INDEPENDENT LABORATORY VALIDATION OF THE ANALYTICAL METHOD DUPONT-16919, "ANALYTICAL METHOD FOR THE DETERMINATION OF LINURON, DIURON, AND RELEVANT METABOLITES IN SOILS USING LC/MS/MS"

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SUMMARY

The objective of this study was to conduct an Independent Laboratory Validation of the Analytical Method, DuPont-16919, for linuron, diuron, N'-(3,4-dichlorophenyl)-N'-methylurea (DCPMU), and 3,4-dichlorophenyl urea (DCPU) in soil. Linuron (DPX-Z0326) and diuron (DPX-14740) are active ingredients in DuPont phenylurea herbicides used to control broadleaf weeds and annual grasses in various field crops, fruit and nut crops, and noncrop areas. DCPMU and DCPU are significant soil metabolites of linuron and diuron, and may be monitored to follow the dissipation of either herbicide in field dissipation studies. The analytical method DuPont-16919 was developed to support the U.S. and EU re-registration and country specific registration effort for DuPont products containing linuron and diuron active ingredients. The method satisfies requirements in European Commission, Directorate General Health and Consumer Protection, and the U.S. EPA OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods (Draft, April, 1996), and is intended as a regulatory method for the determination of residues in soil matrices.

The first trial of Exygen Project P0002038 for the extraction and analysis of linuron, diuron, DCPU, and DCPMU from soil was initially performed using an API 3000 LC/MS/MS, which was an acceptable substitute in the original method.

The first soil validation set consisted of a set of seven standard concentration levels, one unfortified control sample, and one control sample post-fortified at the Limit of Quantitation (LOQ, 0.010 mg/kg). This set was run to establish linearity, instrument sensitivity, and matrix effects.

The second soil validation set consisted of one sand blank, two unfortified control samples, five control samples fortified at the Limit of Quantitation (LOQ, 0.010 mg/kg), and five control samples fortified at 10 × Limit of Quantitation (0.10 mg/kg). The sample size was 5 g of control soil.

The first method trial for the analysis of linuron, diuron, DCPU, and DCPMU from soil was successful. However, the signal to noise ratio at the LOQ was not acceptable at approximately 10:1. The stored extracts were re-injected on a newer-generation LC/MS/MS system and a signal to noise ratio of >10:1 was obtained. The average recoveries for linuron, diuron, DCPU, and DCPMU were within the defined acceptable limits of 70-120%.

The samples were extracted using ASE (accelerated solvent extraction) overnight and analyzed initially the next day by LC/MS/MS. The ASE system can process up to 24 samples in series at a sample-to-sample rate of 27 minutes. The dilution, evaporation, and filtration of the final extracts prior to LC/MS/MS analysis were approximately 2-3 hours. A High-Performance Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) analytical run containing 7 standards and 13 samples took approximately 8 hours. The LC/MS/MS analysis was conducted unattended and could be run overnight.

INTRODUCTION

This report details the results of the confirmatory trial of Independent Laboratory Validation of Analytical Method, DuPont-16919. The study was carried out under GLP according to Study Protocol P0002038.

The first method trial for the analysis of linuron, diuron, DCPU, and DCPMU from soil was successful. However, the signal to noise ratio of the analytes at the LOQ was not acceptable at approximately 10:1. The stored extracts were re-injected on a newer-generation LC/MS/MS system and a signal to noise ratio of >10:1 was obtained. The average recoveries for linuron, diuron, DCPU, and DCPMU were within the defined acceptable limits of 70-120%. The validation set consisted of one sand blank, two unfortified control samples, five control samples fortified at the Limit of Quantitation (LOQ, 0.010 mg/kg), and five control samples fortified at 10 × Limit of Quantitation (0.10 mg/kg).

The study was initiated on February 22, 2006 when the Study Director signed the Protocol for Exygen project P0002038. The experimental start date was February 27, 2006 and the experimental termination date was April 26, 2006.

