

European Commission



**Draft Renewal Assessment Report prepared according to the Commission
Regulation (EU) N° 1107/2009**

INDOXACARB

**Volume 3 – B.5 (PPP) – INDOXACARB 150 g/L
EC**

Rapporteur Member State: France
Co-Rapporteur Member State: Spain

Version History

When	What
2016-12	Initial RAR

Table of contents

B.5. METHODS OF ANALYSIS	4
B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA	4
B.5.1.1. Analysis of the plant protection product	4
B.5.1.2. Methods for the determination of residues	10
B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES	10
B.5.3. REFERENCES RELIED ON.....	11

B.5. METHODS OF ANALYSIS

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

For the Annex I Inclusion in 2006, Steward®30WG (DPX-MP062 30WG) was the representative formulation containing 300 g/kg of the active substance DPX-KN128 and 100 g/kg of the inactive isomer IN-KN127.

Since then DuPont has been able to move from DPX-MP062 to DPX-KN128 technical material (single enantiomeric form).

Today, DuPont is in a transition phase moving all formulated product registrations to indoxacarb (DPX-KN128) technical material and for the renewal of indoxacarb, Indoxacarb 150 g/L EC is the representative formulation.

B.5.1.1. Analysis of the plant protection product

CA 4.1.1/04	Report	Hansen, S.W. (2013); Determination of indoxacarb (DPX-KN128) in technical grade indoxacarb and end-use products DuPont Report No.: DuPont-34638 GLP: No
CA 4.1.1/03	Report	Hansen, S.W. (2004); Technical grade indoxacarb (DPX-KN128) analysis and certification of product ingredients in support of registration of DuPont KN128 technical and DuPont Claridox™ C technical DuPont Report No.: DuPont-13126 and DuPont-13126, Confidential attachment GLP: Yes

Description of the method N°. KN128.220.01.ST(revised)

The method for the assay of indoxacarb in formulation product involves dissolution by ultrasonication of indoxacarb in acetonitrile. A known amount of p-terphenyl internal standard was added to each standard or sample. Samples were filtered before analysis. Analysis was done by reversed-phase liquid chromatography, with quantitation by ultraviolet absorbance at 280 nm. The column used was a Zorbax® RX-C8 column, and the mobile phase was an isocratic mixture of acetonitrile and water. The weight percent of indoxacarb in each sample was determined by comparing peak area ratios of indoxacarb/p-terphenyl with a calibration curve generated from the analysis of standard solutions. Standard solutions were prepared using indoxacarb.

Validation data

Specificity

Chromatograms of acetonitrile blank, sample solution containing DPX-KN128 (standard), Formulation blank solution, formulation solution containing DPX-KN128 and internal standard were presented. No interference was observed at the retention time of indoxacarb.

Analytical method used for determination of the active substance in formulation cannot separate the two enantiomers (not specific for active S-isomer). Nevertheless, an analytical method specific for determination of S-isomer (Chiral column) was developed (see below).

Since method detected both DPX-KN128 and KN127, any IN-KN127 (inactive enantiomer) detected in the sample will be subtracted from the total.

Linearity

The linearity of the method proposed for determination of the active substance in the preparation, was demonstrated. The equation for the calibration line is $y = 468.79456x - 0.00051$. The correlation coefficient for six different concentrations of indoxacarb standards over the range of 30 to 150% of the assay level is 0.99998, and the slope is 468.79456.

Accuracy

The accuracy of the DPX-KN128 150EC formulated was determined by spiking the formulation blank in duplicate with 10% and 20% of DPX-JW062 analytical standard.

The average percent recovery obtained was 100.35% with a standard deviation of 0.088 for the 10% spike and a recovery of 99.72% and standard deviation of 0.126 for the 20% spike. The EU has established that the mean recovery of a formulation method must lie within 98 and 102% if the percent active is above 10%. This method meets the EU requirement for accuracy.

Repeatability

Repeatability testing of the assay method was determined by analysis of six replicate test portions of the test solution at a concentration of 15% w/w. The results were calculated by one analyst on one day. The relative standard deviation was 0.130%. The maximum allowable relative standard deviation calculated from the modified Horwitz equation was 1.77% for the technical material. Therefore, this method fulfils the EU repeatability criteria.

