SUMMARY

Enforcement Method for Soil by LC-MS/MS

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

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

Test commodity
Two Soils: loamy sand and silty loam

Principle of the method
Residues of the sulfonylureas were extracted with acetonitrile / triethylamine 0.02 mol/L (4:1, v/v) from soil. After evaporation to dryness, the residues were reconstituted in water acidified with formic acid (0.01 mol/L). After a liquid/liquid extraction with ethyl acetate / formic acid (0.01 mol/L), the sulfonylureas were determined by LC-MS/MS.

The determination of the residues was done with matrix matched standards. To establish the calibration curve matrix test solutions were injected into the LC-MS/MS-system.

Calibration
A curve of the form $y = a + bx + cx^2$ is applicable over the tested range of 0.1 to 5 ng/mL of matrix matched standards for the tested sulfonylureas.

Recovery efficiency, relative standard deviation (RSD)
Recovery experiments were conducted at 0.01 µg/kg, 0.02 µg/kg, 0.05 µg/kg and 0.5 µg/kg for two different soil types, a loamy sand and silty loam.

4.2 Test and reference substances

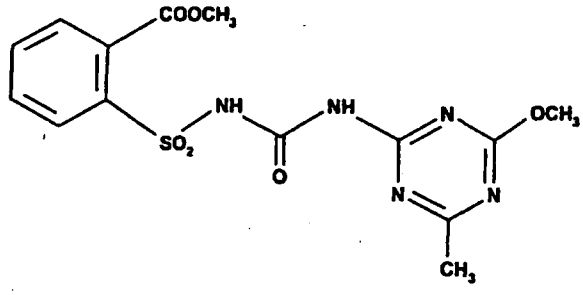
Metsulfuron-methyl (AE F075736)

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

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

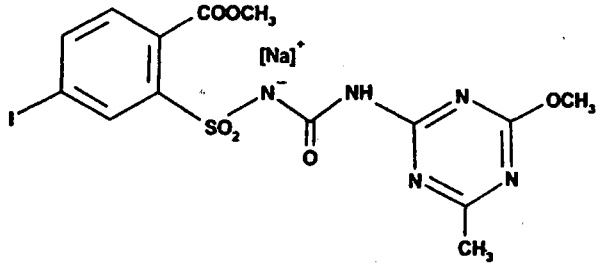
Empirical formula: $C_{14}H_{13}IN_5NaO_6S$

Molecular weight: 529.3

Solubility (20 °C):

Solvent	Solubility		Source
acetone	> 380	g/L	ref. 1
dichloromethane	> 500	g/L	ref. 1
ethyl acetate	23	g/L	ref. 1
n-hexane	$1.2 \cdot 10^{-3}$	g/L	ref. 1
methanol	12	g/L	ref. 1
n-heptane	$1.1 \cdot 10^{-3}$	g/L	ref. 1
2-propanol	4.4	g/L	ref. 1
toluene	2.1	g/L	ref. 1
acetonitrile	52	g/L	ref. 1
DMSO	> 500	g/L	ref. 1
PEG	87	g/L	ref. 1

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

5 Procedures

5.1 Principle of Analytical Method

The method flow sheet is presented in Annex I.

Residues of the sulfonylureas were extracted with acetonitrile / triethylamine 0.02 mol/L (4:1, v/v) from soil. After evaporation to dryness, the residues were reconstituted in water acidified with formic acid (0.01 mol/L). After a liquid/liquid extraction with ethyl acetate / formic acid (0.01 mol/L), the sulfonylureas were determined by LC-MS/MS.

The determination of the residues was done with matrix matched standards. To establish the calibration curve matrix test solutions were injected into the LC-MS/MS-system.

