3.0 MATERIALS

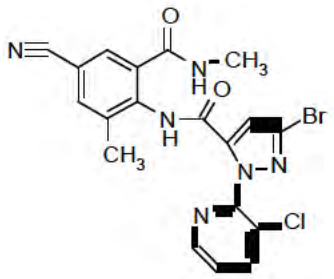
Test Item(s)

Common name: Cyantraniliprole (**DPX-HGW86**)

Chemical name (IUPAC): 3-Bromo-N-[4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide

CAS-Registry-No.: 736994-63-1

Structural formula:



Molecular formula: $C_{19}H_{14}BrClN_6O_2$

Molecular weight: 473.7 g/mol

Common name: **IN-J9Z38**

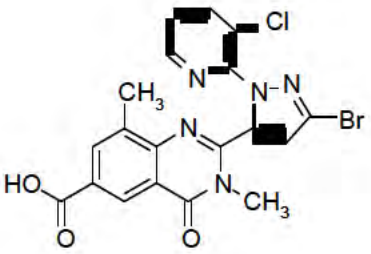
Chemical name (IUPAC): 2-[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]-3,8-dimethyl-4-oxo-3,4-dihydroquinazoline-6-carbonitrile

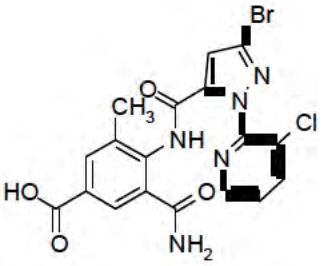
CAS-Registry-No.: not available

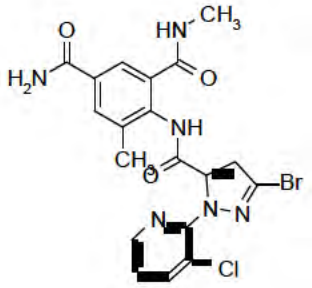
Structural formula:

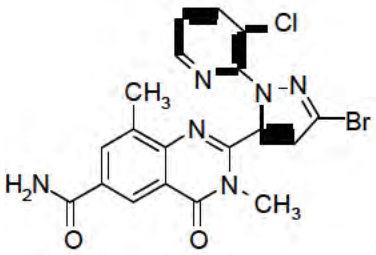
Molecular formula: $C_{19}H_{12}BrClN_6O$

Molecular weight: 455.7 g/mol

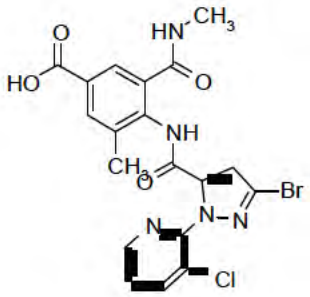
Common name:	**IN-K5A78**
Chemical name (IUPAC):	2-[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]-3,8-dimethyl-4-oxo-3,4-dihydroquinazoline-6-carboxylic acid
CAS-Registry-No.:	not available
Structural formula:	
Molecular formula:	$C_{19}H_{13}BrClN_5O_3$
Molecular weight:	474.7 g/mol

Common name:	**IN-K5A79**
Chemical name (IUPAC):	4-({[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl] carbonyl}amino)-3-carbamoyl-5-methylbenzoic acid
CAS-Registry-No.:	not available
Structural formula:	
Molecular formula:	$C_{18}H_{13}BrClN_5O_4$
Molecular weight:	478.7 g/mol

Common name:	**IN-JCZ38**
Chemical name (IUPAC):	4-({[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]carbonyl}amino)-N3,5-dimethylisophthalamide
CAS-Registry-No.:	not available
Structural formula:	
Molecular formula:	$C_{19}H_{16}BrClN_6O_3$
Molecular weight:	491.7 g/mol

