1. BACKGROUND INFORMATION

The objective of this study was to validate the method of analysis for the determination of BYH 18636 and its metabolites BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636 sulfonamide-carboxylic acid, BYH 18636-MMT and BYH 18636-triazolinone carboxamide in soil and sediment by LC/MS/MS.

In addition, this study included an assessment of the stability of the reference solutions and the final sample extract.

On completion of this study the analytical method GS-003-S06-01: "Analytical Method For The Determination of Residues of BYH 18636 And Its Metabolites BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636 sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide In Soil and Sediment Using LC/MS/MS" was issued.

An ILV has been successfully performed on this method. The method was revised to incorporate the results and recommendations of the ILV study and reissued with a method number of GS-003-S06-02.

The study was performed in accordance with United States Environmental Protection Agency (EPA) Pesticide Assessment Guidelines and Good Laboratory Practices (and Ecological Effects Test Guidelines OPPTS 850.7100[1] and Residue Chemistry Test Guidelines, OPPTS 860.1340[2]). This validation fulfils the requirement that properly validated methods of analysis be utilized for the generation of pesticide residue data and for tolerance enforcement.

Nomenclature for BYH 18636 and its metabolites are presented in Section 3.

2. EXPERIMENTAL DESIGN

This study was conducted following an approved protocol. All amendments to the protocol were signed and dated by the Study Director and the Sponsor's Representative. Any deviations from the protocol were documented and brought to the Study Director's attention when they were noted and maintained with the raw data.

This study was initiated on April 19, 2006. The experimental phase of the study began on April 25 2006 and concluded on May 17, 2006. The following personnel were involved in the conduct of this study:

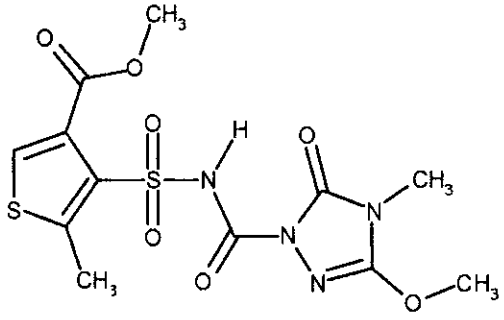
Derek J. Netzband
Senior Scientist
Environmental Chemistry

Jami Wade
R and D Specialist I
Environmental Chemistry

3. TEST AND REFERENCE SUBSTANCES

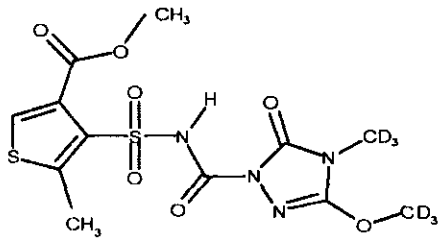
The following compounds were used as test and reference substances, and were supplied by Bayer CropScience. Neat standards were stored in a freezer at approximately –24°C. Standard solutions were stored in a refrigerator at approximately 8°C.

Code Name: BYH 18636 (AE 1162464)
(Parent Molecule)



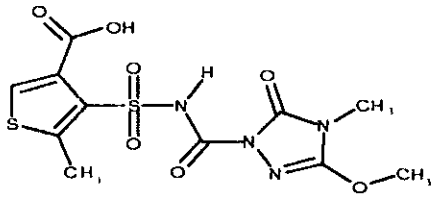
CAS Name:	Methyl 4-[[[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl)carbonyl]amino]sulfonyl]-5-methyl-3-*thiophenecarboxylate*
CAS Number	317815-83-1
Molecular Formula:	$C_{12}H_{14}N_4O_7S_2$
Molecular Weight:	390
ID No.:	K-1439
Reference No.:	0124200503
Purity:	99.3%
Expiration Date:	01/26/11
Storage Conditions:	Frozen
Source:	Bayer CropScience, Kansas City, Missouri

Code Name: BYH 18636-triazolinone-dimethyl-d6
(Parent Molecule, Isotopic Internal Standard)



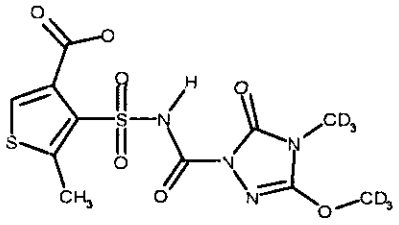
CAS Name: Methyl 4-[[[[4,5-dihydro-3-(methoxy-d_3)-4-(methyl-d_3)-5-oxo-1*H*-1,2,4-triazol-1-yl]carbonyl]amino]sulfonyl]-5-methyl-3-thiophenecarboxylate
Molecular Formula: $C_{17}H_8D_6N_4O_7S_2$
Molecular Weight: 396
ID No.: K-1362
Reference No.: 2003BRP176-249
Purity: 99.6%
Expiration Date: 8/11/14
Storage Conditions: Frozen
Source: Bayer CropScience, Stilwell, Kansas

Code Name: BYH 18636-carboxylic acid (AE 1394083)
(Soil Metabolite)



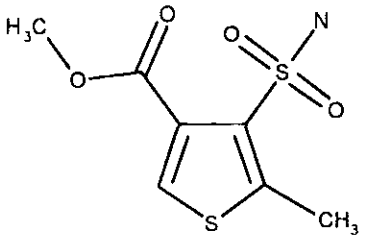
CAS Name: 4-[[[(4,5-Dihydro-3-methoxy-4-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl)carbonyl]amino]sulfonyl]-5-methyl-3-thiophenecarboxylic acid
Molecular Formula: $C_{11}H_{12}N_4O_7S_2$
Molecular Weight: 376
ID No.: K-1596
Reference No.: 0203200604
Purity: 98.2%
Expiration Date: 05/18/09
Storage Conditions: Frozen
Source: Bayer CropScience, Kansas City, Missouri

Code Name: BYH 18636 Acid-triazolinone-dimethyl-d_6 (d_6-AE 1394083)
(Soil Metabolite, Isotopic Internal Standard)



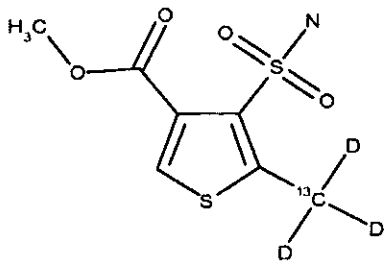
CAS Name: 4-[[[[4,5-Dihydro-3-(methoxy-d_3)-4-(methyl-d_3)-5-oxo-1H-1,2,4-triazol-1-yl]carbonyl]amino]sulfonyl]-5-methyl-3-thiophenecarboxylic acid
Molecular Formula: $C_{11}H_6D_6N_4O_7S_2$
Molecular Weight: 382
ID No.: K-1363
E. R. Ref No.: 2003BRP176-251
Purity: 99.7%
Expiration Date: 08/10/14
Storage Conditions: Frozen
Source: Bayer CropScience, Stilwell, Kansas

