ANALYTICAL METHOD FOR THE DETERMINATION OF DIURON, LINURON, AND METABOLITES IN WATER

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1.0 ABSTRACT

An analytical method was developed for the determination of diuron, linuron, and metabolites DCPMU [3,4- N'-(3,4-dichlorophenyl)-N'-methylurea], DCPU [3,4-dichlorophenyl urea], mCPDMU [3-(3-chlorophenyl)-1,1-dimethylurea] and desmethylated linuron [N'-(3,4-dichlorophenyl)-N'-methoxyurea] in water. The target limit of quantitation (LOQ) for each analyte was 0.05 µg/L. The method was validated at 0.05 µg/L and 0.5 µg/L for all analytes. The extracts were analyzed using a LC/MS/MS system with an electrospray interface (ESI) operating in positive ion mode. The method was validated in two typical water sources (drinking equivalent to ground water and surface water).

Analytical Method

The water sample was filtered through paper and adjusted to 1% methanol . The sample was transferred onto a solid phase extraction cartridge (C18). The cartridge was rinsed with de-ionzed water. The analytes were eluted using methanol/de-ionized water (8+2, v+v). The eluate was concentrated by evaporation. The final extract was filtered prior to analysis by reverse phase HPLC online with ESI-LC/MS/MS for the determination of diuron, linuron, DCPMU, DCPU, mCPDMU, and desmethylated linuron.

The samples were extracted, cleaned-up using solid phase extraction, and typically analyzed overnight by LC/MS/MS. The time required for extraction, evaporation, and filtration of the final extracts prior to LC/MS/MS analysis was approximately 6 hours. A LC/MS/MS analytical run containing 7 standards and 13 samples took approximately 10 hours. The LC/MS/MS analysis was conducted unattended and could be run overnight.

Two specific MS/MS transitions of the molecular ion for each analyte were monitored. The total ion chromatogram (TIC) was used for quantitation and the relative responses of the two fragment ions for each analyte provided confirmatory analysis. Matrix interference was not observed in any of the water specimens examined.

2.0 INTRODUCTION

Diuron (DPX-14740) and linuron (DPX-Z0326) are active ingredients in phenylurea herbicides used to control broadleaf weeds and annual grasses in various field crops, fruit and nut crops, and noncrop areas. This method was developed to support the U.S. and EU re-registration and country specific registration effort for products containing linuron and diuron active ingredients. The method satisfies requirements in European Commission, Directorate General Health and Consumer Protection, "Guidance Document on Residue Analytical Methods", SANCO/825/00 rev. 7, March 17, 2004, and is intended as a regulatory method for the determination of residues in water matrices. DCPMU and DCPU are metabolites of linuron and diuron. The analyte mCPDMU is an anaerobic metabolite of diuron, and desmethylated linuron is a metabolite of linuron.

Water matrix subsamples are extracted using solid-phase extraction (SPE). The extract is concentrated by evaporation. The remaining extract is adjusted to final volume with methanol and filtered prior to the quantitative determination of linuron, diuron, DCPMU, DCPU, mCPDMU and desmethylated linuron by LC/MS/MS analysis. The analytical method was validated at the target method limit of quantitation (LOQ) of 0.05 µg/L and 0.5 µg/L (10×LOQ) for all analytes. The method limit of detection (LOD), based on the least responsive analyte, DCPU, was estimated to be 0.01 µg/L. Confirmatory analysis in this method is possible using relative ratio responses of two molecular ion fragments monitored for each of the analytes.

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified; note any specifications in the following descriptions before making substitutions. Substitutions should only be made if equivalency/suitability has been verified with acceptable control and fortification recovery data.

3.1 Equipment

Analytical balance	Model BP 210 D, serial No 50707526 D95-09-011 – Sartorius
Water jet pump with Woulfe bottle	
Ultra pure water unit	Model Elix 3 UV and Milli-Q 185 Plus – Millipore
Vortex	Model Reax – Heidolph
Nitrogen evaporator	Model 112 and 111 – Organomation
Micro pipette	Model Microman, various sizes – Abimed Analysen-Technik
Centrifuge tube	Graduated glass tube with ground joint NS 14.5 , different sizes

