

**Analytical method for flumiclorac pentyl ester and its acid, IMCA, in soil**

**Reports:** ECM: MRID 48904401. Green, CA. (2012) Flumiclorac Pentyl Ester: Determination of Flumiclorac Pentyl Ester and its Degradate, IMCA, in/on Soil. Laboratory Project ID: RM-29S-1. Unpublished study prepared by Valent Technical Center and submitted by Valent U.S.A., Corporation. 20 pp.

LV: MRID 48753703. Nie, C. (2012) Independent Laboratory Validation of Method RM-29S-1, Determination of Flumiclorac-pentyl Ester and Its Degradate, IMCA in/on Soil. Project Number: 2012000018, 38127. Unpublished study prepared by Valent Technical Center and submitted by Valent U.S.A., Corporation. 72 pp.

ILV: MRID 49175802. Nie, C. (2013) Addendum: Independent Laboratory Validation of Method RM-29S-1, Determination of Flumiclorac-pentyl Ester and Its Degradate, IMCA in/on Soil. Project Number: 201300150, 38127. Unpublished study prepared by Valent Technical Center and submitted by Valent U.S.A., Corporation. 55 pp.

**Document No.:** MRIDs 48904401, 48753703, & 49175802

**Guideline:** 850.6100

**Statements:** All method validations were conducted in compliance with FIFRA GLP standards. Signed and dated Data Confidentiality, GLP Compliance, Quality Assurance, and Authenticity Certification (LVs only) statements were provided for the method, LV, and ILV reports.

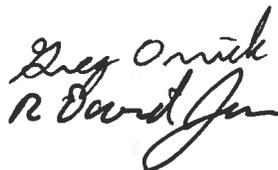
**Classification:** This analytical method is classified as **acceptable**.

**PC Code:** 128724

**Reviewer:** Gregory Orrick, Environmental Scientist

**2<sup>nd</sup> reviewer:** R. David Jones, Ph.D., Senior Agronomist

**Date:** January 30, 2014

**Executive Summary**

This analytical method, RM-29S-1, is designed for the quantitative determination of flumiclorac pentyl ester and its acid, IMCA, in soil using LC/MS/MS (see Table 1). The method is quantitative for the analytes at the stated LOQ of 20 µg/kg. The second laboratory validation (LV) was successful on the first attempt, following confirmation that the instrument to use is the API 4000 LC/MS/MS (MRID 48753703). However, the same Agilent 1200 HPLC was used for the initial and second validations, meaning that MRID 48753703 did not describe a fully independent validation. Therefore, a third laboratory validation (MRID 49175802) was

conducted as the true ILV, using an Agilent 1260 HPLC. The ILV was successful on the second full attempt. (The first attempt failed because the time from extraction to analysis was too long.)

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Flumiclorac-pentyl & Degradate IMCA	48904401	49175802		Soil	8/1/12	Valent U.S.A. Corporation	LC/MS/MS	20 µg/kg

## I. Principle of the Method

Flumiclorac pentyl ester and IMCA residues are extracted from soil with 4:1 acetone:0.1 N HCl (v:v), centrifuged to remove solids, and filtered through glass wool into a separatory funnel. The extract is partitioned with dichloromethane after addition of 5% aqueous sodium chloride. The dichloromethane is removed using rotary evaporation and the residues are dissolved in 0.05% formic acid in methanol and diluted to final volume with 0.05% formic acid in water. A portion of each sample is diluted by a factor of 5 into an autosampler vial for analysis. Sample extracts and standards are analyzed using HPLC with a tandem mass spectrometer. A five-point, 2<sup>nd</sup> order polynomial calibration curve (weighted relative to the largest standard concentration) is used to quantify the analytes in the sample extracts.

## II. Recovery Findings

Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%), *i.e.*, the method is quantitative for each analyte, with an LOQ of 20 µg/kg.

**Table 2. Initial Validation Method Recoveries for Analytes in Soil**

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Flumiclorac pentyl ester	0.02	5	80-84	82	1.8	2.2
	0.2	5	85-88	86	1.1	1.3
IMCA	0.02	5	93-102	96	3.5	3.6
	0.2	5	87-90	89	1.1	1.2

**Table 3. Second Validation Method Recoveries for Analytes in Soil**

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Flumiclorac pentyl ester	0.02	5	96-119	102	9.6	9.4
	0.2	5	91-119	95	3.6	3.8
IMCA	0.02	5	97-104	102	3.5	3.4
	0.2	5	94-104	99	3.7	3.8

