INTRODUCTION

The objective of this study was to independently validate the analytical method in Smithers Viscient, Wareham Study No. 14134.6113 (Validation of an Environmental Chemistry Method for the Determination of PDMU and cPMU in Surface Water), to determine the content of PDMU and cPMU in surface water, in accordance with EPA 850.6100 (2012), EPA 860.1340 (1996) and SANCO/3029/99 rev 4 (2000) guidelines.

Control samples of Fountains Abbey surface water were fortified with PDMU and cPMU at 0.1 and 1 µg/L in quintuplicate and analysed. Samples were diluted with methanol. An aliquot was diluted into calibration range with methanol: surface water (20:80 v/v), if required.

To assess matrix effects, calibration standards were prepared in methanol: surface water (20:80 v/v) and in methanol: water (20:80 v/v).

Samples were analysed for PDMU and cPMU using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

Matrix effects, linearity and specificity of the method were determined. Precision and accuracy were calculated at each validation level in surface water for PDMU and cPMU. One primary and one confirmatory LC-MS/MS transition was analysed for PDMU and cPMU.

The study was initiated on 11 May 2018 (date the protocol was signed by the Study Director) and completed on the date the final report was signed by the Study Director. The practical phase of the study was conducted by Smithers Viscient (ESG) and was started on 15 May 2018 (stock preparation) and completed on 22 May 2018 (LC-MS/MS analysis).

MATERIALS AND METHODS

Protocol Adherence

This study was carried out in accordance with the protocol. Deviations, which did not affect the integrity of the study, are given in Appendix 7.

Test Substances

Test substance name:	**PDMU**
CAS Number	101-42-8
IUPAC Name:	1,1-dimethyl-3-phenylurea
Molecular Formula:	$C_9H_{12}N_2O$
Molecular Mass:	164.204 g/mol
Sponsor Lot Number:	G0612
Purity:	99.4%
Storage Conditions:	Room temperature
Retest Date:	13 November 2019

Test substance name:	**cPMU**
CAS Number	20940-42-5
IUPAC Name:	1-(3-chlorophenyl)-3-methylurea
Molecular Formula:	$C_8H_9ClN_2O$
Molecular Mass:	184.63 g/mol
Sponsor Lot Number:	SMV9059
Purity:	98.8%
Storage Conditions:	Room temperature
Retest Date:	13 November 2018

Certificates of Analysis for the test substances are presented in Appendix 1.

Test System

A control sample of water was sourced by Smithers Viscient (ESG). The water used was CS 14/18 Fountains Abbey surface water.

Water characterisation data are listed in the following table:

Water Name	**Water Type**	**Suspended Solids (mg/L)**	**Conductivity (µS/cm)**	**Hardness (mg/L $CaCO_3$)**	**pH**	**Dissolved Organic Carbon (mg/L)**
Fountains Abbey	Surface	34	154	86	7.44	11.2

The certificate of analysis for the water is presented in Appendix 2.

Reagents

Acetonitrile	HPLC grade, Honeywell
Methanol	HPLC grade, Honeywell
Water	Milli-Q with LCPAK polisher, In House
0.1% Formic acid in water	MS grade, Honeywell
0.1% Formic acid in acetonitrile	MS grade, Honeywell

Equivalent or better reagents may have been used.

Equipment

Shimadzu Nexera series HPLC system with AB Sciex API 5000 MS/MS detector

Analytical Method

Analytical method 14134.6113 was supplied by Smithers Viscient, Wareham on behalf of the sponsor. The method was re-written as SMV 3202043-01D to account for minor differences in instrumentation, reagents and consumables before validation, and re-issued as SMV 3202043-01V after validation. Minor method changes, such as using different equipment or instrumentation, optimisation of instrument conditions and scaling of reagent and stock solutions were written into the primary method, and were therefore not considered to be deviations. Substituted reagents, which were of equivalent grade or higher, were not considered to impact the validity of the study. The calibration range was extended in deviation to the method, which is a discussed in Appendix 7, and was not considered to impact the validity of the study. The full analytical procedure is presented in Appendix 6.

Preparation of Reagents

Methanol: water (20:80 v/v) was prepared by mixing 50 mL HPLC grade methanol with 200 mL Milli-Q water.

