2.0 INTRODUCTION

Independent laboratory validation of soil methods is required to fulfill the requirements under U.S. EPA OPPTS 850.7100, PR Notice 96-1.

The environmental analytical method for DPX-QGU42 and its metabolites in soil as described in DuPont-31005, entitled "Analytical Method for the Determination of DPX-QGU42 and Metabolites IN-E8S72, IN-QPS10, IN-RDT31 and IN-RAB06 in Soil Using LC/MS/MS**"** is applicable for the quantitation of DPX-QGU42 and its metabolites in soil matrix.

DPX-QGU42, IN-E8S72, IN-QPS10, IN-RDT31, and IN-RAB06 were extracted from soil samples using a mixture of formic acid, water and acetonitrile. An aliquot of the extracts were diluted with HPLC grade water and then concentrated under a stream of nitrogen. The concentrated extracts were adjusted to the original volumes with the HPLC grade water and then analyzed using reversed phase liquid chromatography (LC) coupled with electrospray mass spectrometry/mass spectrometry (MS/MS) detector. DPX-QGU42, IN-E8S72, IN-QPS10 and IN-RDT31 were detected by positive ion electrospray MS/MS and IN-RAB06 was detected by negative ion electrospray MS/MS.

The analytical method was designed to achieve an LOQ of 1.0 µg/kg (ppb) for all analytes, and the Limit of Detection (LOD) was estimated to be 0.3 µg/kg (ppb). The independent validation thus evaluated DPX-QGU42, IN-E8S72, IN-QPS10, IN-RDT31 and IN-RAB06 recoveries on samples fortified at 1X and 10X the LOQ level. The method was used as written. No communication between the study monitor and the study director was required during the validation trial.

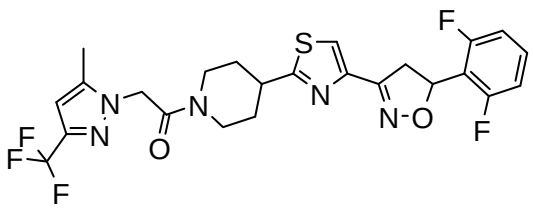
3.0 MATERIALS AND METHODS

3.1 Test Substance

The reference analytical standards (test substances) used for this study were:

DuPont code: DPX-QGU42

Chemical Structure:



DPX-QGU42

Molecular weight: 539.53

Formula: $C_{24}H_{22}F_5N_5O_2S$

Source: E. I. du Pont de Nemours and Company

CAS Number: 1003318-67-9

Batch/Lot Number: E105317-115

Purity: 98.9%

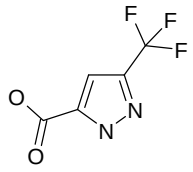
Receipt date: 27 August, 2010

Expiration date: 18 June, 2012

Storage: Ambient temperature

DuPont code: IN-E8S72

Chemical Structure:



IN-E8S72

Molecular weight: 180.09

Formula: $C_5H_3F_3N_2O_2$

Source: E. I. du Pont de Nemours and Company

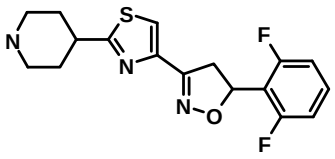
Lot Number: GF1007175

Purity: 99.7%

Receipt date:	15 March, 2011
Expiration date:	12 October, 2013
Storage:	Ambient temperature

DuPont code: I N-QPS10

Chemical Structure:

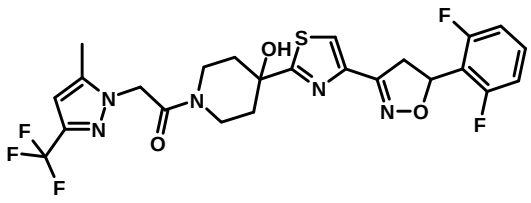


IN-QPS10

Molecular weight:	349.40
Formula:	$C_{17}H_{17}F_2N_3OS$
Source:	E. I. du Pont de Nemours and Company
Lot Number:	113774-081
Purity:	89.4%
Receipt date:	15 March, 2011
Expiration date:	28 July, 2013
Storage:	Ambient temperature

DuPont code: IN-RDT31

Chemical Structure:

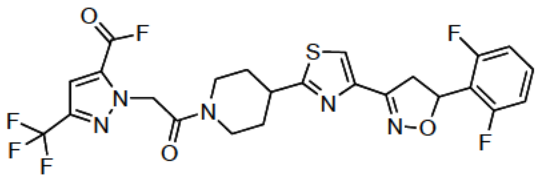


IN-RDT31

Molecular weight:	555.53
Formula:	$C_{24}H_{22}F_5N_5O_3S$
Source:	E. I. du Pont de Nemours and Company
Lot Number:	GF1000813
Purity:	94.0%
Receipt date:	15 March, 2011
Expiration date:	30 June, 2011
Storage:	Freezer ($\leq$ -10 °C)

