2.0 INTRODUCTION

Independent laboratory validation of enforcement methods are required by the U.S. EPA OPPTS 850.7100 (Reference 1) and EU Guidance document SANCO/825/00 rev. 7 (Reference 2).

The subject method is applicable for the quantitation of DPX-YT669, IN-QDK50, IN-QDY62, or IN-QDY63 in soil, as described in DuPont-24804 (Reference 3). Clay loam soil was chosen to validate the analytical method as a representative matrix.

Fortification concentrations in this study were chosen to provide method performance data at the method LOQ and 40×LOQ for the matrix examined. The stated method LOQ was 0.010 ppm for all analytes in soil.

The analytical method was performed without any significant modifications using the extraction without SPE clean-up and with SPE clean-up. The method without SPE clean-up was successfully validated for DPX-YT669, IN-QDK50, IN-QDY62, and IN-QDY63 in soil in one trial. The method with SPE clean-up was successfully validated for DPX-YT669, IN-QDY62, and IN-QDY63 in soil in one trial and was not successful in validating IN-QDK50. This independent laboratory validation study demonstrated that the analytical method DuPont-24804 is acceptable for the quantitation of DPX-YT669, IN-QDK50, IN-QDY62 and IN-QDY63 in soil, according to guidelines set forth by US EPA Ecological Effects Guidelines, OPPTS 850.7100 "Data Reporting for Environmental Chemistry Methods" (Reference 1) and EU Guidance document SANCO/825/00 rev. 7 (Reference 2).

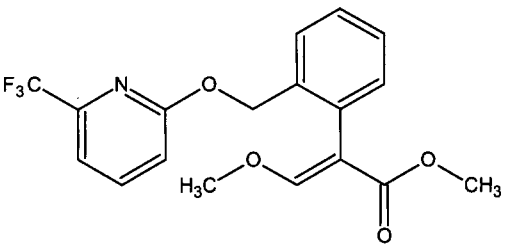
3.0 MATERIALS AND METHODS

3.1 Test and Reference Substances

The DPX-YT669, IN-QDK50, IN-QDY62 and IN-QDY63 analytical standards were received from DuPont frozen or chilled, assigned a unique ABC Laboratories identification code, and stored at approximately -20 °C. The analytical standards were used to prepare mixed fortification and calibration standards for LC/MS/MS instrumental analyses. The following lots were used for method verification for this Independent Laboratory Validation study.

TEST SUBSTANCE	GRADE	SOURCE	DATE RECEIVED	ABC LABS ID#	% PURITY	LOT OR CODE	EXP DATE	PHYSICAL APPEARANCE
Picoxystrobin (DPX-YT669	Technical Grade	DuPont	8/5/08	PS-22035	99.9	ASJ1009 9-03	10/01/19	Crystalline Powder
IN-QDK50	Technical Grade	DuPont	9/10/08	PS-22203	99.5	E110768	3/8/2014	Solid
IN-QDY62	Technical Grade	DuPont	9/10/08	PS-22204	99.2	E110768 -01	7/14/2011	Solid
IN-QDY63	Technical Grade	DuPont	9/10/08	PS-22205	99.5	KI-6905/1M	7/14/2011	Solid

The *Chemical Abstracts* structures (if available) and chemical names of the analytes are shown below:



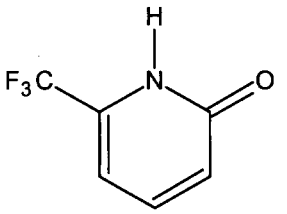
Test Substance: DPX-YT669

Common Name: Picoxystrobin

***Chemical Abstracts* Name**: methyl (E)-α-(methoxymethylene)-2-[[[6-(trifluoromethyl)-2-pyridinyl]oxy]methyl]-benzeneacetate

