2 Objectives

The objective of this study is the validation of the analytical method EM F04/00 – 0 (1) for the determination of residues of the sulfonyl ureas mesosulfuron-methyl and foramsulfuron in surface water by LC-MS/MS.

3 Test commodities

The surface water was taken from the pond at building F821 in Industrie Park Höchst.

The characteristics* of the surface water are:

Pond "F821"	pH	8.2
	DOC [mg/L][1]	5 / 6
	total hardness [° dH][2]	5.9

[1] dissolved organic content (determination was done twice)
[2] degree German hardness [(mg CaO + mg MgO) / 100 mL water]

4 Analytical targets and reference substances

4.1 Analytical targets

The analytical targets are Mesosulfuron-methyl (AE F130060) and Foramsulfuron (AE F130360).

* Determination of the characteristics of the surface water was not done under GLP.

Mesosulfuron-methyl (AE F130060)

Chemical name (CA): methyl 2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-4-methansulfonamidomethylbenzoate

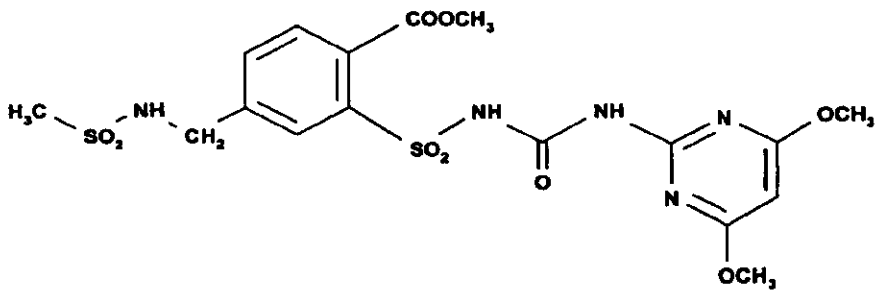
Empirical formula: $C_{17}H_{21}N_5O_9S_2$

Molecular weight: 503.5

Solubility (20 °C):

Solvent	Solubility		Source
water (pH 5.66)	21.4	mg/L	ref. 1
buffer pH 4	2.15	mg/L	ref. 1
buffer pH 5	7.24	mg/L	ref. 1
buffer pH 7	483	mg/L	ref. 1
buffer pH 9	$15.4 \cdot 10^3$	mg/L	ref. 1
buffer pH 10	$13.8 \cdot 10^3$	mg/L	ref. 1
acetone	$13.7 \cdot 10^3$	mg/L	ref. 1
ethyl acetate	$2.03 \cdot 10^3$	mg/L	ref. 1
n-hexane	<1	mg/L	ref. 1
isopropanol	96	mg/L	ref. 1
toluene	12.6	mg/L	ref. 1
acetonitrile	$8.4 \cdot 10^3$	mg/L	ref. 1
methylene chloride	$3.8 \cdot 10^3$	mg/L	ref. 1

Structural formula:



Certificate of analysis: AZ 09762
Drawn up by: Aventis CropScience GmbH
Produktanalytik
D-65926 Frankfurt am Main, Germany
Purity: 98.1 % (w/w)
Expiry date (d/m/y): 18 Jan 2006

Foramsulfuron (AE F130360)

Chemical name (UPAC): N,N-dimethyl-2-[3-(4,6-dimethoxypyrimidin-2-yl) ureidosulfonyl]-4-formylaminobenzamide

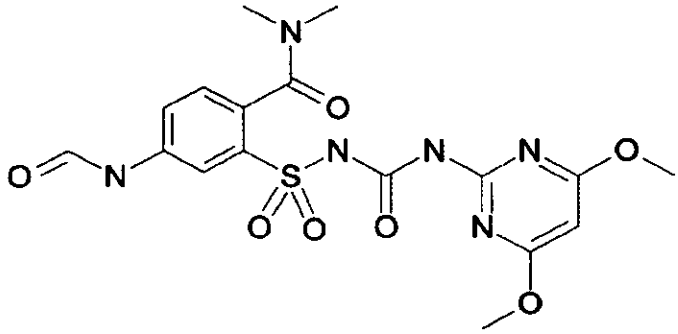
Empirical formula: $C_{17}H_{20}N_6O_7S$

Molecular weight: 452.5

Solubility (20 °C):