DuPont code: IN-RAB06

Chemical Structure:



IN-RAB06

Molecular weight:	569.51
Formula:	$C_{24}H_{20}F_5N_5O_4S$
Source:	E. I. du Pont de Nemours and Company
Lot Number:	E112392-135A
Purity:	96.4%
Receipt date:	27 August, 2010
Expiration date:	14 July, 2012
Storage:	Ambient temperature

DPX-QGU42, IN-E8S72, IN-QPS10, IN-RDT31, and IN-RAB06 were supplied by E. I. du Pont de Nemours and Company, DuPont Crop Protection, Newark, DE. Information pertaining to the characterization and stability of the test substances is archived by DuPont Crop Protection, E. I. du Pont de Nemours and Company, Newark, Delaware.

3.2 Test System

In this study, the analytical method was validated in the soil matrix obtained from a field located in 17 Lee Blvd., Malvern, PA 19355. The soil control sample was dried by the exposure in the chemical hood over night and then ground before used.

Fortifications of the samples were made to 5.0 g of soil using 0.010-µg/mL and 0.10-µg/mL standard solutions.

The samples were assigned unique identification by the laboratory, an alpha-numeric sample ID along with additional designations such as "control," and "LOQ" as appropriate.

3.3 Equipment

Equipment used was either the same as that specified in the analytical method or equivalent.

3.4 Reagents

Reagents used were either the same as that specified in the analytical method or equivalent grade of the quality.

3.5 Principles of the Analytical Method

The analyses in the study followed the analytical method for DPX-QGU42 and metabolites IN-E8S72, IN-QPS10, IN-RDT31 and IN-RAB06 in soil, as described in DuPont Study No. DuPont-31005. The following is a summary of the method conducted at Alliance Pharma. The complete description of the method is described in the original method (DuPont-31005).

A 5.0-gram soil sample was weighed and fortified with a standard solution. To each sample was added 2-mL of deionized water and was allowed to soak for 5 minutes. The samples were extracted with 15 mL of acetonitrile and 0.5 mL of formic acid using a high-speed homogenizer. After centrifugation for 5 minutes at 3000 RPM, the supernatant was transferred into 50-mL centrifuge tubes. The extraction and process was repeated twice using 15-mL of acetonitrile and 0.5-mL of formic acid except the addition 2 mL water which was not required. The three extracts were combined and the volume in the centrifuge tubes was adjusted to 50 mL using acetonitrile and vortexed. A 2.0-mL aliquot of each extract was removed and diluted with 1-mL of HPLC grade water. The volume was then reduced to just under 2-mL and was diluted to 2-mL with HPLC grade water. An aliquot of each sample was loaded into an auto-sampler vial for LC/MS/MS analysis.

Method validation was accomplished by analyzing each of the five analytes in the validation sets each consisting of 1 blank control specimen, 5 replicate specimens fortified at LOQ or 5 replicate specimens fortified at 10xLOQ.

3.6 Modifications, Interpretations, and Critical Steps

The analytical method was run exactly as written except a six-port electronically activated switching valve was not used to direct the flow to waste prior to and following the elution of the analytes.

There was no critical step that appeared to impact analytical results. However, optimizing LC gradient conditions to increase the retention time interval between the negative and positive monitoring modes will be helpful to prevent the chromatogram peaks of interest from out of the monitoring windows in case of the retention time shifting.

3.7 Instrumentation

HPLC Conditions:

System:	MDS Sciex Applied Biosystems 4000 Q TRAP; Shimadzu LC-20AD pumps
Column:	3.0 mm i.d. × 15 cm, 3.5 µm Agilent SB-phenyl
Column Temperature:	40°C
Injection Volume:	15- 25 µL
Autosampler Temperature:	Ambient or 4 ^{0}C
Flow Rate:	0.600 mL/minute
Conditions:	A: 0.05% aq. Formic Acid
	B: 0.01% Formic Acid in Methanol
	Flow in mL/minute
	Time %A %B Flow 0.0 75 25 0.60 4.0 40 60 0.60 5.0 30 70 0.60 10.0 20 80 0.60 10.2 1.0 99 0.60 14.0 1.0 99 0.60 15.0 75 25 0.60 20.0 75 25 0.60
IN-QGU42 RETENTION TIME:	9.12 minutes
IN-RAB06 RETENTION TIME:	9.17 minutes
IN-RDT31 RETENTION TIME:	8.11 minutes
IN-QPS10 RETENTION TIME:	4.90 minutes
IN-E8S72 RETENTION TIME	4.18 minutes
Total Run Time:	20.0 minutes