CAS Registry No.: 117428-22-5

Storage: ≤ -10 °C

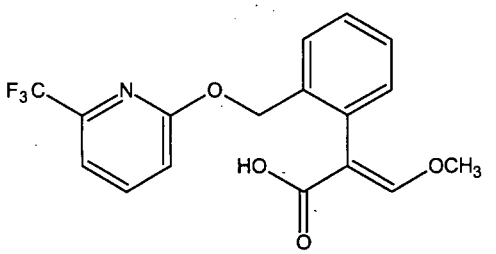


Test Substance: IN-QDK50

***Chemical Abstracts* Name**: 6-(trifluoromethyl)pyridine-1H-2-one

CAS Registry No.: 34486-06-1

Storage: ≤ -10 °C

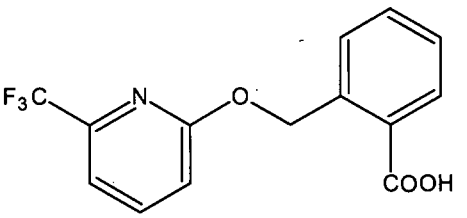


Test Substance: IN-QDY62

***Chemical Abstracts* Name**: (E)-3-methoxy-2-{2-[6-(trifluoromethyl)pyridine-2-yloxymethyl]phenyl}acrylic acid

CAS Registry No.: N/A

Storage: ≤ -10 °C



Test Substance: IN-QDY63

***Chemical Abstracts* Name**: 2-[6-(trifluoromethyl)pyridine-2-yloxymethyl]-benzoic acid

CAS Registry No.: N/A

Storage: ≤ -10 °C

Information pertaining to the characterization and stability of the test substances is archived by E. I. du Pont de Nemours and Company, DuPont Crop Protection, Newark, Delaware. Characterization data were provided by E.I. du Pont de Nemours and Company, DuPont Agricultural Products, Newark, Delaware. Certificates of Analysis, including lot numbers and purity, are included with the study raw data file that will be archived by E. I. du Pont de Nemours and Company.

3.2 Test System

The subject method is applicable for the quantitation of DPX-YT669, IN-QDK50, IN-QDY62, and IN-QDY63 in soil. Clay loam soil (Texas) was chosen to validate the analytical method because it had been previously characterized by Agvise Laboratories.

All control matrices were acquired from sample ID 57.TX.BA.SL.999.A.10.12-20". The control matrix was stored frozen and processed to verify that the control was free of interferences at the appropriate retention times.

SOIL NAME (LOCATION, DUPONT STUDY NO./NOTEBOOK NO.)	TYPE	pH_w	SAND(%)	SILT (%)	CLAY(%)	OM(%)
Texas, DuPont-17457 (Reference 4)	Clay Loam	8.1	39	22	39	0.7

3.3 *Equipment*

The following equipment items were used in the conduct of this independent laboratory validation.

3.3.1 *Instrumentation/Chromatography*

Agilent Series 1100/1200 Liquid Chromatograph with degasser, binary pump, column compartment, (All Agilent, Little Falls, DE) and Leap chilled autosampler

Zorbax® XDB C18 analytical column, 4.6 mm × 50 mm, 1.8-µm diameter packing, (Agilent, Little Falls, DE)

API4000 triple quadrupole mass spectrometer using an electrospray interface (ESI) and Analyst Version 4.1 software (Applied Biosystems, Framingham, MA)

3.3.2 *General Lab Equipment/Devices*

Mettler Analytical Balance, Model XP205DR, for weighing solid standards (Mettler Instrument Corporation, Hightstown, NJ)

Mettler Top-Loading Balance, Model BB2440, for weighing soil samples and salts (Mettler Instrument Corporation)

Platform Shaker - Eberbach Model 6010 - Eberbach Corporation (Ann Arbor, MI)

Nitrogen Evaporator – N-Evap, Model 112 (Organomation Assoc., Berlin, MA)

Gilson Microman positive displacement pipettes, various sizes (Gilson)

Gilson Disposable Pipette Tips, various sizes (Gilson)

Eppendorf repeater pipettes, various sizes (Eppendorf)

Eppendorf Disposable Pipette Tips, various sizes (Eppendorf)

Sonicator - 5200 Ultrasonic cleaner - Branson Ultrasonics Corp. (Danbury, CT)

