

European Commission

**Renewal Assessment Report of the Inclusion of the
Active Substance in Annex I of the
Regulation (EC) 1107/2009**



Oxamyl 10GR

**Volume 3 (CP)
ANNEX B.5 Methods of analysis**

Rapporteur Member State: Italy
Co-Rapporteur Member State: France

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B.5 METHODS OF ANALYSIS

Introduction

Samples of the pure active substance and preparations as manufactured are available and can be provided upon request. Analytical standards for the pure active substance, reference standards for the quantitatively significant impurities (>1 g/kg), and for the relevant metabolites and other components included in the residue definition are available and can be provided upon request.

Oxamyl 10GR was the representative formulation for the first approval review of oxamyl in EU.

Unless specifically indicated, all reports in this section are submitted to address mandatory data requirements for the approval of active substance.

B.5.1 Methods used for the generation of pre-authorisation data

B.5.1.1 Analysis of the plant protection product

Studies submitted to the EU for the first time in this submission.

B.5.1.1/01

Reference: CA 5.1.1	Report	<p>Pandey, S. (2015); Validation of the Analytical Method for the Determination of Oxamyl (DPX-D1410) in Technical Grade Oxamyl and Oxamyl End-Use Products by Reverse Phase High Performance Liquid Chromatography (RPLC) and Ultra Performance Liquid Chromatography (UPLC)</p> <p>DuPont Report No.: DuPont-36605</p> <p>GLP: Yes</p>
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Method: Method D1410.220.01.ES. 3rd Revision, March 2015. Update and validation of the DuPont analytical method D1410.220.01.ES for the analysis of Oxamyl in the end use products Oxamyl TGAI, Oxamyl technical 42 % w/w, Oxamyl 42 SL, Oxamyl 24 SL, Oxamyl 10SL and Vydate 10G.

Principle of the method: A solution of the sample, containing DPX-D1410 and a known amount of acetanilide internal standard, is separated by reverse-phase, high performance liquid chromatography. The eluted compounds are detected and quantitated with a 240nm ultraviolet detector and a digital integrator. Quantitation is performed employing a calibration curve (i.e. peak area ratio vs. concentration) drawn from the analysis of standard solutions.

The validation results are presented in Table 1 below.

Table 1 Validation data for method D1410.220.01.ES.

Precision:	Oxamyl DPX-D1410	HPLC			UHPLC		
		X, %	S,±	%RSD	X, %	S,±	%RSD
	Oxamyl Technical DPX-D1410-535	98.45	0.42	0.43	98.73	0.45	0.46
	Oxamyl Technical 42 % w/w (DPX-D1410-550)	42.04	0.23	0.55	42.22	0.18	0.43
	Oxamyl 42SL DPX-D1410-526	39.62	0.44	1.11	39.68	0.45	1.13
	Oxamyl 24SL DPX-D1410-524	23.99	0.12	0.50	24.04	0.12	0.50
	Oxamyl 10SL DPX-D1410-527	9.84	0.50	0.51	9.85	0.05	0.51
	Vydate® 10G	10.04	0.11	1.10	10.04	0.10	1.00
This repeatability data was determined on the basis of 8 replicate analyses of each sample type by one analyst over 1 day.							
Linearity:	Linearity has been demonstrated for both HPLC and UHPLC, by the analyses of seven standard solutions within the nominal ranges; 0.2 - 1.40 mg/mL for DPX-D1410. The resulting least square linear equations and correlation coefficients are as follows:						
	System	Slope	y-intercept	Correlation Coefficient (R²)			
	HPLC	32.198	0.0042	0.9999			
	UHPLC	32.004	0.0066	0.9999			

Accuracy:	OXAMYL DPX-D1410	HPLC			UHPLC		
		X, %	S, ±	%RSD	X, %	S, ±	%RSD
	Oxamyl Technical DPX-D1410-535	100.57	0.80	0.80	100.55	0.84	0.84
	Oxamyl Technical 42 % w/w (DPX-D1410-550)	99.19	1.10	1.11	99.31	1.08	1.09
	Oxamyl 42SL DPX-D1410-526	98.47	1.08	1.10	98.81	1.11	1.12
	Oxamyl 24SL DPX-D1410-524	98.15	0.63	0.64	98.33	0.63	0.64
	Oxamyl 10SL DPX-D1410-527	96.56	0.79	0.82	96.86	0.79	0.82
	Vydate® 10G	94.08	0.67	0.71	95.98	0.87	0.91
<p>The accuracy for the determination of DPX-D1410 in the Technical and Technical 42 was determined by mean recoveries of the active ingredient as a surrogate sample over the calibration range at levels of 75, 100 and 125% of DPX-D1410, the samples were injected in duplicate at each level and an average calculated for all six recoveries was obtained.</p> <p>The accuracy of the method for the analysis of Oxamyl 42SL, Oxamyl 24 SL, Oxamyl 10SL and Vydate 10G formulations was determined by spiking respective formulation placebos with analytical standard to levels at, 25% above and 25% below the nominal concentration of the active ingredient. Duplicate samples were prepared for each level and an average value was calculated for all six recoveries obtained.</p>							
Specificity:	<p>The specificity of the chromatographic separation was established by demonstrating the purity of the HPLC peak(s) of interest versus potential interferences. Individual solutions of active ingredient and internal standard were prepared using standard materials and injected in duplicate. Chromatograms were examined to determine if there was complete separation between the active ingredient and the internal standard. No interferences were observed.</p>						

