The user must first ensure that all instrumentation is turned on and ready for use. This entails starting Analyst software and ensuring the correct project and hardware configuration is selected and activated. Refer to "4063.08 SOP for Sample Analysis" for additional details.

#### (2) Building an Acquisition Batch

Because of the number of steps involved in building a new batch file, it is acceptable for the user to use a previous batch file and modify it to suit the current analysis by changing the necessary information (e.g., sample names, sample IDs, data file names, comments, etc..). In brief, the analyst must create a sample set to accommodate the following: the startup methods; equilibration injections, unknown samples, and shutdown method. These samples should be run in the order as presented above. Refer to "4063.08 SOP for Sample Analysis" for additional details.

#### (3) Instrument Equilibration

The instrument needs to be equilibrated for at least 30 minutes prior to starting an analysis. Though instrument equilibration is presented following the building of the acquisition batch, the acquisition batch can be built while the instrument is equilibrating.

This procedure assumes that the user is starting a new analysis after the instrument has successfully completed a previous analysis. The user may deviate from this procedure if special circumstances present themselves (e.g., restarting an instrument run that was interrupted).

Refer to "4063.08 SOP for Sample Analysis" for additional details.

#### (4) Submitting and Starting a Batch

Once the instrument has been properly equilibrated and the acquisition batch has been created and saved, the user may submit the batch to the analysis queue and start the analysis sequence. Refer to "4063.08 SOP for Sample Analysis" for additional details.

#### D. Quantitation and Data Review

The UHPLC- MS/MS system software (Analyst 1.6.2) is used for quantitating analysis data. Quantified results are then imported into Starlims for data review by the analyst and team lead, then finally by supervisor or quality assurance officer.

The quantitation of instrument results can be done either at the instrument computer or a different location (e.g., desktop PC) where the LC-MS/MS software is installed. In order to review data at a location other than the instrument, the user will have to create an identical project and copy all required files over to this location.

The following instructions assume that a complete analysis was performed. If the user is only interested in certain samples from an instrument run, the user may deviate from this procedure as necessary.

#### (1) Review Peak Integration

The quantitation method is set up to identify and integrate analyte and internal standard peaks based on specifications such as retention time windows and minimum peak area thresholds. The user should review all peak integrations and correct any integration errors where necessary. Refer to "4063.08 SOP Starlims Data Review"

#### (2) Review Calibration Curves

The analyst should review the calibration curve for each analyte, ensuring that the correct regression model and weighting are used in each case. If a calibration point appears to be erroneous, it may be removed from the curve in consultation with the team lead (Note: the analyst should be aware of the implications of removing the highest or lowest calibration point as this may affect the reportable range of values for an instrument run).

## E. System Maintenance

Agilent UHPLC - Preventative maintenance is performed on an annual basis by a qualified service engineer. Routine maintenance should be performed as indicated in this document and in the Agilent User's Manual.

Applied Biosystems API 6500 MS/MS - Preventative maintenance, tuning and mass calibration is performed on an annual basis by a qualified service engineer. Routine maintenance should be performed as indicated in this document and in the Applied Biosystems User's Manual.

Hamilton Microlab Starlet - Preventative maintenance is performed on an annual basis by a qualified service engineer. Routine maintenance should be performed as indicated in the Hamilton User's Manual.

# 9. Reportable Range of Analytical Results

Table IV: Reportable Range of Analytical Results

Analyte	Reportat	ole ra	nge (μM)
1X	0.03	_	400
3X	0.04	-	300
7X	0.02	-	600
13X	0.01	-	20
17X	0.006	-	200
37X	0.004	-	250
137X	0.003	-	50
1U	0.05	_	500
3U	0.1	-	15
7U	0.04	-	200
13U	0.02	_	50
17U	0.02	-	300
37U	0.03	-	20
137U	0.005	-	40
AAMU	0.1	-	500

Samples with concentrations exceeding the highest calibrator are diluted, re-prepared, and reanalyzed so that the measured value is within the range of the calibration. There is no known maximum acceptable dilution. When possible, avoid small volume pipetting and minimize use of serial dilutions when generating diluted samples. Changes in LOD or concentration of highest calibrator concentration will affect the reportable range.

# 10. Quality Control (QC) Procedures

### A. Blind Quality Controls

Blind QC specimens are inserted prior to the arrival of the samples in the Nutritional Biomarkers Branch. These specimens are prepared at two levels so as to emulate the patient samples; the labels used are identical to those used for patient samples. One blind QC specimen randomly selected for concentration is included at a randomly selected location in every 20 specimens analyzed.

Alternatively, open label blind QC specimens can be used where the analyst knows that the sample is a blind QC, but they do not know what pool the sample is from. Open label blind QCs are used only if they can be selected from at least 5 different pools and the analyte concentrations are similar to those found in patient samples.

#### B. Bench Quality Controls

Bench QC specimens are prepared from three urine pools that represent low, medium and high levels of urine caffeine and caffeine metabolites. Samples from these pools are prepared in the same manner as patient samples and analyzed in duplicate as part of each run.

The results from the pools are checked after each run using a multi-rule quality control system [33] based their characterization data, namely: the pool mean; the pooled within-run standard deviation associated with individual QC results measured in the same run ( $S_w$ ); the standard deviation associated with individual QC results ( $S_i$ ); and the standard deviation associated with run mean QC results ( $S_m$ ). QC rules have been designed to accommodate the use of 1–3 different QC pools during a run, the use of 1–2 measurements of each pool per run, and as many instruments as needed. In the case of three QC pools per run with two QC results per pool:

(1) If all three QC run means are within 2  $S_m$  limits and individual results are within 2 Si limits, accept the run

- (2) If one of the three QC run means is outside a 2  $S_m$  limit, reject run if:
  - (a) 1 3S Rule—Run mean is outside a 3 S<sub>m</sub> limit or
    - (b) 2 2S Rule—Two or more of the three run means are outside the same 2  $S_m$  limit or
    - (c) 10 X bar Rule—Current and previous nine run means are on the same side of the characterization mean
- (3) If one of the six QC individual results is outside a 2  $S_i$  limit, reject run if:
  - (a) Outlier—One individual result is beyond the characterization mean  $\pm 4 S_i$  or
  - (b) R 4S Rule—two or more of the within-run ranges in the same run exceeds 4  $S_w$  (i.e. 95 % range limit).

A QC program written in SAS is available from the DLS Quality Assurance Officer and should be used to apply these rules to QC data and generate Shewhart QC charts. No results for a given analyte are to be reported from an analytical run that has been declared "out of control" for that analyte as assessed by internal (bench) QC.

The initial limits are established by analyzing pool material at least 20 consecutive runs and then are reevaluated periodically. When necessary, limits are updated to include more runs.

While a study is in progress, QC results are stored in STARLIMS. For the runs that are not imported into the database (i.e., R&D, troubleshooting, research-type runs), QC results are stored electronically in the analyte-specific folder on the DLS network. At the conclusion of studies, complete QC records are prepared for review by a DLS statistician.

### C. Sample QC Criteria

Each individual sample result is checked against established sample QC criteria limits to assure data quality. The method also uses the following sample QC criteria:

- Relative retention time (retention time quantitation ion/retention time ISTD)
- Confirmation ion ratio (confirmation ion area/quantitation ion area)
- Percent difference of Individual ISTD area from within-run average

For additional details and criteria, see "4063.08 SOP Sample QC Criteria."

# 11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

The following steps are provided as a general guideline for identifying possible problems resulting in "out of control" values for QC materials. The troubleshooting process should be done in consultation with the supervisor or team lead and may involve additional experiments beyond what is indicated below. Analytical results for runs not in statistical control should not be reported. The following remedial actions should be considered:

- Look for possible sample preparation errors, specimen, and reagents used, etc.
- Check whether the QC samples are handled properly.
- Check all HPLC reagents, any leaks or air bubbles in tube line.
- Check to make sure that the ESI probe position is correct and optimized, and other instrument hardware is functioning properly. Run PPGs in Q1 Scan to check the instrument calibration.
- Run standards in Q1 Scan to see if the molecular ion is detectable.
- Check the proper gas flow for curtain, exhaust, and source from the nitrogen generator.

Caffeine and Caffeine Metabolites NHANES 2014-2014

- Check the auto-sampler for evidence of correct sample injections.
- Check the calibrations of the pipettes and robotic liquid handler.

# 12. Limitations of Method; Interfering Substances and Conditions

The most common cause of poor method performance is a pipetting error. All buffers, reagents and mobile phases should be made fresh whenever possible and verified for performance. Occasionally, the concentration of caffeine or caffeine metabolites in urine will exceed the highest calibrator. In this case, a dilution run will be performed. When using a quadratic equation for calibration, care must be taken to minimize excessive "roll-over" of the curve at higher concentrations. This phenomenon is typically indicative of too much analyte being injected. If it is observed, reducing the sample injection volume is recommended.

This method has also undergone a series of in-house ruggedness testing experiments designed to assess how much method accuracy changes when certain experimental parameters are varied. A total of five parameters judged to most likely affect the accuracy of the method have been identified and tested. Testing generally consisted of performing replicate measurements on a test specimen with the selected parameter set at a value substantially lower and higher than that specified in this method while holding all other experimental variables constant. The ruggedness testing findings for this method are presented in **Appendix B**. Please refer to Chapter 20 of the 2017 DLS Policies and Procedures Manual for further information on ruggedness testing.

# 13. Reference Ranges (Normal Values)

Reference ranges (2.5<sup>th</sup>–97.5<sup>th</sup> percentile) for the representative US population (NHANES 2009–10) [34] are as follows (Table V):

**Table V** Reference range of caffeine metabolites

Analista		Percentile (μΜ	)
Analyte	2.5 <sup>th</sup>	median	97.5 <sup>th</sup>
1X	0.986	27.6	276
3X	0.693	30.9	305
7X	1.13	51.3	546
13X	<0.05	1.63	11.4
17X	<0.1	15.2	105
37X	0.406	20.3	186
137X	<0.01	3.39	33.8
1U	4.58	58.6	508
3U	<0.1	0.560	6.60
7U	0.368	15.5	182
13U	<0.05	6.42	62.9
17U	0.066	24.8	224
37U	<0.05	1.24	13.1
137U	<0.05	1.42	16.2
AAMU	0.339	49.9	539

# 14. Critical Call Results ("Panic Values")

There are no established critical values for urine caffeine and caffeine metabolites, i.e. there is no definition of a safe, normal or acceptable concentration of urine phytoestrogens versus one that would be considered abnormal or life-threatening.

# 15. Specimen Storage and Handling during Testing

Urine samples may be stored overnight in the refrigerator to expedite thawing prior to aliquotting. Samples should be allowed to warm to and be maintained at room temperature during preparation and testing and then returned to frozen storage (typically at ≤-70C) as soon as possible. Ambient light exposure should be avoided if the samples are kept on the working bench more than 2 hours.

#### 16. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

There are no acceptable alternative methods for the analysis of urine caffeine and caffeine metabolites in the Nutritional Biomarker Branch. If the analytical system fails, we recommend that the specimens or prepared samples be stored (typically at  $\leq$ -70C) until the analytical system is restored to functionality.

# 17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Test results are reported to the collaborating agency at a frequency and by a method determined by the supervisor. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as a spreadsheet file (e.g., Microsoft Excel), either through email or via transfer to an ftp site.

For NHANES 1999+, all data are reported electronically on a periodic basis to Westat who in turn transfers the results to NCHS. For smaller studies, electronic copies of a data report are sent; a hard copy of the data report may also be sent if requested.

# 18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

The LIMS is used to keep records and track specimens for all studies. For studies other than NHANES, additional records may be kept in Excel files on the network.

We recommend that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual urine from these analyses for non-NHANES studies are retained for at least 1 year after results have been reported and may then be returned or discarded at the request of the principal investigator. Very little residual material will be available after NHANES analyses are completed, however residual urine is retained for at least 2 years after results have been publicly released; at that point, samples with sufficient volume (>0.2 mL) are returned to NHANES and samples with insufficient may be autoclaved.

The exact procedure used to track specimens varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are kept by the supervisor. In general, when specimens are received, the specimen ID number is entered into a database and the specimens stored in a freezer at -80°C. The specimen ID is read off of the vial by a barcode reader used to prepare the electronic specimen table for the analytical system. When the analyses are completed, the results file is loaded into the database, and the analytical results are linked to the database by ID number. The analyst is responsible for documenting and keeping a record of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. In general, these are documented using codes in LIMS.

# 19. Method performance documentation

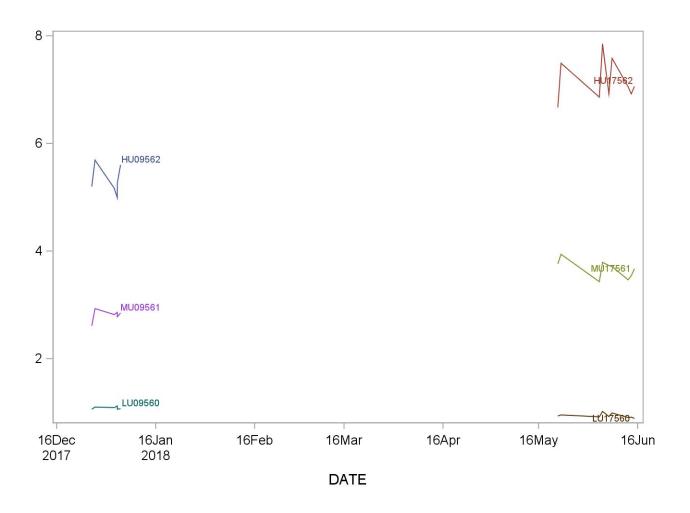
Method performance documentation for this method including accuracy, precision, sensitivity, specificity and stability is provided in **Appendix A** of this method documentation. **The signatures of the branch chief and director of the Division of Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method.** 

## 20. Summary Statistics and QC Graphs

See following pages.

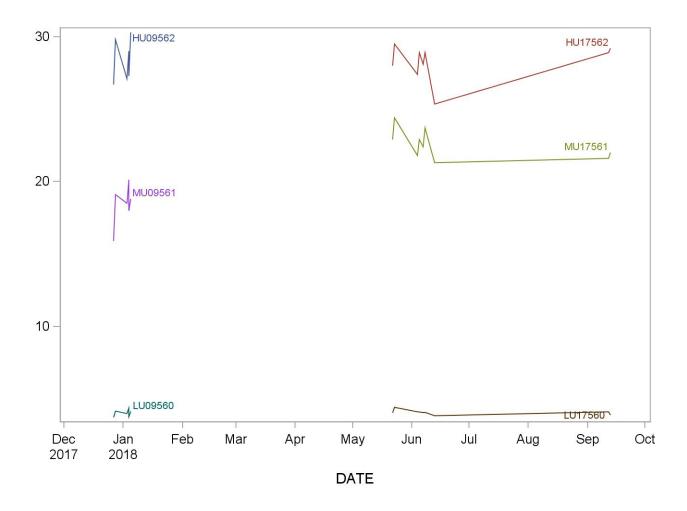
2014 Summary Statistics and QC Chart for 1,3,7-Trimethyluric acid, U 2nd C umol/L

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	6	27DEC17	05JAN18	5.3183	0.2701	5.1
LU09560	6	27DEC17	05JAN18	1.0833	0.0242	2.2
MU09561	6	27DEC17	05JAN18	2.8083	0.1091	3.9
HU17562	9	22MAY18	15JUN18	7.1561	0.3917	5.5
LU17560	9	22MAY18	15JUN18	0.9392	0.0421	4.5
MU17561	9	22MAY18	15JUN18	3.6706	0.1647	4.5



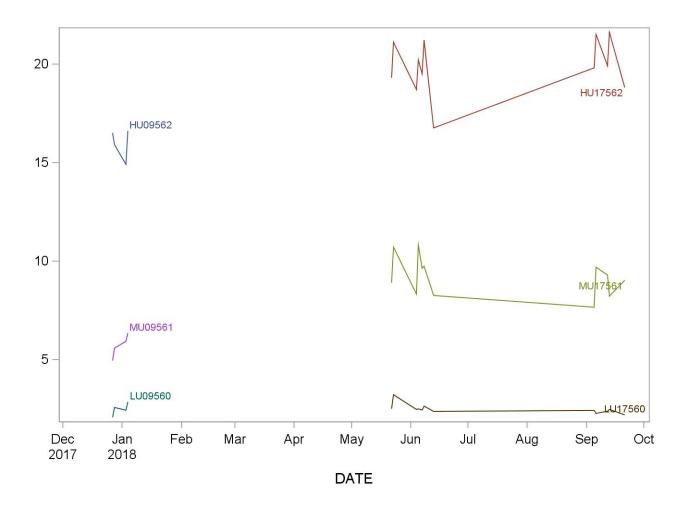
2014 Summary Statistics and QC Chart for 1,3,7-trimethylxanthine, U 2nd C umol/L

Lot	N	Start Date	End Date	Mean		Coefficient of Variation
HU09562	6	27DEC17	05JAN18	28.3667	1.5306	5.4
LU09560	6	27DEC17	05JAN18	4.0183	0.2444	6.1
MU09561	6	27DEC17	05JAN18	18.4000	1.4114	7.7
HU17562	9	22MAY18	13SEP18	28.2500	1.2723	4.5
LU17560	9	22MAY18	13SEP18	4.0611	0.1631	4.0
MU17561	9	22MAY18	13SEP18	22.5556	1.0212	4.5



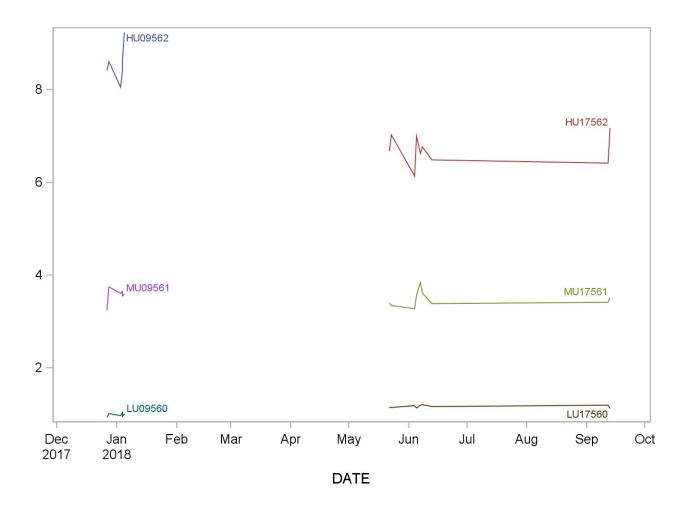
2014 Summary Statistics and QC Chart for 1,3-Dimethyluric acid, U 2nd Col(umol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	4	27DEC17	04JAN18	15.9750	0.7805	4.9
LU09560	4	27DEC17	04JAN18	2.4700	0.3243	13.1
MU09561	4	27DEC17	04JAN18	5.6925	0.5922	10.4
HU17562	12	22MAY18	21SEP18	19.8625	1.4063	7.1
LU17560	12	22MAY18	21SEP18	2.4804	0.2569	10.4
MU17561	12	22MAY18	21SEP18	9.1792	0.9866	10.7



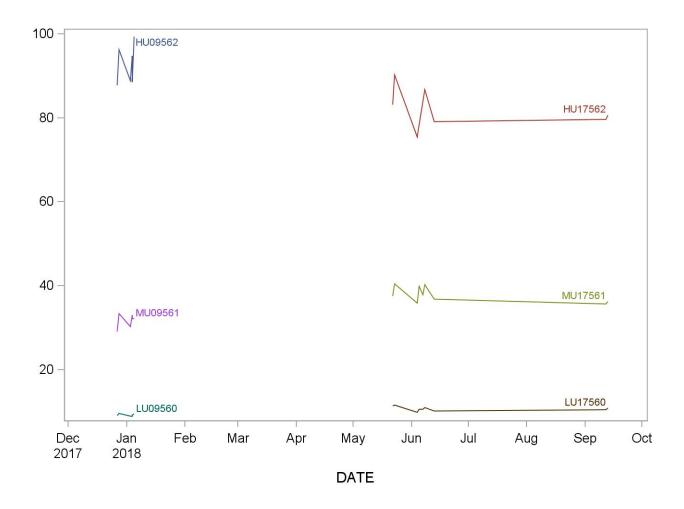
2014 Summary Statistics and QC Chart for 1,3-dimethylxanthine, Urn 2nd C (umol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	6	27DEC17	05JAN18	8.5533	0.3907	4.6
LU09560	6	27DEC17	05JAN18	0.9812	0.0385	3.9
MU09561	6	27DEC17	05JAN18	3.5600	0.1696	4.8
HU17562	9	22MAY18	13SEP18	6.6944	0.3308	4.9
LU17560	9	22MAY18	13SEP18	1.1611	0.0298	2.6
MU17561	9	22MAY18	13SEP18	3.4800	0.1728	5.0



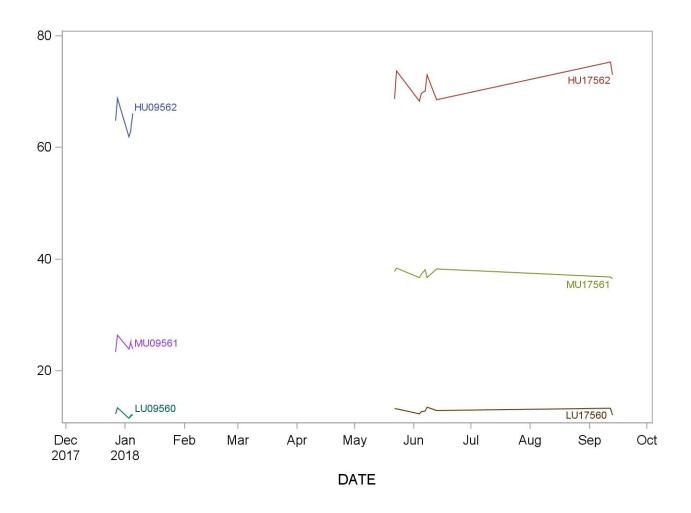
2014 Summary Statistics and QC Chart for 1,7-Dimethyluric acid, U 2nd Col(umol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	6	27DEC17	05JAN18	92.5333	4.8475	5.2
LU09560	6	27DEC17	05JAN18	9.1233	0.2820	3.1
MU09561	6	27DEC17	05JAN18	31.6000	1.6613	5.3
HU17562	9	22MAY18	13SEP18	81.8722	4.5781	5.6
LU17560	9	22MAY18	13SEP18	10.6394	0.5511	5.2
MU17561	9	22MAY18	13SEP18	37.7833	1.9076	5.0



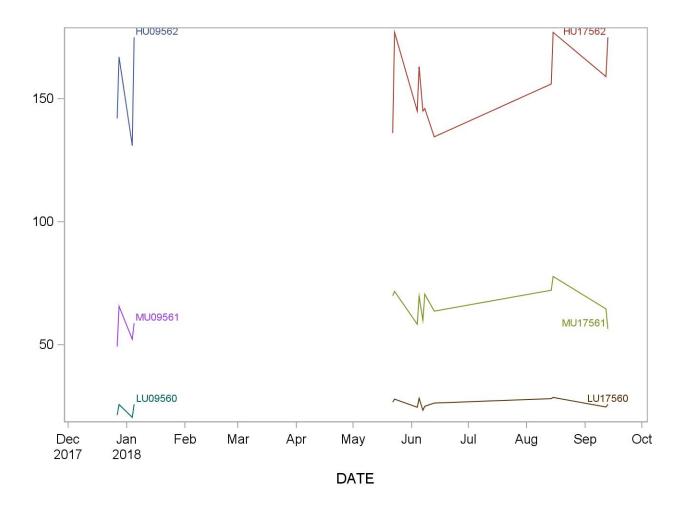
2014 Summary Statistics and QC Chart for 1,7-dimethylxanthine, Urn 2nd C (umol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	6	27DEC17	05JAN18	64.6500	2.5082	3.9
LU09560	6	27DEC17	05JAN18	12.2167	0.6432	5.3
MU09561	6	27DEC17	05JAN18	24.6167	1.1053	4.5
HU17562	9	22MAY18	13SEP18	71.1500	2.6139	3.7
LU17560	9	22MAY18	13SEP18	12.8889	0.4676	3.6
MU17561	9	22MAY18	13SEP18	37.3944	0.7527	2.0



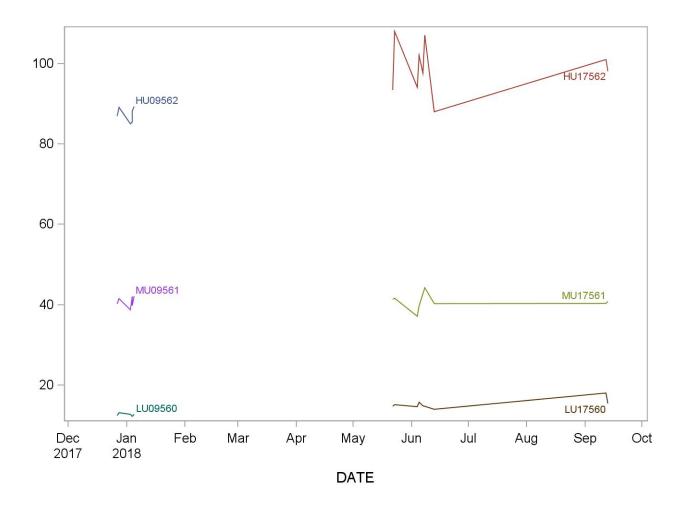
# 2014 Summary Statistics and QC Chart for 1-methyluric acid, Urine 2nd Col(umol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	4	27DEC17	05JAN18	153.7500	20.6781	13.4
LU09560	4	27DEC17	05JAN18	23.2500	2.7958	12.0
MU09561	4	27DEC17	05JAN18	56.4250	7.2894	12.9
HU17562	11	22MAY18	13SEP18	155.7727	15.8262	10.2
LU17560	11	22MAY18	13SEP18	26.2000	1.7510	6.7
MU17561	11	22MAY18	13SEP18	66.7364	6.6677	10.0



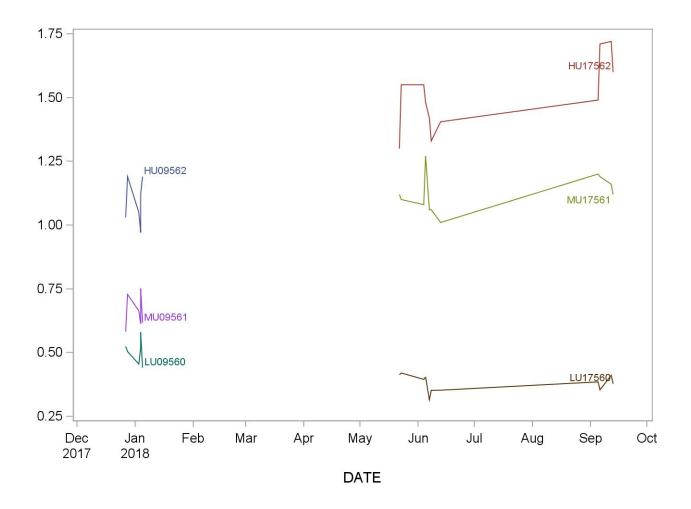
2014 Summary Statistics and QC Chart for 1-methylxanthine, Urine 2nd Col (umol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	6	27DEC17	05JAN18	87.3167	1.8498	2.1
LU09560	6	27DEC17	05JAN18	12.5500	0.3507	2.8
MU09561	6	27DEC17	05JAN18	40.7167	1.3674	3.4
HU17562	9	22MAY18	13SEP18	98.8000	6.4883	6.6
LU17560	9	22MAY18	13SEP18	15.2167	1.1576	7.6
MU17561	9	22MAY18	13SEP18	40.8833	1.9827	4.8



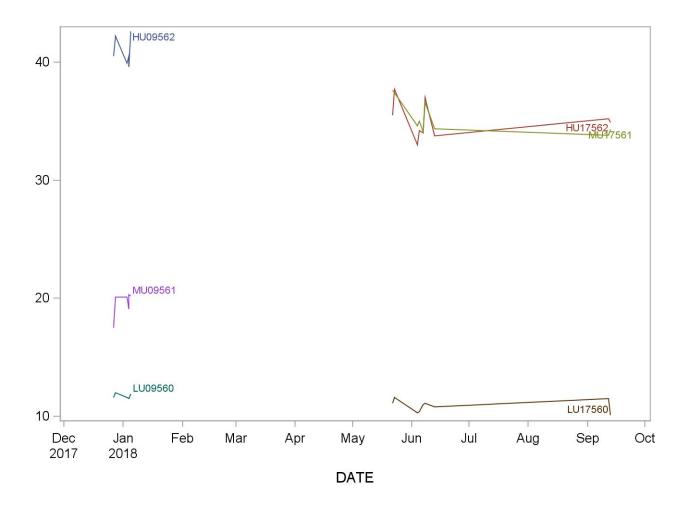
2014 Summary Statistics and QC Chart for 3,7-Dimethyluric acid, U 2nd Col(umol/L)

Lot	N	Start Date	End Date	Mean		Coefficient of Variation
HU09562	6	27DEC17	05JAN18	1.0918	0.0897	8.2
LU09560	6	27DEC17	05JAN18	0.5035	0.0502	10.0
MU09561	6	27DEC17	05JAN18	0.6593	0.0678	10.3
HU17562	11	22MAY18	13SEP18	1.5050	0.1386	9.2
LU17560	11	22MAY18	13SEP18	0.3799	0.0330	8.7
MU17561	11	22MAY18	13SEP18	1.1245	0.0751	6.7