Volumetric pipette	different sizes, quality "AS" or equivalent – Hirschmann or Brand
Pasteur pipette	different sizes
Volumetric flask	different sizes
Erlenmeyer flask	different sizes
Glass bottle	different sizes
Funnel	different sizes
Graduated cylinder	different sizes
Sample vial	1 mL with PTFE sealed crimp-on caps – CS-Chromatographie Service AG
Sample vial	40 mL
SPE manifold	Supelco
Reservoir	30 mL
Adapter	
Single use syringe	different sizes
Membrane filter	0.2 µm PTFE, Macherey-Nagel

HPLC

Unit	**Producer**	**Model**	**Serial No.**
Degasser	Agilent	G1322A	JP05033668
Pump	Agilent	G1311A	DE14918680
Injection system	CTC Analytics	HTS Pal	111374
Column oven with 6-portvalve	Agilent	G1316A	DE14927302
Control module	Agilent	G1323B	DE14903149

MS/MS

Unit	**Producer**	**Model**	**Serial No.**
LC-MS/MS	Applied Biosystems	API 3000	D10340204
Vacuum pump	Varian	DS602	205612
Data system	Applied Biosystems	Analyst, Version 1.4.1	

3.2 Reagents and Standards

3.2.1 Reagents

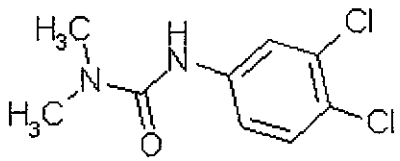
The equivalency/suitability of substituted reagents should be verified.

Methanol for HPLC	Part No. T169.1 – Roth
Deionized water	Produced by Elix 3UV/Milli-Q 185 Plus – Millipore
Formic acid	Part No. 1.00264 – Merck
Supelchem LC-18	0.5 g, 3 mL 57012 – Supelco
Folded filter paper, 185 mm diameter	Part No. 10311647 – Schleicher and Schuell

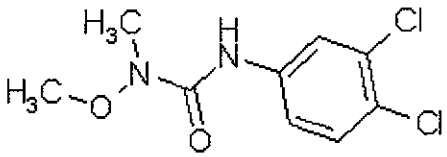
3.2.2 Reference Analytical Standards

The structures and specific information for diuron, linuron, DCPMU, DCPU, mCPDMU and desmethylated linuron are detailed below:

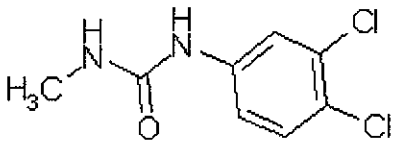
DPX-14740, Herbicide

Common Name	Diuron
C.A. Name:	N'-(3,4-dichlorophenyl)-N,N-dimethylurea
IUPAC Name:	3-(3,4-dichlorophenyl)-1,1-dimethylurea
CAS No.:	330-54-1
Structure:	
Lot (Dash):	235
DuPont Stock No.:	1002061
Appearance:	Solid powder
Date of Last Analysis:	23 April 2004
Purity:	98.7%
Storage Advice:	Room temperature at SGS INSTITUT FRESENIUS
Expiry Date:	23 April 2007

DPX-Z0326, Herbicide

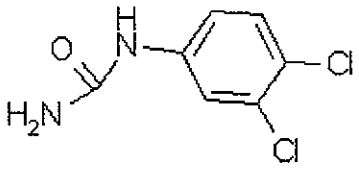
Common Name	Linuron
C.A. Name:	N'-(3,4-dichlorophenyl)-N-methoxy-N-methylurea
IUPAC Name:	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea
CAS No.:	330-55-2
Structure:	
Lot:	236
DuPont Stock No.:	4004110
Appearance:	Solid
Date of Last Analysis:	18 Jan 2006
Purity:	99.9%
Storage Advice:	Room temperature at SGS INSTITUT FRESENIUS
Expiry Date:	18 Jan 2009

DCPMU (IN-15654)

Common Name	3,4-dichlorophenylmethylurea
C.A. Name:	N'-(3,4-dichlorophenyl)-N'-methylurea
IUPAC Name:	3-(3,4-dichlorophenyl)-1-methylurea
CAS No.:	3567-62-2
Structure:	
Lot:	012
DuPont Stock No.:	1000022
Appearance:	White powder
Date of Last Analysis:	25 July 2003
Purity:	99.9%
Storage Advice:	Room temperature at SGS INSTITUT FRESENIUS
Expiry Date:	24 July 2009

DCPU (IN-R0915)

