## SUMMARY

**Enforcement Method of Iodosulfuron-methyl-sodium and its metabolite Metsulfuron-methyl in Surface Water by HPLC incl. Validation**

**Extension of the enforcement method EM F 01/98 - 0 for Iodosulfuron-methyl-sodium in Drinking Water to its metabolite Metsulfuron-methyl incl. Validation**

**Iodosulfuron-methyl-sodium (AE F115008)**
**Metsulfuron-methyl (AE F075736)**

**Relevant residue**
Iodosulfuron-methyl-sodium (AE F115008)
Metsulfuron-methyl (AE F075736)

**Test commodity**
Drinking water
Surface water

**Principle of the method**
Metsulfuron-methyl in drinking water:

The water sample is adjusted to pH 2.5 with phosphoric acid (2 N) and sucked through an C18-cartridge (conditioned with 5 mL methanol and 5 mL water). AE F075736 is eluated with 5 mL methanol. Metsulfuron-methyl in the final solution in acetonitrile/water (1/1, v/v) is determined by HPLC/UV.

Metsulfuron-methyl and iodosulfuron-methyl-sodium in surface water:

The water sample is adjusted to pH 2.5 with phosphoric acid (2 N) and filtered through a glass microfibre filter and a cellulose nitrate filter (0.45 µm). The sample is sucked through a NH2 / C18-cartridge (conditioned with 5 mL methanol and 5 mL water). AE F115008 and AE F075736 are eluated with 15 mL methanol/water (60:40, v/v). After reducing to dryness, the residue is dissolved in 20 mL toluene and sucked through a Silicagel-cartridge (conditioned with 5 mL toluene). AE F115008 and AE F075736 are eluted with 30 mL toluene/methanol (95:5, v/v). Iodosulfuron-methyl-sodium and metsulfuron-methyl in the final solution in acetonitrile/water (1/1, v/v) are determined by HPLC/UV.

**Calibration**
A curve of the form $y = a + bx + cx^2$ is applicable over the tested range of 0.1 to 2.0 µg metsulfuron-methyl/mL and 0.1 to 2.0 µg iodosulfuron-methyl-sodium/mL.

Analytical method flow sheet

**Metsulfuron-methyl in drinking water**

*Extraction AE F075736 and C18-cartridge clean-up*

1000 mL water
is adjust to pH 2.5 with phosphoric acid (2 N)
and sucked through an Chromabond 500 mg C18-cartridge
(conditioned with 5 mL methanol and 5 mL water)
with a flow rate of ca. 10 – 20 mL/min

↓

Wash used glassware with 200 mL Millipore water
and suck the washing water through the cartridge.

↓

Suck the C18-cartridge to dryness within ca. 5 min.

↓

Eluate AE F075736 with 5 mL methanol
into a test tube

↓

Reduce the eluate to dryness using a vacuum rotary evaporator
(bath temperature ca. 50 °C).

↓

*HPLC*

Dissolve the residue in 1.0 mL water/acetonitile (1/1, v/v) for quantification
with UV/HPLC

**Iodosulfuron-methyl-sodium and metsulfuron-methyl in surface water**

| | |
|---|---|
| *Extraction AE F115008 and AE F075736 and NH2 / C18-cartridge clean-up* | 1000 mL water<br>is adjust to pH 2.5 with phosphoric acid (2 N),<br>filtered through a glass microfibre filter and a cellulose nitrate filter (0.45 µm)<br>the sample is sucked through a Chromabond 500 mg NH2 / 500 mg C18-cartridge<br>(conditioned with 5 mL methanol and 5 mL water)<br>with a flow rate of ca. 10 – 20 mL/min |
| | Wash used glassware with 200 mL Millipore water<br>and suck the washing water through the cartridge. |
| | Suck the NH2 / C18-cartridge to dryness within ca. 5 min. |
| | Wash the NH2 / C18-cartirdge with 10 mL methanol/water (30:70, v/v). |
| | Eluate AE F115008 and AE F075736 with 15 mL methanol/water (60:40, v/v) |
| | Reduce the eluate to dryness using a vacuum rotary evaporator<br>(bath temperature ca. 50 °C). |
| *Silicagel-cartridge clean-up* | Dissolve the residue in 20 mL toluene (if necessary use an ultrasonic bath)<br>suck through a Silicagel-cartridge (conditioned with 5 mL toluene) |
| | Discard the eluate, suck the Silicagel-cartridge to dryness |
| | Wash the round-bottom flask with 30 mL toluene/methanol (95:5, v/v)<br>and elute AE F115008 and AE F075736 with this solution<br>Reduce the eluate to dryness using a vacuum rotary evaporator<br>(bath temperature ca. 40 °C). |
| *HPLC* | Dissolve the residue in 1.0 mL water/acetonitile (1/1, v/v) for quantification with UV/HPLC |

