2 Objective

The objective of this study was to demonstrate that method AI-002-S05-01 ("AE 0317309: Analytical Method for the Determination of AE 0317309 and its Metabolite AE B197555 in Soil and Sediment by LC-MS/MS") can be performed with acceptable recoveries for determination of the compounds AE 0317309 and AE B197555 at an independent laboratory having no prior experience with the method. The method was developed by Bayer CropScience LP, Stilwell, USA, and validated in that laboratory with results reported as Method AI-002-S05-01, by D.J. Netzband & D.M. Smith, in report dated June 13, 2005. Soil Höfchen (Germany) and soil Laacher Hof (Germany) were chosen as representative matrices for validation within the present study.

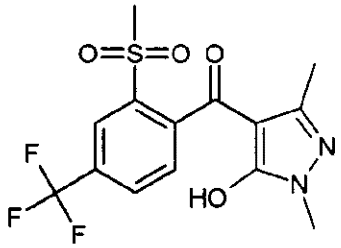
This study was performed in accordance with EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 7 of March 17, 2004, Commission Directive 96/46/EC amending Council Directive 91/414/EEC of July 16, 1996, and BBA Guideline on Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998 and Ecological Effects Test Guidelines, OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods, EPA 712-C-96-348, April 1996.

3 Materials

3.1 Test and Reference Items

AE 0317309:

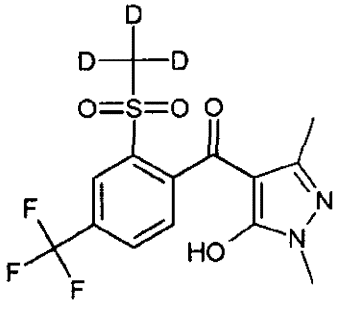
Structural formula:



Chemical code:	AE 0317309
Chemical name (CAS):	(5-hydroxy-1,2-dimethyl-1H-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone
Empirical formula:	$C_{14}H_{13}F_3N_2O_4S$
Molecular weight:	362.33 g/mol
Reference standard:	
Certificate of analysis:	AZ 11214
Purity:	99.5 %
Expiry date:	November 2005
Origin:	Bayer CropScience GmbH, BCS-RD-R-PT, Analytics Frankfurt D-65926 Frankfurt am Main, Germany

AE 0317309-methylsulfonyl-d3 (used as internal standard for the active ingredient AE 0317309):

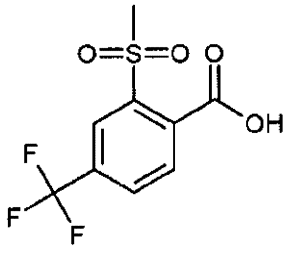
Structural formula:



Code name:	AE 0317309-methylsulfonyl-d3
Chemical name (CAS):	(5-hydroxy-1,3-dimethyl-1H-pyrazol-4-yl)[2[(methyl-d3)sulfonyl]-4-(trifluoromethyl)phenyl)methanone
Empirical formula:	$C_{14} H_{10} D_3 F_3 N_2 O_4 S$
Molecular weight:	365.34 g/mol
Reference standard:	
Standard no.:	K-1409
Reference no.:	2004BRP049-0004
Origin:	Bayer CropScience, Stilwell, KS, USA

AE B197555 (or RPA203328):

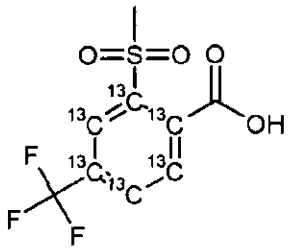
Structural formula:



Chemical code:	AE B197555
Chemical name (CAS):	2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic acid
Empirical formula:	$C_9 H_7 F_3 O_4 S$
Molecular weight:	268.22 g/mol
Reference standard:	
Certificate of analysis:	AZ 11989
Purity:	99.6 %
Expiry date:	September 2009
Origin:	Bayer CropScience GmbH, BCS-RD-R-PT, Analytics Frankfurt D-65926 Frankfurt am Main, Germany

AE B197555-phenyl $^{13}C_6$ (used as internal standard for the test item AE B197555):

Structural formula:



Code name:	AE B197555-phenyl 13C6, RPA203328-phenyl 13C6
Chemical name (CAS):	2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic-1,2,3,4,5,6-$^{13}C_6$ acid
Empirical formula:	$C_3\,^{13}C_6\,H_7\,F_3\,O_4\,S$
Molecular weight:	274.14 g/mol

Reference standard:

Standard no.:	K-1217
Purity:	96.7%
Expiry date:	January 2006
Origin:	Bayer CropScience, Stilwell, KS, USA

3.2 Test System

The method was validated using two German soils *Höfchen* and *Laacher Hof*. Two different soils were used in order to assess a possible influence of different soil characteristics. The soil samples were classified according to DIN and/or USDA specifications. Soil characteristics of the used soils are summarised in Table 1. Complete soil parameterisation is reported in Table 7 and Table 8.