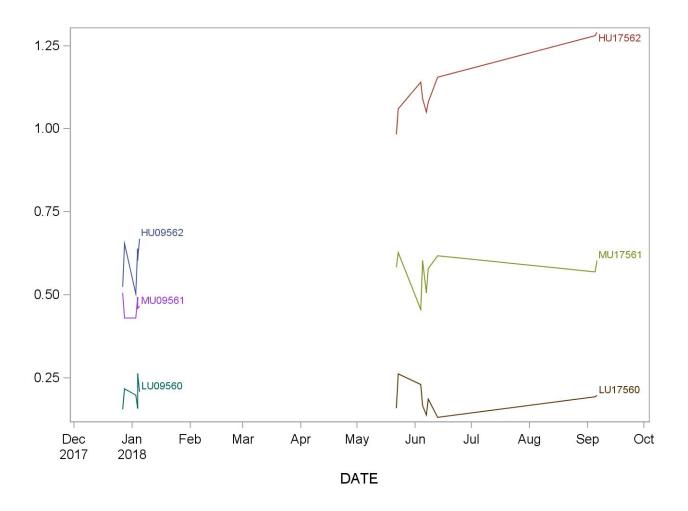
# 2014 Summary Statistics and QC Chart for 3,7-dimethylxanthine, Urn 2nd C (umol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	6	27DEC17	05JAN18	40.8833	1.2319	3.0
LU09560	6	27DEC17	05JAN18	11.6833	0.2137	1.8
MU09561	6	27DEC17	05JAN18	19.5500	1.0950	5.6
HU17562	9	22MAY18	13SEP18	35.0278	1.5324	4.4
LU17560	9	22MAY18	13SEP18	10.8778	0.5239	4.8
MU17561	9	22MAY18	13SEP18	35.3056	1.4816	4.2



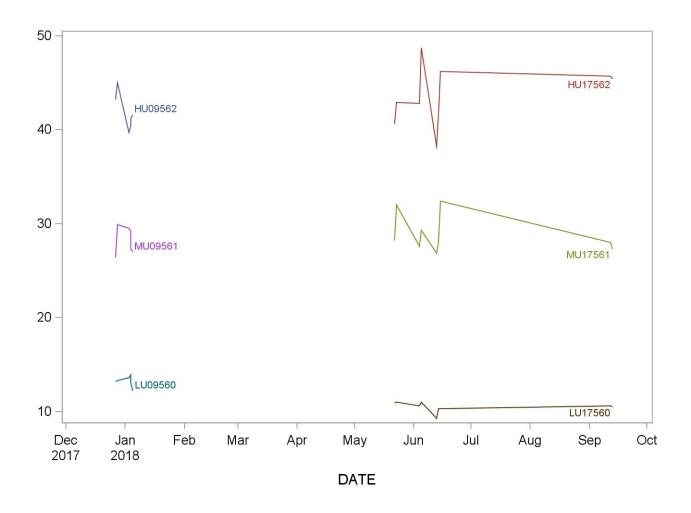
2014 Summary Statistics and QC Chart for 3-methyluric acid, Urine 2nd Col(umol/L)

Lot	N	Start Date	End Date	Mean		Coefficient of Variation
HU09562	6	27DEC17	05JAN18	0.5988	0.0698	11.7
LU09560	6	27DEC17	05JAN18	0.1998	0.0403	20.2
MU09561	6	27DEC17	05JAN18	0.4640	0.0315	6.8
HU17562	9	22MAY18	06SEP18	1.1253	0.1035	9.2
LU17560	9	22MAY18	06SEP18	0.1847	0.0423	22.9
MU17561	9	22MAY18	06SEP18	0.5715	0.0559	9.8



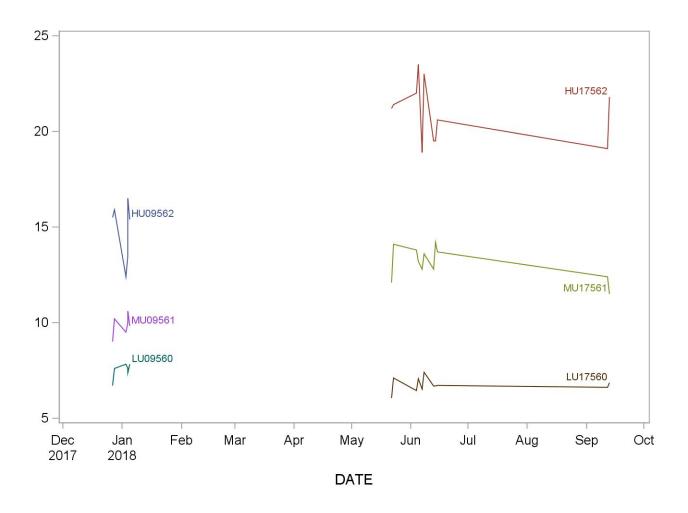
# 2014 Summary Statistics and QC Chart for 3-methylxanthine, Urine 2nd Col (umol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	6	27DEC17	05JAN18	41.8667	1.9325	4.6
LU09560	6	27DEC17	05JAN18	13.2000	0.5831	4.4
MU09561	6	27DEC17	05JAN18	28.2167	1.4878	5.3
HU17562	9	22MAY18	13SEP18	43.5944	3.2096	7.4
LU17560	9	22MAY18	13SEP18	10.4972	0.5316	5.1
MU17561	9	22MAY18	13SEP18	28.8611	2.0124	7.0



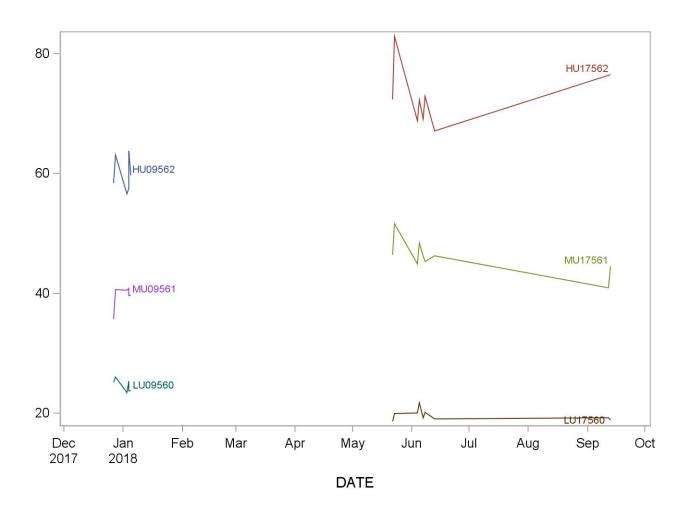
2014 Summary Statistics and QC Chart for 7-methyluric acid, Urine 2nd Col(umol/L)

Lot	N	Start Date	End Date	Mean		Coefficient of Variation
HU09562	6	27DEC17	05JAN18	14.8667	1.5731	10.6
LU09560	6	27DEC17	05JAN18	7.4967	0.4191	5.6
MU09561	6	27DEC17	05JAN18	9.8567	0.5542	5.6
HU17562	11	22MAY18	13SEP18	20.9545	1.5731	7.5
LU17560	11	22MAY18	13SEP18	6.7468	0.3631	5.4
MU17561	11	22MAY18	13SEP18	13.1091	0.8689	6.6



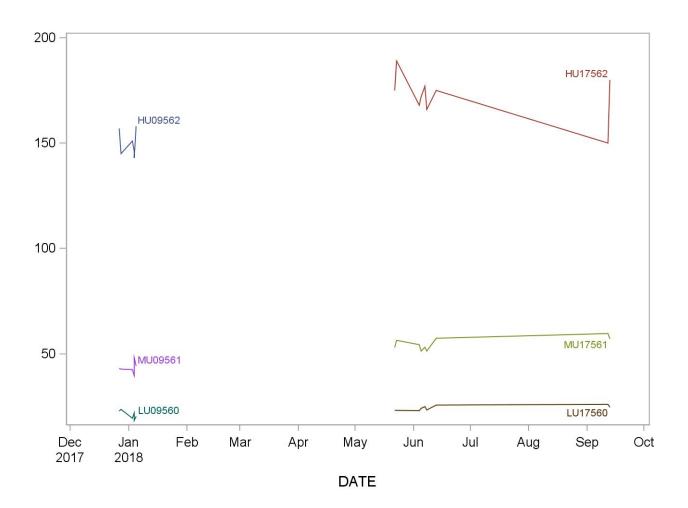
2014 Summary Statistics and QC Chart for 7-methylxanthine, Urine 2nd Col (umol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	6	27DEC17	05JAN18	59.8500	2.9791	5.0
LU09560	6	27DEC17	05JAN18	24.5333	1.0708	4.4
MU09561	6	27DEC17	05JAN18	39.4833	1.9177	4.9
HU17562	9	22MAY18	13SEP18	73.1556	4.8750	6.7
LU17560	9	22MAY18	13SEP18	19.6111	0.9506	4.8
MU17561	9	22MAY18	13SEP18	46.0500	2.9009	6.3



2014 Summary Statistics and QC Chart for AAMU, Urine 2nd collection (umol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU09562	6	27DEC17	05JAN18	150.000	6.387	4.3
LU09560	6	27DEC17	05JAN18	21.067	2.029	9.6
MU09561	6	27DEC17	05JAN18	43.350	2.718	6.3
HU17562	9	22MAY18	13SEP18	172.444	10.783	6.3
LU17560	9	22MAY18	13SEP18	24.206	1.144	4.7
MU17561	9	22MAY18	13SEP18	54.828	2.921	5.3



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# **Appendix A – Method Performance Validation**

# A. Accuracy

(1) AAMU

Accuracy using Sp	ike Recovery	- fill in yellow sh	haded ce	ells									
Recovery = (final	l concentration	on – initial conce	entratio	n)/added	d concentration	1							
Recovery should	be 85-115%	except at 3*LOD	where c	an be 80	-120%								
Method name:	Caffeine an	d Metabolites in	n Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC poo	l: low and mediu	ım QC										
Run date: Dec 13	3, 2017 (day 1	); Dec 15, 2017 (d	lay 2)										
Analyte:	AAMU						AAMU						
				LU17560	)	<u></u>		MU1	7560				
		Spike	Meas	ured cond	entration		Spike	Measured co	ncentratio	n		Mean	
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	recovery (%)	SD (%)
Sample	1	_	25.0	22.7		(70)	_	49.3	59.0		(70)	(///	(70)
	2	0	25.8	25.1	24.7		0	56.8	56.6	56.1		86.9	3.7
	3		24.0	25.5				54.7	60.3				
Sample + Spike 1	1		36.5	39.3									
	2	15	37.4	36.2	37.6	86.2							
	3		37.2	39.1									
Sample + Spike 2	1		46.6	45.1				75.2	75.0				
	2	25	48.1	46.3	46.0	85.1	25	77.3	82.5	76.8	82.7		
	3		43.3	46.3				72.7	78.1				
Sample + Spike 3	1		76.6	74.8				94.6	101				
	2	50	65.9	72.5	71.1	92.8	50	99.2	104	99.9	87.6		

### (2) 1-Methyluric Acid (1U)

Accuracy using Sp	ike Recovery	- fill in yellow sh	naded ce	ells									
Recovery = (fina	concentration	on – initial conce	entratio	n)/added	d concentration	ı							
Recovery should	be 85-115%	except at 3*LOD	where c	an be 80	-120%								
Method name:	Caffeine an	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC pool	l: low and mediu	ım QC										
Run date: Dec 1	3, 2017 (day 1	); Dec 15, 2017 (d	lay 2)										
Analyte:	1U						1U						
				LU17560				MU1	7560				
		Spike	Meas	ured cond	entration		Spike	Measured co	oncentratio	n			
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0	24.7	26.9			0	59.7	62.6				
	2	0	23.9	24.9	25.6		0	70.6	62.9	64.4		103	4.93
	3		27.3	25.7				67.5	63.3				
Sample + Spike 1	1	10	35.1	37.1			15	76.7	79.3				
	2	10	36.8	34.3	36.0	104	15	84.2	80.5	80.9	110		
	3		37.6	34.8				86.3	78.5				
Sample + Spike 2	1	25	49.8	49.3			25	95.4	82.9				
	2	20	53.8	51.1	51.1	102	25	96.1	84.3	90.9	106		
	3		51.6	51.2				93.8	92.9				
Sample + Spike 3	1	50	76.2	69.8			50	112	116				
	2	30	77.8	72.1	73.3	95.4	30	121	103	115	100		
	3		70.7	72.9				119	117				

# (3) 3-Methyluric Acid (3U)

Assurant using Su	sika Basawani	- fill in yellow sh	22424	alle									
, , ,		on – initial conce			d concontration								+
													+
Recovery should	ne 85-115%	except at 3*LOD	where c	an be 80	-120%								+
	C. ((.)	d Advis by division to	. 112										+
	4063	d Metabolites in	Urine										+
Method #: Matrix:	Urine												+
Units:	umol/L												+
		l: low and mediu	ım 00										+
													+
ruii date: Dec 1	5, 2017 (day 1	); Dec 15, 2017 (d	1d y 2)										+
													+
Analyte:	3U						3U						+
Analyte.	30			LU17560	1		30	Mila	7560				+
		Spike	Mose		entration		Spike	Measured co		n			
		opo	Wicas	urea com	entiation	B	Орто	Wicasureu Co	Jili Ceriti atio		<b>D</b>	Mean	SD
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	recovery (%)	(%)
Sample	1	0	0.20	0.15			0	0.61	0.58				
	2		0.21	0.17	0.20		0	0.54	0.65	0.59		104	2.84
	3		0.28	0.18				0.52	0.62				
Sample + Spike 1	1	1	1.10	1.32			1	1.64	1.75				
	2	'	1.14	1.26	1.23	103	'	1.70	1.63	1.68	109		
	3		1.21	1.35				1.76	1.57				
Sample + Spike 2	1	2	2.24	2.04			2	2.72	2.69				
	2	2	2.22	2.22	2.21	101	2	2.52	2.94	2.67	104		
	3		2.28	2.28				2.46	2.69				
Sample + Spike 3	1	3	3.54	3.34			3	3.70	3.73				
Sample + Spike 3	1 2	3	3.54 2.73	3.34 3.38	3.27	102	3	3.70 3.75	3.73 3.69	3.75	106		

# (4) 7-Methyluric Acid (7U)

													_
Accuracy using Sp	ike Recovery	- fill in yellow sh	naded ce	lls									
Recovery = (final	concentration	on – initial conce	entration	n)/added	l concentration	1							
Recovery should	be 85-115%	except at 3*LOD	where ca	n be 80-	120%								
Method name:	Caffeine an	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC poo	: low and mediu	ım QC										
Run date: Dec 13	3, 2017 (day 1	); Dec 15, 2017 (d	ay 2)										
Analyte:	7U						7U						
				LU17560	,			MU	17560				
		Spike	Meas	ured conc	entration		Spike	Measured c	oncentratio	1			
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		7.52	6.95				13.3	14.7			(70)	
	2	0	7.04	6.91	7.19		0	14.9	15.0	14.3		101.0	3.73
	3		8.16	6.54					40.0				
Sample + Spike 1								15.2	12.9				
Campio i Opino i	1		10.0	9.81				15.2 25.4	25.1				
Campio i Opino i	1 2	3	10.0 10.8		10.1	96.4	10			24.9	106		
Campio i Opino i		3		9.81	10.1	96.4	10	25.4	25.1	24.9	106		
Sample + Spike 2	2		10.8	9.81 9.25	10.1	96.4		25.4 25.8	25.1 24.7	24.9	106		
	2	3	10.8 10.9	9.81 9.25 9.71	10.1	96.4	10	25.4 25.8 25.1	25.1 24.7 23.3	24.9	106 97.2		
	2 3 1		10.8 10.9 12.2	9.81 9.25 9.71 12.2				25.4 25.8 25.1 29.2	25.1 24.7 23.3 26.8				
	2 3 1 2	5	10.8 10.9 12.2 13.0	9.81 9.25 9.71 12.2 11.0			15	25.4 25.8 25.1 29.2 30.3	25.1 24.7 23.3 26.8 28.6				
Sample + Spike 2	2 3 1 2 3		10.8 10.9 12.2 13.0 12.9	9.81 9.25 9.71 12.2 11.0				25.4 25.8 25.1 29.2 30.3 28.1	25.1 24.7 23.3 26.8 28.6 30.5				

### (5) 1,3-Dimethyluric Acid (13U)

Accuracy using Sp	oike Recovery	fill in yellow sh	naded ce	ells									
Recovery = (fina	l concentratio	on – initial conce	ntration	n)/added	d concentration								
Recovery should	l be 85-115% e	except at 3*LOD v	where ca	an be 80-	-120%								
Method name:	Caffeine and	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC pool	: low and mediu	ım QC										
Run date: Dec 1	3, 2017 (day 1	); Dec 15, 2017 (d	a y 2)										
Analyte:	13U						13U						
				LU17560				MU	17560				
		Spike	Meas	ured conc	entration		Spike	Measured o	oncentratio	n			
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		2.17	2.55			0	8.34	8.81			<b>\</b>	
	2	0	2.26	2.72	2.47		0	9.15	10.6	9.26		103	4.39
	3		2.52	2.57				9.02	9.63				
Sample + Spike 1	1	2	4.45	4.55			-	14.4	15.5				
	2		4.25	4.65	4.45	99	5	14.7	14.5	14.8	112		
	3		4.13	4.65				15.0	14.9				
Sample + Spike 2	1	3	4.88	5.56			10	20.1	20.4				
	2	3	5.42	6.00	5.49	101	10	19.0	19.4	19.4	102		
	3		5.6	5.49				17.3	20.3				
Sample + Spike 3	1	5	6.81	8.27			15	22.3	26.3				
Campie i Opike o							10			1			
Campie i Opike o	2	5	6.87	8.14	7.54	101		24.9	25.4	24.7	103		

### (6) 1,7-Dimethyluric Acid (17U)

Accuracy using Sp	ika Basayanı	fill in vallow sh	2 40 4 5	alle									
Recovery = (fina						1				-			
Recovery should	l be 85-115% e	except at 3*LOD v	where c	an be 80	-120%								
Method name:		d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC pool	: low and mediu	m QC										
Run date: Dec 13	3, 2017 (day 1)	; Dec 15, 2017 (d	a y 2)										
Analyte:	17U						17U						
			-	LU17560	)			MU1	7560				
		Spike	Meas	ured cond	centration		Spike	Measured co	oncentratio	n			
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		10.8	10.8				33.2	37.1			(13)	
	2	0	10.0	11.1	10.6		0	35.8	37.2	36.2		98.2	1.82
	3		10.8	10.3				36.4	37.2				
Sample + Spike 1	1		14.9	17.4				51.5	53.0				
	2	5	14.9	16.1	15.5	98.0	15	49.9	51.1	51.3	101		
	_			15.3				49.8	52.2				
	3		14.6										
Sample + Spike 2	3		14.6 19.7	21.4					63.7				
Sample + Spike 2	1	10	19.7	21.4	20.4	97.2	25	61.4	63.7	61.2	100		
Sample + Spike 2	1 2	10	19.7 20.2	21.4	20.4	97.2	25	61.4 58.5	63.7 60.4	61.2	100		
	1 2 3		19.7 20.2 19.9	21.4 20.6 20.3	20.4	97.2	25	61.4 58.5 59.4	63.7 60.4 63.6	61.2	100		
Sample + Spike 2 Sample + Spike 3	1 2 3	10	19.7 20.2 19.9 24.7	21.4 20.6 20.3 25.9			25 50	61.4 58.5 59.4 86.2	63.7 60.4 63.6 84.2				
	1 2 3		19.7 20.2 19.9	21.4 20.6 20.3	20.4	97.2 96.0		61.4 58.5 59.4	63.7 60.4 63.6	61.2 84.8	100 97		

# (7) 3,7-Dimethyluric Acid (37U)

•		6.11.		. 11 .									
, , ,	•	- fill in yellow sh											
, ,		on – initial conce				1							
Recovery should	be 85-115%	except at 3*LOD	where c	an be 80	-120%								
Method name:	Caffeine an	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC pool	:low and mediu	ım QC										
Run date: Dec 13	3, 2017 (day 1	); Dec 15, 2017 (d	ay 2)										
Analyte:	37U						37U						
			•	LU17560	)			MU	17560				
		Spike	Meas	ured cond	entration		Spike	Measured c	oncentratio	n			
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		0.35	0.41				1.00	1.22			(70)	
•	2	0	0.36	0.45	0.39		0	1.21	1.13	1.13		93.8	4.40
	3		0.38	0.35				1.19	1.05				
Sample + Spike 1	1		1.31	1.35				1.88	1.95				
		1 1											_
	2	'	1.20	1.57	1.39	101	1		1.98	2.00	86.8		
	2	,	1.20 1.50		1.39	101	1	2.15	1.98 1.99	2.00	86.8		
Sample + Spike 2	3		1.50	1.42	1.39	101		2.15 2.06	1.99	2.00	86.8		
Sample + Spike 2	3 1	2	1.50 2.36	1.42 2.32	1.39	101 93.1	2	2.15 2.06 3.3	1.99	3.01	94.0		
Sample + Spike 2	3 1 2		1.50 2.36 2.01	1.42 2.32 2.39				2.15 2.06 3.3 3.13	1.99 3.13 2.89				
	3 1 2 3		1.50 2.36 2.01 2.05	1.42 2.32 2.39 2.35				2.15 2.06 3.3 3.13 2.76	1.99 3.13 2.89 2.87				
Sample + Spike 2 Sample + Spike 3	3 1 2 3		1.50 2.36 2.01 2.05 3.39	1.42 2.32 2.39 2.35 3.23	2.25	93.1		2.15 2.06 3.3 3.13 2.76 3.96	1.99 3.13 2.89 2.87 3.93	3.01	94.0		
	3 1 2 3	2	1.50 2.36 2.01 2.05	1.42 2.32 2.39 2.35			2	2.15 2.06 3.3 3.13 2.76	1.99 3.13 2.89 2.87				

# (8) 1,3,7-Trimethyluric Acid (137U)

Accuracy using Sp	ike Recovery	- fill in yellow sh	naded co	ells									
		on – initial conce			l concentration	1							
Recovery should	l be 85-115%	except at 3*LOD v	where c	an be 80-	-120%								
,													
Method name:	Caffeine an	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC pool	:low and mediu	ım QC										
Run date: Dec 1	3, 2017 (day 1	); Dec 15, 2017 (d	ay 2)										
Analyte:	137U						137U						
				LU17560				MU	17560				
		Spike	Meas	ured cond	entration		Spike	Measured o	concentratio	n			
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0	0.95	0.93			0	3.55	3.68			(15)	
	2		0.92	1.00	0.96		0	3.67	3.84	3.71		98.1	1.72
	3		0.98	0.99				3.74	3.78				
Sample + Spike 1	1	1	1.88	1.95			2	5.7	5.72				
	2	'	1.97	1.91	1.92	96.2	2	5.79	5.75	5.73	101.0		
								5.58	5.84				
	3		1.92	1.91				5.56	5.64				
Sample + Spike 2		2	1.92 2.92	2.82			2	6.73	6.68				
Sample + Spike 2		2	<b>-</b>		2.91	97.2	3			6.67	98.7		
Sample + Spike 2	1	2	2.92	2.82	2.91	97.2	3	6.73	6.68	6.67	98.7		
Sample + Spike 2  Sample + Spike 3	1 2		2.92 2.96	2.82	2.91	97.2		6.73 6.7	6.68	6.67	98.7		
	1 2 3	2	2.92 2.96 2.97	2.82 2.84 2.92	2.91 3.87	97.2 96.9	3	6.73 6.7 6.63	6.68 6.6 6.69	6.67 8.65	98.7 98.7		

### (9) 1-Methylxanthine (1X)

						1						1	
Accuracy using Sp	ike Recovery	- fill in yellow sh	naded co	ells									
Recovery = (final	concentration	on – initial conce	entratio	n)/addeo	l concentration	1							
Recovery should	be 85-115%	except at 3*LOD	where c	an be 80	120%								
Method name:	Caffeine an	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC poo	l: low and mediu	ım QC										
Run date: Dec 13	3, 2017 (day 1	); Dec 15, 2017 (d	ay 2)										
Analyte:	1X						1X						
				LU17560				MU	17560		-		
		Spike	Meas	ured cond	entration		Spike	Measured o	oncentratio	n			
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	_	14.8	16.5			_	39.0	44.1			(1-5)	
	2	0	14.7	16.2	15.6		0	41.3	42.2	41.7		100	4.88
	3		45.0										
	-		15.2	16.4				38.6	45.2				
Sample + Spike 1	1		25.2	16.4 27.0				38.6 55.4	45.2 57.5				
Sample + Spike 1	1 2	10	1		25.6	100	15			57.8	107		
Sample + Spike 1		10	25.2	27.0	25.6	100	15	55.4	57.5	57.8	107		
	2		25.2 23.8	27.0 26.2	25.6	100		55.4 59.7	57.5 58.3	57.8	107		
Sample + Spike 1  Sample + Spike 2	2	10	25.2 23.8 25.0	27.0 26.2 26.6	25.6 29.7	100	15 25	55.4 59.7 54.9	57.5 58.3 61.2	57.8 67.4	107		
	2 3 1		25.2 23.8 25.0 28.9	27.0 26.2 26.6 30.9				55.4 59.7 54.9 66.8	57.5 58.3 61.2 69.2				
	2 3 1 2	15	25.2 23.8 25.0 28.9 29.5	27.0 26.2 26.6 30.9 29.2			25	55.4 59.7 54.9 66.8 62.8	57.5 58.3 61.2 69.2 68.9				
Sample + Spike 2	2 3 1 2 3		25.2 23.8 25.0 28.9 29.5 30.2	27.0 26.2 26.6 30.9 29.2 29.3				55.4 59.7 54.9 66.8 62.8 65.5	57.5 58.3 61.2 69.2 68.9 71.4				

### (10) 3-Methylxanthine (3X)

Accuracy using Sn	ike Recovery	- fill in yellow sh	naded ce	olls									
		on – initial conce			L concentration	1							
		except at 3*LOD v											
Recovery silouru	DE 03-113/6 (	Except at 3 LOD	wilele C	111 DE 80-	120%								
Method name:	Caffeine an	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC pool	: low and mediu	ım QC										
Run date: Dec 13	3, 2017 (day 1	); Dec 15, 2017 (d	ay 2)										
Analyte:	3X						3X						
				LU17560				MU	17560				
		Spike	Meas	ured conc	entration		Spike	Measured o	oncentratio	n			
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0	12.1	11.3			0	30.4	29.8			(/0)	
	2	0	11.6	10.6	11.4		0	29.6	28.0	29.5		102	4.36
	3		11.5	11.1				28.5	30.7				
Sample + Spike 1	1	5	17.1	15.9			15	44.6	45.8				
	2	5	16.7	15.0	16.2	95.7	15	45.7	43.6	45.2	105		
	3		16.3	15.9				45.6	45.9				
Sample + Spike 2	1	10	21.6	21.3			25	60.6	53.9				
	2	10	21.8	21.0	21.8	104	25	57.0	52.7	56.5	108		
	3		22.7	22.2				57.0	57.6				
Sample + Spike 3	1	15	25.3	26.1			50	87.0	77.4				
	2	15	27.8	26.6	26.3	99.2	30	78.7	76.3	79.8	101		

### (11) 7-Methylxanthine (7X)

													_
Accuracy using Sp	ike Recovery	- fill in yellow sh	naded c	ells									
Recovery = (final	concentration	on – initial conce	entratio	n)/added	l concentration	ı							
Recovery should	be 85-115%	except at 3*LOD	where c	an be 80-	-120%								
Method name:	Caffeine an	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC poo	:low and mediu	ım QC										
Run date: Dec 13	3, 2017 (day 1	); Dec 15, 2017 (d	ay 2)										
Analyte:	7X						7X						
				LU17560				MU1	7560				
		Spike	Meas	ured cond	entration		Spike	Measured c	oncentratio	n			
						Recovery					Recovery	Mean	SD
	Replicate	concentration	Day 1	Day 2	Mean	(%)	concentration	Day 1	Day 2	Mean	(%)	recovery (%)	(%)
Sample	1	_	20.7	22.5						_		(,,,)	
	2							46.3	48.8				
		0	21.1	21.5	21.4		0	46.3 48.1	48.8 49.0	47.8		96.6	2.83
	3	0	21.1		21.4		0			47.8		96.6	2.83
Sample + Spike 1				21.5	21.4			48.1	49.0	47.8		96.6	2.83
Sample + Spike 1	3	15	22.0 35.5	21.5 20.4	21.4	92.4	0	48.1 48.2 63.6	49.0 46.1	47.8 62.4	97.3	96.6	2.83
Sample + Spike 1	3		22.0	21.5 20.4 35.7		92.4		48.1 48.2	49.0 46.1 63.4		97.3	96.6	2.83
	3 1 2	15	22.0 35.5 35.0	21.5 20.4 35.7 37.0		92.4	15	48.1 48.2 63.6 61.8	49.0 46.1 63.4 63.1		97.3	96.6	2.83
Sample + Spike 1 Sample + Spike 2	3 1 2 3		22.0 35.5 35.0 35.2	21.5 20.4 35.7 37.0 33.0		92.4		48.1 48.2 63.6 61.8 61.7	49.0 46.1 63.4 63.1 60.5		97.3 98.1	96.6	2.83
	3 1 2 3 1 2	15	22.0 35.5 35.0 35.2 44.8 43.0	21.5 20.4 35.7 37.0 33.0 42.4	35.2		15	48.1 48.2 63.6 61.8 61.7 74.0 69.4	49.0 46.1 63.4 63.1 60.5 71.5 73.8	62.4		96.6	2.83
Sample + Spike 2	3 1 2 3 1 2 3	15 25	22.0 35.5 35.0 35.2 44.8 43.0 46.1	21.5 20.4 35.7 37.0 33.0 42.4 46.2 47.1	35.2		15	48.1 48.2 63.6 61.8 61.7 74.0 69.4 69.8	49.0 46.1 63.4 63.1 60.5 71.5 73.8 75.1	62.4		96.6	2.83
	3 1 2 3 1 2 3	15	22.0 35.5 35.0 35.2 44.8 43.0 46.1 72.4	21.5 20.4 35.7 37.0 33.0 42.4 46.2 47.1 69.5	35.2		15	48.1 48.2 63.6 61.8 61.7 74.0 69.4 69.8 99.5	49.0 46.1 63.4 63.1 60.5 71.5 73.8 75.1 98.0	62.4		96.6	2.83
Sample + Spike 2	3 1 2 3 1 2 3	15 25	22.0 35.5 35.0 35.2 44.8 43.0 46.1	21.5 20.4 35.7 37.0 33.0 42.4 46.2 47.1	35.2 44.9	94.3	15	48.1 48.2 63.6 61.8 61.7 74.0 69.4 69.8	49.0 46.1 63.4 63.1 60.5 71.5 73.8 75.1	62.4 72.3	98.1	96.6	2.83