Conclusions: The analytical method for the analysis of 4 different PPP formulations [Vydate L, Vydate 10G, Vydate 5G and Vydate 10L] containing Oxamyl at a concentration of from 5 to 25% w/w has been validated. The validation data is considered to be acceptable.

RMS comments and conclusion

The analysis of the plant protection product study DuPont-5020, originally submitted under EU Rev8 Point IIA 5.1, was conducted under guideline Directive Dir98-03, Directive 96/46/EC, OPPTS 830.1800. This study has been superseded by DuPont-36605, which was issued to upgrade the technology in the method.

IT: the study presented is considered acceptable

B.5.1.1.1 Methods for the determination of the active substance in plant protection products

Studies submitted to the EU for the first time in this submission.

B.5.1.1.1/01

Reference: CP 5.1.1/02	Report	Pandey, S., McNally, M.E.P. (2015) Determination of oxamyl (DPX-D1410) in technical grade oxamyl and end-use products DuPont Report No.: DuPont-42001 GLP: No
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B.5.1.1.1/02

Reference: CP 5.1.1/01	Report	Pandey, S. (2015) Validation of the analytical method for determination of oxamyl (DPX- D1410) in technical grade oxamyl and oxamyl end-use products by reverse phase high performance liquid chromatography (RPLC) and ultra performance liquid chromatography (UPLC) DuPont Report No.: DuPont-36605 and DuPont-36605 Confidential attachment GLP: Yes
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Description of the method

The method for assay of oxamyl in Oxamyl 10GR involves dissolution by ultrasonication of Oxamyl 10GR in a solution of 5% methanol and 95% water adjusted to pH 3.0 with H₃PO₄. A known amount of acetanilide internal standard was added to each standard or sample. Samples were shaken well before weighing and filtered before analysis. Analysis was done by reversed-phase liquid chromatography (HPLC) and ultra-high pressure liquid chromatography (UPLC), with quantitation by ultraviolet absorbance at 240 nm. The column used was a 1.8-µm particle size, 4.6 mm × 100 mm Agilent Zorbax-XDB C18 column for UPLC and 5.0 µm particle size, 4.6 mm × 150 mm Agilent Zorbax-Aqua C18 column for HPLC. The mobile phase was a gradient mixture of acetonitrile and water that had been adjusted to pH 3.0. The weight percent of oxamyl in each sample was determined by comparing peak area ratios of oxamyl/acetanilide with a calibration curve generated from the analysis of standard solutions.

Specificity, linearity, accuracy, repeatability

The information reported below concerns the method for the determination of the active substance in Oxamyl 10GR, Oxamyl 100 g/kg granular formulation.

Specificity

Details regarding the specificity of the method (DuPont-36605 Confidential attachment) are claimed as confidential and are included in Oxamyl EU Renewal Dossier, Document J, Part 1, DuPont-40926 EU. As the disclosure of this information would also disclose the impurity profile of the technical product, we request them to be treated as confidential information according to Article 63 of Regulation EC 1107/2009.

The method was evaluated for interferences from formulation inerts. None of the known formulation ingredients present in Oxamyl 10GR co-elute with oxamyl or the internal standard, acetanilide. Known impurities would not be present at concentration levels that would represent a chromatographic interference. Since the EU requires that any interference present does not contribute more than ±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 the active substance, oxamyl, as manufactured was demonstrated. Linearity has been demonstrated for both HPLC and UPLC, by the analyses of seven standard solutions within the nominal ranges; 0.2–1.40 mg/mL for oxamyl. The resulting least square linear equations and correlation coefficients are as follows.

Table 2 Least square linear equations and correlation coefficients

System	Slope	y-intercept	Correlation coefficient (R ²)
HPLC	32.198	0.0042	0.9999
UPLC	32.004	0.0066	0.9999

Accuracy

For HPLC, the accuracy of this method for the analysis of oxamyl technical in Oxamyl 10GR as manufactured samples was evaluated by analysing standard material as surrogate samples as duplicate determinations at three concentrations, in the range of 75–125% of the nominal sample active concentration. The average percent recovery obtained was 94.08% with a standard deviation of 0.67%. The accuracy of this method is adequate for HPLC.

For UPLC, the accuracy of this method for the analysis of oxamyl technical in Oxamyl 10GR as manufactured samples was evaluated by analysing standard material as surrogate samples as duplicate determinations at three concentrations, in the range of 75–125% of the nominal sample active concentration. The average percent recovery obtained was 95.98% with a standard deviation of 0.87%. The accuracy of this method is adequate for HPLC.

