European Commission

VOLUME 3 – Annex B (PPP)

- Flutolanil -

B.5 Methods of analysis

Rapporteur Member State: The Netherlands

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Draft Assessment Report and Proposed decision of the Netherlands prepared in the context of the possible approval of flutolanil under Regulation (EC) 1107/2009

Version history page

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

B.5.1.1 Analysis of the plant protection product

Methods for the determination of the active substance and/or variant in the plant protection product

Data, on the analytical method for the determination of the active ingredient in formulated materials, was submitted for the first inclusion of flutolanil into Annex I of Council Directive 91/414/EEC and was reviewed under uniform principles. The method described in the DAR is considered adequate to address this endpoint. Summaries of these methods are presented in the section called “available data”.

Overview of Methods used for Determination of flutolanil in formulated materials

EU agreed method (EFSA Scientific report No. 126 (2008))		
Material	**Method**	**References (DAR)**
Formulation	HPLC/UV	**Volume 3, Annex B, B.5.1.3** **CP 5.1.1/01** Le Gren, 1998

A new analytical method has been developed and validated for the determination of flutolanil in formulated materials. A full summary of this method validation is presented in the section called “new data”.

Overview of New Methods used for Determination of flutolanil in formulated materials

New methods used in support of physchem studies		
Matrix	**Method**	**References**
Formulation	HPLC/UV	**CP 5.1.1/02** Weatherhead P, 2015

AVAILABLE DATA:

Report:	**CP 5.1.1/01: Le Gren I., 1998**
Title:	Flutolanil: Determination by HPLC analysis in formulation EXP10066A (FS)
Document No:	R&D/CRLD/AN/981649 (A-3043)
Guidelines:	Directive 96/46/EC
GLP	yes
Comment:	This information has previously been presented in the approval submissions made in 2006 and therefore have already been reviewed under Uniform Principles. This information is re-submitted in this application for the sake of completeness.

Principle:

The active ingredient, flutolanil, is extracted from the formulation by macerating with acetonitrile and analysed by reverse phase HPLC with a mixture of acetonitrile/water as eluent and using external standardisation and UV detection at 240 nm.

HPLC/UV conditions:

Column:	Nucleosil C18, 125 x 3 mm, 5 µm	
Solvent	Acetonitrile	
Eluent	Acetonitrile	700 mL
	Milli Q water	300 mL
Flow rate	0.5 mL/min	
Volume of injection	5 µL	
Detector UV	240 nm	

Validation:

The method validation data are summarised in the table below:

Parameters		**Flutolanil**
Linearity	**Concentration Range (mg/L)**	5 calibration points: 0.06 – 0.6 g/L
	Intercept (a)	-1.899
	Slope of the line (b)	19060
	Correlation Coefficient (r)	1.000
Limit of Quantification (LOQ)	**% w/w (in samples based on % recovery)**	n.a.
Precision	**Mean Content (% w/w)**	464 g/L
	% RSD	0.3%
	Acceptance criteria	<1%
Accuracy (% Recovery)	**Mean Recovery (n=6)**	99.6%
	% RSD	0.4%
	Acceptance criteria	100 ± 2%
Stability	The standard solution was proved to be stable for at least 7 days in acetonitrile at ambient temperature (99.9%).	
Specificity (Non-analyte interference)	The examination of control solution of the formulation without active ingredient (formulation “blank”), revealed that there is no interference.	

n.a. = not applicable

Conclusion:

The method is sufficiently validated in accordance with SANCO/3030/99 rev. 4.

RMS comment: Although the analytical method described above is sufficiently validated, it is not clear if this method is suitable for the determination of active substance in the representative formulation. Validation has been done for an FS formulation, of which the composition is currently unclear. However, there is a newer method available, that has been validated for the representative formulations. Therefore, the available data is considered to be additional information only.

NEW DATA:

A new analytical method has been developed and validated for the determination of flutolanil in formulated materials.

Report:	**CP 5.1.1/02: Weatherhead P, 2015**
Title:	Validation of an Analytical method for the Determination of the Active Ingredient in Flutolanil 40 SC EU-D and Flutolanil 40 SC EU Formulations
Document No:	XG150181 (A-3071)
Guidelines:	SANCO/3030/99 rev 4
GLP	yes

Principle:

The HPLC method of analysis, Analytical Method XG150181 for the determination flutolanil in Flutolanil 40 SC EU-D and Flutolanil 40 SC EU SC Formulations (Flutolanil 460 g/L) has been developed and validated.