Code Name: BYH 18636-sulfonamide
(Soil Metabolite)



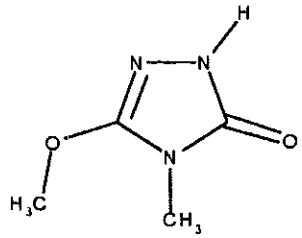
CAS Name: Methyl 4-(aminosulfonyl)-5-methyl-3-thiophenecarboxylate
CAS Number: 317815-81-9
Molecular Formula: $C_7H_9NO_4S_2$
Molecular Weight: 235
ID No.: K-1550
Reference No.: 0210200501
Purity: 99.3%
Expiration Date: 3/6/09
Storage Conditions: Frozen
Source: Bayer CropScience, Kansas City, Missouri

Code Name: Sulfonamide -5-methyl-$^{13}Cd_3$
(Soil Metabolite, Isotopic Internal Standard)



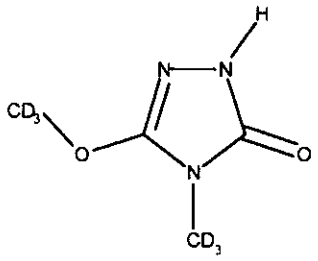
CAS Name: Methyl 4-(aminosulfonyl)-5-(methyl-^{13}C-d_3)-3-thiophenecarboxylate
Molecular Formula: $C_7H_6D_3NO_4S_2$
Molecular Weight: 239
ID No.: K-1441
Ref. No.: 0213200601
Purity: 100%
Expiration Date: 02/10/16
Storage Conditions: Frozen
Source: Bayer CropScience, Stilwell, Kansas

Code Name: BYH 18636-MMT (AE 1277106)
(Soil Metabolite)



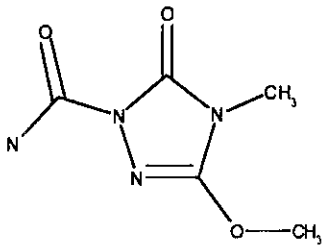
CAS Name: 2,4-Dihydro-5-methoxy-4-methyl-3*H*-1,2,4-triazol-3-one
CAS Number: 135302-13-5
Molecular Formula: $C_4H_7N_3O_2$
Molecular Weight: 129
ID No.: K-1443
E.R. Ref. No.: 95R-31-150A
Purity: 87.8%
Expiration Date: 6/27/07
Storage Conditions: Frozen
Source: Bayer CropScience, Stilwell, Kansas

Code Name: Triazolinone-dimethyl-d6
(Soil Metabolite, Isotopic Internal Standard)



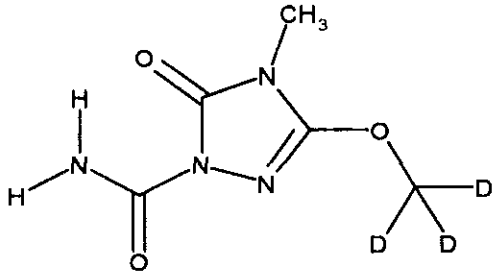
CAS Name: 2,4-Dihydro-5-(methoxy-d_3)-4-(methyl-d_3)-3H-1,2,4-triazol-3-one
Molecular Formula: $C_4HD_6N_3O_2$
Molecular Weight: 135
ID No.: K-1361
E.R. Ref. No.: 2003BRP176-246
Purity: 95.9%
Expiration Date: 9/13/09
Storage Conditions: Frozen
Source: Bayer CropScience, Stilwell, Kansas

Code Name: BYH 18636-triazolinone carboxamide (AE 1430601)
(Soil Metabolite)



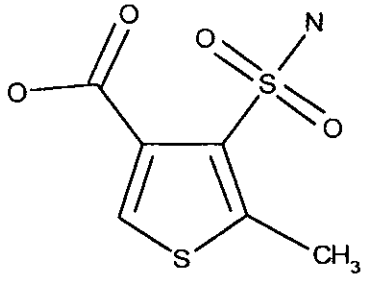
CAS Name: 4,5-Dihydro-3-methoxy-4-methyl-5-oxo-1*H*-1,2,4 triazole-1-carboxamide
Molecular Formula: $C_5H_8N_4O_3$
Molecular Weight: 172
ID No.: K-1495
E.R. Ref. No.: 0524200501
Purity: 95.3%
Expiration Date: 07/03/09
Storage Conditions: Frozen
Source: Bayer CropScience, Stilwell, Kansas

Code Name: BYH 18636-triazolinone carboxamide-methoxy-d_3
(Soil Metabolite, Isotopic Internal Standard)



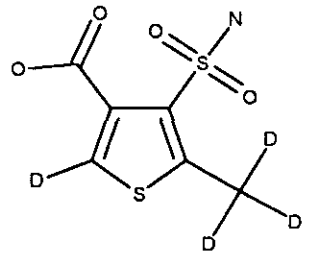
CAS Name: Unknown
Molecular Formula: $C_5H_5D_3N_4O_3$
Molecular Weight: ~75
ID No.: K-1516
E.R. Ref. No.: 2005BRP005-131
Purity: 96.3%
Expiration Date: 6/03/15
Storage Conditions: Frozen
Source: Bayer CropScience, Stilwell, Kansas

Code Name: BYH 18636-sulfonamide-carboxylic acid (AE 1395853)
(Soil Metabolite)



CAS Name: 4-(Aminosulfonyl)-5-methyl-3-thiophenecarboxylic acid
Molecular Formula: $C_6H_7NO_4S_2$
Molecular Weight: 221
ID No.: K-1380
Ref. No.: 0615200405
Purity: 99.8%
Expiration Date: 1/19/09
Storage Conditions: Frozen
Source: Bayer CropScience, Stilwell, Kansas

Code Name:	Sulfonamide Carboxylic acid-2-d-methyl-d3 (Soil Metabolite, Isotopic Internal Standard)



CAS Name:	4-(Aminosulfonyl)-5-(methyl-d_3)-3-thiophene-2-d-carboxylic acid
Molecular Formula:	$C_6H_3D_4NO_4S_2$
Molecular Weight:	225
ID No.:	K-1442
Ref. No.:	0213200602
Purity:	100%
Expiration Date:	2/10/16
Storage Conditions:	Frozen
Source:	Bayer CropScience, Stilwell, Kansas

4. TEST SYSTEM – SOIL AND SEDIMENT SAMPLES

The method was validated using two soil samples and one sediment sample. The untreated soil samples used in this study were collected for Bayer CropScience Study Number MEGSP002[3]: Terrestrial Field Dissipation of BYH 18636 in Nebraska Soil, 2005 and MEGSM013[4]: Terrestrial Field Dissipation of BYH 18636 in California Soil, 2005. The Sediment samples used in this study was obtained from the Sandhill Research Station Lake at the Clemson Extension located in Columbia, SC (sample ID: SR-L), which was collected during Study Number EBFIY003[5].

