

2 Objective

The objective of this study was to demonstrate that method AI-003-W05-01 ("Analytical Method For The Determination of AE 0317309 And Its Metabolite AE B197555 in Water 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 reported as Method AI-003-W05-01, by Derek J. Netzband, dated September 12, 2005. Drinking water (taken from the Institute BCS-RD-D-ROCS, Building 6610, Germany) and River Rhine water (collected at Leverkusen Hitdorf, Germany) were chosen as representative matrices for validation within the present study.

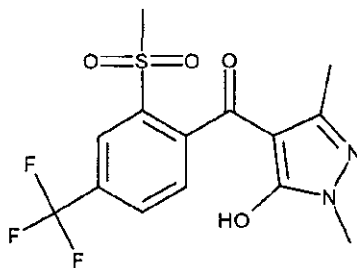
This study was performed in accordance with US EPA Ecological Effects Test Guidelines, OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods, EPA 712-C-96-348, April 1996.

3 Materials

3.1 Test and Reference Items

AE 0317309:

Structural formula:



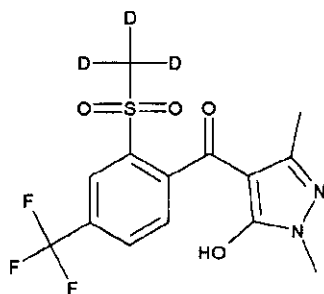
Chemical code:	AE 0317309
Chemical name (CAS):	(5-Hydroxy-1,3-dimethyl-1H-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone
Empirical formula:	C ₁₄ H ₁₃ F ₃ N ₂ O ₄ S
Molecular weight:	362.33 g/mol

Reference standard:

Certificate of analysis:	AZ 11214
Purity:	99.5 %
Expiry date:	November 2005
Origin:	Bayer CropScience GmbH, PT – Analytics Frankfurt, D-65926 Frankfurt am Main, Germany

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

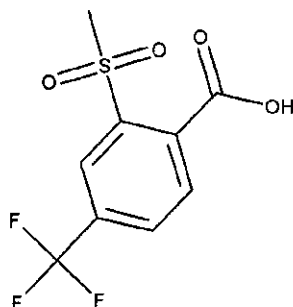
Structural formula:



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: C₁₄H₁₀D₃F₃N₂O₄S
Molecular weight: 365.35 g/mol
Reference standard:
Standard no.: K-1409
Reference no.: 2004BRP049-0004
Purity: 98.3%
Expiry date: December 2006
Origin: Bayer CropScience, Stilwell, KS, USA

AE B197555:

Structural formula:

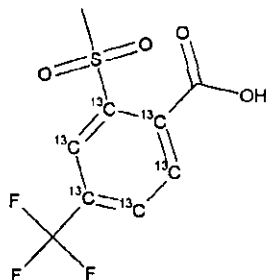


Chemical code: AE B197555 (or RPA203328)
Chemical name (CAS): 2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic acid
Empirical formula: C₉H₇F₃O₄S
Molecular weight: 268.22 g/mol

Reference standard:
Certificate of analysis: AZ 11989
Purity: 99.6%
Expiry date: September 2009
Origin: Bayer CropScience GmbH, PT – Analytics Frankfurt,
D-65926 Frankfurt am Main, Germany

AE B197555-phenyl-¹³C₆ (used as internal standard for the test item
AE B197555):

Structural formula:



Code name: RPA203328-phenyl-¹³C₆,
Chemical name (CAS): 2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic-
1,2,3,4,5,6-¹³C₆ acid
Empirical formula: C₃¹³C₆H₇F₃O₄S
Molecular weight: 274.14 g/mol

Reference standard:

Standard no.: K-1217
Reference no.: GAR 1892/05
Purity: 96.7%
Expiry date: January 2006
Origin: Bayer CropScience, Stilwell, KS, USA

3.2 Test System

The method was validated using River Rhine water Hitdorf and drinking water (tap) Monheim. Two different water types were used in order to assess a possible influence of different water characteristics. The water samples were analysed for TOC, DOC, conductivity, water hardness, dry residue after filtration and pH by Bayer Industry Services, BIS LEV UMG, Building W15. Water types are summarised in Table 1. Complete water parameters are reported in Table 7 and Table 8.