### (12) Theophylline (13X)

	611.											T
•	,											
					1							-
be 85-115% e	except at 3*LOD	where ca	an be 80-	120%								
Caffeine and	d Metabolites in	Urine										
4063												
Urine												
μmol/L												
ench QC pool	: low and mediu	ım QC										
3, 2017 (day 1)	; Dec 15, 2017 (d	ay 2)										
13X						13X						
			LU17560				MU	17560				
	Spike	Meas	ured conc	entration		Spike	Measured c	oncentratio	n			
Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
1		1.13	1.23				3.23	3.66			(,,,	
2	0	1.13	1.27	1.21		0	3.46	3.54	3.47		95.1	1.55
3		1.23	1.26				3.40	3.53				
1		2.18	2.26			_	5.29	5.17				
2	1	2.23	2.18	2.18	97.5	2	5.29	5.40	5.34	93.3		
3		2 12	2 13				5.36	5.50				
		2.12										
1		2.99	3.34				6.56	6.22				
	2	<del>                                     </del>		3.13	95.9	3	6.56 6.20	6.22 6.40	6.29	94.1		
1	2	2.99	3.34	3.13	95.9	3			6.29	94.1		
1 2		2.99 2.98	3.34 3.18	3.13	95.9		6.20	6.40	6.29	94.1		
1 2 3	2	2.99 2.98 3.16	3.34 3.18 3.11	3.13	95.9 95.7	3 5	6.20 6.25	6.40 6.13	6.29 8.18	94.1		
	Concentration be 85-115% of Caffeine and 4063 Urine µmol/L ench QC pool 3, 2017 (day 1)  13X  Replicate  1 2 3 1 2	concentration – initial conce be 85-115% except at 3*LOD  Caffeine and Metabolites in 4063  Urine  µmol/L  ench QC pool: low and medic 3, 2017 (day 1); Dec 15, 2017 (d  13X  Spike  Replicate concentration  1 0 2 3 1 1 2 1	Concentration – initial concentration be 85-115% except at 3*LOD where concentration  Caffeine and Metabolites in Urine 4063  Urine  µmol/L ench QC pool: low and medium QC 3, 2017 (day 1); Dec 15, 2017 (day 2)  13X  Spike Meas Replicate concentration Day 1  1 0 1.13 2 1.13 3 1.23 1 1 2.18 2 2.3	be 85-115% except at 3*LOD where can be 80-  Caffeine and Metabolites in Urine  4063  Urine  μmol/L  ench QC pool: low and medium QC  3, 2017 (day 1); Dec 15, 2017 (day 2)  13X  LU17560  Spike Measured concentration  Replicate concentration  Day 1 Day 2  1 0 1.13 1.23 1.26  1 1 2.18 2.26  2 2.3 2.18	concentration – initial concentration)/added concentration be 85-115% except at 3*LOD where can be 80-120%  Caffeine and Metabolites in Urine 4063  Urine  µmol/L ench QC pool: low and medium QC 3, 2017 (day 1); Dec 15, 2017 (day 2)  13X  LU17560  Spike Measured concentration  Replicate concentration Day 1 Day 2 Mean  1 0 1.13 1.23 1.26 1.23 1.26 1.21 1.21 1.21 1.21 1.21 1.23 1.26	Concentration – initial concentration)/added concentration be 85-115% except at 3*LOD where can be 80-120%  Caffeine and Metabolites in Urine 4063  Urine  µmol/L ench QC pool: low and medium QC 3, 2017 (day 1); Dec 15, 2017 (day 2)  13X  LU17560  Spike Measured concentration Pay 1 Day 2 Mean Replicate concentration Day 1 Day 2 Mean Recovery (%)  1 0 1.13 1.23 1.26 1 1 2.18 2.26 2.23 2.18 2.18 97.5	Caffeine and Metabolites in Urine  4063  Urine  µmol/L ench QC pool: low and medium QC 3, 2017 (day 1); Dec 15, 2017 (day 2)  13X  LU17560  Replicate  Concentration  Day 1  Day 2  Mean  Mean  Recovery (%)  1.13  1.23  2  1.13  1.23  1.21  3  1.23  1.23  2  1.21  3  1.23  2  2  1.21  3  2.18  2.18  97.5	Caffeine and Metabolites in Urine  4063  Urine  µmol/L  ench QC pool: low and medium QC  3, 2017 (day 1); Dec 15, 2017 (day 2)  13X  LU17560  Spike  Measured concentration  Day 1  Day 2  Mean  Replicate  Concentration  Day 1  1  0  1.13  1.23  2  1.13  1.27  1.21  3  0  3.23  3.46  3.40  1  1  1  1  2.18  2.28  2.18  97.5  2  5.29  5.29	Caffeine and Metabolites in Urine  4063  Urine  µmol/L ench QC pool: low and medium QC 3, 2017 (day 1); Dec 15, 2017 (day 2)  13X  LU17560  Spike Measured concentration  Replicate  Concentration  Day 1  Day 2  Mean  Recovery (%)  0  3.23  3.66  3.46  3.54  3  1 1  1 2.18  2.18  97.5  2  5.29  5.40	Caffeine and Metabolites in Urine	Concentration - initial concentration   Jadded concentration	Concentration - initial concentration)/added concentration   be 85-115% except at 3*LOD where can be 80-120%   Caffeine and Metabolites in Urine   4063   Urine

### (13) Paraxanthine (17X)

											_		_
Accuracy using Sp	ike Recovery	- fill in yellow sh	naded ce	ells									
Recovery = (final	concentration	on – initial conce	ntratio	n)/addec	l concentration	ı							
Recovery should	be 85-115%	except at 3*LOD	where c	an be 80-	-120%								
Method name:	Caffeine an	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC pool	: low and mediu	ım QC										
Run date: Dec 13	3, 2017 (day 1	); Dec 15, 2017 (d	ay 2)										
Analyte:	17X						17X						
				LU17560				MU1	7560				
		Spike	Meas	ured conc	entration		Spike	Measured co	oncentratio	n			
	Replicate	concentration	Day 1	D 0		Recovery	concentration	Day 1	D 0	Mean	Recovery	Mean	SD
	Replicate	concentration	Day I	Day 2	Mean	(%)	concentration	Day I	Day 2	wean	(%)	recovery	(%)
Sample	1										(,-,	(%)	(,
		0	14.7	15.4			0	37.4	40.6		(1.9)	(%)	(,
	2	0	14.7 14.8	15.4 15.1	15.1		0	37.4 39.9	40.6 41.1	40.0	(1.5)	99.3	4.04
	2	0			15.1		0			40.0	(iii)		,
Sample + Spike 1			14.8	15.1	15.1			39.9	41.1	40.0			,
Sample + Spike 1	3	5	14.8 15.1	15.1 15.2	15.1 20.3	105	0	39.9 39.4	41.1 41.5	40.0 54.5	96.9		,
Sample + Spike 1	3		14.8 15.1 20.1	15.1 15.2 21.1		105		39.9 39.4 54.4	41.1 41.5 56.2				,
Sample + Spike 1 Sample + Spike 2	3 1 2	5	14.8 15.1 20.1 19.6	15.1 15.2 21.1 21.0		105	15	39.9 39.4 54.4 52.9	41.1 41.5 56.2 55.1				,
	3 1 2 3		14.8 15.1 20.1 19.6 19.6	15.1 15.2 21.1 21.0 20.5		105		39.9 39.4 54.4 52.9 53.9	41.1 41.5 56.2 55.1 54.6				,
	3 1 2 3	5	14.8 15.1 20.1 19.6 19.6 24.8	15.1 15.2 21.1 21.0 20.5 26.6	20.3		15	39.9 39.4 54.4 52.9 53.9 64.3	41.1 41.5 56.2 55.1 54.6 64.3	54.5	96.9		,
	3 1 2 3 1 2	5	14.8 15.1 20.1 19.6 19.6 24.8 24.5	15.1 15.2 21.1 21.0 20.5 26.6 25.8	20.3		15	39.9 39.4 54.4 52.9 53.9 64.3	41.1 41.5 56.2 55.1 54.6 64.3 64.8	54.5	96.9		,
Sample + Spike 2	3 1 2 3 1 2 3	5	14.8 15.1 20.1 19.6 19.6 24.8 24.5 24.8	15.1 15.2 21.1 21.0 20.5 26.6 25.8 25.7	20.3		15	39.9 39.4 54.4 52.9 53.9 64.3 63 61.9	41.1 41.5 56.2 55.1 54.6 64.3 64.8 65.9	54.5	96.9		,

### (14) Theobromine (37X)

													1
Accuracy using Sp		•											
Recovery = (final	concentratio	on – initial conce	ntratio	n)/added	d concentration	1							
Recovery should	be 85-115% e	except at 3*LOD	where ca	n be 80-	-120%								
Method name:	Caffeine and	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC pool	: low and mediu	m QC										
Run date: Dec 13	3, 2017 (day 1	); Dec 15, 2017 (d	a y 2)										
Analyte:	37X						37X						
			-	LU17560	)			MU1	17560				
		Spike	Meas	ured conc	entration		Spike	Measured c	oncentratio	n			
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		44.0		_							(,0)	
·		_	11.8	11.6			_	36.4	37.4				
	2	0	11.8	11.6 11.7	11.7		0	36.4 37.3	37.4 37.6	37.4		99.4	1.18
	3	0			11.7		0			37.4		99.4	1.18
Sample + Spike 1			11.5	11.7	11.7			37.3	37.6	37.4		99.4	1.18
Sample + Spike 1	3	5	11.5 11.9	11.7 11.4	11.7	99.0	0	37.3 37.6	37.6 37.9	37.4 52.3	99.6	99.4	1.18
Sample + Spike 1	3		11.5 11.9 16.5	11.7 11.4 17.0		99.0		37.3 37.6 53.6	37.6 37.9 51.0		99.6	99.4	1.18
	3 1 2 3	5	11.5 11.9 16.5 16.5 16.6	11.7 11.4 17.0 16.5 16.5		99.0	15	37.3 37.6 53.6 51.3 50.7	37.6 37.9 51.0 52.7 54.5		99.6	99.4	1.18
Sample + Spike 1 Sample + Spike 2	3 1 2 3		11.5 11.9 16.5 16.5	11.7 11.4 17.0 16.5		99.0		37.3 37.6 53.6 51.3	37.6 37.9 51.0 52.7		99.6	99.4	1.18
	3 1 2 3 1 2	5	11.5 11.9 16.5 16.5 16.6 21.0 21.2	11.7 11.4 17.0 16.5 16.5 20.9 21.8	16.6		15	37.3 37.6 53.6 51.3 50.7 64.4 62.8	37.6 37.9 51.0 52.7 54.5 62.7 63.3	52.3		99.4	1.18
Sample + Spike 2	3 1 2 3 1 2 3	5	11.5 11.9 16.5 16.5 16.6 21.0 21.2 22.0	11.7 11.4 17.0 16.5 16.5 20.9 21.8 21.5	16.6		15	37.3 37.6 53.6 51.3 50.7 64.4 62.8 60.7	37.6 37.9 51.0 52.7 54.5 62.7 63.3 61.9	52.3		99.4	1.18
	3 1 2 3 1 2 3 1	5	11.5 11.9 16.5 16.5 16.6 21.0 21.2 22.0 26.5	11.7 11.4 17.0 16.5 16.5 20.9 21.8 21.5 26.9	16.6		15	37.3 37.6 53.6 51.3 50.7 64.4 62.8 60.7	37.6 37.9 51.0 52.7 54.5 62.7 63.3 61.9 88.3	52.3		99.4	1.18
Sample + Spike 2	3 1 2 3 1 2 3	5	11.5 11.9 16.5 16.5 16.6 21.0 21.2 22.0	11.7 11.4 17.0 16.5 16.5 20.9 21.8 21.5	16.6	97.5	15	37.3 37.6 53.6 51.3 50.7 64.4 62.8 60.7	37.6 37.9 51.0 52.7 54.5 62.7 63.3 61.9	52.3	101	99.4	1.18

### (15) Caffeine (137X)

Accuracy using Sp	ike Recovery	fill in yellow sh	aded c	ells									
Recovery = (fina	l concentratio	on – initial conce	ntratio	n)/added	d concentration								
Recovery should	l be 85-115% e	except at 3*LOD v	where c	an be 80	-120%								
Method name:	Caffeine an	d Metabolites in	Urine										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Samples: 2017 b	ench QC pool	: low and mediu	m QC										
Run date: Dec 13	3, 2017 (day 1	); Dec 15, 2017 (d	a y 2)										
Analyte:	137X						137X						
				LU17560	)			MU	17560				
		Spike	Meas	ured cond	entration		Spike	Measured	concentratio	n			
	Replicate	concentration	Day 1	Day 2	Mean	Recovery (%)	concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0	4.01	4.18			0	21.7	23.3			(12)	
	2	U	4.01	4.14	4.10			23.1	23.6	23.1		103	3.18
	3		4.15	4.13				23.4	23.4				
Sample + Spike 1	1	2	6.19	6.13			10	34.0	33.2				
	2	2	6.19	6.25	6.16	103	10	34.1	34.2	33.9	108		
	3		5.89	6.29				34.0	33.6				
Sample + Spike 2	1	5	9.08	9.35			15	38.7	38.6				
	2	3	9.09	9.01	9.11	100	15	39.6	37.8	38.7	104		
	3		9.09	9.05				38.2	39.1				
	J									_			
Sample + Spike 3	1	10	13.8	14.1			25	49.9	49.7				
Sample + Spike 3		10	13.8 14.0	14.1 14.1	14.0	99.0	25	49.9 49.9	49.7 48.7	49.3	105		

### A. Stability

# (1) AAMU

Stability - fill in y						-				
Freeze and thaw st	tability = Assess	tor a minimum of 3 t	reeze-thaw cyc	les; conditions shou	ıld mimic intended sample	handling condition	is.			
		en at -80°C and the			·					
Bench-top stability	y = Assess short-t	term stability for len	gth of time nee	ded to handle study	samples (typically at room	temperature)				
Describe condition:	original sample	s (not yet prepared t	for instrument a	nalysis) stored at ro	oom temperature for 8 hou	rs				
					esident time in autosample					
Describe condition:	processed sam	ples (ready for instr	ument analysis	stored at 15°C for 2	24 hours then stored at 5°C	for 1 month				
All stability sample	results should be	within ±15% of non	ninal concentra	tion.						
Run date										
		nch-top stability were r run on 12/07/2017	un on 11/16/2017							
Method name:	Caffeine and M	etabolites in Urine								
Method #:	4063									
Matrix:	Urine									
Units:	μmol/L									
Analyte:	AAMU				AAMU					
MU09561					HU09562		i			
11.303301	Initial	Three freeze-	Bench-top	Processed sample		Initial	Three freeze-thaw	Bench	-top	Processed
	measurement	thaw cycles	stability	stability		measurement	cycles	stabi	•	sample stability
Replicate 1	45.1	51.9	48.1	52.9	Replicate 1	155	145	173	-	198
Replicate 2	52.9	51.3	52.8	60.2	Replicate 2	180	183	170		200
Replicate 3	54.8	46.4	54.4	57.6	Replicate 3	174	169	164		187
		49.9	51.8	56.9	Mean	170	166	17:	1	195
Mean	50.9	45.5								
% difference from initial measurement		-2.09	1.64	11.7	% difference from initial measuremen	 t	-2.36	0.7	79	14.9
% difference from initial measurement Long-term stal between dat Describe con	bility = Asses te of first sau dition:		tability tha n and date amples sto	t equals or ex of last samp ored at -80°C fo	initial measurement acceeds time le analysis or 2 years		-2.36	0.7	79	14.9
% difference from initial measurement  Long-term stal between dat Describe condall stability s	bility = Asses te of first sai dition: ample resul	-2.09 as long-term simple collection example: QC solls should be solls.	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurement acceeds time le analysis or 2 years	ı			79	14.9
% difference from initial measurement  Long-term stal between dat Describe con All stability s  Method name	bility = Asses te of first sai dition: ample resul	-2.09 as long-term simple collection example: QC solts should be sold.	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurements acceeds time le analysis or 2 years concentration	t Init	iial Measurme	nt		
% difference from initial measurement  Long-term stal between dat Describe con All stability s  Method name Method #:	chility = Assesse of first saidition: ample resul	-2.09 as long-term somple collection example: QC solls should be sold to complete the collection of th	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurements acceeds time le analysis or 2 years concentration Repli	Init	ial Measurme Replicate	nt 2	Re	plicate 3
% difference from initial measurement  Long-term stal between dat Describe con All stability s  Method name	chility = Assesse of first saidition: ample resul	-2.09 as long-term simple collection example: QC solts should be sold.	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurements acceeds time le analysis or 2 years concentration Repli	t Init	iial Measurme	nt 2	Re	
% difference from initial measurement  Long-term stal between dat Describe con All stability s  Method name Method #:	bility = Asseste of first saidition: ample resule:	-2.09 as long-term somple collection example: QC solls should be sold to complete the collection of th	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurements acceeds time le analysis or 2 years concentration Repli	Init cate 1 0/2015	ial Measurme Replicate	nt 2	Re	plicate 3
% difference from initial measurement  Long-term stal between dat Describe cone All stability s  Method name Method #: Matrix:	bility = Asseste of first saidition: ample resule:	-2.09  as long-term somple collection example: QC solts should be solted  Caffeine and 14063 Urine	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurement conceds time le analysis or 2 years concentration  Repli 10/20	Init cate 1 0/2015	Replicate 10/22/201	nt 2 .5	Re 1:	plicate 3
% difference from initial measurement  Long-term stal between dat Describe cone All stability s  Method name Method #: Matrix:	bility = Asseste of first saidition: ample resule:	-2.09  as long-term somple collection example: QC solts should be solted  Caffeine and 14063 Urine	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurement conceds time le analysis or 2 years concentration  Repli 10/20	Init cate 1 0/2015	Replicate 10/22/201	nt 2   .5   tty 2	Re 1:	plicate 3 1/9/2015
% difference from initial measurement Long-term stal between dat Describe cone All stability s Method name Method #: Matrix: Units:	bility = Asses e of first san dition: ample resul	-2.09  as long-term simple collection example: QC s Its should be a  Caffeine and a  4063  Urine  µmol/L	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurement in the content of the content o	Initicate 1 0/2015 Lor	Replicate 10/22/201 ng-term stabilit	nt 2   .5   tty 2	Re 1:	plicate 3 1/9/2015
% difference from initial measurement Long-term stal between dat Describe con All stability s Method name Method #: Matrix: Units: Analyte:	bility = Asses e of first san dition: ample resul	-2.09  as long-term somple collection example: QC solts should be solted  Caffeine and 14063 Urine	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurement (ceeds time) le analysis or 2 years concentration  Repli 10/20  Repli 11/14	Initicate 1 0/2015 Lor	Replicate 10/22/201 ng-term stabilit	nt 2   .5   tty 2	Re 1:	plicate 3 1/9/2015 plicate 3
% difference from initial measurement Long-term stal between dat Describe cone All stability s Method name Method #: Matrix: Units:	bility = Asses e of first san dition: ample resul	-2.09  as long-term simple collection example: QC solts should be sold should b	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurement in the content of the content o	Initicate 1 0/2015 Lor	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   .5   tty 2	Ree 11:	plicate 3 1/9/2015 plicate 3 /16/2017
% difference from initial measurement Long-term stal between dat Describe con All stability s Method name Method #: Matrix: Units: Analyte:	bility = Asses e of first san dition: ample resul	-2.09  as long-term simple collection example: QC s Its should be a  Caffeine and a  4063  Urine  µmol/L	1.64 tability tha in and date amples sto within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurement (ceeds time) le analysis or 2 years concentration  Repli 10/20  Repli 11/14	Initicate 1 0/2015 Lor	Replicate 10/22/201 ng-term stabilit	nt 2   .5   tty 2	Ree 11:	plicate 3 1/9/2015
% difference from initial measurement between dat bescribe con All stability s Method name Method #: Matrix: Units: Analyte: MU09561	bility = Assesse of first saudition: ample resule:	-2.09  as long-term simple collection example: QC solts should be sold should b	tability tha n and date amples ste within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal o	initial measurement in the content of the content o	Init cate 1 0/2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2	Ree 11	plicate 3 1/9/2015 plicate 3 /16/2017
% difference from initial measurement Long-term stal between dat Describe con All stability s Method name Method #: Matrix: Units: Analyte:	bility = Assesse of first saudition: ample resule:	-2.09  as long-term strongle collection example: QC solts should be sold should	tability tha n and date amples ste within ±159	t equals or ex of last samp ored at -80°C fo 6 of nominal of s in Urine	initial measurement in the content of the content o	Initicate 1 0/2015 Lor	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2	Ree 11	plicate 3 1/9/2015 plicate 3 /16/2017
% difference from initial measurement Long-term stal between dat Describe cone All stability s Method name Method #: Matrix: Units: Analyte: MU09561 Replica	bility = Assesse of first saudition: ample resulte:	-2.09  as long-term strongle collection example: QC solts should be solds shou	tability tha n and date amples ste within ±159	t equals or exected for the state of last samp pred at -80°C for 6 of nominal control of the state of the sta	initial measurement icceeds time le analysis or 2 years concentration Repliation 10/20 AAMU HU09562	Init cate 1 0/2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2	Ree 11	plicate 3 1/9/2015 plicate 3 /16/2017
% difference from initial measurement between dat bescribe con All stability s Method name Method #: Matrix: Units: Analyte: MU09561	bility = Assesse of first saidition: ample resulte:  ate 1 ate 1 ate 2	-2.09  as long-term strongle collection example: QC solts should be solds shou	tability tha n and date amples ste within ±159	t equals or exected of last sample or ed at -80°C for 6 of nominal of 5 in Urine  s in Urine  term stability  44.8	initial measurement icceeds time le analysis or 2 years concentration Repli 10/20  Repli 11/14  AAMU  HU09562	Init cate 1 0/2015 Lor cate 1 1/2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme	nt 2	Ree 11	plicate 3 1/9/2015 plicate 3 /16/2017
% difference from initial measurement Long-term stal between dat Describe con All stability s Method name Method #: Matrix: Units:  Analyte:  Replica Replica	bility = Assesse of first saidition: ample resulte:  e:  atte 1 atte 2 atte 3	-2.09  as long-term strongle collection example: QC solts should be sold sho	tability tha n and date amples ste within ±159	t equals or exected at the same of last samp or ed at -80°C for 6 of nominal of sin Urine  seem stability 44.8 43.5 38.2	initial measurement icceeds time le analysis or 2 years concentration Repli 10/20  Repli 11/14  AAMU  HU09562  Repli Repli Repli Repli Repli	Init cate 1 0/2015 Lor cate 1 4/2017	Replicate 10/22/201 1g-term stabilit Replicate 11/15/201  Initial measureme 177 180 162	nt 2	Ree 11	plicate 3 1/9/2015 plicate 3 /16/2017 ong-term stability 161 159 151
% difference from initial measurement Long-term stal between dat Describe con All stability s Method name Method #: Matrix: Units: Analyte: MU09561  Replica Replica	bility = Assesse of first saidition: ample resulte:  e:  atte 1 atte 2 atte 3	-2.09  as long-term strongle collection example: QC solts should be sold sho	tability tha n and date amples ste within ±159	t equals or exected for the state of last sample or ed at -80°C for for formula of the state of	initial measurement icceeds time le analysis or 2 years concentration Repli 10/20  Repli 11/14  AAMU  HU09562  Repli Repli Repli Repli Repli	Init cate 1 0/2015 Lor cate 1 1/2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 177 180	nt 2	Rec 11	plicate 3 1/9/2015 plicate 3 /16/2017 ong-term stability 161 159

# (2) 1-Methyluric Acid (1U)

Stability - fill in ye Freeze and thaw st		for a minimum of 3	freeze-thaw cycl	es; conditions should	mimic intended sample h	andling condition	is.		
		zen at -80°C and the							
					amples (typically at room				
					m temperature for 8 hours				
				-	dent time in autosampler hours then stored at 5°C t				
Describe condition.	processed san	ipies (ready for mist	rument analysis)	310164 81 13 6 101 24	flouis their stored at 5 C	TOT I MONUT			
All stability sample	results should b	e within ±15% of nor	minal concentrat	ion.					
Run date	eeze-thaw, and he	nch-top stability were i	rup op 11/16/2017						
Data for processed sa			1411 011 117 1072017						
Method name:		letabolites in Urine							
Method #: Matrix:	4063 Urine								
Units:	μmol/L								
Analyte:	10				<b>1</b> U				
MU09561					HU09562				
	Initial	Three freeze-	Bench-top	Processed sample		Initial	Three freeze-thaw	Bench-to	-
Replicate 1	measurement 61.7	thaw cycles 61.6	stability 61.2	stability 62.8	Replicate 1	measurement 153	cycles 151	stability 160	y sample stabilit
Replicate 2	65.6	64.0	63.6	61.6	Replicate 2	147	150	162	162
Replicate 3	62.5	62.0	60.4	63.4	Replicate 3	153	147	152	150
		62.5	61.7	62.6	Mean	151	149	158	159
Mean	63.3	02.5	01.7		0/ -1166				
% difference from initial measurement Long-term stab between date	 <b>bility</b> = Asse e of first sa	-1.16 ss long-term s mple collection	-2.42 tability that	-1.05  t equals or exce of last sample pred at -80°C for	analysis		-1.10	4.64	5.08
% difference from initial measurement Long-term stab between date Describe cond	 vility = Asse: e of first sa dition:	-1.16 ss long-term s mple collectic example: QC s	tability that	t equals or exce	initial measurement eeds time analysis 2 years		-1.10	4.64	5.08
% difference from initial measurement  Long-term stab between data Describe cond	 bility = Asse: e of first sa dition: ample resu	-1.16 ss long-term s mple collectic example: QC s	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement eeds time analysis 2 years		-1.10		5.08
% difference from initial measurement  Long-term stab between date Describe cond All stabilitys:	 bility = Asse: e of first sa dition: ample resu	-1.16 ss long-term s mple collectic example: QC s Its should be	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement eeds time analysis 2 years encentration	Init	tial Measurme	nt	
% difference from initial measurement  Long-term stable between date one of the concentration	 bility = Asse: e of first sa dition: ample resu	-1.16  as long-term s mple collectic example: QC s Its should be  Caffeine and 4063	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement eeds time analysis 2 years incentration Replic	Init	tial Measurme Replicate	nt 2	Replicate 3
% difference from initial measurement  Long-term stable between date one of the concentration	 bility = Asse: e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement eeds time analysis 2 years encentration	Init cate 1 /2015	ial Measurme Replicate 10/22/201	nt 2	
% difference from initial measurement  Long-term stab between date Describe cond All stability so Method name	 bility = Asse: e of first sa dition: ample resu	-1.16  as long-term s mple collectic example: QC s Its should be  Caffeine and 4063	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	eeds time analysis 2 years Incentration Replic	Init cate 1 /2015	ial Measurmer Replicate 10/22/201	nt 2   1.5   ty	Replicate 3 11/9/2015
% difference from initial measurement  Long-term stable between date one of the concentration	 bility = Asse: e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement eeds time analysis 2 years incentration  Replic 10/20,	Initicate 1 /2015 Lor	Replicate 10/22/201 1g-term stabilit	nt 2   1.5	Replicate 3 11/9/2015
% difference from initial measurement  Long-term stable between date one of the concentration	 bility = Asse: e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	eeds time analysis 2 years Incentration Replic	Initicate 1 /2015 Lor	ial Measurmer Replicate 10/22/201	nt 2   1.5	Replicate 3 11/9/2015
% difference from initial measurement Long-term stable between date Describe conc All stability so Method name Method #: Matrix: Units:	 bility = Asse: e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement eeds time analysis 2 years incentration  Replic 10/20,	Initicate 1 /2015 Lor	Replicate 10/22/201 1g-term stabilit	nt 2   1.5	Replicate 3 11/9/2015
% difference from initial measurement  Long-term stable between date Describe concentrate with the concentration of the concentration o	 bility = Asse: e of first sa dition: ample resu	example: QC solution and 4063 Urine  µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement eeds time analysis 2 years Incentration Replic 10/20, Replic 11/14,	Initicate 1 /2015 Lor	Replicate 10/22/201 1g-term stabilit	nt 2   1.5	Replicate 3 11/9/2015
% difference from initial measurement Long-term stab between date Describe concentration of the concentration of t	 bility = Asse: e of first sa dition: ample resu	example: QCs Its should be Caffeine and 4063 Urine  µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement eeds time e analysis 72 years encentration Replic 10/20, Replic 11/14,	Initicate 1 /2015 Lor	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   1.5	Replicate 3 11/9/2015 Replicate 3 11/16/2017
% difference from initial measurement Long-term stab between date Describe concentration of the concentration of t	 bility = Asse: e of first sa dition: ample resu	-1.16 ss long-term s mple collectic example: QCs lts should be Caffeine and 4063 Urine μmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co s in Urine	initial measurement eeds time e analysis 72 years encentration Replic 10/20, Replic 11/14,	Initicate 1 /2015 Lor	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015 Replicate 3 11/16/2017
% difference from initial measurement Long-term stab between date Describe conc All stability so Method name Method #: Matrix: Units: Analyte: MU09561	oility = Asse: e of first sa dition: ample resu	-1.16  ss long-term s mple collectic example: QCs lts should be  Caffeine and 4063  Urine μmol/L  1U  Initial measureme	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co s in Urine	initial measurement  eeds time analysis 2 years Incentration  Replic 10/20,  Replic 11/14,  1U  HU09562	Initional to Initi	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015 Replicate 3 11/16/2017  Long-term stability
% difference from initial measurement Long-term stable between date Describe concentrate with the stability set that the stability set th	uility = Asse: e of first sa dition: ample resu	example: QCs Its should be Caffeine and 4063 Urine µmol/L  U  Initial measureme 56.5	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co s in Urine erm stability 48.8	initial measurement  eeds time analysis 2 years Incentration  Replic 10/20,  Replic 11/14,  1U  HU09562	Initioate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 123
**difference from initial measurement*  Long-term stab between date Describe conc All stability	uility = Asse: e of first sa dition: ample resu e:	-1.16 ss long-term s mple collectic example: QCs lts should be Caffeine and 4063 Urine μmol/L  1U Initial measureme 56.5 50.4	tability that on and date samples sto within ±15%	erm stability 48.8 52.3	initial measurement  eeds time analysis 2 years Incentration  Replic 10/20,  Replic 11/14,  1U  HU09562	Initional Control of C	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 127 139	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 123 132
% difference from initial measurement Long-term stable between date Describe concentrate with the stability set that the stability set th	uility = Asse: e of first sa dition: ample resu e:	example: QCs Its should be Caffeine and 4063 Urine µmol/L  U  Initial measureme 56.5	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co s in Urine erm stability 48.8	initial measurement  eeds time analysis 2 years Incentration  Replic 10/20,  Replic 11/14,  1U  HU09562	Initional Control of C	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 123
% difference from initial measurement Long-term stab between date Describe conc All stability so Method name Method #: Matrix: Units: Analyte: MU09561  Replica Replica	arte 1	-1.16 ss long-term s mple collectic example: QCs lts should be Caffeine and 4063 Urine μmol/L  1U Initial measureme 56.5 50.4	tability that on and date samples sto within ±15%	erm stability 48.8 52.3	initial measurement  eeds time analysis 2 years Incentration  Replic 10/20,  Replic 11/14,  1U  HU09562	Initicate 1 /2015 Lor cate 1 /2017  cate 1 cate 2 cate 3	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 127 139	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 123 132