5.2 Reagents

- acetonitrile Chromasolv p.A. (Riedel-de Haën, Germany)
- triethylamine, 0.02 mol/L
- formic acid, 0.01 mol/L
- ethyl acetate Pestanal (Riedel-de Haën, Germany)
- AE F075736, analytical standard (AgrEvo GmbH, Germany)
- AE F115008, analytical standard (AgrEvo GmbH, Germany)

Stock solutions of the analytical standards were prepared by dissolving about 50 mg of analytical standard of AE F075736 and 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. The working solutions contain all needed analytical standards.

5.3 Apparatus

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

- standard laboratory glassware
- rotary vacuum evaporator with water bath
- centrifuge tube
- ultrasonic bath
- centrifuge (e.g. Heraeus Labofuge GL with rotor)
- Ultraturrax Typ 18/10
- LC-MS/MS system Quattro LC-Z (Micromass) (see section 5.6)
- chromatography column, Hypersil BDS, 5 µm, 250 mm x 3 mm

5.4 Preparation of samples and storage

Sample handling and preparation of the samples should be done following procedures mentioned in the relevant guidelines.

Soil samples should be mixed thoroughly and were stored deep-frozen.

5.5 Laboratory Steps

5.5.1 Extraction

Weigh 50 g of the homogenised soil sample into a centrifuge beaker. Fortify at this stage for recovery experiments. Add 100 mL acetonitrile / triethylamine 0.02 mol/L (4:1, v/v). Shake for 20 min. on the shaking machine or treat the sample for 2 min. with a desintegrator (the sample will be pulsed).

Centrifuge the mixture for 5 min at 4000 rpm and give the liquid phase into a 500 mL round bottom flask via a funnel with cotton wool. Repeat the extraction with 100 mL acetonitrile / triethylamine 0.02 mol/L (4:1, v/v).

Combine the extracts. Reduce to dryness using a vacuum rotary evaporator (bath temperature ca. 60 °C).

5.5.2 Liquid-liquid clean-up

Dissolve the residue in 20 mL formic acid (0.01 mol/L water) using an ultrasonic bath. Transfer the solution in the centrifuge beaker (Falcon beaker) and centrifuge (5 min at 4000 rpm). Transfer the solution into a 100 mL separation funnel. Repeat the first step with 10 mL formic acid (0.01 mol/L water).

Wash the round bottom flask with 15 mL ethyl acetate, transfer the ethyl acetate into the centrifuge beaker (Falcon beaker). Shake hardly. Centrifuge for 5 min at 4000 rpm. Transfer the ethyl acetate into the separatory funnel filled with the 30 mL formic acid (see above).

Shake the formic acid (0.01 mol/L water) / ethyl acetate mixture for 1 min. Transfer the formic acid phase into a second separatory funnel. Give the ethyl acetate phase into a 100 mL round bottom flask. Repeat extraction of the formic acid twice with 15 mL ethyl acetate, each. Combine the ethyl acetate phases and reduce to dryness using a vacuum rotary evaporator (bath temperature ca. 60 °C).

(In the case of bad separation, combine the ethyl acetate phases with the mixed phases into a centrifuge beaker (Falcon beaker). Centrifuge the mixture for 1 min at 4000 rpm. Transfer the aqueous phase to waste using a 10 mL single syringe stainless steel cannula. Transfer the ethyl acetate phase into a round bottom flask. Reduce to dryness using a vacuum rotary evaporator (bath temperature ca. 60 °C).)

Dissolve the residue in acetonitrile / water (1 : 1; v/v). The final volume should be 2.0 to 10.0 mL. This solution is ready for quantification with LC-MS/MS. If necessary filter the final solution over an injection filter (0.45 µm).

5.5.3 Matrix calibration

To 900 µL matrix solution 100 µL of a test solution with known amounts of sulfonylureas, e.g. metsulfuron-methyl (AE F075736) and iodosulfuron-methyl-sodium (AE F115008), was added. This gives a final volume of 1 mL. If smaller volumes were needed, aliquots of the matrix solution and test solution were used. However, the ratio matrix solution / test solution should be 9 : 1. For the matrix solutions a worked up control sample, diluted in acetonitrile / water (1 : 1; v/v) was used. To establish a calibration curve matrix test solutions were injected into the LC-MS/MS-system.