Repeatability

Repeatability testing of the assay method was determined by calculating the standard deviation of the average percent oxamyl in Oxamyl 10GR obtained from the analysis of eight replicate test portions of the same sample of Oxamyl 10GR. The results were calculated with one analyst on one day.

For HPLC, the relative standard deviation was 1.10% for the analysis of Oxamyl 10GR as manufactured. The maximum allowable relative standard deviation calculated from the modified Horwitz equation was 1.90%. Therefore, the HPLC method fulfils the EU repeatability criteria.

For UPLC, the relative standard deviation was 1.00% for the analysis of Oxamyl 10GR as manufactured. The maximum allowable relative standard deviation calculated from the modified Horwitz equation was 1.90%. Therefore, the UPLC method fulfils the EU repeatability criteria.

There were no outliers during this testing.

Overall suitability for enforcement purposes

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

Applicability of existing CIPAC methods

There is a CIPAC method for oxamyl; it is FAO specification 342/TK (April, 2008). The analytical method for determination of oxamyl (including identity tests) is based on reversed-phase HPLC with UV detection at 240 nm and internal standardization with acetanilide. The method was adopted by CIPAC with provisional status in 2006 and full CIPAC method status in 2007. DuPont and CIPAC have noted that the method can be used with external standardization. The method is applicable for technical concentrate, granules, and soluble concentrates.

RMS comments and conclusion: the methods presented are considered acceptable.

B.5.1.1.2 Methods for the determination of impurities in the preparation

There are no impurities known to be of neither toxicological or environmental relevance, nor isomers in oxamyl as manufactured or formulating ingredients in Oxamyl 10GR that would justify the submission and disclosure of enforcement methods.

B.5.1.1.3 Methods for the determination of formulants or constituents of formulants in the plant protection product

There are no formulating ingredients in Oxamyl 10GR of toxicological or ecotoxicological concern that justify the need for the submission and disclosure of enforcement methods.

B.5.1.2 Methods for the determination of residues

All methods for the determination of oxamyl residues are included in the Oxamyl RAR Volume 3 – CA – B.5.

B.5.2 Methods for post-authorisation control and monitoring purposes

All enforcement methods for the determination of oxamyl residues are included in the Oxamyl RAR Volume 3 – CA – B.5.

B.5.3 References relied on

List of information, tests and studies which are considered as relied upon by the RMS for the evaluation with a view to the approval of the active substance.

Studies marked in yellow are submitted for the first time.

List of studies submitted sorted by Annex Point

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
B.5.1.1/01	Platz, S.J.	2000	Up-date and validation of the DuPont analytical method (No.: D1410.220.01 ES) to verify certified limits of the active ingredient oxamyl (D1410) in the end-use products: (1) Vydate 10L, (2) Vydate 5G and (3) Vydate 10G DuPont Stine-Haskell Research Center DuPont-5020 Superceded by DuPont-36605 Published: No	N			DuPont
B.5.1.1.1/01	Pandey, S.	2015	Validation of the analytical method for determination of oxamyl (DPX- D1410) in technical grade oxamyl and oxamyl end-use products by reverse phase high performance liquid chromatography (RPLC) and ultra performance liquid chromatography (UPLC) Syngene DuPont-36605 and DuPont-36605 Confidential attachment GLP: Yes Published: No	N	Y	New method for the analysis of oxamyl technical and formulated product. The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted.	DuPont
B.5.1.1.1/02	Pandey, S., McNally, M.E.P.	2015	Determination of oxamyl (DPX-D1410) in technical grade oxamyl and end-use products Syngene DuPont-42001 GLP: No Published: No	N	N		DuPont

List of studies submitted sorted by Author

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
B.5.1.1/01	Platz, S.J.	2000	Up-date and validation of the DuPont analytical method (No.: D1410.220.01 ES) to verify certified limits of the active ingredient oxamyl (D1410) in the end-use products: (1) Vydate 10L, (2) Vydate 5G and (3) Vydate 10G DuPont Stine-Haskell Research Center DuPont-5020 Superseded by DuPont-36605 Published: No	N			DuPont
B.5.1.1.1/02	Pandey, S., McNally, M.E.P.	2015	Determination of oxamyl (DPX-D1410) in technical grade oxamyl and end-use products Syngene DuPont-42001 GLP: No Published: No	N	N		DuPont
B.5.1.1.1/01	Pandey, S.	2015	Validation of the analytical method for determination of oxamyl (DPX- D1410) in technical grade oxamyl and oxamyl end-use products by reverse phase high performance liquid chromatography (RPLC) and ultra performance liquid chromatography (UPLC) Syngene DuPont-36605 and DuPont-36605 Confidential attachment GLP: Yes Published: No	N	Y	New method for the analysis of oxamyl technical and formulated product. The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted.	DuPont