TEST SYSTEM

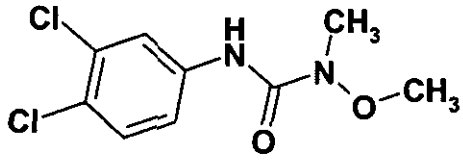
A control sample of soil was obtained from DuPont Crop Protection. The control soil was identified by Exygen as L0007386-0001, container number C0146920.

The control sample was placed in a freezer at a temperature of -20 ± 5°C for storage. The sample was removed from the freezer for extraction, and promptly returned to a freezer after use. Sample login and procurement information can be found in the raw data package associated with this study. Storage records will be kept at Exygen Research.

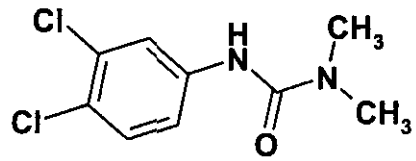
TEST AND REFERENCE MATERIALS

The test substances are linuron, diuron, DCPU, and DCPMU. They were supplied by DuPont. Characterization data for the test substances are maintained by DuPont Crop Protection. The Exygen ExyLIMS number, lot number, purity, expiration date, structure, and CAS registry number for the test substances are listed below.

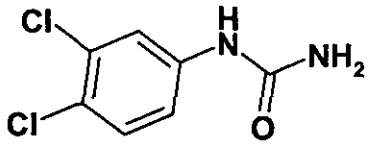
Name or Code:	DPX-Z0326, Linuron
ExyLIMS ID:	SP0007073
Lot:	236
Purity:	99.9%
Expiration Date:	01/18/09
Storage Condition:	Room Temperature
CAS No.:	330-55-2
Molecular Weight:	249.10



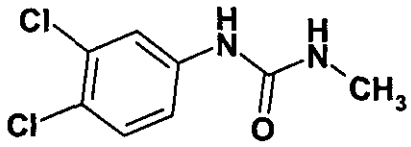
Name or Code:	DPX-14740, diuron
ExyLIMS ID:	SP0007072
Lot:	235
Purity:	98.7%
Expiration Date:	04/23/07
Storage Condition:	Room Temperature
CAS No.:	330-54-1
Molecular Weight:	233.10



Name or Code:	IN-R0915, DCPU
ExyLIMS ID:	SP0007075
Lot:	008
Purity:	99.0%
Expiration Date:	10/22/10
Storage Condition:	Room Temperature
CAS No.:	2327-02-8
Molecular Weight:	205.04



Name or Code:	IN-15654, DCPMU
ExyLIMS ID:	*SP0007074*
Lot:	012
Purity:	99.9%
Expiration Date:	07/24/09
Storage Condition:	Room Temperature
CAS No.:	3567-62-2
Molecular Weight:	219.07



Stock standard solutions were prepared on February 27, 2006. Fortification solutions were prepared on February 27, 2006 and April 6, 2006 by making appropriate dilutions to the stock standard solutions. Calibration solutions were prepared on February 27, 2006 and April 6, 2006 by making appropriate dilutions to the fortification solutions. All stock standard, fortification, and calibration solutions were stored in amber vials in a refrigerator at a temperature of 2-8°C when not in use. Documentation of standard preparation can be found in the raw data associated with this report.

DESCRIPTION OF ANALYTICAL METHOD

Method Information: DuPont-16919, "Analytical Method for the Determination of Linuron, Diuron, and Relevant Metabolites in Soils Using LC/MS/MS" (Reference 1).