Table 1: Soil Types

Soil	Texture of Soil	Organic Matter [%]
Höfchen	silt loam (USDA)	1.58
Laacher Hof	sandy loam (USDA)	2.06

4 Experimental

4.1 Analytical Method

The recovery data for the study were generated using the following method, which gives full details of preparing the analytical sample extracts and the conditions for high performance liquid chromatography (HPLC):

Number of the method:	AI-002-S05-01
Title of the method:	AE 0317309: Analytical Method for the Determination of AE 0317309 and its Metabolite AE B197555 in Soil and Sediment by LC-MS/MS, June 2005
Author of the method:	Derek J. Netzband David M. Smith Bayer CropScience LP 17745 S Metcalf Avenue Stilwell, Kansas 66085
Reference:	Method AI-002-S05-01
Limit of quantitation:	0.5 µg/kg

The following sample sets were analysed:

Table 2: Level and Number of Recoveries per Fortification Level

Soil	Control sample	Level 0.5 µg/kg	Level 5 µg/kg
Höfchen	2	5	5
Laacher Hof	2*	5	5

* only one control sample in case of AE 0317309

Additionally, a solvent blank with internal standards added (LC-MS/MS) were analysed.

4.1.1 Outline of the Method

AE 0317309 and its associated metabolite are extracted from soils using an Accelerated Solvent Extractor (ASE) with 65:35 acetonitrile/water at 100°C / 1500 psi pressure. Following extraction, the extracts are fortified with an isotopic internal standard solution containing AE 0317309-d_3 and AE B197555-$^{13}C_6$, an aliquot is evaporated to reduce volume on a Zymark Turbovap LV, cleaned up using a RP-102 SPE cartridge, and diluted to 5 mL in 90:10 0.1% acetic acid in water/acetonitrile to await analysis by LC/MS/MS for AE 0317309 and AE B197555.

4.1.2 Instruments

Extraction Equipment:	ASE 200 Dionex GmbH Am Woertzgarten 10 65510 Idstein, Germany
Balances:	PC 4400, PM 4800 and AT 261 Mettler Instruments GmbH 35387 Giessen, Germany
Evaporator:	Turbovap LV Zymark GmbH 65510 Idstein, Germany
Ultrasonic Bath:	Transsonic 890/H Heinrich Faust 51145 Cologne, Germany
Liquid Chromatograph:	HP 1100 Column Compartment G1316A HP 1100 Binary Pump G1312A HP 1100 Isocratic Pump G1310A HP 1100 Degasser G1322A Agilent 40880 Ratingen, Germany
Autosampler:	HTC PAL System CTC Analytics AG 4222 Zwingen, Switzerland
Mass Spectrometer:	IONICS EP 10+ with turbo-ionspray interface mass selective detector (MS/MS) performance-enhanced Sciex API-365 Ionics Concord, ON, Canada
Note:	Some mass spectrometric conditions are instrument specific. The spectrometric conditions were optimised by a competent operator prior to analysis.

4.1.3 Reagents and Equipment

Column:	Prodigy 5µ C8, length 50 mm, i.d. 2 mm Order No. 00B-3301-B0 Phenomenex 63741 Aschaffenburg, Germany
SPE cartridges:	RP-102 Resin Spe-ed SPE Cartridges 200 mg/3 mL Catalog # 4208 Applied Separations
Acetonitrile:	for HPLC, super gradient grade Riedel de Haen, No. 34998 30926 Seelze, Germany
Acetic acid (100%):	p.a. Merck, No.1.00063.1011 64271 Darmstadt, Germany
Water:	purified in a Milli-Q unit Milli-Pore GmbH 65731 Eschborn, Germany

Volumetric flasks, pipettes and other equipment commonly used in the laboratory.

4.1.4 Chromatographic Conditions and Mass Spectrometric Parameters

MS/MS parameter settings were optimized for the instrument being used and therefore not identical with those reported in method AI-002-S05-01.

4.1.5 Calculation

Calculations were performed using the computer software MS-EXCEL. In general, the program uses nine decimal places for calculations. The results given are rounded values. Thus, rounding "errors" may occur if recalculations are made using the listed figures.

4.1.5.1 Calculation of AE 0317309 and AE B197555 Residues

For calculation of the concentrations, calibration curves were used. These curves were calculated using linear regression automatically after each sequence run with the Applied Biosystems quantitation software Analyst (vers. 1.4). Further calculations were performed using the software Microsoft® EXCEL 2002. Matrix effects are eliminated by using an internal standard solution of the isotopically labelled reference substance.

