ANALYTICAL METHOD FOR THE DETERMINATION OF LINURON, DIURON, AND RELEVANT METABOLITES IN SOILS USING LC/MS/MS

Anne M. Pentz and Frederick Q. Bramble, Jr.

1.0 ABSTRACT

An analytical method was developed for the determination of linuron, diuron, and relevant metabolites (DCMPU, DCPU) residues in soil. The target limit of quantitation (LOQ) for each analyte was 0.010 mg/kg (ppm). The method was validated at 0.010 mg/kg and 0.10 mg/kg for all analytes. In addition, the method was validated at 5.0 mg/kg for linuron and diuron. The extracts were analyzed using a LC/MS/MS system with an electrospray interface (ESI) operating in positive ion mode. The method was specifically developed to support data generation for field studies and was validated in six typical agricultural soils, selected to cover a broad range of soil characteristics.

Soil matrix subsamples were extracted in methanol/aqueous 0.38% formic acid-0.1% nonionic surfactant (9/1, v/v) using accelerated solvent extraction (ASE) at elevated pressure and temperature. The methanol was evaporated from an aliquot of the extract, then the aqueous extract was diluted and filtered prior to analysis by reverse phase HPLC online with ESI-LC/MS/MS for the determination of linuron, diuron, DCPMU, and DCPU.

After samples are weighed into ASE extraction vessels, the automated extraction processes up to 24 samples in series at a rate of one sample/27 minutes. Samples can be extracted overnight and recovered the next day prior to dilution, evaporation, filtration, and LC/MS/MS analysis. The sample to sample runtime for LC/MS/MS analysis was 23 minutes.

MS/MS transitions of the molecular ion to two specific mass fragments 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 inteference was not observed in any of the six soils examined.

2.0 INTRODUCTION

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. This method 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, "Guidance Document on Residue Analytical Methods", SANCO/825/00 rev. 7, March 17, 2004 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. DCPMU (IN-15654) and DCPU (IN-R0915) are significant soil metabolites of linuron and diuron, and may be monitored to follow the dissipation of either herbicide in field dissipation studies.

Soil matrix subsamples are extracted using an accelerated solvent extraction (ASE) system at elevated pressure and temperature. The methanol is evaporated from an aliquot of the extract. The remaining aqueous extracted is diluted and filtered prior to the quantitative determination of linuron, diuron, DCPMU, and DCPU by LC/MS/MS analysis. The analytical method was validated at the target method limit of quantitation (LOQ) of 0.010 mg/kg and 0.10 mg/kg (10×LOQ) for all analytes. The method was also validated at 5.0 mg/kg for linuron and diuron to accommodate residue levels greater than 10×LOQ for the parent herbicides. The method limit of detection (LOD), based on the least responsive analyte, diuron, was estimated to be 0.002 mg/kg. 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

EQUIPMENT DESCRIPTION	PRODUCT ID	SUPPLIER
Analytical Balance	AE163 Dual Range Balance; PM460 Toploading Balance	Mettler Instrument Corp. (Hightstown, N.J.)
Analytical Evaporator	N-Evap® Model 111 with stainless steel luer fit needles	Organomation Assoc. (South Berlin, Mass.)
Vortex Mixer	Vortex Genie® K-550-G or Vortex-2 Genie®	VWR, Inc. (West Chester, Pa.)
Filtration	Gelman Acrodisc® 13 CR, 0.2-µm PTFE 13 mm dia. membrane syringe filter, Cat. No. 4423	VWR (Bridgeport, N.J.)
Automated Solvent Extraction (ASE) System	ASE™ 200 Extraction Apparatus and the following parts: 22 mL stainless steel extraction cells, #49560; 60 mL collection vials, #49466; septa for collection vial lids, #49464; O-rings, #049457; PEEK seals, #049455; cellulose filters, #49458	Dionex (Sunnyvale, Calif.)
Labware	Pyrex® Brand Single Metric Scale Graduated Cylinders (TC), 1-L and 50-mL capacity; SAMCO® transfer pipets, Cat. No. 336 B/B-PET	VWR (Bridgeport, N.J.)
Labware	Electronic EDP-10mL variable pipet	Rainin (Walnut Creek, Calif.)
Labware	Manual, variable volume pipettors, 100-µL, 200-µL and 1000-µL Pipettors	Gilson Inc. (Middletown, Wis.)
Labware	Falcon® 2096 (15 mL) Polypropylene Centrifuge Tubes; 5-mL Disposable Syringe, Cat. No. BD304074	Becton Dickinson (Franklin Lakes, N.J.)