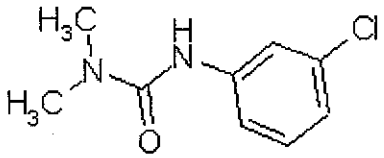
Common Name	3,4-dichlorophenylurea
C.A. Name:	3,4-dichlorophenyl urea
IUPAC Name:	N-(3,4-dichlorophenyl)urea
CAS No.:	2327-02-8
Structure:	



Lot (Dash):	008
DuPont Stock No.:	1001368
Appearance:	Solid powder
Date of Last Analysis:	22 October 2004
Purity:	99.9%
Storage Advice:	Room temperature at SGS INSTITUT FRESENIUS
Expiry Date:	22 October 2010

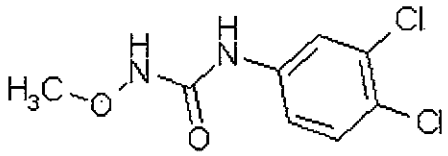
mCPDMU (IN-12894)

Common Name	chlorophenyldimethylurea
C.A. Name:	N'-(3-chlorophenyl)-N,N-dimethyl urea
IUPAC Name:	3-(3-chlorophenyl)-1,1-dimethylurea
CAS No.:	587-34-8
Structure:	



Lot (Dash):	004
Stock No.:	1001187
Appearance:	Solid powder
Date of Last Analysis:	24 February 2004
Purity:	99.1%
Storage Advice:	Room temperature at SGS INSTITUT FRESENIUS
Expiry Date:	24 February 2007

desmethylated-linuron (IN-Z0513)

Common Name	3,4-dichlorophenylmethoxyurea
C.A. Name:	N'-(3,4-dichlorophenyl)-N'-methoxyurea
IUPAC Name:	1-(3,4-dichlorophenyl)-3-methoxyurea
CAS No.:	17356-61-5
Structure:	
Lot (Dash):	001
DuPont Stock No.:	1000759
Appearance:	Solid
Date of Last Analysis:	08 September 2004
Purity:	99.8%
Storage Advice:	Room temperature at SGS INSTITUT FRESENIUS
Expiry Date:	08 September 2010

3.3 Safety and Health

Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment should be used.

4.0 METHODS

4.1 Principle of the Analytical Method

This method was developed for the determination of diuron, linuron, DCPMU, DCPU, mCPDMU and desmethylated linuron in water matrices to support regulatory studies. The method was validated in drinking and surface water for all analytes at a target LOQ of 0.05 µg/L and 0.5 µg/L (10×LOQ).

A 0.2 mL aliquot of methanol was added to 20 mL of paper filtered water sample. A solid phase extraction cartridge (reversed phase, C-18) was conditioned with 5 mL of methanol and 2×5 mL of de-ionized water (the cartridge was not allowed to go to dryness). The sample was eluted through the cartridge. The cartridge was rinsed twice with de-ionzed water (2 mL each) and dried using vacuum. A 0.5 mL aliquot of methanol/de-ionized water (8+2, v+v) was added to the cartridge and incubated on column for 2 minutes without applied vacuum. The analytes were eluted using 2 mL of methanol/de-ionized water (8+2, v+v) into a graduated centrifuge tube. The eluate was concentrated by evaporation using a gentle stream of nitrogen (water bath temperature 40°C) to a volume below 1 mL. The extract was diluted with methanol to a final volume of 1 mL and subsequently filtered (0.2 µm) prior to analysis by

reverse phase HPLC online with ESI-LC/MS/MS for the determination of linuron, diuron, DCPMU, DCPU, mCPDMU, and desmethylated linuron. Quantitative analysis is accomplished using the total ion chromatogram (TIC) from two molecular ion transitions for each analyte. The relative abundance of the MS/MS fragment ions provides confirmatory evidence for each analyte.

4.2 Analytical Procedure

4.2.1 Glassware & Equipment Cleaning Procedures

The effectiveness of any cleaning procedure used should be demonstrated by preparation and analysis of reagent blanks. In general, all reusable glassware and plasticware should be washed in hot tap water with laboratory grade detergent, rinsed several times with tap water, rinsed several times with deionized water, rinsed once with acetone, and allowed to fully dry before use. Care should be taken to avoid working with high levels of the analyte in the same laboratory where samples are being extracted and analyzed.

4.2.2 Preparation & Stability of Reagent Solutions

Aqueous 0.2% formic acid solution
Add 2 mL of formic acid to 998 mL of de-ionized water and mix.