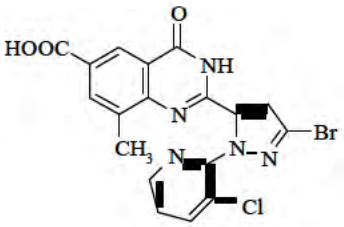
Common name:	**IN-K5A77**
Chemical name (IUPAC):	2-[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]-3,8-dimethyl-4-oxo-3,4-dihydroquinazoline-6-carboxamide
CAS-Registry-No.:	not available
Structural formula:	
Molecular formula:	$C_{19}H_{14}BrClN_6O_2$
Molecular weight:	473.7 g/mol

Common name:	**IN-JSE76**
Chemical name (IUPAC):	4-({[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]carbonyl}amino)-3-methyl-5-(methylcarbamoyl)benzoic acid
CAS-Registry-No.:	not available
Structural formula:	



Molecular formula:	$C_{19}H_{15}BrClN_5O_4$
Molecular weight:	492.7 g/mol

Common name:	**IN-PLT97**
Chemical name (IUPAC):	2-[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]-8-methyl-4-oxo-3,4-dihydroquinazoline-6-carboxylic acid
CAS-Registry-No.:	not available
Structural formula:	



Molecular formula:	$C_{18}H_{11}BrClN_5O_3$
Molecular weight:	460.7 g/mol

Reference Items

The certified reference items of DPX-HGW86 (cyantraniliprole) and its metabolites IN-J9Z38, IN-JCZ38, IN-JSE76, IN-K5A77, IN-K5A78, IN-K5A79, IN-PLT97 were supplied by DuPont Crop Protection, Stine Haskell Research Center, Newark, DE, USA.

Sponsor Code Name:	DPX-HGW86-307
Batch-No.:	D100082-045A
Purity:	99.2 %
Certificate of:	29 Oct 2009
Storage at test facility:	≤ -18 °C
Expiration Date:	23 Sep 2015

Sponsor Code Name:	IN-J9Z38-002
Batch-No.:	2000902-00012C
Purity:	96.4 %
Certificate of:	15 June 2009
Storage at test facility:	< -18 °C
Expiration Date:	14 Sep 2013

Sponsor Code Name:	IN-K5A78-002
Batch-No.:	E112180-96A
Purity:	96.5 %
Certificate of:	21 May 2008
Storage at test facility:	< -18 °C
Expiration Date:	28 Mar 2011

Sponsor Code Name:	IN-JCZ38-005
Batch-No.:	2000902-00413A
Purity:	94.4 %
Certificate of:	06 Oct 2008
Storage at test facility:	< -18 °C
Expiration Date:	29 Aug 2014

Sponsor Code Name:	IN-K5A77-002
Batch-No.:	D100109-024A
Purity:	92.3 %
Certificate of:	22 Oct 2008
Storage at test facility:	< -18 °C
Expiration Date:	16 Sep 2011

Sponsor Code Name:	IN-JSE76-005
Batch-No.:	D100109-0013A
Purity:	93.8 %
Certificate of:	09 Mar 2010
Storage at test facility:	< -18 °C
Expiration Date:	27 Jan 2016

Sponsor Code Name:	IN-K5A79-002
Batch-No.:	D100109-15A
Purity:	84.4 %
Certificate of:	29 Apr 2010
Storage at test facility:	< -18 °C
Expiration Date:	09 Mar 2016

Sponsor Code Name:	IN-PLT97-002
Batch-No.:	D100109-018E
Purity:	92.8 %
Certificate of:	05 Mar 2010
Storage at test facility:	< -18 °C
Expiration Date:	17 May 2013

4.0 METHODS

4.1 Principle of the Analytical Method

Specimen origin

The untreated specimens of drinking (tap) water, taken from the local drinking water supply, and of surface water and ground water were provided by the test facility. Certificates were provided by sub-contractor GBA / Dr. Kaiser & Dr. Woldmann GmbH, Hamburg, Germany (Appendix 5).