Methanol: surface water (20:80 v/v) was prepared by mixing 50 mL HPLC grade methanol with 200 mL Fountains Abbey surface water.

Preparation of Stock Solutions

Primary stock solutions of PDMU and cPMU were prepared as described in the following table:

Stock ID	Test Substance	Amount Weighed (mg)	Purity (%)	Solvent	Final Volume (mL)	Concentration (µg/mL)[1]
Stock 1	PDMU	10.82	99.4	Acetonitrile	10.755	1000
Stock 2		10.30	99.4		10.239	1000
Stock 3	cPMU	10.43	98.8		10.305	1000
Stock 4		10.49	98.8		10.365	1000
Stock 5		10.54	98.8		10.414	1000
Stock 6		11.10	98.8		10.967	1000
Stock 7		10.59	98.8		10.460	1000
Stock 8		10.56	98.8		10.430	1000

[1] Corrected for Purity.

Duplicate stocks were prepared for correlation purposes.

cPMU Stock 3 and 4 failed correlation and were therefore re-prepared. Stock 5 and 6 also failed correlation. Stock 7 and 8 were prepared directly into a disposable amber glass vial, without using a volumetric flask, and the correlation passed. cPMU Stock 3 was used for the matrix assessment, which was analysed at the same time as the first failed correlation. This matrix assessment was still reported, as the absolute concentrations were not considered to be critical.

Primary stocks were stored refrigerated in amber glass bottles and given a nominal expiry of three months.

Secondary stock solutions were prepared as described in the following table:

Test Substance	Fortifying Stock Concentration (µg/mL)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/mL)
PDMU	1000	0.1	Acetonitrile	10	10
cPMU	1000	0.1		10	10

Secondary stock solutions were stored refrigerated in amber glass bottles and given a nominal expiry of one month.

Sub-stock solutions were prepared as described in the following table:

Test Substance	Fortifying Stock Concentration (µg/mL)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/mL)[1]
PDMU	10	0.1	Methanol	10	0.1
cPMU	10	0.1			
Mixed	0.1	1		10	0.01[1]

[1] Mixed stock of PDMU and cPMU.

Sub-stock solutions were prepared on the day of use and stored refrigerated until the corresponding analysis was complete.

Preparation of Calibration Standards
Matrix-matched calibration standards of PDMU and cPMU were prepared directly into disposable glass vials (without using a glass volumetric flask) as described in the following table:

Fortifying Stock Concentration (µg/L)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/L)
100	0.05	Methanol: surface water (20:80 v/v)	10	0.5
0.5	0.8		1	0.4
0.5	0.6		1	0.3
0.5	0.4		1	0.2
0.5	0.2		1	0.1
0.5	0.15		1	0.075
0.5	0.1		1	0.05
0.5	0.048		1	0.024

A single set of calibration standards was prepared for the validation batch. Each standard was analysed in duplicate during the run sequence, in random order interspersed with the samples.

Preparation of Matrix-Matched Standards for Matrix Assessment
Matrix-matched standards of PDMU and cPMU were prepared in methanol: surface water (20:80 v/v).

Stock Concentration (µg/L)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/L)
100	0.02	Methanol: surface water (20:80 v/v)	10	0.2
100	0.02		10	0.2
100	0.02		10	0.2

Preparation of Non-Matrix-Matched Standards for Matrix Assessment
Non-matrix-matched standards of PDMU and cPMU were prepared in blank solvent for comparison of peak areas with matrix matched standards.

Stock Concentration (µg/L)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/L)
100	0.02	Methanol: water (20:80 v/v)	10	0.2
100	0.02		10	0.2
100	0.02		10	0.2

Sample Fortification
An 8-mL aliquot of water was measured into a disposable glass vial. Quintuplicate water samples were fortified at the LOQ (0.1 µg/L) and at 10 × LOQ (1 µg/L) with a mixed stock solution of PDMU and cPMU. Duplicate control water samples and a reagent blank (methanol: surface water (20:80 v/v) was also prepared, as described in the following table:

CS 14/18 Fountains Abbey surface water

Sample ID	Sample Volume (mL)	Stock Concentration (µg/L)	Volume Added (mL)	Fortified Concentration (µg/L)
Reagent Blank A	N/A	N/A	N/A	N/A
Control A-B	8	N/A	N/A	N/A
F0.1 A-E	8	10	0.08	0.1
F1 A-E	8	10	0.8	1

N/A = Not applicable.