Solution for SPE elution (methanol/de-ionized water)
Add 80 mL of methanol to 20 mL of de-ionized water and mix.

4.2.3 Stock Standard Preparation and Stability

If possible, use standards with purity greater than 95%. A minimum of approximately 10 mg of standard should be weighed on an analytical balance that provides a weight precision to three significant figures, or the amount of standard should be increased to satisfy this condition.

Individual stock solutions for diuron, linuron, DCPMU, DCPU, mCPDMU, and desmethylated linuron are prepared at a target concentration of 1 mg/mL in methanol. For example, 1 mg/mL stock standards can be prepared by weighing approximately 10 mg (adjusted for purity) of analyte in a tared 10-mL volumetric flask. The volumetric flasks are diluted to volume in methanol, capped, and well-shaken to prepare stock solutions. These solutions are stored at or below 4°C and are stable for at least six months. Stock standards use may be extended if supported by stability test data.

A 1 mL aliquot of each stock solution was transferred into a 10 mL volumetric flask. The flask was diluted to 10 mL with methanol. The concentration for each analyte was approximately 100 µg/mL in the standard mixture solution (SM).

4.2.4 *Fortification Standard Preparation and Stability*

Fortification solutions are prepared from dilutions of the 100 µg/mL standard mixture solution (SM).

0.1 µg/mL Fortification Solution
Dilute the 100 µg/mL SM with methanol/de-ionized water/0.2% formic acid (30+70+0.2, v+v+v) to a concentration of approximately 0.1 µg/mL for each analyte.

0.02 µg/mL Fortification Solution
Dilute the 100 µg/mL SM with methanol/de-ionized water/0.2% formic acid (30+70+0.2, v+v+v) to a concentration of approximately 0.02 µg/mL for each analyte.

Fortification Standards use may be extended if supported by stability test data.

4.2.5 *Calibration Standard Preparation and Stability*

Calibration standards are prepared from dilutions of 100 µg/mL standard mixture solution (SM). A minimum of five calibration standards over a range from ≤30% of LOQ equivalent final concentration to ≥120% of the highest expected final sample concentration analyzed are required for quantification. Seven or more calibration standards are recommended.

For example, calibration standard of 15 ng/mL can be prepared from the 0.1 µg/mL (100 ng/mL) fortification solutions, by diluting a 3 mL aliquot to final volume of 20.0 mL in methanol/de-ionized water/0.2% formic acid (30+70+0.2, v+v+v). This standard is further diluted in methanol/de-ionized water/0.2% formic acid (30+70+0.2, v+v+v) using pipettes according to the following table to prepare 0.3, 0.5, 1.0, 2.5, 5.0 and 10.0 ng/mL calibration solutions.

Code of standard	Aliquot (mL)	Dilute from	Final volume (mL)	Analyte (ng/mL)
Mix E	-	-	-	100
Mix F	3	Mix E	20	15
Mix G	2.5	Mix E	25	10
Mix H	1	Mix E	20	5
Mix K	5	Mix G	20	2.5
Mix L	2	Mix G	20	1
Mix M	1	Mix G	20	0.5
Mix N	3	Mix L	10	0.3

Calibration standards can be prepared concurrently with sample fortifications using this procedure.

4.2.6 *Source (& Characterization) of Samples*

The drinking water sample (tap water generated from ground water) was taken at SGS INSITUT FRESENIUS in D-65232 Taunusstein.

The surface water sample was taken from a ditch in D-65549 Limburg.

The samples were natural water specimens (drinking and surface water). The surface water specimen was transported in polyethylene bottles. During sampling the air temperature was about 20°C. The specimen arrived in good condition on June 07, 2006 and were stored until analysis at -18°C. The drinking and surface samples were given individual internal specimen identification. The surface water specimens were characterised by pH-value, conductivity, total organic carbon. The measurement of the conductivity is based on the DIN EN 27888*. The determination of the TOC is based on the DIN EN 1484†. The chemicals and the equipment used for the characterisation are specified in the raw data of the study.

Surface water

Specimen code:	010/6202452
Name of the river:	Großbach
Location:	D-65549 Limburg (Hesse)
pH-value:	8.03
Conductivity:	759 µs/cm
Total organic carbon:	5.4 mg/L

Drinking water

Specimen code:	010/6202451
Location:	D-65232 Taunusstein. (Hesse)
pH-value:	7.75
Conductivity:	503 µs/cm
Total organic carbon:	2.3 mg/L

4.2.7 *Sample Fortification Procedure*

Fortify aliquots (20 mL) of the water samples as required. The following table is provided as an example for fortification levels used in this study.

