Laboratory Procedure Manual

Analyte: **Volatile Organic Compounds (VOCs) Metabolites**

Matrix: **Urine**

Method: **Ultra Performance Liquid Chromatography with Electro Spray Tandem Mass Spectrometry**

[UPLC ESI/MSMS]

As performed by:

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Important Information for Users

The Centers for Disease Control and Prevention (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.

Public Release Data Set Information

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

Data File Name	Variable Name	SAS Label
UVOC_J	URX2MH	2-Methylhippuric acid (ng/mL)
	URX34M	3-methipurc acd & 4-methipurc acd(ng/mL)
	URXAAM	N-Ace-S-(2-carbamoylethyl)-L-cys(ng/mL)
	URXAMC	N-Ace-S-(N-methlcarbamoyl)-L-cys(ng/mL)
	URXATC	2-amnothiazolne-4-carbxylic acid(ng/mL)
	URXBMA	N-Acetyl-S-(benzyl)-L-cysteine(ng/mL)
	URXBPM	N-Acetyl-S-(n-propyl)-L-cysteine(ng/mL)
	URXCEM	N-Acetyl-S-(2-Carbxyethyl)-L-Cys(ng/mL)
	URXCYHA	N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine
	URXCYM	N-acetyl-S-(2-cyanoethyl)-L-cyst(ng/mL)
	URXDHB	N-Ace-S- (3,4-Dihydxybutl)-L-Cys(ng/mL)
	URXGAM	N-ac-S-(2-carbmo-2-hydxel)-L-cys(ng/mL)
	URXHEM	N-Ace-S-(2-Hydroxyethyl)-L-cys(ng/mL)
	URXHP2	N-Ace-S-(2-hydroxypropyl)-L-cys(ng/mL)
	URXHPM	N-Ace-S-(3-Hydroxypropyl)-L-Cys(ng/mL)
	URXIPM3	N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine
	URXPMM	N-A-S-(3-hydrxprpl-1-metl)-L-cys(ng/mL)
	URXMAD	Mandelic acid(ng/mL)
	URXMB3	N-ace-S-(phenl-2-hydxyetl)-L-cys(ng/mL)
	URXPHE	N-ace-S-(phenl-2-hydxyetl)-L-cys(ng/mL)
	URXPHG	Phenylglyoxylic acid(ng/mL)
	URXTTC	2-thoxothazlidne-4-carbxylic acid(ng/mL)

1. Clinical Relevance and Summary of Test Principle

A. Clinical relevance

Volatile organic compounds (VOCs) are ubiquitous in the environment, originating from many different natural and anthropogenic sources. Human exposure to VOCs occurs through inhalation, ingestion, and dermal contact [1]. VOCs are present in virtually all homes and workplaces. Long-term exposure to certain VOCs may increase the risk for leukemia [2], bladder cancer [3], birth defects [4], and neurocognitive impairment [5]. In the United States, tobacco smoke is the major non-occupational source of exposure to a number of harmful VOCs. Tobacco smoke contains over 8000 chemicals, including a number of carcinogenic and toxic VOCs (e.g., benzene, vinyl chloride, ethylene oxide, 1,3-butadiene, and acrolein) [6-8]. Regardless of exposure source, high levels of toxic VOCs is an area of significant public health concern [9]. Monitoring urinary metabolites of VOCs provides complimentary data to measuring VOCs in exhaled breath or blood, and a longer time window during which biomarkers are elevated following cessation of exposure to VOCs. The non-invasive sampling of urine, longer physiological half-lives of mercapturic acids, and relatively high degree of specificity make urinary mercapturic acids useful biomarkers of exposure to VOCs. Mercapturic acids are formed primarily through the metabolism of VOCs via the glutathione pathway. VOCs and/or their metabolites can react with *glutathione* (GSH), and undergo further metabolism to form mercapturic acids. These metabolites are then removed from the blood by the kidneys and excreted into urine.

Table 1 shows the urinary VOC metabolites monitored using the current method. We also list the parent compound(s) from which these metabolites can be formed. Except for perchloroethylene (PERC; also known as tetrachloroethylene), 1-bromopropane, and trichloroethylene (TCE) all other parent compounds are constituents of tobacco smoke.

Acrolein is present in various cooked foods and in the environment. It is formed from carbohydrates, vegetable oils, animal fats, and amino acids during heating of foods, and by combustion of petroleum fuels and biodiesel. Smoking tobacco products is typically the largest source of acrolein exposure [10]. Acrolein induces necrotic and apoptotic cell death in humans. Acrylamide is used to produce polymers, formulation of cosmetics and body care products, and in the textile industry. Acrylamide is also a constituent of a normal diet. Acrylamide is formed during the heating of carbohydrate rich food (e.g., French fries, potato chips). It is also a component of cigarette smoke [11]. The acrylamide metabolite, glycidamide, is a putative mutagen and most directly related to acrylamide's carcinogenicity. Acrylonitrile is widely used in the manufacture of plastics, acrylic fibers, and synthetic rubber, and is considered as a probable human carcinogen [12]. Benzene is a group 1 carcinogen [13]. It is found in crude oil, gasoline, and tobacco smoke. 1,3-Butadiene is mainly used for production of synthetic rubber alone or as a copolymer with styrene. Environmental sources of 1,3-butadiene are automobile exhaust, exhaust from heating, and cigarette smoke [14]. 1,3-Butadiene is characterized as being carcinogenic to humans by inhalation. Carbon disulfide exposure can affect cardiovascular and nervous systems [15]. A major source of exposure to crotonaldehyde is mainstream and side stream tobacco smoke [16]. It also occurs naturally in food and forms during combustion of organic materials. A recent study reported that crotonaldehyde exposure induces oxidative stress and apoptosis in human bronchial epithelial cells [17]. There are multiple sources of exposure to cyanide other than tobacco smoke (e.g., cyanide from food and from amino acid catabolism) [18]. N,N-Dimethylformamide (DMF) is a solvent that is used in the production of electronic compounds, pharmaceutical products, and textile coatings; and in the manufacture of synthetic leather, polyurethane, and polyacrylonitrile fibers [19]. Ethylene oxide, which is used as an intermediate in the production of ethylene glycol and other oxide derivatives, has been associated with leukemia [20]. Propylene oxide, which is used in industry as a chemical intermediate in the production of propylene glycols and glycol ethers, has been classified as a probable human carcinogen (group 2B) by the IARC [21]. Styrene is one of the most important chemicals used worldwide to manufacture plastics, synthetic rubber, and resins; and it is an environmental contaminant present in food, tobacco, and engine exhaust. The IARC classified styrene as possibly carcinogenic to humans [22]. Xylenes and toluene are widely used in industry as organic solvents, ingredients of thinners, and in the synthesis of other chemicals [23]. Acute toluene exposure can cause disorientation, euphoria, exhilaration, and tinnitus [24]. Vinyl chloride exposure can cause angiosarcoma [25]. Isoprene, the 2-methyl analog of 1,3-butadiene, has been classified as possibly carcinogenic to humans (group 2B) by IARC. It is mainly used in synthetic rubber production. Tobacco smoke also imposes significant isoprene exposure to humans [29]. PERC and 1-bromopropane are widely used as dry cleaning and metal degreasing solvents. PERC is a hazardous air pollutant, a common contaminant detected at superfund waste sites, and is a surface and ground water pollutant [26]. Over 400 million pounds of PERC are produced annually in the United States. 1-Bromopropane is reported to cause reproductive toxicity in male rats and neurotoxicity in both rats and humans [27]. Trichloroethylene (TCE) is an important industrial chemical widely used because of its favorable solvent characteristics, chemical stability, and relatively low acute toxicity. However, the studies show that the mutagenic and nephrotoxic metabolite formed in human trichloroethylene metabolism could be a risk of nephrocarcinogenesis associated with trichloroethylene exposure [28].

Urinary VOC metabolite biomonitoring data will provide useful baseline information about VOC exposures in the US population.

Table 1. VOC metabolites and their parent compounds

Parent compound	VOC metabolite	Acronym	Code
Acrolein	*N*-Acetyl-S- (2-carboxyethyl)-L-cysteine	2CoEMA	CEMA
	N-Acetyl-S- (3-hydroxypropyl)-L-cysteine	3HPMA	HPMA
Acrylamide	*N*-Acetyl-S-(2-carbamoylethyl)-L-cysteine	2CaEMA	AAMA
	N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	2CaHEMA	GAMA
Acrylonitrile	*N*-Acetyl-S-(2-cyanoethyl)-L-cysteine	2CyEMA	CYMA
	N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine	1CyHEMA	CYHA
Acrylonitrile, vinyl chloride, ethylene oxide	*N*-Acetyl-S- (2-hydroxyethyl)-L-cysteine	2HEMA	HEMA
1-Bromopropane	*N*-Acetyl-S-(*n*-propyl)-L-cysteine	1PMA	BPMA
1,3-Butadiene	*N*-Acetyl-S- (3,4-dihydroxybutyl)-L-cysteine	34HBMA	DHBM
	N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine	4HBeMA	MHB3
Carbon disulfide	2-Thioxothiazolidine-4-carboxylic acid	TTCA	TTCA
Crotonaldehyde	*N*-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine	3HMPMA	HPMM
Cyanide	2-Aminothiazoline-4-carboxylic acid	2ATCA	ATCA
N, N- Dimethylformamide, methyl isocyanate	*N*-Acetyl-S-(N-methylcarbamoyl)-L-cysteine	MCaMA	AMCA
Ethylbenzene, styrene	Phenylglyoxylic acid	PhGA	PHGA
Isoprene	*N*-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine	4HMBeMA	IPM3
Propylene oxide	*N*-Acetyl-S-(2-hydroxypropyl)-L-cysteine	2HPMA	HPM2
Styrene	*N*-Acetyl-S-(1-phenyl-2-hydroxyethyl-L-cysteine + *N*-Acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine	2HPhEMA	PHEM
Styrene, ethylbenzene	Mandelic acid	MADA	MADA
Toluene, benzyl alcohol	*N*-Acetyl-S-(benzyl)-L-cysteine	BzMA	BMA
Xylene	2-Methylhippuric acid	2MHA	2MHA
	3-Methylhippuric acid + 4-Methylhippuric acid	3MHA+4MHA	34MH

B. Test principle

This method is a quantitative procedure for the measurement of VOC metabolites in human urine using ultra performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS) [30]. Currently, chromatographic separation is achieved by using a C18 reversed phase column with 15 mM ammonium acetate and acetonitrile as the mobile phases. The choice of column and mobile phases should be such that it ensures adequate baseline separation among the metabolites and minimizes any background interferences. The eluate from the column is ionized using an electrospray interface to generate and transmit negative ions into the mass spectrometer. Comparison of relative response factors (ratio of native analyte to stable isotope labeled internal standard) with known standard concentrations yields individual analyte concentrations.

2. Safety Precautions

A. Reagent toxicity or carcinogenicity

The chemical, physical, and toxicological properties of most of the VOC metabolites have not been thoroughly investigated. Contact of VOC metabolites with strong oxidizing agents should be avoided as this could generate toxic fumes of carbon monoxide, carbon dioxide, nitrogen oxides, and sulfur oxides. However, aqueous solutions of VOC metabolites do not present a fire or explosion hazard. These compounds may cause respiratory tract, skin, and eye irritation. Gloves, lab coat, and safety glasses must be worn while preparing solutions and handling human urine. Disposable plastics (e.g., pipette tips, autosampler tubes, gloves, etc.), glass, and paper that come in contact with urine are placed in a biohazard autoclave bag. These bags are kept in appropriate containers until sealed and autoclaved. All work surfaces are wiped down with 70% ethanol solution (or equivalent) when work is finished.

Observe Universal Precautions. All biological samples and diluted specimens are disposed in a biohazard autoclave bag at the end of the analysis according to CDC/EHLS guidelines for disposal of hazardous waste.

Special precautions must be followed while handling acetonitrile. Acetonitrile is a flammable liquid and a mucous membrane, skin, and eye irritant. **If acetonitrile comes in contact with any part of the body, it is to be quickly washed with lots of water.**

B. Radioactive hazards

None

C. Microbiological hazards

Follow Universal Precautions. Because of the possibility of exposure to various microbiological hazards, appropriate measures are to be taken to avoid any direct contact with the urine specimen. Gloves, lab coats, and safety glasses must be worn while handling all human urine products. The Hepatitis B vaccination series is recommended for health care and laboratory workers who are exposed to human fluids and tissues.

D. Mechanical hazards

There are only minimal mechanical hazards when performing this procedure using standard safety practices. The manufacturer's information regarding safe operation of the equipment should be read and followed by the laboratory users. Direct contact with the mechanical and electronic components of the mass spectrometer must be avoided unless all power to the instrument is off. Generally, mechanical and electronic maintenance and repair are performed only by qualified technicians. The autosampler and the mass spectrometer contain a number of areas that are hot enough to cause burns. Precautions are to be taken when working in these areas.

E. Protective equipment

Standard safety precautions are followed when performing this procedure, including the use of a lab coat/disposable gown, safety glasses, appropriate gloves, and chemical fume hood.

F. Training

Users are required to demonstrate safe and proper techniques in performing the method and to generate data with acceptable accuracy and precision based on their calibration curves, QCs, and PTs.

G. Personal hygiene

Follow Universal Precautions. Care has to be taken when handling chemicals or any biological specimen. Routine use of gloves, personal protective equipment, and proper hand washing must be practiced. The laboratory Chemical Hygiene Plan and CDC Division of Laboratory Sciences safety policies and procedures are to be consulted for details related to specific activities, reagents, or agents.

H. Disposal of waste

Waste materials must be disposed in compliance with laboratory, federal, state, and local regulations. Solvents and reagents are disposed in an appropriate container clearly marked for waste products and are temporarily stored in a chemical fume hood. All disposable items that come in direct contact with the biological specimens are placed in a biohazard autoclave bag that is kept in appropriate container until sealed and autoclaved. Used unshielded needles, glass Pasteur pipettes, and disposable syringes are immediately placed into a sharps container and autoclaved when this container becomes full. All surfaces are wiped down with 70% ethanol solution (or equivalent) when work is finished.

3. Computerization; Data-System Management

A. Software and knowledge requirements

Different software packages (e.g., Analyst, MultiQuant) are used to control the UPLC system and the mass spectrometer during data acquisition and to analyze chromatograms after the run. Final reportable results are exported to a LIMS database. Knowledge and expertise of these software packages (or their equivalent) are required to utilize and maintain the data management structure.

B. Sample information

Information pertaining to particular specimens is entered into the database either manually or electronically.

C. Data maintenance

All samples and analytical data are checked for transcription errors and overall validity prior to being entered into the LIMS database. The data are routinely backed up locally onto a computer hard drive and in the NCEH network. The local area network manager should be contacted for emergency assistance.

D. Information security

Information security is managed at multiple levels. The information management systems that contain the final reportable results are restricted through user ID and password security access. The computers and instrument systems that contain the raw and processed data files require specific knowledge of software manipulation techniques and physical location. Site security is provided at multiple levels through restricted access to the individual laboratories, buildings, and site.

4. Specimen Collection, Storing and Handling Procedures; Criteria for Specimen Rejection

- **A.** No special instructions such as fasting or special diets are required.
- **B.** The matrix type is urine.
- **C.** A total sample volume of 0.25–0.5 mL is required to allow for repeated analysis. An aliquot of at least 50 µL is needed for typical analysis. However, if the calculated concentration of the analyte is greater than the concentration of the highest calibrator, higher order dilution is required.
- **D.** Acceptable containers include polystyrene cryovial tubes or polypropylene (PP) centrifuge tubes. Sterile collectors should be used for specimen acquisition.
- **E.** The criteria for unacceptable specimen are any suspected contamination due to improper collection procedures or collection devices. In all cases, a second urine specimen should be requested.
- **F.** Specimen characteristics that may compromise test results are as indicated above including contamination of urine by contact with dust, dirt, etc. from improper handling.
- **G.** Detailed instructions for urine collection and processing are outlined in the DLS Policies and Procedures Manual (PPM). Collection, transport, and special requirements are discussed. In general, urine specimens should be transported and stored chilled or frozen at -20ºC. Once received, the samples can be frozen at -70ºC until time for analysis. Portions of the sample that remain after analytical aliquots are refrozen at -20 or -70ºC. Freeze-thawing of samples more than five times is to be avoided.

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

Not applicable to this assay

6. Preparation of Reagents, Calibration (Standards), Controls, and all other Materials; Equipment and Instrumentation

A. Reagents and sources

Reagents that were used during the development, validation, and application of this method are listed in **Table 2** along with their suggested sources. All chemicals and solvents are used without further purification**.** Stable isotopically labelled internal standards listed in the table are for reference purpose only. Other isotopic analogs may be used when there are availability or cost limitations as long as the internal standard is stable and there are no chromatographic or mass spectral interferences.

Table 2. Reagents and sources

Reagent	Code	Suggested source
Solvents		
Acetonitrile (Optima LCMS grade)		Fisher Scientific, Fairlawn, NJ
Ammonium acetate		Sigma Chemicals, St. Louis, MO
Methanol (Optima LCMS grade)		Fisher Scientific, NJ
Isopropyl alcohol (Optima LCMS grade)		Fisher Scientific, NJ
Water (LCMS grade)		Fisher Scientific, Fairlawn, NJ
Native Calibration and Control Materials		
N-Acetyl-S-(benzyl)-L-cysteine	BMA	Battelle Research, Columbus, Ohio
N-Acetyl-S-(2-carbamoylethyl)-L-cysteine	AAMA	C/D/N Isotopes Inc, Quebec, Canada
N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	GAMA	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(2-carboxyethyl)-L-cysteine	CEMA	Cambridge Isotopes, Andover, MA
N-Acetyl-S-(2-cyanoethyl)-L-cysteine	CYMA	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine	DHBM	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(2-hydroxyethyl)-L-cysteine	HEMA	Cambridge Isotopes, Andover, MA
N-Acetyl-S-(2-hydroxypropyl)-L-cysteine	HPM2	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(3-hydroxypropyl)-L-cysteine	HPMA	Cambridge Isotopes, Andover, MA
N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine	MHB3	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(3-hydroxypropyl-1 methyl)-L-cysteine	HPMM	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine	AMCA	Sigma Chemicals, St. Louis, MO
N-Acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine	PHEM1	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine	PHEM2	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(*n*-propyl)-L-cysteine	BPMA	Toronto Research Chemicals, Toronto, Canada
2-Aminothiazoline-4-carboxylic acid	ATCA	Chem-Impex International Inc., Woodale, IL
Mandelic acid	MADA	Sigma Chemicals, St. Louis, MO
2-Methylhippuric acid	2MHA	Sigma Chemicals, St. Louis, MO
3-Methylhippuric acid	3MHA	Sigma Chemicals, St. Louis, MO
4-Methylhippuric acid	4MHA	Sigma Chemicals, St. Louis, MO
Phenylglyoxylic acid	PHGA	Sigma Chemicals, St. Louis, MO
2-Thioxothiazolidine-4-carboxylic acid	TTCA	Sigma Chemicals, St. Louis, MO
N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine	CYHA	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine	IPM3	Toronto Research Chemicals, Toronto, Canada
Isotopically Labeled Internal Standards		
N-Acetyl-S-(benzyl-$^{13}C_6$)-L-cysteine	BMA-$^{13}C_6$	Battelle Research Institute, Columbus, Ohio
N-Acetyl-S-(2-carbamoylethyl-D_4)-L-cysteine	AAMA- D_4	C/D/N Isotopes Inc, Quebec, Canada
N-Acetyl-D_3-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	GAMA- D_3	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(2-carboxyethyl-$^{13}C_3$)-L-cysteine	CEMA-$^{13}C_3$	Cambridge Isotopes, Andover, MA
N-Acetyl-D_3-S-(2-cyanoethyl)-L-cysteine	CYMA- D_3	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(3,4-dihydroxybutyl-$^{13}C_4$)-L-cysteine	DHBM-$^{13}C_4$	Cambridge Isotopes, Andover, MA
N-Acetyl-S-(2-hydroxyethyl-D_4)-L-cysteine	HEMA- D_4	Cambridge Isotopes, Andover, MA
N-Acetyl-S-(2-hydroxypropyl-D_3)-L-cysteine	HPM2- D_3	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-^{13}C-S-(3-hydroxypropyl)-L-cysteine-$^{13}C_3$-^{15}N	HPMA-$^{13}C_4$-^{15}N	Cambridge Isotopes, Andover, MA

Reagent	Code	Suggested source
N-Acetyl-D_3-S-(4-hydroxy-2-buten-1-yl)-L-cysteine	MHB3- D_3	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-^{13}C-S-(3-hydroxypropyl-1-methyl)-L-cysteine-$^{13}C_3$-^{15}N	HPMM-$^{13}C_4$-^{15}N	Cambridge Isotopes, Andover, MA
N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine -$^{13}C_3$-^{15}N	AMCA-$^{13}C_3$-^{15}N	Kalexsyn Inc., Kalamazoo, MI
N-Acetyl-S-(1-phenyl-$^{13}C_6$-2-hydroxyethyl)-L-cysteine	PHEM1-$^{13}C_6$	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(2-phenyl-$^{13}C_6$-2-hydroxyethyl)-L-cysteine	PHEM2-$^{13}C_6$	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(*n*-propyl-D_7)-L-cysteine	BPMA-D_7	Toronto Research Chemicals, Toronto, Canada
2-Aminothiazoline-D_3-4-carboxylic acid	ATCA-D_3	Dr. Bill Draper's Lab, CDPH, CA
Mandelic-$^{13}C_8$ acid	MADA-$^{13}C_8$	Cambridge Isotopes, Andover, MA
2-Methylhippuric-$^{13}C_6$ acid	2MHA-$^{13}C_6$	Toronto Research Chemicals, Toronto, Canada
3-Methylhippuric-D_7 acid	3MHA-D_7	C/D/N Isotopes Inc, Quebec, Canada
4-Methylhippuric-$^{13}C_2$-^{15}N acid	4MHA-$^{13}C_2$-^{15}N	Cambridge Isotopes, Andover, MA
Phenylglyoxylic-$^{13}C_8$ acid	PHGA-$^{13}C_8$	Toronto Research Chemicals, Toronto, Canada
2-Thioxothiazolidine-$^{13}C_3$-4-carboxylic acid	TTCA-$^{13}C_3$	Cambridge Isotopes, Andover, MA
N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine-D_3	CYHA-D_3	Toronto Research Chemicals, Toronto, Canada
N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine-$^{13}C_3$-D_3	IPM3-$^{13}C_3$-D_3	IsoSciences, Ambler, PA

I. Solvents

LCMS grade solvents (e.g., water, acetone, methanol, isopropyl alcohol) are used to prepare mobile phases. Every run contains a water sample with 15 mM ammonium acetate, referred to as a double blank, to monitor the quality of the mobile phase and to detect any contamination.

II. Calibration and control materials

Currently used calibration and quality control materials, including native compounds and isotopically labeled internal standards, are at least 95% pure. Isotopically labeled compounds are checked for any spectral overlap with corresponding native analogs before use. Each run contains a blank sample (internal standard and 15 mM ammonium acetate) to monitor any changes in quality.

B. Reagent preparation

I. 15mM ammonium acetate

15 mM ammonium acetate in LCMS-grade water is used as Solvent A (mobile phase of UPLC), to prepare working calibration standards, and to dilute urine and quality control (QC) samples.

II. Standards solutions preparation

a) Native analytical standards

i. Individual primary stock solutions

The primary stock solutions are prepared by dissolving the neat compounds individually in appropriate solvents (Table 3) being sure to account for any salt components in the calculations as noted by the Certificate of Analysis from the manufacturer. For hygroscopic compounds (i.e. 2DCV, AAMA, AMCA, BPMA, CYHA, DHBM, GAMA, HPM2, HPMM, MBH3, PHEM), special procedures, such as drying the neat material in a desiccator before use, are to be taken. The prepared stocks are stored at -20 ºC for future use.

Table 3. Solvent used to prepare initial stock solution

Analyte; Internal standard	Solvent used to prepare primary stock
AAMA; AAMA-D_4	water
AMCA; AMCA-$^{13}C_3$-^{15}N	water
ATCA; ATCA-D_3	water
BMA; BMA-$^{13}C_6$	water
BPMA; BPMA-D_7	methanol:water (1:1)
CEMA; CEMA-$^{13}C_3$	water
CYMA; CYMA-D_3	water
DHBM; DHBM-$^{13}C_4$	water
GAMA; GAMA-D_3	water
HEMA; HEMA-D_4	water
HPMA; HPMA-$^{13}C_4$-^{15}N	water
HPM2; HPM2-D_3	water
HPMM; HPMM-$^{13}C_4$-^{15}N	water
MADA; MADA-$^{13}C_8$	methanol:water (1:1)
2MHA; 2MHA-$^{13}C_6$	methanol:water (1:1)
3MHA; 3MHA-D_7	methanol:water (1:1)
4MHA; 4MHA-$^{13}C_2$-^{15}N	methanol:water (1:1)
MHB3; MHB3-D_3	methanol:water (1:1)
PHGA; PHGA-$^{13}C_8$	water
PHEM; PHEM-$^{13}C_6$	methanol
TTCA; TTCA-$^{13}C_3$	water
CYHA; CYHA-D_3	methanol:water (1:1)
IPM3; IPM3-D_3-$^{13}C_3$	methanol:water (1:1)

ii. **Mixed intermediate stock solutions**

Intermediate stock solutions are prepared for at least five levels and concentrations are 10 times higher than the corresponding working standards. A representative sample composition is given in **Table 4**. To prepare each level, the appropriate volume of each analyte is pipetted from the individual primary stock solutions into a volumetric flask and the mixture is diluted with LCMS-grade water to attain the required final concentration. The solutions are aliquoted in vials and are stored at -70ºC. Each set is thawed once, and the remaining solution is discarded after use.

Table 4. A sample composition of mixed intermediate stock solutions (ng/mL)

Analyte	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	STD 9
CYMA	0.50	0.75	1.58	5.00	15.8	50.0	158	500	1581
HPMM	3.04	4.56	9.61	30.40	96.1	304.0	961	3040	9613
MHB3	0.55	0.83	1.74	5.50	17.4	55.0	174	550	1739
HPM2	2.64	3.96	8.35	26.42	83.5	264.2	835	2642	8355
3MHA	3.10	4.65	9.80	31.00	98.0	310.0	980	3100	
4MHA	3.10	4.65	9.80	31.00	98.0	310.0	980	3100	
AAMA	1.10	1.65	3.48	11.00	34.8	110.0	348	1100	
BMA	0.44	0.66	1.39	4.40	13.9	44.0	139	440	
HPMA	12.96	19.44	40.98	129.60	409.8	1296.0	4098	12960	
DHBM	4.00	6.00	12.65	40.00	126.5	400.0	1265	4000	
2MHA	3.10	4.65	9.80	31.00	98.0	310.0	980	3100	
AMCA	3.60	5.40	11.38	36.00	113.8	360.0	1138	3600	
BPMA	0.77	1.15	2.43	7.68	24.3	76.8	243	768	
PHGA	10.07	15.11	31.84	100.70	318.4	1007.0	3184		
CEMA	6.00	9.00	18.97	60.00	189.7	600.0	1897		
GAMA	5.91	8.86	18.67	59.05	186.7	590.5	1867		
HEMA	0.38	0.57	1.19	3.77	11.9	37.7	119		
MADA	12.00	18.00	37.95	120.00	379.5	1200.0	3795		
ATCA	8.90	13.35	28.14	89.00	281.4	890.0	2814		
PHEM	0.50	0.75	1.58	5.00	15.8	50.0	158		
TTCA	11.17	16.75	35.31	111.67	353	1117	3531		
IPM3	0.53	0.80	1.69	5.33	16.9	53.3	169	533	1685
CYHA	2.60	3.90	8.22	26.00	82	260	822	2600	

iii. **Working mixed standard solutions**

Each level of intermediate stock is diluted 10 times with 15 mM ammonium acetate solution to prepare the corresponding working standard level. The preparation of the working standard solutions should follow certain criteria: (a) concentration at each level should be separated from the next level by a maximum factor of √10, (b) the lowest concentration is to be equal to or less than the LOD, and (c) the highest standard should ideally cover the 99th percentile of the expected population level, whenever that information is available.

b) Isotopically labeled internal standard solutions

i. **Individual primary stock solutions**

The appropriate volume of each IS is pipetted from the individual primary stock solutions into a volumetric flask and the mixture is diluted with LCMS-grade water or methanol to attain the required final concentration. These solutions are aliquoted in vials and are stored at -70ºC. Each vial is thawed once, if applicable, and the remaining solution is discarded after use.

ii. **Mixed intermediate stock solutions**

The appropriate volume of each IS is pipetted from the individual primary stock solutions into a volumetric flask and the mixture is diluted with LCMS-grade water or methanol to attain the required final concentration. These solutions are aliquoted in vials and are stored at -70ºC. Each vial is thawed once, if applicable, and the remaining solution is discarded after use.

iii. **Working mixed internal standard solutions**

The intermediate stock is diluted 20 times with 15 mM ammonium acetate solution to prepare the working internal standard (IS). The final concentration of each IS is suggested to be between standard (native analyte) level 3-5.