Sample Extraction

A 2-mL aliquot of methanol (including the fortification stock volume) was added to the water and mixed well. A portion of extract was diluted into the calibration range, if required, with methanol: surface water (20:80 v/v). The final extract was transferred into an HPLC vial for analysis. Sample extracts were stored refrigerated in case further analysis was required. The extraction procedure is summarised in the following table:

CS 14/18 Fountains Abbey surface water

Sample ID	Fortified Concentration (µg/L)	Sample Volume (mL)	Volume of Extract (mL)	Sample Dilution (mL to mL)
Reagent Blank A	N/A	N/A	10	N/A
Control A-B	N/A	8	10	N/A
F0.1 A-E	0.1	8	10	N/A
F1 A-E	1	8	10	0.25-1

N/A = Not applicable.

A single set of matrix matched calibration standards was prepared for the validation batch, and analysed twice during the batch, interspersed with the samples.

Instrument Conditions

LC-MS/MS analysis was performed using the following instrument conditions:

LC Parameters:

Column#	XBridge BEH C18 2.5 µm 2.1 × 50 mm		
Mobile Phase A#	0.1% Formic acid in water		
Mobile Phase B#	0.1% Formic acid in acetonitrile		
Flow Rate	0.4 mL/min		
Gradient	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	90	10
	1.0	90	10
	1.25	50	50
	2.5	0	100
	3.5	0	100
	3.6	90	10
	5.0	90	10
Run Time	5.0 minutes		
Column Temperature	35°C		
Autosampler Temperature	15°C		
Injection Volume	25 µL		
Retention Time	Approx. 1.9 minutes (PDMU)		
	Approx. 2.0 minutes (cPMU)		
Valco Valve Diverter	Time (min)	Position	
	0	A (to waste)	
	1	B (to MS)	
	4	A (to waste)	

MS/MS Parameters:

Instrument	AB Sciex API 5000 Triple Quadrupole Mass Spectrometer			
Ionisation Type#	Electrospray (ESI)			
Polarity#	Positive			
Scan Type#	Multiple reaction monitoring (MRM)			
Ion Spray Voltage	5500 V			
Collision Gas (CAD)	5			
Curtain Gas (CUR)	25			
Gas Flow 1 (GS1)	40			
Gas Flow 2 (GS2)	40			
Vaporiser Temperature (TEM)	450°C			
Interface Heater (ihe)	On			
Entrance Potential (EP)	10			
Collision Exit Potential (CXP)	13			
Compound Name	MRM Transition Ions Monitored	Declustering Potential (DP)	Collision Energy (CE)	Dwell Time (ms)
PDMU (Primary)	165.0/72.0	31	23	65
PDMU (Confirmatory)	165.0/46.1	31	23	65
cPMU (Primary)	185.2/93.0	61	37	65
cPMU (Confirmatory)	185.2/128.0	61	21	65

The instrument conditions were optimised prior to use, therefore parameters not marked "#" above may vary slightly from those given in the primary method (14134.6113) and the MRM transitions given in the protocol. This instrument optimisation is not considered to be a deviation from the method or protocol.

LC-MS/MS data were collected and processed using Analyst 1.6.2.

Calculation of Results

When the calibration fit is linear as in this study, Analyst 1.6.2 uses the following formula to calculate the concentration of test substance present in the sample extract:

$x = (y - c) / m$

Where:

x = concentration of test substance in sample extract (µg/L)
y = peak area due to test substance
c = y intercept on calibration line
m = gradient of the calibration line

The calibration line used a 1/x weighting.

The concentration of test substance in the sample is calculated as follows:

Sample concentration (µg/L) = Extract concentration (µg/L) × Dilution factor

Dilution factor = Final extract volume (mL) / volume of sample in final extract (mL)

Procedural recovery from fortified samples is calculated as follows:

Recovery (%) = Sample concentration / Fortified concentration × 100

Matrix-matched calibration standards were used therefore sample recoveries were automatically corrected for interference in the control matrix by the y-intercept from the calibration line.