There were no outliers during this testing.

Conclusion: Method for determination of active substance in plant production product was evaluated and meets the EU criteria with respect to linearity, precision (repeatability), accuracy (recovery), and specificity. Nevertheless, analytical method for the assay of indoxacarb in formulation product should be able to determine each isomer in the presence of the other one. A direct determination of the active S-isomer Indoxacarb (DPX-KN128) in the plant production product is particularly important for control of formulation. Therefore, a full validation of a specific method for DPX-KN128 should be provided.

Method for the determination of the inactive enantiomer in plant protection products

Study submitted to the EU for the first time in this submission

CA 5.1.1/02	Report	Hansen, S.W. (2004); Technical grade indoxacarb (DPX-KN128) analysis and certification of product ingredients in support of registration of DuPont KN128 technical and DuPont Claridox C technical DuPont Report No.: DuPont-13126 and DuPont-13126, Confidential attachment GLP: Yes
	Method	Indoxacarb (DPX-KN128) technical and 150 g/L EC formulation, Determination of DPX-KN127, Chiral normal-phase liquid chromatography method KN128.220.02.ST

Description of the method

The sample is dissolved in ethyl acetate and analysed by chiral normal-phase liquid chromatography using a 25 cm Chiralcel® OD-H column and UV detection at 310 nm. The weight percent of IN-KN127 in each sample is determined by comparison to a calibration curve (area vs. concentration) prepared from the analysis of DPX-JW062 standard solutions. DPX-JW062 is a racemic (exactly 50:50) mixture of DPX-KN128 and IN-KN127.

Validation data

Specificity

The method was evaluated for the interferences from expected manufacturing impurities and formulation inerts. None of the known impurities is expected to be present in technical grade indoxacarb when coeluting with Indoxacarb 150 g/L EC or the internal standard, p-terphenyl. None of the formulation ingredients in Indoxacarb 150 g/L EC are detected or coelute with indoxacarb or the internal standard. Since the EU requires that any interferences present should not contribute more than $\pm 3\%$ to the total quantity determined, this method satisfies the EU criteria for specificity.

Linearity

The linearity of the method proposed for the determination of IN-KN127 in the preparation, was demonstrated. The equation for the calibration line (peak area vs. concentration in $\mu\text{g/mL}$) is $y = 11.29389x - 0.57691$. The

correlation coefficient for six different concentrations of indoxacarb standards over the range of 0.004 to 0.8% is 1.00, and the slope is 11.29389.

Accuracy

The accuracy of this method for the analysis of the formulated sample (Indoxacarb 150 g/L EC) was evaluated by fortifying the corresponding mixture of the formulation inert ingredients in duplicate with analytical standard to levels of 0.1% and 0.5% concentration, as recommended in the guideline (SANCO/3030/99 rev.4). The average percent recovery obtained was 102.4% for the 0.1% spike and a recovery of 102.0% for the 0.5% spike. The EU has established that the mean recovery of a formulation method must lie within 80% and 120% if the percent active is 0.1-1.0%. This method meets the EU requirement for accuracy.

Repeatability

Repeatability testing of the assay method was determined by calculating the standard deviation of the average weight percent of IN-KN127 obtained from the analysis of six replicate test portions of the same sample of Indoxacarb 150 g/L EC formulation. These analyses were conducted by one analyst in one day. The relative standard deviation was 0.34% for the Indoxacarb 150 g/L EC formulation. The maximum allowable relative standard deviation calculated from the modified Horwitz equation was 3.79%. Therefore, this method fulfils the EU repeatability criteria.

There were no outliers during this testing.

Conclusion

The method was successfully evaluated and meets the EU criteria with respect to linearity, precision (repeatability), accuracy (recovery), and specificity. Method allows separation of two isomers. The method requires instrumentation commonly available in most well equipped analytical laboratories, and no hazardous reagents are required. Therefore, this method is suitable for enforcement purposes.