The drinking (tap) water sample was analyzed as following:

PH-value:	6.5
Total hardness	0.20 mmol/L (1.1 °dH)
DOC	< 1.0 mg/L
Residue after filtration:	< 1.0 mg/L

The surface water sample was analyzed as following:

PH-value:	7.4
Total hardness	1.5 mmol/L (8.6 °dH)
DOC	8.5 mg/L
Slit content:	50 mg/L

The ground water sample was analyzed as following:

PH-value:	7.81
Acid capacity:	1.6 mmol/L

Specimen preparation

The samples were stored under refrigerated conditions in the dark. Particulates were allowed to settle before aliquotting. The specimens were uniquely identified with Specht numbers.

Specimen analysis

Specimens were analysed for residues of DPX-HGW86 (cyantraniliprole) and its metabolites IN-J9Z38, IN-JCZ38, IN-JSE76, IN-K5A77, IN-K5A78, IN-K5A79, IN-PLT97 using the method described in ABC Laboratories Report Number ABC-64921, Sponsor study Number DuPont-18850 [1] with LC-MS/MS detection.

Specimen amounts taken for analysis were 20 mL.

Two control specimens and five fortified specimens for each fortification level, each analyte and each matrix were worked up and analysed.

Representative chromatograms are presented in Appendix 3.

4.2 Test Method

4.2.1 Introduction

The method applied is described in ABC Laboratories Report Number ABC-64921, Sponsor study Number DuPont-18850 [1]. This method contains the analytical procedures for the determination of residues of DPX-HGW86 and its metabolites in water.

4.2.2 Outline of the method

A 20 mL sample is purified using ENV SPE cartridges conditioned with methanol followed by 1 mM formic acid, elution with 0.02 M ammonium hydroxide in acetonitrile and reconstitution in methanol. After addition of 0.02 M formic acid the extracts are analysed by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS).

4.2.3 Apparatus and equipment

- Common laboratory glassware
- Sarstedt tubes, 50 mL
- Graduated cylinder, 10 mL.
- Test tubes, with ground stoppers and graduation marks at 2.5 mL, 5.0 mL, and 10.0 mL
- Ultra sonic bath, Sonorex Super 510, Bandelin electronic GmbH & Co. KG, D-12207 Berlin, Germany
- Nitrogen Evaporator, e.g. N-EVAP No. 11155-RT, Organomation Associates, Inc., 266 River Road West, Berlin, MA 01503-1699, USA.
- Vortex, Reax Top, HEIDOLPH Instruments GmbH & Co. KG, D-91126 Schwabach, Germany
- Dilutor, Microlab 500, Hamilton Deutschland GmbH, Fraunhoferstr. 17, D-82152 Martinsried, Germany.
- Bond Elut ENV SPE Cartridge, 6 mL/500 mg, Varian, USA
- Solid phase extraction vacuum manifold, e.g. Supelco Visiprep No. 57030-U
- Disposable Syringe, 3 mL

- Single use filter, Chromafil-O-45/25 (PTFE), Machery-Nagel GmbH & Co. KG, D-52313 Düren, Germany
- Analytical balance appropriate for 100 mg sample weights.
- Volumetric flasks, e.g. 20 mL, 50 mL, 100 mL
- Volumetric pipettes , e.g. 1.0 mL
- Variable Eppendorf pipettes 0-1000 µL

All glassware must be rinsed with water (to remove detergents) and acetone, and dried before use.