FORTIFICATION LEVEL (µG/L)	CONCENTRATION OF FORTIFICATION SOLUTIONS (µG/ML)	FORTIFICATION SOLUTION ALIQUOT (ML)
0.05	0.02	0.05
0.50	0.10	0.10

Shake the samples after fortification.

* Determination of the electrical conductivity (ISO 7888:1985) DIN EN 27888:1993

† Determination of total organic carbon (TOC) and the dissolved organic carbon (DOC) DIN EN 1484: 1997

4.2.8 *Analyte Extraction Procedure*

1. Filter the water sample through a folded filter paper, if necessary.
2. Connect solid-phase cartridge (SPE) to vacuum manifold (water jet pump with Woulfe bottle). Rinse the cartridge subsequently with 5 mL of methanol and twice with 5 mL of de-ionized water, each. Do not allow cartridge to go to dryness. Mount a reservoir on top of the SPE cartridge.
3. Fortify aliquots (20 mL) of the control samples as required. Add 0.2 mL of methanol to the sample and mix.
4. Transfer the sample onto the SPE cartridge and elute the sample through the cartridge using vacuum. Rinse the cartridge using 2x2 mL of de-ionized water. Continue vacuum until cartridge is completely dry (approximately 10 minutes).
5. Break vacuum and transfer 0.5 mL of methanol/de-ionized water (80+20, v+v) onto the cartridge and incubate for 2 minutes with only gravity elution. Elute the analytes with 2 mL of methanol/de-ionized water (80+20, v+v).
6. Evaporate the eluate to less than 1 mL by means of a gentle stream of nitrogen. Dilute the extract with methanol to a final volume of 1 mL. Dilute the extracts of the 10-fold LOQ fortification to a calculated final volume of 10 mL. Filter (0.2 µm PTFE) an aliquot for LC/MS/MS analysis.

4.3 ***Instrumentation***

The following analytical conditions were used.

HPLC-Conditions

Unit	**Producer**	**Model**	**Serial No.**
Degasser	Agilent	G1322A	JP05033668
Pump	Agilent	G1311A	DE14918680
Injection system	CTC Analytics	HTS Pal	111374
Column oven with 6-portvalve	Agilent	G1316A	DE14927302
Control module	Agilent	G1323B	DE14903149

Column	**Analytical Column**
Length×ID [mm×mm]	150×3.0
Sorbent	HYPURITY C8
Particle size [µm]	5.0
Producer/supplier	Thermo
internal IF-no.	225

Eluent (channel) A	-
Eluent (channel) B	-
Eluent (channel) C	Methanol
Eluent (channel) D	0.2% formic acid

Gradient Program

Time [min]	Flow [mL/min]	Eluent C [%]	Eluent D [%]
0.00	0.5	30	70
10.00	0.5	90	10
15.00	0.5	99	1
15.10	0.5	99	1
17.10	0.5	30	70
23.00	0.5	30	70

Oven temperature [°C]	40	Switching intervals	
Injection volume [µL]	20	to waste [min]	from 0 to 5
Temperature of sample thermostat [°C]	10	to LC-MS/MS [min]	from 5 to 11
Post-column Split (detector/waste)	1:1	to waste [min]	from 11 to 23

MS/MS-Conditions

Unit	Producer	Model	Serial No.
LC-MS/MS	Applied Biosystems	API 3000	D10340204
Vacuum pump	Varian	DS602	205612
Data system	Applied Biosystems	Analyst, Version 1.4.1	

Turbo Ion Spray	Position	Resolution
X-axis	5	Q1
Y-axiy	-1	Q3
Nebulizer (gas)	10	
Curtain gas, nitrogen	9	
Collision gas, nitrogen	7	
Auxiliary (gas 2),	5500	