LC/MS/MS System

HPLC	HP1100: G1322A degasser, G1311A quaternary pump; G1330A chilled well plate autosampler; G1316A column unit; G1314A variable wavelength detector	Agilent Technologies, Inc. (Palo Alto, Calif.)
Autosampler Vials	Screw cap vials, write on spot, 100/pk, Part number: 5182-0865	Agilent Technologies, Inc. (Palo Alto, Calif.)
HPLC Column	Pursuit® C8; 4.6 mm × 150 mm, 3 µm particle size diameter, Part# A3031-100X0.46	Varian, Inc. (Palo Alto, Calif.)
Triple Quadrupole MS	Waters MicroMass Quattro Premier triple quadrupole mass spectrometer using an electrospray interface (ESI) and MassLynx NT version 4.0 SP4 software	Waters Corporation (Milford, Mass.)

3.2 Reagents and Standards

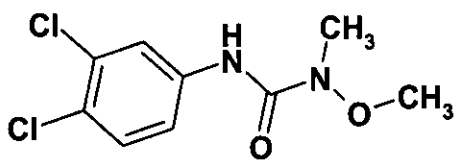
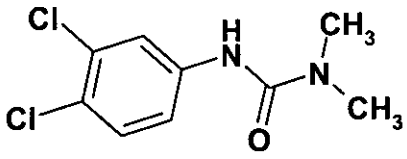
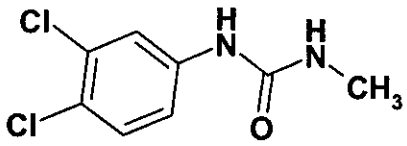
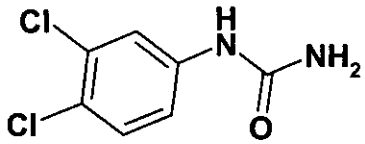
3.2.1 Reagents

The equivalency/suitability of substituted reagents should be verified.

Reagents	Product Description	Product ID	Supplier
Methanol	OmniSolv®, 4L	MX0488-1	EM Science (Gibbstown, N.J.)
Formic Acid	Suprapur® Formic Acid, 98-100% *or* Formic Acid 98-100%	EM-11670-1 B10115-74	EM Science (Gibbstown, N J.) EMD Chemicals (Gibbstown, N.J.)
Water	OmniSolv®, 4L	WX0004-1	EM Science (Gibbstown, N.J.)
Non-Ionic Surfactant	Triton X 100® non ionic surfactant, 500 mL, 'Baker Analyzed'	DX0831-6	J. T. Baker, Phillipsburg, N.J.
Sand	Sand, Standard Ottawa	SX0070-3	EM Science (Gibbstown, N.J.)

3.2.2 Reference Analytical Standards

Reference analytical standards of linuron (DPX-Z0326-229, purity 98.3%), diuron (DPX-14740-235, purity 98.7%), DCMPU (IN-15654-012, purity 99.9%), and DCPU (IN-0915-008, purity 99%) were synthesized at E.I. du Pont de Nemours and Company, DuPont Agricultural Products, Wilmington, DE. Characterization data are archived by DuPont Agricultural Products, E.I. du Pont de Nemours and Company, Wilmington, DE. The structures and specific information for linuron, diuron, DCMPU, and DCPU follow:

	**DuPont Code:** DPX-Z0326
 diuron	**DuPont Code:** DPX-14740 **CAS Chemical Name:** *N*'-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea **CAS Registry Number:** 330-54-1 **IUPAC Chemical Name:** 3-(3,4-dichlorophenyl)-1,1-dimethylurea Molecular weight = 233.10 g/mole Monoisotopic Mass = 232.02 g/mole
 DCPMU (desmethoxy linuron)	**DuPont Code:** IN-15654 **CAS Chemical Name:** N'-(3,4-dichlorophenyl)-N'-methylurea **CAS Registry Number:** 3567-62-2 **IUPAC Chemical Name:** 3-(3,4-dichlorophenyl)-1-methylurea Molecular weight = 219.07 g/mole Monoisotopic Mass = 218.00 g/mole
 DCPU (norlinuron)	**DuPont Code:** IN-R0915 **CAS Chemical Name:** 3,4-dichlorophenyl urea **CAS Registry Number:** 2327-02-8 **IUPAC Chemical Name:** *N*-(3,4-dichlorophenyl)urea Molecular weight = 205.04 g/mole Monoisotopic Mass = 203.99 g/mole

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 linuron, diuron, and their common metabolites, DCPMU and DCPU, in soil matrices to support regulatory studies. The method was validated in a variety of soil types for all analytes at a target LOQ of 0.010 mg/kg (ppm) and 0.10 mg/kg (10×LOQ), and at 5.0 mg/kg for linuron and diuron to support potentially higher residues from maximum labeled application rates.