Solvent	Solubility		Source
Acetone	1925	mg/L	ref. 1
Acetonitrile	1111	mg/L	ref. 1
1,2-Dichloroethane	185	mg/L	ref. 1
Ethyl acetate	362	mg/L	ref. 1
n-Heptan	<10	mg/L	ref. 1
Methanol	1660	mg/L	ref. 1
p-Xylene	<10	mg/L	ref. 1

Structural formula:



Certificate of analysis: AZ 09074

Drawn up by: Aventis CropScience GmbH
Produktanalytik
D-65926 Frankfurt am Main, Germany

Purity: 99.0 % (w/w)

Expiry date (d/m/y): 26 Apr 2006

5 Procedures

5.1 Principle of analytical method

The flow sheet of the analytical method EM F04/00 – 0 (1) is presented in Annex I.

The water sample can optionally be filtered and is afterwards adjust to pH 3-4 with a few droplets of acetic acid. The sample is enriched on a RP C18-cartridge (conditioned with methanol and water). The sulfonyl ureas are eluted with acetonitrile / water 6:4 (v:v). The concentration of the substances in the final solution is determined by LC-MS/MS.

5.2 Reagents

- methanol Chromasolv (Riedel-de Haën, Germany)
- acetonitrile Chromasolv p.A. (Riedel-de Haën, Germany)
- deionized water (prepared with Milli-Q-Plus, Millipore)
- triethylamine, 0.02 mol/L (Riedel-de Haën, Germany)
- conc. acetic acid
-
- AE F130060, analytical standard (Aventis CropScience GmbH, Germany)
- AE F130360, analytical standard (Aventis CropScience GmbH, Germany)
- RP C18 – cartridge, SEP-Pak®Plus, Waters Part No. WAT023635
- glass microfibre filters, 934-AH, 70 mm ∅, Cat. No. 1827 070 (Whatman)

Stock solutions of the analytical standard AE F130360 were prepared by dissolving about 50 mg of analytical standard in ca. 50 mL acetonitrile / triethylamine (0.02M) (4:1, v/v). The stock solution of AE F130060 was prepared by dissolving about 50 mg of analytical standard in ca. 50 mL acetonitrile. Concentration of the stock solutions was 1.00 mg/mL each. Working solutions specially for this validation were prepared from the working solutions above by further dilution with acetonitrile / water (3:2, v/v).

5.3 Apparatus

The following list contains the apparatus used for validation. Suitable alternatives can be taken.

- standard laboratory glassware
- HPLC system with MS/MS-detector

5.4 Preparation of samples and storage

Surface water samples from the pond in the surrounding of the laboratory were freshly taken within 1 day before start of the work up procedure. Within these 1 day the water samples were stored at room temperature.

5.5 Laboratory steps

5.5.1 Sample preparation

250 mL of the surface water sample is adjusted to pH 3 – 4 by adding several droplets of acetic acid.
The water samples can be filtered through a glass fibre filter (e.g. Whatman 934-AH™) if necessary.

5.5.2 Conditioning of the RP C18- cartridges

The RP C18-extraction cartridges are conditioned with 10 mL methanol and washed with 10 mL deionized water prior to extraction. A 70 mL reservoir is set on top of the cartridge.

5.5.3 Extraction

The water samples are sucked through the C18-cartridge with a flow rate of ca. 4 – 5 mL/min. The cartridge and the reservoir are washed with 10 mL of deionized water and the cartridge is sucked to dryness within 1 to 2 min.

5.5.4 Elution and preparation of the final solution

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

5.6 Determination of residues

The following conditions have been used successfully during validation of the analytical method. If different equipment and columns are used, modifications of the given conditions may be necessary.

HPLC-conditions

Column:	Hypersil BDS, 5 µm, 250 mm x 3 mm
Column temperature:	30 °C
Injection volume :	50 µL
Flow:	0.25 mL / min
Pump A:	Formic acid 0.01 mol/L
Pump B:	Acetonitrile

Gradient

Time [min]	Formic acid [% A]	Acetonitrile [% B]
0	80	20
3	80	20
13	20	80
22	20	80
24	80	20
29	80	20

MS/MS Conditions

Analytical standards of all compounds should be taken to determine the most sensitive mass-transition from parent to daughter ion. Afterwards all relevant parameters of the MS/MS-system have to be optimized regarding a maximum sensitivity. Tabulated values below were chosen during this validation study but may vary depending on the system used.