Bench top Centrifuge – Beckman GP Benchtop– Beckman Coulter (Fullerton, CA)

Multitube Vortexer – VX2500, (VWR)

3.3.3 *Solid-Phase Extraction Equipment/Supplies*

Solid-Phase Extraction Boxes – unknown make

Solid-Phase Extraction Valve Liners – unknown make

Solid-Phase Extraction Reservoirs – unknown make

3.3.4 *Labware*

Disposable Pasteur Pipets, 9-inch length glass, (Fisher Scientific)

VWR Disposable Skirted Centrifuge Tube, 50-mL, Polypropylene, (VWR International)

Glass Centrifuge Tubes – Pyrex® Centrifuge Tubes, graduated, 40-mL capacity, (VWR Scientific)

Syringes – BD, 3cc disposable plastic syringes

Syringe Filters Acrodisc® CR 13mm disposable filter, 0.2µm PFTE & Fisher 25mm disposable filter, 0. 45µm PFTE

HPLC Vials – Perkin Elmer, 1.8mL clear screw thread vial with pre-slit screw caps

3.4 Reagents

Acetonitrile - HPLC grade (Acros)

Formic Acid – 99+%, (Acros)

Formic Acid, Ammonium Salt – 99.995%, (Sigma Aldrich)

Concentrated Hydrochloric Acid, Trace Metal Grade, (Fisher Scientific)

Methanol - Optima® HPLC grade (Fisher Scientific)

Acetone – HPLC grade (Fisher Scientific)

Ethyl Acetate – Optima® HPLC grade (Fisher Scientific)

Ultrapure Water –Milli-Q water, 0.22µ type GV filter (Millipore)

Soil – Texas Clay Loam Soil characterized by Agvise Laboratories (12-20")

3.5 Principles of the Analytical Method

Method using SPE clean-up:

Picoxystrobin and metabolites were extracted from a 5.0-g soil sample with 75/25 acetone/1M HCl and acetone solution at ambient temperature using a wrist-action shaker. Following centrifugation, an aliquot of the extract was purified by solid-phase extraction using an Oasis™ HLB cartridge. The analytes were retained in the cartridge and eluted with methanol and 0.1% formic acid in ethyl acetate. Exactly 1.0 mL of water was added to the eluate and it was evaporated until aqueous (1.0 mL) in an N_2-vap at 30°C. The extract was diluted with 1.0 mL of methanol, 0.8 mL of acetonitrile and appropriate amount of water to bring the volume to 5.0 mL and filtered through a 0.45-µm PTFE disk. The purified extract was analyzed by reversed-phase HPLC using a Zorbax® XDB C18 (4.6 × 50 mm, 1.8-µm particle) column and a mobile phase of 0.1% formic acid-0.1 mM ammonium formate (aq) and methanol. Detection of the analytes was by electrospray mass spectrometry/mass spectrometry (ESI-MS/ MS) in the positive ion mode. Two parent-to-daughter ion transitions per analyte were monitored during analysis.

Before blowing down the eluate, water was added in order to avoid the complete drying of the eluate which will result to losses of the volatile pyridine metabolite IN-QDK50.

Method without SPE clean-up:

Picoxystrobin and metabolites were extracted from a 5.0-g soil sample with 75/25 acetone/1M HCl and acetone solution at ambient temperature using a wrist-action shaker. Following centrifugation, an aliquot of the extract was filtered through a 0.45-µm PTFE disk. The purified extract was analyzed by reversed-phase HPLC using a Zorbax® XDB C18 (4.6 × 50 mm, 1.8-µm particle) column and a mobile phase of 0.1% formic acid-0.1 mM ammonium formate (aq) and methanol. Detection of the analytes was by electrospray mass spectrometry/mass spectrometry (ESI-MS/MS) in the positive ion mode. Two parent-to-daughter ion transitions per analyte were monitored during analysis.

Only low concentrations of formic acid and ammonium formate in the mobile phase were necessary to obtain good MS response for all analytes. Formic acid assisted in the formation of molecular ions; ammonium formate, in reducing sodium adducts of most analytes.

