ANALYTICAL METHOD FOR THE DETERMINATION OF DPX-QGU42 IN WATER USING LC/UV

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1.0 SUMMARY

The purpose of this study was to develop an analytical method for the detection and quantitative analysis of DPX-QGU42 in water.

DPX-QGU42 was extracted from water samples using solid phase extraction (SPE). The extract was evaporated under a stream of nitrogen to dryness. The extracts were reconstituted using acetonitrile and diluted with water. An aliquot of the extract was transferred to an auto-sampler vial for analysis. DPX-QGU42 was separated from co-extracts by reversed phase liquid chromatography (LC) and detected using ultraviolet (UV) detection. The Limit of Quantitation (LOQ) was 0.010 μg/g (ppm). The Limit of Detection (LOD) was estimated to be 0.003 μg/g (ppm).

2.0 INTRODUCTION

The structure, CAS name, CAS registry number, and various physical properties of DPX-QGU42 can be found in Appendix 1. The method was validated on ground and surface water.

DPX-QGU42 was extracted from water samples using Solid Phase Extraction (SPE). The extract was evaporated under a stream of nitrogen to dryness. The extracts were reconstituted using acetonitrile and diluted with water. An aliquot of the extract was transferred to an auto-sampler vial for analysis. DPX-QGU42 was separated from co-extracts by reversed phase liquid chromatography (LC) and detected using ultraviolet (UV) detection. The Limit of Quantitation (LOQ) was 0.010 μg/g (ppm). The Limit of Detection (LOD) was estimated to be 0.003 μg/g (ppm).

A separate confirmation method has been developed (Reference 1).

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified. Note any specification 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

Instrumentation

LC system, HP1200 with temperature controlled autosampler (Agilent Technologies, Wilmington, DE)

VWR brand Vortex Geni 2 Mixer, Cat. No. 58815-178 (VWR Scientific Co., Bridgeport, NJ)

Biohit Proline Electronic Pipettors, Variable Volume with Tip Ejector, Vanguard, 5.0-100 µL Cat. No. 53495-200, 50-1000 µL Cat. No. 53495-205 and 0.10-5.0 mL Cat. No. 53495-290 (VWR Scientific Co., Bridgeport, NJ)

Chromatographic Supplies

HPLC Column: 3.0 mm i.d. × 15 cm, MacMod ACE 3 C18-PFP analytical column Part # ACE-1110-1503 (MacMod, Chadds Ford, PA)

HPLC Vials, Target DP Amber Kit, T/S/T Septa, 100 PK, Part # 5182-0556 (Hewlett-Packard, Wilmington, DE)

Solid-Phase Extraction Equipment

Supelclean™ Envi™-Carb cartridge, 0.25g/ 6-mL, Lot SP1759D, PN 57092 (Bellefonte, PA). **Do not substitute.**

Solid Phase Extraction Plastic Reservoir – 75-mL size, Catalog No. 1213-1012 (Varian, Harbor City, CA)

Reservoir Adapters – Catalog No. 1213-1003 (Varian, Harbor City, CA)

Visiprep 12 port SPE vacuum manifold, PN 5-7030 (Supelco, Bellefonte, PA)

Labware

Pyrex Brand Single Metric Scale Graduated Cylinders, 10-mL and 100-mL capacity, Cat. No. 24709-715 and 24709-748, respectively (VWR Scientific Co., Bridgeport, NJ)

VWR brand Disposable Pasteur Pipettes, Borosilicate Glass, 9 in, Cat. No. 53283-914 equipped with 2 mL, 13 X 32 mm rubber bulbs, Cat. No. 56310-240 (VWR Scientific Co., Bridgeport, NJ)

Erlenmeyer Flasks, Polycarbonate 125-mL capacity, Cat. No. 89095-258 (VWR Scientific Co., Bridgeport, NJ)

Centrifuge tubes, Polystyrene 15-mL capacity, Cat. No. 21008-930 (VWR Scientific Co., Bridgeport, NJ)

3.2 Reagents and Standards

Equivalent reagents may be substituted for those listed below. To determine if impurities in substituted reagents interfere with analyses, appropriate amounts of the solvents should be taken through the entire method using the chromatographic conditions specified in this report.