To minimize contamination of the MS/MS system the capillary outlet behind the HPLC-column was connected to a switch valve. This construction ensures that only the flow within a certain time window (expected retention time ± ca. 1.5 min) enters the system while the rest is discarded. During the discarding phase the MS/MS system is stabilised with a flow of 0.25 mL/min of formic acid 0.01 mol/L / Acetonitrile (1 : 1, v/v), provided by an additional HPLC pump.

Tune parameter MS/MS

Modus:	MRM; Electrospray positive	Analyser:	
Capillary:	3.50 kV	LM Res 1	10.0
Extractor:	2 V	HM Res 1	10.0
RF Lens:	0.20 V	I Energy 1	1.0 V
Source block temp:	150 °C	Entrance	5
Desolvation temp.:	350 °C	Exit	7
		LM Res 2	12.0
		HM Res 2	12.0
Nebuliser gas	ca. 60 L/h	I Energy 2	2.0 V
Drying gas	ca. 600 L/h	Multiplier	650 V

Scanning method

Substance	Parent [m/z]	Daughter [m/z]	Dwell [s]	Coll. Energy	Cone Voltage [V]
AE F130060	504.20	182.00	0.3	20	27
AE F130360	453.20	182.00	0.3	22	24

Retention time

Substance	Retention time [min]	Detection time windows [min]
AE F130060	ca. 17.5	14.3 – 20.2
AE F130360	ca. 15.5	14.3 – 20.2

5.7 Calibration

Concentrations of the sulfonyl ureas were calculated using external standards (matrix matched standards as well as standards in pure solvent) at 6 different concentrations over a range from 0.1 up to 4 ng/mL.

The recommended order of samples / test solutions for setting up a sequence for LC-determination is 'test solution – sample – test solution– sample '. If different equipment is used and /or more or less samples are worked up, modifications of this order may be necessary.

5.8 Calculations

Determination of concentration of the analytical target in the final solution
The concentrations of the analytes in control samples, fortified samples and treated samples were calculated using external standard procedures with multi level calibration.

Multi level calibration (calibration function):

For the calibration peak areas (heights) of the standards were plotted versus the corresponding concentrations. An optimized calibration curve of the following form

$$f(Cs) = a + b\,C_s + c\,C_s^2 \qquad (1)$$

is calculated, where $f(C_S)$ is the peak area (height), C_S the concentration of the analyte in the final sample extract and a, b, c are constants.

Determination of residues

Calculation of residues was carried out by a data handling software according to the following procedure

$$Res = \frac{C_S \bullet V_{end} \bullet f}{W} \qquad \left[\mu g/L = \frac{(ng/mL) \bullet mL \bullet 1}{mL}\right] \qquad (2)$$

$$f = \frac{V_1 \bullet V_2 \bullet V_n}{T_1 \bullet T_2 \bullet T_n} \qquad \left[1 = \frac{mL \bullet mL \bullet mL}{mL \bullet mL \bullet mL}\right] \qquad (3)$$

Res	Residue	[µg/L]
C_S	Concentration in final sample solution V_{end} (treated, untreated and recovery)	[ng/mL]
W	Sample volume	[mL]
f	Dilution factor	without dimension
V_1	Volume for primary extraction	[mL]
V_2	Volume after making up of aliquot T_1	[mL]
V_n	Volume after making up of aliquot T_{n-1} (n = 3, 4 and so on)	[mL]
V_{end}	Final sample solution (identical with V_2 or V_3 or V_n depending on the method)	[mL]
T_1	Aliquot of V_1	[mL]
T_2	Aliquot of V_2	[mL]
T_n	Aliquot of V_n (n = 3, 4 and so on)	[mL]

Determination of recovery rates

Calculation of recovery rates were carried out by a data handling software according to the following procedure

$$Res_d = Res_{(Rec)} - Res_{(Unt)} \qquad \left[\frac{\mu g}{L} = \frac{\mu g}{L} - \frac{\mu g}{L}\right] \qquad (4)$$

$$Rec = \frac{Res_d}{Res_f} \bullet 100 \qquad \left[\% = \frac{\mu g/L}{\mu g/L} \bullet \%\right] \qquad (5)$$