3.6 Modifications, Interpretations, and Critical Steps

DuPont-24804 analytical method was run as written with the following changes:

An amendment was written to the protocol to change the 10X LOQ fortification samples to 40X LOQ fortification samples so as to include this high fortification level.

For trial 3 of IN-QDK50 using the method with SPE clean-up, a minor modification was made to second blow down step. Before evaporation, 2mL of water was added to the extract instead of 1mL. The extract was then blown down to 2mL instead of 1mL.

For the sample analysis using the method with SPE clean-up, per the Sponsor, the HPLC conditions (with SPE cleanup) and the API 4000 LC/MS/MS conditions (without SPE cleanup) were used.

The weighout for stock solution of IN-QDY63 was done using ~7.00 mg (instead of the 10.00mg) due to limited compound. This did not affect the fortification solutions as the aliquot volume was adjusted to make up the difference. All aliquot volumes used to prepare the fortification solutions were adjusted to get as close to the concentrations that the method outlines.

3.7 Instrumentation

3.7.1 Chromatography

Reversed-phase liquid chromatography was used to separate the analytes from co-extractants. A Zorbax® XDB C18 (4.6 × 50 mm, 1.8-µm particle) column was used.

HPLC Conditions with SPE Cleanup

System:	Agilent 1100/1200 HPLC
Column:	Zorbax® XDB C18 (4.6 × 50 mm, 1.8-µm particle)
Column Temperature:	40°C
Injection Volume:	20 µL
Autosampler Temperature	4°C
Conditions:	A: 0.1% formic acid in 0.1 mM ammonium formate B: methanol

Time	%A	%B	Flowrate (mL/min)	Comments
0.00	80	20	1.0	
2.00	80	20	1.0	
2.10	40	60	1.0	
7.10	23	77	1.0	
7.20	5. 0	95	1.0	
9.20	5. 0	95	1.0	
9.30	80	20	1.0	
11.5	80	20	1.0	End Run

DPX-YT669 Retention Time:	~ 6.6 min
IN-QDK50 Retention Time:	~ 3.3 min
IN-QDY62 Retention Time:	~ 5.6 min
IN-QDY63 Retention Time:	~ 6.1 min
Total Run Time:	11.5 min

HPLC Conditions without SPE Cleanup

System:	Agilent 1100/1200 HPLC
Column:	Zorbax® XDB C18 (4.6 × 50 mm, 1.8-µm particle)
Column Temperature:	40°C
Injection Volume:	25 µL
Autosampler Temperature	4°C
Conditions:	A: 0.1% formic acid in 0.1 mM ammonium formate B: methanol

Time	%A	%B	Flowrate (mL/min)	Comments
0.00	70	30	1.0	
1.00	70	30	1.0	
1.10	70	30	1.0	
2.00	60	40	1.0	
2.10	40	60	1.0	
7.50	23	77	1.0	
7.60	5. 0	95	1.0	
8.60	5. 0	95	1.0	
8.70	70	30	1.0	
10.00	70	30	1.0	End Run

DPX-YT669 Retention Time:	~ 6.5 min
IN-QDK50 Retention Time:	~ 2.6 min
IN-QDY62 Retention Time:	~ 5.5 min
IN-QDY63 Retention Time:	~ 6.0 min
Total Run Time:	10.0 min

3.7.2 *LC/MS/MS Analysis*

Analysis of DPX-YT669, IN-QDK50, IN-QDY62 and IN-QDY63 was performed using a Sciex API 4000 LC-MS/MS, equipped with a TurboIonSpray source, and operated in MRM, positive ion mode. Quantitation was based on an average response factor using peak areas supplied by Analyst software version 1.4.1. Calculations were performed using Microsoft Excel. A summary of representative experimental conditions is provided in the following table:

Sciex API 4000 LC/MS/MS Mass Spectrometer Conditions with SPE Cleanup

Analytes	Ions Monitored (amu)	CXP (Collision Cell Exit Potential)	DP (Declustering Potential)	Dwell Time (msec)	Collision Energy
DPX-YT669	368.2 → 145.0	24V	85V	100	32V
DPX-YT669	368.2 → 205.0	35V			15V
IN-QDK50	163.9 → 116.0	19V	70V	100	34V
IN-QDK50	163.9 → 144.0	24V			25V
IN-QDY62	354.1 → 191.0	31V	43V	100	13V
IN-QDY62	354.1→ 145.0	24V			30V
IN-QDY63	298.2 → 135.0	24V	39V	100	30V
IN-QDY63	298.2 → 164.0	27V			18V

Scan Type/Polarity:	MRM/Positive
Ion Source Voltage:	ESI, 5500
Collision Gas (CAD):	Medium
Curtain Gas (CUR):	30 psig
Nebulizer Gas (GS1):	45 psig
Heater Gas (GS2):	70 psig
Source Heater (TEM):	600°C
Interface Heater (IHE):	ON
Resolution Q1:	Low
Resolution Q3:	Low

Sciex API 4000 LC/MS/MS Mass Spectrometer Conditions without SPE Cleanup

Analytes	Ions Monitored (AMU)	CXP (Collision Cell Exit Potential)	DP (Declustering Potential)	Dwell Time (msec)	Collision Energy
DPX-YT669	368.2 → 145.0	24V	85V	100	32V
DPX-YT669	368.2 → 205.0	35V			15V
IN-QDK50	163.9 → 116.0	19V	70V	100	34V
IN-QDK50	163.9 → 144.0	24V			25V
IN-QDY62	354.1 → 191.0	31V	43V	100	13V
IN-QDY62	354.1→ 145.0	24V			30V
IN-QDY63	298.2 → 135.0	24V	39V	100	30V
IN-QDY63	298.2 → 164.0	27V			18V
Scan Type/Polarity:	MRM/Positive				
Ion Source Voltage:	ESI, 4500				
Collision Gas (CAD):	10 psig				
Curtain Gas (CUR):	20 psig				
Nebulizer Gas (GS1):	45 psig				
Heater Gas (GS2):	45 psig				
Source Heater (TEM):	400°C				
Interface Heater (IHE):	ON				
Resolution Q1:	Unit				
Resolution Q3:	Unit				

3.7.3 *Calibration Procedure*

Calibration standards were analyzed at the beginning, middle, and end of each batch and progressed from low to high concentrations. The response factor of each calibration standard was calculated by dividing the analyte peak area of each standard by the analyte concentration for that standard. The average response was calculated for calibration standards injected with each batch.

3.8 Calculations

A standard curve was determined by linear regression (y = mx) for each compound by plotting the peak area response obtained from DPX-YT669, IN-QDK50, IN-QDY62, and IN-QDY63 standards against the corresponding concentration (ng/mL) of each analyte in the standards. The correlation coefficient (R^2) was ≥0.98 for all analyses. The concentration (mg/kg) found in the samples was determined by the following calculation:

$$X = \frac{A \times Rf_{avg}\ (\text{ng/mL/area counts}) \times \text{Extract Volume (mL)} \times \text{Final Volume (mL)} \times \text{Dilution Factor}}{\text{Sample Weight (g)} \times \text{Aliquot Volume (mL)} \times 1000\ \text{ng/µg}}$$

Where: A = Corrected Peak Area Counts

= Peak Area Counts in Sample (ac) – Peak Area Counts in Control (ac)

Rf_{avg} = Average Response Factor

= (∑ Standard Response)/n

$$\text{Standard Response} = \frac{\text{Concentration (ng/mL) of Standard}}{\text{Peak Area Counts (ac)}}$$

n = total number of standards analyzed in a sample set

Percent Recovery was calculated as:

$$\%\ \text{Recovery} = \frac{\text{Analyte Found (mg/kg)}}{\text{Fortification Level}} \times 100$$

For example, the calculation for the concentration (mg/kg) found and percent recovery of DPX-YT669 in soil fortified at 0.01 mg/kg (sample LOQ-2), which was prepared and analyzed on February 16-18, 2010, is shown below:

RF_{avg} of six standards	= 7.28×10^{-5} ng/mL/area counts (ac)
Peak Area Counts of fortified sample	= 17166
Peak Area Counts of Control sample	= 0 (average)
Sample Weight	= 4.99 g
Total Extract Volume	= 35.0 mL
Aliquot Volume	= 5.0 mL
Final Volume	= 5.0 mL
Fortification Level	= 0.010 mg/kg

DPX-YT669 Found =

$$\frac{(17166 - 0) \times 7.28e^{-05} \times 35.0\ \text{mL} \times 5.00\ \text{mL} \times 1}{(4.99\ \text{g} \times 5.00\ \text{mL}) / 1000\ \text{ng/mL}}$$

= 0.0088 mg/kg = 0.01 mg/kg

$$\%\ \text{Recovery} = \frac{0.0088\ \text{mg/kg}}{0.010\ \text{mg/kg}} \times 100 = 87\%$$

APPENDIX 2 COMMUNICATION LOG

The following is a listing of all contacts regarding performance of the method, which took place between the confirmatory laboratory and the Sponsor Representative. Included are reasons for the contact, any changes that resulted, and time of this communication with respect to the progress of the confirmatory trial (i.e., before the first trial, during the first trial, etc.):

(1) 02 Dec 09 – An email from the Sponsor (Elena M. Cabusas) was forwarded to Michele Rudroff (PI) with comments regarding the discussion on the method. Sponsor comments on the method were: 1) Any equivalent equipment substitution may be made as long as the column is not substituted; 2) Any substitution that may not be equivalent will be cleared with the Sponsor before the substitute is made; 3) Glassware can be washed according to ABC's SOP and does not need to follow the method; 4) 75/25 Acetone/1M Hydrochloric Acid will be used as the extraction solution; 5) All stock solutions will be prepared in acetonitrile; 6)Texas clay loam soil will be used as the control soil; 7) A post fortified LOQ will be included in each set; 8) The ILV will be set up and run the same as the method outlines for sample analysis; 9) Sample extracts will be considered stable for 3 days if stored frozen. This communication took place before the first SPE clean-up trial.

(2) 18 Dec 09 – An email from the Sponsor was forwarded to the (PI) with comments regarding the discussion on the quantitation approach. Sponsor comments on the equipment substitution sheet were: 1) N-evap water—not certain of room temperature, but drying can be done up to 30°C; 2) PTFE syringe filter – a 0.45 µm can also be used; 3) With regard to the MS operating conditions, the total ion current for the quantitation /molecule is correct. With the use of TIC for quantitation, which sums the signal of 2 transition ions per molecule, typically there is signal improvement for QDK50 (the least responsive analyte). This communication took place before the first SPE clean-up trial.

(3) 22 Dec 09 – An email was sent to the Sponsor by the PI about additional questions regarding the ILV. The Sponsor replied on 28Dec09 with the following comments: 1) Please clarify if the washes ABC proposes to use are after every LC injection; 2) Injection volume can be increased up to 25 µL if there is an issue with the limiting analyte's response; 3) Quantification by average response factor will be used; 4) Methanol will be used as mobile phase B. This communication took place before the first SPE clean up trial.

(4) 28Dec09 – The PI talked to the Sponsor on the phone and discussed that the washes do occur after every LC injection. The Sponsor indicated that these washes will be acceptable. Also, it was discussed that there would be no weighting on the curve, only the average response factor is to be used. The PI asked the Sponsor to send the updated method. This communication took place before the first SPE clean-up trial.

APPENDIX 2 COMMUNICATION LOG (CONTINUED)

(5) 19 Jan 10 - An email was sent by the Sponsor to the PI attaching an updated draft of the soil method and stated the actual field soil dissipation work used a fortification of 40 ng/g instead of the 10 ng/g in the full method validation of 2 soils and we may have to raise the highest fortification of 40 ng/g. The PI replied and attached the LCMS data packet that shows the conditions and integrations of the standards for all 4 analytes. IN-QDK50 was very close to the retention listed in DuPont's method, but IN-QDY62, IN-QDY63 and DPX-YT669 were eluting close together and are not the same retention listed in the method. The Sponsor replied back suggesting to increase the injection volume to 15-20 µL using the conditions (LC/SM/MS with SPE cleanup) stated in the recent draft method, to run only the low and high standards (0.5 and 50 ng/mL), send chromatograms of the transitions ions of QDK50 of the 0.5 ng/mL, send the R2 value of calibration curves from the previous run, and with regard to data processing to use the TIC, using the sum of 2 ions. This took place before the first SPE clean-up trial.