Soil aliquots (5.0 g, fresh weight) are extracted in mostly methanol (90%) solution mixed with aqueous 0.38% formic acid containing 0.1% non-ionic surfactant (10%) using an automated accelerated solvent extraction (ASE) system. The ASE system applies elevated pressure (1000 psi) and temperature (100°C) to the solution flowing through the soil sample to extract the analytes that is collected at ambient room conditions. A 10% aliquot of the extract is evaporated in a stream of nitrogen at 50°C to an aqueous solution and diluted to final solution composition of 30% methanol/70% aqueous 0.01M formic acid solution, that is consistent with initial HPLC mobile phase conditions. An aliquot is filtered (0.2 µm) prior to LC/MS/MS analysis. The analytes are resolved by HPLC reverse-phase chromatography and detected by electrospray MS/MS in positive ion mode. 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. Only dilution and filtration are required for sample extract purification due to the sensitivity and specificity of LC/MS/MS.

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 glass- and plasticware should be washed in hot tap water with laboratory grade, non-phosphate 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 being monitored in the same laboratory where samples are being extracted and analyzed.

4.2.2 Preparation & Stability of Reagent Solutions

The following procedures describe the preparation of liter (L) volumes. Adjustments to prepare different volumes may be applied.

Aqueous 0.38% formic acid-0.1% Triton X-100 Solution

Add 3.8 mL of formic acid and 1.0 mL of Triton X-100 surfactant to a 1-L graduated cylinder partially filled with distilled, deionized water, then dilute to 1 L final volume

with distilled, deionized water. Solution may be stored at ambient room temperature for up to 30 days after preparation.

Extraction Solution *(methanol/aqueous 0.38% formic acid-0.1% Triton X-100)*
Add 100 mL of aqueous 0.38% formic acid-0.1% Triton X-100 solution to a 1-L graduated cylinder and dilute to 1 L final volume with methanol. Solution may be stored at ambient room temperature for up to 30 days after preparation.

Aqueous 0.01M Formic Acid (HPLC mobile phase)
Add 0.43 mL of formic acid to a 1-L graduated cylinder partially filled with distilled, deionized water, then dilute to 1 L final volume with distilled, deionized water. Solution may be stored at ambient room temperature for up to 30 days after preparation.

Standard Prep Solution (aqueous 0.01M formic acid/methanol, 7/3, v/v)
Add 700 mL of aqueous 0.01 M formic acid to a 1-L graduated cylinder, then dilute to 1 L final volume with methanol. Solution may be stored at ambient room temperature for up to 30 days after preparation.

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 linuron, diuron, DCPMU, and DCPU are prepared at a target concentration of 100 µg/mL in methanol*. For example, 100 µg/mL stock standards can be prepared by weighing approximately 10 mg (adjusted for purity) of analyte in a tared 100-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.

4.2.4 *Fortification Standard Preparation and Stability*

Fortification solutions are prepared from dilutions of the individual stock solutions. Individual linuron and diuron 100 µg/mL stock solutions were used to fortify 5.0 mg/kg samples. 10.0 µg/mL and 1.0 µg/mL fortification solutions are prepared for sample fortification at the 10×LOQ and LOQ, respectively. Alternative concentrations may be prepared as needed for other fortification levels.

10.0 µg/mL Fortification Solution
Dilute the stock solution for each analyte appropriately into a common volumetric flask, dilute to volume with methanol, cap and mix well. For example, 10 mL of a

* Stock solutions may be prepared at higher concentrations (not to exceed 1 mg/mL). A minimum standard weight of approximately 10 mg and final standard volume of at least 10 mL should be observed.

100 µg/mL stock solution diluted in a 100 mL volumetric. Store refrigerated and replace monthly.

1.0 µg/mL Fortification Solution

Dilute the 10.0 µg/mL fortification solution (preferred) or the stock solution for each analyte appropriately with methanol into a common volumetric flask. For example, transfer 10.0 mL of the 10.0 µg/mL fortification solution to a 100-mL volumetric flask, dilute to volume with methanol, cap and mix well. Store refrigerated and replace monthly.

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 fortification standards or individual stock standards. A minimum of four calibration standards over a range from ≤70% of LOQ equivalent final concentration to ≥120% of the highest expected final sample concentration analyzed are required for quantification. Five or more calibration standards are recommended.