$Res_{(Rec)}$	Residue in the sample solution of the recovery test calculated with equation (2) and (3)	[µg/L]
$Res_{(Unt)}$	Residue in the sample solution of the corresponding untreated control sample calculated with equation (2) and (3)	[µg/L]
Rec	Recovery rate	[%]
Res_f	Concentration spiked for fortification	[µg/L]
Res_d	Concentration detected by analytical method	[µg/L]

Annex I: Analytical method flow sheet

Surface water

Extraction of sulfonyl ureas on RP C_{18}

take 250 mL of the water sample

(filter if necessary)

adjust to pH 3-4 with acetic acid

condition RP C_{18}-cartridge with 10 mL methanol and 10 mL deionized water

add 70 mL reservoir to cartridge

suck sample through RPC_{18}-cartridge with a flow rate of ca. 4 – 5 mL/min

wash reservoir and cartridge with 10 mL deionized water

suck the C18-cartridge to dryness within 1-2 min

elute with acetonitrile/water (6/4, v/v) into a 5 mL graduated flask until the mark is reached

LC-MS/MS

quantification with LC-MS/MS

Annex II: Deviation of the method

5.2 Reagents

Old (original method EM F04/00-0)
Stock solutions of the analytical standard AE F130360 were prepared by dissolving about 50 mg of analytical standard in ca. 50 mL acetonitrile / triethylamine (0.02M) (4:1, v/v). The stock solution of AE F130060 was prepared by dissolving about 50 mg of analytical standard in ca. 50 mL acetonitrile. Concentration of the stock solutions was 1.00 mg/mL each. Working solutions were prepared from the stock solution by further dilution with **acetonitrile / triethylamine (0.02M) (4:1, v/v).**

New (this validation)

Stock solutions of the analytical standard AE F130360 were prepared by dissolving about 50 mg of analytical standard in ca. 50 mL acetonitrile / triethylamine (0.02M) (4:1, v/v). The stock solution of AE F130060 was prepared by dissolving about 50 mg of analytical standard in ca. 50 mL acetonitrile. Concentration of the stock solutions was 1.00 mg/mL each. Working solutions specially for this validation were prepared from the working solutions above by further dilution with **acetonitrile / water (3:2, v/v).**

5.5.1 Sample preparation

Old (original method EM F04/00-0)

100 mL of the surface water sample is adjusted to pH 3 – 4 by adding **2 to 3** droplets of acetic acid.
The water samples can be filtered through a glass fibre filter (e.g. Whatman 934-AH™) if necessary.

New (this validation)

250 mL of the surface water sample is adjusted to pH 3 – 4 by adding **several** droplets of acetic acid.
The water samples can be filtered through a glass fibre filter (e.g. Whatman 934-AH™) if necessary.

5.5.4 Elution and preparation of the final solution

Old (original method EM F04/00-0)

The sulfonyl ureas are eluted with acetonitrile/water (3:2, v/v) into a **10 mL** graduated flask until the mark is reached.

New (this validation)

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

5.6 Determination of residues

Old (original method EM F04/00-0)

Tune parameter MS/MS

Modus:	MRM; Electrospray positive	Analyser:	
Capillary:	3.50 kV	LM Res 1	10.0
Extractor:	2 V	HM Res 1	10.0
RF Lens:	0.20 V	I Energy 1	1.0 V
Source block temp:	150 °C	**Entrance**	**10**
Desolvation temp.:	350 °C	**Exit**	**15**
		LM Res 2	**15.0**
		HM Res 2	**15.0**
Nebuliser gas	ca. 60 L/h	I Energy 2	2.0 V
Drying gas	ca. 600 L/h	Multiplier	650 V

New (this validation)

Tune parameter MS/MS

Modus:	MRM; Electrospray positive	Analyser:	
Capillary:	3.50 kV	LM Res 1	10.0
Extractor:	2 V	HM Res 1	10.0
RF Lens:	0.20 V	I Energy 1	1.0 V
Source block temp:	150 °C	**Entrance**	**5**
Desolvation temp.:	350 °C	**Exit**	**7**
		LM Res 2	**12.0**
		HM Res 2	**12.0**
Nebuliser gas	ca. 60 L/h	I Energy 2	2.0 V
Drying gas	ca. 600 L/h	Multiplier	650 V