6 Nature and Purpose of the Study

The purpose of this study was to perform an independent laboratory validation of an analytical method for the determination of mesosulfuron-methyl (AE F130060) and foramsulfuron (AE F130360) in surface water (Lit. 1).

The study was aimed to fulfil the European requirements for enforcement residue analytical methods as defined in Council Directive 91/414/EEC, Commission Directive 96/46/EC of July 16, 1996 and Guidance document SANCO/825/00 rev.6 of the 20/06/2000 (Lit. 2, 3).

7 Communication with the Sponsor Concerning the Analytical Method

Number of contacts: 1

October 02, 2002
Item: Results of the recovery rates of a first attempt for LOQ and 10 fold LOQ were discussed with the sponsor. As a result from the discussion with the sponsor the pH value of the surface water must adjusted precisely to pH 3 - 4 by adding several droplets of acetic acid.

8 Test System

The test system for the study consists of surface water. The test system was selected by the sponsor.

9 Test and Reference Item

The test items were also be used as reference item. The test and reference item and the information were supplied by the sponsor.

Mesosulfuron-methyl (AE F130060)

Name:	Mesosulfuron-methyl (AE F130060)
Chemical name:	Methyl 2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-4-methanesulfonamidomethylbenzoate
Batch No.:	CIW 205
Purity:	98.1 %
Number of the certificate:	AZ 09762
Expiration date:	18-Jan-06
Appearance:	White powder
Storage conditions:	<-18°C
Stability of the pure material:	See expiration date
Stability under test conditions:	Was checked within the study

Foramsulfuron (AE F130360)

Name:	Foramsulfuron (AE F130360)
Chemical name:	N,N-dimethyl-2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-4-formylaminobenzamide
Batch No.:	32746-115
Purity:	98.7 %
Number of the certificate:	AZ 09268
Expiration date:	12-Jul-05
Appearance:	Beige powder
Storage conditions:	<-18°C
Stability of the pure material:	See expiration date
Stability under test conditions:	Was checked within the study

10 Specimens

The surface water specimen was taken on July 02, 2002 and transported in polypropylene bottles. During sampling the air temperature was 18°C. They were arrived in good condition on July 03, 2002 and were stored until analysis at <-18°C. The surface water specimens were characterised by pH-value, conductivity and total organic carbon. The measurement of the conductivity is based on the DIN EN 27888[2]. The determination of the TOC is based on the DIN EN 1484[3]. The sampling location is presented in a map (Appendix I).

[2] Determination of the electrical conductivity (ISO 7888:1985) DIN EN 27888:1993

[3] Determination of total organic carbon (TOC) and the dissolved organic carbon (DOC) DIN EN 1484: 1997

10.1 Surface water

Specimen code:	010/2051607
Name of the river:	Scherbach
Location:	Kemading, 94086 Griesbach (Bavaria)
pH-value:	7.9
Conductivity at 8.6°C:	323 µs/cm
Total organic carbon:	11.93 mg/L

11 Residue Analysis

11.1 Analytical Method

mesosulfuron-methyl (AE F130060) and foramsulfuron (AE F130360) were analysed according to "Validation of the Enforcement Method EM F04/00-0 for Surface Water by LC-MS/MS - Mesosulfuron-methyl (AE F130060), Foramsulfuron (AE F130360)" (Lit. 1).

11.2 Summary of the Method

mesosulfuron-methyl (AE F130060) and foramsulfuron (AE F130360) were extracted by solid-phase extraction. The sulfonyl ureas were eluted with acetonitrile / water (6:4, v:v) and determined by LC-MS/MS measurement.

11.3 Minor Modifications/Adaptations

The following main items of the method were modified and/or adapted. The section numbers refer to the original method:

Section 5.2:

Old (original method 02F026)
List of reagents used in the original method.

New (this validation)
Equivalent reagents were used. They are listed in detail in Paragraph 11.4.

Section 5.5.4:

Old (original method 02F026)
The sulfonyl ureas are eluted with acetonitrile / water (3:2, v/v) into a 5 mL graduated flask until the mark is reached.

New (this validation)
The sulfonyl ureas were eluted with acetonitrile / water (6:4, v/v) into a 10 mL graduated flask.

Reason for the modifications:
These minor modifications were necessary because of slightly different laboratory procedures and different instrumentation.