4.2.4 *Reagents*

- Methanol, Chromasolv®, e.g. Riedel de Haën No. 34885.
- Acetonitrile; Chromasolv®; e.g. Riedel de Haën No. 34881
- Ammonium hydroxide, e.g. Riedel-de Haën
- Formic acid, 100 %, e.g. Merck
- Water, Aqua ad iniectabilia, e.g. Braun Melsungen BR370345/2
- 1 M formic acid: Add 4.5 mL of concentrated formic acid to approximately 80 mL of de-ionized water, and dilute to 100 mL with de-ionized water.
- 1 mM formic acid: Add 1.0 mL of 1 M formic acid solution to approximately 800 mL of de-ionized water, and dilute to 1 L with de-ionized water.
- 0.02 M formic acid: Add 20 mL of 1 M formic acid solution to approximately 800 mL of de-ionized water, and dilute to 1 L with de-ionized water.
- 1 M ammonium hydroxide: Add 7 mL of concentrated ammonium hydroxide to 93 mL of de-ionized water.
- 0.02 M ammonium hydroxide in acetonitrile: Add 20 mL of 1 M ammonium hydroxide solution to 980 mL of acetonitrile.

4.2.5 *Sample work up*

1. Measure 20 mL of water sample into a 50 mL-Sarstedt tube.
2. Fortify, if necessary, and mix sample rigorously using a vortex mixer.
3. Condition the ENV SPE cartridges with 6 mL methanol followed by 6 mL 1 mM formic acid (aq) allowing the eluate to go to waste.
4. Attach a 25-mL reservoir to the cartridge and add additional 5 mL of 1 mM formic acid allowing the eluate to go to waste.
5. Add the samples from Step 2 to the reservoirs and allow them to flow through the cartridges using gravity flow.
6. When the samples are through the cartridges, remove the reservoirs and wash the cartridges with 5 mL water.
7. Apply vacuum for 20-30 seconds allowing the eluate to go to waste.
8. Elute the analytes with 3 x 5 mL of 0.02 M ammonium hydroxide in acetonitrile into a Sarstedt tube using gravity flow.

9. Evaporate to dryness on an evaporator using a heated water bath (around 40°C)
10. Reconstitute the extracts in 1.0 mL of methanol. Mix the extracts using a vortex mixer for approximately 20 seconds and sonicate for 2 minutes.
11. Add 1.0 mL of 0.02 M formic acid (aq), mix using a vortex mixer for approximately 20 seconds and sonicate for 2 minutes.
12. Filter a portion of the sample through a PTFE filter into an autosampler vial for the LC-MS/MS determination.

4.2.6 *Detection method for LC-MS/MS*

The final solutions were analysed for DPX-HGW86 (cyantraniliprole) and its metabolites IN-J9Z38, IN-JCZ38, IN-JSE76, IN-K5A77, IN-K5A78, IN-K5A79, IN-PLT97 Agilent Series 1200 HPLC (Agilent Technologies) coupled to a PE-Sciex API 5000 tandem mass spectrometer with a Turbo Ion Spray (ESI) interface. The mass spectrometer was operated in MS/MS-Multiple Reaction Monitoring (MRM) positive ion mode. The HPLC and mass spectral operating conditions are summarized in the following tables.

Chromatographic conditions

HPLC system: Agilent Series 1200

Column: Synergi Polar-RP, C18, 2 x 150 mm, 4 µm particle size

Eluent: A: 0.1 % formic acid in methanol
B: 0.1% formic acid in water

TIMES [MIN]	FLOW RATE [ML/MIN]	A [%]	B [%]
0.0	0.7	50	50
3.0	0.7	70	30
6.0	0.7	70	30
7.0	0.7	95	5
9.0	0.7	95	5
9.3	0.7	50	50
11.0	0.7	50	50
11.1	0.7	50	50

Injection volume: 20 µL
Column oven temperature: 30°C
Retention times: DPX-HGW86 approx. 4.3 min, IN-J9Z38 approx. 8.3 min, IN-JCZ38 approx. 2.7 min, IN-JSE76 approx. 3.6 min, IN-K5A77 approx. 5.3 min, IN-K5A78 approx. 6.3 min, IN-K5A79 approx. 3.1 min, IN-PLT79 approx. 5.4 min
Valco Valve: 0.0 to 0.5 min to waste; 0.5 to 9.0 min to MS

Mass spectrometric conditions:

Instrument:	API 5000™ LC/MS/MS System, Applied Biosystems
Type of interface:	Electrospray ionization
Ionisation mode:	positive
Capillary voltage (IS):	5500 V
Collision gas (CAD):	5 (arbitrary units)
Ionspray Turbo Heater	(TEM): 600 °C
Curtain gas (CUR):	15 (arbitrary units)
Gas Flow 1 (GS1):	60 (arbitrary units)
Gas Flow 2 (GS2):	60 (arbitrary units)
Entrance potential (EP):	10 V

ANALYTE MONITORED	TRANSITIONS MONITORED	DECLUSTERING POTENTIAL (DP) [V]	COLLISION ENERGY (CE) [EV]	CELL EXIT POTENTIAL (CXP) [V]	DWELL TIME [MSEC]
DPX-HGW86	475 / 286 473 / 284	101 71	29 21	18 20	50 50
IN-J9Z38	457 / 188 455 / 186	116 176	41 43	14 14	100 100
IN-JCZ38	493 / 286 491 / 284	86 76	33 19	20 24	50 50
IN-JSE76	494 / 463 492 / 461	116 66	31 25	36 30	50 50
IN-K5A77	475 / 299 473 / 297	141 191	53 53	20 22	50 50
IN-K5A78	474 / 186 476 / 188	211 206	45 51	14 16	100 100
IN-K5A79	480 / 463 478 / 461	131 111	29 23	34 20	50 50
IN-PLT97	460 / 424 462 / 426	186 196	33 37	16 30	50 50

External Standards: ranging from 0.2 ng/mL to 50 ng/mL

Integrated peak areas were used for quantification.

Standards were prepared for DPX-HGW86 (cyantraniliprole) and its metabolites IN-J9Z38, IN-JCZ38, IN-JSE76, IN-K5A77, IN-K5A78, IN-K5A79, IN-PLT97 in one solution: The calibration standard concentrations were: 0.20 ng/mL, 0.50 ng/mL, 1.0 ng/mL, 2.0 ng/mL, 5.0 ng/mL, 10 ng/mL, 20 ng/mL and 50 ng/mL for all analytes.

The evaluation of the results was based on a linearity curve (see below).

Calculation for analysis by LC-MS/MS:

The concentrations in the specimen extracts were determined using an average response factor calculated from the linear 8-point calibration curve.

The residues (R) of DPX-HGW86 (cyantraniliprole) and its metabolites IN-J9Z38, IN-JCZ38, IN-JSE76, IN-K5A77, IN-K5A78, IN-K5A79, IN-PLT97, expressed in µg/L, are calculated according to the following equations:

$$R = \frac{A \times aRF \times V_{END} \times DF}{G}$$

where:

A: Analyte peak area in counts

aRF: Average response factor: average of individual standard concentrations / individual standard peak areas

aRF = (Cstd1/Astd1 + …. + Cstd5/Astd5) / 5

G: Amount of the analytical specimen (20 mL)

V_{END}: final volume of the extract (2.0 mL)

DF: Dilution factor (1 for no dilution)

Recovery

The recovery is calculated from the following equation:

$$\text{Recovery (\%)} = \frac{R_{fortified}}{F} \times 100\ \%$$

$R_{fortified}$: Residues of fortified specimen, in µg/L

F: Fortification, in µg/L

Example of evaluation

Calculation for DPX-HGW86 residues in Specimen Specht No. 12 (MRM 475 / 286)

(Please compare with Residue Analysis Raw Data Sheet Tab. 1 in Appendix 1.)

A: 1580000 counts

aRF: 6.0052E-6

DF: 1

G: 20 mL

V_{END}: 2.0 mL

$$R = \frac{1580000 \times 6.0052 \cdot 10^{-6} \times 2 \times 1}{20}$$

µg/L found = 0.95

$$\text{Recovery} = \frac{0.95\ \mu g/L}{1.00\ \mu g/L} \times 100\ \% = 95\%$$

4.3 Stock, Standard and Fortification Solutions

Stock solution 6102: 400 µg DPX-HGW86 /mL

10.08 mg DPX-HGW86 (99.2 %; page 32) were dissolved in acetonitrile and diluted to 25 mL in a volumetric flask.