5. STORAGE

The untreated soil samples were stored at room temperature. The sediment sample was refrigerated.

6. REAGENTS AND EQUIPMENT

6.1 Reagents and General Equipment

The reagents and equipment used in this study are listed in Sections 5 and 6 of the method of analysis presented in Appendix 3.

Appropriate Material Safety Data Sheets were available to the study personnel during the conduct of the study. General laboratory safety precautions were taken.

6.2 Liquid Chromatographic/Mass Spectrometer Detection System

Residues of BYH 18636 and its metabolites BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide in soil and sediment were determined using an Applied Biosystems Sciex API-4000 LC/MS/MS system; Shimadzu LC-10AD VP HPLC pumps (2) with a high pressure mixer, a Shimadzu SCL-10A VP Pump Controller; and a Gilson 215 Series Autosampler. The Applied Biosystems instrument software applications used was Analyst 1.4.1.

The LC conditions used for the soil and sediment validation and MS/MS operating parameters used are outlined in Appendix 1 of the analytical method presented in Appendix 3 of this report.

Chromatograms using these LC conditions are presented in Appendix 1 of this report.

7. CALCULATIONS

7.1 Calibration Curves

At least six different standard concentrations were run with each set of samples.

Standard concentrations of BYH 18636 and its metabolites typically ranged from 0ng/mL to 20.0ng/mL (ppb), each with 5.0ng/mL isotopic internal standard added. The calibration standards were interspersed with the samples. All calculations were performed using Applied Biosystems Analyst software (Version 1.4.1) or Microsoft® Excel worksheets. Linear regression coefficients were calculated for the ratio of analyte to internal standard area plotted versus the area of analyte in the calibration standards.

7.2 Quantification of Residues

The calculation technique and an example calculation is presented in Appendix 2 of this report.

2. BACKGROUND

The herbicide BYH 18636 is currently being developed by Bayer CropScience.

An analytical method was developed for the analysis of BYH 18636 and its associated metabolites for BYH 18636, BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636 sulfonamide-carboxylic acid, BYH 18636-MMT and BYH 18636-triazolinone carboxamide in soil and sediment, and the method validated in Bayer CropScience Study Number RAGSP007[1].

The structures for these compounds are presented in Section 4. This analytical method was prepared based on the results obtained in the validation study.

Typical recovery results are presented in Appendix 3, and the data shown was obtained from the method validation study.

3. PRINCIPLE

BYH 18636 and its associated metabolites were extracted from soil and sediment by adding 35:65 v/v acetonitrile: deionized water and using microwave extraction. An isotopic internal standard containing BYH 18636-d_6, BYH 18636-carboxylic acid-d_6, BYH 18636-sulfonamide$^{13}Cd_3$, BYH 18636 sulfonamide-carboxylic acid-d_4, BYH 18636-MMT-d_6, and BYH 18636-triazolinone carboxamide-d_3 was added to the extract, which is acidified and partitioned with ethyl acetate. The ethyl acetate extract is evaporated to dryness using a Zymark TurboVap, reconstituted in water:acetonitrile, and an aliquot analyzed by LC/MS/MS for BYH 18636, BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636 sulfonamide-carboxylic acid, BYH 18636-MMT and BYH 18636-triazolinone carboxamide.

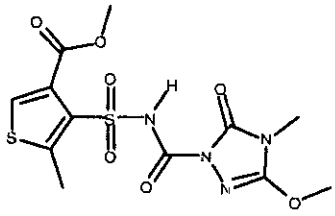
The LOQ of the method is 2ppb(ng/g) for of BYH 18636 and its metabolites.

A flow-chart outlining the procedure summarizes the method in Appendix 6.

4. COMPOUNDS

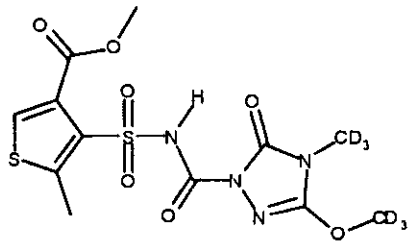
The structures for BYH 18636, its metabolites and the associated internal standards are presented below:

Code Name: **BYH 18636 (AE 1162464)**
(Parent Molecule)



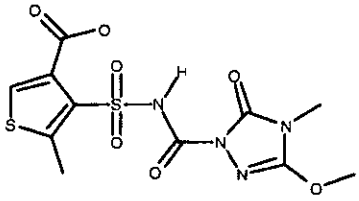
CAS Name: Methyl 4-[[[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl)carbonyl]amino]sulfonyl]-5-methyl-3-thiophenecarboxylate
CAS Number 317815-83-1
Molecular Formula: $C_{12}H_{14}N_4O_7S_2$
Molecular Weight: 390

Code Name: **BYH 18636-triazolinone-dimethyl-d6**
(Parent Molecule, Isotopic Internal Standard)



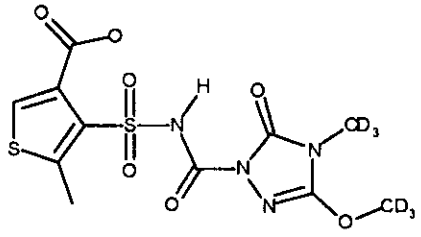
CAS Name: Methyl 4-[[[[4,5-dihydro-3-(methoxy-d_3)-4-(methyl-d_3)-5-oxo-1*H*-1,2,4-triazol-1-yl]carbonyl]amino]sulfonyl]-5-methyl-3-thiophenecarboxylate
Molecular Formula: $C_{12}H_8D_6N_4O_7S_2$
Molecular Weight: 396

Code Name: **BYH 18636-carboxylic acid (AE 1394083)**
(Soil Metabolite)



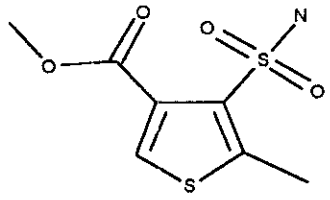
CAS Name: 4-[[[(4,5-Dihydro-3-methoxy-4-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl)carbonyl]amino]sulfonyl]-5-methyl-3-thiophenecarboxylic acid
Molecular Formula: $C_{11}H_{12}N_4O_7S_2$
Molecular Weight: 376