Method for the determination of relevant impurities

- **Method for the determination of IN-C0800, IN-06439, and IN-R1T94**

CA 5.1.1/01	Report	Gravelle, W.D. (2015); Description and validation of the analytical methods for the determination of IN-J1063, IN-C0800, IN-06439, and IN-R1T94 impurities in indoxacarb (DPX-KN128) technical and indoxacarb (DPX-KN128) 150 g/L EC formulation DuPont Report No.: DuPont-38062, Revision No. 1 and DuPont-38062, Revision No. 1, Confidential attachment GLP: Yes
--------------------	---------------	---

Description of the method

A solution of the sample is separated by reversed-phase high-performance liquid chromatography (RPLC) using a 15 cm × 4.6 mm ID Zorbax® Extend-C18 analytical column with 3.5-µm particle size. Tetraethyl ketone, IN-C0800 (CAS 90-93-7); tetraethyl base, IN-06439 (CAS 135-91-1); and tetraethyl hydrol, IN-R1T94 (CAS 134-91-8) are detected and quantitated with LC-MS-MS detection. The liquid chromatograph is coupled to a triple quadrupole mass spectrometer by an atmospheric pressure chemical ionization (APCI) source.

COMPOUND CHEMICAL NAME, CAS REGISTRY NO.

IN-C0800	4,4'-Bis(Diethylamino)benzophenone, CAS 90-93-7
IN-06439	N1,N1-diethyl-4-[4-(diethylamino)benzyl]aniline, CAS 135-91-1
IN-R1T94	tetraethyl hydrol, bis [4-(diethylamino)phenyl]methanol, CAS 134-91-8

Ion transitions:

m/z IN-C0800: 325.22 → 176.24 and 176.24 → 133

m/z IN IN-R1T94: 309.33 → 265.17 and 309.33 → 221.0

m/z IN-R1T94: 311.3 → 267.24 and 267.24 → 223.0

Specificity

The method was evaluated for interferences from expected manufacturing impurities and formulation inerts. None of the impurities coelute with indoxacarb (DPX-KN128) and none of the formulation ingredients in Indoxacarb 150 g/L EC are detected or coelute with the impurities. Since the EU requires that any interference present does not contribute more than $\pm 3\%$ to the total quantity determined this method satisfies the EU criteria for specificity.

Confirmation of identity of the analytes detected by DuPont Method No KN128.220.10.ST was accomplished by comparison of chromatographic peak retention times generated from standard and technical material sample solutions. Method utilizes MS/MS detection and is highly specific for the Tetraethyl Ketone (IN-C0800), Tetraethyl Base (IN-06439) and Tetraethyl Hydrol (IN-R1T94) impurities.

HPLC/MS/MS chromatograms are presented to confirm the identities of the impurities for a commercially-produced indoxacarb sample.

HPLC/MS/MS chromatograms for indoxacarb impurities were presented.

Typical chromatograms of the blank, standard solutions and sample solution were provided

Linearity

Linearity was evaluated by determining the slope, intercept, and correlation coefficient of a generated standard curve (peak area versus concentration in ng/mL) for each impurity. Six different concentration levels of each component were analysed with duplicate injections. The reported results were obtained within the linear calibration ranges. The concentration ranges were converted from ng/mL to parts-per-million (ppm) based on a nominal Indoxacarb 150 g/L EC sample solution.

Calibration curves prepared from external standard solutions are used to determine the amount of each impurity in a sample.

Method reference	Component	Concentration range (ng/ml)	Slope	Intercept	Correlation coefficient
KN128.220.10.ST/03	IN-C0800	2.6-104	8223.9	-5174	0.9992
	IN-06439	2.5-101	10412	5687	0.9998
	IN-R1T94	3.0-117	1183.6	-76.9	0.9996

Accuracy Repeatability

The accuracy of this method for the analysis of samples of Indoxacarb 150 g/L EC was evaluated by analysing blanks formulation that were spiked (fortified) in duplicate at two levels for each of the impurities. The fortification target levels of **0.5 ng/mg** and 5.0 ng/mg correspond to LOQ and 10X LOQ.

The average percent recoveries for IN-06439, IN-R1T94, and IN-C0800 obtained were 101.7, 94.8, and 118.8%, respectively, respectively. The EU has established that the mean recovery of a formulation method must lie within 75 and 125% if the percent impurity is below 0.1%. This method meets the EU requirement for accuracy.