95% confidence intervals were calculated for each validation level as follows:

95% confidence interval (±) = $t_{n-1}s/\sqrt{n}$

Where:

t_{n-1} = 2.78
s = standard deviation
n = number of samples (5)

The Limit of Detection (LOD) based upon the sample concentration equivalent to three times the baseline noise of a control sample was calculated as follows:

LOD (µg/L) = 3 × height of control baseline noise × control dilution factor × calibration standard concentration (µg/mL) / height of calibration standard peak

The Method Detection Limit (MDL) based upon the sample concentration equivalent to the lowest calibration standard was calculated as follows:

MDL (µg/L) = lowest calibration standard concentration × control dilution factor

Validation Pass Criteria
The validation was deemed acceptable if the following criteria were met for the primary and confirmatory transitions monitored for each compound:

Mean Recovery and Precision
Recovery and precision were acceptable if each fortification level had a mean recovery between 70 and 120% and a %RSD (relative standard deviation) ≤ 20%.

Specificity/Selectivity
Specificity was acceptable if the concentrations found in blank samples were ≤ 30% of the LOQ.

Linearity
Linearity was acceptable if the lowest calibration standard concentration was ≤ 80% of the equivalent LOQ final extract concentration. The highest calibration standard concentration was ≥ 120% of the 10 × LOQ extract concentration (after dilution if applicable). Matrix-matched calibration standards were used for consistency with the primary method (14134.6113). The correlation coefficient (r) was acceptable if it was ≥ 0.995.

LOD (Limit of Detection) Assessment
An estimate of the LOD was made at 3 × baseline noise for primary and confirmatory transitions for all compounds.

MDL (Method Detection Limit)
The MDL was calculated as the sample concentration equivalent to the lowest calibration standard.

Matrix Assessment
An assessment of matrix effects was made by comparison of peak areas for triplicate standards prepared in methanol: water (20:80 v/v) and in methanol: surface water (20:80 v/v). This was assessed for all compounds and for the primary and confirmatory transitions.

Results were presented as a % difference from the mean non-matrix-matched standard value.

A difference of ≥ 20% was considered significant.

REFERENCES

EPA 850.6100 (2012) Environmental Chemistry Methods and Associated Independent Laboratory Validation.

EPA 860.1340 (1996) Residue Analytical Method

SANCO/3029/99 rev 4 (2000) Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414.

Smithers Viscient (Wareham) Study No. 14134.6113 Validation of an Environmental Chemistry Method for the Determination of PDMU and cPMU in Surface Water (Author: Stephen R. DeVellis).

Appendix 6
Analytical Procedure

Analytical Procedure

Procedure Title	Determination of PDMU and cPMU in Surface Water by LC-MS/MS
Procedure Code	SMV 3202043-01V
Issue Date	08 June 2018
Page Number	1 of 11

The methodology described in this procedure was validated in Fountains Abbey surface water at 0.1 and 1 µg/L.

SMITHERS VISCIENT

REVISION HISTORY

SMV 3202043-01D	New method for independent laboratory validation based upon Smithers Viscient, Wareham study 14134.6113
SMV 3202043-01V	Re-issued following validation with minor corrections. Removal of references to volumetric flasks. Stated that stock preparations and dilutions should be performed directly into disposable glass bottles or vials. Corrected number of control samples. Stated that the volume of methanol added to extracts includes the fortification stock already added. Updated MS/MS conditions with actual optimised settings used.

SAFETY PRECAUTIONS

Operators should take the normal precaution of wearing gloves, laboratory coats and safety glasses when handling compound and matrix samples.

Safety assessments (Control of Substances Hazardous to Health, COSHH) have been made of those procedural steps involving preparation of solutions, reagents and analysis of matrix samples. Appropriate safety codes have been included in the text and are defined in the section titled General Handling Control Categories.

The hazards and risks of the substances hazardous to health used in this method have been considered. Provided the method is accurately followed and the control measures specified in the method are correctly used, there should be no foreseeable hazards to health.