## 4.2 Test and reference substances

**Iodosulfuron-methyl-sodium (AE F115008)**

Chemical name (IUPAC): methyl 4-iodo-2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)ureido-sulfonyl]benzoate, sodium salt

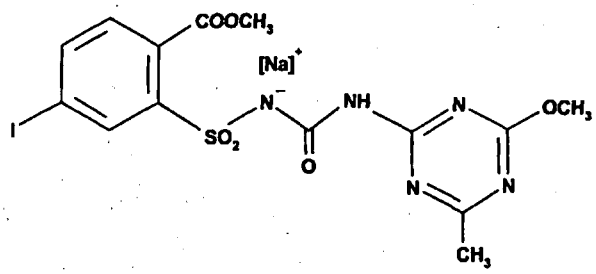
Empirical formula: $C_{14}H_{13}IN_5NaO_6S$

Molecular weight: 529.3

Solubility (20 °C):

| Solvent | Solubility | | Source |
|---|---|---|---|
| acetone | > 380 | g/L | ref. 2 |
| dichloromethane | > 500 | g/L | ref. 2 |
| ethyl acetate | 23 | g/L | ref. 2 |
| n-hexane | $1.2 \bullet 10^{-3}$ | g/L | ref. 2 |
| methanol | 12 | g/L | ref. 2 |
| n-heptane | $1.1 \bullet 10^{-3}$ | g/L | ref. 2 |
| 2-propanol | 4.4 | g/L | ref. 2 |
| toluene | 2.1 | g/L | ref. 2 |
| acetonitrile | 52 | g/L | ref. 2 |
| DMSO | > 500 | g/L | ref. 2 |
| PEG | 87 | g/L | ref. 2 |

Structural formula:




Certificate of analysis: AZ 07931

Drawn up by: Hoechst Schering AgrEvo GmbH
Produktanalytik
D-65926 Frankfurt am Main, Germany

Purity: 97.3 % (w/w)

Expiry date (d/m/y): 30 May 2000

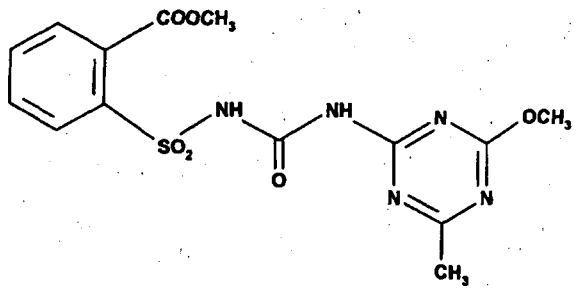
**Metsulfuron-methyl (AE F075736)**

Chemical name (IUPAC): methyl 2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) ureidosulfonyl]-benzoate

Empirical formula: $C_{14}H_{15}N_5O_6S$

Molecular weight: 381.4

Structural formula:




Certificate of analysis: AZ 06892
Drawn up by: Hoechst Schering AgrEvo GmbH
Produktanalytik
D-65926 Frankfurt am Main, Germany
Purity: 98.4 % (w/w)
Expiry date (d/m/y): 07 May 2000

## 5 Procedures

### 5.1 Principle of analytical method

The method flow sheets are presented in Appendix I.

Metsulfuron-methyl in drinking water:

The water sample is adjust to pH 2.5 with phosphoric acid (2 N) and sucked through an C18-cartridge (conditioned with 5 mL methanol and 5 mL water). AE F075736 is eluated with 5 mL methanol. AE F075736 in the final solution in acetonitrile/water (1/1, v/v) is determined by HPLC/UV.