For example, calibration standards of 50 ng/mL and 5 ng/mL can be prepared from the 10.0 and 1.0 µg/mL fortification solutions, respectively, by diluting a 50-µL aliquot to final volume of 10.0 mL in standard prep solution. These standards are further diluted in standard prep solution using adjustable pipettors according to the following table to prepare 0.25, 0.5, 2.5, 10.0, and 25.0 ng/mL calibration solutions.

Diluting Standard (ng/mL)	Diluting Standard Aliquot (mL)	Final Volume (mL)	Final Concentration (ng/mL)
50	2.00	4.0	25.0
50	2.00	10.0	10.0
5	5.00	10.0	2.5
5	1.00	10.0	0.5
5	0.500	10.0	0.25

Calibration standards can be prepared concurrently with sample fortifications using this procedure. Keep calibration standards refrigerated and replace by at least biweekly. Calibration standards use may be extended if supported by stability test data.

4.2.6 *Source (& Characterization) of Samples*

The California and Louisiana soils were collected from test sites used in the DuPont-16918 linuron field soil dissipation study (this method will be applied to the analysis of soil samples for this study). Additional soils (4) were obtained from DuPont Crop Protection soil bank. The following table contains background information and characteristics for these test soils.

Soil Names	Texture or Type	Location	State or Country	Sand (%)	Silt (%)	Clay (%)	pH_w	OM_{ash} (%)	CEC (meq/100g)
Cajun	Silt Loam	Porterville	California	38.0	58.0	4.0	8.1	0.8	11.0
Baldwin	Silty Clay Loam	Washington	Louisiana	19.2	47.8	33.0	5.5	2.3	17.5 *
Eau Gallie	Sand	Bradenton	Florida	97.0	3.0	0.0	8.0	1.0	3.8
Sassafras #16	Sandy Loam	Chesapeake Farms	Maryland	58	35	7	5.9	1	5.1
Drummer #7	Clay Loam	Rochelle	Illinois	24	43	33	6.1	4.8	32.5
Nambsheim	Loam	Nambsheim	France	51	38	10	7.9	1.4	6.7

* Soil data are representative properties for Baldwin soil (0-12") provided by the Missouri Cooperative Soil Survey. Actual characterization data can be found in DuPont-16918 study report.

4.2.7 *Storage & Preparation of Samples*

Soil samples were received from field test sites (California and Louisiana) or the DuPont soil bank and stored at ambient room temperature in plastic zip-lock bags.

For test samples from regulatory studies, soil samples should be stored frozen at approximately -20 ℃. Allow the sample to thaw, remove sticks, rocks, and leaves by hand. The soil should be mixed extensively to ensure homogeneity. An example procedure for the homogenization soil samples is briefly described below.

All subsamples from each plot to be tested are combined. The entire combined soil sample of the cores is blended mechanically using a food chopper (ADE cutter). Dry ice is added to keep the soil frozen. After approximately 10 minutes, the blending procedure is stopped and the entire soil portion is sieved (2-3 mm). Thereafter the mechanical blending procedure is continued until a homogeneous soil mixture is obtained. A portion of the blended soil is transferred into a container.

4.2.8 *Sample Fortification Procedure*

Fortify the soil of control samples in ASE extraction vessels as required. The following table is provided as an example for fortification levels used in this study.

Fortification Level (ppm)	Methanol Fortification Solutions (µg/mL)	Fortification Solution Aliquot (mL)
0.010	1.0	0.050
0.10	10.0	0.050
5.0	100	0.25

Allow solvent to dissipate in a fume hood for at least 15 minutes. At least one sample should be fortified at 0.010 ppm (LOQ) in a sample set. Fortification at levels above 1.0 ppm are intended for parent (linuron and diuron) only.

4.2.9 *Analyte Extraction Procedure*

1. For each sample, install endcap + 10 µm steel frit in a 22 mL ASE extraction vessel. Insert 2 cellulose filters (19.1 mm dia., type D28) and press filters to bottom (a plunger from a 10 mL disposable syringe may be used).

2. 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.
3. Fortify control samples as required*. Allow to stand in fume hood for at least 15 minutes.
4. Add sand to fill each of the vessels and cap sample vessels.
5. 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).
6. Remove vessels and glass collection tubes containing the extracts from the ASE extractor.
   Note: 1 cm ≈ 5 mL; expect approximately 40 mL or 8 cm extract solution. ASE extracts are stable for at least four days when stored under refrigeration.