Scanning Method

Period	1	1	1	1
Experiment	1	1	1	1
Retention window [min]	0-23	0-23	0-23	0-23
Ion spray voltage [V]	5500	5500	5500	5500
Temperature [°C]	350	350	350	350
Analyte	Diuron	Diuron	Linuron	Linuron
Ionisation mode	ES+	ES+	ES+	ES+
Scan type	MRM	MRM	MRM	MRM
Q1 Mass [amu]	233	233	249	249
Q3 Mass [amu]	72	46	160	182
Declustering potential [V]	35	35	30	30
Focusing potential [V]	140	140	110	110
Collision energy [V]	37	33	33	19
Collision cell exit pot [V]	2	10	10	12
Entrance potential [V]	10	10	10	10
Ion energy 1 [V]	1.000	1.000	1.000	1.000
Ion energy 3 [V]	0.400	0.400	0.400	0.400
Dwell [msec]	100	100	100	100
Deflector [V]	-220	-220	-220	-220
Channel electron multiplier [V]	2300	2300	2300	2300

Scanning Method

Period	1	1	1	1
Experiment	1	1	1	1
Retention Window [min]	0-23	0-23	0-23	0-23
Ion spray voltage [V]	5500	5500	5500	5500
Temperature [°C]	350	350	350	350
Analyte	DCPMU	DCPMU	DCPU	DCPU
Ionisation mode	ES+	ES+	ES+	ES+
Scan type	MRM	MRM	MRM	MRM
Q1 Mass [amu]	219	219	205	205
Q3 Mass [amu]	127	162	127	162
Declustering potential [V]	30	30	30	30
Focusing potential [V]	140	140	120	120
Collision energy [V]	37	23	35	21
Collision cell exit pot [V]	8	10	10	10
Entrance potential [V]	10	10	10	10
Ion energy 1 [V]	1.000	1.000	1.000	1.000
Ion energy 3 [V]	0.400	0.400	0.400	0.400
Dwell [msec]	100	100	100	100
Deflector [V]	-220	-220	-220	-220
Channel electron multiplier [V]	2300	2300	2300	2300

Period	1	1	1	1
Experiment	1	1	1	1
Retention window [min]	0-23	0-23	0-23	0-23
Ion spray voltage [V]	5500	5500	5500	5500
Temperature [°C]	350	350	350	350
Analyte	Des.-linuron	Des.-linuron	mCPDMU	mCPDMU
Ionisation mode	ES+	ES+	ES+	ES+
Scan type	MRM	MRM	MRM	MRM
Q1 Mass [amu]	235	235	199	199
Q3 Mass [amu]	160	161	72	46
Declustering potential [V]	30	30	35	35
Focusing potential [V]	120	120	130	130
Collision energy [V]	25	27	35	29
Collision cell exit pot [V]	10	8	2	6
Entrance potential [V]	10	10	10	10
Ion energy 1 [V]	1.000	1.000	1.000	1.000
Ion energy 3 [V]	0.400	0.400	0.400	0.400
Dwell [msec]	100	100	100	100
Deflector [V]	-220	-220	-220	-220
Channel electron multiplier [V]	2300	2300	2300	2300

Approximate Analyte Retention Times:

diuron =	8.7 min
linuron =	9.4 min
DCPMU =	8.6 min
DCPU =	8.2 min
mCPDMU =	6.9 min
Desm. linuron =	8.7 min

4.3.1 *Calibration Procedures*

Use standard mass spectrometer tuning and calibration techniques. If confidence in the mass calibration needs to be established (modern mass spectrometers under digital control generally do not need frequent mass calibration, especially for quantitative modes), use vendor recommended calibrating solution. Optimization tuning of MS system may be accomplished by infusion of one or more of the test analytes. This method uses external standards, prepared as described in Section *4.2.5*.

Linear regression without weighting was used. The linear regression response of external calibration standards using Excel® functions SLOPE, INTERCEPT, and RSQ were monitored to establish calibration curve linearity. Acceptance criteria for valid quantitation was: R value (correlation coefficient) >0.99.

The instrument calibrated range was 0.3 ng/mL (0.3 × LOQ of 0.010 µg/L equivalent final concentration) to 15.0 ng/mL with 20 µL injection volume. Generally, seven calibration solutions were analyzed for quantitative LC/MS/MS analysis (a minimum of five calibration solutions are required).

Net recoveries may be calculated for fortified samples only (not acceptable for field samples). Net recoveries may be calculated and reported only when residues in the control sample are integrable and <30% of the LOQ. When the control residues are >30% of the LOQ, the recovery samples prepared at the LOQ using that control are invalidated. When the control residues are <30% of the LOQ, corrected µg/L found in fortified samples are calculated by subtracting area counts found in the control from area counts found in fortified samples. If net recoveries are calculated, those results must be uniquely identified or presented in a separate spreadsheet column heading for corrected µg/L.