Procedure

For each sample, install endcap + 10 µm steel frit in a 22 mL ASE extraction vessel. Insert 2 cellulose filters and press filters to bottom (a plunger from a 10 mL disposable syringe may be used). Place vessel on a toploading analytical balance and tare the balance. Weigh ~1 g of sand into vessel and re-tare the balance. Weigh 5.0 ± 0.05 g of soil into vessel. Record exact weight of test soil. Fortify control samples as required. Allow to stand in fume hood for at least 15 minutes. Add sand to fill each of the vessels and cap sample vessels. Extract samples on the ASE extraction system using the following conditions. Heat: 5 minutes, Static: 3 minutes, Flush%: 100, Purge: 60 seconds, Cycle 3 (times), Temp: 100°C, Pressure: 1000 psi. Extraction solvent: Methanol/0.38% formic acid-0.1% Triton X 100 (9/1, v/v). Remove vessels and glass collection tubes containing the extract from the ASE extractor. Transfer extract to 50 mL graduated cylinder (TC) and dilute to final volume of 50.0 mL with methanol rinses of the extract collection vial. Transfer 5.0 mL of final extract to a 15 mL polypropylene centrifuge tube. Add 1 mL water to the centrifuge tube and evaporate to ~1 mL in a gentle stream of nitrogen with sample heated to ~50°C. Add 3 mL of methanol and dilute to final volume of 10.0 mL using the gradation on the tube with aqueous 0.01M formic acid solution. Filter an aliquot for LC/MS/MS analysis.

Extracts were filtered using 0.2 µm AcroDisc PTFE 13 mm syringe filters and injected onto an Agilent HP1100 HPLC system with a Varian, Pursuit C8, 4.6 x 150 mm, 3-µm HPLC analytical column using a 0.01M formic acid (aq)/0.01M formic acid in methanol mobile phase system. Detection is on a PE Sciex API 3000 or API 5000 LC/MS/MS by positive electrospray in a multiple reaction monitoring (MRM) mode mass spectrometry.

The following reagents/materials/instrumentation were used in the study.

1. Methanol, EMD, HPLC Grade
2. Water, EMD, HPLC Grade
3. Non-ionic Surfactant, Triton X 100, EMD
4. Sand, Standard Ottawa, EMD
5. Formic Acid, EM Science, 90%
6. Analytical Balance, Mettler-Toledo, Inc. Model MX5
7. Top-loader Balance, Mettler-Toledo, Inc. Model PG2002
8. Organomation Associates, Inc N-EVAP 112
9. VWR Vortex Genie 2 Vortexer
10. AcroDisc 13-mm, 0.2-µm, PTFE Syringe Filters, PALL Life Sciences
11. 10-mL Disposable Syringes, BD
12. 15-mL Disposable Polypropylene Centrifuge Tubes, VWR
13. Rainin Digital Autopipettes, 10-100 µL and 100-1000 µL
14. Socorex 1-10 mL Autopipette with Wheaton Tips

15. Pyrex Graduated Cylinders (TC), 50-mL and 1-L
16. National Scientific Autosampler Vials, Amber
17. ASE 200 Extraction Apparatus, Dionex
18. 22-mL Stainless Steel Extraction Cells, Dionex
19. 60-mL Collection Vials with Septa and Cap, VWR
20. Cellulose Filters, Type D28, Dionex

The following are the analytical conditions that were employed for the procedure taken from ABC Labs trial run as described in DuPont-16919 Appendix 5 and initially used in this validation study.

Instrument:	PE Sciex API 3000 Triple Quadrapole LC/MS/MS Turbo Ion Spray Liquid Introduction Interface
Computer:	Intel Pentium 4
Software:	Analyst 1.4.1
HPLC Equipment:	Agilent Series HP1100 Quaternary Pump Vacuum Degasser WPALS Autosampler Column Compartment
HPLC Column:	Varian Pursuit C8, 150 x 4.6 mm x 3µm
Temperature:	40°C
Mobile Phase (A):	0.01M Formic Acid (aq)
Mobile Phase (B):	0.01M Formic Acid in Methanol

TIME (MIN)	% A	% B	FLOW (ML/MIN)
0.00	70	30	0.500
10.00	10	90	0.500
15.00	1	99	0.500
15.10	1	99	0.500
17.10	70	30	0.500
23.00	70	30	0.500

Injected Volume: 50 µL

Divert Valve:

Time (min)	Column Eluate Flow
0.00 – 10.0	Waste
10.0 – 16.0	MS Source
16.0 – 23.0	Waste

Polarity: Positive (MRM)

Ions monitored:

Analyte	Precursor Ion	Product Ion	Source Temp	NEB Gas	CUR Gas	CAD Gas	DP	FP	EP	CE	CXP
DCPU	204.8	126.7	400	12.00	9.00	6.00	20.00	94.00	14.00	35.00	15.00
	204.8	161.7	400	12.00	9.00	6.00	25.00	89.00	14.00	20.00	10.00
DCPMU	219.0	126.6	400	12.00	9.00	6.00	41.00	102.0	10.00	35.00	15.00
	219.0	161.5	400	12.00	9.00	6.00	25.00	111.0	10.00	25.00	15.00
Diuron	232.8	45.6	400	12.00	9.00	6.00	20.00	86.60	14.00	35.00	10.00
	232.8	71.4	400	12.00	9.00	6.00	20.00	70.00	14.00	20.00	10.00
Linuron	248.9	159.6	400	12.00	9.00	6.00	30.00	100.0	14.00	25.00	13.50
	248.9	181.6	400	12.00	9.00	6.00	20.00	100.0	14.00	20.00	13.50

The following are the analytical conditions that were employed for the newer-generation LC/MS/MS for the re-injection of stored samples from trial 1.

Instrument: MDS Sciex API 5000 Triple Quadrapole LC/MS/MS
Turbo Ion Spray Liquid Introduction Interface

Computer: Dell Precision 370

Software: Analyst 1.4.1

HPLC Equipment: Agilent Series HP1100
Quaternary Pump
Vacuum Degasser
WPALS Autosampler
Column Compartment

HPLC Column: Varian Pursuit C8, 150 x 4.6 mm x 3µm

Temperature: 40°C

Mobile Phase (A): 0.01M Formic Acid (aq)

Mobile Phase (B): 0.01M Formic Acid in Methanol

TIME (MIN)	% A	% B	FLOW (ML/MIN)
0.00	70	30	0.500
10.00	10	90	0.500
15.00	1	99	0.500
15.10	1	99	0.500
17.10	70	30	0.500
23.00	70	30	0.500

Injected Volume: 50 µL

Divert Valve:

Time (min)	Column Eluate Flow
0.00 – 10.0	Waste
10.0 – 16.0	MS Source

16.0 – 23.0 Waste

Polarity: Positive (MRM)

Ions monitored:

Analyte	Precursor Ion	Product Ion	Source Temp	CUR Gas	GS1 Gas	GS2 Gas	CAD Gas	DP	EP	CE	CXP
DCPU	205.0	127.1	550	30.00	40.00	40.00	10.00	60.00	10.00	27.00	10.00
	205.0	162.1	550	30.00	40.00	40.00	10.00	60.00	10.00	27.00	10.00
DCPMU	219.0	127.1	550	30.00	40.00	40.00	10.00	60.00	10.00	27.00	10.00
	219.0	162.1	550	30.00	40.00	40.00	10.00	60.00	10.00	27.00	10.00
Diuron	233.0	46.3	550	30.00	40.00	40.00	10.00	70.00	10.00	30.00	10.00
	233.0	72.3	550	30.00	40.00	40.00	10.00	70.00	10.00	30.00	10.00
Linuron	249.0	160.2	550	30.00	40.00	40.00	10.00	79.00	10.00	25.00	10.00
	249.0	182.2	550	30.00	40.00	40.00	10.00	79.00	10.00	25.00	10.00

D. Quantitation and Example Calculations

Fifty microliters of sample or calibration solution was injected into the LC/MS/MS. The peak area was measured and the standard curve was generated by the mean response factor of the analyte peak area versus the concentration of analyte using Microsoft Excel software. Percent recovery, average response factor, standard deviation, and relative standard deviation were calculated for each analyte across all fortification levels. The residue concentration was determined from the following equations:

Equation 1 was used to calculate the amount found in residue samples by average response factor analysis:

Equation 1:

$$\text{mg/kg (ppm) found} = \frac{PA}{ARF} \times \frac{(FV \times XV)}{(AV \times SW)} \times UC$$

where,

PA is analyte Peak Area,
ARF is Average Response Factor (area/ng/mL),
FV is Final Volume of extract (10.0 mL),
XV is eXtract Volume recovered from the ASE extraction (50.0 mL),
AV is Aliquot Volume (5.0 mL),
SW is Sample Weight (5.0 g) of sample aliquot extracted, and