4.2.10 *Post Extraction Sample Processing*

1. 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.
2. 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.
3. 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.
   Note: LOQ (0.010 ppm) equivalent concentration = 0.5 ng/mL
4. For samples fortified at 5.0 ppm, extracts are diluted within the calibration range, e.g., 0.2 mL extract/10 mL standard prep solution (a 50-fold dilution, equivalent to 5.0 ng/mL).
   Note: Extracts require additional dilution and a second analysis when one or more analyte concentrations are expected or found to be outside the calibrated range.
5. Filter (0.2 µm AcroDisc PTFE 13 mm) an aliquot for LC/MS/MS analysis.
   Note: If sample extracts cannot be analyzed immediately, store extracts in a refrigerator prior to analysis. Sample extracts are stable for at least four days when stored under refrigerated conditions.

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* A recommended practice during sample fortification is to prepare a **fortification standard** from each fortification solution used by pipetting an aliquot of the fortification solution (preferably the same volume applied in the fortification) to a tube or volumetric flask and diluting each fortification standard into the range of calibration standards analyzed (e.g., LOQ fortification: 0.05 mL of 1.0 µg/mL fortification soution diluted to 100 mL = 0.50 ng/mL). The fortification standards would be analyzed with the analysis set to demonstrate consistency of standards used in fortified samples and calibration standards.

4.3 *Instrumentation*

4.3.1 *Description*

An Agilent HP1100 HPLC and a Waters Quattro Premier triple quadrupole mass spectrometer were used for LC/MS/MS analysis.

4.3.2 *Operating Conditions*

Typical equipment components and operating conditions follow:

Agilent HP1100 HPLC:	G1322A vacuum degasser, G1311A quaternary pump, G1367A chilled autosampler, G1330A chiller, G1316A column compartment
Injection Volume:	25 µL (may be varied to correct for MS sensitivity)
HPLC Column:	Varian Pursuit C8 (15.0 cm x 4.6 mm i.d., 3 µm diameter particle)
Column Temperature:	40°C
Mobile Phases:	A = aqueous 0.01M formic acid
	B = methanol
Waters Quattro Premier:	MassLynx Version 4 SP4 software
Interface:	electrospray (ESI)
Polarity:	positive ion
Mode:	MRM

HPLC Conditons:

TIME	FLOWRATE (ML/MIN)	%A	%B	COMMENTS
Initial	0.5	70	30	No post-column split to MS
10.0	0.5	10	90	
15.0	0.5	1	99	
15.1	0.5	1	99	
17.1	0.5	70	30	
23.0	0.5	70	30	End Run

Approximate Analyte Retention Times:

linuron = 11.9 min
diuron = 11.1 min
DCMPU = 11.1 min
DCPU = 10.7 min

Quattro Premier Triple Quadrupole MS operating conditions follow:

Interface:	electrospray (ESI)	
Mode:	positive ion	
Divert Valve:	0.0–8.0 min	to waste
	8.0–13.4 min	to source
	13.4–23.0 min	to waste

Voltages			Temperatures		Gas Flow	
Capillary (V)	Extractor (V)	RF Lens (V)	Source (°C)	Desolv. (°C)	Desolv. (L/hr)	Cone (L/hr)
2.0	4.0	0.1	125	400	700	100

Q1		Q2		Q3	
LM Res	12.0	Entrance	Exit	12.0	LM Res
HM Res	12.0	1	1	12.0	HM Res
Ion Energy	0.4			1.5	Ion Energy

Collision Cell:	mL/min:	0.35	mbar:	3.28E-03

MRM Functions

Analyte (retention window)	Parent (m/z)	Daughter (m/z)	Dwell (secs)	Cone (volts)	Coll Energy (eV)
DCPU	204.80	126.70	0.05	23.00	25.00
(9-11.5 min)	204.80	161.70	0.05	23.00	15.00
diuron	232.80	45.60	0.05	28.00	15.00
(9.1-12.1 min)	232.80	71.40	0.05	23.00	16.00
DCPMU	219.00	126.60	0.05	25.00	27.00
(10-12 min)	219.00	161.50	0.05	25.00	15.00
linuron	248.90	159.60	0.05	26.00	16.00
(10.9-13.5 min)	248.90	181.60	0.05	26.00	16.00

Mass assignment on other instruments may vary ± 0.5 amu.

4.3.3 *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*.