III. Preparation of quality control material

a) Quality control pools

Quality Control (QC) materials are prepared at two concentration levels, QC low (Q_L) and QC high (Q_H), in urine. Q_L is suggested to be between standard levels 3 and 5, and Q_H between 5 and 7. The urine matrix can have high backgrounds for certain analytes. In those cases, the amount of analyte to be spiked should be adjusted to meet the target concentration. Aliquots of Q_L and Q_H are stored separately in cryovials at -70°C until use. Each vial is thawed once, and the remaining solution is discarded after use. At least 20 separate QC samples are analyzed using different sample runs and instruments to characterize the QCs and to determine the mean values and coefficient of variation (CV) for individual analytes.

b) Proficiency testing samples

Proficiency testing materials at four (native analyte concentration) levels are prepared in a manner similar to the mixed intermediate stocks using individual primary stock solutions separate from those used to make standard solutions. Also, Proficiency testing materials are prepared by an external source other than that used to make standard solutions whenever available. Aliquots are stored in cryovials; at -70°C until use. Proficiency testing samples are run at least two times a year. A proficiency testing coordinator, independent from the sample analysis team, blind-codes the PT stock vials and verifies accuracy of quantified results of four PT samples at each of the four concentration levels and one sample at any of the four different levels.

C. Instrumentation and operation

I. Liquid chromatography (LC)

Chromatographic separation of the analytes is achieved with a UPLC system (e.g., Waters Acquity) fitted with a reversed phase C18 column (e.g., Acquity UPLC® HSS T3). A guard column is mounted upstream to protect the analytical column from impurities. The column and the sample manager are set at optimum temperatures, for example, 40°C and 25°C respectively.

The mobile phase consists of 15 mM ammonium acetate (Solvent A) and acetonitrile (Solvent B). The separation conditions are optimized to obtain good resolution among VOC metabolites, a representative example is given in **Table 5**. Before each run, the column is equilibrated with the initial mobile phase composition for at least 10 column volumes. After each sample injection, the needle is first cleaned with a strong wash and subsequently with a weak wash (**Table 5)**. At the end of each run, the column is washed with an aqueous solution (e.g., A:B = 97:3) followed by 100% acetonitrile and is stored in acetonitrile (shutdown method).

Table 5. Chromatography parameters for the UPLC

Parameter	**Details**
Weak Wash	LCMS grade water
Strong Wash	25% LCMS grade water 25% Optima LCMS grade acetonitrile 25% Optima LCMS grade methanol 25% Optima LCMS grade isopropyl alcohol
Gradient: Time, flow, Solvent A: Solvent B	initial, 250 µL/min, 97%: 3% 2 min, 250 µL/min, 95%: 5% 3 min, 300 µL/min, 90%:10% 5 min, 300 µL/min, 70%: 30% 6.5 min, 300 µL/min, 60%:40% 7 min, 300 µL/min, 85%:15% 7.5 min, 300 µL/min, 90%:10% 8 min, 300 µL/min, 97%:3% 9 min, 300 µL/min, 97%:3%

II. Mass spectrometer (MS)

A triple quadrupole mass spectrometer (e.g., AB Sciex Triple Quad 5500) with an electrospray ion source is used for the detection of urinary VOC metabolites. The mass spectrometer is operated under Scheduled Multiple Reaction Monitoring (MRM) mode. The instrument parameters are optimized to obtain the maximum signal intensity, dynamic range, and signal to noise (S/N) ratio. Compounds (native analytes and internal standards) are optimized individually to select transitions and associated mass spectrometric parameters (e.g., declustering potential, collision energy, etc.) for maximum selectivity and signal intensity. These parameters should be re-optimized when transferring the method to a new instrument. Ideally, the m/z value for the precursor ion should match between the quantitation and the confirmation ions whenever possible. Similarly, the internal standard transition should correspond to the quantitation ion transition to avoid any quantitation bias. In some instances (e.g., BMA), alternate transitions have been chosen because of the presence of co-eluents or spectral overlap. **Table 6** lists suggested transitions for the VOC metabolites measured by this method.

Table 6. Example of MRM transitions for VOC metabolites

Analyte	Transition		Internal standard	Transition
	Quan. ion[a]	Conf. ion[b]		
AAMA	233/104	233/58	AAMA-D_4	237/108
AMCA	219/162	219/84	AMCA-^{15}N-$^{13}C_3$	223/166
ATCA	145/67	145/58	ATCA-D_3	148/70
BMA	252/123	253/124	BMA-$^{13}C_6$	258/84
BPMA	204/84	204/75	BPMA-D_7	211/84
CEMA	234/162	234/105	CEMA-$^{13}C_3$	237/162
CYHA	231/84	231/102	CYHA-D_3	234/84
CYMA	215/162	215/86	CYMA-D_3	218/165
DHBM	250/121	250/75	DHBM-$^{13}C_4$	254/125
GAMA	249/120	249/128	GAMA-D_3	252/120
HEMA	206/77	206/75	HEMA-D_4	210/81
HPMA	220/91	220/89	HPMA-$^{13}C_4$-^{15}N	225/91
HPM2	220/91	221/91	HPM2-D_3	223/91
HPMM	234/105	235/105	HPMM-$^{13}C_4$-^{15}N	239/105
IPM3	246/117	246/87	IPM3-D_3-$^{13}C_3$	252/123
MADA	151/107	151/77	MADA-$^{13}C_8$	159/114
2MHA	192/148	192/91	2MHA-$^{13}C_6$	198/154
34MH	192/148	192/91	4MHA-$^{13}C_2$-^{15}N	195/150
MHB3	232/103	233/103	MHB3-D_3	235/103
PHGA	149/77	149/105	PHGA-$^{13}C_8$	157/83
PHEM	282/153	282/123	PHEM-$^{13}C_6$	288/159
TTCA	162/58	162/118	TTCA-$^{13}C_3$	165/58

[a]Quantitation ion. [b]Confirmation ion.
Note: Analytes with same SMRM transitions (e.g., 2MHA and 34MH) elute at different retention times.

Mass spectrometers are tuned following any repair or performance maintenance. The curtain plate is cleaned as needed to remove any deposition from previous runs. The performance of the instrument is also checked before every scheduled run by injecting a low standard (e.g., std 2) three times and by calculating the S/N ratio, which should be at least 10. Additionally, the overall intensity and resolution between peaks is evaluated.

III. Robotic liquid handling system

All calibration standards, QCs, and urine samples are aliquoted, prepared, and mixed by a robotic liquid handling system such as Hamilton Microlab Star. **Table 7** exemplifies a sample preparation protocol. Preventive maintenance of liquid handling system is performed annually.

Table 7. An example of a sample preparation protocol using robotic liquid handler

Sample	Vol. of sample (µL)	Vol. of IS (µL)	Vol. of 15 mM ammonium acetate (µL)
Double blank	0	0	500
Blank	0	25	475
Calibration standard[a]	50	25	425
Calibration standard[b]	50 + 50	25	375
Quality control	50	25	425
Urine	50	25	425
Proficiency testing	50	25	425

[a]Using one source vial of all calibration material. [b]Using two source vials of calibration material (ie. reactives and nonreactives).

7. Calibration and calibration verification

Different urine samples contain varying background levels of VOC metabolites and hence urine cannot be used as a reliable matrix to prepare calibration standards. Instead, 15 mM ammonium acetate solution is used for this purpose. Matrix validation experiments were performed to verify that the calibration curves in urine and in ammonium acetate had the same slope (Appendix B, **Table B1**) [30].

A. Calibration curve

At least one set of calibrators is used for the quantitation of analytes in all urine samples from a batch. The calibration curve for each analyte is constructed from the response ratio, which is the area ratio of the unlabeled analyte to its corresponding internal standard. The slope and intercept of curves are determined by least squares regression of 1/x weighted data. Calibration curves should be composed of at least five standard levels that span the range of all detectable unknown samples and should achieve an R-squared coefficient of at least 0.98.

B. Calibration verification

Calibration accuracy is tested with each run by analysis of blank (15 mM ammonium acetate and IS) and quality control samples. A full set of calibrators is analyzed with each batch of urine samples. Absolute accuracy is verified by proficiency testing at least twice a year.

8. Procedure Operation Instructions; Calculations; Interpretation of Results

A. Sample preparation

An analytical run consists of double blank (15 mM ammonium acetate), blank (15 mM ammonium acetate and internal standard), calibration standards, low level QC, high level QC, and unknown urine samples. Prior to analysis, all samples including urine, standards, IS, and QCs are completely thawed using a thawing station for approximately 20 minutes. The thawing time varies depending on the room temperature. The urine samples are mixed thoroughly in a rugged rotator for 15 minutes at setting 60. The mixing step can go up to an hour without any significant changes in measured analyte concentrations. A robotic liquid handling system prepares the samples following the protocol as shown in **Table 7**. Briefly, urine samples and QCs are diluted 1:10 with 15 mM ammonium acetate. Each sample is immediately spiked with the internal standard solution and mixed properly.

B. Data analysis

Unknown samples are quantified by the ratio of the analyte peak area to the internal standard peak area. Use of internal standard compensates for analyte-dependent selectivity biases, such as matrix effects associated with the ionization process, and confirms the presence of a native target when there is any shift in chromatographic retention time. Urine and QC sample concentrations are multiplied by the appropriate dilution factor.

C. Data processing

I. peak integration

Each peak is visually inspected, and peak integration is corrected if the software erroneously integrates a peak. For each analyte, the confirmation ion signal is quantified above a certain concentration threshold.

II. Excluding calibrators

A particular calibrator is only excluded if it significantly affects (>10% change) the detectable results in QC and the cause behind the anomaly is identified. Scenarios that might only affect a single standard include no or low addition of native analyte or internal standard and missed injection because of instrument failure. However, the highest standard level can be excluded if the calibration curve is nonlinear over this region because all QCs fall below standard level 7. In that case, analysis of unknown samples which exceed the calibration range are diluted and repeated.

III. Excluding sample data

Absolute internal standard response is evaluated for consistency among the standards, blanks, QCs, and urine samples. Sample data is excluded if low or excess IS is added to the urine sample, which is identified by the unusually high or low absolute IS response compared to similar sample types. Poorly resolved co-eluents can cause an unusually high internal standard response, which also warrants elimination of the sample.

9. Reportable range of results

A. Reportable limits

Only data above or at LOD are reported, unless <LOD results are requested. The upper reportable limit corresponds to the concentration of the highest standard times additional dilutions. If the analyte level exceeds the upper calibration range, the sample is repeated by diluting it with 15 mM ammonium acetate such that the analyte concentration falls within the standard curve.

B. Limit of detection

Refer to the DLS PPM for calculation of LOD.

C. Accuracy

The accuracy of the assay is established by blind analysis of Proficiency Testing (PT) samples and whenever necessary, by spike recovery experiment in which urine is spiked at three different concentration levels.

D. Precision

The precision of the method is reflected in the variance of quality control samples analyzed over time. The coefficient of variation (CV) of the method was determined based on 20 independent analyses of the QC samples.

E. Analytical specificity

LC-MS/MS is a highly selective analytical method for quantifying the target analytes in complex aqueous matrices. Reversed phase liquid chromatography reproducibly resolves the target analytes, even in the most concentrated urine samples. Analytical specificity is established by comparing the retention times of an analyte relative to its internal standard. Tandem mass spectrometry provides a further degree of selectivity, by filtering out all ions except a specific transition of precursor-to-product ions for each analyte. Additionally, qualifier ratios, the area ratios of quantitation ion to the confirmation ion, are determined for the standards and QC samples. The average value of this ratio is typically within ± 25%.

10. Quality Assessment and Proficiency Testing

A. Quality assessment

Quality assessment procedures follow standard practices [32]. Daily experimental checks are made on the stability of the analytical system. Blanks, standards, and QC materials are added to each run sequence. A blank is analyzed at the beginning of each run to check the system for possible contamination. Relative retention times are examined for the internal standard to ensure the choice of the correct chromatographic peak. A calibration curve is developed for the batch using a complete set of calibration standards. The calibration curve must have a coefficient of determination, R-squared coefficient of at least 0.98. The results from the analysis of QC materials obtained using these calibration curves are compared using the acceptance criteria given below to assure precision of the analysis.

B. Quality control procedures

I. Establishing QC limits

Two different pools of quality control material are used, one at a low and the other at a high concentration. Quality control limits are established by characterizing assay precision with 20 distinct analyses of each QC pool. Different variables are included in the characterization analyses (e.g., different analysts, columns, instruments, etc.) to capture realistic assay variation over time. One instrument characterizes no more than two samples from one pool per day. The mean, standard deviation, coefficient of variation, and confidence limits are calculated from this QC characterization data set. Individual quality control charts for the characterization runs are created and examined. Quality control limits are used to document assay precision and accuracy on a daily basis. Limits are based on statistical calculation accounting for two QCs analyzed in each analytical run.

II. Quality control evaluation

After the completion of a run, the calculated results from the analysis of quality control samples are compared to the established quality control limits to determine if the run is “in control”. The quality control rules apply to the average of the beginning and ending analyses of each of the QC pools. The quality control results are evaluated according to the DLS Policies and

Procedures Manual. If a QC result is declared “out of control”, the results for all patient samples analyzed during that run are invalid for reporting.

C. Proficiency testing

I. Scope of PT

The proficiency testing (PT) scheme for this method is administered by an in-house proficiency testing coordinator. Externally prepared aqueous proficiency testing materials are blind-coded by the in-house PT coordinator. The samples are analyzed and the results are evaluated by the in-house PT coordinator.

II. Frequency of PT

Five samples of unknown PT concentrations are analyzed at least twice a year using the same method described for unknown samples.

III. Documentation of PT

Analytical PT results are reviewed by the analyst and laboratory supervisor and submitted to the in-house PT coordinator electronically. The PT results are evaluated by the PT coordinator; if the value falls between 75% and 125% of the expected value, then the analysis passes the proficiency test. A summary report of the PT evaluation is maintained by the laboratory quality control officer. If the assay fails proficiency testing, then the sample preparation and instrumentation are thoroughly examined to identify and correct the source of assay error. Analyte data for unknown specimens may only be reported if that analyte successfully passes proficiency testing.

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

If an analyte result for a quality control material falls outside the acceptable range, then it fails the QC criteria; and following steps should be taken.

A. **Calibration standards:** If R-squared coefficient is less than 0.98 for the fitted curve, then the individual calibration standards are evaluated for any obvious error (e.g., missed IS or native analyte or injection, improper peak integration, etc.). If not, then a new calibration set (working standard) is prepared and acquisition and analysis of the entire batch, including QCs & unknown samples, is repeated.

B. **Quality control material:** If the QC material is the suspected cause of the error, then a fresh QC sample is prepared and analyzed.

C. **Internal standard response:** If no missed IS aliquoting or missed injection is detected, then the absolute IS response should be compared to earlier runs. If the observed change exceeds 25%, then a new IS working solution is prepared and the run is repeated.

D. **Contamination:** Blank (internal standard and ammonium acetate) and double blank (ammonium acetate only) samples should be investigated for any contamination, e.g, presence of a ghost co-eluate peak or high background of unlabeled analyte in blank. The mobile phase is to be prepared fresh and the LC system needs to be cleaned prior to any measurement.

E. **Intermediate stock solution:** Occasionally the composition of the intermediate stock solution for native analytes or internal standard could be erroneous. In that case, new intermediate stock solutions followed by the working standards should be prepared and used for further measurements.

If these steps do not result in correction of the "out of control" values for QC materials, the supervisor should be consulted for other appropriate corrective actions. Analytical results are not reported for runs that are out of statistical control.

12. Limitations of Method, Interfering Substances and Conditions

The described method is highly selective. Because of excellent chromatographic and mass spectrometric resolution, we typically do not find other interfering substances that have similar chromatographic and mass spectrometric characteristics. However, in some urine samples, chromatography can be distorted by unknown co-eluates; usually, this problem is resolved by further diluting the sample and re-analyzing it. In those situations, where a co-eluate cannot be resolved from the target analyte, the data are not reported.

13. Reference Ranges (Normal Values)

Reference ranges for smokers and non-smokers are presented in **Table 8**.

Table 8. VOC metabolites in urine collected from non-smokers and smokers.

Analyte Code (other Acronym)	Analytical limit of detection (LOD)	Range		Ref.
		Non-smokers	Smoker	
AAMA	2.5	12.7-171 µg/L	30.3-447 µg/L	[33]
		9.8-171 µg/g creatinine	35.1-401 µg/g creatinine	
AMCA (AMCC)	5	38.9-498 µg/L	122-1453 µg/L	[33]
		47.3- 449 µg/g creatinine	196-1153 µg/g creatinine	
ATCA	25	85 ± 47 ng/mL	233 ± 237 ng/mL	[34]
BMA	0.02	2.4-81.4 µg/g creatinine	1.7-31.2 µg/g creatinine	[35]
CEMA	0.15	ND-94 µg/L	27-744 µg/L	[36]
		ND-158 µg/g creatinine	ND-744 µg/g creatinine	
CYMA*	0.5	0.14-1.83 pmol/mL	390-1257 pmol/mL	[38]
DHBM (DHBMA)	0.14	ND-329 µg/L	113-1830 µg/L	[36]
		ND-582 µg/g creatinine	166-1092 µg/g creatinine	
HEMA	0.03	ND-1.44 µg/L	ND-20.8 µg/L	[36]
		ND-1.05 µg/g creatinine	ND-16 µg/g creatinine	
HPMA	0.2	ND-128 µg/L	80.9-4030 µg/L	[36]
		ND-245 µg/g creatinine	75-3678 µg/g creatinine	
HPM2 (2HPMA)	5	<5-49.3 µg/L	<5- 252 µg/L	[33]
		<5-73.6 µg/g creatinine	<5-206 µg/g creatinine	
HPMM (HPMMA)	28	192-1740 µg/24hr	815-5457 µg/24hr	[16]
PMA	0.01	ND-0.26 µg/L	ND-37.7 µg/L	[36]
		ND-0.45 µg/g creatinine	ND-18.4 µg/g creatinine	

*interquartile range

14. Critical Call Results ("Panic Values")

Mercapturic acids are specific biomarkers of VOC exposure. High levels of urinary VOC metabolites could indicate excessive exposure to VOCs. However, the stoichiometric relationship of VOCs and many of the urinary VOC metabolites has not been established. Therefore, there are no critical call values for VOC metabolites at this time. The biological exposure indices (BEI) reported by ACGIH [38] for some of the VOC metabolites in this method are given in **Table 9** as the maximum values allowable in urine samples collected from workers.

Table 9. Biological exposure indices.

VOC Metabolite	BEI	Parent Compound
AMCA	15 mg/L	*N, N* dimethylformamide
DHBM	2.5 mg/L	1,3-butadiene
2MHA+3MHA+4MHA	1.5 g/g creatinine	*o-, m-, p-* xylenes
MADA + PHGA	400 mg/g creatinine	styrene
TTCA	0.5 mg/g creatinine	carbon disulfide

15. Specimen Storage and Handling During Testing

Specimens must be stored at ≤ -20°C until analysis; however, they may be kept at ambient temperature during analysis.

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

Alternate methods have not been evaluated for measuring VOC metabolites in urine.

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

Results are reported to three significant figures based on assay sensitivity calculations. Study subject data is reported in both concentration units (ng/mL) and as adjusted values based on creatinine excretion (µg/g creatinine).

Once the validity of the data is established by the QC/QA system outlined above, results are verified by a DLS statistician, and the data is reported in both hard and electronic forms. This data, a cover letter, and a table of method specifications and reference range values will then be routed through the appropriate channels for approval (i.e. supervisor, branch chief, division director). After approval at the division level, the report will be sent to the contact person who requested the analyses.

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

If greater than 0.25 mL of sample remains following successful completion of analysis, this material should be returned to storage at ≤ -70°C in case further analysis is required. These samples should be retained until valid results have been obtained, reported, and sufficient time has passed for review of the results.

Standard record keeping (e.g., database, notebooks, and data files) is used to track specimens. Records are maintained for 3 years, including related QA/QC data. Additionally, duplicate records will be kept off-site in electronic format. Study subject confidentiality is protected by providing personal identifiers only to the medical officer.

19. Method Performance Documentation

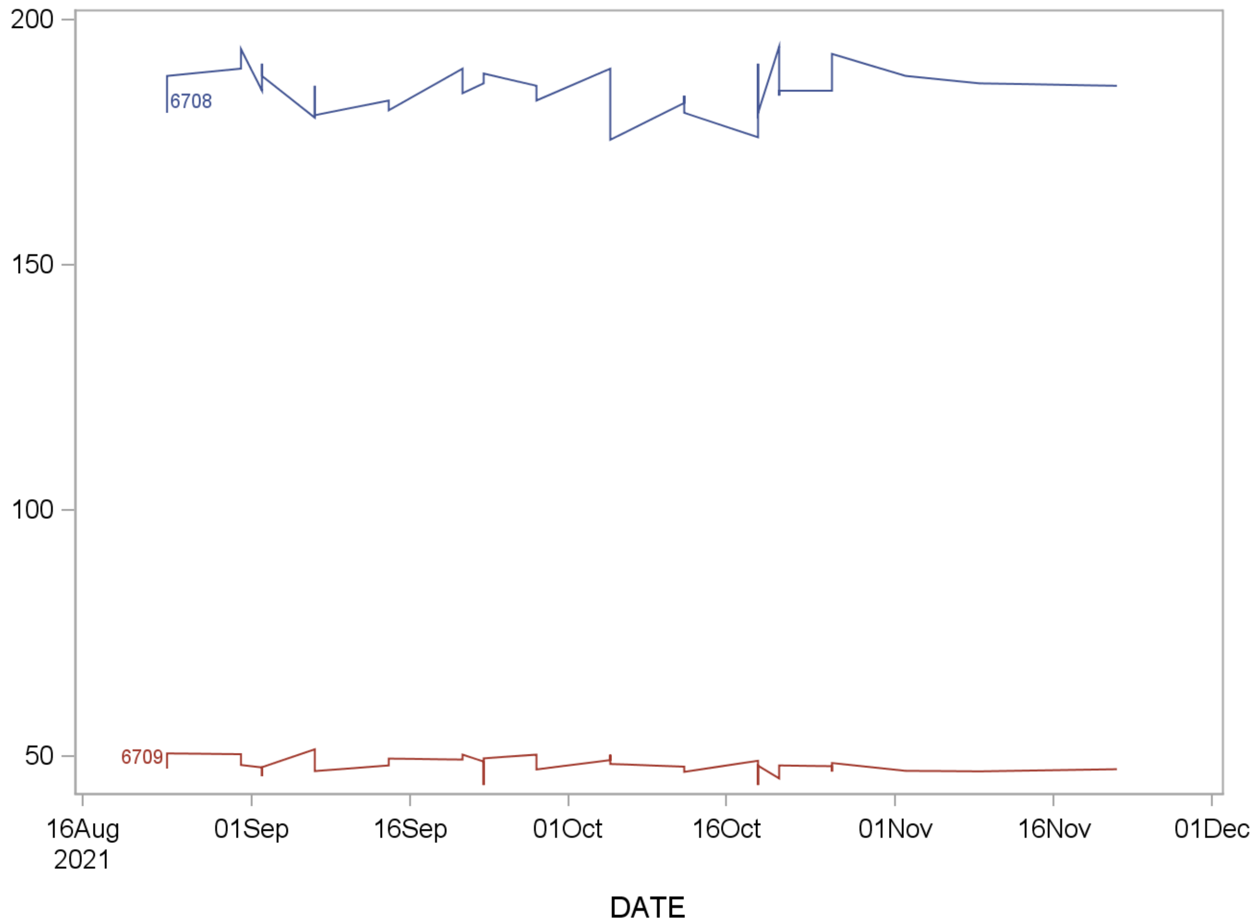
Method performance documentation for this method including accuracy, precision, sensitivity, specificity, and stability is provided in Appendix C of this method documentation. The signatures of the branch chief and director of the Division of Laboratory Sciences on the first page of this procedure denote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

20. Summary Statistics and QC Graphs

See next pages.