**Table 4. Independent Validation Method Recoveries for Analytes in Soil**

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Flumiclorac pentyl ester	0.02	5	89-96	92	2.7	3.0
	0.2	5	93-104	99	4.0	4.1
IMCA	0.02	5	94-107	99	5.5	5.5
	0.2	5	99-103	101	1.8	1.8

### III. Method Characteristics

The LOQ was defined as the lowest concentration tested at which acceptable recoveries were obtained with an RSD of  $\leq 20\%$ . The results for both flumiclorac pentyl ester and IMCA support the stated LOQ of 0.02 ppm (20  $\mu\text{g}/\text{kg}$ ). The LOD of 0.01 ppm was based on a 2.5-g sample, a 10-mL final volume, a dilution factor of 5, and a 0.0005- $\mu\text{g}/\text{mL}$  standard as the lowest concentration in the set of instrument calibration standards ( $[0.0005 \mu\text{g}/\text{mL} \times 10 \text{ mL} \times 5] \div 2.5 \text{ g} = 0.01 \text{ ppm}$ ).

**Table 5. Method Characteristics**

	Flumiclorac pentyl ester	IMCA
Limit of Quantitation (LOQ)	20 $\mu\text{g}/\text{kg}$	20 $\mu\text{g}/\text{kg}$
Limit of Detection (LOD)	10 $\mu\text{g}/\text{kg}$	10 $\mu\text{g}/\text{kg}$
Linearity (calibration curve $R^2$ and concentration range)	$R^2 > 0.99$ 0.0005-0.05 ppm	$R^2 > 0.99$ 0.0005-0.05 ppm
Repeatable	Yes	Yes
Reproducible	Yes	Yes
Specific	Yes	Yes

A second-order polynomial was used to fit the calibration curve. If the second-order term is significant, care should be taken with this approach to regularly re-fit the curve and to not use it to extrapolate beyond the concentration range. In this case, however, the second-order term was seven orders of magnitude less than the first-order term, indicating that the curve was linear. Therefore, in this case, use of a second-order polynomial to fit the calibration curve was not problematic.

### IV. Method Deficiencies and Reviewer's Comments

There are no deficiencies with the analytical method, laboratory validations, or their documentation.

Sample analysis was performed at Valent Technical Center, a subsidiary of the registrant, Valent, U.S.A Corporation. OCSPP Guideline 850.6100 indicates that an independent laboratory can be privately or publicly owned or in the registrant's own organization. However, if the "laboratory is located in the registrant's organization, or is in anyway associated with the development of the original environmental chemistry method (ECM), the same people, equipment, instruments, and supplies should not be utilized to validate the ECM." The second laboratory validation (LV) was

successful on the first attempt, after confirming the instrument to use is the API 4000 LC/MS/MS (MRID 48753703). However, the same Agilent 1200 HPLC was used for the initial and second validations, meaning that MRID 48753703 did not describe a fully independent validation (the study director, staff, and other laboratory equipment were independent of those that conducted the initial validation).

Therefore, a third laboratory validation (MRID 49175802) was conducted as the true ILV, using an Agilent 1260 HPLC. The ILV was successful on the second full attempt. (The first attempt failed because the time from extraction to analysis was too long.)

### Attachment 1: Chemical Names and Structures

**Table 1. Flumiclorac-pentyl and Its Environmental Degradates**

Code Name/ Synonym	Chemical Name	Chemical Structure
<b>Flumiclorac-pentyl</b> <b>S-23031</b> <b>V-23031</b>	Pentyl 2-chloro-4-fluoro-5-(3,4,5,6-tetrahydrophthalimido)phenoxyacetate  <b>CAS No.:</b> 87546-18-7 <b>Formula:</b> C <sub>21</sub> H <sub>23</sub> ClFNO <sub>5</sub> <b>MW:</b> 423.87 g/mol <b>SMILES:</b> <chem>FC1=C(C=C(C(=C1)Cl)OCC(=O)OCCCC)N3C(=O)C2=C(CCC2)C3=O</chem>	
<b>Flumiclorac acid</b> <b>IMCA</b>	2-chloro-4-fluoro-5-(3,4,5,6-tetrahydrophthalimido)phenoxyacetic acid  <b>CAS No.:</b> 87547-04-4 <b>Formula:</b> C <sub>16</sub> H <sub>13</sub> ClFNO <sub>5</sub> <b>MW:</b> 353.74 g/mol <b>SMILES:</b> <chem>FC1=C(C=C(C(=C1)Cl)OCC(=O)O)N3C(=O)C2=C(CCCC2)C3=O</chem>	