Code Name: **BYH 18636 Acid-triazolinone-dimethyl-d6**
(Soil Metabolite, Isotopic Internal Standard)



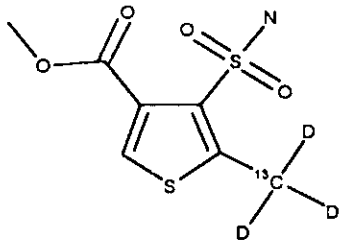
CAS Name: 4-[[[[4,5-Dihydro-3-(methoxy-d_3)-4-(methyl-d_3)-5-oxo-1H-1,2,4-triazol-1-yl]carbonyl]amino]sulfonyl]-5-methyl-3-thiophenecarboxylic acid
Molecular Formula: $C_{11}H_6D_6N_4O_7S_2$
Molecular Weight: 382

Code Name: **BYH 18636-sulfonamide**
(Soil Metabolite)



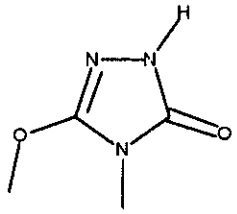
CAS Name: Methyl 4-(aminosulfonyl)-5-methyl-3-thiophenecarboxylate
CAS Number: 317815-81-9
Molecular Formula: $C_7H_9NO_4S_2$
Molecular Weight: 235

Code Name: **Sulfonamide -5-methyl-$^{13}Cd_3$**
(Soil Metabolite, Isotopic Internal Standard)



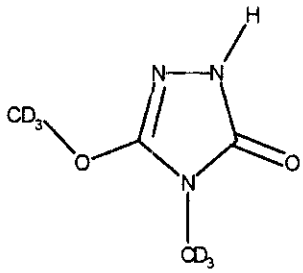
CAS Name: Methyl 4-(aminosulfonyl)-5-(methyl-^{13}C-d_3)-3-thiophenecarboxylate
Molecular Formula: $C_7H_6D_3NO_4S_2$
Molecular Weight: 239

Code Name: **BYH 18636-MMT (AE 1277106)**
(Soil Metabolite)



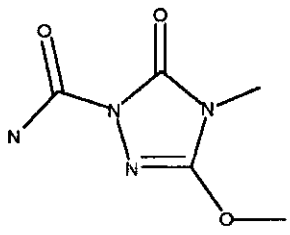
CAS Name: 2,4-Dihydro-5-methoxy-4-methyl-3*H*-1,2,4-triazol-3-one
CAS Number: 135302-13-5
Molecular Formula: $C_4H_7N_3O_2$
Molecular Weight: 129

Code Name: **Triazolinone-dimethyl-d6**
(Soil Metabolite, Isotopic Internal Standard)



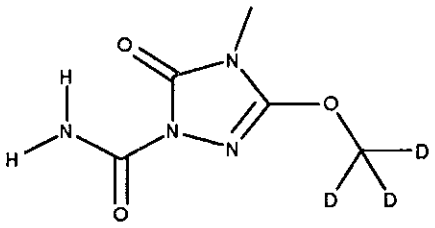
CAS Name: 2,4-Dihydro-5-(methoxy-d_3)-4-(methyl-d_3)-3H-1,2,4-triazol-3-one
Molecular Formula: $C_4HD_6N_3O_2$
Molecular Weight: 135

Code Name: **BYH 18636-triazolinone carboxamide (AE 1430601)**
(Soil Metabolite)



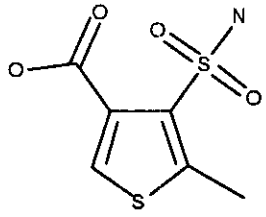
CAS Name: 4,5-Dihydro-3-methoxy-4-methyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
Molecular Formula: $C_5H_8N_4O_3$
Molecular Weight: 172

Code Name: **BYH 18636-triazolinone carboxamide-methoxy-d_3**
(Soil Metabolite, Isotopic Internal Standard)



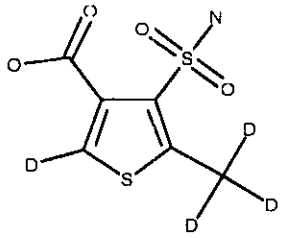
CAS Name: Unknown
Molecular Formula: $C_5H_5D_3N_4O_3$
Molecular Weight: 175

Code Name: **BYH 18636-sulfonamide-carboxylic acid (AE 1395853)**
(Soil Metabolite)



CAS Name: 4-(Aminosulfonyl)-5-methyl-3-thiophenecarboxylic acid
Molecular Formula: $C_6H_7NO_4S_2$
Molecular Weight: 221

Code Name: **Sulfonamide Carboxylic acid-2-d-methyl-d3**
(Soil Metabolite, Isotopic Internal Standard)



CAS Name: 4-(Aminosulfonyl)-5-(methyl-d_3)-3-thiophene-2-d-carboxylic acid
Molecular Formula: $C_6H_3D_4NO_4S_2$
Molecular Weight: 225

5. APPARATUS

Use as a guide; equivalent apparatus may be substituted.

- VWR Pyrex® Brand volumetric pipets, glass class A (Assorted Volumes)
- Eppendorf Reference Series 2000 pipettes (Cat. No.: 05-402-48 and 05-402-50)
- VWR Pyrex® Brand volumetric flasks, glass class A (Assorted Volumes)
- VWR Pyrex® Brand glass funnels
- VWR Pyrex® Brand disposable Pasteur pipets (Cat. No.: 53283-910 & 53283-914)
- National Scientific LC vials, Snap-Its (Cat. No.: C4011-5)
- National Scientific LC vial Snap-It Seals, (Cat. No.: C4011-55)
- LiChrospher® 60 RP-select B 5µ 125 x 3.00 mm Column (Part No.: 79925SB-563)
- Applied Biosystems PE Sciex 4000 LC/MS/MS System with Analyst Software Version 1.4.1 or higher installed.
- Shimadzu LC-10AD VP HPLC pumps (two), Shimadzu SCL-10A VP Controller with a Gilson 215 series autosampler
- VICI Cheminert Valve and 2 position actuator Controller.
- Disposable, 1"-long, 5/16"-diameter magnetic stir bars (Fisher catalog # 1451394)
- Fisherbrand 125-mL glass jar (Cat. No. 02-911-455)
- Fisherbrand 50-mL polypropylene disposable centrifuge tube (Cat. No. 06-443-18)
- Acrodisc 0.45µm 13mm syringe filter, Pall Life Sciences, Part No. 4426T
- Milestone Ethos E Microwave Labstation, equipped with a Model 320 Touch Screen Controller and automatic temperature control with fiber optic sensor.
- Zymark TurboVap II with Zymark 200mL evaporation flasks.