Instrument calibration was based on the average response factor (analyte peak area response/analyte concentration) of external calibration standards using Excel® functions AVERAGE, STDEV, and RSD. For average response factor calibration, a %RSD of less than or equal to 20% should be observed. 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 are: (1) RSQ value >0.99 for calibration curve and (2) the %RSD ≤20% for the individual calibration standard response

factors. Alternative approaches including linear regression with or without weighting (e.g., 1/X) may be used if they provide an equivalent or more consistent fit of sample response to the response of calibration standards.

The instrument calibrated range was 0.25 ng/mL (0.5 × LOQ of 0.010 ppm equivalent final concentration) to 50.0 ng/mL. 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 <50% of the LOQ. When the control residues are >50% of the LOQ, the recovery samples prepared at the LOQ using that control are invalidated. When the control residues are <50% of the LOQ, corrected ppm (mg/kg) 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 ppm (mg/kg).

4.3.4 *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 1–3 samples in each analytical set. Extracts and calibration standards should be refrigerated if stored. Generally, fortification sample recoveries (70-120%) are required for acceptable quantitation results in a analysis set.

4.4 Calculations

4.4.1 *Methods*

Linuron, diuron, DCPMU, and DCPU residues were measured as mg/kg (ppm) in soil. Quantitation was based on an average response factor determined from the multiple calibration standards concurrently analyzed with sample extracts. All calculations were made using unrounded values that were reported to two significant figures. In field studies, residues reported at or above the LOQ are rounded to two significant figures. Integrable residues detected below the LOQ are reported to one significant figure. Fortified sample recoveries are reported to the nearest whole number percentage (%).

The calculation to determine mg/kg found in residue samples by average response factor analysis follows:

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

where,

PA is analyte **P**eak **A**rea,
ARF is **A**verage **R**esponse **F**actor (area/ng/mL),
FV is **F**inal **V**olume of extract (10.0 mL unless final extracts are diluted, then volume = dilution factor × 10 mL , e.g., 5 ppm samples diluted 50×10 mL = 500 mL),
XV is e**X**tract **V**olume recovered from the ASE extraction (50.0 mL),
AV is **A**liquot **V**olume (5.0 mL),
SW is **S**ample **W**eight (5.0 g) of sample aliquot extracted, and

UC is Units **C**onversion: $ng/g \times \frac{10^3 \text{ g}}{1 \text{ kg}} \times \frac{1 \text{ mg}}{10^6 \text{ ng}} = \text{mg/kg}$ or $\left(\frac{ng/g}{1000}\right)$

The % Recovery of linuron, diuron, DCPMU, or DCPU in fortified samples is determined as follows:

% Recovery = (mg/kg found) × 100 / (mg/kg applied)

4.4.2 *Examples*

Louisiana LOQ Fortification Sample 0518 LA LOQ 1, Linuron Recovery
(reference Appendix 1, Figure 10, Table 2)

$$\left(\frac{1073 \text{ area counts}}{2279 \text{ area/ng/mL}}\right) \times \left(\frac{10.0 \text{ mL} \times 50.0 \text{ mL}}{5.0 \text{ mL} \times 5.0 \text{ g}}\right) \times \frac{10^3 \text{ mg·g}}{10^6 \text{ ng·kg}} = 0.00942 = 0.009 \text{ mg/kg}$$

% Recovery = 0.00942 mg/kg × 100 / 0.010 mg/kg = 94%

California 5.0 ppm Fortification Sample 0518 CA L-D 500X 1, Diuron Recovery
(reference Appendix 1, Figure 8, Table 1)

$$\left(\frac{20282 \text{ area counts}}{4042 \text{ area/ng/mL}}\right) \times \left(\frac{500 \text{ mL} \times 50.0 \text{ mL}}{5.0 \text{ mL} \times 5.0 \text{ g}}\right) \times \frac{10^3 \text{ mg·g}}{10^6 \text{ ng·kg}} = 5.02 = 5.0 \text{ mg/kg}$$

% Recovery = 5.02 mg/kg × 100 / 5.0 mg/kg = 100%

5.1.5.a *Background Evaluation*

Background levels experienced in tandem mass spectrometry analyses are minimal. Generally, the chromatographic profiles of a sample extract solution and a calibration standard solution appear the same. The control sample chromatograms for each soil tested are provided in Figure 8 through Figure 19.

5.1.5.b *Limit of Detection (LOD)*

A method limit of detection (LOD) was estimated to be 0.002 mg/kg based on the limiting response analyte, diuron. The LOD is defined as the analyte concentration in matrix with a response equivalent to a signal-to-noise ratio of approximately 3 to 1. The LOD was estimated from the signal to noise response of each analyte in matrix at LOQ level using the following equation.

$$\frac{\text{LOD signal to noise response (3/1)}}{\text{Observed LOQ signal to noise response}} \times \text{LOQ} = \frac{3/1}{12/1} \times 0.010 \text{ mg/kg} = 0.002 \text{ mg/kg}$$

Variation in the LOD was observed and each lab using this method should estimate an LOD value.