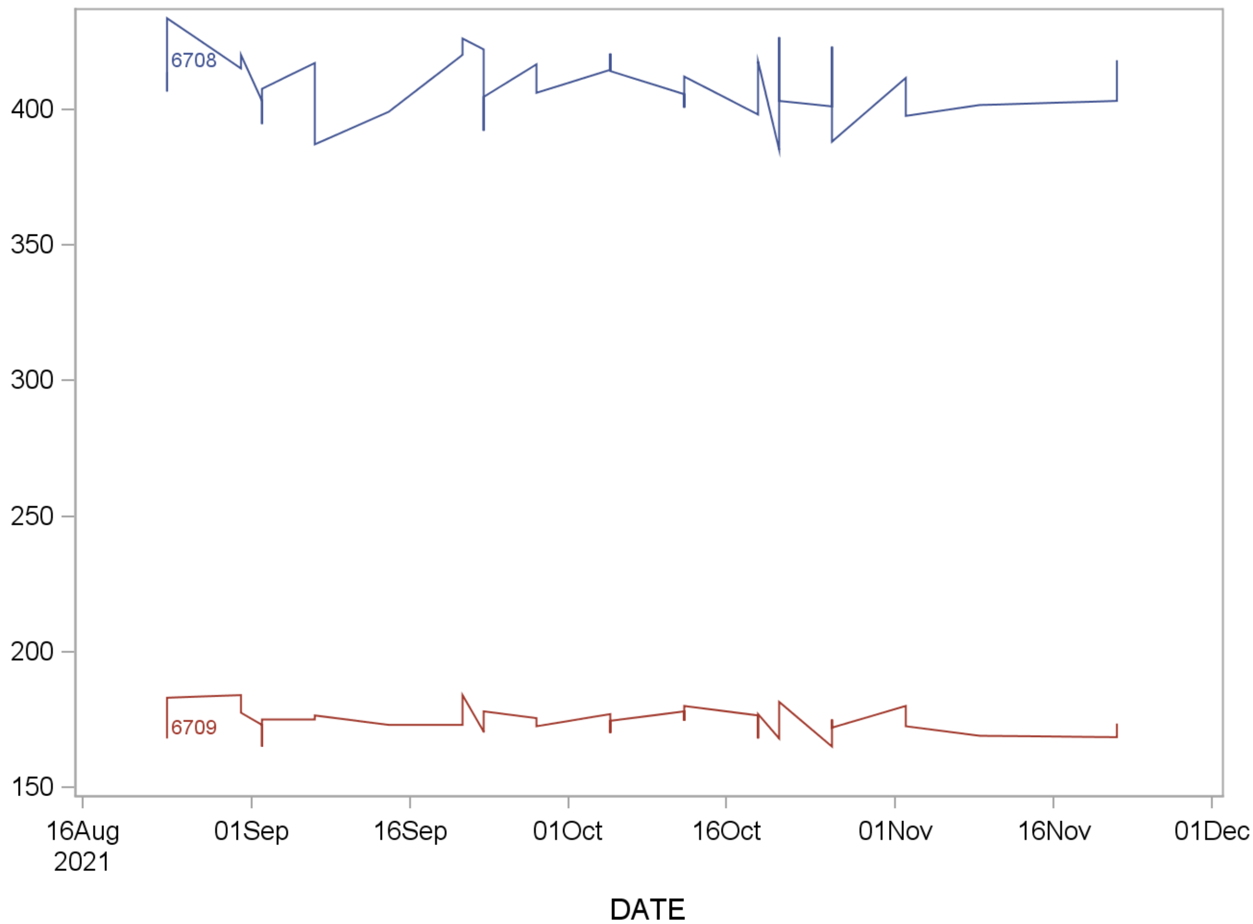
2017-2018 Summary Statistics and QC Chart
URX2MH (2-methylhippuric acid (ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	38	24AUG21	22NOV21	185.9868	4.4804	2.4
6709	38	24AUG21	22NOV21	47.9447	1.6421	3.4



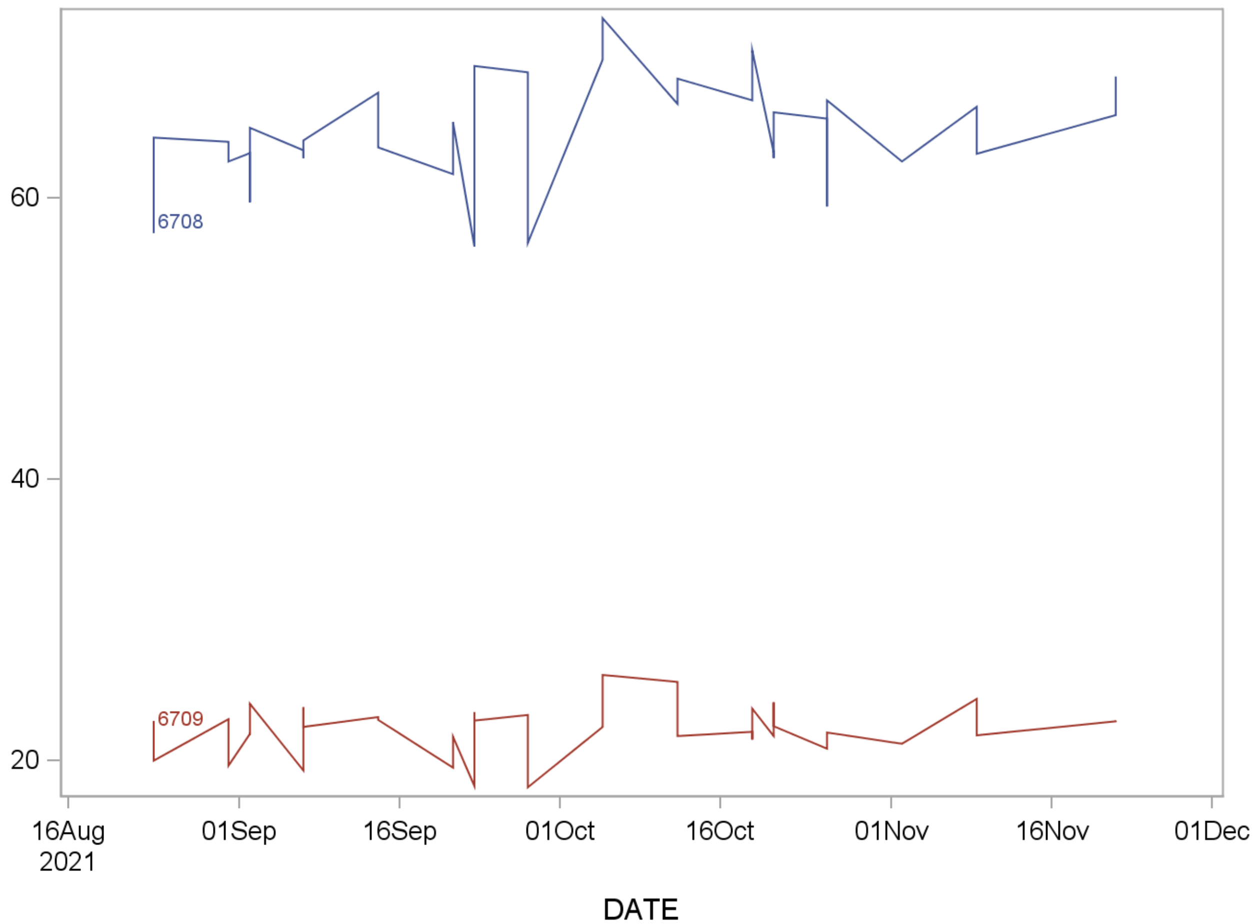
2017-2018 Summary Statistics and QC Chart
URX34M (3-methipurc acd & 4-methipurc acd(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	408.5385	11.5573	2.8
6709	39	24AUG21	22NOV21	174.3718	4.7845	2.7



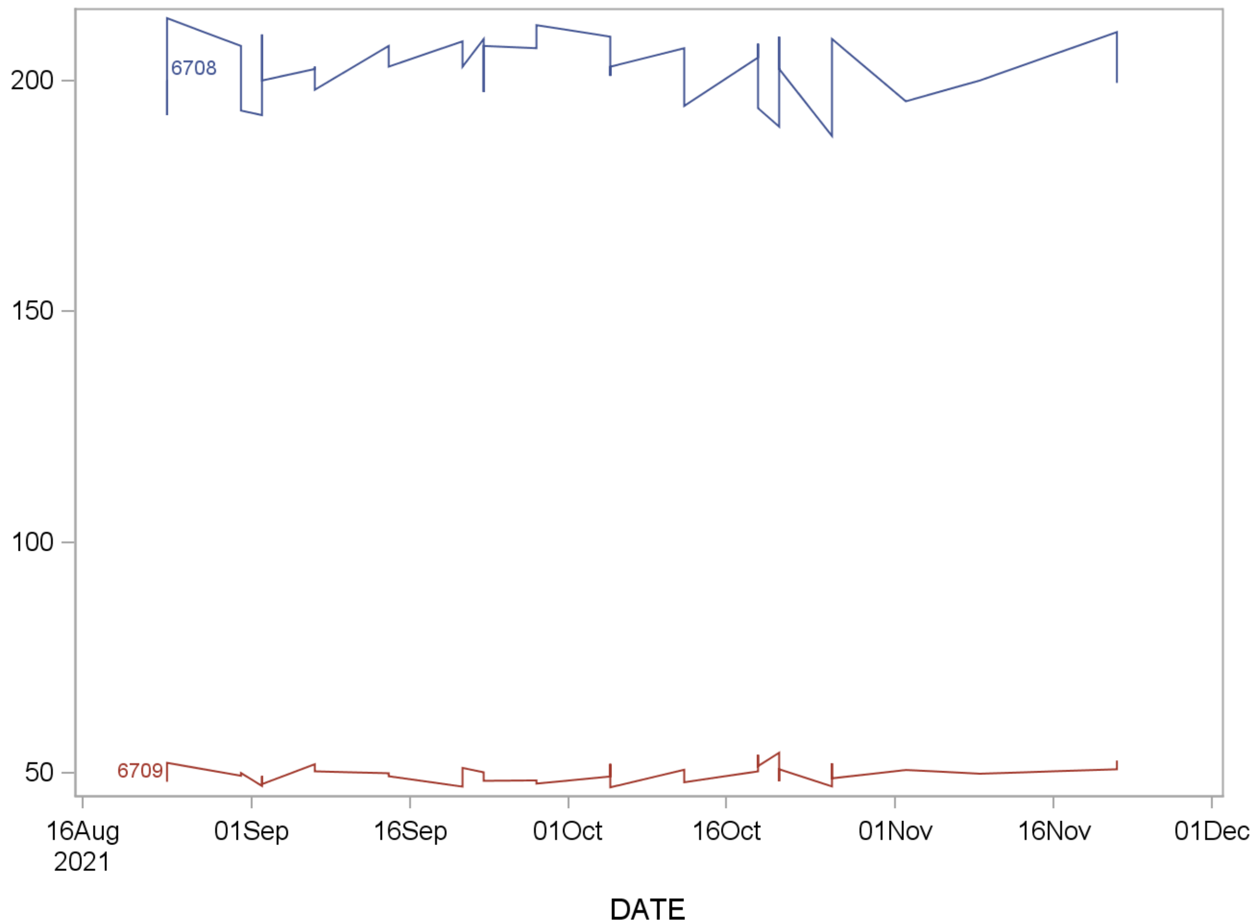
2017-2018 Summary Statistics and QC Chart
URXAAM (N-ace-S-(2-carbamoylethyl)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	40	24AUG21	22NOV21	64.7650	3.6971	5.7
6709	40	24AUG21	22NOV21	22.1738	1.7439	7.9



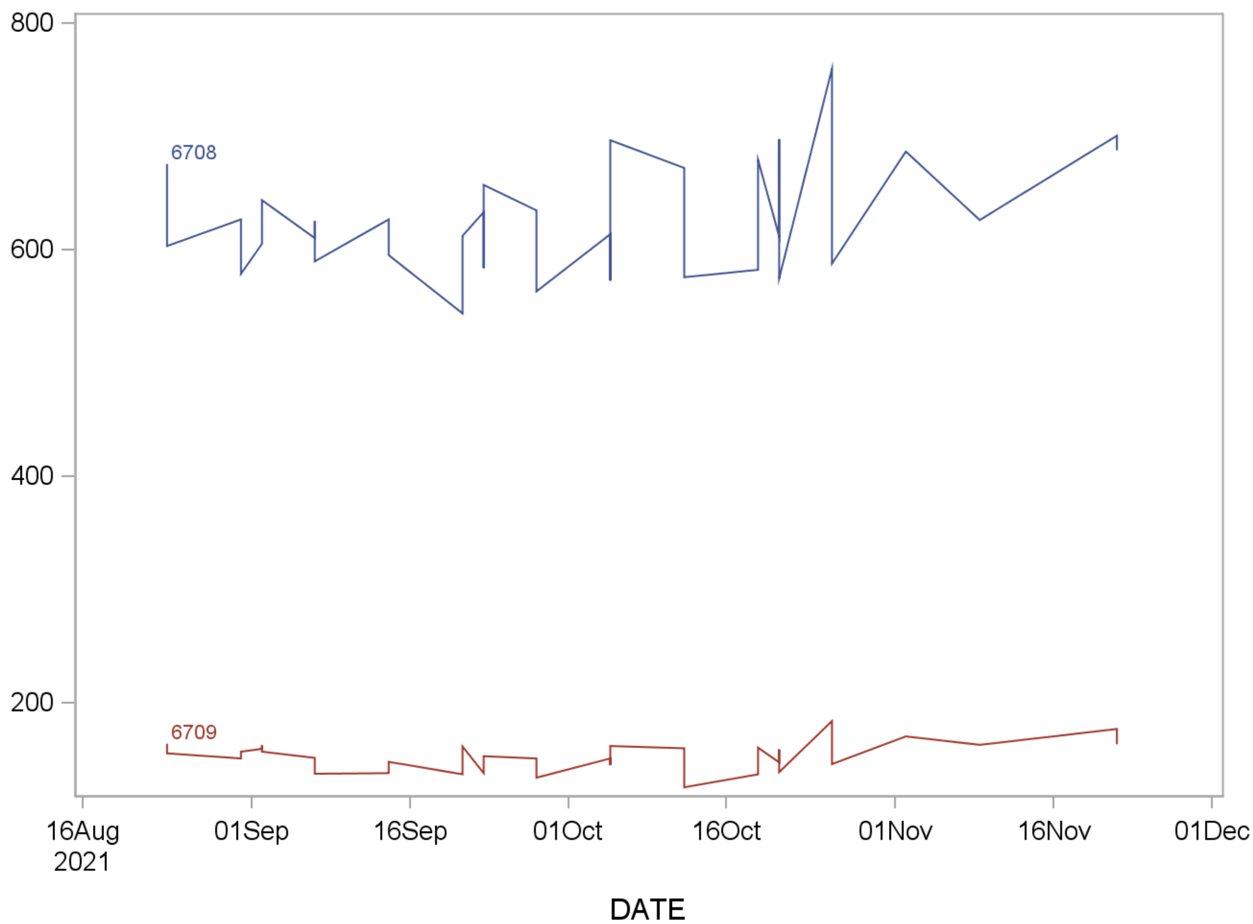
2017-2018 Summary Statistics and QC Chart
URXAMC (N-ace-S-(N-methlcarbamoyl)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	202.308	6.605	3.3
6709	39	24AUG21	22NOV21	49.850	1.870	3.8



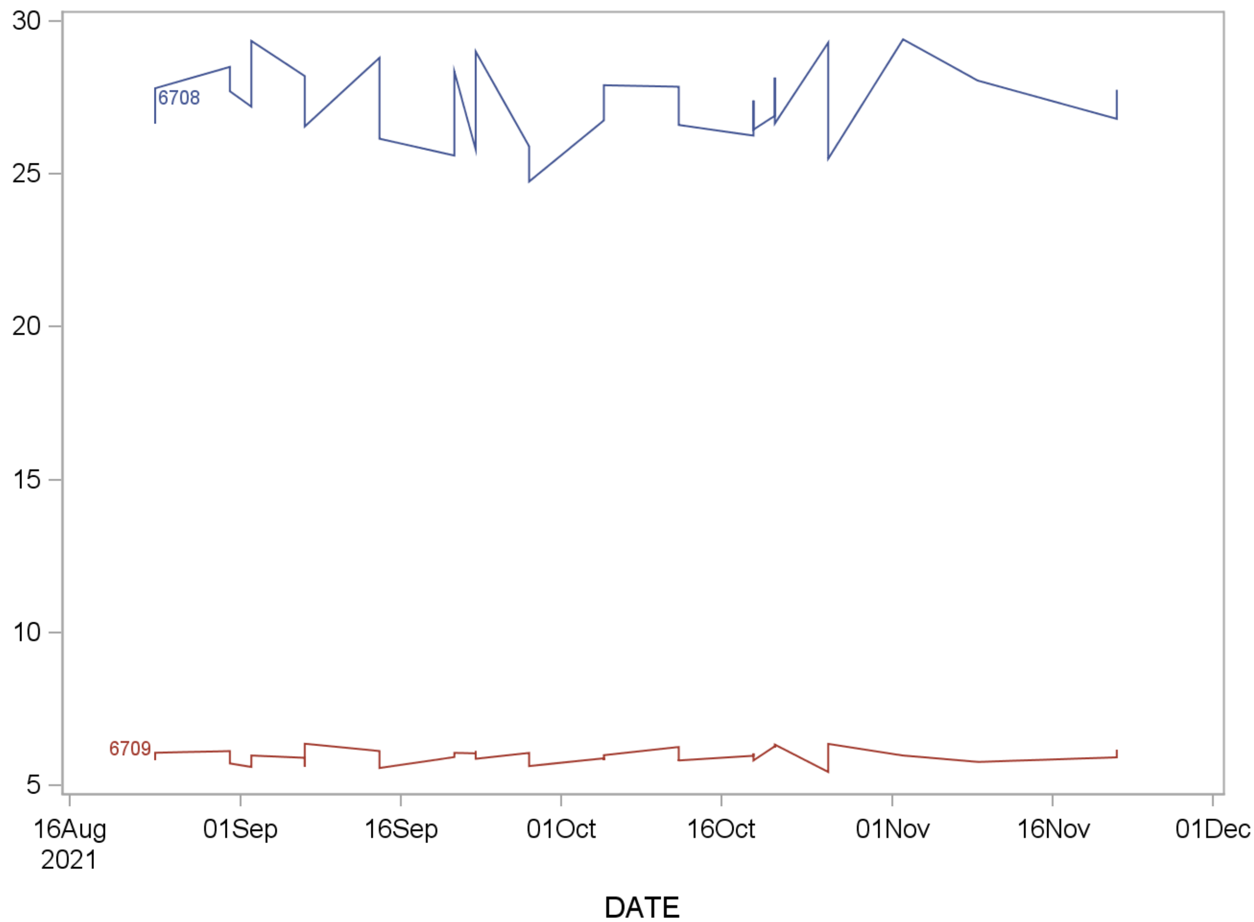
2017-2018 Summary Statistics and QC Chart
URXATC (2-amnothiazolne-4-carbxylic acid(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	626.7051	46.6931	7.5
6709	39	24AUG21	22NOV21	151.6538	12.3630	8.2



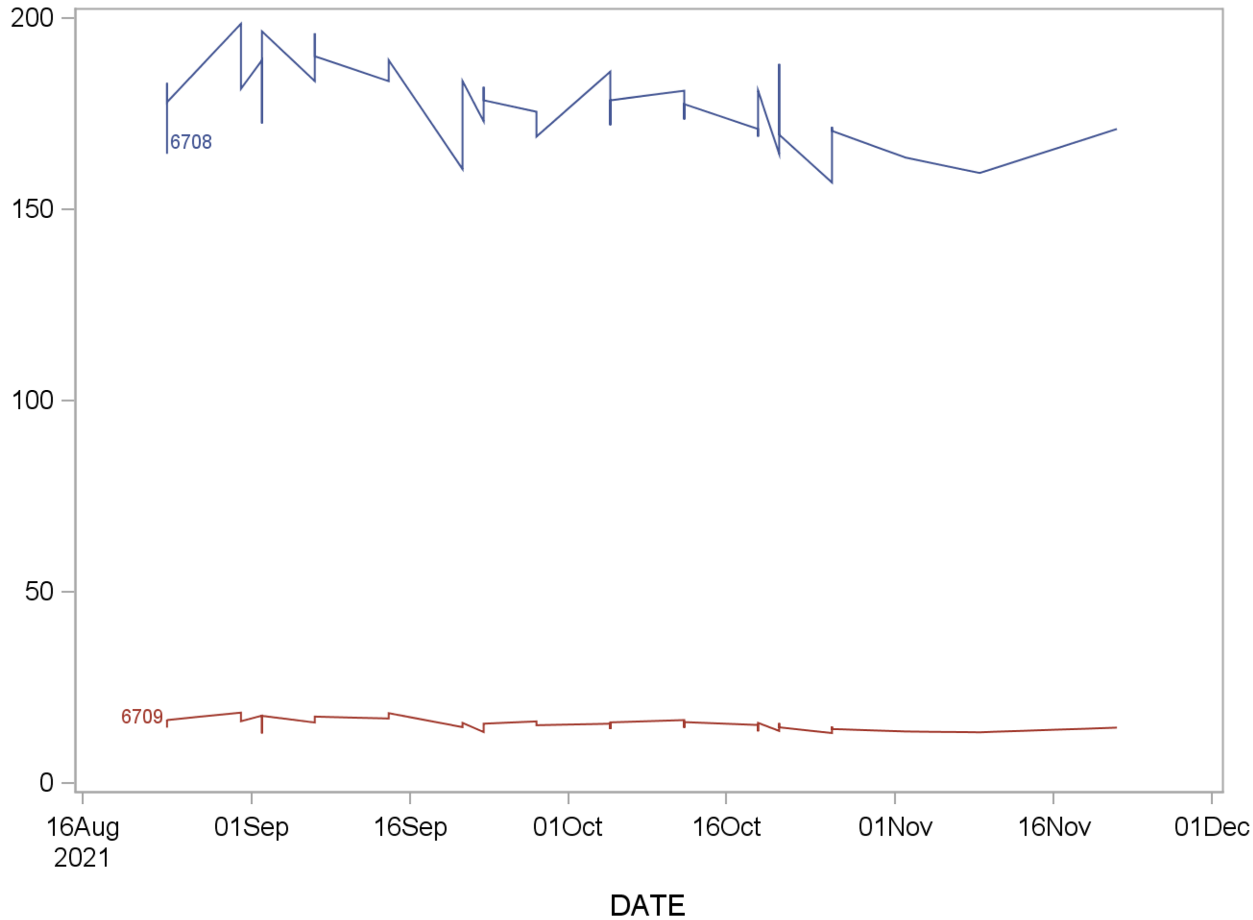
2017-2018 Summary Statistics and QC Chart
URXBMA (N-acetyl-S-(benzyl)-L-cysteine(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	27.3500	1.1621	4.2
6709	39	24AUG21	22NOV21	5.9572	0.2243	3.8



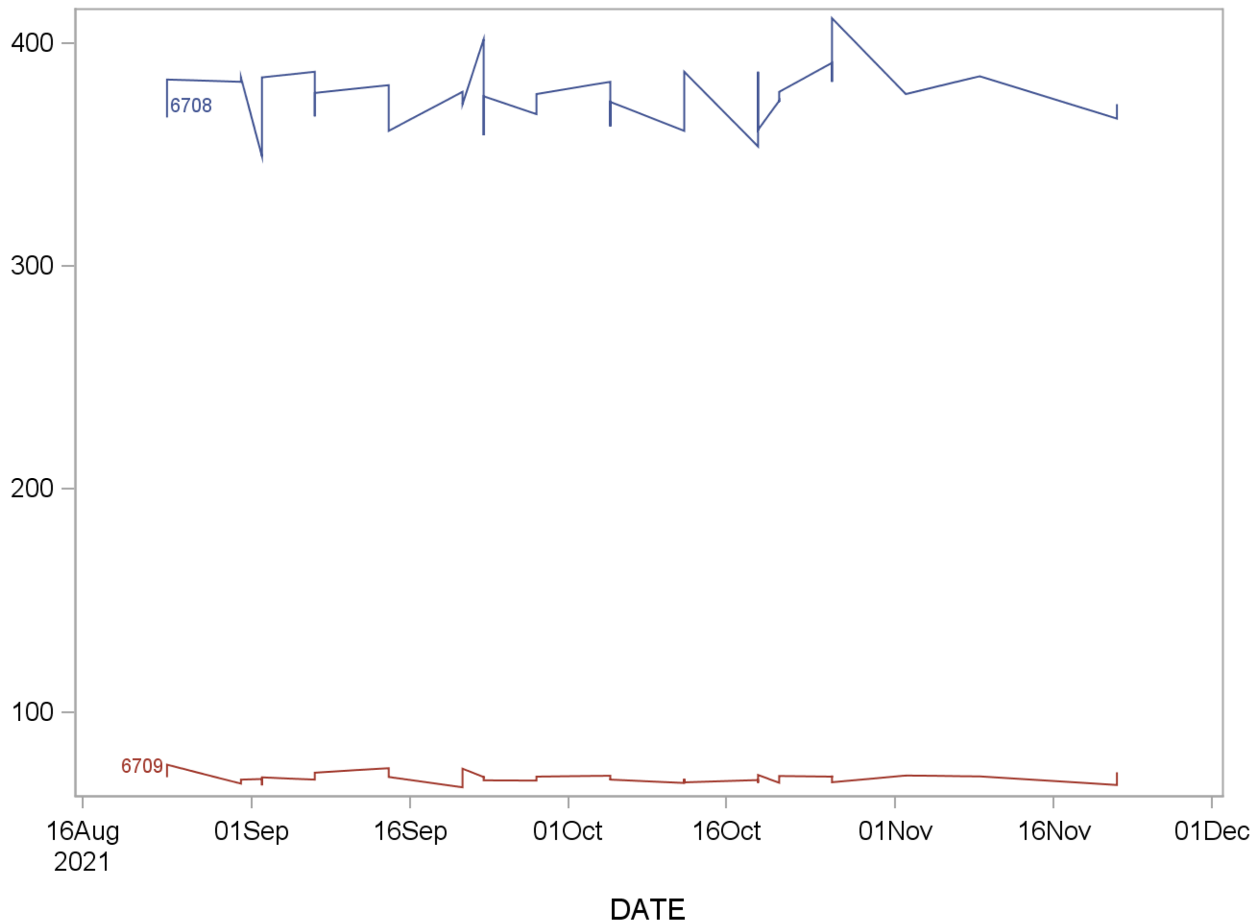
2017-2018 Summary Statistics and QC Chart
URXBPM (N-acetyl-S-(n-propyl)-L-cysteine(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	38	24AUG21	22NOV21	177.1579	10.3781	5.9
6709	38	24AUG21	22NOV21	15.4053	1.4928	9.7



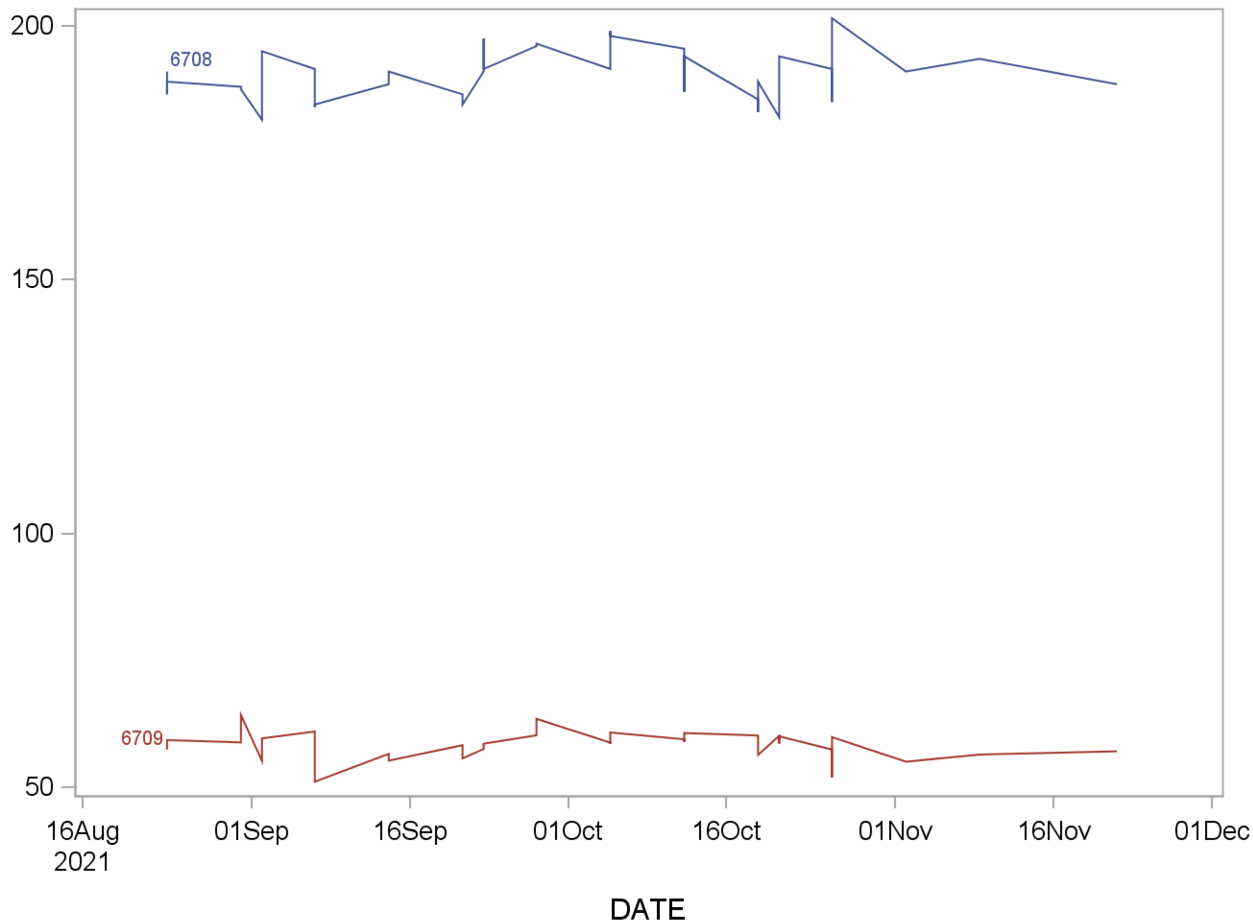
2017-2018 Summary Statistics and QC Chart
URXCEM (N-acetyl-S-(2-carbxyethyl)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	375.9487	12.3180	3.3
6709	39	24AUG21	22NOV21	70.4205	2.0965	3.0