### (3) 3-Methyluric Acid (3U)

Stability - fill in ye	امم لممامم بينمالم	le .							
	ellow shaded cel	15							
Freeze and thaw st	ability = Assess	for a minimum of 3	freeze-thaw cyc	les; conditions shoul	ld mimic intended sample h	andling condition	is.		
Describe condition:	three times fro	zen at -80°C and the	n thawed (3 free	eze-thaw cycles)					
					samples (typically at room				
					om temperature for 8 hours				
					sident time in autosampler				
Describe condition:	processed san	nples (ready for instr	rument analysis)	stored at 15°C for 2	4 hours then stored at 5°C t	for 1 month			
All stability sample	results should be	e within ±15% of nor	minal concentra	ion.					
Run date Data for Initial, three fro Data for processed sai		nch-top stability were r	run on 11/16/2017						
Method name:		letabolites in Urine							
Method #:	4063								
Matrix:	Urine								
Units:	μmol/L								
Analyte:	3U				3U				
MU09561					HU09562				
	Initial	Three freeze-	Bench-top	Processed sample		Initial	Three freeze-thaw	Bench-top	Processed
	measurement		stability	stability		measurement	cycles	stability	sample stabilit
Replicate 1	0.56	0.61	0.59	0.64	Replicate 1	1.06	1.28	1.11	1.09
Replicate 2	0.59	0.54	0.71	0.77	Replicate 2	0.95	1.15	1.11	1.34
Replicate 3	0.57	0.60	0.50	0.55	Replicate 3	1.04	1.24	0.98	1.14
		0.58	0.60	0.65	Mean	1.02	1.22	1.07	1.19
Mean	0.58	0.50							
Mean % difference from	0.58				% difference from		20.2	4 80	17.0
% difference from initial measurement Long-term stab	 oility = Asses	0.87 ss long-term s	3.59 tability tha	13.1 t equals or ex	<u>initial measurement</u> ceeds time		20.3	4.89	17.0
% difference from initial measurement Long-term stab between date Describe cond	 vility = Asses e of first sa dition:	o.87 ss long-term s mple collection	a.59 tability tha on and date	t equals or exectly of last sample ored at -80°C for	initial measurement ceeds time e analysis or 2 years		20.3	4.89	17.0
% difference from initial measurement Long-term stab between date Describe cond	 bility = Asses e of first sa dition: ample resu	0.87 ss long-term s mple collectic example: QC s	a.59 tability tha on and date samples sto within ±159	t equals or exe of last sampl ored at -80°C fo 6 of nominal c	initial measurement ceeds time e analysis or 2 years	 Init	20.3		17.0
% difference from initial measurement  Long-term stab between date Describe conc All stabilitys:	 bility = Asses e of first sa dition: ample resu	os long-term s mple collectic example: QC s lts should be	a.59 tability tha on and date samples sto within ±159	t equals or exe of last sampl ored at -80°C fo 6 of nominal c	initial measurement ceeds time le analysis or 2 years concentration		ial Measurme	nt	
% difference from initial measurement  Long-term stable between date Describe concount All stabilitys:  Method name Method #:	 bility = Asses e of first sa dition: ample resu	o.87  as long-term s mple collectic example: QC s lts should be  Caffeine and 4063	a.59 tability tha on and date samples sto within ±159	t equals or exe of last sampl ored at -80°C fo 6 of nominal c	initial measurement ceeds time le analysis or 2 years concentration Replic	cate 1	ial Measurme Replicate	nt 2 I	Replicate 3
% difference from initial measurement  Long-term stable between date Describe conc All stabilitys:  Method name Method #: Matrix:	 bility = Asses e of first sa dition: ample resu	o.87  ass long-term s mple collectio example: QC s Its should be  Caffeine and 4063 Urine	a.59 tability tha on and date samples sto within ±159	t equals or exe of last sampl ored at -80°C fo 6 of nominal c	initial measurement ceeds time le analysis or 2 years concentration	cate 1 /2015	ial Measurme Replicate 10/22/201	nt 2 F	
% difference from initial measurement  Long-term stable between date Describe concount All stabilitys:  Method name Method #:	 bility = Asses e of first sa dition: ample resu	o.87  as long-term s mple collectic example: QC s lts should be  Caffeine and 4063	a.59 tability tha on and date samples sto within ±159	t equals or exe of last sampl ored at -80°C fo 6 of nominal c	initial measurement ceeds time le analysis or 2 years concentration  Replic 10/20	cate 1 /2015 Lor	ial Measurmer Replicate 10/22/201 g-term stabilit	nt 2 F	Replicate 3 11/9/2015
% difference from initial measurement  Long-term stable between date Describe conc All stabilitys:  Method name Method #: Matrix:	 bility = Asses e of first sa dition: ample resu	o.87  ass long-term s mple collectio example: QC s Its should be  Caffeine and 4063 Urine	a.59 tability tha on and date samples sto within ±159	t equals or exe of last sampl ored at -80°C fo 6 of nominal c	initial measurement ceeds time le analysis or 2 years concentration  Replic 10/20,	cate 1 /2015 Lor cate 1	ial Measurmer Replicate 10/22/201 g-term stabilit Replicate	nt 2 F	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement  Long-term stable between date Describe conc All stabilitys:  Method name Method #: Matrix:	 bility = Asses e of first sa dition: ample resu	o.87  ass long-term s mple collectio example: QC s Its should be  Caffeine and 4063 Urine	a.59 tability tha on and date samples sto within ±159	t equals or exe of last sampl ored at -80°C fo 6 of nominal c	initial measurement ceeds time le analysis or 2 years concentration  Replic 10/20	cate 1 /2015 Lor cate 1	ial Measurmer Replicate 10/22/201 g-term stabilit	nt 2 F	Replicate 3 11/9/2015
% difference from initial measurement  Long-term stable between date Describe conc All stabilitys:  Method name Method #: Matrix:	 bility = Asses e of first sa dition: ample resu	o.87  ass long-term s mple collectio example: QC s Its should be  Caffeine and 4063 Urine	a.59 tability tha on and date samples sto within ±159	t equals or exe of last sampl ored at -80°C fo 6 of nominal c	initial measurement ceeds time le analysis or 2 years concentration  Replic 10/20,	cate 1 /2015 Lor cate 1	ial Measurmer Replicate 10/22/201 g-term stabilit Replicate	nt 2 F	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement Long-term stab between date Describe cond All stability so Method name Method #: Matrix: Units:	 bility = Asses e of first sa dition: ample resu	o.87  ss long-term s mple collectic example: QC s Its should be  Caffeine and 4063 Urine  µmol/L	a.59 tability tha on and date samples sto within ±159	t equals or exe of last sampl ored at -80°C fo 6 of nominal c	initial measurement ceeds time e analysis or 2 years concentration  Replic 10/20 Replic 11/14	cate 1 /2015 Lor cate 1	ial Measurmer Replicate 10/22/201 g-term stabilit Replicate	nt 2 F	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement Long-term stable between date Describe conc All stability so Method name Method #: Matrix: Units:	 bility = Asses e of first sa dition: ample resu	o.87  as long-term s mple collection example: QC s Its should be  Caffeine and 4063 Urine  µmol/L	a.59 tability tha on and date samples sto within ±159	t equals or exe of last sampl ored at -80°C fo 6 of nominal c	initial measurement ceeds time le analysis or 2 years concentration  Replic 10/20,  Replic 11/14,	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2 F	Replicate 3 11/9/2015 Replicate 3 11/16/2017
% difference from initial measurement Long-term stab between date Describe cond All stability so Method name Method #: Matrix: Units:	 bility = Asses e of first sa dition: ample resu	o.87  as long-term s mple collection example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  3U	tability tha on and date samples sto within ±159	t equals or exe of last sampl pred at -80°C fo 6 of nominal co s in Urine	initial measurement ceeds time e analysis or 2 years concentration  Replic 10/20 Replic 11/14	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   F   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017
% difference from initial measurement Long-term stab between date Describe concentration of the concentration of t	 bility = Asses e of first sa dition: ample resu	o.87  as long-term s mple collectio example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  3U  Initial measurement	tability tha on and date samples sto within ±159	t equals or exected for the state of last sample or ed at -80°C for 6 of nominal cost in Urine	initial measurement  ceeds time le analysis or 2 years concentration  Replic 10/20,  Replic 11/14,  3U  HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   F   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability
% difference from initial measurement Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units: Analyte: MU09561 Replica	oility = Assese of first sa dition: ample resu	o.87  as long-term s mple collection example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  3U	tability tha on and date samples sto within ±159	t equals or exected for the stability of	initial measurement  ceeds time le analysis or 2 years concentration  Replic 10/20,  Replic 11/14,  3U  HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 g-term stabilit Replicate 11/15/201  Initial measureme 0.52	nt 2   F   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 0.56
% difference from initial measurement Long-term stab between date Describe conc All stabilitys: Method name Method #: Matrix: Units: Analyte: MU09561	oility = Assese of first sa dition: ample resu	o.87  as long-term s mple collectio example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  3U  Initial measurement	tability tha on and date samples sto within ±159	t equals or except of last sample or ed at -80°C for 6 of nominal cost in Urine  term stability  0.38  0.52	initial measurement  ceeds time le analysis or 2 years concentration  Replic 10/20,  Replic 11/14,  3U  HU09562	cate 1 //2015 Lor cate 1 //2017  cate 1 cate 1 cate 2	Replicate 10/22/201 g-term stabilit Replicate 11/15/201  Initial measureme 0.52 0.81	nt 2   F   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability
% difference from initial measurement Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units: Analyte: MU09561 Replica	oility = Assese of first sa dition: ample resu	o.87  as long-term s mple collectio example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  3U  Initial measuremen 0.46	tability tha on and date samples sto within ±159	t equals or exected for the stability of	initial measurement  ceeds time le analysis or 2 years concentration  Replic 10/20,  Replic 11/14,  3U  HU09562	cate 1 //2015 Lor cate 1 //2017  cate 1 cate 1 cate 2	Replicate 10/22/201 g-term stabilit Replicate 11/15/201  Initial measureme 0.52	nt 2   F   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 0.56
% difference from initial measurement Long-term stab between date between date bescribe concerns and stability so the second s	oility = Assese e of first sa dition: ample resu e:  tite 1 ate 2 ate 3	o.87  as long-term s mple collectic example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  3U  Initial measureme 0.46 0.44 0.44	tability tha on and date samples sto within ±159	t equals or excording to the equals of excording the equals of excording the equals of the equal of the equals of the equals of the equals of the equal of the equals of the equal of	initial measurement  ceeds time le analysis or 2 years concentration  Replic 10/20,  Replic 11/14,  3U  HU09562  Replic Replic Replic	cate 1 //2015 Lor cate 1 //2017  cate 1 cate 1 cate 2 cate 3	Replicate 10/22/201 g-term stabilit Replicate 11/15/201  Initial measureme 0.52 0.81 0.56	nt 2   F   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 0.56 0.53 0.69
% difference from initial measurement Long-term stab between date Describe conc All stabilitys: Method name Method #: Matrix: Units: Analyte: MU09561  Replica Replica	oility = Assese of first sa dition: ample resu	o.87  as long-term s mple collectic example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  3U  Initial measuremen 0.46 0.44	tability tha on and date samples sto within ±159	t equals or except of last sample or ed at -80°C for 6 of nominal cost in Urine  term stability  0.38  0.52	initial measurement  ceeds time le analysis or 2 years concentration  Replic 10/20,  Replic 11/14,  3U  HU09562	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 2 cate 3 an	Replicate 10/22/201 g-term stabilit Replicate 11/15/201  Initial measureme 0.52 0.81	nt 2   F   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 0.56 0.53

# (4) 7-Methyluric Acid (7U)

Stability - fill in ye		IIS							
rreeze and thaw st			freeze-thaw cyc	les; conditions should	d mimic intended sample h	andling condition	ns.		
Describe condition:	three times fro	ozen at -80°C and the	n thawed (3 free	eze-thaw cycles)					
Bench-top stability	= Assess short-	-term stability for len	gth of time nee	ded to handle study s	amples (typically at room	temperature)			
	-				om temperature for 8 hours				
					ident time in autosampler				
Describe condition:	processed san	npies (ready for instr	ument analysis)	stored at 15 C for 22	hours then stored at 5°C f	for 1 month			
All stability sample	esults should b	e within ±15% of non	ninal concentrat	ion					
Run date Data for Initial, three fre Data for processed sar		ench-top stability were r run on 12/07/2017	un on 11/16/2017						
Method name:	Caffoing and N	Metabolites in Urine							
Method #:	4063	vietabolites ili offile							
Matrix:	Urine								
Units:	μmol/L								
Analyte:	70			+	7U				
MU09561					HU09562				
	Initial measurement	Three freeze-	Bench-top	Processed sample		Initial measurement	Three freeze-thaw	Bench-top	Processed
Replicate 1	12.4	thaw cycles 13.9	stability 13.9	stability 12.4	Replicate 1	21.1	cycles 21.9	stability 24.3	sample stabilit
Replicate 2	13.9	14.1	14.1	12.4	Replicate 2	22.0	22.8	20.8	21.3
Replicate 3	14.1	14.0	14.7	13.3	Replicate 3	22.6	21.8	21.3	20.6
Mean	13.5	14.0	14.2	12.7	Mean	21.9	22.2	22.1	21.5
% difference from initial measurement		3.96	5.69	-5.69	% difference from initial measurement		1.22	1.07	-1.98
between date	of first sa	ss long-term si	n and date	of last sample	e analysis				
between date Describe cond	of first sa lition:	mple collectio	and date	of last sample ored at -80°C fo	e analysis <mark>r 2 years</mark>				
between date Describe cond	of first sa lition: ample resu	mple collection example: QC s	and date amples sto within ±15%	of last sample ored at -80°C fo 6 of nominal co	e analysis <mark>r 2 years</mark>	Init	ial Measurme	nt	
between date Describe cond All stabilitysa Method name	of first sa lition: ample resu	mple collection example: QC solls should be sold to comple the collection of the col	and date amples sto within ±15%	of last sample ored at -80°C fo 6 of nominal co	e analysis r 2 years oncentration		1		eplicate 3
between date Describe cond All stability sa Method name Method #:	of first sa lition: ample resu	mple collection example: QC solls should be collected.  Caffeine and 4063	and date amples sto within ±15%	of last sample ored at -80°C fo 6 of nominal co	e analysis r 2 years oncentration Replic	cate 1	Replicate	2 R	eplicate 3
between date Describe conc All stability sa Method name Method #: Matrix:	of first sa lition: ample resu	mple collection example: QC solls should be sold Caffeine and 4063 Urine	and date amples sto within ±15%	of last sample ored at -80°C fo 6 of nominal co	e analysis r 2 years oncentration	cate 1 /2015	Replicate 10/22/201	2 R	eplicate 3 11/9/2015
between date Describe cond All stability sa Method name Method #:	of first sa lition: ample resu	mple collection example: QC solls should be collected.  Caffeine and 4063	and date amples sto within ±15%	of last sample ored at -80°C fo 6 of nominal co	e analysis r 2 years concentration Replic 10/20	cate 1 /2015 Lor	Replicate 10/22/201 ng-term stabili	2 R 15 :	11/9/2015
between date Describe conc All stability sa Method name Method #: Matrix:	of first sa lition: ample resu	mple collection example: QC solls should be sold Caffeine and 4063 Urine	and date amples sto within ±15%	of last sample ored at -80°C fo 6 of nominal co	e analysis r 2 years concentration Replic 10/20,	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 R 15 : ty 2 R	11/9/2015 eplicate 3
between date Describe conc All stability sa Method name Method #: Matrix:	of first sa lition: ample resu	mple collection example: QC solls should be sold Caffeine and 4063 Urine	and date amples sto within ±15%	of last sample ored at -80°C fo 6 of nominal co	e analysis r 2 years concentration Replic 10/20	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili	2 R 15 : ty 2 R	11/9/2015
between date Describe conc All stability sa Method name Method #: Matrix:	of first sa lition: ample resu	mple collection example: QC solls should be sold Caffeine and 4063 Urine	and date amples sto within ±15%	of last sample ored at -80°C fo 6 of nominal co	e analysis r 2 years concentration Replic 10/20,	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 R 15 : ty 2 R	11/9/2015 eplicate 3
between date Describe conc All stability sa Method name Method #: Matrix: Units:	of first sa lition: ample resu	mple collection example: QC sollection exampl	and date amples sto within ±15%	of last sample ored at -80°C fo 6 of nominal co	e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 R 15 : ty 2 R	11/9/2015 eplicate 3
between date Describe conc All stability sa Method name Method #: Matrix: Units: Analyte:	of first sa lition: ample resu	mple collection example: QC solls should be sold to the sold to th	and date amples sto within ±15%	of last sample ored at -80°C fo 6 of nominal co	e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate 11/15/201	2 R 1.5 : ty 2 R 1.7 1	eplicate 3 1/16/2017
between date Describe conc All stability sa Method name Method #: Matrix: Units: Analyte:	of first sa lition: ample resu	mple collection example: QC sollection exampl	on and date camples sto within ±159  Metabolite	of last sample pred at -80°C fo 6 of nominal co s in Urine	e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 R 1.5 : ty 2 R 1.7 1	eplicate 3 1/16/2017
between date Describe conc All stability sa Method name Method #: Matrix: Units: Analyte:	of first sa lition: ample resu	mple collection example: QC solls should be sold to the sold to th	on and date camples sto within ±159  Metabolite	of last sample ored at -80°C fo 6 of nominal co	e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate 11/15/201	2 R 1.5 : ty 2 R 1.7 1	eplicate 3 1/16/2017 Long-term stability
between date Describe conc All stability sa Method name Method #: Matrix: Units: Analyte:	e of first sa lition: ample resu :	mple collection example: QC sollection exampl	on and date camples sto within ±159  Metabolite	of last sample pred at -80°C fo 6 of nominal co s in Urine	e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabili Replicate 11/15/201	2 R 1.5 : ty 2 R 1.7 1	eplicate 3 1/16/2017
between date Describe conc All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561	e of first sa lition: ample resu :	mple collection example: QC sollection (Caffeine and 4063) Urine  µmol/L  7U  Initial measurement	on and date camples sto within ±159  Metabolite	of last sample ored at -80°C for 6 of nominal constitutions in Urine	e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,  7U  HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabili Replicate 11/15/201	2 R 1.5 : ty 2 R 1.7 1	eplicate 3 1/16/2017 Long-term stability
between date Describe conc All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561	e of first sa lition: ample resu : te 1 te 2	mple collection example: QC sollection example: QC sollection caffeine and 4063 Urine µmol/L  7U  Initial measurement 11.0	on and date camples sto within ±159  Metabolite	of last sample ored at -80°C for 6 of nominal constitutions in Urine serm stability 8.77	e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,  7U  HU09562	Cate 1 /2015 Lor cate 1 /2017  Cate 1 cate 2	Replicate 10/22/201 ng-term stabili Replicate 11/15/201  Initial measureme 14.3	2 R 1.5 : ty 2 R 1.7 1	eplicate 3 1/16/2017 Long-term stability 15.2
between date Describe conc All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561  Replica Replica	te 1 te 2 te 3	caffeine and 4063 Urine  µmol/L  Initial  measurement  11.0  10.4	on and date camples sto within ±159  Metabolite	pred at -80°C fo 6 of nominal co s in Urine serm stability 8.77 9.81	e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,  7U  HU09562	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 1 cate 2 cate 3	Replicate 10/22/201 ng-term stabili Replicate 11/15/201  Initial measureme 14.3 15.2	2 R 1.5 : ty 2 R 1.7 1	eplicate 3 1/16/2017 Long-term stability 15.2 16.2

### (5) 1,3-Dimethyluric Acid (13U)

Stability - fill in ye									
			froozo thaw evel	os: conditions should	d mimic intended sample h	andling condition	20		
	-	zen at -80°C and the			a mimic mtended sample n	anding condition	15.		
					amples (typically at room	temperature)			
					om temperature for 8 hours				
					sident time in autosampler				
					4 hours then stored at 5°C				
All stability sample	results should be	e within ±15% of nor	minal concentrat	ion.					
Run date									
Data for Initial, three from Data for processed sar		nch-top stability were r run on 12/07/2017	run on 11/16/2017						
Method name:	Caffeine and M	letabolites in Urine							
Method #:	4063								
Matrix:	Urine								
Units:	μmol/L								
A I I .	4011				4011				
Analyte:	13U				13U				
MU09561					HU09562				
	Initial .	Three freeze-	Bench-top	Processed sample		Initial .	Three freeze-thaw	Bench-top	Processed
Popliests 1	measurement		stability	stability	Donlinsta 1	measurement	cycles	stability	sample stabilit
Replicate 1 Replicate 2	8.68 9.15	8.81 9.25	8.26 9.47	8.41 8.78	Replicate 1 Replicate 2	19.0 18.9	18.4 19.6	19.1 19.6	18.8
Replicate 3	9.15	8.92	9.47	8.36	Replicate 3	17.6	18.9	19.0	19.2
		7.72							
		0.00	9.02	8.52	Mean	18.5	19.0	19.2	18.7
Mean	9.21	8.99	J.02						
% difference from initial measurement Long-term stab between date	 vility = Asses e of first sa	-2.35 ss long-term s mple collection	-2.06 tability that on and date	-7.53 t equals or exc of last sample	e analysis		2.52	3.96	1.08
% difference from initial measurement  Long-term stab between date Describe cond	 vility = Asses e of first sa dition:	-2.35 ss long-term s mple collectic example: QC s	tability that	-7.53 t equals or exc	initial measurement needs time e analysis r 2 years		2.52	3.96	1.08
% difference from initial measurement  Long-term stab between date Describe cond	 illity = Asses e of first sa dition: ample resu	-2.35 ss long-term s mple collectic example: QC s	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement needs time e analysis r 2 years		2.52		1.08
% difference from initial measurement  Long-term stab between date Describe conc All stabilitys:  Method name	 illity = Asses e of first sa dition: ample resu	-2.35  as long-term s mple collectic example: QC s lts should be  Caffeine and	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement seeds time e analysis r 2 years oncentration	Init	tial Measurme	nt	
% difference from initial measurement  Long-term stable between date Describe concentrate All stability something method name Method #:	 illity = Asses e of first sa dition: ample resu	-2.35 as long-term s mple collectic example: QC s lts should be Caffeine and 4063	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement seeds time e analysis r 2 years oncentration Replic	Init	tial Measurme Replicate	nt 2 R	eplicate 3
% difference from initial measurement  Long-term stable between date of the concentration of	 illity = Asses e of first sa dition: ample resu	-2.35 ss long-term s mple collectic example: QC s Its should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement seeds time e analysis r 2 years oncentration	Init cate 1 /2015	tial Measurme Replicate 10/22/201	nt 2 R	
% difference from initial measurement  Long-term stable between date one one of the concentration of the concentra	 illity = Asses e of first sa dition: ample resu	-2.35 as long-term s mple collectic example: QC s lts should be Caffeine and 4063	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement seeds time e analysis r 2 years oncentration  Replic 10/20	Init cate 1 /2015	tial Measurme Replicate 10/22/201 ng-term stabili	nt 2 R	eplicate 3 11/9/2015
% difference from initial measurement Long-term stable between date Describe concall stability so Method name Method #: Matrix:	 illity = Asses e of first sa dition: ample resu	-2.35 ss long-term s mple collectic example: QC s Its should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement seeds time e analysis r 2 years oncentration  Replic 10/20	Initicate 1 /2015 Lor	tial Measurme Replicate 10/22/201 ng-term stabili Replicate	nt 2 R	eplicate 3 11/9/2015
% difference from initial measurement Long-term stable between date Describe concall stability so Method name Method #: Matrix:	 illity = Asses e of first sa dition: ample resu	-2.35 ss long-term s mple collectic example: QC s Its should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement seeds time e analysis r 2 years oncentration  Replic 10/20	Initicate 1 /2015 Lor	tial Measurme Replicate 10/22/201 ng-term stabili	nt 2 R	eplicate 3 11/9/2015
% difference from initial measurement  Long-term stab between date Describe conc All stability so Method name Method #: Matrix: Units:	 illity = Asses e of first sa dition: ample resu	-2.35 ss long-term s mple collectic example: QC s Its should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement seeds time e analysis r 2 years oncentration  Replic 10/20	Initicate 1 /2015 Lor	tial Measurme Replicate 10/22/201 ng-term stabili Replicate	nt 2 R	eplicate 3 11/9/2015 eplicate 3
% difference from initial measurement  Long-term stab between date Describe conce All stability so Method name Method #: Matrix: Units:  Analyte:	 illity = Asses e of first sa dition: ample resu	-2.35 as long-term s mple collectio example: QCs Its should be Caffeine and 4063 Urine  µmol/L	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement seeds time e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,	Initicate 1 /2015 Lor	tial Measurme Replicate 10/22/201 ng-term stabili Replicate	nt 2 R	eplicate 3 11/9/2015 eplicate 3
% difference from initial measurement Long-term stab between date Describe conce All stability so Method name Method #: Matrix: Units:	 illity = Asses e of first sa dition: ample resu	-2.35 as long-term s mple collectio example: QCs Its should be Caffeine and 4063 Urine µmol/L	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement seeds time e analysis r 2 years oncentration  Replic 10/20 Replic 11/14	Initicate 1 /2015 Lor	tial Measurmer Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2 R 1.5 : ty 2 R 1.7 1	Replicate 3 11/9/2015 Replicate 3 1/16/2017
% difference from initial measurement  Long-term stab between date Describe conce All stability so Method name Method #: Matrix: Units:  Analyte:	 illity = Asses e of first sa dition: ample resu	-2.35 as long-term s mple collectio example: QCs Its should be Caffeine and 4063 Urine  µmol/L	tability that on and date samples sto within ±15%	t equals or exc of last samplored at -80°C fo 6 of nominal co s in Urine	initial measurement seeds time e analysis r 2 years oncentration  Replic 10/20 Replic 11/14	Initicate 1 /2015 Lor	tial Measurme Replicate 10/22/201 ng-term stabili Replicate	nt 2 R 1.5 : ty 2 R 1.7 1	Replicate 3 11/9/2015 Replicate 3 1/16/2017
% difference from initial measurement Long-term stab between date Describe conce All stability so Method name Method #: Matrix: Units:	 illity = Asses e of first sa dition: ample resu	-2.35 as long-term s mple collectio example: QCs Its should be Caffeine and 4063 Urine µmol/L	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co	initial measurement seeds time e analysis r 2 years oncentration  Replic 10/20 Replic 11/14	Initicate 1 /2015 Lor	tial Measurmer Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2 R R L S R L T 1	Replicate 3 11/9/2015 Replicate 3 1/16/2017
% difference from initial measurement Long-term stab between date Describe conc All stability st	ility = Asses e of first sa lition: ample resu	-2.35 as long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L  13U  Initial	tability that on and date samples sto within ±15%	t equals or exc of last samplored at -80°C fo 6 of nominal co s in Urine	initial measurement seeds time e analysis r 2 years oncentration  Replic 10/20 Replic 11/14	Init cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2 R R L S R L T 1	Replicate 3 11/9/2015 Replicate 3 1/16/2017
% difference from initial measurement Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units: Analyte: MU09561 Replica	vility = Assesse of first sa dition: ample resu	-2.35 as long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L  13U  Initial measurement	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co s in Urine	initial measurement reeds time e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,  13U  HU09562	Init cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2 R R L S R L T 1	deplicate 3 11/9/2015 deplicate 3 1/16/2017
% difference from initial measurement Long-term stab between date Describe conc All stability set Method name Method #: Matrix: Units: Analyte: MU09561	ility = Asses e of first sa lition: ample resu ::	-2.35 as long-term s mple collectic example: QC s Its should be Caffeine and 4063 Urine µmol/L  13U  Initial measurement 5.28	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co s in Urine erm stability 5.13	initial measurement seeds time e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,  13U  HU09562	Initicate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme	nt 2 R R L S R L T 1	Long-term stability
% difference from initial measurement  Long-term stab between date between date bescribe concentration of the conc	te 1 te 2 te 3	-2.35 as long-term s mple collectic example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  13U  Initial measureme 5.28 5.26	tability that on and date samples sto within ±15%	t equals or exc of last sample ored at -80°C fo 6 of nominal co s in Urine erm stability 5.13 5.33	initial measurement leeds time le analysis r 2 years concentration Replic 10/20, Replic 11/14, 13U HU09562 Replic Replic Replic	Initicate 1 /2015 Lor cate 1 /2017  cate 1 cate 2 cate 3	Replicate 10/22/201 Replicate 11/15/201 Replicate 11/15/201 Initial measureme 14.5 15.8	nt 2 R R L S R L T 1	Long-term stability 16.0 15.8