11.4 Equipment and Materials

Analytical balance	Model BP 210 D, serial No 50707526 D95-09-011 - Sartorius Model AE 166; serial No 38680 - Mettler Model BA 4100S, serial No 20603530 - Sartorius
Top loading balance	Model BP 3100S, serial no. 40608510 - Sartorius Model GS 3200-2, serial no. 81103161 - Kern
Magnetic stirrer	Model 11 MR 2002 - Heidolph
Water jet pump with Woulfe bottle	
Ultra pure water unit	Seralpur Pro 90 CN
Micro pipette	Model Microman, M1000 (1000 µL) - Abimed Analysen-Technik
pH meter	Model 530, WTW - with pH-electrode model Sentix 61 - WTW Model pH 325, serial No. 7130352 – WTW
Conductance meter	Model LF 96, serial No 9811367 - WTW
DOC analyser	Model LiquiTOC, serial No. 30921127 - Foss-Heraeus

11.4.1 Glass- and Plastic ware

Volumetric pipette	different sizes, quality "AS" or equivalent - Hirschmann or Brand
Graduated pipette	different sizes
Glass dispenser	different sizes
Pasteur pipette	
Volumetric flask	different sizes
Graduated cylinder	different sizes
Glas bottle	different sizes
Funnel	different sizes
Sample vial	1 mL with PTFE sealed crimp-on caps – CS-Chromatographie Service
SPE manifold	Supelco
Reservoir	75 mL - Baker or equivalent
Adapter	Baker or equivalent
Single use syringe	different sizes
Membrane filter 0.2 µm (PTFE)	Macherey-Nagel
PE bottle	500 mL - Burdich (local glass blower)

11.4.2 Solvents and Reagents

Acetonitrile	Part No. 1.14291 - Merck
Methanol	Part No.. T169.1 - Roth
Acetic acid (conc.)	Part No. 3738.2 - Roth

Triethylamine	Part No. 90340 - Fluka
SPE cartridges (C 18, SEP Pak Plus)	Part No. WAT 023635 - Waters
Potassium chloride	Batch No. TA 114336612 - Merck
Potassium hydrogenphthalate	Batch No. A 297274132 - Merck
Sodium carbonate	Batch No. A311693127 - Merck
Sodium peroxodisulphate	Batch No. K30619609 - Merck
Ortho phosphoric acid	Batch No. K30371373 - Merck
Water	Ultra pure water from the Seralpur Pro 90 CN apparatus - Seral

11.4.3 LC-MS/MS Equipment

HPLC	Serie 1100 - Hewlett-Packard
Degasser	Model G1322A, serial No.: DP73011145
Pump	Model G1312A, serial No.: DE83102448
Autosampler	Model G1329A, serial No.: DE82201949
Thermostat for autosampler	Model G1330A, serial No.: DE82201965
Column oven with 6 port valve	Model G1316A, serial No.: DE82205517
LC-MS/MS	Model Quattro, serial No.: 9151 – Micromass
Vacuum pump	Model E2M28, serial No.: 802207 – Edwards
Cryostat	Model RTE-101, serial No.: 198075118 – Neslab
Compressor	Model 4000-40M, serial No.: 473110 – Jun-Air
Nitrogen generator	Model 75-72, serial No.: 7572-0668, Whatman

11.4.4 Mobil Phase

Mobile Phase:	A	0.01 mol/L Formic acid
	B	Acetonitrile

11.5 Solutions of the Test and Reference Items

11.5.1 Stock Solution

Stock solutions for calibration and fortification were prepared according to the following typical procedure:

Example:

54.55 mg of the test and reference item AE F130060 were weighed into a 50 mL volumetric flask and dissolved in acetonitrile. Taking into account the purity of the reference item, the concentration of the stock solution was calculated to be 1.070 mg/mL. The stock solution was transferred into a glass vial and stored at 4-8°C in a refrigerator.

43.95 mg of the test and reference item AE F130360 were weighed into a 50 mL volumetric flask and dissolved in acetonitrile / triethylamine (4/1, v/v). Taking into account the purity of the reference item, the concentration of the stock solution was calculated to be 0.8676 mg/mL. The stock solution was transferred into a glass vial and stored at 4-8°C in a refrigerator.

1 mL of the stock solution of AE F130060 and AE F130360 each were mixed into a 100 mL volumetric flask and filled up with a mixture of acetonitrile / water (6/4, v/v). The nominal concentration of the stock solution mix was calculated to be 10.70 µg/mL for AE F130060 and 8.676 µg/mL AE F130360.