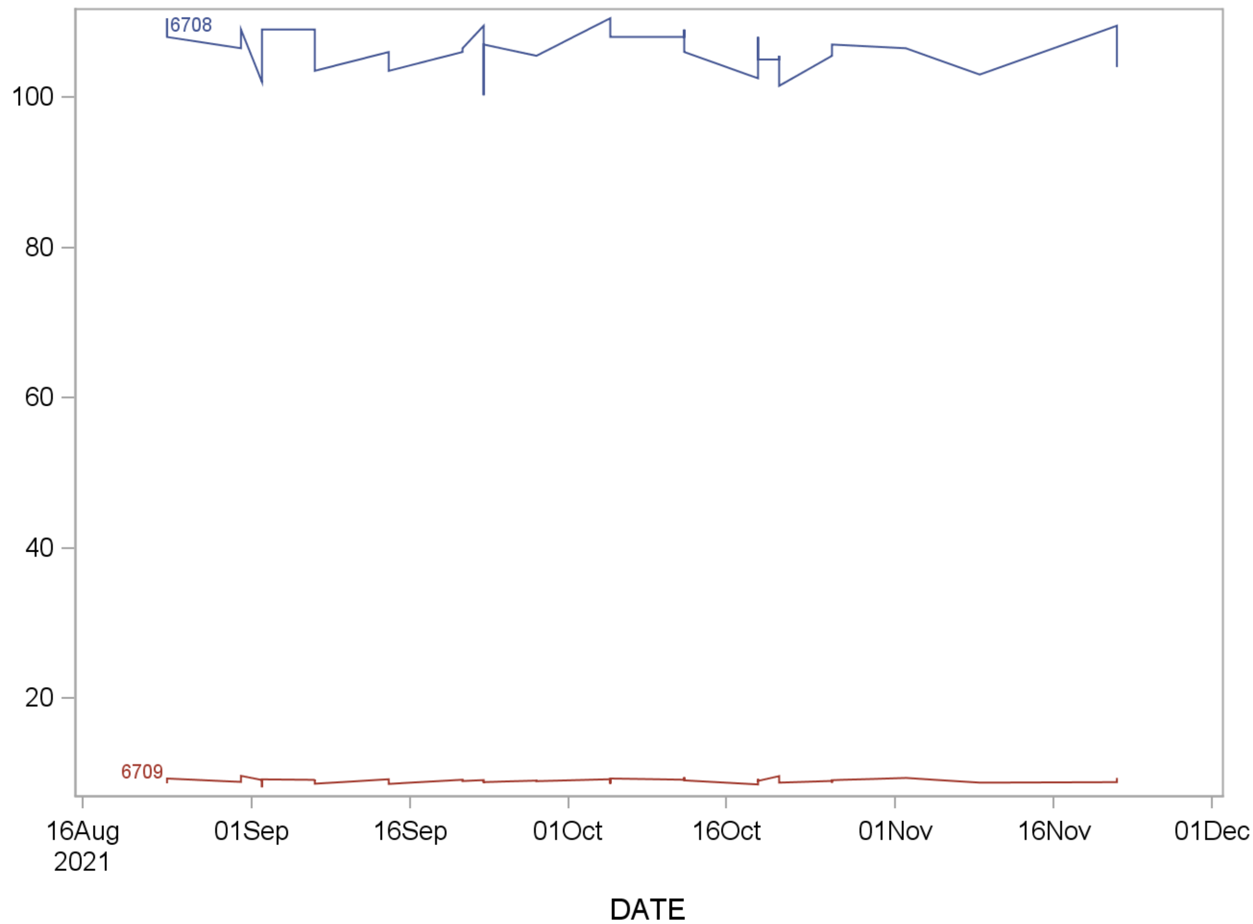
2017-2018 Summary Statistics and QC Chart
URXCYHA (CYHA cysteine (ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	38	24AUG21	22NOV21	190.2763	4.9288	2.6
6709	38	24AUG21	22NOV21	58.3289	2.6049	4.5



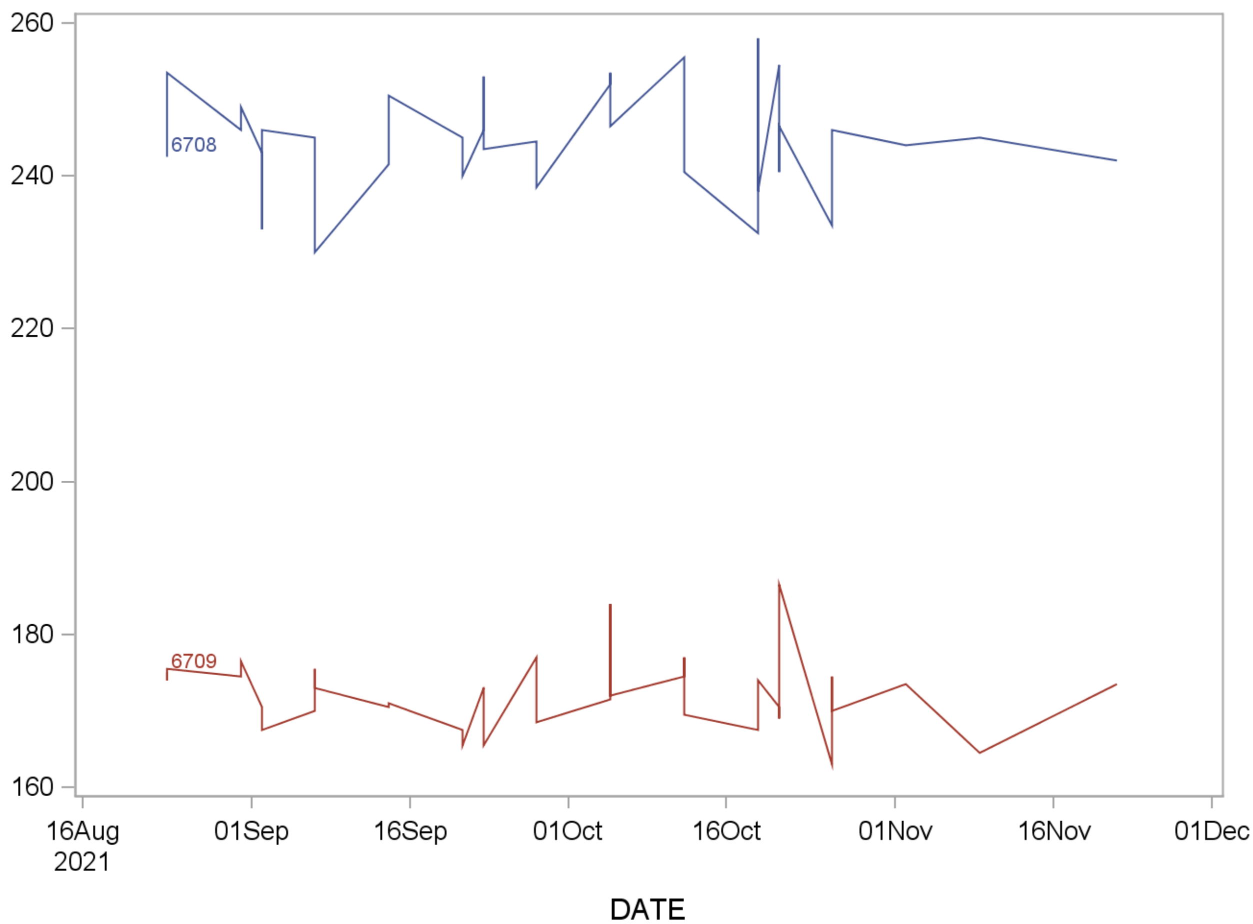
2017-2018 Summary Statistics and QC Chart
URXCYM (N-acetyl-S-(2-cyanoethyl)-L-cyst(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	106.3526	2.5724	2.4
6709	39	24AUG21	22NOV21	9.0317	0.3154	3.5



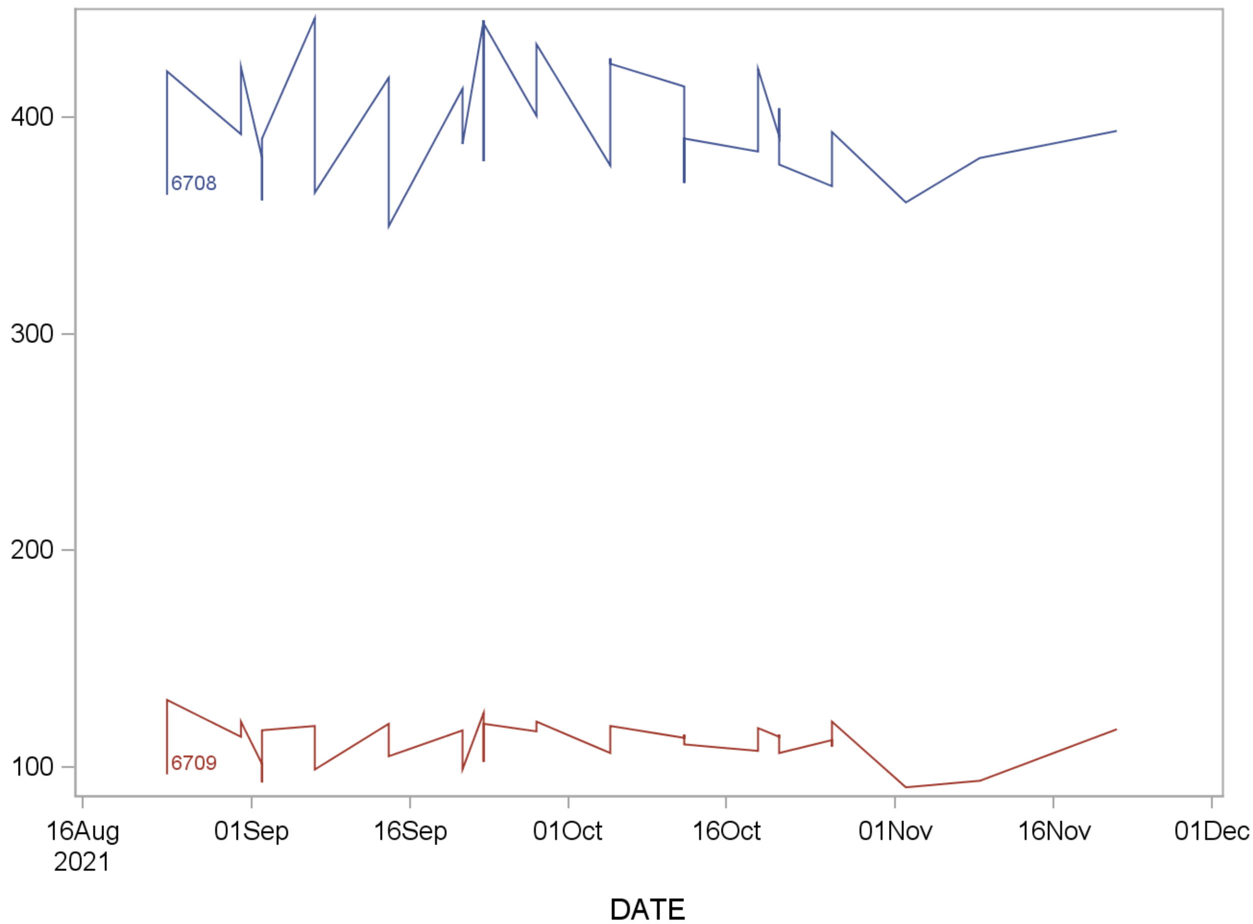
2017-2018 Summary Statistics and QC Chart
URXDHB (N-ace-S- (3,4-Dihidxybutl)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	38	24AUG21	22NOV21	244.7105	6.4751	2.6
6709	38	24AUG21	22NOV21	171.9868	4.7782	2.8



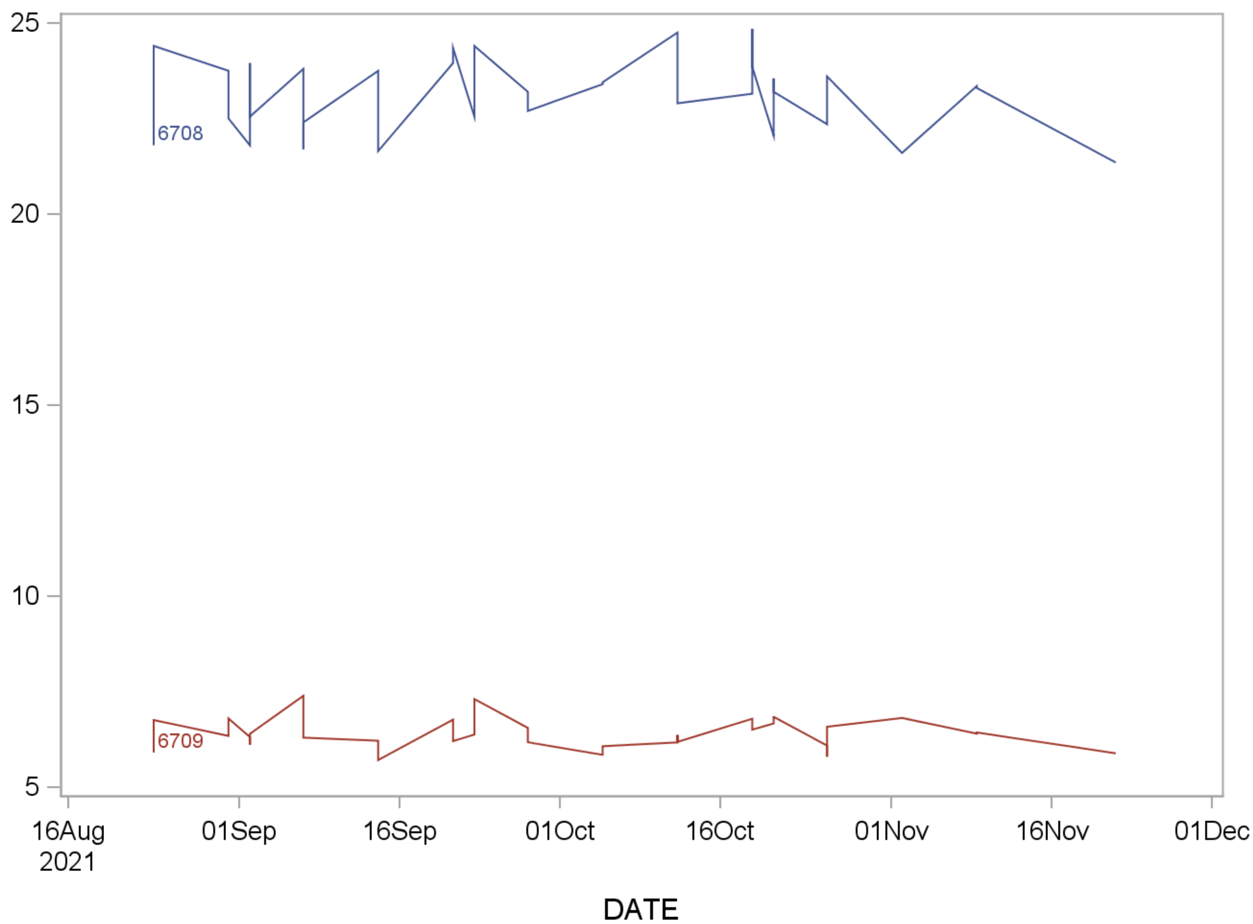
2017-2018 Summary Statistics and QC Chart
URXGAM (N-ac-S-(2-carbmo-2-hydxel)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	38	24AUG21	22NOV21	395.7500	25.4640	6.4
6709	38	24AUG21	22NOV21	111.7895	9.4027	8.4



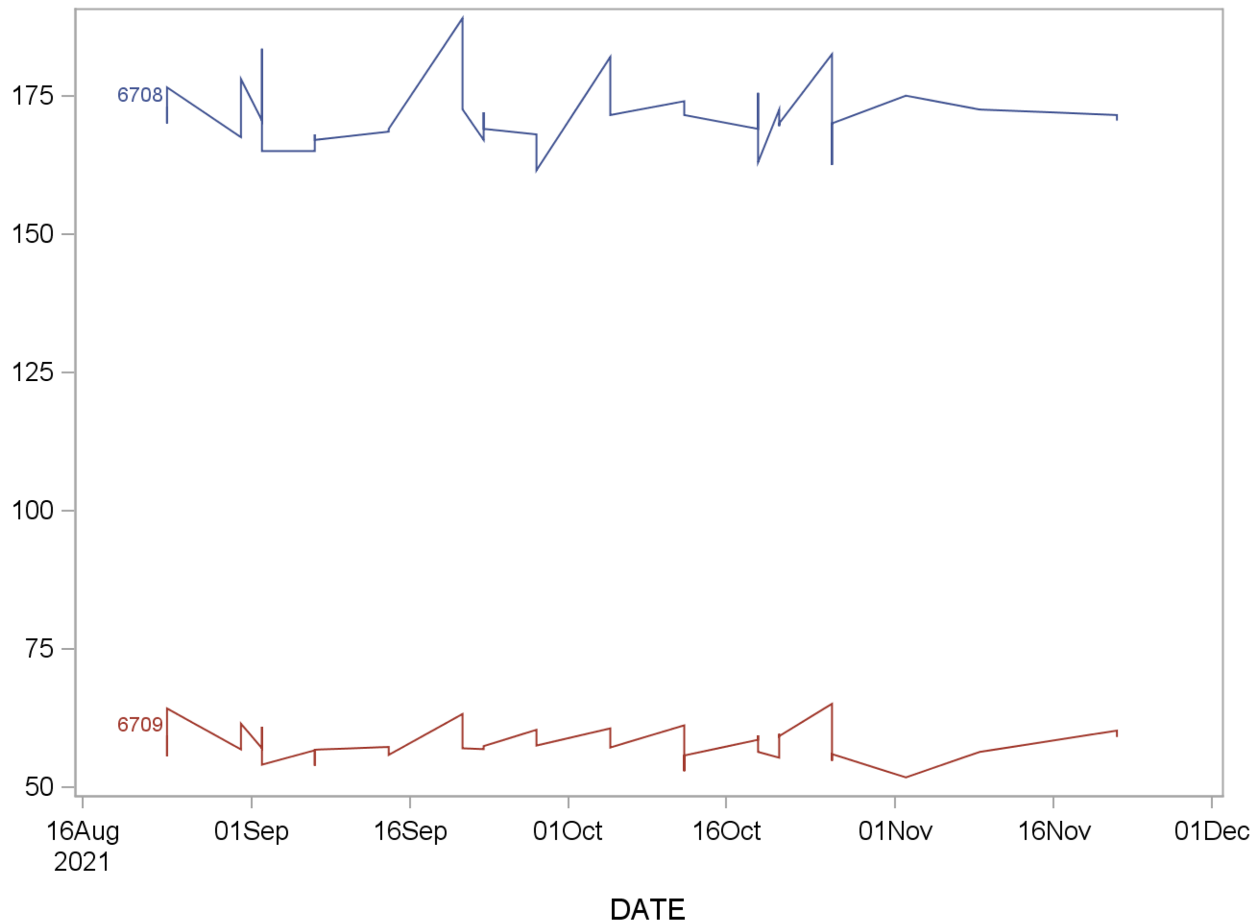
2017-2018 Summary Statistics and QC Chart
URXHEM (N-ace-S-(2-hydroxyethyl)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	38	24AUG21	22NOV21	23.1158	0.9299	4.0
6709	38	24AUG21	22NOV21	6.4293	0.3852	6.0



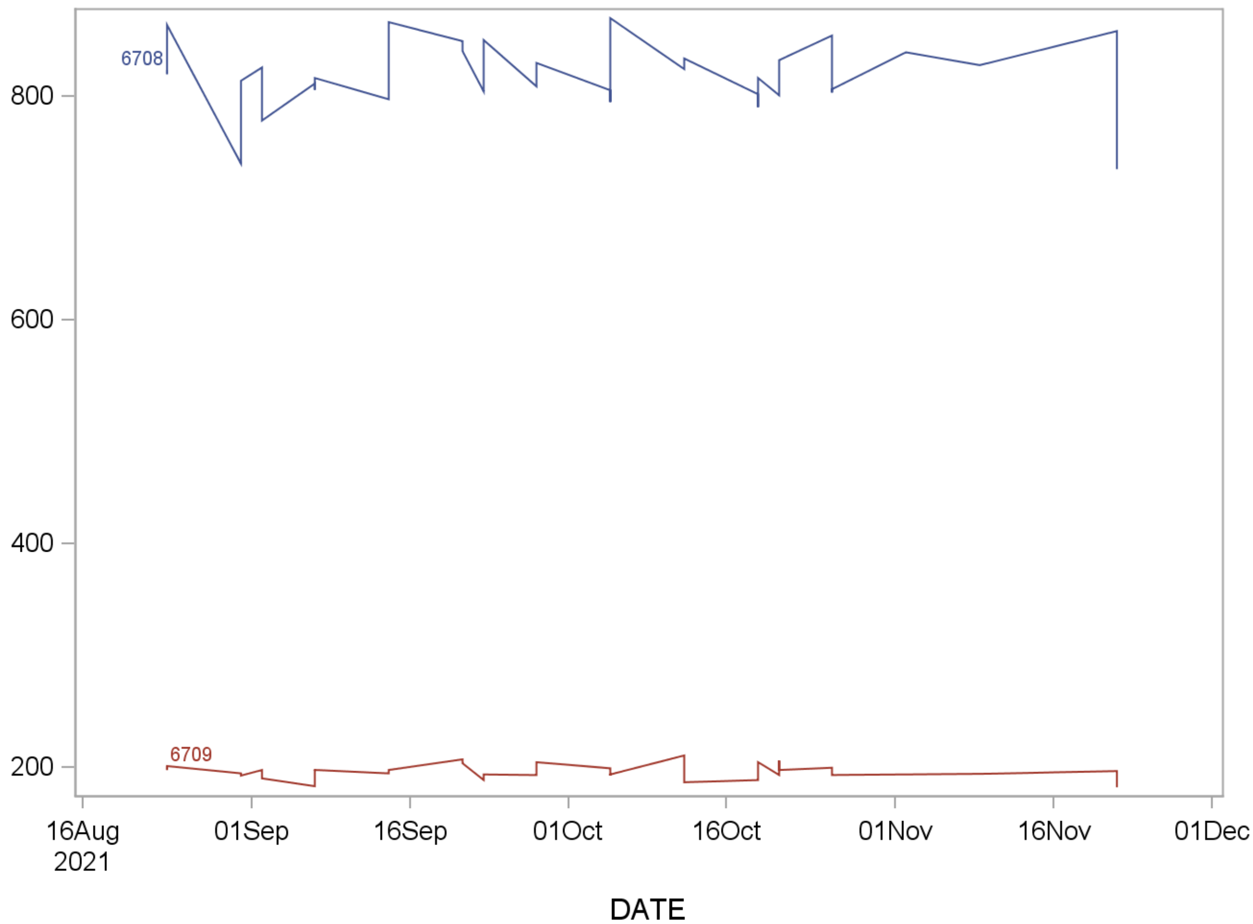
2017-2018 Summary Statistics and QC Chart
URXHP2 (N-ace-S-(2-hydroxypropyl)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	171.5128	5.7382	3.3
6709	39	24AUG21	22NOV21	57.9192	2.9235	5.0



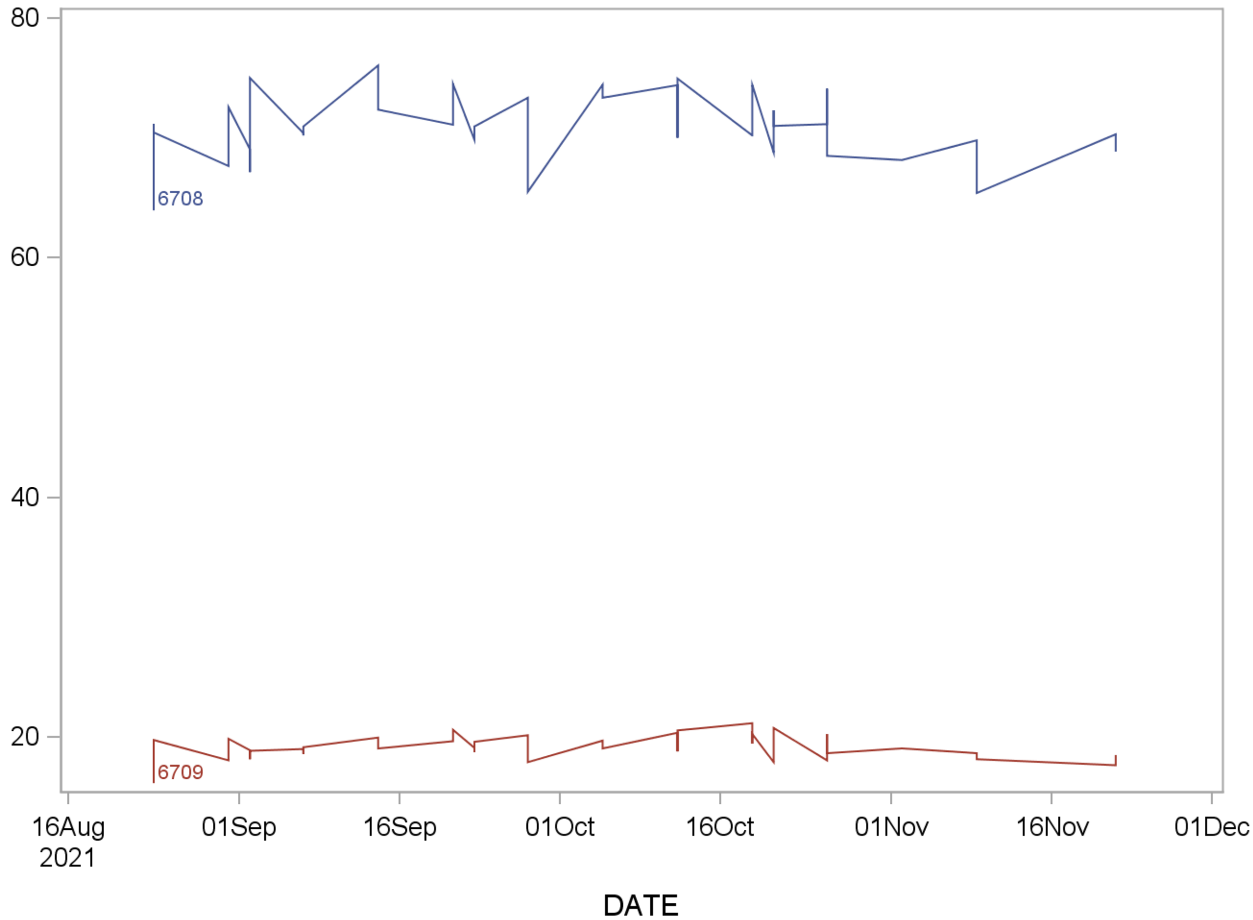
2017-2018 Summary Statistics and QC Chart
URXHPM (N-ace-S-(3-hydroxypropyl)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	817.4744	29.2554	3.6
6709	39	24AUG21	22NOV21	195.1923	6.1533	3.2



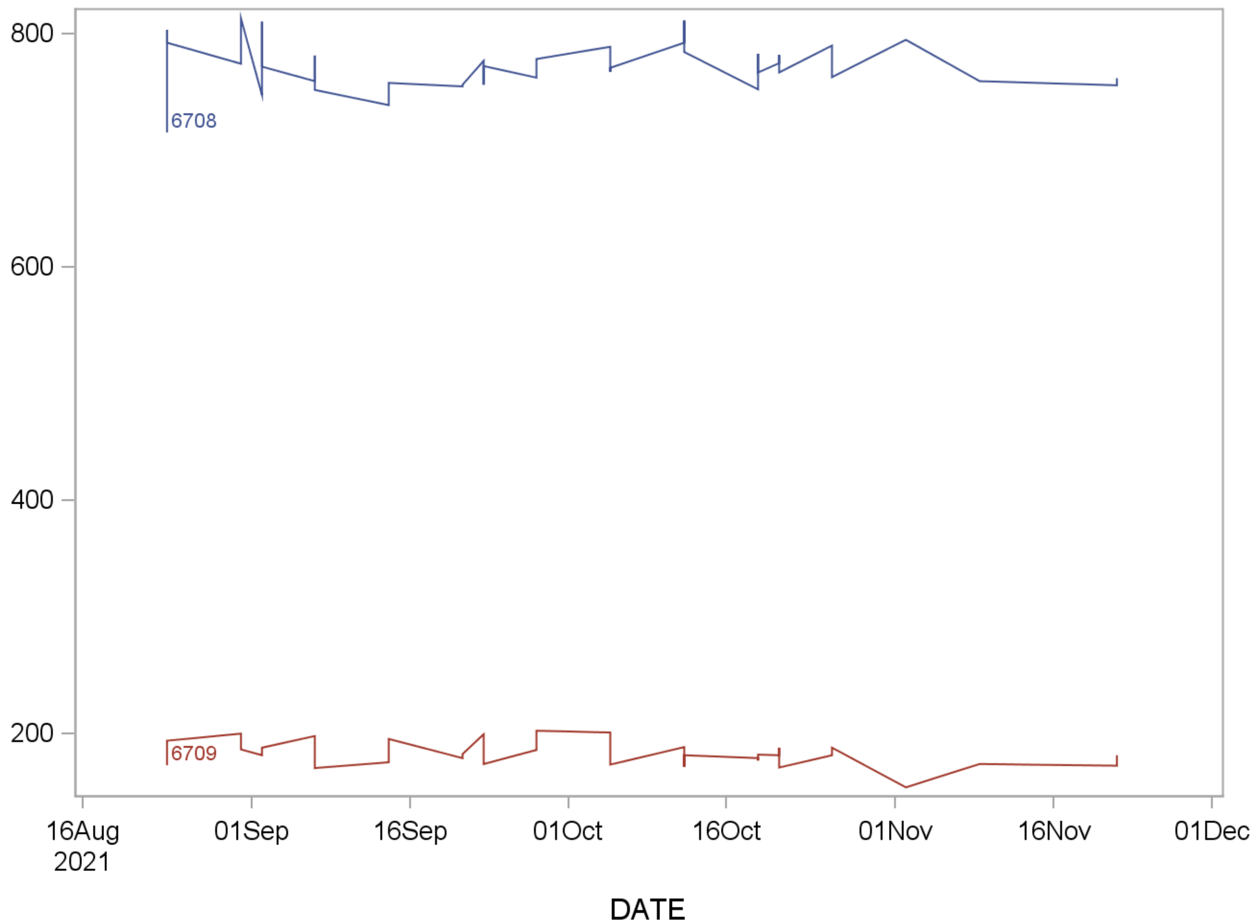
2017-2018 Summary Statistics and QC Chart
URXIPM3 (IPM3 cysteine (ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	70.8564	2.8193	4.0
6709	39	24AUG21	22NOV21	19.1321	1.0372	5.4