The detection method utilized was LC-MS/MS employing atmospheric pressure chemical ionization (APCI) interface in the positive mode on a triple quadrupole instrument. The acquisition method was adjusted to maximize the response of the

fragment ions detected. The ion transitions for DPX-QGU42 and metabolites are shown below:

ANALYTES	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-QGU42	**540.2→ 500.3 AMU**	**85**	**36**	**10**
	540.2→ 163.2 AMU	85	65	14
IN-RAB06	**570.2→ 167.2 AMU**	**85**	**40**	**10**
	570.2→ 177.1 AMU	85	67	15
IN-QPS10	**350.2→ 210.1 AMU**	**85**	**41**	**16**
	350.2→ 82.2 AMU	85	62	13
IN-RDT31	**556.2→ 191.1 AMU**	**70**	**62**	**15**
	556.2→ 331.2 AMU	70	44	15
Time:	4.5 – 12 minutes			
Ion Mode:	Positive			
Turbo Spray Voltage:	5500 V			
Source Temperatures:	550 ^{0}C			
CUR:	30			
CAD:	High			
GS1:	50			
GS2:	55			
Dwell	0.15 Seconds			

ANALYTES	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-E8S72	**179.0→ 65.0 AMU**	**-40**	**-30**	**-9**
	179.0→ 135.0 AMU	-40	-15	-9
Time:	0 - 4.5 minutes			
Ion Mode:	Negative			
Turbo Spray Voltage:	-4500 V			
Source Temperatures:	550 ^{0}C			
CUR:	30			
CAD:	High			
GS1:	50			
GS2:	55			
Dwell	0.15 Seconds			

The instrument was operated in the MS/MS (MRM) positive and negative ion modes for quantitative analysis. The ion chromatograms were integrated and the peak area used for quantitation.

For each analytical run, a six-point standard curve was prepared by injecting constant volumes of standard solutions of each analyte of interest. Constant volume injections were used for sample extracts as well. The relative ratio of the fragment ions was evaluated to confirm the presence of an analyte in an unknown sample.

3.8 Calculations

R*esidues of DPX-QGU42 and its metabolites IN-E8S72, IN-QPS10, IN-RAB06 and IN-RDT31 were quantitated by external standards. A calibration curve for each analyte was generated by plotting the detector's response in peak area versus the amount (ng) of standard injected. The data system derived an equation for the fit of the standard curve and this equation was used to calculate intercept and slope of the linear regression curve.*

The calibration curve was obtained by direct injection of 15 µL of the standard of each analyte into LC-MS/MS in the range of 0.05 ng/mL to 5.0 ng/mL. In a given injection run, the same injection volume was used for all samples and standards.

Peak integration and quantitation were performed using Applied Biosystems' Analyst software with version 1.4.2. Calculations and Recovery results were computed for each set of samples by Microsoft's Excel® spreadsheet. Equations used for quantitation are shown below:

For the calculation of residues the following formula was used:

$R = C_{End} X V_F / (AF*DF) / W$

Where:

R: Analyte residue in ng/g (ppb)

C_{End}: Final concentration of analyte derived from calibration curve in ng/mL

AF: Aliquot factor = Aliquot volume (V_{aliq}) / Extraction volume (V_{Ex})

DF: Dilution factor = Aliquot extract volume (V_{aliq}) / Final volume (V_F)

W: Soil sample weight: 5 g

Recoveries (Rec.) were calculated for the fortified specimens as follows:

$Rec. = (R / R_{fortified}) X 100 \%$

Example: Sample 100812DPX-V01-LOQ-5, DPX-QGU42, Soil, Fortified @ 1.0 ppb (Figure 11):

$C_{End} = 0.091$ ng/mL

$R_{fortified} = 1.0$ ppb

$AF = V_{aliq} / V_{Ex} = 2$ mL / 50 mL $= 0.04$

$DF = V_{aliq} / V_F = 2\ mL / 2\ mL = 1$

$R = C_{End} X V_F / (AF*DF) / W$

$= 0.091\ (ng/mL) * (2\ mL) / (0.04*1) / 5\ (g) = 0.91\ ng/g = 0.91\ ppb$

$Rec. = (R / R_{fortified}) X\ 100\ \% = (0.91 / 1.0) X\ 100\% = 91\%$