INTRODUCTION

This method describes the procedure for determining concentrations of PDMU and cPMU in surface water by LC-MS/MS. Surface water is diluted with methanol to give a composition of methanol: surface water (20:80 v/v). The extract is further diluted into calibration range, if required with methanol: surface water (20:80 v/v).
The extracts are quantified by LC-MS/MS.

Matrix effects for PDMU and cPMU in aqueous matrices are determined by comparing peak areas of calibration standards prepared in methanol: surface water (20:80 v/v) and in methanol: water (20:80 v/v). Matrix effects are significant if the matrix matched standard area is $\geq$ 20% different to the non-matrix standard area. Matrix matched calibration standards should be used for method validation for consistency with the primary method (14134.6113).

cPMU is thought to be affected by re-usable glass volumetric flasks, therefore stock preparations and dilutions should be carried out directly into disposable glass bottles or vials, without using glass volumetric flasks.

APPARATUS, MATERIALS, REAGENTS AND SOLUTIONS

Apparatus and Glassware

- Shimadzu Nexera series HPLC system with AB Sciex API 5000 MS/MS detector.
- HPLC column: Waters XBridge BEH C18, 2.5 µm, 2.1 × 50 mm
- Analytical balance
- Positive displacement pipettes
- Amber glass vials
- Disposable glass vials
- HPLC vials

Equivalent equipment may be used if required

Materials

• Acetonitrile	HPLC grade, Honeywell
• Methanol	HPLC grade, Honeywell
• Water	Milli-Q (with LCPAK polisher)
• 0.1% Formic acid in water	LC-MS grade, Honeywell
• 0.1% Formic acid in acetonitrile	LC-MS grade, Honeywell

Equivalent materials may be used if required

Reagents

Methanol: water (20:80 v/v)

Mix 50 mL HPLC grade methanol with 200 mL Milli-Q water.

Methanol: surface water (20:80 v/v)

Mix 50 mL methanol with 200 mL Fountains Abbey surface water.

Standard Solution Preparation [1b, 4a]

Primary Standard Stock

Separately prepare duplicate stock solutions of PDMU and cPMU at 1000 µg/mL in acetonitrile. Accurately weigh approximately 10 mg test substance into an amber glass bottle. Add 10 mL acetonitrile, adjusted for purity and the actual amount weighed to give exactly 1000 µg/mL. The primary stocks should be stored refrigerated and given a nominal expiry date of 3 months.

Standard Correlation

Dilute the duplicate primary stocks to the mid-point of the calibration line. Correlate the standard solutions by injecting each of the two calibration standards 5 times into the LC/MS/MS. Ensure that the two solutions are injected alternately in the run sequence. The results for the correlation should be ± 5% of the overall mean calculated by peak areas.

Review of Results

Review the data and document the correlation calculations. If the correlation is out of specification, either repeat the injections, re-dilute, or prepare two new stock standards and repeat the procedures in sections *<<Initial Weighing of Stock Solutions>>* to *<<Review of Results>>*.

If the acceptance criteria from section <<*Standard Correlation*>> have been met, then the calibration solutions are acceptable for use. If required, fortification solutions for method validation will be made from the same stock standard, or its dilutions, from which the calibration line has been prepared.

Secondary Standard Stocks

Prepare secondary stock solutions of PDMU and cPMU directly into amber glass bottles as described in the following table:

Test substance	Primary stock concentration (µg/mL)	Volume taken (mL)	Solvent	Final volume (mL)	Concentration (µg/mL)
PDMU	1000	0.1	Acetonitrile	10	10
cPMU	1000	0.1		10	10

The secondary stocks should be stored refrigerated and given a nominal expiry date of 1 month.

Sub-Stocks

Prepare sub-stock solutions of PDMU and cPMU directly into disposable glass vials as described in the following table:

Test Substance	Fortifying stock concentration (µg/mL)	Volume taken (mL)	Solvent	Final volume (mL)	Concentration (µg/mL)
PDMU	10	0.1	Methanol	10	0.1[1]
cPMU	10	0.1			
Mixed	0.1	1		10	0.01

[1] Mixed stock of PDMU and cPMU.

The sub-stock solutions should be prepared on the day of use.