Metsulfuron-methyl and iodosulfuron-methyl-sodium in surface water:

The water sample is adjust to pH 2.5 with phosphoric acid (2 N) and filtered through a glass microfibre filter and a cellulose nitrate filter (0.45 µm). The sample is sucked through a NH2 / C18-cartridge (conditioned with 5 mL methanol and 5 mL water). AE F115008 and AEF075736 are eluated with 15 mL methanol/water (60:40, v/v). After reducing to dryness, the residue is dissolved in 20 mL toluene and sucked through a Silicagel-cartridge (conditioned with 5 mL toluene). AE F115008 and AE F075736 are eluted with 30 mL toluene/methanol (95:5, v/v). AE F115008 and AE F075736 in the final solution in acetonitrile/water (1/1, v/v) are determined by HPLC/UV.

### 5.2 Reagents

- methanol Chromasolv, cat. No. 34860 (Riedel-de Haën, Germany)
- acetonitrile Chromasolv p.A., cat. No. 34851(Riedel-de Haën, Germany)
- deionized water
- water (e.g. prepared with Milli-Q-Plus, Millipore)
- phosphoric acid 2 N, cat. No. 30417 (Riedel-de Haën, Germany)
- toluene Pestanal, cat. No. 34494 (Riedel-de Haën, Germany)
- AE F075736, analytical standard (AgrEvo GmbH, Germany)
- AE F115008, analytical standard (AgrEvo GmbH, Germany)
- C18 – cartridge, 500 mg, cat. No. 730013 (Chromabond)
- Glass microfibre filter, cat. No. 1827070 (Whatman)
- Cellulose nitrate filter (0.45 µm), cat. No. 11306-50-N (Satorius)
- NH2/C18 – cartridge, 500mg NH2, 500mg C18, cat. No. 730618 (Chromabond)
- Silicagel-cartridge, 500 mg, ISOLUTE, cat. No. 460-0050-H (ICT)

Stock solutions of the analytical standards were prepared by dissolving about 50 mg of analytical standard of AE F075736 and 50 mg of the analytical standard of AE F115008 in ca. 50 mL acetonitrile / triethylamine (0.02 mol/L), 4:1, v/v. Concentration of the stock solutions was 1.0 mg/mL. Working solutions were prepared from the stock solution by further dilution with acetonitrile / water, 1:1, v/v.

### 5.3 Apparatus

The following list contains the apparatus used in the laboratory of the author for validation. Suitable alternatives can be taken.

- standard laboratory glassware
- rotary vacuum evaporator with water bath
- HPLC system with UV-detector
- chromatography column, Prodigy ODS, 150 mm x 4.6 mm, 5 µm
- chromatography column, Nucleosil C18, 5 µm, 250 mm x 4 mm (confirmation method)

### 5.4 Preparation of samples and storage

The samples of drinking water (Vittel) were bought November 1999.
The samples of surface water were taken from the small lake at building F821 (Industriepark Höchst) on 14 Jan 2000.

Samples were stored at room temperature.

### 5.5 Laboratory steps

#### 5.5.1 Metsulfuron-methyl in drinking water

##### 5.5.1.1 Extraction and C18-cartridge clean up

1000 mL of the water sample is adjust to pH 2.5 with phosphoric acid (2 N) and sucked through a Chromabond 500 mg C18-cartridge (conditioned with 5 mL methanol and 5 mL water) with a flow rate of ca. 10 – 20 mL/min. Wash used glassware with 200 mL Millipore water and suck the washing water through the cartridge. Suck the C18-cartridge to dryness within ca. 5 min. Eluate AE F075736 with 5 mL methanol into a test tube. Reduce the eluate to dryness using a vacuum rotary evaporator (bath temperature ca. 50 °C).

##### 5.5.1.2 Preparation of the final solution

Dissolve the residue in 1.0 mL acetonitrile/water (1/1, v/v).

### 5.5.1.3 Determination of residues

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

**HPLC-conditions**

| | |
|---|---|
| Instrument: | Beckmann |
| System | IBM-PC System 2 8570 Model 70386 |
| Pump: | 226 Beckmann |
| Detector: | Diode Array Detector 168 Beckmann |
| Injector: | Autosampler 507 Beckmann |
| Injection volume: | 100 µL |
| Column temperature: | 30 °C |
| Column | Prodigy ODS, 5 µm, 150 mm x 4,6 mm |
| Wavelength: | 233 nm |
| Flow rate: | 1.0 mL/min |
| Mobile phase: | |
| Eluent A | Acetonitrile Chromasolv |
| Eluent B | Phosphoric acid $c_{H3PO4}$= 0.01mol/L |