Acetone - EM Omni Solv®, HPLC-grade acetone, #AX0116-1 (EM Science, Gibbstown, NJ)

Acetonitrile (ACN) - EM Omni Solv®, HPLC-grade acetonitrile, #AX0142-1 (EM Science, Gibbstown, NJ)

Formic Acid - Guaranteed Reagent 98% minimum, #FX0440-5 (EM Science, Gibbstown, NJ)

Methanol - EM Omni Solv®, HPLC-grade methanol, #MX0488-1 (EM Science, Gibbstown, NJ)

Phosphoric Acid – Baker Analyzed, #0260-01 (JT Baker, Phillipsburg, NJ)

Toluene – Drisolve, TX0732-6 (EM Science, Gibbstown, NJ)

Water - EM Omni Solv®, HPLC-grade water, #WX0004-1 (EM Science, Gibbstown, NJ)

DPX-QGU42 reference substance (Dash 126, 98.9% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

3.3 Safety and Health

No unusually hazardous materials are used in this method. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment used. An MSDS sheet for the analytes is available from DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company.

4.0 METHOD

4.1 Principles of the Analytical Method

DPX-QGU42 was extracted from water samples using SPE. The extract was evaporated under a stream of nitrogen to dryness. The extracts were reconstituted using acetonitrile and diluted with water. An aliquot of the extract was transferred to an auto-sampler vial for analysis. DPX-QGU42 was separated from co-extracts by reversed phase liquid chromatography and detected using UV detection.

4.2 Analytical Procedure

4.2.1 Glassware and Equipment Cleaning

Glassware should be scrubbed with a brush using a laboratory soap solution, rinsed two to five times with tap water, rinsed with distilled or deionized water and finally rinsed with acetone or another suitable solvent and allowed to air dry prior to each use.

4.2.2 *Preparation of Solutions*

The following solutions should be prepared monthly and stored at room temperature unless stated otherwise:

Mobile Phase A: 1.0 % aqueous phosphoric acid solution - Add 10-mL of phosphoric acid to 990 mL of water and mix the resulting solution to homogeneity.

1.0% Aqueous Formic Acid: Add 10-mL of concentrated formic acid to 990 mL of water and mix the resulting solution to homogeneity.

50% 1.0% Aqueous Formic Acid/ 50% Acetonitrile: Add 500 mL of 1.0% aqueous formic acid to 500 mL of acetonitrile and mix the resulting solution to homogeneity.

90% Toluene / 10% Methanol/ 1% Formic Acid: Add 450 mL of toluene to 50 mL of methanol and add 5-mL of formic acid and mix the resulting solution to homogeneity.

4.2.3 *Preparation and Stability of Stock Standard*

Use Class A volumetric flasks when preparing standard solutions.

Prepare standard stock solutions by accurately weighing 10 ± 0.01 mg of DPX-QGU42 into a 100-mL volumetric flask using an analytical balance. Record the accurate weight of the standard. Dissolve the standards in approximately 50 mL of HPLC-grade acetonitrile. After dissolving, bring the solution to a volume of 100 mL using HPLC-grade acetonitrile and invert the volumetric flask to mix the solution to homogeneity. The standard solutions are stable for approximately 3 months when stored in a freezer at approximately -20°C immediately after each use. The concentration of DPX-QGU42 in solution is 100 µg/mL.

4.2.4 *Preparation and Stability of Intermediate and Fortification Standards*

Use Class A volumetric flasks when preparing standard solutions.

Prepare a 1.0-µg/mL DPX-QGU42 intermediate standard in acetonitrile by pipetting 1.00 mL of the 100.0-µg/mL stock standard into a 100-mL volumetric flask. Dilute the standard to approximately 50-mL with acetonitrile and add 1.0-mL of concentrated formic acid. Bring to volume using HPLC-grade acetonitrile and mix to homogeneity.

Alternate or additional solutions may be prepared as needed. All standard solutions prepared in acetonitrile or acetonitrile are stable for approximately 3 months if stored in a freezer at approximately -20°C immediately after each use.