6. REAGENTS

Use as a guide; equivalents or different manufactures (brands) may be substituted.

- *Acetonitrile, Fisher Scientifc Optima, , (Cat. No. A996-4)*
- Deionized Water filtered through a Milli-Q water system or Water, Fisher Scientific Optima, (Cat. No.: W7-4)
- Acetic Acid, Guaranteed Reagent, (VRW Cat. No.: EM-AX0073-14)
- Formic Acid, 88% (J.T. Baker Cat No.0128-01)
- Anhydrous sodium sulphate, Certified A.C.S. (Fisher Scientific Cat. No.: S421-3)
- Glass wool
- Certified analytical reference standards of BYH 18636 and its metabolites BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide
- Certified internal standards of BYH 18636-triazolinone-dimethyl-d_6 and its metabolites BYH 18636 Acid-triazolinone-dimethyl-d_6, BYH 18636 sulfonamide acid-2-d-methyl-d_3, BYH 18636 sulfonamide-5-methyl-$^{13}C,d_3$, triazolinone-dimethyl-d_6, and BYH 18636-triazolinone carboxamide-methoxy-d_3

7. PREPARATION OF ANALYTICAL STANDARDS

NOTE: The following procedure is an example description of how standard solutions may be prepared. Standards may be prepared as mixed solutions by dilution from individual stock solutions or prepared individually. Alternate or additional standards of appropriate weight and volume may be prepared as needed.

Class "A" volumetric glassware or calibrated pipets should be used in the preparation of all analytical standards. All standard solutions should be stored in a refrigerator in amber glass bottles when not in use. Solutions should be allowed to warm to room temperature prior to use.

7.1 Primary Stock Standard Solutions

Prepare individual 100µg/mL stock solutions of BYH 18636 and its metabolites BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide by transferring 0.0100 grams of each analyte in separate 100mL volumetric flasks. Dilute to volume with acetonitrile and mix well.

Prepare a mixed stock 2.0µg/mL (2000ng/mL) solution containing a mixture of BYH 18636 and its metabolites by taking a 2mL aliquot of each of the six 100µg/mL stock solutions and diluting to 100mL with acetonitrile.

The stock standard solutions are stored in the dark at ≤-18°C.

NOTE: Corrections for standard purities should be applied when expressing standard concentrations.

7.2 Fortification Standard Solutions

Prepare a 0.2µg/mL (200ng/mL) fortification solution containing a mixture of BYH 18636 and its metabolites BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide by taking a 10mL aliquot of the 2µg/mL standard solution and diluting to 100mL with acetonitrile.

Further dilutions of this mixed fortification solution may be made as needed.

7.3 Isotopic Internal Standard Solutions

Prepare individual 100µg/mL stock solutions of BYH 18636-triazolinone-dimethyl-d_6 and its metabolites BYH 18636 acid-triazolinone-dimethyl-d_6, BYH 18636 sulfonamide acid-2-d-methyl-d_3, BYH 18636 sulfonamide-5-methyl-13C-d_3, triazolinone-dimethyl-d_6, and BYH 18636-triazolinone carboxamide-methoxy-d_3 by transferring 0.005 grams of each analyte in separate 50mL volumetric flasks. Dilute to volume with acetonitrile.

Prepare a 5.0µg/ml mixed stock internal standard solution of BYH 18636-triazolinone-dimethyl-d_6 and its metabolites BYH 18636 Acid-triazolinone-dimethyl-d_6, BYH 18636 sulfonamide acid-2-d-methyl-d_3, BYH 18636 sulfonamide-5-methyl-13C-d_3, triazolinone-

dimethyl-d_6, and BYH 18636-triazolinone carboxamide-methoxy-d_3 by taking a 5mL aliquot of 100.0µg/mL stock solution and diluting to 100mL with acetonitrile
Prepare a 0.5 µg/mL (500ng/mL) solution containing a mixture of BYH 18636-triazolinone-dimethyl-d_6 and its metabolites BYH 18636 Acid-triazolinone-dimethyl-d_6, BYH 18636 sulfonamide acid-2-d-methyl-d_3, BYH 18636 sulfonamide-5-methyl-^{13}C-d_3, triazolinone-dimethyl-d_6, and BYH 18636-triazolinone carboxamide-methoxy-d_3 by taking a 10.0 mL aliquot of 5 µg/ml mixed stock internal standard solution and diluting to 100 mL with acetonitrile.

Further dilutions of this mixed fortification solution may be made as needed.

7.4 Calibration Standard Solutions

Prepare working calibration solutions consisting of 0.0, 1.0, 2.0, 5.0, 10.0, and 20.0ng/mL of BYH 18636 and its metabolites diluted to 100mL with 99:1 v/v deionized water:acetonitrile. Before bringing the calibration solutions to volume, add by pipet 1.0mL of the 0.5µg/mL internal standard solution prepared in acetonitrile to each of the calibration solutions. (see Section 7.3 Internal Standard Solutions).

Further calibration solutions may be prepared as needed.

Concentration of Standard Solution used for dilution (ng/mL)	Concentration of Internal Standard Solution used for dilution (ng/mL)	Aliquot Native mix Taken (mL)	Aliquot Internal Standard Taken (mL)	Dilution Volume (mL)	Concentration of Calibration Solution (ng/mL)
2000	500	1	1	100	20
2000	500	0.5	1	100	10
2000	500	0.25	1	100	5
200	500	1	1	100	2
200	500	0.5	1	100	1
N/A	500	N/A	1	100	0

The standard solutions are stable for a minimum of one month when stored below 10°C in the dark.

Representative calibration curves for each of the analytes are presented in Appendix 5.

8. ANALYTICAL PROCEDURE FOR ANALYSIS OF SOIL AND SEDIMENT

A method flow chart is presented in Appendix 6, and a summary of the analytical method parameters is presented in Table 1.

Stopping points in the analytical method are designated by the following symbol: §

8.1 Sample Preparation

Treated samples of soil and sediment should be thoroughly homogenized and stored frozen until sampled for extraction.