Table 1: Water Types

Water Type	Source of Water
Surface Water	River Rhine Water Hitdorf
Drinking Water	Drinking Water Monheim

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) and tandem mass spectrometric determination (MS/MS):

Number of the method: AI-003-W05-01
Title of the method: Analytical Method For The Determination of AE 0317309 And Its Metabolite AE B197555 in Water by LC/MS/MS
Author of the method: Derek J. Netzband
Bayer CropScience LP
17745 S Metcalf Avenue
Stilwell, Kansas 66085
Reference: Method AI-003-W05-01
Limit of quantitation: 0.05 µg/L

The following sample sets were analysed:

Table 2: Level and Number of Recoveries per Fortification Level

Water	Control sample	Level 0.05 µg/L	Level 0.5 µg/L
Drinking Water Monheim	2	5	5
Surface Water Hitdorf	2	5	5

Additionally, a solvent blank without internal standards added was analysed.

4.1.1 Outline of the Method

AE 0317309 and AE B197555 are extracted from the water using solid phase extraction (SPE) procedure and analyzed by LC/MS/MS. The LC/MS/MS technique allows quantitation of all analytical targets with a high inherent specificity and without the need of derivatisation for the more polar analytes. An aliquot of water is fortified with an isotopic internal standard containing AE 0317309-d₃ and AE B197555-¹³C₆, acidified, cleaned up using a RP-102 SPE cartridge, and diluted to 5 mL in 10 % acetonitrile / 90 % 0.1% acetic acid in water to await analysis by LC/MS/MS for AE 0317309 and AE B197555.

4.1.2 Instruments

Liquid Chromatograph: HP 1100 Column Compartment G1316A
HP 1100 Binary Pump G1312A
HP 1100 Isocratic Pump G1310A
HP 1100 Degasser G1322A
Agilent Technologies
40880 Ratingen, Germany

Autosampler: HTC PAL System
CTC Analytics AG
4222 Zwingen, Switzerland

Mass Spectrometer: Ionics EP10+ with turbo-ionspray interface
mass selective detector (MS/MS)
upgrade API 365, Applied Biosystems
Ionics, Concord, Ontario 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 (HPLC): Prodigy C8, 5 µm, length 50 mm, i.d. 2 mm
Order No. 00B-3301-B0
Phenomenex
63741 Aschaffenburg, Germany

Acetonitrile: for HPLC, super gradient grade
Riedel de Haën, No. 34998
30926 Seelze, Germany

Methanol: for HPLC
Promochem GmbH
46469 Wesel, Germany

Acetic acid (100%): p.a.
Merck, No.1.00063.1011
64271 Darmstadt, Germany

Formic acid (98 - 100%): purest
Riedel-de Haën, No.27001
30926 Seelze, 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

Liquid chromatographic conditions were identical to those described in Appendix 1 of the original method report AI-003-W05-01.

MS/MS parameter settings were in general as described in method AI-003-W05-01 but optimized for the instrument being used.

4.1.5 Calculation

For calculation of the concentrations, six-point calibration curves were used. These curves were calculated using linear regression automatically after each sequence run with the Perkin-Elmer quantitation software Analyst (vers. 1.4). Further calculations were performed using the software MS-EXCEL 2002.

Matrix effects for AE 0317309 and AE B197555 were eliminated by using an internal standard solution of the isotopically labelled reference substance. This solution was added to the sample solutions prior to analysis.

The linear equation is expressed as:

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

$$y = \text{Area}, x = \text{Concentration}$$

When an internal standard is used the calibration equation is defined as:

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

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

Since the concentration of the isotopically labelled internal standard added was the same in each sample, the Conc_{IS} is not considered in the formula.

By means of the linear equation, the content of the compounds AE 0317309 and AE B197555 in water samples can be calculated as follows:

$$\text{Conc}_{\text{Sample}} = \frac{\text{Int. Ratio} - \text{Intercept}}{\text{Slope}}, \quad \text{Int. Ratio} = \frac{\text{Area}_{\text{Sample}}}{\text{Area}_{\text{Internal Standard}}}$$

Intercept: intercept of the linear regression curve
Slope: slope of the linear regression curve
Area_{Sample}: area of the analyte in the sample solution
Conc_{Sample}: concentration of the analyte in the water sample [µg/L]

Table 7: Characteristics of the Surface Water from River Rhine, Sampled in Leverkusen-Hitdorf (Germany)

Parameter	Value
Total organic carbon (TOC)	3 mg/L
Dissolved organic carbon (DOC)	3 mg/L
Conductivity	535 μ S/cm
pH	7.3
Water hardness	11.2 °dH
Dry residue after filtration	144 mg/L

Table 8: Characteristics of the Drinking Water Sampled in Monheim (Germany)

Parameter	Value
Total organic carbon (TOC)	< 2 mg/L
Dissolved organic carbon (DOC)	< 2 mg/L
Conductivity at 25 °C	622 μ S/cm
pH	7.6
Water hardness	14.8 °dH