The stability of stock solutions used during the analytical phase of the study was investigated. The solutions of AE F130060 and AE F130360 were stable for this period of time when stored at 4-8°C (see Tables 4 to 6).

11.5.2 Calibration and Fortification

Solutions for calibration and fortification were prepared according to the following typical procedure:

The stock solution mix was used to prepare standard solutions in the range of approximately 0.04 to 214 ng/mL for AE F130060 and approximately 0.03 to 174 ng/mL for AE F130360 in a mixture of acetonitrile / water (6/4, v/v). The exact concentrations of the standards taking into account the precise weight were used for the calculation of the residue data.

For matrix matched calibration, standard solutions were prepared by evaporation of 100 µL of a final extract of an untreated specimen to dryness using a gentle stream of nitrogen and re-dissolving with 100 µL of the corresponding standard solution.

11.6 Specimen Preparation

Homogenised specimen materials (by shake) were used for the validation.

11.7 Fortification

The fortification levels for AE F130060 and AE F130360 were calculated using following equation:

$$C_{FOR} = \frac{c \cdot V_{Std}}{V} \left[\frac{\mu g}{L}\right]$$

where

C_{FOR}: amount of test item fortified [µg/L]
c: concentration of the standard solution for fortification [µg/mL]
V_{Std}: volume of the standard solution added to the specimen [mL]
V: specimen volume [L]

Examples:
0.25 L of surface water specimen 010/2051607-A were fortified with 0.59 mL of standard solution of AE F130060 (0.00214 µg/mL). The fortification level of AE F130060 was calculated using equation above:

$$C_{FOR} = \frac{0.00214 \cdot 0.59}{0.25} = 0.00505 \left[\frac{\mu g}{L}\right]$$

where

C_{FOR}: amount of test item fortified [µg/L]

c: 0.00214 [µg/mL]
V_{Std}: 0.59 [mL]
V: 0.25 [L]

11.8 Extraction

250 mL of the surface water specimen were adjusted precisely to pH 3 - 4 by adding several droplets of acetic acid. The pH value was measured with a pH electrode.

RP C18 extraction cartridges were equilibrated with 10 mL of methanol and washed with 10 mL of water prior to extraction. A 70 mL reservoir was set on top of the cartridge.

The water specimens were sucked through the C18 cartridge with a flow rate of ca. 4 - 5 mL / min. The cartridge and the reservoir were washed with 10 mL of water and the cartridge was sucked to dryness within 1 to 2 min.

The sulfonyl ureas were eluted with acetonitrile / water (6/4, v/v) into a 10 mL graduated flask until the mark is reached. The specimen was filtered over a 0.2 µm single-use filter prior to LC-MS/MS analysis.

11.9 LC-MS/MS – Chromatographic Conditions

Tuning Parameters: ES+		
Source Page (ESI)		
Capillary	3.5	kV
Cone	24	V
Extractor	2	V
RF Lens	0.20	V
Source Block Temperature	120	°C
Desolvation Temperature	350	°C
MS / MS		
Entrance	10	V
Exit	15	V
Ion Energy 1	2	V
LM Resolution 1	10	
HM Resolution 1	10	
Ion Energy 2	2	V
LM Resolution 2	15	
HM Resolution 2	15	
Multiplier	650	V
Pressures		
Analyser vacuum	$3.1*10^{-5}$	mbar
Gas cell	$1.4*10^{-3}$	mbar
Flows		

Neb Gas Flow	68	L/h
Desol Gas Flow	455	L/h
MRM of Mass Pair: 504.13 > 182.17 (AE F130060)		
Dwell	0.3	sec
Cone	27	V
Col. Energy	20	eV
MRM of Mass Pair: 369.79 >261.09 (AE F075032)		
Dwell	0.3	sec
Cone	23	V
Col. Energy	13	eV
MRM of Mass Pair: 453.27 >182.17 (AE F130360)		
Dwell	0.3	sec
Cone	24	V
Col. Energy	22	eV

HPLC Conditions		
Column	250 mm * 3 mm, 5 µm Hypersil BDS C18 - MZ Analysentechnik	
Column oven	30	°C
Eluent	A	B
	0.01 mol/L Formic acid	Acetonitrile
Gradient program (time table)		
Time (min)	% A	% B
0	80	20
3.0	80	20
13.0	20	80
22.0	20	80
24.00	80	20
29.00	80	20
Flow	0.25	mL/min
Injection volume	50	µL
Split (detector/waste)	1 : 1	
Retention time window		