# (6) 1,7-Dimethyluric Acid (17U)

Describe condition: Bench-top stability	bility = Assess								
Describe condition: Bench-top stability		for a minimum of 3	freeze-thaw cycl	es; conditions should	mimic intended sample h	andling condition	ıs.		
	three times from				·				
					amples (typically at room				
					m temperature for 8 hours				
					dent time in autosampler hours then stored at 5°C t				
Describe condition.	processed sail	ipies (ready for mistr	unient analysis)	Stored at 15 C for 24	nours then stored at 5 C	TOT I HIOHUI			
All stability sample re	esults should be	e within ±15% of nor	minal concentrat	ion.					
Run date Data for Initial, three fre	eze-thaw and be	nch-top stability were r	aup op 11/16/2017						
Data for processed sam			uli 011 11/10/2017						
		letabolites in Urine							
	4063 Urine								
	μmol/L								
	<b></b>								
Analyte:	17U				17U				
MU09561					HU09562				
	Initial	Three freeze-	Bench-top	Processed sample		Initial	Three freeze-thaw	Bench-top	
Replicate 1	measurement 34.9	thaw cycles 35.5	stability 34.9	stability 32.2	Replicate 1	measurement 80.6	cycles 81.1	stability 79.4	sample stabilit 81.8
Replicate 2	35.7	35.9	36.3	35.4	Replicate 2	83.7	80.0	80.0	79.4
Replicate 3	35.3	35.2	35.9	35.0	Replicate 3	80.2	77.9	80.0	79.4
Mean	35.3	35.5	35.7	34.2	Mean	81.5	79.7	79.8	80.2
% difference from initial measurement		0.66	1.13	-3.12	% difference from initial measurement		-2.25	-2.09	-1.60
		mple collectio	n and date	t equals or exco of last sample p <mark>red at -80°C for</mark>	analysis				
Describe cond	ition:	mple collection example: QC s	on and date samples sto	of last sample	analysis 2 years				
Describe cond	ition: Imple resu	mple collection example: QC s	on and date samples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	analysis 2 years	Init	tial Measurme	nt	
Describe cond All stability sa Method name	ition: imple resu :	mple collection example: QC sollers should be	on and date samples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	analysis 2 years ncentration				Replicate 3
Describe cond All stability sa Method name Method #:	ition: imple resu :	mple collection example: QC solls should be conferned and 4063	on and date samples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	analysis 2 years Incentration Replic	cate 1	Replicate	2	Replicate 3 11/9/2015
Describe cond All stability sa Method name Method #: Matrix:	ition: imple resu :	mple collection example: QC s lts should be Caffeine and 4063 Urine	on and date samples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	analysis 2 years ncentration	cate 1 /2015	Replicate 10/22/201	2	Replicate 3 11/9/2015
Describe cond All stability sa Method name Method #:	ition: imple resu :	mple collection example: QC solls should be conferned and 4063	on and date samples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	ranalysis r2 years racentration Replic	cate 1 /2015 Lor	Replicate 10/22/201 ng-term stabili	2 .5 ty	11/9/2015
Describe cond All stability sa Method name Method #: Matrix:	ition: imple resu :	mple collection example: QC s lts should be Caffeine and 4063 Urine	on and date samples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	ranalysis r 2 years	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 .5 ty 2	11/9/2015 Replicate 3
Describe cond All stability sa Method name Method #: Matrix:	ition: imple resu :	mple collection example: QC s lts should be Caffeine and 4063 Urine	on and date samples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	ranalysis r2 years racentration Replic	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili	2 .5 ty 2	11/9/2015
Describe cond All stability sa Method name Method #: Matrix:	ition: imple resu :	mple collection example: QC s lts should be Caffeine and 4063 Urine	on and date samples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	ranalysis r 2 years	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 .5 ty 2	11/9/2015 Replicate 3
Describe cond All stability sa Method name Method #: Matrix: Units:	ition: imple resu :	mple collection example: QC selfs should be selfeine and 4063 Urine  µmol/L	on and date samples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	Replication Replic	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 .5 ty 2	11/9/2015 Replicate 3
Describe cond All stability sa Method name Method #: Matrix: Units:	ition: imple resu :	mple collection example: QC sets should be set to the collection of the collection o	on and date samples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	Replication Replic	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	2 .5 ty 2	11/9/2015 Replicate 3 11/16/2017
Describe cond All stability sa Method name Method #: Matrix: Units:	ition: imple resu :	mple collection example: QC sets should be set to the s	on and date samples sto within ±15%  Metabolite	of last sample ored at -80°C for 6 of nominal co s in Urine	Replication Replic	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	2	11/9/2015  Replicate 3 11/16/2017  Long-term
Describe cond All stability sa Method name Method #: Matrix: Units: Analyte: MU09561	ition: imple resu :	mple collection example: QC s lts should be s  Caffeine and 4063 Urine  µmol/L  37U  Initial measurement	on and date samples sto within ±15%  Metabolite	of last sample ored at -80°C for 6 of nominal costs in Urine	Replication  Replication  Replication  Replication  Replication  Replication  11/14,  37U  HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201 Initial measureme	2	11/9/2015  Replicate 3 11/16/2017  Long-term stability
Describe cond All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561  Replica:	ition: imple resu :	mple collection example: QC s lts should be s  Caffeine and 4063 Urine  µmol/L  37U  Initial measurement 0.66	on and date samples sto within ±15%  Metabolite	of last sample ored at -80°C for 6 of nominal co s in Urine  erm stability 0.61	Replication  Replication  Replication  Replication  Replication  Replication  11/14,  37U  HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 0.99	2	Replicate 3 11/16/2017  Long-term stability 0.95
Describe cond All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561  Replica: Replica:	ition: imple resu : te 1 te 2	mple collection example: QC s lts should be s  Caffeine and 4063 Urine  µmol/L  37U  Initial measurement 0.66 0.50	on and date samples sto within ±15%  Metabolite	of last sample ored at -80°C for 6 of nominal co s in Urine  erm stability 0.61 0.63	Replication  Replication  Replication  Replication  Replication  Replication  11/14,  37U  HU09562	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 2	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 0.99 1.10	2	11/9/2015  Replicate 3 11/16/2017  Long-term stability 0.95 0.91
Describe cond All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561  Replica:	ition: imple resu : te 1 te 2	mple collection example: QC s lts should be s  Caffeine and 4063 Urine  µmol/L  37U  Initial measurement 0.66	on and date samples sto within ±15%  Metabolite	of last sample ored at -80°C for 6 of nominal co s in Urine  erm stability 0.61	Replication  Replication  Replication  Replication  Replication  Replication  11/14,  37U  HU09562	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 2	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 0.99	2	Replicate 3 11/16/2017  Long-term stability 0.95
Describe cond All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561  Replica: Replica:	ition: imple resu : te 1 te 2 te 3	mple collection example: QC s lts should be s  Caffeine and 4063 Urine  µmol/L  37U  Initial measurement 0.66 0.50	on and date samples sto within ±15%  Metabolite	of last sample ored at -80°C for 6 of nominal co s in Urine  erm stability 0.61 0.63	Replication  Replication  Replication  Replication  Replication  Replication  11/14,  37U  HU09562	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 1 cate 2 cate 3	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 0.99 1.10	2	11/9/2015  Replicate 3 11/16/2017  Long-term stability 0.95 0.91

# (7) 3,7-Dimethyluric Acid (37U)

Stability - fill in ye Freeze and thaw st		IS							
	ability = Assess		freeze-thaw cvc	les; conditions should	d mimic intended sample h	nandling condition	is.		
Describe condition:		zen at -80°C and the							
					amples (typically at room	temperature)			
Describe condition:	original sample	s (not yet prepared	for instrument a	nalysis) stored at roo	m temperature for 8 hours	5			
Processed sample s	tability = Assess	s short-term stabilit	y of processed s	amples, including res	ident time in autosampler				
Describe condition:	processed sam	ples (ready for inst	rument analysis	stored at 15°C for 24	hours then stored at 5°C	for 1 month			
All stability sample	results should be	e within ±15% of no	minal concentra	tion.					
Run date Data for Initial, three fr Data for processed sa			run on 11/16/2017						
Method name:	Caffeine and M	letabolites in Urine							
Method #:	4063								
Matrix:	Urine								
Units:	μmol/L								
Analyte:	37U				37U				
MU09561					HU09562				
	Initial	Three freeze-	Bench-top	Processed sample		Initial	Three freeze-thaw	Bench-top	Processed
B 11	measurement	thaw cycles	stability	stability		measurement	cycles	stability	sample stabilit
Replicate 1	1.09	1.13	1.07	0.989	Replicate 1	1.51	1.31	1.48	1.44
Replicate 2 Replicate 3	1.05 0.97	1.17 0.99	1.02	1.11	Replicate 2 Replicate 3	1.43	1.38 1.33	1.47	1.28
Replicate 3	0.97	0.99	1.24	1.15	Nephrate 3	1.41	1.55	1.37	1.45
Mean	1.04	1.10	1.11	1.08	Mean	1.45	1.34	1.44	1.39
% difference from		5.85	7.07	3.83	% difference from		-7.59	-0.69	-4.14
initial measurement		5.05	7.07	5.05	initial measurement	-	-7.55	-0.05	-4.14
between dat	e of first sa	mple collection	on and date	t equals or exce of last sample	e analysis				
between dat Describe cond	e of first sau dition:	mple collection example: QC s	on and date samples sto	•	e analysis <mark>r 2 years</mark>				
between dat Describe cond	e of first sau dition: ample resul	mple collection example: QC s	on and date samples sto within ±159	of last sample ored at -80°C for 6 of nominal co	e analysis <mark>r 2 years</mark>	Init	ial Measurme	nt	
between dat Describe cond All stability s	e of first sau dition: ample resul	mple collection example: QC solution like the collection with the	on and date samples sto within ±159	of last sample ored at -80°C for 6 of nominal co	e analysis <mark>r 2 years</mark>		ial Measurme Replicate		Replicate 3
between dat Describe cond All stability s Method name Method #:	e of first sai dition: ample resul	mple collection example: QC solution less should be confering and confer	on and date samples sto within ±159	of last sample ored at -80°C for 6 of nominal co	e analysis r 2 years oncentration Replii	cate 1	ı	2 F	•
between dat Describe cond All stability s Method name Method #: Matrix:	e of first san dition: ample resul	mple collection example: QC solls should be Caffeine and 4063 Urine	on and date samples sto within ±159	of last sample ored at -80°C for 6 of nominal co	e analysis r 2 years oncentration	cate 1 /2015	Replicate 10/22/201	2 F	Replicate 3 11/9/2015
between dat Describe cond All stability s Method name Method #:	e of first san dition: ample resul	mple collection example: QC solution like the collection with the collection with the collection with the collection with the collection like the collection with the	on and date samples sto within ±159	of last sample ored at -80°C for 6 of nominal co	e analysis r 2 years oncentration Replic	cate 1 /2015 Lor	Replicate 10/22/201 ng-term stabilit	2 F .5	11/9/2015
between dat Describe cond All stability s Method name Method #: Matrix:	e of first san dition: ample resul	mple collection example: QC solls should be Caffeine and 4063 Urine	on and date samples sto within ±159	of last sample ored at -80°C for 6 of nominal co	e analysis r 2 years oncentration Replic 10/20	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate	2 F	11/9/2015 Replicate 3
between dat Describe cond All stability s Method name Method #: Matrix:	e of first san dition: ample resul	mple collection example: QC solls should be Caffeine and 4063 Urine	on and date samples sto within ±159	of last sample ored at -80°C for 6 of nominal co	e analysis r 2 years oncentration Replic	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit	2 F	11/9/2015
between dat Describe cond All stability s Method name Method #: Matrix:	e of first sau dition: ample resul	mple collection example: QC solls should be Caffeine and 4063 Urine	on and date samples sto within ±159	of last sample ored at -80°C for 6 of nominal co	e analysis r 2 years oncentration Replic 10/20	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate	2 F	11/9/2015 Replicate 3
between dat Describe cond All stability s Method name Method #: Matrix: Units:	e of first sau dition: ample resul	mple collection example: QC sollection example: QC sollection lts should be Caffeine and 4063 Urine	on and date samples sto within ±159	of last sample ored at -80°C for 6 of nominal co	Replication Replic	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate	2 F	11/9/2015 Replicate 3
between dat Describe conc All stability s Method name Method #: Matrix: Units: Analyte:	e of first sau dition: ample resul	mple collection example: QC sollection example: QC sollection lts should be Caffeine and 4063 Urine	on and date samples str within ±159 Metabolite	of last sample pred at -80°C for 6 of nominal co s in Urine	e analysis r 2 years concentration Replic 10/20 Replic 11/14	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	2 F 55 ty 2 F 77 :	11/9/2015 Replicate 3 11/16/2017 Long-term
between dat Describe cond All stability s Method name Method #: Matrix: Units: Analyte: MU09561	e of first sau dition: ample resul	mple collection example: QC solls should be Caffeine and 4063 Urine  µmol/L  17U  Initial measureme	on and date samples str within ±159 Metabolite  nt Long-1	of last sample pred at -80°C for 6 of nominal constitutions in Urine	Replication  Replication  Replication  Replication  Replication  Replication  10/20  Replication  11/14  17U  HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201 Initial measureme	2 F 55 ty 2 F 77 :	11/9/2015 Replicate 3 11/16/2017  Long-term stability
between dat Describe cond All stability s Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	e of first saudition: ample resul	mple collection example: QC solls should be  Caffeine and 4063 Urine  µmol/L  17U  Initial measureme 34.8	on and date samples str within ±159 Metabolite  nt Long-1	e of last sample ored at -80°C for 6 of nominal constitutions in Urine seem stability 31.2	Replication  TOU  HU09562  Replication	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 91.2	2 F 55 ty 2 F 77 :	11/9/2015 Replicate 3 11/16/2017  Long-term stability 90.9
between dat Describe cond All stability s Method name Method #: Matrix: Units:  Analyte: MU09561  Replica Replica	e of first saudition: ample resul	mple collection example: QC solls should be  Caffeine and 4063 Urine  µmol/L  17U  Initial measureme 34.8 32.0	on and date samples str within ±159 Metabolite  nt Long-1	s of last sample ored at -80°C for 6 of nominal costs in Urine serm stability 31.2 29.9	Replication  T7U  HU09562  Replication	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 2	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 91.2 100	2 F 55 ty 2 F 77 :	11/9/2015  Replicate 3 11/16/2017  Long-term stability 90.9 87.5
between dat Describe cond All stability s Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	e of first saudition: ample resul	mple collection example: QC solls should be  Caffeine and 4063 Urine  µmol/L  17U  Initial measureme 34.8	on and date samples str within ±159 Metabolite  nt Long-1	e of last sample ored at -80°C for 6 of nominal constitutions in Urine seem stability 31.2	Replication  TOU  HU09562  Replication	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 2	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 91.2	2 F 55 ty 2 F 77 :	11/9/2015 Replicate 3 11/16/2017  Long-term stability 90.9
between dat Describe cond All stability s Method name Method #: Matrix: Units:  Analyte: MU09561  Replica Replica	e of first saudition: ample result: :: :: :: :: :: :: :: :: :: :: :: :: :	mple collection example: QC solls should be  Caffeine and 4063 Urine  µmol/L  17U  Initial measureme 34.8 32.0	on and date samples str within ±159 Metabolite  nt Long-1	s of last sample ored at -80°C for 6 of nominal costs in Urine serm stability 31.2 29.9	Replication  T7U  HU09562  Replication	cate 1 /2015  Lor cate 1 /2017  cate 1 cate 1 cate 2 cate 3	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 91.2 100	2 F 55 ty 2 F 77 :	11/9/2015  Replicate 3 11/16/2017  Long-term stability 90.9 87.5

# (8) 1,3,7-Trimethyluric Acid (137U)

Freeze and thaw st		ls							
	ability = Assess		freeze-thaw cvc	les: conditions shoul	d mimic intended sample h	nandling condition	ıs.		
Describe condition:		zen at -80°C and the							
Bench-top stability	= Assess short-1	term stability for ler	ngth of time nee	ded to handle study s	samples (typically at room	temperature)			
Describe condition:	original sample	es (not yet prepared	for instrument a	inalysis) stored at ro	om temperature for 8 hours	S			
Processed sample s	stability = Assess	s short-term stabilit	y of processed s	amples, including re	sident time in autosampler	-			
Describe condition:	processed sam	nples (ready for insti	rument analysis	stored at 15°C for 2	4 hours then stored at 5°C	for 1 month			
All stability sample	results should be	e within ±15% of no	minal concentra	tion.					
Run date Data for Initial, three fr Data for processed sa		nch-top stability were run on 12/07/2017	run on 11/16/2017						
Method name:	Caffeine and M	letabolites in Urine							
Method #:	4063	ictabolites ill offile							
Matrix:	Urine								
Units:	μmol/L								
Analyte:	137U				137U				
MU09561					HU09562				
	Initial	Three freeze-	Bench-top	Processed sample		Initial	Three freeze-thaw	Bench-top	Processed
	measurement	thaw cycles	stability	stability		measurement	cycles	stability	sample stabilit
Replicate 1	3.47	3.79	3.71	3.49	Replicate 1	7.53	7.33	6.99	7.47
Replicate 2	3.68	3.70	3.63	3.66	Replicate 2	7.18	7.37	7.61	7.52
Replicate 3	3.64	3.52	3.77	3.70	Replicate 3	7.66	7.26	7.25	7.81
Mean	3.60	3.67	3.70	3.62	Mean	7.46	7.32	7.28	7.60
% difference from initial measurement		2.04	2.97	0.56	% difference from initial measurement		-1.83	-2.32	1.92
between dat	e of first sa	mple collection	on and date	t equals or exc of last sampl	e analysis				
between dat Describe cond	e of first sa dition:	mple collection example: QC s	on and date samples sto	•	e analysis or 2 years				
between dat Describe cond	e of first sa dition: ample resu	mple collection example: QC s	on and date samples sto within ±159	of last sampl ored at -80°C fo % of nominal c	e analysis or 2 years	Init	tial Measurme	nt	
between dat Describe cond All stability s Method name	e of first sa dition: ample resu	mple collection example: QC solution like the collection with the collection with the collection with the collection like the collection like the collection like the collection with the collection like the	on and date samples sto within ±159	of last sampl ored at -80°C fo % of nominal c	e analysis or 2 years oncentration				Replicate 3
between dat Describe cond All stability s Method name Method #:	e of first sa dition: ample resu	mple collection example: QC sollection example: QC sollection example: QC sollection example collection exam	on and date samples sto within ±159	of last sampl ored at -80°C fo % of nominal c	e analysis or 2 years oncentration Repli	cate 1	Replicate	2	Replicate 3
between dat Describe cond All stability s Method name Method #: Matrix:	e of first sa dition: ample resu	mple collection example: QC s lts should be Caffeine and 4063 Urine	on and date samples sto within ±159	of last sampl ored at -80°C fo % of nominal c	e analysis or 2 years oncentration	cate 1 /2015	Replicate 10/22/201	2	Replicate 3 11/9/2015
between dat Describe cond All stability s Method name Method #:	e of first sa dition: ample resu	mple collection example: QC sollection example: QC sollection example: QC sollection example collection exam	on and date samples sto within ±159	of last sampl ored at -80°C fo % of nominal c	e analysis or 2 years oncentration  Replic 10/20	cate 1 /2015 Lor	Replicate 10/22/201 ng-term stabili	2 .5 ty	11/9/2015
between dat Describe cond All stability s Method name Method #: Matrix:	e of first sa dition: ample resu	mple collection example: QC s lts should be Caffeine and 4063 Urine	on and date samples sto within ±159	of last sampl ored at -80°C fo % of nominal c	e analysis or 2 years oncentration Repli	cate 1 /2015 Lor	Replicate 10/22/201 ng-term stabili Replicate	2 .5 ty 2	•
between dat Describe cond All stability s Method name Method #: Matrix:	e of first sa dition: ample resu	mple collection example: QC s lts should be Caffeine and 4063 Urine	on and date samples sto within ±159	of last sampl ored at -80°C fo % of nominal c	e analysis or 2 years oncentration  Replic 10/20	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili	2 .5 ty 2	11/9/2015
between dat Describe cond All stability s Method name Method #: Matrix: Units:	e of first sa dition: ample resu	mple collection example: QC solls should be Caffeine and 4063 Urine  µmol/L	on and date samples sto within ±159	of last sampl ored at -80°C fo % of nominal c	e analysis or 2 years oncentration  Replic 10/20  Replic 11/14	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 .5 ty 2	11/9/2015 Replicate 3
between dat Describe cond All stability s Method name Method #: Matrix: Units: Analyte:	e of first sa dition: ample resu	mple collection example: QC s lts should be Caffeine and 4063 Urine	on and date samples sto within ±159	of last sampl ored at -80°C fo % of nominal c	e analysis or 2 years oncentration  Replic 10/20  Replic 11/14	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 .5 ty 2	11/9/2015 Replicate 3
between dat Describe cond All stability s Method name Method #: Matrix:	e of first sa dition: ample resu	mple collection example: QC solls should be Caffeine and 4063 Urine  µmol/L	on and date samples sto within ±159	of last sampl ored at -80°C fo % of nominal c	e analysis or 2 years oncentration  Replic 10/20  Replic 11/14	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 .5 ty 2	11/9/2015 Replicate 3
between dat Describe cond All stability s Method name Method #: Matrix: Units: Analyte:	e of first sa dition: ample resu	mple collection example: QC solls should be Caffeine and 4063 Urine  µmol/L  137U	on and date samples str within ±159 Metabolite	e of last sampl pred at -80°C fo % of nominal co es in Urine	e analysis or 2 years oncentration  Replic 10/20  Replic 11/14	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	2 55 tty 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	11/9/2015  Replicate 3 11/16/2017  Long-term
between dat Describe cond All stability s Method name Method #: Matrix: Units: Analyte: MU09561	e of first sa dition: ample resu	mple collection example: QC solls should be Caffeine and 4063 Urine  µmol/L  137U  Initial measureme	on and date samples str within ±159 Metabolite  nt Long-1	e of last sample ored at -80°C for 60	e analysis or 2 years oncentration  Replic 10/20  Replic 11/14  137U  HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201 Initial measureme	2 55 tty 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	11/9/2015  Replicate 3 11/16/2017  Long-term stability
between dat Describe cond All stability s Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	e of first sa dition: ample resu	mple collection example: QC solls should be Caffeine and 4063 Urine  µmol/L  137U  Initial measureme 2.78	on and date samples str within ±159 Metabolite  nt Long-1	term stability	e analysis or 2 years oncentration  Replication  Replication  Replication  10/20  Replication  11/14  137U  HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 5.14	2 55 tty 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	Replicate 3 11/16/2017  Long-term stability 5.14
between dat Describe cond All stability s Method name Method #: Matrix: Units:  Analyte: MU09561  Replica Replica	e of first sa dition: ample resule:	mple collection example: QC solls should be Caffeine and 4063 Urine  µmol/L  137U  Initial measureme 2.78 2.74	on and date samples str within ±159 Metabolite  nt Long-1	term stability 2.71 2.71	e analysis or 2 years oncentration  Replication  Replication  Replication  10/20  Replication  11/14  137U  HU09562	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 1 cate 2	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 5.14 5.24	2 55 tty 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	11/9/2015  Replicate 3 11/16/2017  Long-term stability 5.14 5.06
between dat Describe cond All stability s Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	e of first sa dition: ample resule:	mple collection example: QC solls should be Caffeine and 4063 Urine  µmol/L  137U  Initial measureme 2.78	on and date samples str within ±159 Metabolite  nt Long-1	term stability	e analysis or 2 years oncentration  Replication  Replication  Replication  10/20  Replication  11/14  137U  HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 5.14	2 55 tty 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	Replicate 3 11/16/2017  Long-term stability 5.14
between dat Describe cond All stability s Method name Method #: Matrix: Units:  Analyte: MU09561  Replica Replica	e of first sa dition: ample result: e:	mple collection example: QC solls should be Caffeine and 4063 Urine  µmol/L  137U  Initial measureme 2.78 2.74	on and date samples str within ±159 Metabolite  nt Long-1	term stability 2.71 2.71	e analysis or 2 years oncentration  Replication  Replication  Replication  10/20  Replication  11/14  137U  HU09562	cate 1 /2015  Lor cate 1 /2017  cate 1 cate 2 cate 3	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 5.14 5.24	2 55 tty 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	11/9/2015  Replicate 3 11/16/2017  Long-term stability 5.14 5.06

### (9) 1-Methylxanthine (1X)

	ellow shaded cel								
•			freeze-thaw cycl	es; conditions should	mimic intended sample h	andling condition	ıs.		
		zen at -80°C and the							
Bench-top stability	= Assess short-	term stability for ler	ngth of time need	ded to handle study sa	mples (typically at room t	temperature)			
					n temperature for 8 hours				
					dent time in autosampler				
Describe condition:	processed sam	nples (ready for insti	rument analysis)	stored at 15°C for 24	hours then stored at 5°C f	for 1 month			
All stability sample	results should be	e within ±15% of nor	minal concentrat	ion.					
Run date Data for Initial, three fro Data for processed sar			run on 11/16/2017						
Method name:	Caffeine and M	letabolites in Urine							
Method #:	4063								
Matrix:	Urine								
Units:	μmol/L								
Analyte:	1X				1X				
MU09561					HU09562				
	Initial	Three freeze-	Bench-top	Processed sample		Initial	Three freeze-thaw	Bench-to	•
Replicate 1	measurement 37.6	thaw cycles 45.2	stability 40.9	stability 39.0	Replicate 1	measurement 93.8	cycles 106	stability 99.1	y sample stabilit 93.4
Replicate 2	40.1	38.7	38.7	43.2	Replicate 2	103	93.9	97.8	98.8
Replicate 3	41.0	41.5	42.4	41.3	Replicate 3	97.7	98.2	102	101
			40.7	44.2	N4	00.2	00.4	00.5	07.7
	20.5			41.2	Mean	98.2	99.4	99.6	97.7
Mean	39.6	41.8	40.7		% difference from				
% difference from nitial measurement Long-term stab between date	 <b>bility</b> = Asses e of first sa	5.64 ss long-term s mple collection	2.78 tability tha	4.04  t equals or exce of last sample pred at -80°C for	analysis		1.22	1.49	-0.44
% difference from initial measurement Long-term stab between date Describe cond	 vility = Asses e of first sa dition:	5.64 ss long-term s mple collection example: QC s	tability that on and date	4.04 t equals or exce of last sample	initial measurement eeds time analysis 2 years		1.22	1.49	-0.44
% difference from initial measurement Long-term stab between date Describe cond	 vility = Asses e of first sa dition: ample resu	5.64 ss long-term s mple collection example: QC s	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement eeds time analysis 2 years	 Init	1.22		-0.44
% difference from initial measurement  Long-term stab between date Describe conc All stabilitys:	ility = Asses e of first sa lition: ample resu	5.64 as long-term s mple collectio example: QCs lts should be	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement eeds time analysis 2 years			nt	-0.44  Replicate 3
% difference from initial measurement  Long-term stab between date Describe conc All stabilitys: Method name	ility = Asses e of first sa lition: ample resu	5.64 as long-term s mple collectic example: QC s lts should be	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	eeds time analysis 2 years ncentration	cate 1	ial Measurme	nt 2	
% difference from initial measurement  Long-term stab between date Describe conc All stability sa Method name Method #:	ility = Asses e of first sa lition: ample resu	5.64 as long-term s mple collectic example: QC s lts should be Caffeine and 4063	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	eeds time analysis 2 years ncentration Replic	cate 1 /2015	ial Measurme Replicate	nt 2	Replicate 3
% difference from initial measurement  Long-term stable between date Describe concept All stability so Method name Method #: Matrix:	ility = Asses e of first sa lition: ample resu	5.64 ass long-term s mple collectic example: QC s Its should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	eeds time analysis 2 years ncentration Replic	cate 1 /2015 Lor	ial Measurme Replicate 10/22/201	nt 2   1.5   ty	Replicate 3
% difference from initial measurement Long-term stable between date Describe concall stability sa Method name Method #: Matrix:	ility = Asses e of first sa lition: ample resu	5.64 ass long-term s mple collectic example: QC s Its should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	eds time analysis 2 years ncentration  Replic 10/20/	cate 1 /2015 Lor cate 1	ial Measurmer Replicate 10/22/201 ng-term stabilit	nt 2	Replicate 3 11/9/2015
% difference from initial measurement  Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units:	ility = Asses e of first sa lition: ample resu	5.64 ss long-term s mple collectic example: QC s Its should be Caffeine and 4063 Urine  µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	nitial measurement  eds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/	cate 1 /2015 Lor cate 1	Replicate 10/22/201 1g-term stabilit Replicate	nt 2	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement  Long-term stab between date Describe conce All stability say Method name Method #: Matrix: Units:  Analyte:	ility = Asses e of first sa lition: ample resu	5.64 ass long-term s mple collectic example: QC s Its should be Caffeine and 4063 Urine	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	reds time analysis  2 years ncentration  Replic 10/20/ Replic 11/14/	cate 1 /2015 Lor cate 1	Replicate 10/22/201 1g-term stabilit Replicate	nt 2	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement  Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units:	ility = Asses e of first sa lition: ample resu	5.64 ss long-term s mple collectic example: QC s Its should be Caffeine and 4063 Urine  µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	nitial measurement  eds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/	cate 1 /2015 Lor cate 1	Replicate 10/22/201 1g-term stabilit Replicate	nt 2	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement  Long-term stab between date Describe conce All stability say Method name Method #: Matrix: Units:	ility = Asses e of first sa lition: ample resu	5.64 as long-term s mple collectio example: QCs Its should be Caffeine and 4063 Urine µmol/L  1X Initial	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co s in Urine	reds time analysis  2 years ncentration  Replic 10/20/ Replic 11/14/	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015 Replicate 3 11/16/2017
% difference from initial measurement Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units: Analyte: MU09561	vility = Asses e of first sa lition: ample resu	5.64  as long-term s mple collection example: QCs Its should be  Caffeine and 4063 Urine  µmol/L  1X  Initial measureme	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co s in Urine	initial measurement  eeds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/ 1X HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015 Replicate 3 11/16/2017  Long-term stability
% difference from initial measurement  Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	vility = Asses e of first sa dition: ample resu ::	5.64  as long-term s mple collection example: QCs Its should be  Caffeine and 4063 Urine  µmol/L  1X  Initial measureme 40.2	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co s in Urine erm stability 34.9	initial measurement  eeds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/  1X HU09562	cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015 Replicate 3 11/16/2017  Long-term stability 80.9
% difference from initial measurement  Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units:  Analyte:  MU09561  Replica Replica	sility = Asses e of first sa lition: ample resu ::	5.64  as long-term s mple collectio example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  1X  Initial measureme 40.2 41.9	tability that on and date samples sto within ±15%	erm stability 34.9 39.9	initial measurement  eeds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/  1X  HU09562  Replic Replic Replic	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 1 cate 2	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 78.7 90.3	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 80.9 84.3
% difference from initial measurement  Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	sility = Asses e of first sa lition: ample resu ::	5.64  as long-term s mple collection example: QCs Its should be  Caffeine and 4063 Urine  µmol/L  1X  Initial measureme 40.2	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co s in Urine erm stability 34.9	initial measurement  eeds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/  1X HU09562	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 1 cate 2	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015 Replicate 3 11/16/2017  Long-term stability 80.9
% difference from initial measurement  Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units:  Analyte:  MU09561  Replica Replica	sility = Assesse of first sa dition: ample resu  ::  tte 1 tte 2 tte 3	5.64  as long-term s mple collectio example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  1X  Initial measureme 40.2 41.9	tability that on and date samples sto within ±15%	erm stability 34.9 39.9	initial measurement  eeds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/  1X  HU09562  Replic Replic Replic	cate 1 //2015 Lor cate 1 //2017  cate 1 cate 1 cate 2 cate 3	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 78.7 90.3	nt 2   1.5   1.7   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 80.9 84.3