The linear equation is expressed as:

$$y = \text{Intercept} + \text{Slope} \cdot x$$

y = Int. Ratio, x = Conc.Ratio

$$y = \frac{\text{Area}_{\text{Standard}}}{\text{Area}_{\text{Internal Standard}}} = \text{Int.Ratio} \quad \text{and} \quad x = \frac{\text{Conc}_{\text{Standard}}}{\text{Conc}_{\text{IS}}} = \text{Conc.Ratio}$$

Int.Ratio: *intensity ratio*

$Conc_{Standard}$: *concentration of standard solution [µg/L]*

$Conc_{IS}$: *concentration of internal standard solution [µg/L]*

Conc.Ratio: *concentration ratio*

Because the concentration of the isotopically labelled internal standard was the same in all solutions that were injected into the HPLC instrument, it has not to be taken into consideration. However, the concentration of the internal standard should be similar to the analyte concentration.
By means of the linear equation, the content of AE 0317309 and AE B197555 in dry soil can be calculated as follows:

$$\text{Dilution}_{\text{Factor}} = \frac{\text{Volume}_{\text{Extraction}} \times \text{Volume}_{\text{End}}}{\text{Weight} \times \text{Volume}_{\text{Aliquot}}}$$

$$\text{Conc}_{\text{Analyte}} = \frac{\text{Int.Ratio} - \text{Intercept}}{\text{Slope}}$$

$$\text{Conc}_{\text{Soil Wet}} = \text{Conc}_{\text{Analyte}} \times \text{Dilution}_{\text{Factor}}$$

$$\text{Conc}_{\text{Soil Dry}} = \text{Conc}_{\text{Soil Wet}} \times \frac{100\%}{100\% - \text{Moisture}}$$

$Conc_{Analyte}$: *concentration of the analyte in the sample solution [µg/L]*
$Conc_{Soil\ Wet}$: *concentration of the analyte in wet soil [µg/kg]*
$Conc_{Soil\ Dry}$: *concentration of the analyte in dry soil [µg/kg]*
Intercept: *intercept of the linear regression curve*
Slope: *slope of the linear regression curve [L/µg]*
Volume: *volumen of the extraction solvent, the aliquot and final solution [L]*
Weight: *weight of the soil sample [kg]*

The recovery is calculated according to the following equation:

$$\text{Recovery} = \frac{\text{Conc}_{\text{Soil Wet}} \times 100\%}{\text{Conc}_{\text{Soil Spiked}}}$$

$Conc_{Soil\ Spiked}$: *concentration of the spiked reference substance [µg/kg]*

4.1.6 Deviations from the Method

According to method AI002-S05-01 a flush volume of 100% for the ASE-procedure was used. However, within the present ILV the ASE stopped the extraction procedure owing to overflow of the collection vials. Therefore, the flush volume was reduced to 80%.

4.2 Linearity of the Detector

The linearity of the detector response for AE 0317309 and its metabolite AE B197555 were tested by injections of standard solutions. The following concentrations were measured:

Table 3: Standard Concentrations for the Determination of Detector Linearity

	Concentration [µg/L]					
AE 0317309	0.2	0.5	2.0	5.0	10	20
AE B197555	0.2	0.5	2.0	5.0	10	20

Table 7: *Characteristics of Soil Höfchen*

Soil Höfchen, plot 4011:	0 – 30 cm soil layer
pH (in $CaCl_2$ solution)	6.7
pH (in H_2O)	7.4
Organic Carbon [%]	0.92
Organic Matter [%] *	1.58
Cation Exchange Capacity [meq / 100 g dry soil]	12.4
max. Water Holding Capacity [g / 100 g dry soil]	39.5

Textural Description according to USDA	
Fraction	[%]
Clay (<0.002 mm)	19.4
Silt (0.002-0.050 mm)	76.3
Sand (0.050-2.000 mm)	4.3
Soil type: silt loam	

* Organic matter = Organic carbon x 1.72

Table 8: Characteristics of Soil Laacher Hof

Soil Laacher Hof, plot 712/718:	0 – 30 cm soil layer
pH (in $CaCl_2$ solution)	6.8
pH (in H_2O)	7.4
Organic Carbon [%]	1.20
Organic Matter [%]	2.06
Cation Exchange Capacity [meq / 100 g dry soil]	9.8
max. Water Holding Capacity [g / 100 g dry soil]	37.9

Textural Description according to USDA	
Fraction	[%]
Clay (<0.002 mm)	12.0
Silt (0.002-0.050 mm)	18.3
Sand (0.050-2.000 mm)	69.7
Soil type: sandy loam	

* Organic matter = Organic carbon x 1.72