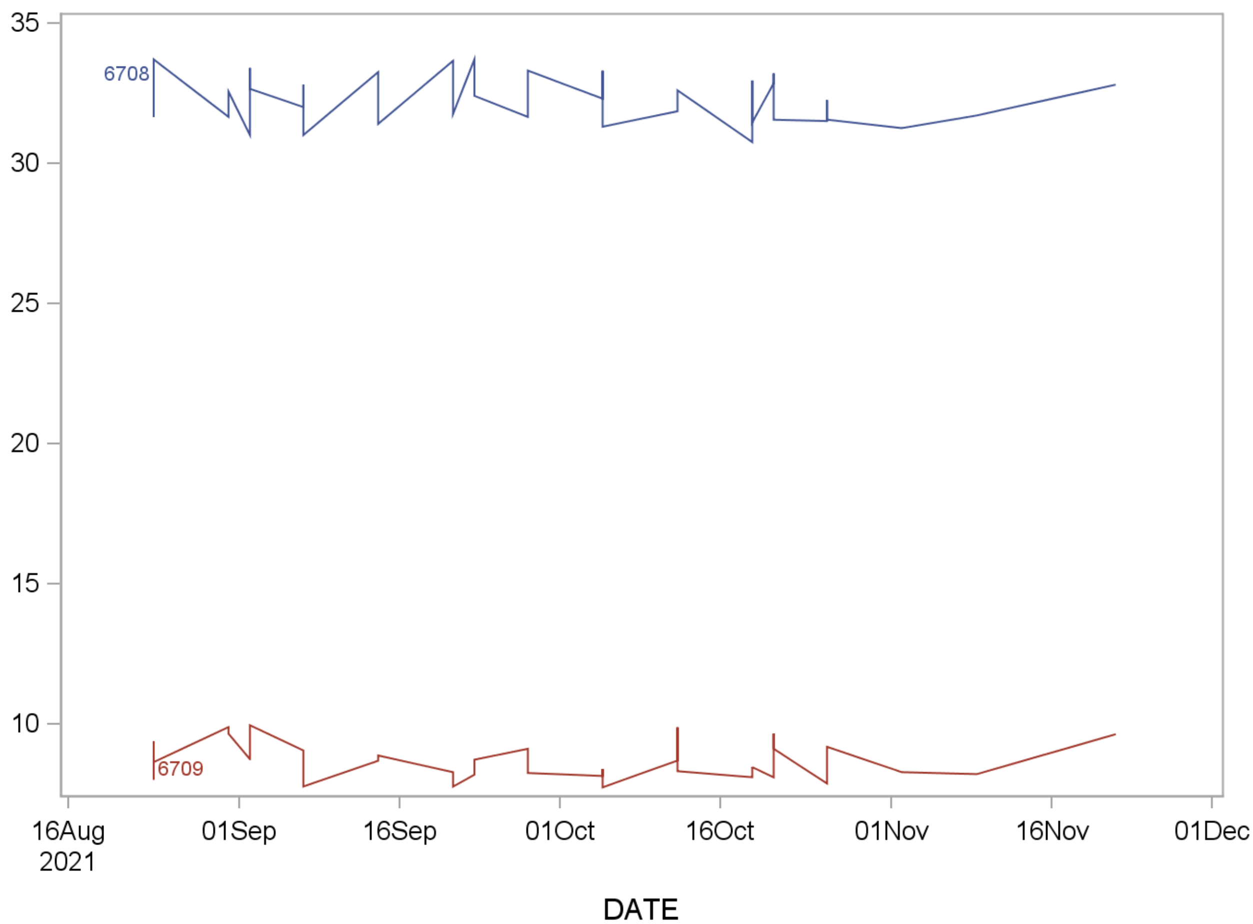
2017-2018 Summary Statistics and QC Chart
URXMAD (Mandelic acid(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	772.0385	20.4796	2.7
6709	39	24AUG21	22NOV21	182.6410	10.1040	5.5



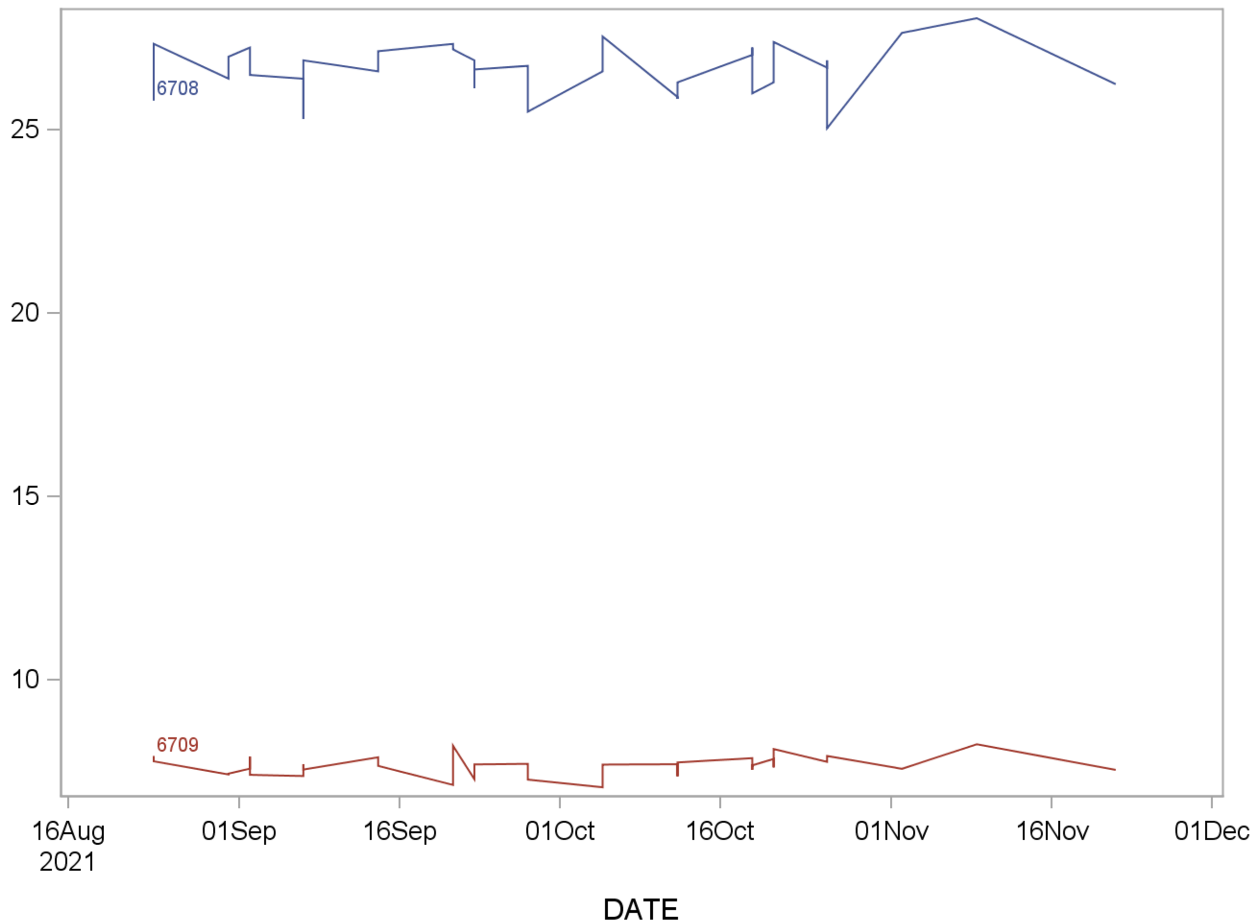
2017-2018 Summary Statistics and QC Chart
URXMB3 (N-A-S-(4-hydrxy-2-butenyl)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	38	24AUG21	22NOV21	32.2776	0.8394	2.6
6709	38	24AUG21	22NOV21	8.6363	0.6383	7.4



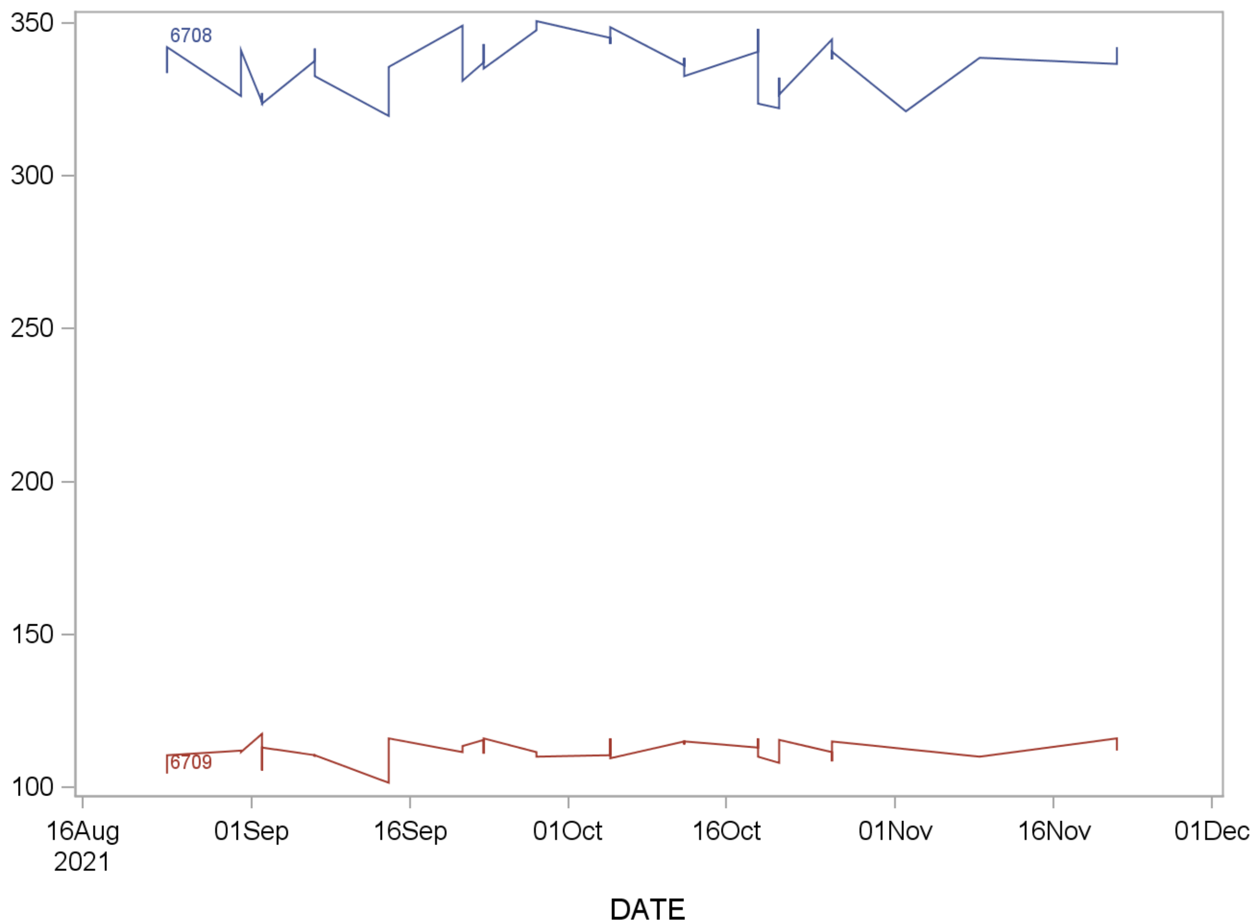
2017-2018 Summary Statistics and QC Chart
URXPHE (N-ace-S-(phenl-2-hydxyetl)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	38	24AUG21	22NOV21	26.6750	0.6714	2.5
6709	38	24AUG21	22NOV21	7.6496	0.2667	3.5



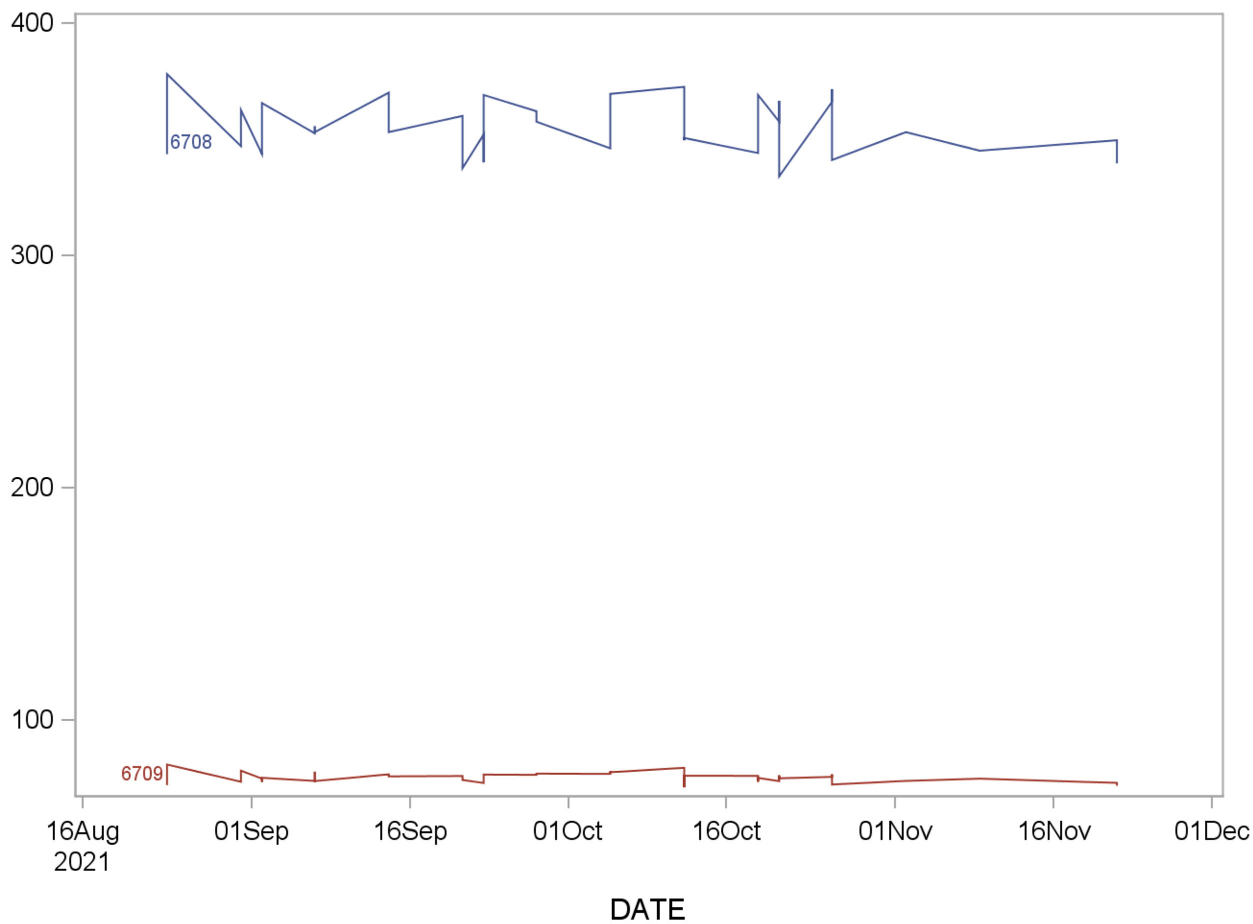
2017-2018 Summary Statistics and QC Chart
URXPHG (Phenylglyoxylic acid(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	336.2949	8.5492	2.5
6709	39	24AUG21	22NOV21	111.7949	3.4311	3.1



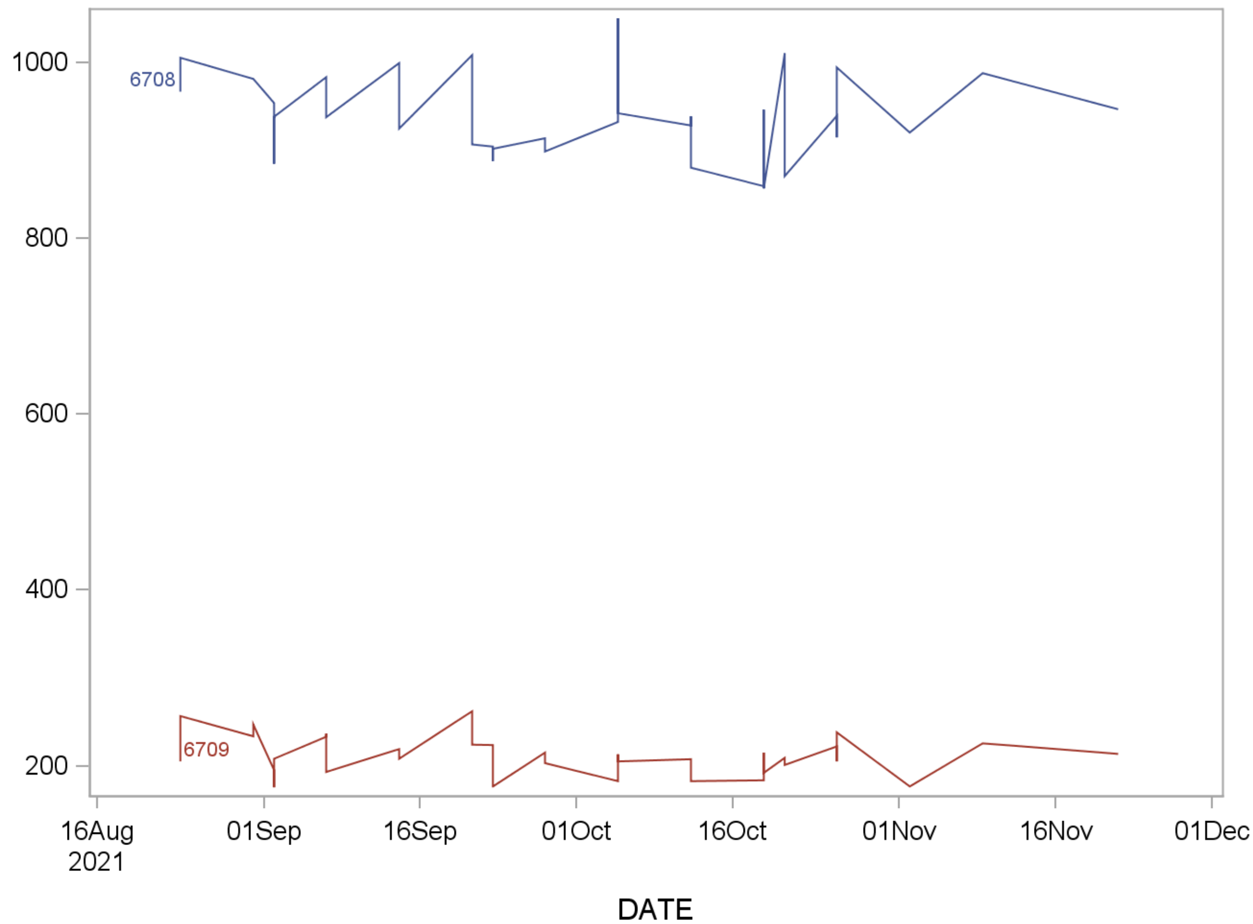
2017-2018 Summary Statistics and QC Chart
URXPMM (N-A-S-(3-hydrxprpl-1-metl)-L-cys(ng/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	39	24AUG21	22NOV21	354.9487	11.2219	3.2
6709	39	24AUG21	22NOV21	75.0231	2.1810	2.9



2017-2018 Summary Statistics and QC Chart
URXTTC (2-Thioxothiazolidine-4-carboxylic acid)

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6708	38	24AUG21	22NOV21	941.434	46.150	4.9
6709	38	24AUG21	22NOV21	210.000	22.222	10.6



APPENDIX A: Ruggedness Testing

The ruggedness of the method was evaluated by the following parameters that were assessed through independent experiments:

i. Methanol as organic phase (Solvent B):

Acetonitrile was favored over methanol for mobile phase B because the latter created higher backpressure.

ii. Stability at 4°C and -20°C:

No statistically significant difference among data for any analyte was observed when samples were stored at 4°C and -20°C for a week. For long-term, samples should be stored at -70°C.

iii. Samples run at 1:10 and 1:50 dilutions:

Samples were prepared at 1:10 and 1:50 dilutions and were analyzed for all the analytes. The percentage differences among final estimates were < 10%.

iv. Samples run at 1:500, 1:1000, 1:2000, and 1:5000 dilutions for 34MH, MADA, PHGA, and 2MHA:

Samples were prepared at 1:500, 1:1000, 1:2000, and 1: 5000 dilutions and were analyzed for the selected analytes. The percentage differences among final estimates were within 26% with the exception of 1:5000 dilutions.

v. Samples run at different pH values:

Spiked urine samples were adjusted to different pH values and analyte concentrations were measured. All analytes were stable within the pH range from 2-11 (Table A1).

Table A1: Effect of pH on urinary metabolite concentrations

pH	Analyte	Target Concentration (ng/mL)	Measured Concentration (ng/mL)	% Error
2	CEMA	223.95	219.77	-2%
2	ATCA	291.69	300.73	3%
2	GAMA	182.03	207.45	14%
2	AAMA	50.80	53.58	5%
2	HEMA	11.82	10.15	-14%
2	DHBM	157.56	168.45	7%
2	AMCA	125.58	112.79	-10%
2	TTCA	355.88	387.49	9%
2	HPMA	488.78	448.66	-8%
2	HPM2	90.85	106.88	18%
2	MADA	365.70	377.98	3%
2	CYMA	17.33	18.36	6%
2	MHB3	19.37	22.18	14%
2	HPMM	136.56	131.83	-3%
2	PHGA	368.26	352.52	-4%
2	2MHA	125.69	116.28	-7%

pH	Analyte	Target Concentration (ng/mL)	Measured Concentration (ng/mL)	% Error
2	BPMA	21.17	22.97	9%
2	34MH	105.12	106.36	1%
2	PHEM	16.78	14.52	-13%
2	BMA	16.71	18.06	8%
2	CYHA	88.63	89.81	1%
2	IPM3	17.08	20.28	19%
3	CEMA	223.95	230.59	3%
3	ATCA	291.69	307.95	6%
3	GAMA	182.03	220.03	21%
3	AAMA	50.80	58.07	14%
3	HEMA	11.82	13.42	14%
3	DHBM	157.56	168.42	7%
3	AMCA	125.58	120.68	-4%
3	TTCA	355.88	391.20	10%
3	HPMA	488.78	480.16	-2%
3	HPM2	90.85	87.44	-4%
3	MADA	365.70	397.78	9%
3	CYMA	17.33	20.40	18%
3	MHB3	19.37	16.64	-14%
3	HPMM	136.56	134.05	-2%
3	PHGA	368.26	371.34	1%
3	2MHA	125.69	122.95	-2%
3	BPMA	21.17	22.69	7%
3	34MH	105.12	107.47	2%
3	PHEM	16.78	15.58	-7%
3	BMA	16.71	17.44	4%
3	CYHA	88.63	82.80	-7%
3	IPM3	17.08	19.13	12%
4	CEMA	223.95	227.65	2%
4	ATCA	291.69	286.64	-2%
4	GAMA	182.03	192.95	6%
4	AAMA	50.80	53.68	6%
4	HEMA	11.82	11.74	-1%
4	DHBM	157.56	161.28	2%
4	AMCA	125.58	127.33	1%
4	TTCA	355.88	366.90	3%
4	HPMA	488.78	536.26	10%
4	HPM2	90.85	94.02	3%
4	MADA	365.70	370.62	1%
4	CYMA	17.33	16.40	-5%
4	MHB3	19.37	17.61	-9%
4	HPMM	136.56	133.18	-2%
4	PHGA	368.26	354.84	-4%
4	2MHA	125.69	128.76	2%
4	BPMA	21.17	22.44	6%
4	34MH	105.12	103.80	-1%
4	PHEM	16.78	14.81	-12%

pH	Analyte	Target Concentration (ng/mL)	Measured Concentration (ng/mL)	% Error
4	BMA	16.71	19.56	17%
4	CYHA	88.63	99.58	12%
4	IPM3	17.08	19.55	14%
5	CEMA	223.95	205.16	-8%
5	ATCA	291.69	274.52	-6%
5	GAMA	182.03	194.48	7%
5	AAMA	50.80	44.83	-12%
5	HEMA	11.82	10.99	-7%
5	DHBM	157.56	155.80	-1%
5	AMCA	125.58	128.50	2%
5	TTCA	355.88	372.35	5%
5	HPMA	488.78	437.26	-11%
5	HPM2	90.85	94.19	4%
5	MADA	365.70	374.83	2%
5	CYMA	17.33	17.41	0%
5	MHB3	19.37	18.61	-4%
5	HPMM	136.56	121.27	-11%
5	PHGA	368.26	345.49	-6%
5	2MHA	125.69	114.29	-9%
5	BPMA	21.17	22.58	7%
5	34MH	105.12	102.02	-3%
5	PHEM	16.78	16.15	-4%
5	BMA	16.71	18.37	10%
5	CYHA	88.63	95.40	8%
5	IPM3	17.08	17.02	0%
6	CEMA	223.95	206.78	-8%
6	ATCA	291.69	282.28	-3%
6	GAMA	182.03	188.66	4%
6	AAMA	50.80	51.21	1%
6	HEMA	11.82	11.06	-6%
6	DHBM	157.56	158.73	1%
6	AMCA	125.58	119.40	-5%
6	TTCA	355.88	343.18	-4%
6	HPMA	488.78	465.91	-5%
6	HPM2	90.85	79.93	-12%
6	MADA	365.70	352.12	-4%
6	CYMA	17.33	17.82	3%
6	MHB3	19.37	17.65	-9%
6	HPMM	136.56	127.44	-7%
6	PHGA	368.26	344.67	-6%
6	2MHA	125.69	117.61	-6%
6	BPMA	21.17	20.30	-4%
6	34MH	105.12	104.17	-1%
6	PHEM	16.78	15.09	-10%
6	BMA	16.71	20.11	20%
6	CYHA	88.63	77.53	-13%
6	IPM3	17.08	19.89	16%

pH	Analyte	Target Concentration (ng/mL)	Measured Concentration (ng/mL)	% Error
7	CEMA	223.95	208.71	-7%
7	ATCA	291.69	294.32	1%
7	GAMA	182.03	167.40	-8%
7	AAMA	50.80	47.67	-6%
7	HEMA	11.82	12.97	10%
7	DHBM	157.56	141.00	-11%
7	AMCA	125.58	130.01	4%
7	TTCA	355.88	355.25	0%
7	HPMA	488.78	403.29	-17%
7	HPM2	90.85	93.09	2%
7	MADA	365.70	339.19	-7%
7	CYMA	17.33	17.74	2%
7	MHB3	19.37	19.00	-2%
7	HPMM	136.56	110.33	-19%
7	PHGA	368.26	339.88	-8%
7	2MHA	125.69	112.43	-11%
7	BPMA	21.17	21.97	4%
7	34MH	105.12	101.98	-3%
7	PHEM	16.78	17.50	4%
7	BMA	16.71	18.20	9%
7	CYHA	88.63	93.64	6%
7	IPM3	17.08	19.59	15%
8	CEMA	223.95	212.62	-5%
8	ATCA	291.69	318.47	9%
8	GAMA	182.03	192.92	6%
8	AAMA	50.80	49.10	-3%
8	HEMA	11.82	12.12	3%
8	DHBM	157.56	146.62	-7%
8	AMCA	125.58	130.64	4%
8	TTCA	355.88	360.97	1%
8	HPMA	488.78	444.95	-9%
8	HPM2	90.85	82.88	-9%
8	MADA	365.70	387.61	6%
8	CYMA	17.33	16.17	-7%
8	MHB3	19.37	18.68	-4%
8	HPMM	136.56	118.43	-13%
8	PHGA	368.26	352.75	-4%
8	2MHA	125.69	114.11	-9%
8	BPMA	21.17	22.57	7%
8	34MH	105.12	102.31	-3%
8	PHEM	16.78	17.42	4%
8	BMA	16.71	19.52	17%
8	CYHA	88.63	86.57	-2%
8	IPM3	17.08	18.84	10%
9	CEMA	223.95	212.58	-5%
9	ATCA	291.69	299.61	3%
9	GAMA	182.03	201.87	11%

pH	Analyte	Target Concentration (ng/mL)	Measured Concentration (ng/mL)	% Error
9	AAMA	50.80	46.93	-8%
9	HEMA	11.82	12.09	2%
9	DHBM	157.56	153.24	-3%
9	AMCA	125.58	98.41	-22%
9	TTCA	355.88	345.07	-3%
9	HPMA	488.78	501.72	3%
9	HPM2	90.85	85.72	-6%
9	MADA	365.70	400.78	10%
9	CYMA	17.33	18.89	9%
9	MHB3	19.37	16.56	-15%
9	HPMM	136.56	122.98	-10%
9	PHGA	368.26	366.67	0%
9	2MHA	125.69	119.87	-5%
9	BPMA	21.17	24.52	16%
9	34MH	105.12	102.54	-2%
9	PHEM	16.78	15.34	-9%
9	BMA	16.71	15.63	-6%
9	CYHA	88.63	91.47	3%
9	IPM3	17.08	19.15	12%
10	CEMA	223.95	209.07	-7%
10	ATCA	291.69	301.94	4%
10	GAMA	182.03	185.12	2%
10	AAMA	50.80	43.03	-15%
10	HEMA	11.82	11.04	-7%
10	DHBM	157.56	146.21	-7%
10	AMCA	125.58	101.21	-19%
10	TTCA	355.88	330.45	-7%
10	HPMA	488.78	521.40	7%
10	HPM2	90.85	86.73	-5%
10	MADA	365.70	354.00	-3%
10	CYMA	17.33	18.61	7%
10	MHB3	19.37	19.37	0%
10	HPMM	136.56	114.17	-16%
10	PHGA	368.26	321.19	-13%
10	2MHA	125.69	118.43	-6%
10	BPMA	21.17	25.19	19%
10	34MH	105.12	104.14	-1%
10	PHEM	16.78	16.31	-3%
10	BMA	16.71	17.45	4%
10	CYHA	88.63	88.91	0%
10	IPM3	17.08	17.68	4%
11	CEMA	223.95	217.72	-3%
11	ATCA	291.69	300.91	3%
11	GAMA	182.03	185.76	2%
11	AAMA	50.80	39.76	-22%
11	HEMA	11.82	12.65	7%
11	DHBM	157.56	143.28	-9%

pH	Analyte	Target Concentration (ng/mL)	Measured Concentration (ng/mL)	% Error
11	AMCA	125.58	102.62	-18%
11	TTCA	355.88	323.33	-9%
11	HPMA	488.78	440.49	-10%
11	HPM2	90.85	91.36	1%
11	MADA	365.70	347.19	-5%
11	CYMA	17.33	16.22	-6%
11	MHB3	19.37	17.25	-11%
11	HPMM	136.56	122.82	-10%
11	PHGA	368.26	323.43	-12%
11	2MHA	125.69	108.07	-14%
11	BPMA	21.17	22.68	7%
11	34MH	105.12	93.93	-11%
11	PHEM	16.78	15.45	-8%
11	BMA	16.71	18.62	11%
11	CYHA	88.63	73.34	-17%
11	IPM3	17.08	19.00	11%

