ANALYTICAL METHOD FOR THE DETERMINATION OF DPX-MP062 [75% DPX-KN128 (INDOXACARB) AND 25% IN-KN127] AND METABOLITES IN-MS775, IN-JT333, IN-MP819, IN-JU873, AND IN-KG433 IN GROUND, SURFACE, AND DRINKING WATERS USING LC/MS/MS

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REASON FOR SUPPLEMENT NO. 1

This supplement adds confirmation data for the analysis of DPX-MP062 and metabolites IN-MS775, IN-JT333, IN-MP819, IN-JU873, and IN-KG433 in water to the method described in DuPont-9605, Revision No. 1. This supplement also recalculates recovery data for DPX-MP062 and IN-MS775 using a single ion transition. The original method report used total ion current to calculate recoveries for DPX-MP062 and IN-MS775. Calibration curves are presented for the quantitative and confirmatory ion transitions.

1.0 SUMMARY

The purpose of this supplement is to add second ion confirmation data for the analysis of DPX-MP062 and metabolites IN-MS775, IN-JT333, IN-MP819, IN-JU873, and IN-KG433 in water. This supplement also recalculates recovery data for DPX-MP062 and IN-MS775 in water using a single ion transition. The original method report used total ion current to calculate recoveries for DPX-MP062 and IN-MS775.

All data presented in this supplement was collected as part of DuPont-9605, Revision No. 1. The electronic data was reanalyzed to generate the result presented in this supplement report, no new experiments were conducted. A complete summary of the analytical method used to generate this data is included in DuPont-9605, Revision No. 1.

The Limit of Quantitation (LOQ) was 0.050 µg/L (ppb) for all six analytes. The Limit of Detection (LOD) was estimated to be 0.02 µg/L (ppb).

3.0 METHOD

3.1 Principles of the Analytical Method

The quantitative analysis of DPX-MP062, IN-MS775, IN-JT333, IN-MP819, IN-JU873, and IN-KG433 using one analytical method required some special sample handling procedures as described below.

DPX-MP062, IN-MS775, IN-JT333, IN-MP819, IN-JU873, and IN-KG433 were extracted from the water samples by filtration through an Oasis HLB (0.50 g) solid phase extraction (SPE) cartridge. DPX-MP062 and IN-JT333 have a tendency to adhere to glass and plastic surfaces when in water. Therefore, the samples were diluted with acetonitrile (80:20 water:acetonitrile) prior to loading onto the SPE cartridge. In addition, it is good practice to measure out all washes and elution steps using the graduated 50-mL sample centrifuge tube. The use of extra sample handling accessories such as SPE solvent reservoirs and adapters should be avoided to minimize surface area. All samples or extracts should be loaded directly into the SPE cartridges. The pH of the samples was adjusted by the addition of 40 µL of acetic acid. When the pH of the samples was not adjusted low recoveries were observed for IN-KG433 and IN-JU873 for some water types. Following the load step, the cartridges were washed with 70:30 water:acetonitrile followed by hexane. The analytes were eluted in 25-mL of acetonitrile. The extracts were evaporated under a flow of nitrogen to a volume of approximately 100-µL. One mL of acetonitrile was added to the extracts. The extracts were vortexed, sonicated, and then diluted to 2 mL using water. DPX-MP062, IN-MS775, IN-JT333, IN-JU873, and IN-KG433 were detected by positive ion electrospray (ESI) mass spectrometry/mass spectrometry (MS/MS). IN-MP819 did not produce sufficient single in ESI it was detected by positive ion Atmospheric Pressure Chemical Ionization (APCI) mass spectrometry/mass spectrometry (MS/MS).

3.2 Analytical Procedure

3.2.1 LC/MS/MS Analysis

The quantitative analysis of DPX-MP062 and metabolites was performed using a Micromass Quattro II LC/MS/MS system. The system parameters were adjusted while a solution of each analyte was infused directly into the ion source. The solution

composition was 50% acetonitrile/50% water, so that it would approximate the composition of the mobile phase at the retention time of the analyte. The solution concentration was approximately 2 µg/mL. Extensive in-source fragmentation was observed for IN-MP819 in the APcI source. As a result, fragments generated in the source were used for quantitative analysis. A summary of the experimental conditions is provided in the following table:

Micromass Quattro LC ESI-LC/MS/MS Mass Spectrometer Conditions

Analytes	Ions Monitored	Cone Voltage	Collision Energy	Dwell (Seconds)
DPX-MP062	**528.0→ 217.8 ± 0.5 AMU**	**32V**	**22V**	**0.30**
	528.0→ 202.9 ± 0.5 AMU	32V	40V	0.30
IN-MS775	**411.9→ 208.8 ± 0.5 AMU**	**40V**	**14V**	**0.30**
	411.9→ 190.8 ± 0.5 AMU	40V	22V	0.30
IN-JT333	470.0→ 149.8 ± 0.5 AMU	25V	48V	0.30
	470.0→ 266.9 ± 0.5 AMU	**25V**	**12V**	**0.30**
IN-JU873	458.0→ 149.0 ± 0.5 AMU	42V	45V	0.30
	458.0→ 204.8 ± 0.5 AMU	**42V**	**22V**	**0.30**
IN-KG433	516.0→ 220.9 ± 0.5 AMU	35V	30V	0.30
	516.0→ 280.8 ± 0.5 AMU	**35V**	**18V**	**0.30**
Ion Mode:	Positive			
Capillary Voltage:	3.50 KV			
Detector Voltage:	750 V			
Source Temperature:	80°C			
Collision Gas Pressure:	1.3e-3 to 2.6e-3mBar			
Drying Gas Flow:	350 L/h			

Micromass Quattro LC APcI-LC/MS/MS Mass Spectrometer Conditions

Analytes	Ions Monitored	Cone Voltage	Collision Energy	Dwell (Seconds)
IN-MP819	238.5→ 194.2 ± 0.5 AMU	23V	11V	0.20
	238.5→ 131.2 ± 0.5 AMU	**23V**	**19V**	**0.20**
Ion Mode:	Positive			
Corona Voltage:	3.25 KV			
Detector Voltage:	750 V			
Probe Temperature:	600°C			
Source Temperature:	150°C			
Collision Gas Pressure:	1.6e-3 to 2.6e-3mBar			
APCI Sheath Gas Flow:	125 L/h			
Drying Gas Flow:	350 L/h			

The instrument was operated in MS/MS-(MRM) positive ion mode for quantitative analysis. Peak area was used for quantitation. **In this supplement report the**

quantitation ion transition is displayed in bold face print. The confirmation ion transition is displayed in plain text.