**Gradient program for the determination of AE F075736**

| Time [min] | Total flow pump A + B [mL/min] | Pump A (eluent A) Acetonitrile Chromasolv [%] | Pump B (eluent B) phosphoric acid $c_{H3PO4}$= 0.01mol/L [%] |
|---|---|---|---|
| 0 | 1.0 | 20 | 80 |
| 10 | 1.0 | 50 | 50 |
| 20 | 1.0 | 50 | 50 |
| 30 | 1.0 | 80 | 20 |
| 35 | 1.0 | 80 | 20 |
| 45 | 1.0 | 20 | 80 |
| 47 | 1.0 | 20 | 80 |
| 55 | 1.0 | | |

Under these conditions the retention time for AE F075736 is about 21.0 min.

The chromatography data were recorded and evaluated with TURBOCHROM© Client/Server system, PERKIN ELMER.

### 5.5.2 Iodosulfuron-methyl-sodium and metsulfuron-methyl in surface water

#### 5.5.2.1 Extraction and NH2/C18-cartridge clean up

1000 mL of the water sample is adjust to pH 2.5 with phosphoric acid (2 N) and filtered through a glass microfibre filter and a cellulose nitrate filter (0.45 µm). The sample is sucked through a Chromabond 500 mg NH2 / 500 mg C18-cartridge (conditioned with 5 mL methanol and 5 mL water) with a flow rate of ca. 10 – 20 mL/min. Wash used glassware with 200 mL Millipore water and suck the washing water through the cartridge. Suck the NH2 / C18-cartridge to dryness within ca. 5 min. and wash the NH2 / C18-cartirdge with 10 mL methanol/water (30:70, v/v). Eluate AE F115008 and AE F075736 with 15 mL methanol/water (60:40, v/v) into a round-bottom flask. Reduce the eluate to dryness using a vacuum rotary evaporator (bath temperature ca. 50 °C).

#### 5.5.2.2 Silicagel-cartridge clean up

Dissolve the residue in 20 mL toluene (if necessary use an ultrasonic bath) and suck through a Silicagel-cartridge (conditioned with 5 mL toluene). Discard the eluate, suck the Silicagel-cartridge to dryness. Wash the round-bottom flask with 30 mL toluene/methanol (95:5, v/v), if necessary use an ultrasonic bath, and elute AE F115008 and AE F075736 with this solution. Reduce the eluate to dryness using a vacuum rotary evaporator (bath temperature ca. 40 °C).

#### 5.5.2.3 Preparation of the final solution

Dissolve the residue in 1.0 mL acetonitrile/water (1/1, v/v).

#### 5.5.2.4 Determination of residues

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

**HPLC-conditions**

| | |
|---|---|
| Instrument: | Beckmann |
| System | IBM-PC System 2 8570 Model 70386 |
| Pump: | 226 Beckmann |
| Detector: | Diode Array Detector 168 Beckmann |
| Injector: | Autosampler 507 Beckmann |
| Injection volume: | 100 µL |
| Column oven: | Beckmann |
| Column temperature: | 30 °C |
| Column | Prodigy ODS, 5 µm, 150 mm x 4,6 mm (validation) |
| Column | Nucleosil C18, 5 µm, 250 mm x 4 mm (confirmation method) |
| Wavelength: | 233 nm |
| Flow rate: | 1.0 mL/min |
| Mobile phase: | |
| Eluent A | Acetonitrile Chromasolv |
| Eluent B | Phosphoric acid $c_{H3PO4}$= 0.01mol/L |

**Gradient program for the determination of AE F075736**

| Time [min] | Total flow pump A + B [mL/min] | Pump A (eluent A) Acetonitrile Chromasolv [%] | Pump B (eluent B) phosphoric acid $c_{H3PO4}$= 0.01mol/L [%] |
|---|---|---|---|
| 0 | 1.0 | 20 | 80 |
| 10 | 1.0 | 50 | 50 |
| 20 | 1.0 | 50 | 50 |
| 30 | 1.0 | 80 | 20 |
| 35 | 1.0 | 80 | 20 |
| 45 | 1.0 | 20 | 80 |
| 47 | 1.0 | 20 | 80 |
| 55 | 1.0 | | |

Under these conditions the retention time for AE F075736 is about 21.0 min and for AE F115008 about 25.4 min.