### (10) 3-Methylxanthine (3X)

		lc							
Stability - fill in ye									
					mimic intended sample h	andling condition	ns.		
		zen at -80°C and the			malas (tunisallu at room t	tomporatura)			
					mples (typically at room t n temperature for 8 hours				
					dent time in autosampler				
					hours then stored at 5°C f				
			, , , , , , , , , , , , , , , , , , , ,						
All stability sample r	esults should b	e within ±15% of nor	minal concentrat	ion.					
Run date									
Data for Initial, three fre		nch-top stability were r	run on 11/16/2017						
Data for proceeded our	inpro otability was	1011 011 12/01/2011							
Method name:	Caffeine and N	letabolites in Urine							
Method #:	4063								
Matrix:	Urine								
Units:	μmol/L								
Analyta	2V				2V				
Analyte:	3X				3X				
MU09561					HU09562				
	Initial	Three freeze-	Bench-top	Processed sample		Initial .	Three freeze-thaw	Bench-top	
Poplicate 1	measurement	thaw cycles	stability	stability	Porliente 1	measurement	cycles	stability	sample stabilit
Replicate 1 Replicate 2	27.9 28.7	28.1 28.2	30.1 28.9	28.8 30.2	Replicate 1 Replicate 2	45.2 50.5	48.1 42.9	45.5 50.0	54.1 49.6
Replicate 3	29.8	28.6	29.7	31.6	Replicate 3	44.8	47.2	50.8	49.0
				1	- p				
Mean	28.8	28.3	29.6	30.2	Mean	46.8	46.1	48.8	51.0
TVIC GIT	20.0								
% difference from initial measurement  Long-term stab between date	 ility = Asse: e of first sa	mple collectio	n and date	4.86 t equals or exce of last sample pred at -80°C for	analysis		-1.64	4.13	8.83
% difference from initial measurement  Long-term stab between date Describe cond	ility = Asses of first sa lition:	ss long-term s mple collection example: QC s	tability that on and date samples sto	t equals or exce of last sample	eds time analysis 2 years		-1.64	4.13	8.83
% difference from initial measurement  Long-term stab between date Describe cond All stability sa	ility = Assese of first sa lition: ample resu	ss long-term s mple collection example: QC s lts should be	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	eds time analysis 2 years				8.83
% difference from initial measurement  Long-term stab between date Describe cond All stability sa  Method name	ility = Assese of first sa lition: ample resu	ss long-term s mple collection example: QC s lts should be	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	eds time analysis 2 years ncentration	Init	tial Measurme	nt	
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #:	ility = Assese of first sa lition: ample resu	ss long-term somple collection example: QC solls should be confident and 4063	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement reds time analysis 2 years ncentration Replic	Init	tial Measurme Replicate	nt 2	Replicate 3
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #:	ility = Assese of first sa lition: ample resu	ss long-term s mple collection example: QC s lts should be	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	eds time analysis 2 years ncentration	Init	tial Measurme	nt 2	
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #: Matrix:	ility = Assese of first sa lition: ample resu	ss long-term somple collection example: QC solls should be confident and 4063	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement reds time analysis 2 years ncentration Replic	Init cate 1 /2015	tial Measurme Replicate	nt 2	Replicate 3
% difference from initial measurement  Long-term stab between date Describe cond All stability sa  Method name	ility = Assese of first sa lition: ample resu	ss long-term somple collection example: QC solls should be a collection of the colle	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement reds time analysis 2 years ncentration Replic	Init cate 1 /2015	tial Measurme Replicate 10/22/201	nt 2	Replicate 3
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #: Matrix:	ility = Assese of first sa lition: ample resu	ss long-term somple collection example: QC solls should be a collection of the colle	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	nitial measurement  eds time analysis 2 years ncentration  Replic 10/20/	Initicate 1 /2015 Lor	tial Measurme Replicate 10/22/201 ng-term stabili Replicate	nt 2 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #: Matrix:	ility = Assese of first sa lition: ample resu	ss long-term somple collection example: QC solls should be a collection of the colle	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	eds time analysis 2 years ncentration  Replic 10/20/	Initicate 1 /2015 Lor	tial Measurme Replicate 10/22/201 ng-term stabili	nt 2 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Replicate 3 11/9/2015
**difference from initial measurement  Long-term stab between date Describe cond All stability sa  Method name Method #: Matrix: Units:	ility = Assese of first sa lition: ample resu	ss long-term somple collection example: QC solls should be a collection of the colle	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	nitial measurement  eds time analysis 2 years ncentration  Replic 10/20/	Initicate 1 /2015 Lor	tial Measurme Replicate 10/22/201 ng-term stabili Replicate	nt 2 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:	ility = Assese of first sa lition: ample resu	ss long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	nitial measurement  eds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/	Initicate 1 /2015 Lor	tial Measurme Replicate 10/22/201 ng-term stabili Replicate	nt 2 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:	ility = Assese of first sa lition: ample resu	ss long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement  leds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/	Initicate 1 /2015 Lor	tial Measurmer Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Replicate 3 11/9/2015 Replicate 3 11/16/2017
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:	ility = Assese of first sa lition: ample resu	ss long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement  leds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/	Initicate 1 /2015 Lor	tial Measurme Replicate 10/22/201 ng-term stabili Replicate	nt 2 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:	ility = Assese of first sa lition: ample resu	ss long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal co	initial measurement  leds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/	Initicate 1 /2015 Lor	tial Measurmer Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2	Replicate 3 11/9/2015 Replicate 3 11/16/2017
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561	ility = Assese of first sa lition: ample resu	ss long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal con s in Urine	nitial measurement  eds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/ 3X  HU09562	Init cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2	Replicate 3 11/9/2015 Replicate 3 11/16/2017  Long-term stability
**difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #:  Matrix: Units:  Analyte:  MU09561	ility = Assete of first sa lition: ample resu	ss long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal con s in Urine  erm stability 28.0	nitial measurement  eds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/ 3X  HU09562	Init cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 41.9	nt 2	Replicate 3 11/9/2015 Replicate 3 11/16/2017  Long-term stability 42.0
**difference from initial measurement  Long-term stab between date Describe cond All stability sa  Method name Method #: Matrix: Units:  Analyte:  MU09561  Replica Replica	ility = Assete of first sa lition: ample resu :	ss long-term simple collection example: QC solls should be a constant of the collection of the collect	tability that on and date samples sto within ±15%	erm stability 28.0 28.1	nitial measurement  eds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/ 3X  HU09562  Replic Replic Replic	Init cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 Replicate 11/15/201 Initial measureme 41.9 39.7	nt 2	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 42.0 39.1
**difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	ility = Assete of first sa lition: ample resu :	ss long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L	tability that on and date samples sto within ±15%	t equals or exce of last sample ored at -80°C for 6 of nominal con s in Urine  erm stability 28.0	nitial measurement  eds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/ 3X  HU09562	Init cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 41.9	nt 2	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 42.0
**difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #:  Matrix: Units:  Analyte:  MU09561  Replica Replica	ility = Assete of first salition: ample resu : te 1 te 2 te 3	ss long-term simple collection example: QC solls should be a constant of the collection of the collect	tability that on and date samples sto within ±15%	erm stability 28.0 28.1	nitial measurement  eds time analysis 2 years ncentration  Replic 10/20/ Replic 11/14/ 3X  HU09562  Replic Replic Replic	Initicate 1 /2015 Lor cate 1 /2017  cate 1 cate 2 cate 3	Replicate 10/22/201 Replicate 11/15/201 Initial measureme 41.9 39.7	nt 2	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 42.0 39.1
% difference from initial measurement  Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:  Analyte:  MU09561  Replica Replica Replica	te 1 te 2 te 3 n	ss long-term simple collection example: QC solls should be a collection and 4063. Urine proof proof with the collection and 4063. Urine proof pr	tability that on and date samples sto within ±15%	erm stability 28.0 28.1 28.9	Initial measurement  Leds time analysis 2 years Incentration  Replic 10/20/ Replic 11/14/  3X  HU09562  Replic Replic Replic	Initiate 1 /2015 Lor cate 1 /2017  cate 1 cate 1 cate 2 cate 3 an from initial	Replicate 10/22/201 ng-term stabilin Replicate 11/15/201  Initial measureme 41.9 39.7 40.6	nt 2	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 42.0 39.1 40.7

### (11) 7-Methylxanthine (7X)

Stability - fill in ye									
	ability = Assess	for a minimum of 3	freeze-thaw cyc	les; conditions should	d mimic intended sample h	andling condition	ns.		
Describe condition:						Į į			
Bench-top stability	= Assess short-	term stability for ler	ngth of time nee	ded to handle study s	amples (typically at room	temperature)			
Describe condition:	original sample	s (not yet prepared	for instrument	analysis) stored at roc	om temperature for 8 hours				
Processed sample s	stability = Assess	s short-term stabilit	y of processed :	amples, including res	sident time in autosampler				
Describe condition:	processed sam	ples (ready for insti	rument analysis	stored at 15°C for 24	4 hours then stored at 5°C t	for 1 month			
All stability sample	results should be	e within ±15% of nor	minal concentra	tion.					
Run date									
Data for Initial, three fre Data for processed sar			run on 11/16/201 <i>i</i>						
	- 44								
Method name:		letabolites in Urine							
Method #:	4063								
Matrix:	Urine								
Units:	μmol/L								
Analyte:	7X				7X				
MU09561					HU09562				
11.303301	Initial	Three freeze-	Bench-top	Processed sample	11003302	Initial	Three freeze-thaw	Bench-top	Processed
	measurement		stability	stability		measurement	cycles	stability	sample stabilit
Replicate 1	43.3	47.8	49.3	45.7	Replicate 1	76.5	78.2	80.1	77.4
Replicate 2	46.7	47.8	49.0	48.4	Replicate 2	77.0	78.4	77.4	77.6
Replicate 3	47.4	48.8	50.0	49.2	Replicate 3	76.5	77.2	81.6	76.7
·									
Mean	45.8	48.1	49.4	47.8	Mean	76.7	77.9	79.7	77.2
% difference from		F 00		4.20	% difference from		1.65	2.00	0.74
initial measurement  Long-term stab  between date	<b>pility</b> = Asses e of first sa	mple collection	7.93 tability tha	t equals or exc	initial measurement seeds time e analysis		1.65	3.96	0.74
Long-term stab between date Describe cond	<b>bility</b> = Asses e of first sa dition:	ss long-term s mple collection example: QC s	tability tha	t equals or exc	initial measurement seeds time e analysis r 2 years		1.65	3.96	0.74
Long-term stab between date Describe conc All stability sa	oility = Asses e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	initial measurement seeds time e analysis r 2 years				0.74
Long-term stab between date Describe cond All stability sa Method name	oility = Asses e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	eeds time e analysis r 2 years oncentration	Init	tial Measurme	nt	
Long-term stab between date Describe cond All stability	oility = Asses e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and 4063	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	eeds time e analysis r 2 years oncentration Replic	Init	tial Measurme Replicate	nt 2	Replicate 3
Long-term stab between date Describe cond All stability sa Method name	oility = Asses e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	eeds time e analysis r 2 years oncentration	Init cate 1 /2015	tial Measurmer Replicate 10/22/201	nt 2   1	
Long-term stab between date Describe cond All stability stab Method name Method #:	oility = Asses e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and 4063	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	eeds time e analysis r 2 years oncentration Replic	Init cate 1 /2015	tial Measurmer Replicate 10/22/201	nt 2   1	Replicate 3
Long-term stab between date Describe cond All stability sa Method name Method #: Matrix:	oility = Asses e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and 4063 Urine	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	eeds time e analysis oncentration  Replic 10/20	Init cate 1 /2015	tial Measurmer Replicate 10/22/201	nt 2   1	Replicate 3 11/9/2015
Long-term stab between date Describe cond All stability sa Method name Method #: Matrix:	oility = Asses e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and 4063 Urine	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	eeds time e analysis oncentration  Replic 10/20,	Initicate 1 /2015 Lor	tial Measurmer Replicate 10/22/201 ng-term stabilit Replicate	nt 2   12.5   15	Replicate 3 11/9/2015 Replicate 3
Long-term stab between date Describe cond All stability sa Method name Method #: Matrix:	oility = Asses e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and 4063 Urine	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	eeds time e analysis oncentration  Replic 10/20	Initicate 1 /2015 Lor	tial Measurmer Replicate 10/22/201	nt 2   12.5   15	Replicate 3 11/9/2015
Long-term stab between date Describe cond All stability sa Method name Method #: Matrix:	pility = Asses e of first sa dition: ample resu	ss long-term s mple collection example: QC s lts should be Caffeine and 4063 Urine	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	eeds time e analysis oncentration  Replic 10/20,	Initicate 1 /2015 Lor	tial Measurmer Replicate 10/22/201 ng-term stabilit Replicate	nt 2   12.5   15	Replicate 3 11/9/2015 Replicate 3
Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:	pility = Asses e of first sa dition: ample resu	example: QCs lts should be Caffeine and 4063 Urine µmol/L	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	eeds time e analysis r 2 years oncentration  Replic 10/20,	Initicate 1 /2015 Lor	tial Measurmer Replicate 10/22/201 ng-term stabilit Replicate	nt 2   12.5   15	Replicate 3 11/9/2015 Replicate 3
Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:	pility = Asses e of first sa dition: ample resu	ss long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	initial measurement seeds time e analysis or 2 years oncentration  Replic 10/20,  Replic 11/14,	Initicate 1 /2015 Lor	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   12.5   15	Replicate 3 11/9/2015 Replicate 3 11/16/2017
Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:	pility = Asses e of first sa dition: ample resu	example: QCs lts should be Caffeine and 4063 Urine µmol/L	tability tha on and date samples st within ±15	t equals or exc e of last sample ored at -80°C fo % of nominal co	initial measurement seeds time e analysis or 2 years oncentration  Replic 10/20,  Replic 11/14,	Initicate 1 /2015 Lor	tial Measurmer Replicate 10/22/201 ng-term stabilit Replicate	nt 2   12.5   15	Replicate 3 11/9/2015 Replicate 3
Long-term stab between date Describe cond All stability sa Method name Method #: Matrix: Units:	pility = Asses e of first sa dition: ample resu	ss long-term s mple collectio example: QC s Its should be Caffeine and 4063 Urine µmol/L	tability that on and date samples st within ±15.	t equals or exc e of last sample ored at -80°C fo % of nominal co	initial measurement seeds time e analysis or 2 years oncentration  Replic 10/20,  Replic 11/14,	Initicate 1 /2015 Lor	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   12.5   12.5   12.7   12	Replicate 3 11/9/2015 Replicate 3 11/16/2017
initial measurement  Long-term stab between date Describe conc All stability sa  Method name Method #: Matrix: Units:  Analyte: MU09561	pility = Asses e of first sa dition: ample resu	ss long-term s mple collectio example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  7X  Initial measureme	tability that on and date samples st within ±15.	t equals or exc e of last sample ored at -80°C fo % of nominal co es in Urine	initial measurement seeds time e analysis or 2 years oncentration  Replic 10/20,  Replic 11/14,  7X  HU09562	Initional Initio	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	nt 2   12.5   12.5   12.7   12	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability
initial measurement  Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	pility = Asses e of first sa dition: ample resul	cs long-term s mple collectio example: QC s lts should be  Caffeine and 4063 Urine  µmol/L  7X  Initial measureme 37.4	tability that on and date samples st within ±15.	t equals or exc e of last sample ored at -80°C fo % of nominal co es in Urine	initial measurement  seeds time e analysis oncentration  Replic 10/20,  Replic 11/14,  7X  HU09562	Initional 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 59.5	nt 2   12.5   12.5   12.7   12	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 60.4
initial measurement  Long-term stab between date Describe conc All stability sa  Method name Method #: Matrix: Units:  Analyte: MU09561  Replica Replica	pility = Asses e of first sa dition: ample results:	cs long-term s mple collectio example: QC s lts should be  Caffeine and 4063 Urine  µmol/L  7X  Initial measureme 37.4 39.5	tability that on and date samples st within ±15.	t equals or except of last sample ored at -80°C for of nominal control of the sample o	initial measurement  seeds time e analysis or 2 years oncentration  Replic 10/20,  Replic 11/14,  7X  HU09562  Replic Replic	Initional 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 59.5 63.3	nt 2   12.5   12.5   12.7   12	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 60.4 58.8
initial measurement  Long-term stab between date Describe conc All stability sa Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	pility = Asses e of first sa dition: ample results:	cs long-term s mple collectio example: QC s lts should be  Caffeine and 4063 Urine  µmol/L  7X  Initial measureme 37.4	tability that on and date samples st within ±15.	t equals or exc e of last sample ored at -80°C fo % of nominal co es in Urine	initial measurement  seeds time e analysis oncentration  Replic 10/20,  Replic 11/14,  7X  HU09562	Initional 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 59.5	nt 2   12.5   12.5   12.7   12	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 60.4
initial measurement  Long-term stab between date Describe conc All stability sa  Method name Method #: Matrix: Units:  Analyte: MU09561  Replica Replica	pility = Assesse of first sadition: ample results: atte 1 atte 2 atte 3	ss long-term s mple collectic example: QCs lts should be  Caffeine and 4063 Urine  µmol/L  7X  Initial measureme 37.4 39.5 39.5	tability that on and date samples st within ±15.	t equals or except of last sample ored at -80°C for some of some of the some o	reeds time e analysis r 2 years oncentration  Replic 10/20,  Replic 11/14,  7X  HU09562  Replic Replic Replic	Initioate 1 /2015 Lor cate 1 /2017  cate 1 cate 2 cate 3	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 59.5 63.3 57.5	nt 2   12.5   12.5   12.7   12	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 60.4 58.8 62.2
initial measurement  Long-term stab between date Describe conc All stability sa  Method name Method #: Matrix: Units:  Analyte: MU09561  Replica Replica Replica Mea	pility = Assese of first sadition: ample results: atte 1 atte 2 atte 3	cs long-term s mple collectio example: QC s lts should be  Caffeine and 4063 Urine  µmol/L  7X  Initial measureme 37.4 39.5	tability that on and date samples st within ±15.	t equals or except of last sample ored at -80°C for of nominal control of the sample o	reeds time e analysis r 2 years concentration  Replic 10/20,  Replic 11/14,  7X  HU09562  Replic Replic Replic Replic Replic	Initional Control Cont	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 59.5 63.3	nt 2   12.5   12.5   12.7   12	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 60.4 58.8
initial measurement  Long-term stab between date Describe conc All stability sa  Method name Method #: Matrix: Units:  Analyte: MU09561  Replica Replica	pility = Assese of first sadition: ample results: atte 1 atte 2 atte 3	ss long-term s mple collectic example: QCs lts should be  Caffeine and 4063 Urine  µmol/L  7X  Initial measureme 37.4 39.5 39.5	tability that on and date samples st within ±15.	t equals or except of last sample ored at -80°C for some of some of the some o	reeds time e analysis r 2 years oncentration  Replic 10/20,  Replic 11/14,  7X  HU09562  Replic Replic Replic	Initional Control Cont	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 59.5 63.3 57.5	nt 2   12.5   12.5   12.7   12	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 60.4 58.8 62.2

### (12) Theophylline (13X)

Stability - fill in yel	llow shaded cel	ls							
•			freeze-thaw cyc	es; conditions shoul	d mimic intended sample h	andling condition	is.		
Describe condition:						Į i			
Bench-top stability	= Assess short-	term stability for ler	gth of time need	ded to handle study s	samples (typically at room	temperature)			
Describe condition:	original sample	s (not yet prepared	for instrument a	nalysis) stored at roo	om temperature for 8 hours	;			
Processed sample st	ability = Asses	s short-term stabilit	y of processed s	amples, including res	sident time in autosampler				
Describe condition:	processed sam	ples (ready for instr	rument analysis)	stored at 15°C for 24	4 hours then stored at 5°C	for 1 month			
All stability sample re	esults should be	within ±15% of nor	minal concentrat	ion.					
Run date Data for Initial, three free Data for processed sam			run on 11/16/2017						
Method name:	Caffeine and M	letabolites in Urine							
	4063	ctabolites in office							
	Urine								
	μmol/L								
Analyte:	13X				13X				
MU09561					HU09562				
	Initial	Three freeze-	Bench-top	Processed sample		Initial	Three freeze-thaw	Bench-top	Processed
	measurement	thaw cycles	stability	stability		measurement	cycles	stability	sample stabilit
Replicate 1	3.12	3.47	3.63	3.18	Replicate 1	6.56	6.80	6.90	6.38
Replicate 2	3.50	3.51	3.50	3.37	Replicate 2	6.54	6.93	6.57	6.58
Replicate 3	3.62	3.56	3.52	3.32	Replicate 3	6.54	6.64	6.77	6.11
Mean	3.41	3.51	3.55	3.29	Mean	6.55	6.79	6.75	6.36
% difference from		2.93	4.00	-3.61	% difference from		3.72	3.05	-2.90
initial measurement					initial measurement				_
Describe cond All stability sa		•	•	ored at -80°C fo 6 of nominal co	•				
Method name:	:	Caffeine and	Metabolite	s in Urine		Init	ial Measurmei	nt	
Method #:		4063						1.0	
Matrix:		Urine			I Replic	cate 1	Replicate		eplicate 3
						cate 1 /2015	Replicate	2 R	eplicate 3
Units:					10/20,	/2015	10/22/201	2 R	eplicate 3 11/9/2015
		μmol/L			10/20,	/2015 Lor	10/22/201 ng-term stabilit	2 R 5 :	11/9/2015
		μποι/Ε				/2015 Lor	10/22/201	2 R 5 :	•
		μποιγε			10/20, Replic	/2015 Lor cate 1	10/22/201 ng-term stabilit Replicate	2 R 5 :: ty 2 R	11/9/2015 eplicate 3
		µшог/ с			10/20,	/2015 Lor cate 1	10/22/201 ng-term stabilit	2 R 5 :: ty 2 R	11/9/2015
Analyte:		13X			10/20, Replic	/2015 Lor cate 1	10/22/201 ng-term stabilit Replicate	2 R 5 :: ty 2 R	11/9/2015 eplicate 3
·					10/20, Replic 11/14,	/2015 Lor cate 1	10/22/201 ng-term stabilit Replicate	2 R 5 :: ty 2 R	11/9/2015 eplicate 3
·		13X			10/20,  Replic  11/14,	/2015 Lor cate 1	10/22/201 ng-term stabilit Replicate 11/15/201	2 R 5 :: ty 2 R 7 1	eplicate 3 1/16/2017
·		13X Initial	nt loss t	orm stability	10/20,  Replic  11/14,	/2015 Lor cate 1	10/22/201 ng-term stabilit Replicate 11/15/201	2 R 5 :	eplicate 3 1/16/2017 .ong-term
MU09561		13X Initial measureme	_	erm stability	10/20, Replic 11/14,  13X HU09562	/2015 Lor cate 1 /2017	10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme	2 R 5 :	eplicate 3 1/16/2017 Long-term stability
MU09561 Replicat	te 1	Initial measureme 4.29	_	3.36	10/20,  Replic 11/14,  13X  HU09562	/2015 Lor cate 1 /2017	10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 7.76	2 R 5 :	eplicate 3 1/16/2017 ong-term stability 8.11
MU09561  Replication	te 1 te 2	Initial measureme 4.29 4.07	_	3.36 3.30	10/20,  Replic 11/14,  13X  HU09562  Replic Replic	/2015 Lor cate 1 /2017 cate 1 cate 1 cate 2	10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 7.76 8.63	2 R 5 :	eplicate 3 1/16/2017 Long-term stability
MU09561 Replicat	te 1 te 2	Initial measureme 4.29	_	3.36	10/20,  Replic 11/14,  13X  HU09562	/2015 Lor cate 1 /2017 cate 1 cate 1 cate 2	10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 7.76	2 R 5 :	eplicate 3 1/16/2017  cong-term stability 8.11
MU09561  Replicat  Replicat	te 1 te 2 te 3	Initial measureme 4.29 4.07 2.62	_	3.36 3.30 3.32	10/20,  Replice 11/14,  13X  HU09562  Replice Replice Replice	/2015 Lor cate 1 /2017  cate 1 cate 1 cate 2 cate 3	lnitial measureme 7.76 8.63 7.91	2 R 5 :	eplicate 3 1/16/2017 .ong-term stability 8.11 7.91 7.94
Replicat	te 1 te 2 te 3	Initial measureme 4.29 4.07	_	3.36 3.30	10/20,  Replic 11/14,  13X  HU09562  Replic Replic	/2015 Lor cate 1 /2017  cate 1 cate 1 cate 2 cate 3	10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 7.76 8.63	2 R 5 :	eplicate 3 1/16/2017 .ong-term stability 8.11 7.91
MU09561 Replicat Replicat	te 1 te 2 te 3	Initial measureme 4.29 4.07 2.62	_	3.36 3.30 3.32	10/20, Replice 11/14, 13X HU09562  Replice Rep	/2015 Lor cate 1 /2017 cate 1 cate 1 cate 2 cate 3	lnitial measureme 7.76 8.63 7.91	2 R 5 :	eplicate 3 1/16/2017 .ong-term stability 8.11 7.91 7.94