APPENDIX B: Calibration Curve Matrix Validation

Table B1. Typical slopes of matrix based (urine) and solvent based (15 mM ammonium acetate) concentration plots of selected VOC metabolites. The difference in slopes from matrix-matched urine calibrators and non-matrix-matched calibrators meets DLS PPM requirements of less than or equal to 5% difference)

	Slope		
Analyte code	**Urine matrix**	**15 mM Ammonium acetate matrix**	**% Difference**
AAMA	0.9242	0.9262	0.22
AMCA	0.9623	0.9626	0.032
ATCA	1.0047	1.0048	0.010
BMA	1.0111	1.0103	0.079
BPMA	0.9737	0.9737	0.000
CEMA	0.9725	0.9723	0.021
CYMA	0.9992	0.9993	0.010
1DCV	0.9866	0.9868	0.02
2DCV	1.0233	1.0239	0.059
DHBM	0.9529	0.9530	0.010
GAMA	1.0110	1.0110	0.000
HEMA	1.1831	1.1843	0.10
HPMA	1.0149	1.0153	0.040
HPM2	0.9638	0.9640	0.020
HPMM	0.9662	0.9660	0.021
MADA	0.9999	1.0022	0.23
2MHA	0.9646	0.9655	0.093
34MH	0.9904	0.9906	0.020
MHB3	1.2050	1.2040	0.083
PHGA	0.9930	0.9929	0.010
PHEM	0.9839	0.9837	0.020
PMA	0.9925	0.9915	0.10
TCVM	0.9873	0.9877	0.041
TTCA	0.9404	0.9412	0.085
CYHA	0.0704	0.0676	3.97
IPM3	0.0751	0.0764	1.75

APPENDIX C: Method Performance Documentation

Method performance documentation for this method including accuracy, precision, specificity, and stability is provided in Appendix C of this method documentation. **The signatures of the Branch Chief and Director of the Division of the Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method.**

Table C1. Accuracy using spike recovery

Accuracy using Spike Recovery - fill in yellow shaded cells
Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name:	VOC metabolites in urine
Method #:	2103a
Matrix:	Urine
Units:	µg/L
Analyte:	**CEMA**

		Sample 1					Sample 2				
			Measured concentration					Measured concentration			
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)
Sample	1	0.00	2.42	2.34			0.00	2.14	2.22		
	2		2.15	2.17	2.27			2.20	2.42	2.23	
	3		2.18	2.35				2.12	2.30		
Sample + Spike 1	1	41.4	42.4	45.0			131	131	133		
	2		41.4	46.9	43.7	100		131	131	133	99.7
	3		41.9	44.9				137	133		
Sample + Spike 2	1	65.6	67.8	69.9			207	204	211		
	2		66.4	63.7	67.3	99.2		205	214	209	99.6
	3		71.7	64.5				211	209		
Sample + Spike 3	1	85.7	91.5	98.5			271	273	277		
	2		86.0	92.3	91.5	104		259	274	276	101
	3		91.0	89.8				290	282		

Mean recovery (%)	SD (%)
101	1.82

Analyte:	**ATCA**

		Sample 1					Sample 2				
			Measured concentration					Measured concentration			
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)
Sample	1	0.00	3.68	6.69			0.00	2.85	4.83		
	2		2.57	6.66	4.19			1.67	1.49	2.71	
	3		3.99	1.53				3.29	2.15		
Sample + Spike 1	1	61.8	62.8	67.0			195	197	206		
	2		63.5	71.5	69.6	106		212	214	212	107
	3		78.3	74.4				215	228		
Sample + Spike 2	1	98.0	102	114			310	346	340		
	2		94.3	95.7	103	101		314	337	329	105
	3		104	110				304	335		
Sample + Spike 3	1	128	140	158			405	393	444		
	2		140	150	151	115		418	442	425	104
	3		144	175				440	414		

Mean recovery (%)	SD (%)
106	4.60

Analyte:	**GAMA**

		Sample 1					Sample 2				
			Measured concentration					Measured concentration			
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)
Sample	1	0.00	1.01	0.92			0.00	1.05	1.11		
	2		1.15	0.99	1.04			0.99	0.93	1.00	
	3		1.08	1.07				0.97	0.96		
Sample + Spike 1	1	41.0	40.0	39.0			130	122	124		
	2		40.7	40.5	41.0	97.4		117	125	125	95.8
	3		42.8	43.2				129	135		
Sample + Spike 2	1	65.1	62.3	68.2			206	198	197		
	2		62.2	65.4	64.8	97.9		192	205	201	97.0
	3		64	66				211	200		
Sample + Spike 3	1	85.0	87.3	91.6			269	254	258		
	2		81.3	91.2	87.9	102		249	263	258	95.6
	3		90.3	86.0				268	256		

Mean recovery (%)	SD (%)
97.6	2.42

Analyte: **AAMA**

	Replicate	Sample 1: Spike concentration	Sample 1: Measured concentration Day 1	Sample 1: Day 2	Sample 1: Mean	Sample 1: Recovery (%)	Sample 2: Spike concentration	Sample 2: Measured concentration Day 1	Sample 2: Day 2	Sample 2: Mean	Sample 2: Recovery (%)
Sample	1	0.00	0.49	0.46			0.00	0.46	0.41		
	2		0.43	0.43	0.46			0.43	0.43	0.44	
	3		0.45	0.50				0.43	0.49		
Sample + Spike 1	1	7.61	8.01	8.63			24.1	23.4	23.6		
	2		7.62	8.11	8.3	102		23.5	24.1	24.2	98.5
	3		9.00	8.21				25.7	24.6		
Sample + Spike 2	1	12.1	12.9	13.1			38.2	39.7	41.2		
	2		12.5	12.4	12.9	103		36.5	38.5	38.8	100
	3		13.1	13.2				38.4	38.5		
Sample + Spike 3	1	15.8	17.1	18.3			49.9	50.9	51.2		
	2		17.1	17.6	17.3	107		49.4	50.5	50.7	101
	3		16.8	16.6				51.1	51.0		

Mean recovery (%)	SD (%)
102	2.75

Analyte: **HEMA**

	Replicate	Sample 1: Spike concentration	Sample 1: Measured concentration Day 1	Sample 1: Day 2	Sample 1: Mean	Sample 1: Recovery (%)	Sample 2: Spike concentration	Sample 2: Measured concentration Day 1	Sample 2: Day 2	Sample 2: Mean	Sample 2: Recovery (%)
Sample	1	0.00	0.21	0.22			0.00	0.24	0.34		
	2		0.24	0.16	0.24			0.39	0.40	0.31	
	3		0.27	0.36				0.26	0.20		
Sample + Spike 1	1	2.60	3.36	3.17			8.23	8.81	8.98		
	2		2.78	3.39	3.25	116		8.45	7.99	8.51	99.6
	3		3.32	3.49				8.58	8.26		
Sample + Spike 2	1	4.13	4.08	5.04			13.1	13.8	13.0		
	2		3.96	5.09	4.58	105		13.3	12.7	13.2	98.8
	3		5.18	4.15				12.7	13.7		
Sample + Spike 3	1	5.40	5.84	4.91			17.1	17.6	16.9		
	2		5.90	5.91	5.74	102		18.9	16.8	17.7	102
	3		5.84	6.06				17.3	18.6		

Mean recovery (%)	SD (%)
104	6.15

Analyte: **DHBM**

	Replicate	Sample 1: Spike concentration	Sample 1: Measured concentration Day 1	Sample 1: Day 2	Sample 1: Mean	Sample 1: Recovery (%)	Sample 2: Spike concentration	Sample 2: Measured concentration Day 1	Sample 2: Day 2	Sample 2: Mean	Sample 2: Recovery (%)
Sample	1	0.00	2.16	2.03			0.00	1.90	2.05		
	2		1.93	2.00	2.00			1.99	1.91	2.00	
	3		1.92	1.95				1.90	2.27		
Sample + Spike 1	1	27.6	30.5	32.6			87.2	86.1	92.6		
	2		28.7	30.0	30.9	105		89.2	89.6	90.4	101
	3		32.4	30.9				94.0	91.1		
Sample + Spike 2	1	43.7	45.7	48.9			138	138	147		
	2		46.2	44.7	46.6	102		137	141	142	101
	3		49.9	44.1				142	148		
Sample + Spike 3	1	57.1	61.6	68.2			181	181	187		
	2		57.6	61.6	62.1	105		177	195	188	103
	3		61.6	62.2				196	190		

Mean recovery (%)	SD (%)
103	1.69

Analyte: **CYHA**

	Replicate	Sample 1: Spike concentration	Sample 1: Measured concentration Day 1	Sample 1: Day 2	Sample 1: Mean	Sample 1: Recovery (%)	Sample 2: Spike concentration	Sample 2: Measured concentration Day 1	Sample 2: Day 2	Sample 2: Mean	Sample 2: Recovery (%)
Sample	1	0.00	0.00	0.00			0.00	0.00	0.00		
	2		0.00	0.00	0.00			0.00	0.00	0.00	
	3		0.00	0.00				0.00	0.00		
Sample + Spike 1	1	17.8	20.5	18.5			56.3	60.3	62.9		
	2		18.1	19.9	19.4	109		62.9	64.4	62.6	111
	3		18.9	20.3				63.3	62.0		
Sample + Spike 2	1	28.3	28.6	31.0			89.3	107	97.9		
	2		30.2	30.7	29.9	106		95.6	100	101	113
	3		29.7	29.5				101	102		
Sample + Spike 3	1	36.9	40.9	45.2			117	125	132		
	2		38.0	41.6	41.6	113		115	136	127	109
	3		42.8	41.1				126	131		

Mean recovery (%)	SD (%)
110	2.65

Analyte: **AMCA**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Day 2	Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Day 2	Mean	Sample 2 Recovery (%)
Sample	1	0.00	0.42	0.42			0.00	0.32	0.60		
	2		0.48	0.53	0.46			0.47	0.45	0.44	
	3		0.51	0.39				0.40	0.39		
Sample + Spike 1	1	24.9	27.3	25.9			78.9	75.8	83.1		
	2		27.8	25.8	26.4	104		76.1	85.6	78.8	99.3
	3		25.6	26.1				82.4	69.8		
Sample + Spike 2	1	39.6	40.4	43.3			125	136	122		
	2		43.9	39.9	41.5	104		129	124	125.9	100
	3		38.6	42.7				128	116		
Sample + Spike 3	1	51.7	50.9	64.3			163	151	163		
	2		56.9	55.6	56.8	109		175	166	167.2	102
	3		58.5	54.4				180	168		

Mean recovery (%)	SD (%)
103	3.44

Analyte: **TTCA**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Day 2	Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Day 2	Mean	Sample 2 Recovery (%)
Sample	1	0.00	10.2	10.2			0.00	10.1	10.0		
	2		10.3	10.0	10.2			10.2	10.1	10.1	
	3		10.1	10.1				10.2	10.1		
Sample + Spike 1	1	77.2	98.4	90.7			244	249	274		
	2		85.7	82.8	89.1	102		285	276	269	106
	3		89.8	86.9				271	257		
Sample + Spike 2	1	122	135	149			387	417	449		
	2		129	137	136	103		385	414	405	102
	3		129	139				394	374		
Sample + Spike 3	1	160	171	200			506	525	549		
	2		167	185	182	107		484	536	532	103
	3		180	189				525	575		

Mean recovery (%)	SD (%)
104	2.17

Analyte: **HPMA**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Day 2	Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Day 2	Mean	Sample 2 Recovery (%)
Sample	1	0.00	6.77	6.63			0.00	6.41	6.86		
	2		6.68	6.61	6.73			6.38	6.83	6.53	
	3		6.92	6.77				6.20	6.51		
Sample + Spike 1	1	89.4	99.6	97.4			283	304	285		
	2		100	101	102	107		303	310	302	105
	3		106	109				312	302		
Sample + Spike 2	1	142	148	156			448	482	492		
	2		147	162	157	106		453	492	481	106
	3		168	159				493	476		
Sample + Spike 3	1	185	206	221			585	641	645		
	2		197	212	211	110		612	642	635	107
	3		217	210				647	625		

Mean recovery (%)	SD (%)
107	1.86

Analyte: **MADA**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Day 2	Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Day 2	Mean	Sample 2 Recovery (%)
Sample	1	0.00	1.40	24.5			0.00	6.13	0.68		
	2		2.56	9.64	6.99			3.83	0.00	2.89	
	3		2.44	1.41				5.11	1.61		
Sample + Spike 1	1	82.5	97.5	110			261	290	255		
	2		105	97.6	98.5	111		264	264	268	101
	3		102	79.8				267	265		
Sample + Spike 2	1	131	137	159			414	409	432		
	2		134	134	140	102		389	437	426	102
	3		139	140				466	420		
Sample + Spike 3	1	171	199	221			540	586	577		
	2		184	202	202	114		535	604	575	106
	3		196	211				565	584		

Mean recovery (%)	SD (%)
106	5.35

Analyte: **HPM2**

		Sample 1					Sample 2						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0.00	0.00	0.00			0.00	0.00	0.00				
	2		0.00	0.00	0.00			0.00	0.00	0.00		101	2.33
	3		0.00	0.00				0.00	0.00				
Sample + Spike 1	1	18.2	17.2	18.6			57.6	56.7	56.0				
	2		17.9	20.3	18.7	102		55.3	57.4	56.4	98.0		
	3		19.4	18.6				59.7	53.5				
Sample + Spike 2	1	28.9	28.6	30.3			91.4	93.8	91.5				
	2		28.4	28.1	29.1	101		89.3	92.5	92.5	101		
	3		29.3	29.7				94.4	93.4				
Sample + Spike 3	1	37.7	38.4	42.5			119	111	123				
	2		37.4	39.7	39.5	105		115	124	118	99.3		
	3		40.0	38.9				120	120				

Analyte: **CYMA**

		Sample 1					Sample 2						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0.00	0.00	0.00			0.00	0.00	0.00				
	2		0.00	0.00	0.00			0.00	0.00	0.00		109	0.91
	3		0.00	0.00				0.00	0.00				
Sample + Spike 1	1	3.43	3.83	3.90			10.9	10.3	12.2				
	2		3.44	3.34	3.73	109		10.9	11.6	11.7	108		
	3		3.85	4.02				11.9	13.4				
Sample + Spike 2	1	5.45	6.61	5.70			17.2	17.8	19.1				
	2		5.52	5.59	5.83	107		18.2	18.6	18.9	109		
	3		5.42	6.13				19.8	19.6				
Sample + Spike 3	1	7.11	8.52	7.33			22.5	23.9	24.8				
	2		7.54	7.67	7.75	109		22.6	25.2	24.5	109		
	3		7.50	7.93				27.0	23.7				

Analyte: **MHB3**

		Sample 1					Sample 2						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0.00	0.04	0.16			0.00	0.00	0.08				
	2		0.20	0.10	0.19			0.27	0.00	0.12		110	4.28
	3		0.22	0.41				0.20	0.21				
Sample + Spike 1	1	3.81	4.65	4.12			12.0	12.9	13.4				
	2		4.31	4.12	4.26	107		13.1	13.2	13.0	107		
	3		4.05	4.32				12.9	12.6				
Sample + Spike 2	1	6.04	6.72	6.67			19.1	20.0	21.6				
	2		6.81	6.67	6.54	105		19.8	22.4	21.0	109		
	3		6.79	5.57				21.7	20.6				
Sample + Spike 3	1	7.89	9.31	9.31			25.0	28.6	28.7				
	2		8.71	10.47	9.36	116		26.4	30.4	28.4	113		
	3		9.35	8.98				28.6	27.8				

Analyte: **HPMM**

		Sample 1					Sample 2						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0.00	0.44	0.57			0.00	0.54	0.91				
	2		0.52	0.66	0.59			0.62	0.80	0.70		109	2.54
	3		0.60	0.73				0.77	0.57				
Sample + Spike 1	1	20.8	23.5	24.4			65.8	67.1	69.2				
	2		23.9	24.4	24.0	112		72.0	70.8	70.1	106		
	3		25.0	22.6				72.2	69.5				
Sample + Spike 2	1	33.0	34.8	35.9			104	113	113				
	2		35.7	37.7	36.3	108		110	111	113	107		
	3		37.8	35.7				114	114				
Sample + Spike 3	1	43.1	47.3	51.7			136	152	153				
	2		45.1	48.1	48.4	111		142	152	151	110		
	3		50.1	48.3				155	151				

Analyte: **PHGA**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Sample 1 Measured concentration Day 2	Sample 1 Measured concentration Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Sample 2 Measured concentration Day 2	Sample 2 Measured concentration Mean	Sample 2 Recovery (%)
Sample	1	0.00	5.22	4.66			0.00	4.66	4.71		
	2		5.48	5.01	5.11			4.79	4.47	4.74	
	3		5.42	4.85				5.06	4.77		
Sample + Spike 1	1	69.3	72.0	78.0			219	235	228		
	2		70.4	78.3	75.4	101		234	217	229	102
	3		80.3	73.5				219	242		
Sample + Spike 2	1	110	113	116			348	360	367		
	2		117	118	117	102		330	362	358	102
	3		123	113				373	355		
Sample + Spike 3	1	144	149	157			454	457	480		
	2		142	153	153	103		440	478	462	101
	3		162	156				453	464		

Mean recovery (%)	SD (%)
102	0.87

Analyte: **IPM3**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Sample 1 Measured concentration Day 2	Sample 1 Measured concentration Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Sample 2 Measured concentration Day 2	Sample 2 Measured concentration Mean	Sample 2 Recovery (%)
Sample	1	0.00	0.03	0.02			0.00	0.08	0.00		
	2		0.00	0.00	0.05			0.00	0.00	0.07	
	3		0.21	0.05				0.28	0.05		
Sample + Spike 1	1	5.51	5.92	6.44			17.4	19.0	19.6		
	2		5.49	6.67	6.25	112		18.3	19.6	19.4	111
	3		6.60	6.38				20.0	20.2		
Sample + Spike 2	1	8.74	9.93	10.0			27.7	31.1	31.9		
	2		9.38	9.92	9.87	112		28.8	30.9	30.9	112
	3		9.81	10.2				32.1	30.8		
Sample + Spike 3	1	11.4	12.6	13.5			36.1	41.2	40.8		
	2		13.2	13.1	13.2	115		38.8	40.1	40.4	112
	3		13.7	13.1				41.1	40.6		

Mean recovery (%)	SD (%)
112	1.43

Analyte: **2MHA**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Sample 1 Measured concentration Day 2	Sample 1 Measured concentration Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Sample 2 Measured concentration Day 2	Sample 2 Measured concentration Mean	Sample 2 Recovery (%)
Sample	1	0.00	0.00	0.00			0.00	0.00	0.00		
	2		0.00	0.00	0.00			0.00	0.00	0.00	
	3		0.00	0.00				0.00	0.00		
Sample + Spike 1	1	21.3	23.3	20.5			67.3	72.0	74.9		
	2		21.2	22.7	22.7	107		79.4	67.9	74.2	110
	3		26.7	21.8				75.3	75.7		
Sample + Spike 2	1	33.8	38.0	35.6			107	106	121		
	2		30.2	32.6	34.8	103		106	102	112	105
	3		40.2	32.4				119	115		
Sample + Spike 3	1	44.1	48.9	47.5			139	166	157		
	2		43.1	43.3	47.8	109		151	144	153	110
	3		51.5	52.7				148	153		

Mean recovery (%)	SD (%)
107	2.88

Analyte: **BPMA**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Sample 1 Measured concentration Day 2	Sample 1 Measured concentration Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Sample 2 Measured concentration Day 2	Sample 2 Measured concentration Mean	Sample 2 Recovery (%)
Sample	1	0.00	0.67	0.69			0.00	0.90	0.68		
	2		0.73	0.65	0.72			0.74	0.76	0.74	
	3		0.85	0.71				0.69	0.69		
Sample + Spike 1	1	5.31	6.56	6.92			16.8	19.8	16.9		
	2		6.48	7.07	6.77	114		19.4	20.5	19.2	110
	3		6.96	6.64				19.5	19.3		
Sample + Spike 2	1	8.43	9.42	9.16			26.7	29.7	32.0		
	2		9.79	9.94	9.68	106		31.7	30.2	30.8	113
	3		10.13	9.62				30.8	30.3		
Sample + Spike 3	1	11.0	12.3	12.0			34.8	37.9	42.3		
	2		12.1	15.0	13.2	113		35.9	38.3	39.4	111
	3		14.1	13.4				39.8	42.1		

Mean recovery (%)	SD (%)
111	2.77

Analyte: **34MH**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Sample 1 Measured concentration Day 2	Sample 1 Measured concentration Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Sample 2 Measured concentration Day 2	Sample 2 Measured concentration Mean	Sample 2 Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0.00	0.00	0.00			0.00	0.00	0.00				
	2		0.00	0.00	0.00			0.00	0.00	0.00		115	3.78
	3		0.00	0.00				0.00	0.00				
Sample + Spike 1	1	42.8	47.0	46.2			135	139	160				
	2		49.3	48.3	48.2	113		158	151	151	112		
	3		49.8	48.5				150	151				
Sample + Spike 2	1	67.9	72.1	76.6			215	240	238				
	2		74.2	76.4	76.3	112		245	246	248	115		
	3		82.4	75.9				262	255				
Sample + Spike 3	1	88.6	98.7	126			280	317	322				
	2		96.4	110	108	122		313	315	320	114		
	3		115.8	102				326	329				

Analyte: **PHEM**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Sample 1 Measured concentration Day 2	Sample 1 Measured concentration Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Sample 2 Measured concentration Day 2	Sample 2 Measured concentration Mean	Sample 2 Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0.00	0.00	0.00			0.00	0.00	0.00				
	2		0.00	0.00	0.00			0.00	0.00	0.02		104	2.49
	3		0.00	0.00				0.00	0.10				
Sample + Spike 1	1	3.47	3.61	3.76			11.0	10.8	11.1				
	2		3.56	3.69	3.62	104		11.0	11.2	11.2	101		
	3		3.74	3.39				11.8	11.0				
Sample + Spike 2	1	5.50	5.79	6.33			17.4	17.1	17.5				
	2		5.54	5.47	5.73	104		17.9	18.3	18.1	104		
	3		5.66	5.56				19.0	18.6				
Sample + Spike 3	1	7.19	7.82	7.97			22.7	22.5	23.4				
	2		7.19	7.51	7.84	109		22.5	24.2	23.6	104		
	3		8.29	8.27				24.6	24.5				

Analyte: **BMA**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Sample 1 Measured concentration Day 2	Sample 1 Measured concentration Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Sample 2 Measured concentration Day 2	Sample 2 Measured concentration Mean	Sample 2 Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0.00	0.04	0.06			0.00	0.04	0.05				
	2		0.09	0.07	0.07			0.04	0.06	0.05		106	3.04
	3		0.07	0.05				0.04	0.05				
Sample + Spike 1	1	3.00	3.35	3.49			9.50	9.41	9.63				
	2		3.01	3.39	3.25	106		9.58	10.1	9.59	100		
	3		3.13	3.10				9.13	9.65				
Sample + Spike 2	1	4.76	4.74	5.35			15.1	16.7	15.7				
	2		5.09	5.56	5.17	107		15.0	16.1	15.9	105		
	3		5.02	5.28				15.6	16.0				
Sample + Spike 3	1	6.22	7.47	6.89			19.7	20.5	19.2				
	2		6.68	6.67	6.87	109		19.7	20.7	21.1	107		
	3		6.50	7.04				24.3	22.4				

Table C2. Precision

Precision - fill in yellow shaded cells

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	VOC metabolites in urine
Method #:	2103a
Matrix:	Urine
Units:	µg/L
Analyte:	**CEMA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
A17188	47.9	52.4	50.1	5.15	5.15	5.02E+03
A17193	55.0	55.3	55.2	0.02	0.02	6.09E+03
A17087	48.3	57.5	52.9	21.1	21.1	5.59E+03
C17130	48.4	51.4	49.9	2.33	2.33	4.98E+03
C17135	50.8	54.8	52.8	4.04	4.04	5.58E+03
C17138	49.2	54.1	51.6	5.93	5.93	5.33E+03
C17192	49.5	56.2	52.8	11.1	11.1	5.59E+03
C17087	49.4	54.0	51.7	5.34	5.34	5.35E+03
P17135	50.5	42.7	46.6	15.2	15.2	4.34E+03
P17166	48.0	51.5	49.7	2.99	2.99	4.94E+03