UC is Units Conversion: $\text{ng/g} \times \frac{10^3 \text{ g}}{1 \text{ kg}} \times \frac{1 \text{ mg}}{10^6 \text{ ng}} = \text{mg/kg}$

Equation 2 was used to calculate percent recovery for fortified samples:

Equation 2:

Percent recovery = (mg/kg found) / (mg/kg applied) × 100

Sample Data			Standard Data	Peak
Parameter	Value	Units	Conc. (ng/mL)	Area
Peak Area	149476	counts	0.25	76830
Avg Response Factor	317023	area/ng/mL	0.5	159662
Final Volume	10	mL	2.5	800583
Extract Volume	50	mL	5.0	1616946
Aliquot Volume	5	mL	10.0	3296520
Sample Weight	5.00	grams	25.0	7859787
			50.0	15242642

An example calculation is shown below for linuron using soil sample QC 0.010 mg/kg Rep 3 from Data Set: 042606. This sample was fortified with 0.010 mg/kg of linuron.

From Equation 1: mg/kg found

$$\text{mg/kg (ppm) found} = \frac{149476}{317023} \times \frac{(10 \times 50)}{(5 \times 5)} \div 1000$$

$$= 0.00943 \text{ mg/kg}$$

From Equation 2: Percent Recovery (% Rec):

Percent Recovery = (0.00943 mg/kg) / (0.0100 mg/kg) × 100

= 94%

METHOD OBSERVATIONS

A. Problems Encountered

No problems were encountered.

B. Critical Steps

None other than those listed in the subject method were observed.

C. Matrix or Solvent Effects

No matrix or solvent effects were observed.

RECOMMENDED CHANGES TO METHOD

None.

CONCLUSIONS

Analytical Method DuPont-16919 was successfully validated for linuron, diuron, DCPU, and DCPMU at the limit of quantitation (LOQ, 0.010 mg/kg) and at 10 × LOQ (0.10 mg/kg) in control soil using a substitute of a newer-generation LC/MS/MS system according to Exygen Project P0002038.

The method validation meets U.S. EPA Ecological Effects Test Guidelines OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods.

TIME REQUIREMENTS

The samples were extracted using ASE overnight and analyzed the next day by LC/MS/MS. The ASE system can process up to 24 samples in series at a sample-to-sample rate of 27 minutes. The dilution, evaporation, and filtration of the final extracts prior to LC/MS/MS analysis were approximately 2-3 hours. A High-Performance Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) analytical run containing 7 standards and 13 samples took approximately 8 hours. The LC/MS/MS analysis was conducted unattended and could be run overnight. Therefore, a set of 7 standards and 13 samples could be extracted and run in 20 hours over 3 days.

CONTACTS WITH SPONSOR

The Sponsor Study Monitor was notified of the results after each trial. A breakdown of contact with the Sponsor is documented throughout the Results section of this report. The actual correspondences (e-mail and phone logs) can be found in the raw data associated with the study.

CIRCUMSTANCES THAT MAY HAVE AFFECTED THE DATA

No circumstances occurred that might have adversely affected the raw data for the study.

RETENTION OF DATA AND SAMPLES

When the final report is complete, all original paper data generated by Exygen Research will be shipped to and archived by DuPont. Exact copies of all raw data, as well as a signed copy of the final report and all original facility-specific raw data, will be retained in the Exygen Research archives for the period of time specified in 40 CFR 160.195 (b). Sample extracts will be disposed of when the results have been accepted by the Sponsor Study Monitor. The retained sample of the test and reference substance for this study is archived by the Sponsor.

REFERENCE

1. DuPont-16919, "Analytical Method for the Determination of Linuron, Diuron, and Relevant Metabolites in Soils Using LC/MS/MS", Bramble, F.Q., Pentz, A.M. (2005), E.I. du Pont de Nemours and Company, Wilmington, DE.