4.3.2 *Sample Analysis*

Preliminary runs of at least two calibration standards or control sample extracts are routinely made to insure the LC/MS/MS system is equilibrated. If multiple sets are analyzed, a solvent blank injection should be made between the last and first injections of the sets to minimize risk of carryover between sets. Calibration standard analyses should precede the first sample analysis and follow the last sample analysis so sample analyses are contained within the external standard calibration. Generally, the injection sequence was organized from lowest to highest expected analyte concentrations. Calibration standard runs were intermixed with the test samples and

should be analyzed before and after every 3–4 samples in each analytical set. Extracts and calibration standards should be refrigerated if stored. Generally, fortification sample recoveries (70-110%) are required for acceptable quantitation results in a analysis set.

4.4 Calculations

4.4.1 Methods

Diuron, linuron, DCPMU, DCPU, mCPDMU and desmethylated linuron residues were measured as µg/L in water.

The detector signals were registered and integrated using the data systems outlined in section MS/MS conditions. The peak area was taken into account to determine the analyte amounts in the specimens. The calibration curves were calculated from the peak area of the calibration solutions and the corresponding amount of the analyte injected using equation (1).

(1) $y = a + bx$

where:

- y: peak area [integration units iu/sec]
- x: amount of the analyte injected [ng]
- a: ordinate intercept [iu]
- b: slope [iu/ng]

The amount injected (ng) of the analyte in the specimen was calculated using the transformed equation (1):

(2) $x = \frac{y - a}{b}$

The concentration C of the analyte in the specimen was calculated from x using equation (3):

(3) $C = \frac{x \cdot V_E \cdot}{V_i \cdot W} \times \frac{1\mu g}{1000 ng} \times \frac{10^6 \mu L}{L}$

where:

- x: amount of the analyte injected (ng)
- C: analysed concentration of the analyte in the specimen (µg/L)
- V_E: final volume (1 or 10 mL)
- V_i: injection volume (20 µL)
- W: specimen volume (20 mL)

The recovery data was calculated according to equation (4):

$$(4)\ R = \frac{C \cdot 100}{C_{for}}$$

where:

R: recovery [%]

C: analysed concentration of the analyte in the fortified specimen (µg/L)

C_{for}: nominal concentration of the analyte in the fortified specimen (µg/L)

An example calculation is shown below for diuron using water sample 010/6202451-A extracted and analyzed on June 23, 2006. This sample was fortified with 0.050 µg/L of diuron. The peak area of the diuron peak was 24087 integration units iu/sec.

Calibration:

(1) $y = 1100 + 1188649.98x$

The amount of the analyte in the specimen injected was calculated using the transformed equation (1):

$$(2)\ \frac{24087 - 1110}{1188649.98} = 0.0193\,\text{ng}$$

The concentration C of the analyte in the specimen was calculated from x using equation (3):

$$(3)\ C = \frac{0.0193 \cdot 1}{20 \cdot 20} \times 1000 = 0.048 \mu g / L$$

where:

x: amount of the analyte injected (0.0193 ng)

C: analysed concentration of the analyte in the specimen (0.048 µg/L)

V_E: final volume (1 mL)

V_i: injection volume (20 µL)

W: specimen volume (20.03 mL)

The recovery data was calculated according to equation (4):

(4) $$R = \frac{0.048 \cdot 100}{0.05017} = 96\ \%$$

where:

R: recovery [%]

C: analysed concentration of the analyte in the fortified specimen (0.048 µg/L)

C_{for}: nominal concentration of the analyte in the fortified specimen (0.05017 µg/L)

The recovery values reported represent rounded results which were obtained from calculations based on the exact raw data. The detector signals of the control specimens (if any) were subtracted from the fortified specimens.

5.2 Timing

Generally, samples were extracted using SPE and subsequently analyzed by LC/MS/MS. The extraction and clean-up can process up to 24 samples in series within 4 hours. The time required for dilution, evaporation, and filtration of the final extracts prior to LC/MS/MS analysis was approximately 3 hours. The sample to sample LC/MS/MS analysis time was 23 minutes.

5.3 Modifications or Special Precautions

Due to the broad range of residues fortified and potentially found in treated samples (0.05 to 0.5 µg/L), special precautions should be taken to insure reusable labware is clean, test materials are handled to minimize cross-contamination, and instrument (LC-MS/MS) are maintained to avoid carryover or contamination that can affect method performance.