5.2 ***Timing***

Generally, 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 was approximately 2–3 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 (<0.010 to 5.0 mg/kg), special precautions should be taken to insure reusable labware is clean, test materials are handled to minimize cross-contamination, and instruments (ASE and MS) are maintained to avoid carryover or contamination that can affect method performance.

Routinely, a LC/MS/MS end run was setup so that high percentage organic solvent (95-99% methanol or acetonitrile) would continue overnight at a low flowrate (0.25 mL/min) after the final analysis to keep the HPLC column in good condition. The HPLC column may be backflushed with high percentage organic solvent to recondition the column if peak shapes deteriorate.

5.4 ***Method Ruggedness***

5.4.1 *Stability*

All stock and standard solutions prepared in methanol and stored at or below 4°C are stable for at least six months. Standards prepared in mobile phase solution are stable

for at least two weeks when stored at or below 4°C. Extracts are stable for at least four days if stored at or below 4°C.

5.4.2 *Specificity/Potential Interference*

5.4.2.a *Interference from Glassware & Reagents, Matrices*

No interferences attributable to glassware, reagents, or matrices were observed to co-elute with test analytes.

5.4.2.b *Interference from Other Pesticides*

Due to the selective nature of the detection of this method (two individual ion transitions monitored), interference peaks were not observed at the retention time of the analytes. As a result of the selective detection used, interference testing is not necessary for this method.

In the Louisiana soil, a significant peak was detected in the chromatograms for diuron, DCPMU, and DCPU (see Figure 10 and Figure 11). Each of these peaks were resolved from the respective test analytes, and did not interfere with the analysis. Diuron has the same monoisotopic mass as fluometuron and yields the same MS/MS fragment ions. The Louisiana field test site acknowledged that Cotoran® herbicide containing fluometuron had been applied to the soil the previous season.

5.4.3 *Confirmatory Procedure*

Two independent MS/MS transitions of the molecular ion for each analyte were monitored. The relative response ratios of the two fragment ions (base peak/secondary peak) were determined from calibration standard responses for confirmation of analyte in soil matrix samples. Acceptable confirmation criteria are a co-eluting peak and equivalent ion ratio, each within ± 30% of the average response observed in calibration standards at or above the LOQ equivalent concentration concurrently analyzed with the samples. The calculated response ratios and retention times for each analyte determined in an analysis set are shown in Appendix 3.

5.4.4 *Second Lab Tryout*

ABC Laboratories conducted a Second Lab Tryout for this method using the California and Louisiana control soils. Linuron, DCPMU, and DCPU were fortified at 0.010, 0.10, and 5.0 ppm levels in each soil examined. A Sciex API-3000 mass spectrometer was used instead of the Quattro Premier. Injection volumes were increased from 25 to 50 µL to compensate for decreased mass spectrometer sensitivity. Acceptable results were reported for the initial trial and are provided in Appendix 4 (raw data was transcribed into method validation spreadsheet template for consistency). PE Sciex API 3000-LC-MS/MS System instrumental conditions are provided in Appendix 5.

6.0 CONCLUSIONS

- This analytical method is suitable for the quantitation of linuron, diuron, DCPMU, and DCPU residues in soil matrices. The results support an LOQ of 0.010 mg/kg (ppm) and an estimated LOD of 0.002 mg/kg in soil. The method meets European Commission, Directorate General Health and Consumer Protection. "Guidance Document on Residue Analytical Methods", SANCO/825/00 rev. 7, March 17, 2004 and U.S. EPA Ecological Effects Test Guidelines.

- Residue confirmation for each analyte was demonstrated at 0.010 mg/kg (LOQ) and 0.10 mg/kg fortification levels based on retention time and the relative ratios of two MS/MS parent-to-fragment ion transitions detected during sample analysis.