Grand sum 1.03E+03 **Grand mean** 51.3

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	146	14.6	3.83	7.46
Between Run	101	11.3	0.00	0.00
Total	248		3.83	**7.46**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	527	550	539	134	134	5.81E+05
2	540	601	571	937	937	6.51E+05
3	510	513	511	2.48	2.48	5.23E+05
4	538	571	554	273	273	6.14E+05
5	542	575	559	267	267	6.24E+05
6	503	547	525	498	498	5.51E+05
7	488	554	521	1.09E+03	1.09E+03	5.43E+05
8	519	584	551	1.05E+03	1.05E+03	6.08E+05
9	513	563	538	627	627	5.78E+05
10	499	557	528	819	819	5.58E+05

Grand sum 1.079E+04 **Grand mean** 539.7

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	1.14E+04	1.14E+03	33.7	6.25
Between Run	6.38E+03	709	0.00	0.00
Total	1.78E+04		33.7	**6.25**

Analyte: **ATCA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	103	104	104	0.61	0.61	2.15E+04
2	100	93.0	96.4	11.1	11.1	1.86E+04
3	81.0	96.5	88.7	59.8	59.8	1.58E+04
4	89.0	93.0	91.0	4.06	4.06	1.66E+04
5	93.8	116	105	123	123	2.20E+04
6	82.0	100	90.8	77.8	77.8	1.65E+04
7	86.9	99.5	93.2	39.7	39.7	1.74E+04
8	93.6	94.1	93.8	0.06	0.06	1.76E+04
9	90.9	102	96.3	29.4	29.4	1.86E+04
10	89.5	88.7	89.1	0.18	0.18	1.59E+04

Grand sum 1.896E+03 **Grand mean** 94.8

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	692	69.2	8.32	8.78
Between Run	576	64.0	0.00	0.00
Total	1.27E+03		8.32	**8.78**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	913	899	906	48.4	48.4	1.64E+06
2	911	955	933	498	498	1.74E+06
3	826	924	875	2.37E+03	2.37E+03	1.53E+06
4	846	930	888	1.76E+03	1.76E+03	1.58E+06
5	878	990	934	3.14E+03	3.14E+03	1.74E+06
6	831	979	905	5.52E+03	5.52E+03	1.64E+06
7	898	916	907	81.3	81.3	1.65E+06
8	885	958	921	1.34E+03	1.34E+03	1.70E+06
9	858	1002	930	5.18E+03	5.18E+03	1.73E+06
10	948	937	942	29.1	29.1	1.78E+06

Grand sum	1.828E+04	**Grand mean**	914

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	3.99E+04	3.99E+03	63.2	6.91
Between Run	8.49E+03	943	0.00	0.00
Total	4.84E+04		63.2	**6.91**

Analyte: **GAMA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	34.3	34.0	34.2	0.02	0.02	2.33E+03
2	35.3	36.3	35.8	0.24	0.24	2.56E+03
3	32.5	30.2	31.3	1.27	1.27	1.96E+03
4	33.6	34.4	34.0	0.14	0.14	2.31E+03
5	32.3	32.7	32.5	0.06	0.06	2.11E+03
6	32.1	32.7	32.4	0.07	0.07	2.10E+03
7	34.4	37.6	36.0	2.56	2.56	2.59E+03
8	33.3	38.6	35.9	6.84	6.84	2.58E+03
9	31.1	28.1	29.6	2.31	2.31	1.75E+03
10	32.3	34.1	33.2	0.80	0.80	2.20E+03

Grand sum	670	**Grand mean**	33.5

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	28.6	2.86	1.69	5.05
Between Run	80.6	8.95	1.75	5.21
Total	109		2.43	**7.26**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	301	318	310	77.2	77.2	1.92E+05
2	303	325	314	115	115	1.97E+05
3	297	300	298	3.57	3.57	1.78E+05
4	295	324	309	216	216	1.91E+05
5	300	314	307	46.7	46.7	1.89E+05
6	297	315	306	85.4	85.4	1.87E+05
7	321	341	331	106	106	2.19E+05
8	322	330	326	18.5	18.5	2.13E+05
9	282	312	297	223	223	1.76E+05
10	302	324	313	126	126	1.96E+05

Grand sum	6.222E+03	**Grand mean**	311

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	2.04E+03	204	14.3	4.59
Between Run	2.09E+03	233	3.80	1.22
Total	4.13E+03		14.8	**4.75**

Analyte: **AAMA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	13.1	13.5	13.3	0.04	0.04	353
2	13.3	14.0	13.6	0.12	0.12	370
3	14.0	15.7	14.9	0.72	0.72	443
4	14.0	15.5	14.8	0.61	0.61	436
5	14.9	16.8	15.9	0.90	0.90	503
6	14.4	16.0	15.2	0.63	0.63	463
7	12.9	15.4	14.1	1.68	1.68	400
8	13.8	17.2	15.5	2.87	2.87	482
9	14.0	12.8	13.4	0.40	0.40	359
10	14.9	16.2	15.6	0.46	0.46	484
Grand sum	292	**Grand mean**	14.6			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	16.9	1.69	1.30	8.88
Between Run	16.4	1.82	0.26	1.79
Total	33.3		1.32	**9.06**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	104	110	107	11.3	11.3	2.29E+04
2	103	109	106	9.55	9.55	2.26E+04
3	102	134	118	253	253	2.80E+04
4	109	125	117	63.8	63.8	2.75E+04
5	120	130	125	25.3	25.3	3.10E+04
6	117	126	121	17.7	17.7	2.95E+04
7	106	119	113	41.9	41.9	2.53E+04
8	108	128	118	98.8	98.8	2.79E+04
9	114	118	116	4.28	4.28	2.67E+04
10	109	115	112	10.4	10.4	2.51E+04
Grand sum	2.31E+03	**Grand mean**	115			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.07E+03	107	10.4	8.98
Between Run	619	68.8	0.00	0.00
Total	1.69E+03		10.4	**8.98**

Analyte: **HEMA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	4.15	4.04	4.10	0.00	0.00	33.5
2	3.87	4.19	4.03	0.03	0.03	32.5
3	3.75	4.81	4.28	0.28	0.28	36.6
4	3.66	3.87	3.77	0.01	0.01	28.4
5	3.53	4.26	3.90	0.13	0.13	30.3
6	3.91	4.42	4.17	0.07	0.07	34.7
7	3.66	4.26	3.96	0.09	0.09	31.4
8	4.47	4.43	4.45	0.00	0.00	39.6
9	5.16	4.17	4.67	0.25	0.25	43.5
10	4.53	4.21	4.37	0.03	0.03	38.2
Grand sum	83.4	**Grand mean**	4.17			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.76	0.18	0.42	10.1
Between Run	1.37	0.15	0.00	0.00
Total	3.13		0.42	**10.1**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	42.9	45.1	44.0	1.18	1.18	3.87E+03
2	38.8	46.6	42.7	15.2	15.2	3.64E+03
3	44.5	48.0	46.2	3.03	3.03	4.27E+03
4	39.4	42.7	41.1	2.76	2.76	3.37E+03
5	46.7	46.3	46.5	0.03	0.03	4.32E+03
6	42.7	46.7	44.7	4.04	4.04	4.00E+03
7	44.7	47.5	46.1	2.02	2.02	4.25E+03
8	46.9	46.6	46.8	0.02	0.02	4.37E+03
9	46.3	49.2	47.8	2.04	2.04	4.56E+03
10	46.7	48.2	47.5	0.56	0.56	4.50E+03

Grand sum	907	**Grand mean**	45.3

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	61.8	6.18	2.49	5.48
Between Run	84.9	9.44	1.28	2.82
Total	147		2.79	**6.16**

Analyte: **DHBM**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	124	122	123	0.83	0.83	3.01E+04
2	127	124	126	1.22	1.22	3.15E+04
3	115	116	116	0.31	0.31	2.67E+04
4	125	130	127	7.51	7.51	3.25E+04
5	123	132	128	18.8	18.8	3.26E+04
6	126	131	128	6.13	6.13	3.29E+04
7	112	140	126	200	200	3.19E+04
8	133	142	137	21.5	21.5	3.78E+04
9	125	116	120	22.5	22.5	2.90E+04
10	119	132	126	40.1	40.1	3.16E+04

Grand sum	2.51E+03	**Grand mean**	126

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	638	63.8	7.99	6.36
Between Run	579	64.3	0.50	0.40
Total	1.22E+03		8.00	**6.37**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	391	421	406	225	225	3.30E+05
2	376	446	411	1.22E+03	1.22E+03	3.38E+05
3	342	435	389	2.15E+03	2.15E+03	3.02E+05
4	393	415	404	111	111	3.26E+05
5	390	438	414	565	565	3.43E+05
6	413	414	414	0.44	0.44	3.42E+05
7	383	448	415	1036	1036	3.45E+05
8	427	415	421	34.0	34.0	3.55E+05
9	397	434	416	331	331	3.46E+05
10	394	406	400	31.5	31.5	3.20E+05

Grand sum	8.18E+03	**Grand mean**	409

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.14E+04	1.14E+03	33.8	8.26
Between Run	1.62E+03	180	0.00	0.00
Total	1.30E+04		33.8	**8.26**

Analyte: **CYHA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	6.70	7.05	6.88	0.03	0.03	94.5
2	6.84	6.80	6.82	0.00	0.00	93.0
3	6.12	6.72	6.42	0.09	0.09	82.4
4	6.98	7.10	7.04	0.00	0.00	99.1
5	6.24	7.96	7.10	0.74	0.74	101
6	6.65	6.58	6.62	0.00	0.00	87.5
7	6.66	7.76	7.21	0.30	0.30	104
8	6.87	8.10	7.49	0.38	0.38	112
9	7.23	6.11	6.67	0.31	0.31	89.0
10	7.23	6.70	6.97	0.07	0.07	97.0

Grand sum	138	**Grand mean**	6.92

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	3.86	0.39	0.62	8.98
Between Run	1.74	0.19	0.00	0.00
Total	5.60		0.62	**8.98**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	88.3	92.5	90.4	4.43	4.43	1.63E+04
2	85.5	91.3	88.4	8.32	8.32	1.56E+04
3	87.8	87.5	87.6	0.01	0.01	1.54E+04
4	93.0	98.0	95.5	6.05	6.05	1.82E+04
5	85.5	98.0	91.7	39.0	39.0	1.68E+04
6	87.1	94.7	90.9	14.5	14.5	1.65E+04
7	85.4	95.2	90.3	24.1	24.1	1.63E+04
8	92.7	92.9	92.8	0.01	0.01	1.72E+04
9	88.7	90.8	89.7	1.06	1.06	1.61E+04
10	94.1	90.6	92.4	3.10	3.10	1.71E+04

Grand sum	1.82E+03	**Grand mean**	91.0

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	201	20.1	4.48	4.93
Between Run	93.0	10.3	0.00	0.00
Total	294		4.48	**4.93**

Analyte: **AMCA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	26.8	27.7	27.2	0.20	0.20	1.48E+03
2	24.8	27.5	26.2	1.82	1.82	1.37E+03
3	29.7	34.4	32.0	5.64	5.64	2.05E+03
4	29.1	31.8	30.4	1.76	1.76	1.85E+03
5	30.3	31.6	31.0	0.46	0.46	1.92E+03
6	33.4	30.9	32.2	1.53	1.53	2.07E+03
7	31.3	38.2	34.7	11.9	11.9	2.41E+03
8	29.4	35.3	32.4	8.73	8.73	2.09E+03
9	29.2	27.3	28.3	0.96	0.96	1.60E+03
10	30.5	33.9	32.2	2.79	2.79	2.07E+03

Grand sum	613	**Grand mean**	30.7

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	71.6	7.16	2.68	8.73
Between Run	128	14.2	1.87	6.11
Total	199		3.27	**10.7**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	317	355	336	379	379	2.26E+05
2	331	367	349	314	314	2.44E+05
3	342	305	324	327	327	2.09E+05
4	361	372	367	32.1	32.1	2.69E+05
5	359	405	382	518	518	2.92E+05
6	356	377	367	114	114	2.69E+05
7	362	410	386	593	593	2.98E+05
8	363	368	365	5.20	5.20	2.67E+05
9	365	375	370	28.9	28.9	2.74E+05
10	362	375	368	41.3	41.3	2.72E+05
Grand sum	7.23E+03	**Grand mean**	361			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	4.70E+03	470	21.7	6.00
Between Run	6.90E+03	767	12.2	3.37
Total	1.16E+04		24.9	**6.88**

Analyte: **TTCA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
A17188	40.3	46.8	43.6	10.5	10.5	3.79E+03
A17193	50.2	50.3	50.2	0.00	0.00	5.05E+03
3	49.1	56.1	52.6	12.2	12.2	5.52E+03
4	38.5	47.8	43.2	21.5	21.5	3.73E+03
5	46.8	56.3	51.5	22.6	22.6	5.30E+03
6	46.3	58.9	52.6	39.8	39.8	5.54E+03
C17192	36.0	43.0	39.5	12.2	12.2	3.12E+03
8	50.4	54.9	52.7	5.13	5.13	5.55E+03
9	50.5	51.0	50.8	0.07	0.07	5.15E+03
10	39.9	45.8	42.8	8.76	8.76	3.67E+03
Grand sum	959	**Grand mean**	47.9			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	265	26.5	5.15	10.7
Between Run	463	51.4	3.53	7.36
Total	728		6.24	**13.0**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	784	743	763	407	407	1.17E+06
2	717	703	710	45.3	45.3	1.01E+06
3	791	776	783	54.7	54.7	1.23E+06
4	735	778	757	464	464	1.15E+06
5	753	773	763	100	100	1.16E+06
6	682	703	692	117	117	9.59E+05
7	642	586	614	790	790	7.54E+05
8	840	834	837	9.73	9.73	1.40E+06
9	813	752	783	926	926	1.23E+06
10	739	737	738	1.32	1.32	1.09E+06
Grand sum	1.49E+04	**Grand mean**	744			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	5.83E+03	583	24.1	3.24
Between Run	6.66E+04	7.40E+03	58.4	7.84
Total	7.24E+04		63.2	**8.49**

Analyte: **HPMA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	68.6	80.6	74.6	36.3	36.3	1.11E+04
2	84.2	91.4	87.8	12.7	12.7	1.54E+04
3	76.7	89.9	83.3	43.4	43.4	1.39E+04
4	78.9	88.0	83.5	20.7	20.7	1.39E+04
5	81.5	86.2	83.9	5.71	5.71	1.41E+04
6	81.7	93.8	87.7	36.8	36.8	1.54E+04
7	78.4	93.2	85.8	55.4	55.4	1.47E+04
8	75.6	84.9	80.3	21.6	21.6	1.29E+04
9	74.8	73.9	74.4	0.20	0.20	1.11E+04
10	82.7	83.3	83.0	0.11	0.11	1.38E+04

Grand sum	1.65E+03	**Grand mean**	82.4

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	466	46.6	6.83	8.28
Between Run	406	45.1	0.00	0.00
Total	872		6.83	**8.28**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	725	819	772	2.23E+03	2.23E+03	1.19E+06
2	870	1002	936	4.39E+03	4.39E+03	1.75E+06
3	835	808	822	1.85E+02	1.85E+02	1.35E+06
4	849	934	891	1.80E+03	1.80E+03	1.59E+06
5	837	937	887	2.51E+03	2.51E+03	1.57E+06
6	804	925	865	3.63E+03	3.63E+03	1.49E+06
7	811	978	894	6.94E+03	6.94E+03	1.60E+06
8	712	922	817	1.09E+04	1.09E+04	1.33E+06
9	842	896	869	7.52E+02	7.52E+02	1.51E+06
10	887	968	928	1.64E+03	1.64E+03	1.72E+06

Grand sum	1.74E+04	**Grand mean**	868

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	7.01E+04	7.01E+03	83.7	9.64
Between Run	4.76E+04	5.29E+03	0.00	0.00
Total	1.18E+05		83.7	**9.64**

Analyte: **MADA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	80.5	70.9	75.7	23.0	23.0	1.15E+04
2	85.7	100	92.9	52.6	52.6	1.73E+04
3	68.2	82.1	75.2	48.3	48.3	1.13E+04
4	93.0	82.9	88.0	25.5	25.5	1.55E+04
5	101	88.5	94.6	36.2	36.2	1.79E+04
6	87.6	91.7	89.7	4.20	4.20	1.61E+04
7	88.6	83.1	85.8	7.51	7.51	1.47E+04
8	78.1	86.9	82.5	19.4	19.4	1.36E+04
9	81.2	100	90.8	92.0	92.0	1.65E+04
10	80.9	88.9	84.9	16.0	16.0	1.44E+04

Grand sum	1.72E+03	**Grand mean**	86.0

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	649	64.9	8.06	9.37
Between Run	795	88.3	3.42	3.98
Total	1.44E+03		8.75	**10.2**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	877	940	909	991	991	1.65E+06
2	972	961	967	29.8	29.8	1.87E+06
3	789	925	857	4.61E+03	4.61E+03	1.47E+06
4	841	1002	921	6.52E+03	6.52E+03	1.70E+06
5	923	916	920	13.4	13.4	1.69E+06
6	983	991	987	14.8	14.8	1.95E+06
7	913	882	897	249	249	1.61E+06
8	944	924	934	104	104	1.74E+06
9	883	835	859	560	560	1.48E+06
10	832	900	866	1.17E+03	1.17E+03	1.50E+06

Grand sum 1.82E+04 **Grand mean** 912

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	2.85E+04	2.85E+03	53.4	5.86
Between Run	3.49E+04	3.88E+03	22.6	2.48
Total	6.34E+04		58.0	**6.36**

Analyte: **HPM2**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	17.8	17.1	17.4	0.13	0.13	607
2	16.0	19.3	17.7	2.71	2.71	623
3	16.6	19.3	17.9	1.81	1.81	643
4	16.5	17.9	17.2	0.50	0.50	593
5	17.6	18.7	18.2	0.34	0.34	659
6	16.4	18.3	17.4	0.91	0.91	603
7	16.8	18.3	17.6	0.56	0.56	618
8	17.7	17.8	17.8	0.00	0.00	630
9	18.6	16.3	17.4	1.38	1.38	607
10	18.2	16.6	17.4	0.62	0.62	604

Grand sum 352 **Grand mean** 17.6

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	17.9	1.79	1.34	7.60
Between Run	1.48	0.16	0.00	0.00
Total	19.4		1.34	**7.60**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	146	163	154	73.6	73.6	4.76E+04
2	138	158	148	95.2	95.2	4.38E+04
3	129	138	134	19.7	19.7	3.57E+04
4	148	160	154	32.4	32.4	4.74E+04
5	147	160	153	37.7	37.7	4.70E+04
6	146	163	155	74.2	74.2	4.78E+04
7	141	155	148	49.9	49.9	4.40E+04
8	152	159	155	11.2	11.2	4.83E+04
9	141	166	154	164	164	4.72E+04
10	149	161	155	34.3	34.3	4.78E+04

Grand sum 3.02E+03 **Grand mean** 151

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.18E+03	118	10.9	7.21
Between Run	791	87.9	0.00	0.00
Total	1.97E+03		10.9	**7.21**

Analyte: **CYMA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	4.28	5.00	4.64	0.13	0.13	43.1
2	4.24	5.23	4.74	0.25	0.25	44.8
3	4.44	5.58	5.01	0.32	0.32	50.2
4	5.15	5.11	5.13	0.00	0.00	52.6
5	5.49	5.3	5.40	0.01	0.01	58.2
6	4.92	4.86	4.89	0.00	0.00	47.8
7	5.23	5.52	5.38	0.02	0.02	57.8
8	5.01	5.21	5.11	0.01	0.01	52.2
9	5.00	4.20	4.60	0.16	0.16	42.3
10	4.74	5.02	4.88	0.02	0.02	47.6

Grand sum	99.5	**Grand mean**	4.98

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.84	0.18	0.43	8.62
Between Run	1.41	0.16	0.00	0.00
Total	3.25		0.43	**8.62**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	155	161	158	9.7	9.7	5.01E+04
2	157	167	162	20.4	20.4	5.25E+04
3	161	167	164	9.15	9.15	5.35E+04
4	163	171	167	14.8	14.8	5.59E+04
5	156	173	164	68.0	68.0	5.41E+04
6	160	163	161	1.24	1.24	5.21E+04
7	159	164	161	5.76	5.76	5.20E+04
8	156	177	166	115	115	5.53E+04
9	151	172	161	110	110	5.19E+04
10	148	171	160	136	136	5.09E+04

Grand sum	3.25E+03	**Grand mean**	163

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	980	98.0	9.90	6.09
Between Run	144	16.1	0.00	0.00
Total	1.12E+03		9.90	**6.09**

Analyte: **MHB3**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	5.24	4.85	5.05	0.04	0.04	50.9
2	5.00	5.19	5.10	0.01	0.01	51.9
3	4.58	4.54	4.56	0.00	0.00	41.6
4	4.25	4.53	4.39	0.02	0.02	38.5
5	3.49	2.88	3.19	0.09	0.09	20.3
6	4.61	4.19	4.40	0.04	0.04	38.7
7	4.43	4.90	4.67	0.06	0.06	43.5
8	4.52	4.86	4.69	0.03	0.03	44.0
9	4.22	3.83	4.03	0.04	0.04	32.4
10	4.46	4.27	4.37	0.01	0.01	38.1

Grand sum	88.8	**Grand mean**	4.44

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.67	0.07	0.26	5.83
Between Run	5.36	0.60	0.51	11.6
Total	6.03		0.58	**13.0**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	33.4	35.7	34.6	1.36	1.36	2.39E+03
2	32.4	35.7	34.0	2.58	2.58	2.32E+03
3	28.6	32.2	30.4	3.19	3.19	1.85E+03
4	32.7	34.2	33.4	0.54	0.54	2.24E+03
5	34.5	33.9	34.2	0.09	0.09	2.33E+03
6	33.6	33.0	33.3	0.09	0.09	2.22E+03
7	32.1	33.7	32.9	0.60	0.60	2.16E+03
8	31.1	36.7	33.9	7.92	7.92	2.30E+03
9	32.6	36.0	34.3	2.94	2.94	2.35E+03
10	31.9	36.1	34.0	4.47	4.47	2.31E+03

Grand sum	670	**Grand mean**	33.5

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	47.6	4.76	2.18	6.51
Between Run	26.1	2.90	0.00	0.00
Total	73.6		2.18	**6.51**

Analyte: **HPMM**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	87.4	99.8	93.6	38.4	38.4	1.75E+04
2	95.1	103	99.1	15.8	15.8	1.96E+04
3	80.5	99.5	90.0	90.1	90.1	1.62E+04
4	91.3	94.6	92.9	2.72	2.72	1.73E+04
5	95.8	97.3	96.5	0.50	0.50	1.86E+04
6	89.4	104	96.6	52.1	52.1	1.87E+04
7	96.1	103	99.4	10.7	10.7	1.98E+04
8	90.1	97.4	93.8	13.2	13.2	1.76E+04
9	95.5	90.0	92.8	7.51	7.51	1.72E+04
10	92.8	100	96.5	13.4	13.4	1.86E+04

Grand sum	1.90E+03	**Grand mean**	95.1

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	489	48.9	6.99	7.35
Between Run	161	17.9	0.00	0.00
Total	650		6.99	**7.35**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	909	1.04E+03	976	4.44E+03	4.44E+03	1.90E+06
2	958	1.00E+03	982	5.39E+02	5.39E+02	1.93E+06
3	955	879	917	1.44E+03	1.44E+03	1.68E+06
4	889	945	917	7.79E+02	7.79E+02	1.68E+06
5	868	964	916	2.31E+03	2.31E+03	1.68E+06
6	892	947	919	7.73E+02	7.73E+02	1.69E+06
7	910	956	933	5.34E+02	5.34E+02	1.74E+06
8	896	963	930	1.14E+03	1.14E+03	1.73E+06
9	932	1.00E+03	966	1.15E+03	1.15E+03	1.87E+06
10	968	1.00E+03	986	3.32E+02	3.32E+02	1.95E+06

Grand sum	1.89E+04	**Grand mean**	944

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	2.69E+04	2.69E+03	51.8	5.49
Between Run	1.57E+04	1.74E+03	0.00	0.00
Total	4.26E+04		51.8	**5.49**

Analyte: **PHGA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	14.8	16.2	15.5	0.53	0.53	480
2	21.2	21.6	21.4	0.03	0.03	915
3	17.4	16.0	16.7	0.53	0.53	557
4	16.6	17.2	16.9	0.07	0.07	572
5	18.7	20.1	19.4	0.52	0.52	751
6	17.1	17.7	17.4	0.10	0.10	603
7	16.4	15.2	15.8	0.40	0.40	499
8	14.6	16.4	15.5	0.82	0.82	482
9	18.6	16.8	17.7	0.79	0.79	625
10	15.0	14.8	14.9	0.00	0.00	443

Grand sum	342	**Grand mean**	17.1

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	7.56	0.76	0.87	5.08
Between Run	71.8	7.97	1.90	11.1
Total	79.3		2.09	**12.2**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	364	400	382	320	320	2.92E+05
2	404	420	412	60.1	60.1	3.40E+05
3	363	413	388	614	614	3.01E+05
4	462	494	478	256	256	4.56E+05
5	488	524	506	315	315	5.12E+05
6	355	379	367	148	148	2.70E+05
7	413	428	421	51.2	51.2	3.54E+05
8	492	525	508	270	270	5.17E+05
9	487	494	490	13.4	13.4	4.81E+05
10	509	447	478	946	946	4.57E+05

Grand sum	8.86E+03	**Grand mean**	443

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	5.99E+03	599	24.5	5.52
Between Run	5.37E+04	5.96E+03	51.8	11.7
Total	5.97E+04		57.3	**12.9**

Analyte: **IPM3**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
C17206	3.21	2.99	3.10	0.01	0.01	19.2
A17206	3.52	2.34	2.93	0.35	0.35	17.1
P17206	2.81	2.75	2.78	0.00	0.00	15.4
C17207	3.01	3.13	3.07	0.00	0.00	18.9
A17207	3.05	3.20	3.12	0.01	0.01	19.5
P17207	2.94	3.00	2.97	0.00	0.00	17.7
C17213	2.84	2.96	2.90	0.00	0.00	16.8
A17213	2.60	2.88	2.74	0.02	0.02	15.0
P17213	3.21	3.20	3.21	0.00	0.00	20.6
P17214	3.07	3.14	3.10	0.00	0.00	19.3

Grand sum	59.8	**Grand mean**	2.99

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.80	0.08	0.28	9.45
Between Run	0.44	0.05	0.00	0.00
Total	1.24		0.28	**9.45**

[redacted]

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	43.9	47.7	45.8	3.72	3.72	4.20E+03
2	43.4	47.3	45.4	3.69	3.69	4.11E+03
3	41.5	40.2	40.9	0.38	0.38	3.34E+03
4	46.7	47.2	47.0	0.08	0.08	4.41E+03
5	40.0	44.7	42.3	5.66	5.66	3.59E+03
6	41.7	43.4	42.6	0.75	0.75	3.62E+03
7	43.0	46.7	44.8	3.40	3.40	4.01E+03
8	44.5	44.8	44.6	0.02	0.02	3.98E+03
9	43.9	46.0	45.0	1.06	1.06	4.04E+03
10	39.6	47.4	43.5	15.3	15.3	3.78E+03
Grand sum	883	**Grand mean**	44.2			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	68.1	6.81	2.61	5.91
Between Run	61.0	6.77	0.00	0.00
Total	129		2.61	**5.91**

Analyte: **2MHA**

[redacted]

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	30.4	35.2	32.8	5.81	5.81	2.16E+03
2	33.4	30.1	31.7	2.64	2.64	2.01E+03
3	31.9	38.9	35.4	12.5	12.5	2.50E+03
4	29.2	27.9	28.5	0.44	0.44	1.63E+03
5	29.4	32.6	31.0	2.50	2.50	1.92E+03
6	26.9	33.3	30.1	9.95	9.95	1.81E+03
7	29.5	26.9	28.2	1.69	1.69	1.59E+03
8	30.4	32.5	31.4	1.19	1.19	1.98E+03
9	31.0	29.3	30.1	0.75	0.75	1.82E+03
10	31.2	31.6	31.4	0.04	0.04	1.97E+03
Grand sum	621	**Grand mean**	31.1			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	74.9	7.49	2.74	8.81
Between Run	77.3	8.59	0.74	2.38
Total	152		2.84	**9.13**