ES+	14 - 22	min
Temperature (Autosampler)	18°C	

LC-MS/MS – Chromatographic Conditions – Summary

Analyte	Retention time (typical) - min	Source polarity	Parent	Daughter ion
AE F130360	16.8	positive	453.27	182.17
AE F130060	18.5	positive	504.13	182.17

12 Calibration and Calculations

12.1 Calibration

The chromatographic systems were calibrated by measuring calibration solutions interspersed between the extracts of the specimens. The standard solutions were prepared with pure solvent and together with specimen material of an untreated specimen to compensate the impact of the matrix on the peak response (matrix matched calibration). The analytical calibration extended over a range appropriate to the lowest and highest nominal concentration of the analyte ± at least 20 %. Eight different concentrations were measured.

The concentrations of AE F130060 and AE F130360 were determined using peak area. The peak area of each analyte was determined using the Mass Lynx data system. The calibration curve and further calculations such as mean and standard deviation were calculated according to Funk et al. (Lit. 4).

The correlation coefficients (r) of the calibration curves which were used for the evaluation of the reported results were above 0.999.

12.2 Calculations

12.2.1 AE F130060 and AE F130360

A calibration curve was generated from the peak area using equation (1).

$$y_{rel.} = c \cdot x^2 + b \cdot x + a \qquad (1)$$

where

y: Peak area

x: Concentration of reference item injected [ng/mL]

a: Ordinate intercept

b, c: Coefficient

The amounts of AE F130060 and AE F130360 in the specimen were calculated using equation (1), the transformed equation (2).

$$x = -\frac{b}{2 \cdot c} - \sqrt{\frac{b^2}{4 \cdot c^2} - \frac{a - y}{c}} \qquad (2)$$

The concentrations C of AE F130060 and AE F130360 were calculated from x of equation (2) taking into account the injection volume, the specimen volume and the final volume caused by the extraction procedure (3).

$$C = \frac{x \cdot V_{end}}{V_i \cdot V} \left[\frac{\mu g}{L}\right] \qquad (3)$$

where
- C: analysed concentration of AE F130060 or AE F130360 [µg/L]
- x: analysed amount in the specimen portion [ng]
- V: specimen volume [L]
- V_{end}: final volume of the cleaned-up extract used for chromatography [mL]
- V_i: injection volume [µL]

The recovery data was calculated according to equation (4).

$$R = \frac{C \cdot 100}{C_{FOR}} \qquad (4)$$

where
- R: recovery rate [%]
- C: analysed concentration in the fortified specimen [µg/L]
- C_{FOR}: amount of test item fortified [µg/L]

If blank values had to be subtracted the peak size of the blank was subtracted from the fortified peak.

The residue concentrations and recovery data were calculated using Microsoft Excel97 version SR-2. The final results were rounded at the end of the calculation process. Slight deviations may be obtained and can be explained by rounding effects when re-calculating the results from the data given in the report.

12.2.2 Example for Calculation

The fortified surface water specimen 010/2051607-A was extracted on October 15, 2002 and analysed on October 15, 2002.

The coefficients of the calibration curve for AE F130060 were a = -12.130, b = 82150.236 and c = -83749.048. A plot of this curve is provided in Figure 2. The peak area of the analyte corresponded to 547 µV*s. The amount of AE F130060 was calculated according to equation (2):

$$x = -\frac{82150.236}{2 \cdot -83749.048} - \sqrt{\frac{82150.236^2}{4 \cdot -83749.048^2} - \frac{-12.130 - 547}{-83749.048}} = 0.0069\ [ng]$$

The residue concentration of AE F130060 was calculated according to equation (3).

See equation (3)
$$C = \frac{0.0069 \cdot 10}{50 \cdot 0.25} = 0.00552 \left[\frac{\mu g}{L}\right]$$

where
- C: analysed concentration of AE F130060 [µg/L]
- x: 0.0069 [ng]
- V: 0.25 [L]
- V_{end}: 10 [mL]
- V_i: 50 [µL]

The recovery data was calculated according to equation (4).

(4)
$$R = \frac{0.00552 \cdot 100}{0.00505} = 109\%$$

where
R: recovery rate [%]
C: 0.00552 [µg/L]
C_{FOR}: 0.00505 [µg/L]