Stock solution 6103: 400 µg IN-J9Z38 /mL

10.38 mg IN-J9Z38 (96.4 %; page 32) were dissolved in acetonitrile and diluted to 25 mL in a volumetric flask.

Stock solution 6106: 400 µg IN-JCZ38 /mL

10.60 mg IN-JCZ38 (94.4 %; page 32) were dissolved in acetonitrile and diluted to 25 mL in a volumetric flask.

Stock solution 6108: 400 µg IN-JSE76 /mL

10.66 mg IN-JSE76 (93.8 %; page 32) were dissolved in acetonitrile and diluted to 25 mL in a volumetric flask.

Stock solution 6107: 400 µg IN-K5A77 /mL

10.84 mg IN-K5A77 (92.3 %; page 32) were dissolved in acetonitrile and diluted to 25 mL in a volumetric flask.

Stock solution 6104: 400 µg IN-K5A78 /mL

10.36 mg IN-K5A78 (96.5 %; page 32) were dissolved in acetonitrile and diluted to 25 mL in a volumetric flask.

Stock solution 6105: 400 µg IN-K5A79 /mL

11.86 mg IN-K5A79 (84.4 %; page 33) were dissolved in acetonitrile / DMSO (20 / 5, v/v) and diluted to 25 mL in a volumetric flask.

Stock solution 6109: 400 µg IN-PLT97 /mL

11.49 mg IN-PLT97 (92.8 %; page 33) were dissolved in acetonitrile / DMSO (15 / 10, v/v) and diluted to 25 mL in a volumetric flask.

Fortification solutions:

Fortification solutions were prepared in acetonitrile /water (1+4, v+v) using a dilutor, volumetric pipettes and volumetric flasks. Fortification solutions were stored at 4-8 °C in the dark.

FORTIFICATION SOLUTION	SOLUTION USED	PIPETTE VOLUME µL	FINAL VOLUME mL	CONCENTRATION µG/mL
DZ1	Stock solutions 6102, 6103, 6104, 6105, 6106, 6107, 6108, 6109	each 50	100	0.20
Z1	DZ1	2000	20	0.200
Z2	Z1	2000	20	0.0200

Standard solutions

The following external standard solutions (L) were prepared by diluting with methanol / 0.02 M formic acid (1:1, v/v) using volumetric pipettes, a dilutor and volumetric flasks**.** Solvent standard solutions were stored at 4-8 °C in the dark.

STANDARD NAME	SOLUTION USED	PIPETTE VOLUME mL	FINAL VOLUME mL	CONCENTRATION NG/mL
L1	DZ1	0.250	1.0	50
L2	DZ1	0.100	1.0	20
L3	DZ1	0.060	1.2	10
L4	L1	0.100	1.0	5.0
L5	L3	0.200	1.0	2.0
L6	L3	0.100	1.0	1.0
L7	L3	0.050	1.0	0.50
L8	L5	0.100	1.0	0.20

5.0 FORTIFICATIONS

Control (untreated) specimens were fortified prior to extraction with 1.0 mL of fortification solution as follows:

MATRIX	SPECIMEN AMOUNT	FORTIFICATION SOLUTION	FORTIFICATION LEVEL
Tap water	20 mL	Z2 (0.0020 µg/mL)	0.10 µg/L
	20 mL	Z1 (0.020 µg/mL)	1.0 µg/L
Ground water	20 mL	Z2 (0.0020 µg/mL)	0.10 µg/L
	20 mL	Z1 (0.020 µg/mL)	1.0 µg/L
Surface water	20 mL	Z2 (0.0020 µg/mL)	0.10 µg/L
	20 mL	Z1 (0.020 µg/mL)	1.0 µg/L

Results of recoveries are reported in Appendix 1.