8.2 Extraction

NOTE: This method uses internal standards to determine the concentrations of BYH 18636 and its metabolites present in soil and sediment. If the concentration of these components are outside the range of the appropriate calibration curve the analyses will have to be repeated using either a reduced sample weight or by further diluting the sample extract prior to the addition of the internal standard. If a further dilution is made to the final extract, adjust the concentration of internal standard added in step 8.2.10 so that the final concentration of internal standard present in the final sample is 5ppb.

1. Weigh 20 ± 0.1 grams of soil or sediment into a 125-mL glass jar with a screw cap lid. §

2. Fortify the recovery samples at the desired fortification level with the appropriate mixed standard solution prepared in acetonitrile (see Section 7.2 Fortification Stock Solutions).

3. Add ~60mL of 35:65 v/v acetonitrile: deionized water to each jar, cap and shake vigorously for about 10 seconds to break up any large soil aggregates.

4. Remove the cap and place a disposable magnetic stir bar into the glass jar. Loosely attach the lid to the glass jar. NOTE: Over tightening the lids may cause a pressure build up inside the jar, resulting in a potential explosion hazard.

5. Place the jars in the microwave, evenly spaced around the center of the carousel.

6. Insert the thermo-well into the untreated control sample and insert the fiber optic temperature probe into the thermo-well.

7. With the microwave door open, turn on the manual magnetic stirrer control and check to see that the stir bars are turning but without splashing the samples. The manual stirrer will override any program stirring rates.

8. Close the microwave door, and program the microwave with the following method:

Step Number (Nr)	Time Duration (t)	Temperature set point at end of step (T1)	Power Limit to maintain/ control temperature (E)	Comments
1	0.5 min.	50 °C	350 Watts Max	Ramp from ambient to 50 °C
2	5.5 min.	50 °C	350 Watts Max	Maintain 50 °C

Ventilation time:	1 minute
QP limit:	60-80% (Shut off limit in case vapors in oven too concentrated)
Stirrer (speed setting):	Value not used. Manual control overrides program setting.
Rotor control:	On (Rotor rotation is on)
Twist control:	On (Rotor rotates clockwise and then counterclockwise to keep probe cable from twisting)

9. Extract the samples using the above method. On completion of the extraction cycle, allow the glass jars to cool.

10. Remove the jars from the microwave and add by pipet 100µL of the 0.50ppm internal standard solution prepared in 100% acetonitrile (see Section 7.3 Internal Standard Solutions) to the contents of each glass jar. Cap the jar and mix well.

11. Centrifuge the glass jars at approximately 2000 rpm for a minimum of 10 minutes. (If needed glass jars filled with water may be used to balance the centrifuge).

12. Decant ~30mL of the supernatant solution into a second 125ml screw top jar and add 100µL of formic acid.

13. Add 35-45mL of ethyl acetate and shake for ~1min.

14. Using a disposable syringe transfer the ethyl acetate layer into a 200m TurboVap flask through 10 to 20 grams of sodium sulfate in a Pyrex funnel stoppered with glass wool.

15. Add an additional 35-45mL of ethyl acetate ethyl acetate to the screw top jar and shake for ~1min. Repeat step 14. Rinse the sodium sulfate pad with ~25mL of ethyl acetate before discarding. §

16. Evaporate to dryness using a Turbovap® II evaporator set to a temperature of 30 to 35°C. Add 5 mL of 1:99 (v/v) acetonitrile:deionized water. Sonicate the sample and mix well. NOTE: The TurboVap temperature should not exceed see 40°C. See Section 10.3.

17. Filter the sample using an Acrodisc® 0.45µm syringe filter into a LC vial and cap to await analysis by LC/MS/MS.§

9. LC/MS/MS ANALYSIS

9.1 Sample Analysis

BYH 18636 and its metabolites BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide are analyzed by LC/MS/MS using isotopic internal standards.

Inject a 35 µL aliquot of each test sample (or fortified sample matrix) from step 17 in Section 8.2 onto the LC/MS/MS under the conditions presented in Appendix I. Variations in equipment or sample characteristics may require different injection volumes or slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity.

It is often beneficial to make several 'priming' injections of standards and/or samples prior to starting the LC/MS/MS analysis. Typically 4 to 6 priming injections are made. The results from these injections are not included in any calculations used in residue determinations. These injections help stabilize the LC/MS/MS response prior to running the analytical set.

Example chromatograms are shown in Appendix 4.

9.2 LC/MS/MS Standard Calibration and Residue Calculations

The example calculation displayed below was used by the laboratory developing this method. Alternate calculation procedures appropriate to the reporting requirements may be substituted.

Standardize the LC/MS/MS response under the conditions outlined in Appendix 1 by injecting an aliquot of each LC/MS/MS calibration solution interspersed with samples.

BYH 18636 and its metabolites BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide residues were quantified using internal standard linear regression analysis. A separate calibration curve was produced for each set of samples analyzed on the LC/MS/MS. A calibration curve was generated by linear regression of the ratio of standard peak/internal standard peak areas versus the standard concentrations in ng/mL using Applied Biosystems Analyst Software (Version 1.4.1), a computer-programmed data capturing system. The Analyst Software uses the MS/MS standard responses to calculate the regression coefficients for slope, M, and intercept, B, for each analytical set.

The standards were fit to the linear equation: $Y = MX + B$

where: X is the concentration of the reference standard in ng/mL[a]
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area:isotopic peak area ratio

[a] As the reference standards and samples contain the same internal standard concentrations it may be omitted from the calculation

The equation shown below is for the calculation of BYH 18636 residues.

After regression coefficients were calculated, the residue in parts per billion was determined. The parts per billion (ppb) of BYH 18636 in the soil was calculated using the following equation,

$$\text{BYH 18636 found (ppb)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume}(V_1)}{\text{Initial sample wt. (W)}} \times \frac{\text{Final dilution volume } (V_3)}{\text{Aliquot taken } (V_2)}$$

Where: $W =$ 20g
$V_1 =$ 60 mL
$V_2 =$ 30 mL
$V_3 =$ 5 mL

Analyst software was used to calculate the amount of BYH 18636 in ppb for each sample and the percent recovery for the spiked samples.

9.3 Fortification Experiments

Note: Fortification experiments may be performed as needed to monitor method efficiency and reproducibility, but are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing & validating method efficiency.

With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries using the following equation:

$$\text{Recovery (\%)} = \frac{(R - S)}{T} \times 100$$

Where: R = ppb of target analyte found in fortified sample
S = ppb of target analyte found in control sample, real or apparent
T = theoretical ppb in fortified sample

Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at the LOQ of 2ppb in soil and sediment or other appropriate level with fortification solutions. Calculate the final residue R for the control (S) and fortified control (R) samples.