An accurate weight of the formulation (140 mg) was dissolved in 2 mL water. Then, the volume was made up to 100 mL with methanol. The sample was filtered through a 0.45 µm before analysis by HPLC/UV.

HPLC/UV conditions:

Column:	Ascentis Express C18, 150 x 3 mm, 2.7 µm or equivalent		
Column temperature:	45ºC		
Mobile phase	A: 0.05 M Triethylamine (aq) pH 3.0 (H3PO4) / MeOH (95/5 v/v)		
	B: Water / MeOH (5/95 v/v)		
Gradient:	Time (min.)	A%	B%
	0.0	65	35
	15.0	10	90
	20.0	10	90
	20.1	65	35
Flow rate	0.5 mL/min		
Volume of injection	2 µL		
Detector UV	295 nm		

Validation data:

The method validation data are summarised in the table below:

Parameters		**Flutolanil**
Linearity	**Concentration Range (mg/L)**	5 calibration points (in duplicate): 0.25 – 0.75 mg/mL flutolanil (corresponding to 00-600 g/L flutolanil in the test item), covering 50-150%
	Intercept (a)	-10.152
	Slope of the line (b)	2038.2
	Correlation Coefficient (r)	0.9998
Limit of Quantification (LOQ)	**% w/w (in samples based on % recovery)**	n.a.
Precision (repeatability, 6 determinations, 5AE9401F)	**Mean Content (% w/w)**	42.1%
	% RSD	0.94%
	Acceptance criteria	<1%
Accuracy (% Recovery from fortified formulation, 5AE9601F)	**75% Level**	98.7%
	100% Level	101%
	125% Level	99.9%
	Mean	99.9%
	Acceptance criteria	100 ± 2%
Specificity (Non-analyte interference)	No interference peaks are seen in the region of flutolanil in the reagent blank or the formulation blank chromatograms.	
	Retention time and UV spectra recorded for the Flutolanil peaks in the Flutolanil 40 SC EU-D and Flutolanil 40 SC EU test items matched that recorded for the reference standard.	

n.a. = not applicable

Conclusion:

The method was successfully validated and is suitable for the determination of flutolanil in formulated materials, complying with the requirements of SANCO/3030/99 rev 4.

Methods for determination of relevant impurities identified in the technical material or which may be formed during manufacture of the plant protection product or from degradation of the plant protection product during storage

The formulation of Moncut 40 SC contains neither impurities nor co-formulants that are of toxicological, ecotoxicological or environmental concern. However, several methods for the determination of impurities in the TGAI are described in the Confidential Section (please refer to Volume 4). If needed, these methods could be easily adapted to the determination of the same impurities in formulated product.

Methods for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities

It is not considered necessary to provide methods for the determination of formulants or constituents of formulants in the preparation because there are no materials at a level which are of toxicological, ecotoxicological or environment concern.

B.5.1.2 Methods for the determination of residues

Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Please refer to Volume 3, Section CA B5

Methods in soil, water and any additional matrices used in support of efficacy studies

Please refer to Volume 3, Section CA B5

Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicology studies

Please refer to Volume 3, Section CA B5

Methods in body fluids, air, and any additional matrices used in support of operator, worker, resident and bystander exposure studies

Please refer to Volume 3, Section CA B5

Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

Please refer to Volume 3, Section CA B5

Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Please refer to Volume 3, Section CA B5

Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Please refer to Volume 3, Section CA B5

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

Methods for the determination of residues in or on plants, plant products, processed food commodities, food and feed of plant and animal origin.

Monitoring methods for plant and animal matrices are discussed in Vol. 3 CA.B5

Methods for the determination of residues in body fluids and tissues

Monitoring methods for body fluids are addressed in volume 3 CA B5.

Methods for the determination of residues in soil

Monitoring methods for soil are discussed in Vol. 3 CA.B5

Methods for the determination of residues in water

Monitoring methods for water are discussed in Vol. 3 CA.B5

Methods for the determination of residues in air, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

Monitoring methods for air are discussed in Vol. 3 CA.B5

B.5.3 References relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
CP 5.1.1-02	Weatherhead P.	2015	Validation of an Analytical method for the determination of the active ingredient in Flutolanil 40 SC EU-D and Flutolanil 40 SC EU Formulations Battelle UK Ltd. Report No.: XG150181 (A-3071) GLP: yes Published: no	N	Y	New GLP study, provided within the scope of the renewal	Nihon Nohyaku Co. Ltd