### (13) Paraxanthine (17X)

				1 100 1 11					
Freeze and thaw st	tability = Assess	for a minimum of 3	freeze-thaw cy	cles; conditions should	mimic intended sample h	andling condition	is.		
Describe condition:	three times froz	en at -80°C and the	n thawed (3 fre	eze-thaw cycles)					
					mples (typically at room				
					n temperature for 8 hours				
					dent time in autosampler				
Describe condition:	processed sam	iples (ready for insti	rument analysis	s) stored at 15 C for 24 I	hours then stored at 5°C	for 1 month			
All stability sample	results should be	within +15% of nor	minal concentra	ation					
All stability sample	Tesuits siloulu be	WIGHIII 11370 OF HO	minar concerni	ation.					
Run date Data for Initial, three fr Data for processed sa			run on 11/16/201	7					
Method name:	Caffeine and M	etabolites in Urine							
Method #:	4063								
Matrix:	Urine								
Units:	μmol/L								
Analyte:	17X				17X				
MU09561					HU09562				
	Initial	Three freeze-	Bench-top	Processed sample		Initial	Three freeze-thaw	Bench-top	
B 11 4 5	measurement	thaw cycles	stability	stability	5 !!	measurement	cycles	stability	sample stabili
Replicate 1	36.7	40.0	40.2	36.8	Replicate 1	75.3	75.7	76.2	70.3
Replicate 2 Replicate 3	40.6 39.3	40.0 40.0	40.7 41.2	38.7 38.1	Replicate 2 Replicate 3	76.3 77.1	75.9 75.3	75.3 74.9	71.8 69.3
neplicate 5	33.3	40.0	41.2	30.1	Neplicate 3	77.1	13.3	14.3	03.3
Mean	38.9	40.0	40.7	37.9	Mean	76.2	75.6	75.5	70.5
ivieari									
% difference from			4.72	2.57	% difference from		0.70	1.01	7.56
% difference from initial measurement Long-term stal between dat	bility = Asses e of first sai	2.92 s long-term s mple collection	n and dat	-2.57 at equals or exce	initial measurement eeds time analysis		-0.79	-1.01	-7.56
% difference from initial measurement Long-term stal between dat Describe cond	bility = Asses e of first sau dition:	2.92 ss long-term s mple collectic example: QC s	tability tha	at equals or exce	initial measurement eds time analysis 2 years	-	-0.79	-1.01	-7.56
% difference from initial measurement Long-term stal between dat Describe cond	bility = Asses e of first sai dition: ample resul	2.92 ss long-term s mple collectic example: QC s	tability tha on and dat samples st within ±15	at equals or exce e of last sample cored at -80°C for % of nominal co	initial measurement eds time analysis 2 years		-0.79		-7.56
% difference from initial measurement  Long-term stal between dat Describe condall stability s	collity = Assesse of first saidition: ample resul	z.92  as long-term s mple collectic example: QCs ts should be	tability tha on and dat samples st within ±15	at equals or exce e of last sample cored at -80°C for % of nominal co	initial measurement eds time analysis 2 years ncentration	Init	tial Measurme	nt	
% difference from initial measurement  Long-term stal between dat Describe condall stability s  Method name Method #:	coility = Assesse of first saudition: ample resul	s long-term s mple collection example: QC s Its should be Caffeine and 4063	tability tha on and dat samples st within ±15	at equals or exce e of last sample cored at -80°C for % of nominal co	initial measurement eds time analysis 2 years ncentration Replic	Init	tial Measurme Replicate	nt 2	Replicate 3
% difference from initial measurement.  Long-term stal between dat Describe cone All stability s  Method name Method #: Matrix:	pility = Asses e of first sau dition: ample resul	s long-term s mple collection example: QC s Its should be Caffeine and 4063 Urine	tability tha on and dat samples st within ±15	at equals or exce e of last sample cored at -80°C for % of nominal co	initial measurement eds time analysis 2 years ncentration	Init cate 1 /2015	tial Measurme Replicate 10/22/201	nt 2	
% difference from initial measurement  Long-term stal between dat Describe cond All stabilitys  Method name	pility = Asses e of first sau dition: ample resul	s long-term s mple collection example: QC s Its should be Caffeine and 4063	tability tha on and dat samples st within ±15	at equals or exce e of last sample cored at -80°C for % of nominal co	initial measurement reds time analysis 2 years ncentration Replic 10/20	Init cate 1 /2015	Replicate 10/22/201	nt 2   1.5   ty	Replicate 3 11/9/2015
% difference from initial measurement  Long-term stal between dat Describe cone All stability s  Method name Method #: Matrix:	pility = Asses e of first sau dition: ample resul	s long-term s mple collection example: QC s Its should be Caffeine and 4063 Urine	tability tha on and dat samples st within ±15	at equals or exce e of last sample cored at -80°C for % of nominal co	initial measurement reds time analysis 2 years ncentration Replic 10/20	Init cate 1 /2015 Lor cate 1	Replicate 10/22/201 1g-term stabili Replicate	nt 2   15   15   17   17   17   17   17   17	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement  Long-term stal between dat Describe cone All stability s  Method name Method #: Matrix:	pility = Asses e of first sau dition: ample resul	s long-term s mple collection example: QC s Its should be Caffeine and 4063 Urine	tability tha on and dat samples st within ±15	at equals or exce e of last sample cored at -80°C for % of nominal co	initial measurement reds time analysis 2 years ncentration Replic 10/20	Init cate 1 /2015 Lor cate 1	Replicate 10/22/201	nt 2   15   15   17   17   17   17   17   17	Replicate 3 11/9/2015
% difference from initial measurement between dat Describe cone All stability s Method name Method #: Matrix: Units:	oility = Asses e of first san dition: ample resul	s long-term s mple collection example: QC s Its should be Caffeine and 4063 Urine	tability tha on and dat samples st within ±15	at equals or exce e of last sample cored at -80°C for % of nominal co	initial measurement reds time analysis 2 years ncentration Replic 10/20	Init cate 1 /2015 Lor cate 1	Replicate 10/22/201 1g-term stabili Replicate	nt 2   15   15   17   17   17   17   17   17	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement.  Long-term stal between dat Describe cone All stability s  Method name Method #: Matrix:	oility = Asses e of first san dition: ample resul	z.92  as long-term s mple collection example: QC s Its should be  Caffeine and 4063 Urine  µmol/L	tability tha on and dat samples st within ±15	at equals or exce e of last sample cored at -80°C for % of nominal co	initial measurement  eds time analysis 2 years ncentration  Replic 10/20,  Replic 11/14,	Init cate 1 /2015 Lor cate 1	Replicate 10/22/201 1g-term stabili Replicate	nt 2   15   15   17   17   17   17   17   17	Replicate 3 11/9/2015 Replicate 3
% difference from initial measurement between dat Describe condall stability something measurement with the condal stability s	oility = Asses e of first san dition: ample resul	2.92  as long-term s mple collection example: QCs Its should be  Caffeine and 4063 Urine  µmol/L  17X  Initial	tability that on and dat samples st within ±15 Metabolite	at equals or exce e of last sample cored at -80°C for % of nominal con es in Urine	initial measurement  eds time analysis 2 years ncentration  Replic 10/20,  Replic 11/14,	Init cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate 11/15/201	nt 2   1.5   1.7   2   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term
# difference from initial measurement Long-term stal between dat Describe cone All stability s  Method name Method #: Matrix: Units:  Analyte: MU09561	pility = Asses e of first sai dition: ample resul	2.92  as long-term s mple collection example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  17X  Initial measureme	tability that on and dat samples st within ±15 Metabolite	at equals or exce e of last sample cored at -80°C for % of nominal con es in Urine	initial measurement  eds time analysis 2 years ncentration  Replic 10/20,  Replic 11/14,  17X  HU09562	Init cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 Replicate 11/15/201 Initial measureme	nt 2   1.5   1.7   2   1.7	Replicate 3 11/9/2015 Replicate 3 11/16/2017  Long-term stability
% difference from initial measurement between dat between dat Describe cone All stability s Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	pility = Assesse of first sandition: ample resul	2.92  as long-term s mple collection example: QCs Its should be  Caffeine and 4063 Urine  µmol/L  17X  Initial measureme 23.4	tability that on and dat samples st within ±15 Metabolite	at equals or exce e of last sample cored at -80°C for % of nominal cor es in Urine term stability	initial measurement  reds time analysis 2 years ncentration  Replic 10/20, Replic 11/14, 17X HU09562	Init cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 Replicate 11/15/201 Replicate 11/15/201	nt 2   1.5   1.7   2   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 66.2
% difference from initial measurement between dat between dat Describe condall stability something measurement with the condensation of the conden	bility = Assesse of first saidition: ample resulte:	2.92  Is long-term s mple collection example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  17X  Initial measureme 23.4 23.7	tability that on and dat samples st within ±15 Metabolite	at equals or exce e of last sample cored at -80°C for % of nominal cor es in Urine term stability 23.5 24.6	initial measurement  reds time analysis 2 years ncentration  Replic 10/20, Replic 11/14, 17X HU09562  Replic Replic	Init cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 Replicate 11/15/201 Replicate 11/15/201 Initial measureme 58.7 65.3	nt 2   1.5   1.7   2   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 66.2 65.2
% difference from initial measurement between dat between dat Describe concall stability so Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	bility = Assesse of first saidition: ample resulte:	2.92  as long-term s mple collection example: QCs Its should be  Caffeine and 4063 Urine  µmol/L  17X  Initial measureme 23.4	tability that on and dat samples st within ±15 Metabolite	at equals or exce e of last sample cored at -80°C for % of nominal cor es in Urine term stability	initial measurement  reds time analysis 2 years ncentration  Replic 10/20, Replic 11/14, 17X HU09562	Init cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 Replicate 11/15/201 Replicate 11/15/201	nt 2   1.5   1.7   2   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 66.2
**difference from initial measurement   Long-term stal   between dat   Describe cone   All stability s   Method name   Method #:   Matrix:   Units:   Analyte:   MU09561   Replica   Replica	bility = Assesse of first saidition: ample resulte:  atte 1 atte 2 atte 3	2.92  Is long-term s mple collection example: QC s Its should be  Caffeine and 4063 Urine  µmol/L  17X  Initial measureme 23.4 23.7	tability that on and dat samples st within ±15 Metabolite	at equals or exce e of last sample cored at -80°C for % of nominal cor es in Urine term stability 23.5 24.6	initial measurement  reds time analysis 2 years ncentration  Replic 10/20, Replic 11/14, 17X HU09562  Replic Replic	Initicate 1 /2015 Lor cate 1 /2017  cate 1 cate 2 cate 3	Replicate 10/22/201 Replicate 11/15/201 Replicate 11/15/201 Initial measureme 58.7 65.3	nt 2   1.5   1.7   2   1.7	Replicate 3 11/9/2015  Replicate 3 11/16/2017  Long-term stability 66.2 65.2

### (14) Theobromine (37X)

		ells							
Freeze and thaw st	ellow shaded ce ability = Assess		freeze-thaw cyc	es; conditions should	mimic intended sample h	andling condition	IS.		
		ozen at -80°C and the			·	J			
Bench-top stability	= Assess short-	-term stability for len	gth of time nee	ded to handle study sa	amples (typically at room	temperature)			
					m temperature for 8 hours				
					dent time in autosampler				
Describe condition:	processed sar	npies (ready for instr	ument analysis)	stored at 15 C for 24	hours then stored at 5°C f	for 1 month			
All stability sample	results should b	ne within ±15% of nor	ninal concentrat	ion.					
Run date Data for Initial, three fro Data for processed san		ench-top stability were r run on 12/07/2017	un on 11/16/2017						
Method name:	Caffaire and A	Metabolites in Urine							
Method #:	4063	vietabolites III Offfie							
Matrix:	Urine								
Units:	μmol/L								
Analyte:	37X				37X				
MU09561					HU09562				
	Initial measurement	Three freeze-	Bench-top	Processed sample		Initial measurement	Three freeze-thaw	Bench-top	Processed
Replicate 1	32.8	thaw cycles 36.5	stability 36.7	stability 33.6	Replicate 1	35.7	cycles 36.7	stability 36.5	sample stabilit
Replicate 2	34.4	36.5	36.1	36.3	Replicate 2	37.2	36.4	37.0	37.3
Replicate 3	35.8	35.9	35.9	36.3	Replicate 3	36.7	36.8	36.3	35.9
Mean	34.3	36.3	36.2	35.4	Mean	36.5	36.6	36.6	36.3
% difference from initial measurement		5.73	5.53	3.11	% difference from initial measurement		0.27	0.18	-0.73
		mple collectio	n and date	of last sample	analysis				
Describe cond	lition:	mple collection	and date	•	e analysis r 2 years				
Describe cond	lition: ample resu	mple collection	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	e analysis r 2 years	Init	ial Measurme	nt	
Describe cond All stabilitysa Method name	lition: ample resu	example: QC sollection example: QC sollection its should be sollected.	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	e analysis r 2 years encentration		ial Measurme Renlicate		enlicate 3
Describe cond All stabilitys: Method name Method #:	lition: ample resu	example: QC solls should be well to the control of	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	e analysis 72 years Incentration Replic	cate 1	Replicate	2 R	eplicate 3
Describe cond All stability sa Method name Method #: Matrix:	lition: ample resu	example collection example: QC so ults should be well Caffeine and 4063 Urine	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	e analysis r 2 years encentration	cate 1 /2015	Replicate 10/22/201	2 R	eplicate 3 1/9/2015
Describe cond All stability sa Method name Method #:	lition: ample resu	example: QC solls should be well to the control of	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	e analysis 72 years Incentration Replic 10/20	cate 1 /2015 Lor	Replicate 10/22/201 ig-term stabili	2 Ro .5 1 ty	1/9/2015
Describe cond All stability sa Method name Method #: Matrix:	lition: ample resu	example collection example: QC so ults should be well Caffeine and 4063 Urine	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	Replic	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 Ro	1/9/2015 eplicate 3
Describe cond All stability sa Method name Method #: Matrix:	lition: ample resu	example collection example: QC so ults should be well Caffeine and 4063 Urine	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	e analysis 72 years Incentration Replic 10/20	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ig-term stabili	2 Ro	1/9/2015
Describe cond All stability sa Method name Method #: Matrix: Units:	lition: ample resu	example collection example: QC so ults should be well Caffeine and 4063 Urine	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	Replic	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 Ro	1/9/2015 eplicate 3
Describe cond All stability sa Method name Method #: Matrix:	lition: ample resu	example collection example: QC sollection lits should be solded Caffeine and 4063 Urine	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	Replice 11/14/	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 Ro	1/9/2015 eplicate 3
Describe cond All stability sa Method name Method #: Matrix: Units:	lition: ample resu	example collection example: QC sollection exa	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	Replic 11/14,	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate 11/15/201	2 R <sub>1</sub> .5 1 ty 2 R <sub>1</sub> .7 1.	1/9/2015 eplicate 3 1/16/2017
Describe cond All stability sa Method name Method #: Matrix: Units:	lition: ample resu	example collection example: QC sollection lits should be solded Caffeine and 4063 Urine	and date amples sto within ±15%	of last sample ored at -80°C for 6 of nominal co	Replic 11/14,	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate	2 R. 1 ty 2 R. 7 7 1	1/9/2015 eplicate 3 1/16/2017 ong-term
Describe cond All stability sa Method name Method #: Matrix: Units:	lition: ample resu	example collection example: QC sollection exa	on and date camples sto within ±159  Metabolite	of last sample ored at -80°C for 6 of nominal co	Replic 11/14,	cate 1 /2015 Lor cate 1	Replicate 10/22/201 ng-term stabili Replicate 11/15/201	2 R. 1 ty 2 R. 7 7 1	1/9/2015 eplicate 3 1/16/2017
Describe cond All stability sa Method name Method #: Matrix: Units:	lition: ample resu	caffeine and 4063 Urine  µmol/L  Initial	on and date camples sto within ±159  Metabolite	of last sample ored at -80°C for 6 of nominal co s in Urine	Replic 11/14,	Cate 1 /2015 Lor Cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201	2 R. 1 ty 2 R. 7 7 1	1/9/2015 eplicate 3 1/16/2017 ong-term
Describe cond All stability sa Method name Method #: Matrix: Units: Analyte:	lition: ample resu ::	caffeine and 4063 Urine  µmol/L  Initial  measuremen	on and date camples sto within ±159  Metabolite	of last sample pred at -80°C for 6 of nominal co s in Urine  erm stability	Replic 11/14,  37X  HU09562	Cate 1 /2015 Lor cate 1 /2017	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201 Initial measureme	2 R. 1 ty 2 R. 7 7 1	1/9/2015 eplicate 3 1/16/2017 ong-term stability
Describe cond All stability so Method name Method #: Matrix: Units:  Analyte: MU09561  Replica	lition: ample resu :: te 1 te 2	cample collection example: QC sollection example: QC sollection leading to the collection  Caffeine and collection 4063 Urine  µmol/L  37X  Initial measurement 19.5	on and date camples sto within ±159  Metabolite	of last sample ored at -80°C for 6 of nominal co	Replic 11/14/ 37X HU09562	Cate 1 /2015 Lor cate 1 /2017  Cate 1 cate 1 cate 2	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 37.8	2 R. 1 ty 2 R. 7 7 1	1/9/2015 eplicate 3 1/16/2017 ong-term stability 41.4
Describe cond All stability so Method name Method #: Matrix: Units:  Analyte: MU09561  Replica Replica	te 1 te 2 te 3	cample collection example: QC sollection example: QC sollection caffeine and collection 4063 Urine pmol/L  37X  Initial measurement 19.5 21.0	on and date camples sto within ±159  Metabolite	of last sample pred at -80°C for 6 of nominal co s in Urine  erm stability 19.1 19.0	Replic 11/14/ 37X HU09562  Replic Rep	cate 1 /2015 Lor cate 1 /2017  cate 1 cate 1 cate 2 cate 3	Replicate 10/22/201 ng-term stabilit Replicate 11/15/201  Initial measureme 37.8 43.3	2 R. 1 ty 2 R. 7 7 1	1/9/2015 eplicate 3 1/16/2017 ong-term stability 41.4 40.5

### (15) Caffeine (137X)

three times froz - Assess short-t	for a minimum of 3 for a minimum of 3 for at -80°C and there	n thawed (3 freez	e-thaw cycles)	ıld min	nic intended sample h	andling condition	5.		
three times froz - Assess short-t	en at -80°C and ther	n thawed (3 freez	e-thaw cycles)		· ·				
	erm stability for len	ath of time need							
original sample:		igui oi uille lieeu	ed to handle study	sampl	les (typically at room	temperature)			
	s (not yet prepared f	for instrument an	alysis) stored at re	oom te	mperature for 8 hours				
					t time in autosampler				
processed sam	ples (ready for instr	ument analysis)	stored at 15°C for	24 hou	rs then stored at 5°C	for 1 month			
sults should be	within ±15% of non	minal concentrati	on.						
		run on 11/16/2017							
Caffeine and Me	etabolites in Urine								
4063									
Urine									
ımol/L									
137X					137X				
					HU09562				
Initial	Three freeze-	Bench-top	Processed sample			Initial	Three freeze-thaw	Bench-top	Processed
measurement	thaw cycles	stability	stability			measurement	cycles	stability	sample stabilit
20.9	22.2		21.5			30.2	30.1	30.2	29.5
	22.4		23.2			29.8	29.8	29.7	29.9
22.4	22.1	22.3	25.2		керисаte 3	29.5	30.0	29.4	30.0
22.0	22.2	22.3	23.3		Mean	29.8	30.0	29.8	29.8
	0.01	1 21	C 7C		% difference from		0.45	0.22	-0.11
	0.91	1.21	5.75		initial measurement		0.45	-0.22	-0.11
tion:	example: QC s	amples sto	red at -80°C f	or 2 y	years				
	Caffeine and I	Metabolites	in Urine			Init	al Measurme	nt	
	4063				Repli	cate 1	Replicate	2	Replicate 3
	Urine				10/20	/2015	10/22/201	.5	11/9/2015
	umol/L						g-term stabilit	v	
					Renli				Replicate 3
							•		11/16/2017
					11/14	12011	11/15/201	.1	11/10/201/
	137X				137X				
					HU09562				
	1						1121 1		
									Long-term
	measuremer	nt Long-te	rm stability					ent	stability
e 1	18.0		15.9		Repli	cate 1	26.6		26.1
e 2	17.6		16.7		Repli	cate 2	28.2		25.9
e 3	17.8		16.1		Repli	cate 3	27.3		26.6
	47.0		16.2				27.4		26.2
	17.8		16.2		Me	an	27.4		26.2
	cze-thaw, and berole stability was in Caffeine and M 1063 Urine Limol/L 137X  Initial measurement 20.9 22.4 22.0  Iity = Asses of first sail tion: Imple resul  e 1 e 2	Initial measurement 22.9 22.1 22.0 22.2 0.91  Ility = Assess long-terms of first sample collectic tion: example: QC smple results should be carefully a smooth of the collection of the colle	Initial measurement example: QC samples sto mple results should be within ±15%  Caffeine and Metabolites in Urine  137X  Initial measurement 20.9 22.2 22.4 22.4 22.4 22.1 22.3 22.0 24.2 22.4 22.4 22.1 22.3 22.0 25.2 22.2 22.3 25.0 25.2 25.3 25.3 25.3 25.3 25.3 25.3 25.3	Initial measurement than collection and date of last sample collection and date of last sample ton:    Caffeine and Metabolites in Urine   Caffeine and Metabolites in Urine   Caffeine and Metabolites	Caffeine and Metabolites in Urine   Caffeine and Metabolites   Caffeine and Metabolites   Caffeine and Metabolites in Urine   Caffeine a	Caffeine and Metabolites in Urine   Caffeine   Caffein			Caffeine and Metabolites in Urine   Caffeine and Metabolites in

### A. Precision

(1) AAMU

# Caffeine and Caffeine Metabolites NHANES 2014-2014

NHANES 20	714-2014												
Precision - fill in ye	ellow shaded cel	ls											
Total relative star	ndard deviation	should be ≤ 15% (0	CV ≤ 15%)										
Method name:	Caffeine and M	etabolites in Urin	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 2017	bench QC chara	cterization											
6/13/2017 to 8/25/	2017												
AAMU - medi	um bench Q0						AAMU - hig	h bench QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	59.9	57.8	58.9	1.10	1.10	6927	1	201	176	189	156	156	71065
2	59.2	52.6	55.9	10.9	10.9	6250	2	186	175	181	30.3	30.3	65161
3	54.9	68.0	61.5	42.9	42.9	7552	3	197	192	195	6.25	6.25	75661
4	56.6	56.1	56.4	0.06	0.06	6351	4	204	185	195	90.3	90.3	75661
5	61.5	61.3	61.4	0.01	0.01	7540	5	180	188	184	16.0	16.0	67712
6	56.1	60.2	58.2	4.20	4.20	6763	6	204	190	197	49.0	49.0	77618
7	53.6	57.8	55.7	4.41	4.41	6205	7	165	195	180	225	225	64800
8	57.7	60.9	59.3	2.56	2.56	7033	8	189	189	189	0.00	0.00	71442
9	53.8	56.6	55.2	1.96	1.96	6094	9	178	175	177	2.25	2.25	62305
10	47.7	55.0	51.4	13.32	13.32	5274	10	183	180	182	2.25	2.25	65885
Grand sum	1147	Grand mean	57.4				Grand sum	3732	Grand mean	187			
				Rel Std							Rel Std		
	Sum squares	Mean Sq Error	Std Dev						Mean Sq Error	Std Dev	Dev (%)		
Within Run	163	16.3	4.04	7.03			Within Run	1155	116	10.7	5.76		
Between Run	173	19.2	1.21	2.10			Between Run		102	0.00	0.00		
Total	336		4.21	7.34			Total	2071		10.7	5.76		

# (2) 1-Methyluric Acid (1U)

Precision - fill in ye	ellow shaded cel	ls											
Total relative sta	ndard deviation :	should be ≤ 15% (0	CV ≤ 15%)										
Method name:	Caffeine and Me	etabolites in Urin	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 2017	7 bench QC chara	cterization											
6/13/2017 to 8/25/	/2017												
1U - medium l	ench QC						1U - high ber	nch QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	68.3	72.5	70.4	4.41	4.41	9912	1	167	159	163	16.0	16.0	53138
2	68.3	72.5	70.4	4.41	4.41	9912	2	155	160	158	6.25	6.25	49613
3	62.6	71.7	67.2	20.7	20.7	9018	3	155	175	165	100	100	54450
4	65.9	70.7	68.3	5.76	5.76	9330	4	148	158	153	25.0	25.0	46818
5	68.0	64.9	66.5	2.40	2.40	8831	5	152	170	161	81.0	81.0	51842
6	66.7	69.3	68.0	1.69	1.69	9248	6	168	174	171	9.00	9.00	58482
7	67.0	76.2	71.6	21.2	21.2	10253	7	146	147	147	0.25	0.25	42925
8	61.3	66.5	63.9	6.76	6.76	8166	8	169	197	183	196	196	66978
9	67.1	89.6	78.4	127	127	12277	9	142	146	144	4.00	4.00	41472
10	55.4	60.0	57.7	5.29	5.29	6659	10	140	162	151	121	121	45602
Grand sum	1365	Grand mean	68.2				Grand sum	3190	Grand mean	160			
				Rel Std							Rel Std		
		Mean Sq Error							Mean Sq Error		Dev (%)		
Within Run	398	39.8	6.31	9.25			Within Run	1117	112	10.6	6.63		
Between Run	514	57.2	2.94	4.31			Between Run		279	9.16	5.74		
Total	913		6.96	10.2			Total	3631		14.0	8.77		

# (3) 3-Methyluric Acid (3U)

Precision - fill in ye	allaw shadad aal	II.											
Total relative sta			TV < 15%)										
Method name:		etabolites in Urino											
		etabolites in Urini	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 2017	7 bench QC chara	cterization											
6/13/2017 to 8/25/	/2017												
3U - medium l	bench QC						3U - high ber	nch QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	0.587	0.617	0.602	0.00	0.00	0.72	1	1.29	1.24	1.27	0.00	0.00	3.20
2	0.688	0.727	0.708	0.00	0.00	1.00	2	1.24	1.26	1.25	0.00	0.00	3.13
3	0.662	0.448	0.555	0.01	0.01	0.62	3	1.05	1.14	1.10	0.00	0.00	2.40
4	0.686	0.598	0.642	0.00	0.00	0.82	4	1.37	1.05	1.21	0.03	0.03	2.93
5	0.627	0.479	0.553	0.01	0.01	0.61	5	1.14	0.92	1.03	0.01	0.01	2.13
6	0.566	0.671	0.619	0.00	0.00	0.77	6	1.29	1.10	1.20	0.01	0.01	2.86
7	0.556	0.732	0.644	0.01	0.01	0.83	7	1.12	1.19	1.16	0.00	0.00	2.67
8	0.409	0.492	0.451	0.00	0.00	0.41	8	1.28	1.32	1.30	0.00	0.00	3.38
9	0.545	0.620	0.583	0.00	0.00	0.68	9	1.05	1.03	1.04	0.00	0.00	2.16
10	0.604	0.601	0.603	0.00	0.00	0.73	10	1.24	0.99	1.12	0.02	0.02	2.49
Grand sum	11.9	Grand mean	0.596				Grand sum	23.3	Grand mean	1.17			
				Rel Std							Rel Std		
	Sum squares	Mean Sq Error	Std Dev	Dev (%)				Sum squares	Mean Sq Error	Std Dev	Dev (%)		
Within Run	0.066	0.007	0.081	13.7			Within Run	0.13	0.013	0.115	9.90		
Between Run	0.085	0.009	0.037	6.26			Between Run	0.16	0.018	0.047	3.99		
Total	0.151		0.090	15.0			Total	0.29		0.124	10.7		

# (4) 7-Methyluric Acid (7U)

Precision - fill in ye													
Total relative sta	ndard deviation	should be ≤ 15% (	CV ≤ 15%)										
Method name:	Caffeine and M	etabolites in Urin	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 201	7 bench QC chara	cterization											
6/13/2017 to 8/25	/2017												
7U - medium l	bench QC						7U - high ber	nch QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	12.1	13.1	12.6	0.25	0.25	318	1	22.1	18.7	20.4	2.89	2.89	832
2	14.4	15.0	14.7	0.09	0.09	432	2	23.1	22.6	22.9	0.06	0.06	1044
3	13.7	12.7	13.2	0.25	0.25	348	3	23.9	22.1	23.0	0.81	0.81	1058
4	15.8	13.5	14.7	1.32	1.32	429	4	23.6	23.8	23.7	0.01	0.01	1123
5	14.5	12.5	13.5	1.00	1.00	365	5	20.3	21.3	20.8	0.25	0.25	865
6	13.7	14.4	14.1	0.12	0.12	395	6	24.2	23.4	23.8	0.16	0.16	1133
7	13.7	13.2	13.5	0.06	0.06	362	7	21.7	21.2	21.5	0.06	0.06	920
8	14.3	13.5	13.9	0.16	0.16	386	8	22.3	23.8	23.1	0.56	0.56	1063
9	12.9	12.6	12.8	0.02	0.02	325	9	20.5	21.2	20.9	0.12	0.12	869
10	13.7	13.3	13.5	0.04	0.04	365	10	21.1	22.4	21.8	0.42	0.42	946
Grand sum	273	Grand mean	13.6				Grand sum	443	Grand mean	22.2			
				Rel Std							Rel Std		
	Sum squares	Mean Sq Error	Std Dev	Dev (%)				Sum squares	Mean Sq Error	Std Dev	Dev (%)		
Within Run	6.64	0.66	0.81	5.98			Within Run	10.7	1.07	1.03	4.67		
Between Run	9.04	1.00	0.41	3.03			Between Run	28.7	3.19	1.03	4.65		
Total	15.7		0.91	6.70			Total	39.4		1.46	6.59		

### (5) 1,3-Dimethyluric Acid (13U)

Precision - fill in ye	ellow shaded cel	Is											
Total relative sta	ndard deviation	should be ≤ 15% (0	CV ≤ 15%)										
Method name:	Caffeine and Me	etabolites in Urin	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 201	7 bench QC chara	cterization											
6/13/2017 to 8/25/	2017												
13U - medium	bench QC						13U - high be	ench QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	8.90	9.39	9.15	0.06	0.06	167	1	22.0	20.5	21.3	0.56	0.56	903
2	9.67	9.93	9.80	0.02	0.02	192	2	19.2	18.7	19.0	0.06	0.06	718
3	8.48	9.10	8.79	0.10	0.10	155	3	20.9	21.9	21.4	0.25	0.25	916
4	8.83	9.31	9.07	0.06	0.06	165	4	21.0	19.9	20.5	0.30	0.30	836
5	9.12	8.20	8.66	0.21	0.21	150	5	17.9	18.5	18.2	0.09	0.09	662
6	9.38	9.33	9.36	0.00	0.00	175	6	20.5	20.4	20.5	0.00	0.00	836
7	8.77	8.97	8.87	0.01	0.01	157	7	20.2	19.2	19.7	0.25	0.25	776
8	10.20	9.78	9.99	0.04	0.04	200	8	22.5	22.6	22.6	0.00	0.00	1017
9	8.63	9.72	9.18	0.30	0.30	168	9	20.9	19.1	20.0	0.81	0.81	800
10	9.84	9.48	9.66	0.03	0.03	187	10	20.3	19.8	20.1	0.06	0.06	804
Grand sum	185	Grand mean	9.25				Grand sum	406	Grand mean	20.3			
				Rel Std							Rel Std		
		Mean Sq Error		Dev (%)					Mean Sq Error		Dev (%)		
Within Run	1.65	0.17	0.41	4.39			Within Run	4.79	0.48	0.69	3.41		
Between Run	3.56	0.40	0.34	3.67			Between Run	27.9	3.10	1.15	5.64		
Total	5.22		0.53	5.73			Total	32.7		1.34	6.59		

## (6) 1,7-Dimethyluric Acid (17U)

Precision - fill in ye													
Total relative sta	ndard deviation	should be ≤ 15% (0	CV ≤ 15%)										
Method name:	Caffeine and Me	etabolites in Urin	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 201	7 bench QC chara	cterization											
6/13/2017 to 8/25/	/2017												
17U - medium	bench QC						17U - high be	ench QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	35.9	37.0	36.5	0.30	0.30	2657	1	82.4	71.4	76.9	30.3	30.3	11827
2	36.0	35.7	35.9	0.02	0.02	2570	2	86.0	78.8	82.4	13.0	13.0	13580
3	34.7	33.7	34.2	0.25	0.25	2339	3	81.2	80.4	80.8	0.16	0.16	13057
4	36.2	37.9	37.1	0.72	0.72	2745	4	84.8	83.8	84.3	0.25	0.25	14213
5	38.1	37.7	37.9	0.04	0.04	2873	5	82.6	82.4	82.5	0.01	0.01	13613
6	36.7	37.6	37.2	0.20	0.20	2760	6	84.3	79.8	82.1	5.06	5.06	13464
7	35.3	36.6	36.0	0.42	0.42	2585	7	80.2	83.5	81.9	2.72	2.72	13399
8	38.3	41.1	39.7	1.96	1.96	3152	8	85.3	86.8	86.1	0.56	0.56	14809
9	36.0	31.7	33.9	4.62	4.62	2292	9	80.5	75.4	78.0	6.50	6.50	12152
10	36.4	36.1	36.3	0.02	0.02	2628	10	79.5	87.2	83.4	14.8	14.8	13894
Grand sum	728.7	Grand mean	36.4				Grand sum	1636.3	Grand mean	81.8			
				Rel Std							Rel Std		
		Mean Sq Error	Std Dev						Mean Sq Error		Dev (%)		
Within Run	17.1	1.71	1.31	3.59			Within Run	147	14.7	3.83	4.68		
Between Run	52.0	5.77	1.42	3.91			Between Run	135	15.0	0.41	0.50		
Total	69.1		1.93	5.31			Total	282		3.85	4.71		