[redacted]

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	295	270	283	160	160	1.60E+05
2	284	276	280	18.6	18.6	1.57E+05
3	259	272	266	41.5	41.5	1.41E+05
4	253	281	267	192	192	1.43E+05
5	263	270	266	11.7	11.7	1.42E+05
6	256	279	268	142	142	1.43E+05
7	277	270	274	9.99	9.99	1.50E+05
8	263	273	268	26.0	26.0	1.43E+05
9	290	330	310	407	407	1.92E+05
10	299	320	310	111	111	1.92E+05
Grand sum	5.58E+03	**Grand mean**	279			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	2.24E+03	224	15.0	5.36
Between Run	5.38E+03	598	13.7	4.90
Total	7.62E+03		20.3	**7.27**

Analyte: **BPMA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	5.27	5.89	5.58	0.10	0.10	62.3
2	5.11	6.84	5.98	0.75	0.75	71.4
3	5.94	6.92	6.43	0.24	0.24	82.7
4	4.96	6.28	5.62	0.44	0.44	63.2
5	6.96	6.25	6.61	0.13	0.13	87.3
6	5.56	6.01	5.79	0.05	0.05	66.9
7	4.77	5.78	5.28	0.26	0.26	55.7
8	7.00	6.27	6.64	0.13	0.13	88.0
9	7.99	6.30	7.15	0.71	0.71	102
10	8.23	6.52	7.38	0.73	0.73	109
Grand sum	125	**Grand mean**	6.24			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	7.06	0.71	0.84	13.5
Between Run	8.92	0.99	0.38	6.05
Total	16.0		0.92	**14.8**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	173	180	177	9.70	9.70	6.24E+04
2	173	179	176	9.77	9.77	6.22E+04
3	163	174	169	30.3	30.3	5.69E+04
4	182	214	198	270	270	7.84E+04
5	199	200	200	0.27	0.27	7.97E+04
6	192	205	198	43.1	43.1	7.87E+04
7	172	190	181	84.0	84.0	6.56E+04
8	196	195	195	0.28	0.28	7.61E+04
9	221	256	239	297	297	1.14E+05
10	221	226	224	6.63	6.63	1.00E+05
Grand sum	3.91E+03	**Grand mean**	196			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.50E+03	150	12.2	6.26
Between Run	8.67E+03	963	20.2	10.3
Total	1.02E+04		23.6	**12.1**

Analyte: **34MH**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	87.3	91.1	89.2	3.71	3.71	1.59E+04
2	88.7	92.2	90.5	3.13	3.13	1.64E+04
3	90.6	91.4	91.0	0.16	0.16	1.66E+04
4	83.3	92.1	87.7	19.4	19.4	1.54E+04
5	84.8	91.6	88.2	11.3	11.3	1.56E+04
6	84.1	86.9	85.5	1.90	1.90	1.46E+04
7	81.7	95.0	88.4	44.6	44.6	1.56E+04
8	86.9	96.0	91.4	20.5	20.5	1.67E+04
9	82.3	72.8	77.5	22.5	22.5	1.20E+04
10	79.0	89.0	84.0	25.1	25.1	1.41E+04
Grand sum	1.75E+03	**Grand mean**	87.3			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	304	30.4	5.52	6.32
Between Run	312	34.7	1.46	1.67
Total	617		5.71	**6.54**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	598	626	612	1.94E+02	1.94E+02	7.50E+05
2	589	684	636	2.28E+03	2.28E+03	8.10E+05
3	616	580	598	3.27E+02	3.27E+02	7.15E+05
4	592	665	628	1.34E+03	1.34E+03	7.90E+05
5	586	644	615	8.52E+02	8.52E+02	7.57E+05
6	603	652	628	6.10E+02	6.10E+02	7.88E+05
7	591	665	628	1.36E+03	1.36E+03	7.89E+05
8	601	657	629	7.76E+02	7.76E+02	7.91E+05
9	593	647	620	7.30E+02	7.30E+02	7.70E+05
10	592	613	602	1.08E+02	1.08E+02	7.26E+05

Grand sum	1.24E+04	**Grand mean**	620

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.72E+04	1.72E+03	41.4	6.68
Between Run	2.86E+03	318	0.00	0.00
Total	2.00E+04		41.4	**6.68**

Analyte: **PHEM**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	4.77	5.12	4.95	0.03	0.03	48.9
2	5.62	6.15	5.89	0.07	0.07	69.3
3	4.86	5.26	5.06	0.04	0.04	51.2
4	4.90	5.63	5.27	0.13	0.13	55.4
5	4.75	5.39	5.07	0.10	0.10	51.4
6	5.57	5.22	5.40	0.03	0.03	58.2
7	4.71	5.01	4.86	0.02	0.02	47.2
8	5.26	5.20	5.23	0.00	0.00	54.7
9	4.96	4.40	4.68	0.08	0.08	43.8
10	5.10	5.29	5.20	0.01	0.01	54.0

Grand sum	103	**Grand mean**	5.16

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.04	0.10	0.32	6.24
Between Run	1.97	0.22	0.24	4.64
Total	3.00		0.40	**7.78**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	50.0	54.0	52.0	4.00	4.00	5.40E+03
2	57.0	59.4	58.2	1.50	1.50	6.78E+03
3	51.6	51.2	51.4	0.04	0.04	5.28E+03
4	50.2	52.9	51.5	1.89	1.89	5.31E+03
5	49.8	55.0	52.4	6.86	6.86	5.49E+03
6	55.8	54.1	55.0	0.71	0.71	6.05E+03
7	49.9	52.1	51.0	1.23	1.23	5.20E+03
8	52.3	57.9	55.1	7.73	7.73	6.07E+03
9	51.0	52.3	51.6	0.42	0.42	5.33E+03
10	50.1	51.8	50.9	0.74	0.74	5.19E+03

Grand sum	1.06E+03	**Grand mean**	52.9

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	50.2	5.02	2.24	4.24
Between Run	103	11.5	1.79	3.39
Total	153		2.87	**5.43**

Analyte: **BMA**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	4.01	4.13	4.07	0.00	0.00	33.1
2	3.84	4.51	4.18	0.11	0.11	34.9
3	4.69	4.11	4.40	0.08	0.08	38.7
4	3.96	4.54	4.25	0.08	0.08	36.1
5	3.81	4.18	4.00	0.03	0.03	31.9
6	3.81	4.35	4.08	0.07	0.07	33.3
7	3.82	3.91	3.87	0.00	0.00	29.9
8	3.65	4.90	4.28	0.39	0.39	36.6
9	4.17	3.60	3.89	0.08	0.08	30.2
10	3.86	4.27	4.07	0.04	0.04	33.0
Grand sum	82.1	**Grand mean**	4.11			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.81	0.18	0.43	10.4
Between Run	0.53	0.06	0.00	0.00
Total	2.34		0.43	**10.4**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	37.9	40.6	39.3	1.85	1.85	3.08E+03
2	37.0	41.8	39.4	5.83	5.83	3.11E+03
3	33.7	41.8	37.8	16.4	16.4	2.85E+03
4	40.3	42.0	41.2	0.73	0.73	3.39E+03
5	35.7	42.9	39.3	12.9	12.9	3.09E+03
6	37.5	44.4	41.0	12.0	12.0	3.36E+03
7	37.8	47.5	42.7	23.5	23.5	3.64E+03
8	40.5	39.6	40.0	0.22	0.22	3.21E+03
9	38.9	42.8	40.9	3.86	3.86	3.34E+03
10	40.0	40.9	40.5	0.19	0.19	3.27E+03
Grand sum	804	**Grand mean**	40.2			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	155	15.5	3.94	9.80
Between Run	32.8	3.65	0.00	0.00
Total	188		3.94	**9.80**

Table C3. Stability

Stability - fill in yellow shaded cells

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Condition: Three times frozen at -80°C and then thawed (3 freeze-thaw cycles, in-house spiked samples)
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Condition: Original samples (not yet prepared for instrument analysis) stored at room temperature for 1 day
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Condition: Processed samples (ready for instrument analysis) stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Condition: Samples stored at -80°C for 2 years (QCL261722 and QCH261735)

All stability sample results should be within ±15% of nominal concentration

Method name: VOC metabolites in urine
Method #: 2103a adjusted to 96-well plate (7 mm cap mat cover)
Matrix: Urine
Units: µg/L
Analyte: **CEMA**

Quality material 1	P17199	P17202	P17199	P17201	C17228	C17229	A17194 & P17194	P19196
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	24.9	26.2	24.9	28.1	29.1	25.7	48.4	51.5
Replicate 2	27.9	26.2	27.9	25.4	30.1	27.7	54.1	50.7
Replicate 3	25.9	27.2	25.9	29.0	28.9	25.7	49.4	50.9
Mean	26.2	26.5	26.2	27.5	29.4	26.4	50.6	51.0
% difference from initial measurement	--	**1.28**	--	**5.04**	--	**-10.1**	--	**0.75**

Quality material 2	P17199	P17202	P17199	P17201	C17228	C17229	A17194 & P17194	P19196
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	260	250	260	285	262	270	514	532
Replicate 2	265	257	265	275	269	263	585	548
Replicate 3	276	266	276	275	271	284	513	540
Mean	267	258	267	279	267	273	537	540
% difference from initial measurement	--	**-3.56**	--	**4.31**	--	**1.90**	--	**0.51**

Analyte: **ATCA**

Quality material 1								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	47.0	45.7	47.0	34.9	38.2	42.2	96.9	92.0
Replicate 2	35.8	47.0	35.8	44.7	48.6	34.2	105	103
Replicate 3	46.1	35.1	46.1	47.6	43.7	40.1	102	96.1
Mean	43.0	42.6	43.0	42.4	43.5	38.8	101	96.9
% difference from initial measurement	--	**-0.82**	--	**-1.35**	--	**-10.7**	--	**-4.39**

Quality material 2								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	451	419	451	431	381	367	1040	1030
Replicate 2	444	440	444	451	353	386	977	1080
Replicate 3	446	390	446	443	441	395	997	1060
Mean	447	416	447	442	392	383	1005	1057
% difference from initial measurement	--	**-6.80**	--	**-1.09**	--	**-2.32**	--	**5.15**

Analyte: **GAMA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	26.1	26.3	26.1	29.7	29.9	27.7	30.0	29.7
Replicate 2	28.3	25.9	28.3	28.0	32.3	26.2	34.0	28.5
Replicate 3	27.1	28.5	27.1	26.9	27.2	27.5	30.4	30.8
Mean	27.2	26.9	27.2	28.2	29.8	27.1	31.5	29.7
% difference from initial measurement	--	**-1.05**	--	**3.85**	--	**-8.92**	--	**-5.70**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	267	254	267	273	284	290	309	284
Replicate 2	266	269	266	286	264	255	327	303
Replicate 3	262	272	262	268	274	282	299	284
Mean	265	265	265	276	274	276	312	290
% difference from initial measurement	--	**-0.06**	--	**3.95**	--	**0.62**	--	**-6.81**

Analyte: **AAMA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	4.75	4.57	4.75	5.12	5.47	4.84	13.5	14.9
Replicate 2	5.11	5.16	5.11	4.57	5.56	5.75	15.1	13.7
Replicate 3	5.36	5.54	5.36	4.73	5.73	4.30	15.1	13.9
Mean	5.07	5.09	5.07	4.81	5.59	4.96	14.6	14.2
% difference from initial measurement	--	**0.34**	--	**-5.28**	--	**-11.2**	--	**-3.01**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	49.4	49.7	49.4	52.1	46.8	49.3	101	108
Replicate 2	49.8	48.6	49.8	54.3	48.3	44.6	108	114
Replicate 3	51.6	48.2	51.6	53.2	50.8	50.1	114	111
Mean	50.2	48.8	50.2	53.2	48.6	48.0	108	111
% difference from initial measurement	--	**-2.79**	--	**5.95**	--	**-1.34**	--	**3.04**

Analyte: **HEMA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	1.48	1.69	1.48	2.20	1.85	1.74	3.83	4.61
Replicate 2	2.26	1.64	2.26	2.14	1.77	2.00	4.88	4.74
Replicate 3	1.62	1.86	1.62	1.46	1.73	1.53	4.27	5.76
Mean	1.79	1.73	1.79	1.93	1.79	1.75	4.33	5.04
% difference from initial measurement	--	**-3.21**	--	**8.26**	--	**-1.79**	--	**16.4**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	16.5	15.8	16.5	18.4	16.8	15.9	45.9	48.2
Replicate 2	17.8	17.9	17.8	16.8	15.6	17.9	44.9	54.3
Replicate 3	17.8	16.0	17.8	17.0	17.1	17.4	46.0	50.5
Mean	17.4	16.6	17.4	17.4	16.5	17.1	45.6	51.0
% difference from initial measurement	--	**-4.59**	--	**0.27**	--	**3.50**	--	**11.9**

Analyte: **DHBM**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	17.2	20.5	17.2	20.9	16.7	15.4	117	118
Replicate 2	16.9	18.1	16.9	17.8	17.3	21.1	145	123
Replicate 3	17.6	16.7	17.6	17.6	19.1	19.6	129	134
Mean	17.2	18.4	17.2	18.8	17.7	18.7	130	125
% difference from initial measurement	--	**7.10**	--	**9.00**	--	**5.57**	--	**-3.76**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	181	180	181	191	181	186	392	400
Replicate 2	185	185	185	191	184	183	455	426
Replicate 3	185	185	185	180	186	186	403	402
Mean	184	183	184	187	184	185	417	409
% difference from initial measurement	--	**-0.11**	--	**2.11**	--	**0.77**	--	**-1.81**

Analyte: **CYHA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	13.0	12.1	13.0	12.0	13.3	11.4	7.61	6.26
Replicate 2	11.3	12.3	11.3	12.6	13.3	12.4	6.40	7.19
Replicate 3	12.7	13.7	12.7	13.2	12.8	12.3	7.25	8.22
Mean	12.3	12.7	12.3	12.6	13.1	12.0	7.09	7.22
% difference from initial measurement	--	**3.26**	--	**2.32**	--	**-8.59**	--	**1.91**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	123	125	123	120	120	117	88.8	89.5
Replicate 2	122	129	122	117	117	116	99.8	98.2
Replicate 3	123	132	123	129	129	125	92.6	94.9
Mean	123	129	123	122	122	119	93.7	94.2
% difference from initial measurement	--	**4.78**	--	**-0.69**	--	**-2.59**	--	**0.51**

Analyte: **AMCA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	16.3	15.7	16.3	19.0	14.7	15.6	28.3	29.1
Replicate 2	20.5	20.5	20.5	16.3	19.3	16.0	33.0	26.9
Replicate 3	16.2	14.9	16.2	15.1	16.9	16.6	31.7	30.1
Mean	17.7	17.0	17.7	16.8	17.0	16.1	31.0	28.7
% difference from initial measurement	--	**-3.65**	--	**-5.03**	--	**-5.34**	--	**-7.38**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	173	154	173	165	157	154	399	341
Replicate 2	157	166	157	165	154	153	411	380
Replicate 3	165	151	165	171	165	162	331	353
Mean	165	157	165	167	159	157	380	358
% difference from initial measurement	--	**-4.88**	--	**0.98**	--	**-1.34**	--	**-5.82**

Analyte: **TTCA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	58.9	60.7	58.9	56.1	57.0	50.5	41.9	50.1
Replicate 2	64.9	55.6	64.9	55.5	54.7	51.2	58.9	45.1
Replicate 3	66.7	60.7	66.7	59.3	56.1	44.8	44.1	54.5
Mean	63.5	59.0	63.5	57.0	55.9	48.8	48.3	49.9
% difference from initial measurement	--	**-7.13**	--	**-10.3**	--	**-12.7**	--	**3.28**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	646	535	646	574	542	496	763	634
Replicate 2	605	533	605	538	534	451	733	753
Replicate 3	636	549	636	574	559	509	749	636
Mean	629	539	629	562	545	485	748	675
% difference from initial measurement	--	**-14.3**	--	**-10.6**	--	**-11.0**	--	**-9.85**

Analyte: **HPMA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	57.7	63.7	57.7	57.7	56.0	51.8	76.1	79.2
Replicate 2	56.0	55.0	56.0	58.8	59.1	50.6	80.3	83.3
Replicate 3	58.9	54.3	58.9	56.3	56.8	52.7	79.1	87.0
Mean	57.5	57.6	57.5	57.6	57.3	51.7	78.5	83.2
% difference from initial measurement	--	**0.21**	--	**0.13**	--	**-9.82**	--	**5.96**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	584	574	584	596	511	496	701	857
Replicate 2	584	569	584	610	493	496	833	906
Replicate 3	593	565	593	586	522	500	814	871
Mean	587	569	587	597	509	497	783	878
% difference from initial measurement	--	**-3.02**	--	**1.75**	--	**-2.26**	--	**12.2**

Analyte: **MADA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	48.0	51.0	48.0	53.7	51.3	46.0	103	87.8
Replicate 2	47.1	47.1	47.1	63.4	62.0	58.5	105	99.3
Replicate 3	45.8	56.2	45.8	34.8	48.2	51.1	108	103
Mean	47.0	51.4	47.0	50.6	53.9	51.9	105	96.6
% difference from initial measurement	--	**9.55**	--	**7.84**	--	**-3.66**	--	**-8.43**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	516	474	516	550	536	524	1034	878
Replicate 2	577	516	577	540	559	522	993	1120
Replicate 3	559	533	559	565	529	537	935	1010
Mean	551	507	551	551	541	528	987	1003
% difference from initial measurement	--	**-7.85**	--	**0.14**	--	**-2.54**	--	**1.59**

Analyte: **HPM2**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	12.8	12.4	12.8	12.4	13.4	12.0	17.1	17.2
Replicate 2	12.2	12.0	12.2	11.8	12.5	12.4	20.0	16.4
Replicate 3	11.7	11.9	11.7	12.2	12.9	11.2	16.7	16.8
Mean	12.2	12.1	12.2	12.1	13.0	11.9	17.9	16.8
% difference from initial measurement	--	**-1.10**	--	**-0.85**	--	**-8.46**	--	**-6.22**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	144	132	144	146	120	122	161	136
Replicate 2	141	134	141	138	123	126	163	145
Replicate 3	141	135	141	145	133	142	150	134
Mean	142	134	142	143	125	130	158	138
% difference from initial measurement	--	**-5.70**	--	**0.89**	--	**4.03**	--	**-12.5**

Analyte: **CYMA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	2.15	2.94	2.15	2.31	2.58	2.56	4.82	4.46
Replicate 2	2.44	1.89	2.44	2.65	1.88	2.23	6.11	4.70
Replicate 3	2.39	2.34	2.39	2.21	2.61	1.85	4.99	4.87
Mean	2.33	2.39	2.33	2.39	2.36	2.21	5.31	4.68
% difference from initial measurement	--	**2.65**	--	**2.79**	--	**-5.99**	--	**-11.8**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	25.3	24.5	25.3	25.6	24.6	23.0	153.5	164
Replicate 2	25.2	23.8	25.2	25.9	25.5	24.9	165.4	174
Replicate 3	25.3	24.5	25.3	23.9	26.2	25.4	158.7	165
Mean	25.3	24.3	25.3	25.1	25.4	24.5	159	168
% difference from initial measurement	--	**-4.02**	--	**-0.78**	--	**-3.86**	--	**5.29**

Analyte: **MHB3**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	2.33	2.83	2.33	3.16	2.48	2.68	4.46	5.30
Replicate 2	2.30	2.48	2.30	2.05	3.25	2.38	4.68	5.25
Replicate 3	2.36	2.39	2.36	2.43	2.57	2.39	4.90	4.73
Mean	2.33	2.57	2.33	2.55	2.77	2.48	4.68	5.09
% difference from initial measurement	--	**10.1**	--	**9.36**	--	**-10.2**	--	**8.83**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	26.9	25.9	26.9	29.2	27.4	23.2	34.6	35.6
Replicate 2	26.4	27.9	26.4	25.1	23.5	24.7	35.4	35.9
Replicate 3	25.3	26.2	25.3	27.4	27.0	29.0	31.7	33.6
Mean	26.2	26.7	26.2	27.2	26.0	25.7	33.9	35.0
% difference from initial measurement	--	**1.81**	--	**3.96**	--	**-1.24**	--	**3.40**

Analyte: **HPMM**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	13.3	14.7	13.3	14.2	14.4	14.2	91.5	87.5
Replicate 2	14.0	13.1	14.0	12.8	14.8	12.9	104	83.7
Replicate 3	12.7	13.0	12.7	14.3	12.6	12.8	96.0	87.4
Mean	13.3	13.6	13.3	13.8	13.9	13.3	97.0	86.2
% difference from initial measurement	--	**2.37**	--	**3.49**	--	**-4.54**	--	**-11.1**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	148	140	148	147	137	130	899	872
Replicate 2	143	148	143	150	131	132	974	922
Replicate 3	146	142	146	152	140	142	914	857
Mean	146	143	146	150	136	135	929	883
% difference from initial measurement	--	**-1.61**	--	**2.82**	--	**-0.65**	--	**-4.92**

Analyte: **PHGA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	46.4	51.7	46.4	49.8	52.2	46.9	31.4	24.5
Replicate 2	46.5	47.1	46.5	47.3	54.1	49.9	32.0	31.8
Replicate 3	45.9	48.0	45.9	42.7	51.2	46.8	27.7	26.4
Mean	46.3	48.9	46.3	46.6	52.5	47.9	30.4	27.6
% difference from initial measurement	--	**5.79**	--	**0.71**	--	**-8.83**	--	**-9.19**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	452	475	452	473	454	443	357	420
Replicate 2	467	470	467	459	442	439	437	462
Replicate 3	463	447	463	472	483	446	343	312
Mean	461	464	461	468	459	443	379	398
% difference from initial measurement	--	**0.70**	--	**1.59**	--	**-3.63**	--	**5.00**

Analyte: **IPM3**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	3.96	4.18	3.96	4.08	4.27	3.16	0.50	1.47
Replicate 2	3.76	4.37	3.76	4.82	3.78	4.25	0.93	0.313
Replicate 3	4.24	4.10	4.24	3.97	3.89	3.62	0.91	0.687
Mean	3.99	4.22	3.99	4.29	3.98	3.67	0.78	0.82
% difference from initial measurement	--	**5.76**	--	**7.66**	--	**-7.66**	--	**5.74**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	40.0	40.3	40.0	39.7	37.5	39.5	42.0	42.6
Replicate 2	39.5	40.7	39.5	41.6	37.9	37.4	46.4	46.2
Replicate 3	40.2	38.5	40.2	41.7	42.0	42.5	43.9	40.5
Mean	39.9	39.8	39.9	41.0	39.1	39.8	44.1	43.1
% difference from initial measurement	--	**-0.32**	--	**2.68**	--	**1.59**	--	**-2.29**

Analyte: **2MHA**

Quality material 1								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	19.7	16.0	19.7	16.5	14.4	12.6	32.4	39.6
Replicate 2	17.2	14.0	17.2	15.8	15.9	13.4	37.7	38.9
Replicate 3	19.0	18.3	19.0	17.6	14.4	12.9	30.9	33.0
Mean	18.6	16.1	18.6	16.6	14.9	13.0	33.7	37.2
% difference from initial measurement	--	**-13.7**	--	**-10.8**	--	**-13.2**	--	**10.4**

Quality material 2								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	147	145	147	130	159	160	337	340
Replicate 2	138	155	138	155	130	159	302	346
Replicate 3	140	146	140	158	152	154	333	319
Mean	142	148	142	148	147	158	324	335
% difference from initial measurement	--	**4.69**	--	**4.32**	--	**7.32**	--	**3.43**

Analyte: **BPMA**

Quality material 1								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	3.53	3.95	3.53	4.05	3.02	3.62	4.94	6.17
Replicate 2	3.12	3.45	3.12	2.95	3.82	3.68	5.82	5.82
Replicate 3	3.40	3.42	3.40	3.02	3.47	3.24	5.64	6.52
Mean	3.35	3.61	3.35	3.34	3.44	3.51	5.47	6.17
% difference from initial measurement	--	**7.71**	--	**-0.38**	--	**2.14**	--	**12.9**

Quality material 2								A19218
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	34.9	34.9	34.9	31.9	36.1	35.3	176	149
Replicate 2	34.9	34.0	34.9	34.2	31.4	35.9	160	139
Replicate 3	36.6	38.0	36.6	36.9	39.7	38.9	166	150
Mean	35.5	35.6	35.5	34.3	35.7	36.7	167	146
% difference from initial measurement	--	**0.48**	--	**-3.12**	--	**2.60**	--	**-12.9**

Analyte: **34MH**

Quality material 1								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	29.6	31.4	29.6	32.9	29.4	30.8	82.2	80.8
Replicate 2	28.3	32.7	28.3	31.3	32.3	30.1	86.9	85.2
Replicate 3	31.2	31.4	31.2	32.2	28.3	26.6	83.4	87.6
Mean	29.7	31.8	29.7	32.1	30.0	29.1	84.2	84.5
% difference from initial measurement	--	**7.30**	--	**8.32**	--	**-2.90**	--	**0.44**

Quality material 2								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	308	323	308	320	302	299	597	577
Replicate 2	299	318	299	314	301	295	623	622
Replicate 3	313	312	313	310	317	320	596	618
Mean	307	317	307	315	307	305	605	606
% difference from initial measurement	--	**3.48**	--	**2.61**	--	**-0.54**	--	**0.07**

Analyte: **PHEM**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	2.18	2.38	2.18	2.65	2.83	2.32	5.36	5.76
Replicate 2	2.45	2.10	2.45	2.38	2.56	2.05	4.83	5.80
Replicate 3	2.27	2.14	2.27	2.49	2.23	2.26	5.22	5.70
Mean	2.30	2.21	2.30	2.51	2.54	2.21	5.14	5.75
% difference from initial measurement	--	**-4.03**	--	**9.00**	--	**-13.0**	--	**12.0**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	24.0	20.7	24.0	24.4	23.8	23.3	52.0	51.4
Replicate 2	23.8	21.8	23.8	23.3	22.5	22.8	53.2	58.8
Replicate 3	24.3	22.1	24.3	23.6	25.7	23.8	50.8	54.1
Mean	24.0	21.5	24.0	23.8	24.0	23.3	52.0	54.8
% difference from initial measurement	--	**-10.3**	--	**-1.04**	--	**-2.86**	--	**5.42**

Analyte: **BMA**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	2.01	1.94	2.01	2.18	2.09	1.94	3.74	4.12
Replicate 2	2.10	1.90	2.10	1.86	2.32	1.75	4.69	4.54
Replicate 3	2.22	1.84	2.22	2.09	1.99	2.19	3.82	4.05
Mean	2.11	1.89	2.11	2.04	2.13	1.96	4.08	4.24
% difference from initial measurement	--	**-10.3**	--	**-3.35**	--	**-8.00**	--	**3.76**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	19.7	17.5	19.7	19.7	20.2	19.3	35.8	39.4
Replicate 2	19.1	17.5	19.1	19.2	20.7	19.1	41.7	40.6
Replicate 3	18.3	18.3	18.3	20.7	20.0	21.2	38.9	39.4
Mean	19.0	17.8	19.0	19.8	20.3	19.9	38.8	39.8
% difference from initial measurement	--	**-6.72**	--	**4.21**	--	**-2.21**	--	**2.74**

Table C4. LOD, Specificity, Fit for intended use

LOD, specificity and fit for intended use - fill in yellow shaded cells

Method name:	VOC metabolites in urine
Method #:	2103a
Matrix:	Urine
Units:	µg/L

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
CEMA	6.96	yes	yes
ATCA	29.5	yes	yes
GAMA	9.40	yes	yes
AAMA	2.20	yes	yes
HEMA	0.79	yes	yes
DHBM	5.25	yes	yes
CYHA	2.60	yes	yes
AMCA	6.26	yes	yes
TTCA	11.2	yes	yes
HPMA	13.0	yes	yes
MADA	12.0	yes	yes
HPM2	5.30	yes	yes
CYMA	0.50	yes	yes
MHB3	0.60	yes	yes
HPMM	1.70	yes	yes
PHGA	12.0	yes	yes
IPM3	1.20	yes	yes
2MHA	5.00	yes	yes
BPMA	1.20	yes	yes
34MH	8.00	yes	yes
PHEM	1.00	yes	yes
BMA	0.50	yes	yes

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