4.2.5 *Preparation and Stability of Calibration Standards*

Prepare the calibration standards as showed in the table below (alternative or additional standards may be prepared as needed):

Standard Used (µg/mL)	Volume Pippetted (µL)	Volume of 50%Water/ 50% Acetonitrile added (µL)	Final Concentration (µg/mL)
100	200	800	20.0
100	100	900	10.0
100	50	950	5.0
100	10	990	1.0
10	50	950	0.50
10	25	975	0.25

These standard solutions should be freshly prepared with each sample set and stored approximately 4°C prior to use. Each of the calibration standards was vortex mixed for 30 seconds prior to filling the auto-sampler vials.

4.2.6 *Source of Samples*

Water control samples were intended to represent the three water types: ground surface and drinking. The waters selected were:

Water type	Identification
Surface	White Clay Creek, DE
Ground	Kemblesville Well Water, PA

Water characterization information for the surface and ground water are provided in Appendix 3.

4.2.7 *Storage and Preparation of Samples*

Water samples should be in a refrigerator at 4°C until use. Samples were vigorously shaken prior to sub-sampling

4.2.8 *Sample Fortification Procedure*

All fortifications were made directly to the 50-mL water sample. Fortified samples were prepared using a 100-µg/mL standard solution.

Fortification Level (µg/kg)	Volume of Standard (mL)
10	0.005
100	0.050

4.2.9 *Analyte Extraction Procedure*

1. Accurately measure 50.0-mL (± 1%) of water into a 125-mL Erlenmeyer flask. Fortify samples if necessary. Cap and shake the samples vigorously.
2. Add 0.10-mL of concentrated formic acid and 50-mL of acetonitrile to each sample. Mix thoroughly for 30-seconds.
3. Attach a 6-mL, 0.25-g Envi™-Carb cartridge to an SPE manifold. Using an adapter place a 75-mL reservoir above the SPE to aid in loading the sample. Condition the cartridges with 5-mL of acetonitrile followed by 10-mL of 50:50 1.0% aqueous formic acid: acetonitrile. **Do not let the cartridge go to dryness**.
4. Using gravity flow, allow the sample to pass through the cartridge at a flow rate of 2-5 mL/min. Rinse the Erlenmeyer flask with 5-mL of 50:50 1% aqueous formic acid: acetonitrile and load the rinse into the reservoir just before all of the sample passes through. **Do not let the cartridge go to dryness**.
5. Wash the SPE cartridge with 5-mL of acetonitrile. Place a 15-mL centrifuge tube under the SPE and using gravity flow elute with 5-mL of acetone followed by 5-mL of 90% Toluene / 10% Methanol/ 1% Formic Acid solution.
6. Remove the 15-mL centrifuge tubs and evaporate the extract to dryness using a flow of nitrogen in an N-Evap at 30-35°C. Add 0.5-mL of acetonitrile and vortex mix for 30 seconds and sonicate the extract for 5-minutes. Dilute the extract to 1.0-mL with 1% aqueous formic acid. Vortex for 30-seconds and transfer an aliquot of the extract using a disposable pipette into an HPLC vial.
7. Analyze the solution for DPX-QGU42 by LC/UV as described in the following section.

Extracts will be stable for approximately 72 hours if stored at 4°C.

4.3 Instrumentation for the Method

4.3.1 *LC/UV Analysis*

Reversed-phase chromatography was used to separate DPX-QGU42 from co-extracts. The column choice reflected experimental results indicating preferred separation from co-extractants. Alternative chromatographic conditions can be used, provided the analytical method is validated and provides acceptable recoveries as defined by regulatory method guidelines.

SYSTEM:	Agilent 1200 HPLC
COLUMN:	3.0 mm i.d. × 15 cm, MacMod C18-PFP
COLUMN TEMPERATURE:	40 °C
SAMPLE TEMPERATURE	4 °C
INJECTION VOLUME:	0.075 mL
FLOW RATE:	1.00 mL/min
CONDITIONS:	A: 1.0 % aqueous phosphoric acid B: acetonitrile

Time	%A	%B	Flow (mL/Min.)
0.0	60	40	1.0
1.0	60	40	1.0
8.0	10	90	1.0
12.0	10	90	1.0
12.5	60	40	1.0

DPX-QGU42 RETENTION TIME:	5.4 minutes
TOTAL RUN TIME:	20.0 minutes

SIGNALS:	BAND WIDTH	REFERENCE	Band Width
210 NM	4	360	100
255 NM	16	360	100
260 NM	8	360	100

4.3.2 *Calibration Procedure and Sample Analysis*

A 0.25-µg/mL chromatographic standard should be analyzed prior to the start of analyses to establish that the instrument is working properly. Operating parameters must be tailored to the particular instrument used, especially if it is to be an alternate vendor's instrument, and should be checked daily. Begin each sample set by injecting a minimum of 2 calibration standards. The first injection should always be disregarded.