5.6 *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

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
20	20	80
22	80	20
27	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	10
Desolvation temp.:	350 °C	Exit	15
		LM Res 2	15.0
		HM Res 2	15.0
Nebuliser gas	ca. 90 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 F075736	382.20	167.00	0.3	18	20
AE F115008	508.20	167.00	0.3	20	23

Retention time

Substance	Retention time [min]	Detection time windows [min]
AE F075736	16.9	14.5 – 20.5
AE F115008	18.9	14.5 – 20.5

5.7 Calibration

The concentrations of the tested sulfonylureas were calculated using external standards at 5 - 6 different concentrations over a range from 0.1 pg/µL up to 5 pg/µL. The recommended order of samples / test solutions for setting up a sequence for LC-MS/MS-determination is 'test solution – sample – sample – test solution'. 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 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

$$Res = \frac{C_S \bullet V_{end} \bullet f}{W \bullet 1000} \qquad \left[mg/kg = \frac{(ng/mL) \bullet mL \bullet 1}{g \bullet 1000}\right] \qquad (3)$$

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

Res	Residue	[mg/kg]
C_S	Concentration in final sample solution V_{end} (treated, untreated and recovery)	[ng/mL]
W	Sample weight	[g]
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

$$Res_d = Res_{(Rec)} - Res_{(Unt)} \qquad \left[\frac{mg}{kg} = \frac{mg}{kg} - \frac{mg}{kg}\right] \qquad (5)$$

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

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

6.5 Critical steps of the method

- Check the pH-value at „Liquid-liquid clean-up" before adding ethyl acetate. The pH-value must be 3 to 4. At lower pH-values the sulfonylureas can decompose.
- End the evaporation of the „Liquid-liquid clean-up" immediately after reaching dryness.

- Because of the very low LOQ, take care for a contamination of the samples.

6.6 Time for analysis

From extraction of the soil samples to preparation of the final solutions for LC-MS/MS determination, it is normally possible to analyse a batch of 12 samples in one day.

Annex I: Analytical method flow sheet

Extraction sulfonylureas

50 g soil
100 mL acetonitrile / triethylamine 0.02 mol/L (4:1, v/v)
Shake for 20 min. on the shaking machine or treat the sample for 2 min. with a desintegrator (the sample will be pulsed)
centrifuge 5 min at 4000 rpm
filter over cotton wool
repeat with 100 mL acetonitrile / triethylamine 0.02 mol/L (4:1, v/v)
combine the organic phases
Reduce to dryness using a vacuum rotary evaporator (bath temperature ca. 60 °C)

clean up liquid/liquid extraction ethyl acetate / formic acid

Dissolve the residue in 20 mL formic acid (0.01 mol/L water) using an ultrasonic bath
Transfer the solution in the Falcon beaker and centrifuge (5 min at 4000 rpm)
Transfer the solution into a 100 mL separation funnel

Repeat the first step with 10 mL formic acid (0.01 mol/L water)

Wash the round bottom flask with 15 mL ethyl acetate
Transfer the ethyl acetate into the centrifuge beaker (Falcon beaker)
Shake hardly
Centrifuge for 5 min at 4000 rpm
Transfer the ethyl acetate into the separatory funnel

Shake the formic acid (0.01 mol/L water) / ethyl acetate mixture for 1 min
Transfer the formic acid phase into a second separatory funnel
Give the ethyl acetate phase into a 100 mL round bottom flask
Repeat extraction of the formic acid twice with 15 mL ethyl acetate, each

Combine the ethyl acetate phases and reduce to dryness using a vacuum rotary evaporator (bath temperature ca. 60 °C).

LC-MSMS

Dissolve in acetonitrile / water (1 : 1, v/v)
final volume should be 2.0 to 10.0 mL
(if necessary filter the final solution over an injection filter (0.45 µm))
LC-MS/MS