(6) 25 Jan 10 - An email was sent from the PI to the Sponsor with question regarding the updated method. In the materials section 3.1, the Zorbax column lists part number 922975-902, but the column ABC is using is part number 927975-902. The Sponsor responded that they were using that same part number. This took place before the first SPE clean-up trial.

(7) 27 Jan 10 – An email was sent from the Sponsor to the PI indicating that the high fortification level needs to be changed from 0.10 mg/kg to 0.40 mg/kg and an amendment needs to be drafted. This took place before the first SPE clean-up trial.

(8) 2-Feb-10 – An email was sent by the PM (Del Koch) letting the Sponsor know that the data (standards) had been reprocessed using the TIC sum of 2 ions as requested. The Sponsor replied to the PI and said that standards of 0.5–30 ppb should be used. The PI responded back clarifying that the method stated 0.5–50 ng/mL should be used. The instrument conditions were also clarified to the Sponsor. The Sponsor responded and stated that 0.5–50 ng/mL is right to use and we should also check the MS response of the limiting analyte and make sure the S/N is $\geq$3. This took place before the first SPE clean-up trial.

(9) 3 Feb 10 – A telephone call from the Sponsor to the PI had the following action items: to check the S/N ratio (3:1), prepare an amendment to have the high fortification level changed to 40xLOQ (0.40 mg/kg), provide the r^2 data for linear regressed curves, and provide the chromatograms of the ion transitions for DPX-YT669, IN-QDY62. This took place before the first SPE clean-up trial.

APPENDIX 2 COMMUNICATION LOG (CONTINUED)

(10) 10 Feb 10 - The PI then emailed to confirm the Sponsor action items before proceeding. The Sponsor emailed back that calibration curves need to have the line equation and r^2 values of each (concentration and peak area). For residue calculations, use the average response factor method. The acceptance criteria for using average response factor method for quantitation are: % RSD of standards response factor <20% and a linear curve of r^2 >0.98. For the limiting analytes (QDY62 and YT669, least MS responders) of the 0.5 ppb standard (lowest standard concentration) please send the chromatograms (integrated) of each transition ions- e.g. YT669 (368.2) are 205.0 and 145.0; QDY62 (354.1) are 131.0 and 145.0 and need to know if each has S/N ration of ≥3. For the ILV, target S/N of the peaks (TIC; sum of 2 transitions) of limiting analyte(s) at 0.5 ppb is ≥3 and at LOQ, the S/N of these limiting analytes about 10. This took place before the first SPE clean-up trial.

(11) 11 Feb 10 – In an email the PI attached the data the Sponsor requested. The Sponsor emailed back with an attached updated draft version of the method with procedure clarifications. The Sponsor needs to confirm the last 8 chromatograms sent are Confirmatory 1 ions. If those are the analytes' responses from a 0.5 ng/mL standard, they are acceptable. Based on that data, there is no need to adjust injection volume. The Sponsor suggests to have the r^2 value with 3 decimal places (e.g. 0.9990). The PI emailed back with updated r^2 values and chromatograms and asked if they could move forward with the ILV using these 3200 MS conditions on the API4000. The Sponsor replied by email stating to use the 3200 MS conditions. The Sponsor also reviewed the amendment and indicated it is ready for signing. This took place before the first SPE clean-up trial.

(12) 15 Feb 10 – The signed amendment was forwarded to the Sponsor from the PI. The Sponsor returned the amendment with confirmation signature. This took place before the first SPE clean-up trial.

(13) 17 Feb 10 – In an email the PM sent data to the Sponsor regarding the corrected r^2 values. The Sponsor replied back to verify that the purity had been taken into account before preparing the standards. The PI responded to conclude that purity had been taken into account. This took place before the first SPE clean-up trial.