## (7) 3,7-Dimethyluric Acid (37U)

Precision - fill in ye	llow shaded cel	Is											
Total relative star			V ≤ 15%)										
Method name:	Caffeine and Me	etabolites in Urine	e ,										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 2017		cterization											
6/13/2017 to 8/25/		cterization											
6/15/2017 (0 6/25/	2017												
37U - medium	bench QC						37U - high be	nch QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.18	1.10	1.14	0.00	0.00	2.60	1	1.62	1.53	1.58	0.00	0.00	4.96
2	1.15	1.10	1.13	0.00	0.00	2.53	2	1.61	1.20	1.41	0.04	0.04	3.95
3	1.20	1.04	1.12	0.01	0.01	2.51	3	1.67	1.27	1.47	0.04	0.04	4.32
4	1.05	1.11	1.08	0.00	0.00	2.33	4	1.56	1.37	1.47	0.01	0.01	4.29
5	1.10	1.11	1.11	0.00	0.00	2.44	5	1.36	1.22	1.29	0.00	0.00	3.33
6	1.20	1.28	1.24	0.00	0.00	3.08	6	1.49	1.31	1.40	0.01	0.01	3.92
7	1.13	1.14	1.14	0.00	0.00	2.58	7	1.36	1.41	1.39	0.00	0.00	3.84
8	1.15	1.20	1.18	0.00	0.00	2.76	8	1.35	1.37	1.36	0.00	0.00	3.70
9	1.11	1.05	1.08	0.00	0.00	2.33	9	1.53	1.41	1.47	0.00	0.00	4.32
10	1.01	0.98	0.99	0.00	0.00	1.98	10	1.41	1.43	1.42	0.00	0.00	4.03
Grand sum	22.4	Grand mean	1.12				Grand sum	28.5	Grand mean	1.42			
				Rel Std							Rel Std		
		Mean Sq Error							Mean Sq Error				
Within Run	0.026	0.003	0.051	4.55			Within Run	0.22	0.022	0.15	10.4		
Between Run	0.075	0.008	0.053	4.77			Between Run	0.11	0.012	0.00	0.00		
Total	0.10		0.074	6.59			Total	0.33		0.15	10.4		

# (8) 1,3,7-Trimethyluric Acid (137U)

Precision - fill in ye														
Total relative sta	ndard deviation	should be ≤ 15% (0	CV ≤ 15%)											
Method name:	Caffeine and Me	etabolites in Urino	e											
Method #:	4063													
Matrix:	Urine													
Units:	μmol/L													
Data Source: 2017	7 bench QC chara	cterization												
6/13/2017 to 8/25/	/2017													
137U - mediur	m bench QC						137	'U - high b	ench QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2		Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	3.70	3.61	3.66	0.00	0.00	26.7		1	7.45	7.44	7.45	0.00	0.00	111
2	3.85	3.75	3.80	0.00	0.00	28.9		2	7.90	7.48	7.69	0.04	0.04	118
3	3.75	3.82	3.79	0.00	0.00	28.7		3	7.62	7.84	7.73	0.01	0.01	120
4	3.63	3.76	3.70	0.00	0.00	27.3		4	8.02	7.54	7.78	0.06	0.06	121
5	3.72	3.71	3.72	0.00	0.00	27.6		5	7.55	7.59	7.57	0.00	0.00	115
6	3.66	3.65	3.66	0.00	0.00	26.7		6	7.42	7.17	7.30	0.02	0.02	106
7	3.65	3.83	3.74	0.01	0.01	28.0		7	7.48	7.68	7.58	0.01	0.01	115
8	3.65	3.71	3.68	0.00	0.00	27.1		8	7.56	7.58	7.57	0.00	0.00	115
9	3.63	3.68	3.66	0.00	0.00	26.7		9	7.18	7.34	7.26	0.01	0.01	105
10	3.62	3.60	3.61	0.00	0.00	26.1		10	7.14	7.33	7.24	0.01	0.01	105
Grand sum	74.0	Grand mean	3.70				Gi	rand sum	150	Grand mean	7.52			
				Rel Std								Rel Std		
	Sum squares	Mean Sq Error	Std Dev	Dev (%)					Sum squares	Mean Sq Error	Std Dev	Dev (%)		
Within Run	0.040	0.0040	0.063	1.70			W	ithin Run	0.31	0.031	0.18	2.35		
Between Run	0.067	0.0075	0.042	1.14			Bet	ween Run	0.71	0.079	0.15	2.05		
Total	0.107		0.076	2.04				Total	1.02		0.23	3.12		

### (9) 1-Methylxanthine (1X)

Precision - fill in ye	ellow shaded cel	Is											
		should be ≤ 15% (0	CV ≤ 15%)										
Method name:		etabolites in Urino											
Method #:	4063	eta borries in onin											
Matrix:	Urine												
Units:	μmol/L												
Data Source: 201		cterization											
6/13/2017 to 8/25													
0/13/2017 to 6/23/	2017												
1X - medium l	ench QC						1X - high l	ench QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	36.7	41.1	38.9	4.84	4.84	3026	1	102	103	103	0.25	0.25	21013
2	39.9	40.7	40.3	0.16	0.16	3248	2	106	97	102	18.9	18.9	20665
3	44.5	38.2	41.4	9.92	9.92	3420	3	104	107	106	2.25	2.25	22261
4	40.7	40.6	40.7	0.00	0.00	3305	4	105	104	105	0.25	0.25	21841
5	44.8	44.4	44.6	0.04	0.04	3978	5	103	105	104	1.00	1.00	21632
6	39.2	44.4	41.8	6.76	6.76	3494	6	112	102	107	25.0	25.0	22898
7	40.8	42.2	41.5	0.49	0.49	3445	7	101	103	102	1.00	1.00	20808
8	42.5	42.6	42.6	0.00	0.00	3621	8	108	104	106	4.00	4.00	22472
9	40.4	37.8	39.1	1.69	1.69	3058	9	100	91	96	18.1	18.1	18260
10	41.5	40.8	41.2	0.12	0.12	3387	10	101	97	99	4.62	4.62	19543
Grand sum	824	Grand mean	41.2				Grand sui	n 2055	Grand mean	103			
				Rel Std							Rel Std		
		Mean Sq Error							Mean Sq Error		Dev (%)		
Within Run	48.1	4.81	2.19	5.32			Within Ru		15.1	3.88	3.78		
Between Run	49.3	5.48	0.58	1.41			Between R		24.4	2.16	2.10		
Total	97.4		2.27	5.51			Total	370		4.44	4.32		

## (10) 3-Methylxanthine (3X)

Precision - fill in ye													
Total relative sta	ndard deviation	should be ≤ 15% (	CV ≤ 15%)										
Method name:	Caffeine and M	etabolites in Urin	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 201	7 bench QC chara	cterization											
6/13/2017 to 8/25	2017												
3X - medium l	ench QC						3X - high ber	nch QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	26.4	24.1	25.3	1.32	1.32	1275	1	46.3	44.9	45.6	0.49	0.49	4159
2	28.8	27.3	28.1	0.56	0.56	1574	2	48.1	44.5	46.3	3.24	3.24	4287
3	30.5	30.3	30.4	0.01	0.01	1848	3	49.9	48.2	49.1	0.72	0.72	4812
4	29.3	28.3	28.8	0.25	0.25	1659	4	46.5	46.1	46.3	0.04	0.04	4287
5	29.2	30.0	29.6	0.16	0.16	1752	5	46.5	45.1	45.8	0.49	0.49	4195
6	27.8	26.8	27.3	0.25	0.25	1491	6	42.2	43.7	43.0	0.56	0.56	3689
7	29.8	29.0	29.4	0.16	0.16	1729	7	47.5	48.5	48.0	0.25	0.25	4608
8	28.1	30.3	29.2	1.21	1.21	1705	8	46.9	46.7	46.8	0.01	0.01	4380
9	27.7	25.9	26.8	0.81	0.81	1436	9	45.8	45.9	45.9	0.00	0.00	4204
10	28.8	26.7	27.8	1.10	1.10	1540	10	46.5	50.5	48.5	4.00	4.00	4705
Grand sum	565	Grand mean	28.3				Grand sum	930	Grand mean	46.5			
				Rel Std							Rel Std		
	Sum squares	Mean Sq Error	Std Dev	Dev (%)				Sum squares	Mean Sq Error	Std Dev	Dev (%)		
Within Run	11.7	1.17	1.08	3.82			Within Run	19.6	1.96	1.40	3.01		
Between Run	42.5	4.73	1.33	4.72			Between Run	54.5	6.05	1.43	3.08		
Total	54.2		1.72	6.08			Total	74.1		2.00	4.30		

### (11) 7-Methylxanthine (7X)

Precision - fill in ye	ellow shaded cel	lls											
		should be ≤ 15% (0	CV ≤ 15%)										
Method name:	Caffeine and M	etabolites in Urin	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 2017	7 bench QC chara	cterization											
6/13/2017 to 8/25/	/2017												
7X - medium k	ench QC						7X - high ben	ich QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	47.3	48.0	47.7	0.12	0.12	4541	1	86.5	79.5	83.0	12.3	12.3	13778
2	47.1	46.3	46.7	0.16	0.16	4362	2	79.4	72.6	76.0	11.6	11.6	11552
3	47.5	46.4	47.0	0.30	0.30	4409	3	75.2	83.3	79.3	16.4	16.4	12561
4	45.4	46.8	46.1	0.49	0.49	4250	4	80.0	72.9	76.5	12.6	12.6	11689
5	47.4	47.4	47.4	0.00	0.00	4494	5	75.8	75.4	75.6	0.04	0.04	11431
6	49.2	48.3	48.8	0.20	0.20	4753	6	84.5	75.6	80.1	19.8	19.8	12816
7	48.9	50.1	49.5	0.36	0.36	4901	7	74.8	77.6	76.2	1.96	1.96	11613
8	48.2	47.6	47.9	0.09	0.09	4589	8	76.8	74.5	75.7	1.32	1.32	11446
9	46.8	48.3	47.6	0.56	0.56	4522	9	78.1	74.9	76.5	2.56	2.56	11705
10	45.4	44.7	45.1	0.12	0.12	4059	10	70.6	70.3	70.5	0.02	0.02	9926
Grand sum	947	Grand mean	47.4				Grand sum	1538	Grand mean	76.9			
				Rel Std							Rel Std		
		Mean Sq Error							Mean Sq Error		Dev (%)		
Within Run	4.83	0.48	0.69	1.47			Within Run	157	15.7	3.96	5.15		
Between Run	28.9	3.21	1.17	2.47			Between Run	198	22.0	1.78	2.31		
Total	33.7		1.36	2.87			Total	355		4.34	5.65		

## (12) Theophylline (13X)

Precision - fill in ye													
		should be ≤ 15% (											
Method name:	Caffeine and M	etabolites in Urin	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 201	7 bench QC chara	cterization											
6/13/2017 to 8/25	/2017												
13X - medium	bench QC						13X - high be	ench QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	3.63	3.41	3.52	0.01	0.01	24.8	1	7.00	6.96	6.98	0.00	0.00	97
2	3.53	3.54	3.54	0.00	0.00	25.0	2	7.43	6.90	7.17	0.07	0.07	103
3	3.56	3.47	3.52	0.00	0.00	24.7	3	7.24	7.48	7.36	0.01	0.01	108
4	3.53	3.48	3.51	0.00	0.00	24.6	4	7.27	7.12	7.20	0.01	0.01	104
5	3.45	3.77	3.61	0.03	0.03	26.1	5	7.17	7.57	7.37	0.04	0.04	109
6	3.41	3.46	3.44	0.00	0.00	23.6	6	7.52	7.10	7.31	0.04	0.04	107
7	3.48	3.68	3.58	0.01	0.01	25.6	7	7.21	7.27	7.24	0.00	0.00	105
8	3.37	3.48	3.43	0.00	0.00	23.5	8	6.92	6.27	6.60	0.11	0.11	87
9	3.42	3.33	3.38	0.00	0.00	22.8	9	6.53	6.01	6.27	0.07	0.07	79
10	3.25	3.38	3.32	0.00	0.00	22.0	10	6.21	5.85	6.03	0.03	0.03	73
Grand sum	69.6	Grand mean	3.48				Grand sum	139	Grand mean	6.95			
				Rel Std							Rel Std		
	Sum squares	Mean Sq Error	Std Dev	Dev (%)				Sum squares	Mean Sq Error	Std Dev	Dev (%)		
Within Run	0.12	0.012	0.11	3.15			Within Run	0.76	0.076	0.28	3.97		
Between Run	0.15	0.017	0.05	1.43			Between Run	4.20	0.47	0.44	6.36		
Total	0.27		0.12	3.46			Total	4.96		0.52	7.50		

### (13) Paraxanthine (17X)

Precision - fill in ye	ellow shaded cel	Is											
Total relative star			CV ≤ 15%)										
Method name:	Caffeine and M	etabolites in Urino	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 2017		cterization											
6/13/2017 to 8/25/	2017												
17X - medium	bench QC						17X - high be	nch QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	38.0	37.4	37.7	0.09	0.09	2843	1	73.6	68.1	70.9	7.56	7.56	10039
2	38.2	37.4	37.8	0.16	0.16	2858	2	74.1	70.7	72.4	2.89	2.89	10484
3	36.2	35.9	36.1	0.02	0.02	2599	3	73.5	72.9	73.2	0.09	0.09	10716
4	37.1	37.4	37.3	0.02	0.02	2775	4	71.8	69.8	70.8	1.00	1.00	10025
5	38.4	40.2	39.3	0.81	0.81	3089	5	70.7	71.7	71.2	0.25	0.25	10139
6	38.0	38.7	38.4	0.12	0.12	2941	6	73.7	69.2	71.5	5.06	5.06	10210
7	38.5	38.8	38.7	0.02	0.02	2988	7	69.2	71.0	70.1	0.81	0.81	9828
8	37.7	37.8	37.8	0.00	0.00	2850	8	73.6	70.3	72.0	2.72	2.72	10354
9	38.0	37.3	37.7	0.12	0.12	2835	9	70.9	71.1	71.0	0.01	0.01	10082
10	38.3	38.2	38.3	0.00	0.00	2926	10	69.4	69.6	69.5	0.01	0.01	9661
Grand sum	757.5	Grand mean	37.9				Grand sum	1424.9	Grand mean	71.2			
				Rel Std							Rel Std		
		Mean Sq Error							Mean Sq Error				
Within Run	2.76	0.28	0.52	1.39			Within Run	40.8	4.08	2.02	2.84		
Between Run	13.6	1.52	0.79	2.08			Between Run	20.9	2.33	0.00	0.00		
Total	16.4		0.95	2.50			Total	61.7		2.02	2.84		

## (14) Theobromine (37X)

Precision - fill in ye													
Total relative sta	ndard deviation	should be ≤ 15% (0	CV ≤ 15%)										
Method name:	Caffeine and M	etabolites in Urin	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 2017	7 bench QC chara	cterization											
6/13/2017 to 8/25/	/2017												
37X - medium	bench QC						37X - high be	ench QC					
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	35.9	35.2	35.6	0.12	0.12	2528	1	38.1	36.1	37.1	1.00	1.00	2753
2	38.5	37.1	37.8	0.49	0.49	2858	2	39.8	37.4	38.6	1.44	1.44	2980
3	35.7	34.2	35.0	0.56	0.56	2443	3	34.8	37.9	36.4	2.40	2.40	2643
4	36.4	37.1	36.8	0.12	0.12	2701	4	37.0	35.8	36.4	0.36	0.36	2650
5	33.3	34.5	33.9	0.36	0.36	2298	5	33.7	33.1	33.4	0.09	0.09	2231
6	35.7	37.1	36.4	0.49	0.49	2650	6	38.3	35.5	36.9	1.96	1.96	2723
7	37.3	35.8	36.6	0.56	0.56	2672	7	35.0	36.1	35.6	0.30	0.30	2528
8	36.0	34.9	35.5	0.30	0.30	2513	8	36.5	33.7	35.1	1.96	1.96	2464
9	35.6	35.1	35.4	0.06	0.06	2499	9	35.0	35.1	35.1	0.00	0.00	2457
10	35.8	34.8	35.3	0.25	0.25	2492	10	35.2	36.2	35.7	0.25	0.25	2549
Grand sum	716	Grand mean	35.8				Grand sum	720	Grand mean	36.0			
				Rel Std							Rel Std		
	Sum squares	Mean Sq Error	Std Dev	Dev (%)				Sum squares	Mean Sq Error	Std Dev	Dev (%)		
Within Run	6.65	0.67	0.82	2.28			Within Run	19.5	1.95	1.40	3.88		
Between Run	21.6	2.40	0.93	2.60			Between Run	35.7	3.96	1.00	2.78		
Total	28.2		1.24	3.46			Total	55.2		1.72	4.77		

### (15) Caffeine (137X)

Precision - fill in ye	ellow shaded ce	lls											
Total relative sta	ndard deviation	should be ≤ 15% (0	V ≤ 15%)										
Method name:	Caffeine and M	etabolites in Urin	e										
Method #:	4063												
Matrix:	Urine												
Units:	μmol/L												
Data Source: 2017	7 bench QC chara	cterization											
6/13/2017 to 8/25/	/2017												
137X - mediur	n bench QC						137X - high b	ench QC					
Quality mater	ial 1 -medium	n bench QC					Quality mate	rial 2 -high b	ench QC				
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	21.6	20.5	21.1	0.30	0.30	886	1	28.6	28.1	28.4	0.06	0.06	1607
2	24.5	24.5	24.5	0.00	0.00	1201	2	32.1	30.8	31.5	0.42	0.42	1978
3	22.9	23.3	23.1	0.04	0.04	1067	3	29.4	31.0	30.2	0.64	0.64	1824
4	23.4	24.2	23.8	0.16	0.16	1133	4	30.4	30.6	30.5	0.01	0.01	1861
5	22.1	22.5	22.3	0.04	0.04	995	5	28.3	27.6	28.0	0.12	0.12	1562
6	22.3	21.6	22.0	0.12	0.12	964	6	28.9	25.9	27.4	2.25	2.25	1502
7	22.3	23.3	22.8	0.25	0.25	1040	7	28.2	28.8	28.5	0.09	0.09	1625
8	23.2	23.3	23.3	0.00	0.00	1081	8	30.6	28.2	29.4	1.44	1.44	1729
9	22.6	25.2	23.9	1.69	1.69	1142	9	29.9	32.2	31.1	1.32	1.32	1928
10	22.7	22.4	22.6	0.02	0.02	1017	10	28.8	28.1	28.5	0.12	0.12	1619
Grand sum	458.4	Grand mean	22.9				Grand sum	586.5	Grand mean	29.3			
				Rel Std							Rel Std		
	Sum squares	Mean Sq Error	Std Dev	Dev (%)				Sum squares	Mean Sq Error	Std Dev	Dev (%)		
Within Run	5.26	0.53	0.73	3.16			Within Run	13.0	1.30	1.14	3.88		
Between Run	18.7	2.08	0.88	3.84			Between Run	35.3	3.92	1.15	3.90		
Total	24.0		1.14	4.98			Total	48.2		1.61	5.51		

## B. LOD, Specificity, and Fit for Intended Use

LOD, specificity and f	it for intended use - fil	l in yellow shaded cells	
Method name:	Caffeine and Metabol	ites in Urine	
Method #:	4063		
Matrix:	Urine		
Units:	μmol/L		
	Limit of Detection (LOD)(µM)	Interferences successfully checked in at least 50 human	Accuracy, precision, LOD, specificity and stability meet performance specifications
Analytes	, ,,, ,	samples	for intended use
AAMU	0.10	yes	yes
1U	0.05	yes	yes
3U	0.10	yes	yes
7U	0.04	yes	yes
13U	0.02	yes	yes
17U	0.02	yes	yes
37U	0.03	yes	yes
137U	0.005	yes	yes
1X	0.03	yes	yes
3X	0.04	yes	yes
7X	0.02	yes	yes
13X	0.01	yes	yes
17X	0.006	yes	yes
37X	0.004	yes	yes
137X	0.003	yes	yes

### **Appendix B: Ruggedness testing**

#### A. Principles and Proposals

#### (1) Conversion of AFMU to AAMU

Principle: 5-acetylamino-6-formylamino-3-methyluracil (AFMU) is an unstable product of caffeine metabolism that will gradually decompose into 5-acetylamino-6-amino-3-methyluracil (AAMU). In order to accurately quantify this metabolite, the conversion of AFMU to AAMU is forced to completion via alkaline sample treatment during sample preparation.

Proposal: The incubation time (i.e., time allowed for alkaline sample treatment) and the concentration of HCl used in the re-acidification of the sample following alkaline treatment were varied.

#### (2) Sample filtration

Principle: All samples, calibrators and quality control materials are filtered using a  $0.2 \mu m$  nylon filter prior to analysis. Filtration removes particulate matter that may interfere with HPLC-MS/MS measurements or cause problems (e.g., reduced HPLC column life).

Proposal: Filtration with a  $0.2~\mu m$  nylon filter was compared with removal of particulates by centrifugation.

#### (3) Sample matrix and mobile phase composition

Principle: Samples are prepared in a buffer that matches the matrix of the starting mobile phase composition. The injection of samples in which the sample matrix differs significantly from the mobile phase may lead to poor chromatographic performance.

Proposal: The relative strength (in terms of formic acid and methanol content) of the buffer solution in which samples were prepared was varied. The formic acid content of the HPLC mobile phases was also varied.

### B. Findings

### (1) Conversion of AFMU to AAMU

	Effect of alkaline con	treatment incul scentration (μΜ		Effect of HCl concentration in re-acidification on concentration (μΜ)			
Analyte	Method specification (30 min)	Low test condition (10 min)	High test condition (60 min)	Method specification (2 N)	Low test condition (1 N)	High test condition (3 N)	
1X	41.2	39.1	42.5	41.2	44.3	44.4	
3X	27.5	27.4	28.4	27.5	29.2	28.7	
7X	36.0	37.0	37.5	36.0	38.4	38.5	
13X	3.6	3.8	3.9	3.6	3.9	3.8	
17X	25.0	23.8	24.6	25.0	25.0	25.3	
37X	21.3	20.0	21.1	21.3	22.0	21.9	
137X	16.3	15.9	16.9	16.3	16.7	17.6	
1U	48.5	48.0	48.7	48.5	52.7	53.0	
3U	0.4	0.4	0.4	0.4	0.4	0.4	
7U	11.2	10.8	11.1	11.2	11.8	12.0	
13U	5.6	5.4	5.7	5.6	6.2	5.8	
17U	33.1	31.5	34.0	33.1	36.0	34.1	
37U	0.8	0.8	0.8	0.8	0.8	0.9	
137U	2.9	2.7	2.7	2.9	3.2	3.3	
AAMU	39.2	37.5	37.4	39.2	41.0	39.6	

No changes were observed for AAMU, caffeine, or any other caffeine metabolite when incubation time and HCl concentration were varied within the range tested.

### (2) Sample filtration:

	Effect of sample filtration on concentration (μΜ)			
Analyte	Method specifies (filtered)	Alternative condition (centrifuge)		
1X	41.2	41.3		
3X	27.5	27.3		
7X	36.0	37.1		
13X	3.6	3.9		
17X	25.0	25.7		
37X	21.3	21.1		
137X	16.3	17.0		
1U	48.5	50.2		
3U	0.4	0.4		
7U	11.2	11.4		
13U	5.6	5.7		
17U	33.1	32.0		
37U	0.8	0.8		
137U	2.9	2.9		
AAMU	39.2	38.1		

No changes were observed when particulates were removed from samples using centrifugation versus filtration.

### (3) Sample matrix and mobile phase composition

	Effect of sample dilution buffer	Effect of formic acid in mobile phase	
Analyte	strength (relative strength to mobile	on concentration	
	phase) on concentration (μM)	on concentration	

2017						
	Method specifies (1X)	Low condition (0.5X)	High condition (2X)	Method specifies (0.05%)	Low condition (0.01%)	High condition (0.1%)
1X	41.2	42.0	41.2	41.2	40.8	40.3
3X	27.5	27.9	28.5	27.5	27.3	27.9
7X	36.0	37.5	36.5	36.0	36.2	36.5
13X	3.6	3.8	3.7	3.6	4.2	4.1
17X	25.0	24.5	24.4	25.0	22.4	24.1
37X	21.3	21.5	20.9	21.3	21.0	20.1
137X	16.3	16.8	16.4	16.3	16.4	16.6
1U	48.5	50.5	51.2	48.5	50.2	48.4
3U	0.4	0.4	0.4	0.4	0.4	0.4
7U	11.2	11.3	11.6	11.2	11.5	10.9
13U	5.6	5.6	5.8	5.6	5.3	5.6
17U	33.1	33.5	33.0	33.1	31.3	32.9
37U	0.8	0.8	0.8	0.8	0.8	0.9
137U	2.9	2.9	2.9	2.9	2.8	3.0
AAMU	39.2	38.4	37.4	39.2	37.6	39.7

Samples are prepared in a buffer that matches the matrix of the starting mobile phase composition. The injection of samples in which the sample matrix differs significantly from the mobile phase may lead to poor chromatographic performance.

## **Appendix C: Extinction Coefficients**

Analyte	Extinction coefficient (m <sup>-1</sup> cm <sup>-1</sup> )	Wavelength (nm)	рН	Reference
AAMU	18000	264	<7.0	35
1U	11400	284	3.0	36
3U	11100	287	3.0	36
7U	11400	286	3.0	36
13U	11600	287	3.0	36
17U	11000	286	3.0	36
1X	10200	266	5.0	37
3X	10000	271	5.0	37
7X	9600	269	5.0	37
13X	10407	270	6.0	37
17X	9549	267	2	38
37 X	10100	273	7.0	39
137X	9900	273	7.0	39

### **Appendix D: Analysis Parameters**

# MS/MS transitions\*

	Positive ion mode <sup>†</sup>				Negative ion mode <sup>‡</sup>			
Compound	RT (min)	MRM transition (m/z)		MS parameter (V)	RT (min)	MRM transition (m/z)		MS parameter (Volt)
		Precursor	Product	CE		Precursor	Product	CE
1X	1.86	167	110	24	1.86	165	108	-24
3X	1.69	167	124	24	1.69	165	122	-24
7X	1.46	167	150	24	-	-	-	_
		167	124	24	-	-	-	_
13X	-	-	-	-	4.19	179	164	-26
	-	-	-	-		179	122	-28
17X	3.98	181	124	24	-	-	-	_
		181	96	32	-	-	-	_
37X	2.93	181	138	24	-	-	-	_
		181	163	24	-	-	-	_
137X	6.36	195	138	24	-	-	-	_
		195	110	32	-	-	-	_
1U	-	-	-	-	1.50	181	138	-22
	-	-	-	-		181	110	-24
3U	-	-	-	-	1.00	181	138	-20
	-	-	-	-		181	110	-26
7U	-	-	-	-	1.28	181	138	-20
	-	-	-	-		181	110	-24
13U	-	-	-	-	2.59	195	110	-30
	-	-	-	-		195	180	-24
17U	-	-	-	-	3.74	195	137	-32
	-	-	-	-		195	180	-24
37U	-	-	-	-	1.82	195	124	-26
	-	_	_	-		195	180	-24
137U	-	_	_	_	5.13	209	194	-24
	-	_	_	-		209	137	-32
AAMU	-	_	_	-	0.74	197	140	-16
	-	-	-	-		197	127	-20

<sup>\*</sup> MS/MS transition used for quantitation appears in bold.

<sup>&</sup>lt;sup>†</sup> For positive ion mode, the following global conditions were used: ionization voltage = 1850 V; interface temperature = 700 °C; entrance potential = 10V; declustering potential = 25; cell exit potential = 11.

<sup>&</sup>lt;sup>‡</sup> For negative ion mode, the following global conditions were used: ionization voltage = -1850V; interface temperature = 700 °C; entrance potential = -10V; declustering potential = -25; cell exit potential = -16.

### Internal standard MS/MS transitions

	(IS) Positive ion mode§				(IS) Negative ion mode**			
Compound (IS)	RT (min)	narameter I			MS parameter (V)			
		Precursor	Product	CE		Precursor	Product	CE
1X (IS)	1.86	174	115	24	1.86	172	113	-24
3X (IS)	1.69	174	129	24	1.69	172	127	-24
7X (IS)	1.46	173	128	24	-	_	-	_
13X (IS)	_	-	-	_	4.08	185	125	-28
17X (IS)	3.98	188	129	24	-	_	-	-
37X (IS)	2.93	187	143	24	-	_	-	-
137X (IS)	6.32	204	144	24	_	_	_	_
1U (IS)	-	_	-	_	1.50	188	143	-22
3U (IS)	-	-	-	_	1.00	188	143	-20
7U (IS)	-	_	-	_	1.28	188	143	-20
13U (IS)	-	_	-	_	2.59	202	114	-30
17U (IS)	-	-	-	_	3.74	202	142	-32
37U (IS)	-	-	-	_	1.82	199	127	-26
137U (IS)	-	-	-	_	5.13	216	142	-32
AAMU (IS)	-	-	-		0.74	204	130	-20

<sup>§</sup> For (IS) positive ion mode, the following global conditions were used: ionization voltage = 1850 V; interface temperature = 700°C; entrance potential = 10V; declustering potential = 25; cell exit potential = 11.

<sup>\*\*</sup> For (IS) negative ion mode, the following global conditions were used: ionization voltage = -1850V; interface temperature = 700°C; entrance potential = -10V; declustering potential = -25; cell exit potential = -16.