The chromatography data were recorded and evaluated with TURBOCHROM© Client/Server system, PERKIN ELMER.

Confirmatory method
For confirmatory purposes a different stationary phase was used:

HPLC-Column: Nucleosil C18, 5 µm, 250 mm x 4 mm

Under these conditions the retention time for AE F075736 is about 21.5 min and for AE F115008 about 25.5 min.

## 5.6 Calibration

The concentration of AE F075736 and AE F115008 were calculated using external standards at 4 different concentrations over a range from 0.1 ng/µL up to 1 or 2 ng/µL. The lowest concentration was 0.1 ng/µL. The highest concentration was 2 ng/µL.

The recommended order of samples / test solutions for setting up a sequence for HPLC-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.

## 5.7 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 or single level calibration.

**Single level calibration (one point calibration):**

$$C_S = \frac{P_S}{P_R} \bullet C_R \bullet \frac{I_R}{T_4} \qquad \left[ pg/\mu L = \frac{counts}{counts} \bullet pg/\mu L \bullet \frac{\mu L}{\mu L} \right] \qquad (1)$$

| | | |
|---|---|---|
| $C_S$ | Concentration in final sample solution $V_{end}$ (identical with conc. in $T_4$) (treated, untreated and recovery) | [pg/µL] = [ng/mL] |
| $C_R$ | Concentration in reference solution | [pg/µL] = [ng/mL] |
| $P_S$ | Peak area or peak height of the sample solution | [counts] |
| $P_R$ | Peak area or peak height of the reference solution | [counts] |
| $T_4$ | Injection volume of the sample solution | [µL] |
| $I_R$ | Injection volume of the reference solutions | [µL] |

**Multi level calibration (calibration curve):**

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

$$f(C_S) = P = a + bC_S + cC_S^2 \qquad (2)$$

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

$$Res = \frac{C_S \bullet V_{end} \bullet f}{W} \qquad \left[\mu g/L = \frac{(ng/mL) \bullet mL \bullet 1}{mL}\right] \qquad (3)$$

$$f = \frac{V_1 \bullet V_2 \bullet V_n}{T_1 \bullet T_2 \bullet T_n} \qquad \left[1 = \frac{mL \bullet mL \bullet mL}{mL \bullet mL \bullet mL}\right] \qquad (4)$$

| | | |
|---|---|---|
| ***Res*** | Residue | [µg/L] |
| $C_S$ | Concentration in final sample solution $V_{end}$ (treated, untreated and recovery) | [ng/mL] |
| ***W*** | Sample weight | [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

$$Res_d = Res_{(Rec)} - Res_{(Unt)} \qquad \left[\frac{\mu g}{L} = \frac{\mu g}{L} - \frac{\mu g}{L}\right] \qquad (5)$$

$$Rec = \frac{Res_d}{Res_f} \bullet 100 \qquad \left[\% = \frac{\mu g/L}{\mu g/L} \bullet \%\right] \qquad (6)$$

| | | |
|---|---|---|
| $Res_{(Rec)}$ | Residue in the sample solution of the recovery test calculated with equation (3) and (4) | [µg/L] |
| $Res_{(Unt)}$ | Residue in the sample solution of the corresponding untreated control sample calculated with equation (3) and (4) | [µg/L] |
| ***Rec*** | Recovery rate | [%] |
| $Res_f$ | Concentration spiked for fortification | [µg/L] |
| $Res_d$ | Concentration detected by analytical method | [µg/L] |

### 6.3 Blank values

Drinking water:
Analysis of control samples has shown that apparent residues of AE F075736 observed were n.d. (not detectable, < 0.3 x LOQ). This demonstrates that 0.1 µg/L is a feasible level for recognition of residues with reasonable certainty.

Surface water:
Analysis of control samples has shown that apparent residues of AE F115008 and AE F075736 observed were n.d. (not detectable, < 0.3 x LOQ). This demonstrates that 0.1 µg/L is a feasible level for recognition of residues with reasonable certainty.

### 6.4 Critical steps of the method

There are no critical steps of the method.

### 6.5 Time for analysis

From extraction of the samples to preparation of the final solutions for HPLC/UV determination, it is normally possible to analyse a batch of 14 samples in three day.