(14) 25-Feb 10 – The PM notified the Sponsor that the first ILV was posted on Quickplace for review. This took place after the first SPE clean-up trial.

(15) 1 Mar 10 – The PM notified the Sponsor that the first ILV data was re-posted on Quickplace showing the data for IN-QDY62 using only one of the ion transitions. The Sponsor indicated these results are acceptable for IN-QDY62 and the trial will be repeated for IN-QDK50. This took place after the first SPE clean-up trial.

(16) 8 Mar 10 – The PM notified the Sponsor that the second trial for IN-QDK50 had been posted to Quickplace and that the recoveries are still low. The Sponsor called the PI and left several questions on the voicemail. This took place after the second SPE clean-up trial.

APPENDIX 2 COMMUNICATION LOG (CONTINUED)

(17) 10 Mar 10 – The PI responded to the Sponsor's questions summed up below: 1) The first blow down was blown down to 2-mL and the second was only blown to 1-mL; 2) The temperature used during the blow down is 30°C; 3) The elution is done using 1 drop a second and the vacuum is used to get the elution started, but then is done using gravity. This took place after the second SPE clean-up trial.

(18) 11 Mar 10 – The PM talked to the Sponsor over the phone. The discussion included a change to the extraction method for the third ILV trial. For the second blowdown, 2 mL of water will be added (rather than 1 mL) and blow down to 2 mL. The N_2 needs to be regulated to avoid any bubbling or splashing at the surface. The temperature will remain at 30°C. The Sponsor also indicated that the elution needs to be started by vacuum but then allow gravity to elute. This took place after the second SPE clean up-trial.

(19) 16 Mar 10 – The PI emailed the Sponsor to let her know that the instrument was having issues and the data will be delayed. The PI emailed the Sponsor again on 18Mar10 to let her know that the instrument was back up and running and would run overnight. This took place after the third SPE clean-up trial.

(20) 19 Mar 10 – The PI emailed the PM with the data and chromatograms from the third ILV trial. The controls have high analyte peaks that could possibly be contamination since we've never seen these peaks before and it is the same lot of soil. This took place after the third SPE clean-up trial.

(21) 22 Mar 10 – The PM contacted the PI to confirm that none of the original extracts (pre clean-up) were kept. The PM had spoken with the Sponsor early that day and had indicated that we would conduct a Trial 1 for all analytes on the extraction without SPE clean-up. A post fortification sample will be prepared as well. This took place before the first trial without SPE clean-up.

(22) 24 Mar 10 – The PI contacted the Sponsor to ask the following questions: 1) Should the curve range be changed to the non-SPE method curve range? Some additional MS work would need to be conducted in order to see the lower range; 2) Clarification is needed on Step 13 of the extraction method. This took place before the first trial without SPE clean-up.

(23) 25 Mar 10 – The Sponsor called the PI to confirm that the lower curve range is to be used with this extraction and that the current extraction procedure is to filter the extracts from Step 8, there is no blow down step. This took place before the first trial without SPE clean-up.

(24) 29 Mar 10 – The PI provided the Sponsor with the data and chromatograms from the lower curve range. The results were discussed and the r^2 values were provided to the Sponsor. The Sponsor agreed to proceed with the ILV. This took place before the first trial without SPE clean-up.

APPENDIX 2 COMMUNICATION LOG (CONTINUED)

(25) 1 Apr 10 – The PI provided the results of the ILV to the Sponsor. The Sponsor agreed that the data looked good and that we could proceed with the report. This took place after the first trial without SPE clean-up. The Sponsor called the PI to discuss the intensity settings on the chromatograms for all analytes. The PI then called the Sponsor back on 2Apr10 with the MS analyst to confirm what the Sponsor had requested. The Sponsor then requested that the intensity be set according to the LOQ for DPX-YT669 at whichever intensity we deemed fit, and the rest of the analytes will either go above this or will be set at the same intensity. This took place after the first trial without SPE clean-up.