Repeatability testing of the impurity method was determined by calculating the standard deviation of the average weight percent of IN-06439, IN-R1T94, and IN-C0800 obtained from the analysis of six replicate test portions of the same sample fortified to 0.5 ng/mg (0.5 ppm) for the 150g/l EC formulation. These analyses were conducted by one analyst in one day. Results are shown in the table below. The relative standard deviations are below the maximum allowable limit calculated from the modified Horwitz equation. Therefore, this method fulfils the EU repeatability criteria.

There were no outliers during this testing.

Method Reference	Component	Mean (ppm)	Standard Deviation (%)	Relative Standard Deviation (%)
KN128.220.10.ST	IN-06439	0.55	0.03	5.71
	IN-R1T94	0.60	0.02	3.44
	IN-C0800	0.78	0.08	9.74

LOQ:

IN-06439 - EC Formulation Results

Sample ID	Inj No.	Response (peak area)	Found Conc. (ng/mg)	Recovery (%)
LOQ	1	26184.11	0.49	98.0
	2	27465.38	0.51	102.7
	3	26892.30	0.50	100.6
	4	28519.73	0.53	106.7
	5	26399.30	0.49	98.8
	6	28703.61	0.54	107.4
Average:				102.4
SD:				4.0
%RSD:				3.9
Horwitz limit:				11.8

Expected concentration = 0.5 ng/mg

IN-R1T94 - EC Formulation Results

Sample ID	Inj No.	Response (peak area)	Found Conc. (ng/mg)	Recovery (%)
LOQ	1	3651.33	0.52	104.2
	2	4015.29	0.57	114.5
	3	4011.61	0.57	114.4
	4	3457.27	0.49	98.6
	5	3940.73	0.56	112.4
	6	3761.54	0.54	107.3
Average:				108.6
SD:				6.4
%RSD:				5.9
Horwitz limit:				11.72

Expected concentration = 0.5 ng/mg

IN-C0800 - EC Formulation Results

Sample ID	Inj No.	Response (peak area)	Found Conc. (ng/mg)	Recovery (%)
LOQ	1	24231.08	0.45	90.6
	2	27183.41	0.51	101.6
	3	25055.16	0.47	93.7
	4	25197.23	0.47	94.2
	5	25187.26	0.47	94.2
	6	24428.03	0.46	91.3
Average:				94.3
SD:				3.92
%RSD:				4.2
Horwitz limit:				12.00

Expected concentration = 0.5 ng/mg

Conclusion

The method was successfully evaluated and meets the EU criteria with respect to linearity, precision (repeatability), accuracy (recovery), and specificity. No hazardous reagents are required. Therefore, this method is suitable for enforcement purposes.

However, LOQ was higher than calculated value for relevant impurities in formulated Indoxacarb 150 g/L EC(<0.375ppm)

- Calculated certified value for impurity a: IN-06439 corresponds to “tetraethyl base” in Indoxacarb 150 g/L EC was <0.375 ppm,
- Calculated certified value for impurity b: IN-R1T94 corresponds to “tetraethyl hydrol” in Indoxacarb 150 g/L EC was; <0.375 ppm,
- Calculated certified value for impurity c: IN-J1063 corresponds to “tetraethyl ketone” in Indoxacarb 150 g/L EC was; <0.375 ppm,

• **Method for the determination of relevant impurity IN-J1063**

CA 5.1.1/01	Report	Gravelle, W.D. (2015); Description and validation of the analytical methods for the determination of IN-J1063 impurity in indoxacarb (DPX-KN128) technical and indoxacarb (DPX-KN128) 150 g/L EC formulation DuPont Report No.: DuPont-40620, Revision No. 1 and DuPont-38062, Revision No. 1, Confidential attachment GLP: Yes
--------------------	---------------	---

Description of the method

A solution of sample is separated by reversed phase high performance liquid chromatography (RPLC) using a 25 cm x 3.0 mm Zorbaw SB-C8 solvent saver analytical column. Ethyl violet is detected with UV detection at 590 nm. A calibration curve (peak area ratio vs. amount ratio), prepared from standard solution of IN-J1063, is used to determine the total amount of ethyl violet in each sample.

Specificity:

Specificity was tested for potential interference with indoxacarb and other impurities. Individual stock of the impurity was prepared, equivalent to approximately 10 times the nominal LOQ concentration of the target sample concentration by weight, and injected. IN-J1063 was separated from the indoxacarb standard and other impurities.