4.4 Calculations

4.4.1 *Methods*

Average Response Factor (RF_{Avg}) was calculated as follows:

$$RF_{Ave} = \frac{(\text{Conc. A} \div \text{A Corrected Area A}) + (\text{Conc. B} \div \text{Corrected Area B}) + (\text{Conc. C} \div \text{Corrected Area C}) + (\text{Conc. D} \div \text{Corrected Area D})}{\text{Total Number of Standards Injected}}$$

Corrected Area = (Area in the standard – Area on the control)

ug/g (ppb) found was calculated as follows:

$$\mu\text{g/g Found} = \frac{(\text{Corrected Peak Area}) \times (RF_{Ave}) \times (\text{Final Volume}) \times (\text{Aliquot Factor})}{(\text{grams of Sample})}$$

In the event a peak was detected in the control, a corrected peak area was used to calculate ppb found for freshly fortified samples. The corrected peak area is the area of the fortified sample minus the area of the control sample.

The percent recovery found was calculated as follows:

$$\% \text{ Recovery} = \frac{(\mu g/g \text{ Found})}{(\mu g/g \text{ Fortified})} \text{ x } 100$$

4.4.2 *Example*

For a Water sample fortified with DPX-QGU42 at 0.010 µg/g) [Date analyzed 25-Jan-12, LOQ 1KWW Fortification (LOQ 1 Well)], the concentration found was calculated as follows:

Average Response Factor was calculated as follows:

$$RF_{Ave} = \frac{(0.25\mu g/mL \div 20) + (0.50\mu g/mL \div 41) + (1.0\mu g/mL \div 80) + (5.0\mu g/mL \div 391) + (10\mu g/mL \div 793) + (20\mu g/mL \div 1587)}{6}$$

(AC ≡ Area Counts)

RF_{Avg} =1.25452e^{-2} µg/mL/AC

µg/g (ppm) found was calculated as follows:

$$\text{ug/g Found} = \frac{(42.72 \text{ AC}) \times (1.25452\text{e} - 2\ \mu g/mL/AC) \times (1.0 \text{ mL}) \times (1)}{(50 \text{ grams})}$$

µg/g Found = 0.0107186

The percent recovery found was calculated as follows:

$$\% \text{ Recovery} = \frac{(0.0107186\ \mu g/g)}{(0.010\ \mu g/g)} \text{ x } 100$$

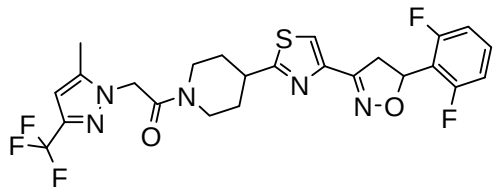
% Recovery = 107%

(percent recoveries are rounded to the nearest whole number in Table 1, without rounding the concentration or ppb found)

9.0 REFERENCES

1. Analytical Method for the Determination of DPX-QGU42 and Metabolites in Water Using LC/MS/MS, DuPont-32124, 2011, E. I. du Pont de Nemours and Company, Wilmington, Delaware.
2. [^{14}C]-DPX-QGU42: Degradability and Fate in the Water/Sediment System, Cleland, H, DuPont-28073, 2011

APPENDIX 1 STRUCTURE AND PROPERTIES OF DPX-QGU42

COMMON NAME	DPX-QGU42
STRUCTURE	
DPX NUMBER	DPX-QGU42
FORMULA	$C_{24}H_{22}F_5N_5O_2S$
MOLECULAR WEIGHT	539.53
CAS NUMBER	1003318-67-9