7.0 RETENTION OF RECORDS

Originals or exact copies of all raw data and pertinent information and the final report will be retained at:

E.I. du Pont de Nemours and Company
DuPont Crop Protection
Global Technology Division
Stine-Haskell Research Center
Newark, Delaware 19714-0030

8.0 REFERENCES

1. Bramble, F.Q.; Norwood, G.I. "The Degradation of [Phenyl(U)-^{14}C] Diuron in Soil Under Aerobic Conditions," DuPont Report No. AMR 2603-93 Supplement No.1. E.I. du Pont de Nemours and Company, Wilmington, DE.
2. Bergström, L.; Bramble, F.Q., *et al.* "Leaching of [Phenyl(U)-^{14}C]Diuron in Scandinavian Soils Using Field Lysimeters," DuPont Report No. AMR 2619-93. E.I. du Pont de Nemours and Company, Wilmington, DE.
3. Bramble, F.Q.; Behmke, F.D., *et al.* "Field Soil Dissipation of Diuron Following Application of Karmex DF Herbicide," DuPont Report No. AMR 4383-97. E.I. du Pont de Nemours and Company, Wilmington, DE.

APPENDIX 5 ABC LABORATORIES METHOD TRYOUT INSTRUMENTAL CONDITIONS

— PE Sciex API 3000-LC-MS/MS System with Analyst Software —

HPLC Conditions:

System:	Agilent HP1100 HPLC
Column:	4.6 mm i.d. × 100 mm, Varian Pursuit C-8 analytical column with 3-µm diameter packing.
Column Temperature:	40 °C
Injection Volume:	50 µL
Autosampler Temperature:	N/A
Flow Rate:	500 µL/min
Conditions:	Time %A %B Flow 0.0 70 30 500 0.1 70 30 500 10.0 10 90 500 15.0 1 99 500 15.1 1 99 500 17.1 70 30 500 23.0 70 30 500 A: 0.01 M aq. Formic Acid (F.A.) B: 0.01 M F.A. in Methanol Flow in µL/min
Approximate Retention Times	(Minutes)
Linuron	11.9
DCPU	10.8
DCPMU	11.2
Total Run Time:	23.0 minutes

The valve switching times are given in the following table.

TIME (MINUTES)	COLUMN ELUATE FLOW
0.0-0.10	Waste
0.10-15.0	MS source
15.0-end	Waste

APPENDIX 5 ABC LABORATORIES METHOD TRYOUT INSTRUMENTAL CONDITIONS (CONTINUED)

APCI-LC/MS/MS Conditions:

ANALYTES MONITORED	ION MODE	IONS MONITORED FOR QUANTIFICATION	ACQUISITION TIMING (MIN.)
Linuron	MRM	248.9 → 159.6 AMU 248.9 → 181.6 AMU	11.7 – 12.4

Polarity	Positive	
Probe Temp	400 °C	
Nebulising Gas Setting	12.00	
Curtain Gas Setting	9.00	
CAD Gas Setting	6.00	
Declustering Potential	30.00 (159.6 AMU)	20.00 (181.6 AMU)
Focusing Potential	120.00 (159.6 AMU)	100.00 (181.6 AMU)
Entrance Potential	10.00	
Collision Cell Exit Potential	15.00	
Collision Energy	25.00 (159.6 AMU)	20.00 (181.6 AMU)

ANALYTES MONITORED	ION MODE	IONS MONITORED FOR QUANTIFICATION	ACQUISITION TIMING (MIN.)
DCPU	MRM	204.8 → 126.7 AMU 204.8 → 161.7 AMU	10.6 – 11.4

Polarity	Positive	
Probe Temp	400°C	
Nebulising Gas Setting	12.00	
Curtain Gas Setting	9.00	
CAD Gas Setting	6.00	
Declustering Potential	25.00	
Focusing Potential	150.00 (126.7 AMU)	120.00 (161.7 AMU)
Entrance Potential	10.00	
Collision Cell Exit Potential	15.00	
Collision Energy	35.00 (126.7 AMU)	20.00 (161.7 AMU)

APPENDIX 5 ABC LABORATORIES METHOD TRYOUT INSTRUMENTAL CONDITIONS (CONTINUED)

ANALYTES MONITORED	ION MODE	IONS MONITORED FOR QUANTIFICATION	ACQUISITION TIMING (MIN.)
DCPMU	MRM	219.0 → 126.6 AMU 219.0 → 161.5 AMU	11.0 – 11.6

Polarity	Positive	
Probe Temp	400 °C	
Nebulising Gas Setting	12.00	
Curtain Gas Setting	9.00	
CAD Gas Setting	6.00	
Declustering Potential	25.00 (126.6 AMU)	20.00 (161.5 AMU)
Focusing Potential	150.00 (126.6 AMU)	100.00 (161.5 AMU)
Entrance Potential	10.00	
Collision Cell Exit Potential	15.00	
Collision Energy	35.00 (126.6 AMU)	25.00 (161.5 AMU)