Confirmation of impurity identities was accomplished by:

✓ comparison of chromatographic peak retention times generated from standard, technical material and sample solutions

✓ collection and comparison of highly specific HPLC/UV DAD spectral data for each component.

HPLC/UV DAD spectral was presented and typical chromatograms of the blank, standard appear and sample solution were provided.

Linearity:

Calibration curve is linear between $4.9 \cdot 10^{-5}$ mg/ml to $9.7 \cdot 10^{-2}$ mg/ml for IN-J1063 standard solution. Eight point calibrations were performed to establish linearity. Coefficient correlation was determinate to be 1.00.

Accuracy:

Samples of DPX-KN128 formulated 150 g/l EC were prepared with two levels of fortification 0.5 and 5.0 ppm. The average percent recoveries for Ethyl violet obtained were 113.1 and 102.3%, respectively. The EU has established that the mean recovery of a formulation method must lie within 75 and 125% if the percent impurity is below 0.1%. This method meets the EU requirement for accuracy.

Repeatability:

Six sample of DPX-KN128 formulated 150 g/l EC with IN-J1063 at the target LIOQ level (0.5ppm) and analysed in replicate. These analyses were conducted by one analyst in one day. Average was 0.607 ppm and the relative standard deviation was determined to be 1.36%.

LOQ: 0.5ppm

LOD: 0.16ppm

Conclusion

The method was successfully evaluated and meets the EU criteria with respect to linearity, precision (repeatability), accuracy (recovery), and specificity. No hazardous reagents are required. Therefore, this method is suitable for enforcement purposes.

However, LOQ was higher than calculated value for relevant impurities in formulated Indoxacarb 150 g/L EC (<0.27ppm)

- Calculated certified value for impurity a: IN-J1063 corresponds to “Ethyl violet” in Indoxacarb 150 g/L EC was <0.327 ppm,

B.5.1.2. Methods for the determination of residues

See Volume 3 – B5

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES

See Volume 3 – B5

B.5.3. REFERENCES RELIED ON

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or Not	Vertebrate Study Y/N	Data Protection Y/N	Justification if Data Protection Is Claimed	Owner	Previous Evaluation
CP, 5.1.1	Kahler, T.W.	2006	Technical grade active ingredient indoxacarb (DPX-KN128) analysis and certification of product ingredients in support of registration of DuPont KN128 technical Exygen Research DuPont-16774 and DuPont-16774, Confidential attachment Published: No	N	Y	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study summarised in evaluation report on the equivalence of the technical material for the active substance Indoxacarb, 2009
CP, 5.1.1/01	Gravelle, W.D.	2015	Description and validation of the analytical methods for the determination of IN-J1063, IN-C0800, IN-06439, and IN-R1T94 impurities in indoxacarb (DPX-KN128) technical and indoxacarb (DPX-KN128) 150 g/L EC formulation Product Safety Labs DuPont-38062, Revision No. 1 and DuPont-38062, Revision No. 1, Confidential attachment GLP: Yes Published: No	N	Y	Conducted to meet guideline requirement. The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A. ^a

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or Not	Vertebrate Study Y/N	Data Protection Y/N	Justification if Data Protection Is Claimed	Owner	Previous Evaluation
CP, 5.1.1/02	Hansen, S.W.	2004	Technical grade indoxacarb (DPX-KN128) analysis and certification of product ingredients in support of registration of DuPont KN128 technical and DuPont Claridox C technical DuPont Stine-Haskell Research Center DuPont-13126 and DuPont-13126, Confidential attachment GLP: Yes Published: No	N	Y	Representative formulation supporting renewal different from PPP supporting initial inclusion. The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CP, 5.1.1/03	Kennedy, A.P.	2014	Description and validation of the analytical method for determination of ethyl violet (IN-J1063) in Indoxacarb (DPX-KN128) 150 g/L EC formulation DuPont Stine-Haskell Research Center DuPont-40620, Revision No. 1 and DuPont-40620, Revision No. 1, Confidential attachment GLP: Yes Published: No	N	Y	Conducted to meet guideline requirement. The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

^a N.A. = not applicable, as this is a new study submitted for the first time at EU level for the purpose of renewal.