Calibration Standards

Prepare mixed calibration standards of PDMU and cPMU directly into disposable glass vials as described in the following table:

Fortifying stock concentration (µg/L)	Volume taken (mL)	Solvent	Final volume (mL)	Concentration (µg/L)
100	0.05	Methanol: surface water (20:80 v/v)	10	0.5
0.5	0.8		1	0.4
0.5	0.6		1	0.3
0.5	0.4		1	0.2
0.5	0.2		1	0.1
0.5	0.15		1	0.075
0.5	0.1		1	0.05
0.5	0.048		1	0.024

A single set of calibration standards should be prepared for each validation batch, and analysed twice during the batch, interspersed with the samples.

Preparation of Matrix Matched Standards for Matrix Assessment

Prepare matrix matched standards of PDMU and cPMU in disposable glass vials as described in the following table:

Stock concentration (µg/L)	Volume taken (mL)	Solvent	Final volume (mL)	Concentration (µg/L)
100	0.02	Methanol: surface water (20:80 v/v)	10	0.2
100	0.02		10	0.2
100	0.02		10	0.2

Preparation of Non-Matrix Matched Standards for Matrix Assessment

Prepare non-matrix matched standards of PDMU and cPMU in disposable glass vials as described in the following table:

Stock concentration (µg/L)	Volume taken (mL)	Solvent	Final volume (mL)	Concentration (µg/L)
100	0.02	Methanol: water (20:80 v/v)	10	0.2
100	0.02		10	0.2
100	0.02		10	0.2

PROCEDURES

All procedures will be carried out in compliance with departmental SOPs, following departmental safety procedures in conjunction with COSHH assessments.

All work should be carried out under the minimum control categories listed under the safety precautions section. Additional controls are listed with the individual steps of the procedure.

Fortification of control samples for method validation [1b, 4a]
Measure 8 mL surface water into a disposable glass vial. Fortify samples with mixed standard in methanol as shown in the following table:

Fountains Abbey surface water:

Number of replicates	Sample type	Stock concentration (µg/L)	Volume added (mL)	Sample volume (mL)	Fortified concentration (µg/L)
1	Reagent blank	N/A	N/A	N/A	N/A
2	Control	N/A	N/A	8	N/A
5	LOQ	10	0.08	8	0.1
5	10 × LOQ	10	0.8	8	1

Sample extraction [1b, 4a]

1. Add 2 mL methanol (corrected for the volume of fortification stock already added) to the sample and mix well.
2. Dilute into calibration range, if required with methanol: surface water (20:80 v/v).
3. Transfer into an HPLC vial for analysis.

Recommended dilution procedure is given in the following table:

Sample type	Fortified Concentration (µg/L)	Sample volume (mL)	Extract volume (mL)	Dilution (mL-mL)	Dilution factor
Reagent blank[1]	N/A	N/A	N/A	N/A	1.25
Control	N/A	8	10	N/A	1.25
LOQ	0.1	8	10	N/A	1.25
10 × LOQ	1	8	10	0.25-1	5

[1] Reagent blank is methanol: surface water (20:80 v/v).

LC-MS/MS CONDITIONS

HPLC Parameters:

Column#	XBridge BEH C18 2.5 µm 2.1 × 50 mm		
Mobile Phase A#	0.1% Formic acid in water		
Mobile Phase B#	0.1% Formic acid in acetonitrile		
Flow Rate	0.4 mL/min		
Gradient	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	90	10
	1.0	90	10
	1.25	50	50
	2.5	0	100
	3.5	0	100
	3.6	90	10
	5.0	90	10
Run Time	5.0 minutes		
Column Temperature	35°C		
Autosampler Temperature	15°C		
Injection Volume	25 µL		
Retention Time	Approx. 1.9 minutes (PDMU) Approx. 2.0 minutes (cPMU)		
Valco Valve Diverter	Time (min)	Position	
	0	A (to waste)	
	1	B (to MS)	
	4	A (to waste)	

MS/MS Parameters:

Instrument	AB Sciex API 5000 Triple Quadrupole Mass Spectrometer			
Ionisation Type#	Electrospray (ESI)			
Polarity#	Positive			
Scan Type#	Multiple reaction monitoring (MRM)			
Ion Spray Voltage	5500 V			
Collision Gas (CAD)	5			
Curtain Gas (CUR)	25			
Gas Flow 1 (GS1)	40			
Gas Flow 2 (GS2)	40			
Vaporiser Temperature (TEM)	450°C			
Interface Heater (ihe)	On			
Entrance Potential (EP)	10			
Collision Exit Potential (CXP)	13			
Compound Name	MRM Transition Ions Monitored	Declustering Potential (DP)	Collision Energy (CE)	Dwell Time (ms)
PDMU (Primary)	165.0/72.0	31	23	65
PDMU (Confirmatory)	165.0/46.1	31	23	65
cPMU (Primary)	185.2/93.0	61	37	65
cPMU (Confirmatory)	185.2/128.0	61	21	65

Parameters marked # may not be modified. Minor adjustments to the remaining parameters may be required in order to fully optimise the system.

CALCULATION OF RESULTS

All peak measurements and calculations are performed on Analyst 1.6.2. From the measured peak area, where the calibration fit is linear as in this study, Analyst uses the following formula to calculate the concentration of test substance present in the sample extract.

$$x = \frac{(y - c)}{m} \times DF$$

Where:-

x = concentration of test substance in sample (µg/L)
y = area of peak due to test substance
m = gradient
c = Y intercept on calibration graph
DF = sample dilution factor

Procedural recovery data from fortified samples are calculated via the following equation:

$$\text{Recovery}(\%) = \frac{A}{S} \times 100$$

Where:-

A = concentration found in fortified sample (µg/L)
S = concentration added to fortified sample (µg/L)

METHOD CRITERIA

For the analysis by LC-MS/MS to be considered successful the following criteria should be met.

- At least 5 calibration standards will be used in the determination of the calibration line.
- The correlation coefficient (r) for the calibration line will be ≥ 0.995 with a 1/x weighting.
- All sample extracts will be within the appropriate range of calibration standards.
- Mean recovery from fortified samples will be considered acceptable within the range of 70 to 120%.
- The control sample should not contain interference > 30% of the LOQ.

GENERAL HANDLING CONTROL CATEGORIES

CATEGORY Main Division	CONTROL Name and Specification
1	GLOVES
a	Disposable latex
b	Disposable nitrile
c	Rubber gloves
d	Specific type for the job (see assessment giving details)
2	PROTECTIVE CLOTHING
a	Laboratory coat or equivalent
b	Disposable overalls
c	Oversleeves
d	Overshoes
e	Plastic apron
3	EYE/FACE PROTECTION
a	Safety glasses to BS 2092/2 C or better
b	Face shield to BS 2092/2 C or better
c	Safety goggles to BS 2092/2 C or better
4	ENGINEERING CONTROLS
a	Open bench in ventilated area
b	Fume cupboard to BS 7258
c	Laminar flow cabinet to BS 5295 Class 1
d	Re-circulating fume chamber
e	Radioisotope lab
f	Biohazard lab
g	Glove box
5	RESPIRATORY PROTECTIVE EQUIPMENT
a	Disposable filtering facemask (HSE approved), i - organic vapour ii - dust iii – combination organic vapour/dust MUST SPECIFY TYPE
b	Powered respirators/helmets with safety visor to BS 2092/2 C or better (HSE approved)
c	Respirator with specified canister (HSE approved)
6	SPECIFIC IMMUNISATION REQUIRED (GIVE DETAILS)
7	ALLERGIC PERSONS PROHIBITED (SPECIFY ALLERGY)
8	REFER TO MATERIAL SAFETY DATA SHEET
9	KNOWN OR SUSPECTED REPRODUCTIVE HAZARD TO EITHER SEX (must specify details)
10	POISON – ensure antidote is available and is within its expiry date (must specify details)

Appendix 7
Deviations

The study protocol incorrectly stated that the required calibration range was ≤ 30% of the LOQ to ≥ 120% of the 10 × LOQ sample concentration. The correct range according to the SANCO 3029/99 guideline is ≤ 80% of the LOQ to ≥ 120% of the 10 × LOQ sample concentration. The calibration range required by the guideline was met, even with the exclusion of the lowest calibration point for cPMU. Therefore this protocol deviation had no impact on the integrity of the study.