10. DISCUSSION

10.1 Method Validation

The method validation has been performed and reported in Bayer CropScience Study RAGSP007[1]. The results from this study are summarized in Appendix 3.

10.2 Independent Laboratory Validation (ILV)

An ILV has been successfully performed on this method[2]. The validation results are summarized in Table 2 of this report.

10.3 Critical Steps And Observations

In Section 8.2.16, the analyst must ensure that the temperature of the TurboVap does not exceed 40°C, as degradation of some of the analytes may occur. Typically the analyst will observe a decrease in the recoveries for the higher molecular mass analytes, while there will be an increase in the BYH 18636-MMT recovery.

10.4 Confirmatory Method

The analytical method employs highly specific and selective detectors (LC/MS/MS), therefore it was not deemed necessary to develop a confirmatory method. However, if unexpected interferences are detected alternate ion transitions may be monitored. The following alternate ions are suggested, and the spectra for each of the analytes are presented in Appendix 4.

Analyte	Primary Ion Transition			Alternate Ion Transition		
	Polarity	Q1	Q3	Polarity	Q1	Q3
BYH 18636	+	391	359	+	391	230
BYH 18636-carboxylic acid	-	375	202	+	377	359
BYH 18636-sulfonamide	+	236	204	+	236	219
BYH 18636-sulfonamide carboxylic acid	-	220	176	-	220	80
BYH 18636-MMT	+	130	115	+	130	58
BYH 18636-triazolinone carboxamide	+	173	130	+	173	115

10.5 Time Considerations

A set of fourteen samples can be weighed and prepared for analysis in 5-6 hours, analyzed overnight and the data processed the following working day.

10.6 Analytical stopping points *(if needed)*

As noted in the method by the symbol §, the procedure may be paused if needed. These should flexibly accommodate the analyst's normal working day or schedule. It is assumed that the analysis will resume during the next working period.

11. SAFETY

All available appropriate Material Safety Data Sheets should be available to the study personnel during the conduct of the method. General laboratory safety precautions should be taken.

12. REFERENCES

No.	Doc. No.	Report No.	**Author(s).** Title. Year.
1	RAGSP007		**Netzband. D.J.**, In House Laboratory Validation Of An Analytical Method For The Determination of Residues of BYH 18636 And Its Metabolites BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide In Soil and Sediment Using LC/MS/MS
2	P611060013	MR-06/155	**Brumhard, B.,Koch, V.**,Independent laboratory validation of method GS-003-S06-01 for the determination of BYH18636 and its metabolites BYH18636-carboxylic acid, BYH18636-sulfonamide, BYH18636-sulfonamide-carboxylic acid, BYH18636-MMT, and BYH18636-triazolinone carboxamide in soil and sediment using LC/MS/MS.

Table 1 Analytical Method Summary Parameters (DER Table B.1.1)

Summary Parameters for the Analytical Method Used for the Quantitation of BYH 18636, BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide Residues in Soil and Sediment.	
Method ID	GS-003-S06-02
Analyte(s)	BYH 18636, BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide
Extraction solvent / Technique	Microwave extraction using 35% acetonitrile/65% water
Cleanup Strategies	Ethyl acetate partition
Instrument Detector Column	- Shimadzu LC-10AD VP HPLC pump with Gilson 215 Liquid handler and Gilson 819 Valve Actuator - Applied Biosystems API 4000 MS/MS - LiChrospher® 60 RP-select B 5 µm 125 x 3.0m
Standardization Method	Multi point calibration curve (Internal standard)
Stability of Standard Solutions	Stock standard solutions are stable for a minimum of 3 months when stored in the dark at =-18°C Fortification and calibration standard solutions are stable for a minimum of 1 month when stored in the dark at =4°C
Retention times	BYH 18636-MMT (~7.0 minutes) BYH 18636-triazolinone-carboxamide(~8.3 minutes) BYH 18636-sulfonamide carbooxylic acid(~10.9 minutes) BYH 18636-sulfonamide(~12.1 minutes) BYH 18636-carboxylic acid(~12.3 minutes) BYH 18636(~13.4 minutes)

Appendix 1 Instrument Conditions For BYH 18636 and its metabolites

Equipment with equivalent or better sensitivity and performance may be substituted.

LC/MS/MS Parameters

NOTE: Variations in equipment or sample characteristics may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Therefore, the given LC/MS/MS parameters listed below are guidelines and may be modified. These parameters should be optimized for the instrument and column actually used. Also, instrument parameters and mobile phase may be adjusted to improve separation from any observed interfering peaks.

Comment:	BYH 18636
Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Acquisition Duration:	19min58sec
Number Of Scans:	1487
Periods In File:	4
Acquisition Module:	Acquisition Method
Software version	Analyst 1.4.1

MS Method Properties:

Period 1:

Scans in Period:	600
Relative Start Time:	0.00 msec
Experiments in Period:	1

Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Polarity:	Positive
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Low
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	5.0070 msec
MCA: No	
Step Size:	0.00 amu

BYH 18636-MMT (~7.0 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
130.00	115.00	250.00	DP	61.00	61.00
			CE	25.00	25.00

BYH 18636-MMT internal standard (~7.1 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
136.00	118.00	250.00	DP	61.00	61.00
			CE	25.00	25.00

BYH 18636-triazolinone-carboxamide (~8.3 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
173.00	130.00	250.00	DP	41.00	41.00
			CE	15.00	15.00

BYH 18636-triazolinone-carboxamide internal standard (~8.3 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
176.00	133.00	250.00	DP	41.00	41.00
			CE	15.00	15.00

Parameter Table (Period 1 Experiment 1):

CUR:	10.00
GS1:	70.00
GS2:	70.00
TEM:	500.00
ihe:	OFF
CAD:	6.00
IS:	5000.00
EP	10.00
CXP	8.00

Period 2:

Scans in Period:	137
Relative Start Time:	10.20 min
Experiments in Period:	1

Period 2 Experiment 1:

Scan Type:	MRM (MRM)
Polarity:	Negative
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Low
Resolution Q3:	Low
Intensity Thres.:	0.00 cps
Settling Time:	700.0000 msec
MR Pause:	5.0070 msec
MCA:	No

BYH 18636-sulfonamide carboxylic acid (~10.9 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param Start	Stop
220.00	176.00	300.00		

BYH 18636-sulfonamide carboxylic acid internal standard (~10.9 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param Start	Stop
224.00	180.00	300.00		

Parameter Table(Period 2 Experiment 1):

CUR:	10.00
GS1:	70.00
GS2:	70.00
TEM:	500.00
ihe:	OFF
CAD:	6.00
IS:	-4200.00
DP	-30.00
EP	-10.00
CE	-16.00
CXP	-15.00

Period 3:

Scans in Period:	62
Relative Start Time:	11.60 min
Experiments in Period:	2

Period 3 Experiment 1:

Scan Type:	MRM (MRM)
Polarity:	Positive
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Low
Resolution Q3:	Low
Intensity Thres.:	0.00 cps
Settling Time:	700.0000 msec
MR Pause:	5.0070 msec
MCA: No	
Step Size:	0.00 amu

BYH 18636-sulfonamide(~12.1 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param Start	Stop
236.00	204.00	300.00		

BYH 18636-sulfonamide internal standard (~12.1 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param Start	Stop
240.00	208.00	300.00		

Parameter Table (Period 3 Experiment 1):

CUR:	10.00
GS1:	70.00
GS2:	70.00
TEM:	500.00
ihe:	OFF
CAD:	6.00
IS:	4200.00
DP	36.00
EP	10.00
CE	15.00
CXP	14.00

Period 3 Experiment 2:

Scan Type:	MRM (MRM)
Polarity:	Negative
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Low
Resolution Q3:	Low
Intensity Thres.:	0.00 cps
Settling Time:	700.0000 msec
MR Pause:	5.0070 msec
MCA:	No
Step Size:	0.00 amu

BYH 18636-carboxylic acid (~12.3 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
375.00	202.00	300.00	DP	-10.00	-10.00

BYH 18636-carboxylic acid internal standard (~12.3 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
381.00	202.00	300.00	DP	-40.00	-40.00

Parameter Table(Period 3 Experiment 2):

CUR:	10.00
GS1:	70.00
GS2:	70.00
TEM:	500.00
ihe:	OFF
CAD:	6.00
IS:	-4200.00
EP	-10.00
CE	-18.00
CXP	-15.00

Period 4:
Scans in Period: 688
Relative Start Time: 12.95 min
Experiments in Period: 1

Period 4 Experiment 1:
Scan Type: MRM (MRM)
Polarity: Positive
Scan Mode: N/A
Ion Source: Turbo Spray
Resolution Q1: Low
Resolution Q3: Low
Intensity Thres.: 0.00 cps
Settling Time: 700.0000 msec
MR Pause: 5.0070 msec
MCA: No
Step Size: 0.00 amu

BYH 18636(~13.4 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param Start	Stop
391.00	359.00	300.00		

BYH 18636 internal standard (~13.4 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param Start	Stop
397.00	365.00	300.00		

Parameter Table(Period 4 Experiment 1):
CUR: 10.00
GS1: 70.00
GS2: 70.00
TEM: 500.00
ihe: OFF
CAD: 6.00
IS: 4200.00
DP 30.00
EP 10.00
CE 14.00
CXP 14.00

Gilson 215 Autosampler Properties

Inject details:
Syringe Size (µl): 1000
Injection Volume (µl): 35

Flush details:
Pre-inject Flushes (#): 0
Post-inject Flushes (#): 4

Inject details (Advanced):
Air Cushion (µl): 10
Excess Volume (µl): 10
Sample Speed (ml/min): 5.00
Needle Level (%): 5
Needle Z-Direction Speed: Very Fast
Inject Delay Time (min): 0.0
Loop Volume (µl): 100
Flush details (Advanced):
Needle Flush Volume (µl): 500
Flush Speed (ml/min):5.00
Port Flush Volume (µl): 500

Valco Valve Method Properties

Valco Valve Diverter

	Total Time (min)	Position
1	0.0	To Waste
2	6.0	To Mass Spec
3	16.0	To Waste

Shimadzu LC Method Properties

Shimadzu LC system Equilibration time = 0.00 min
Shimadzu LC Method Parameters

Pumps

Pump A Model: LC-10ADvp
Pump B Model: LC-10ADvp
Binary Gradient Total Flow: 0.500 mL/min.
Pump B Pct: 1.0
Pressure Range: 0 - 4000 psi

System Controller

Model:	SCL-10Avp
Power:	On
Event 1:	Off
Event 2:	Off
Event 3:	Off
Event 4:	Off

Time Program

Time	Module	Events	Parameter
0.50	Pumps	%B	1
6.50	Pumps	%B	3
13.00	Pumps	%B	65
13.50	Pumps	%B	90
14.50	Pumps	%B	90
14.51	Pumps	%B	1
20.00	Pumps	%B	1
20.10	System Controller	Stop	

Column:

Manufacturer:	LiChrospher®
Type:	60 RP-select B
Particle Size:	5 µm
Diameter:	3.0 mm
Length:	125 mm

Mobile Phase A:	0.5% (v/v) Acetic Acid in Water
Mobile Phase B:	0.1% (v/v) Formic Acid in Acetonitrile

Appendix 2 Example Calculation

An example calculation for BYH 18636 from sample 06GS-NE-SLLOQ-003, which was analyzed during the method validation study is presented below. This sample was fortified with 2.0ppb BYH 18636, BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide . The chromatogram used in this example is presented in Appendix 4 (Chromatogram 5) and the calibration curve for this analysis is presented in Appendix 5.

The standards were fit to the linear equation: $Y = MX + B$

where: X is the concentration of the reference standard in ng/mL
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area:isotopic peak area ratio

The example shown below is for the calculation of BYH 18636 residues. BYH 18636-carboxylic acid, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid, BYH 18636-MMT, and BYH 18636-triazolinone carboxamide residues are calculated in a similar fashion.

After regression coefficients were calculated, the residue in parts per billion was determined. The parts per billion (ppb) of BYH 18636 in the soil was calculated using the following equation,

$$\text{BYH 18636 found (ppb)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume}(V_1)}{\text{Initial sample wt. (W)}} \times \frac{\text{Final dilution volume } (V_3)}{\text{Aliquot taken } (V_2)}$$

W	V_1	V_2	V_3	Native Peak Area	IS Peak Area	Y	M	B
20g	60mL	30mL	5mL	352100.8	406410.1	0.8663	0.223	-0.00928

From the above equations:

$$\text{Dilution Factor (D)} = \frac{60}{20} \times \frac{5}{30} = 0.5$$

$$\text{BYH 18636 found} = \frac{(0.8663-(-0.00928) \times 0.50}{0.223} = 1.963 \text{ ppb}$$

Therefore sample 06GS-NE-SLLOQ-003 contains 1.963ppb BYH 18636.

The % recovery was calculated using the following equation:

$$\text{Recovery (\%)} = \frac{(R - S)}{T} \times 100$$

Where: R = ppb of target analyte found in fortified sample
S = ppb of target analyte found in control sample, real or apparent
T = theoretical ppb in fortified sample

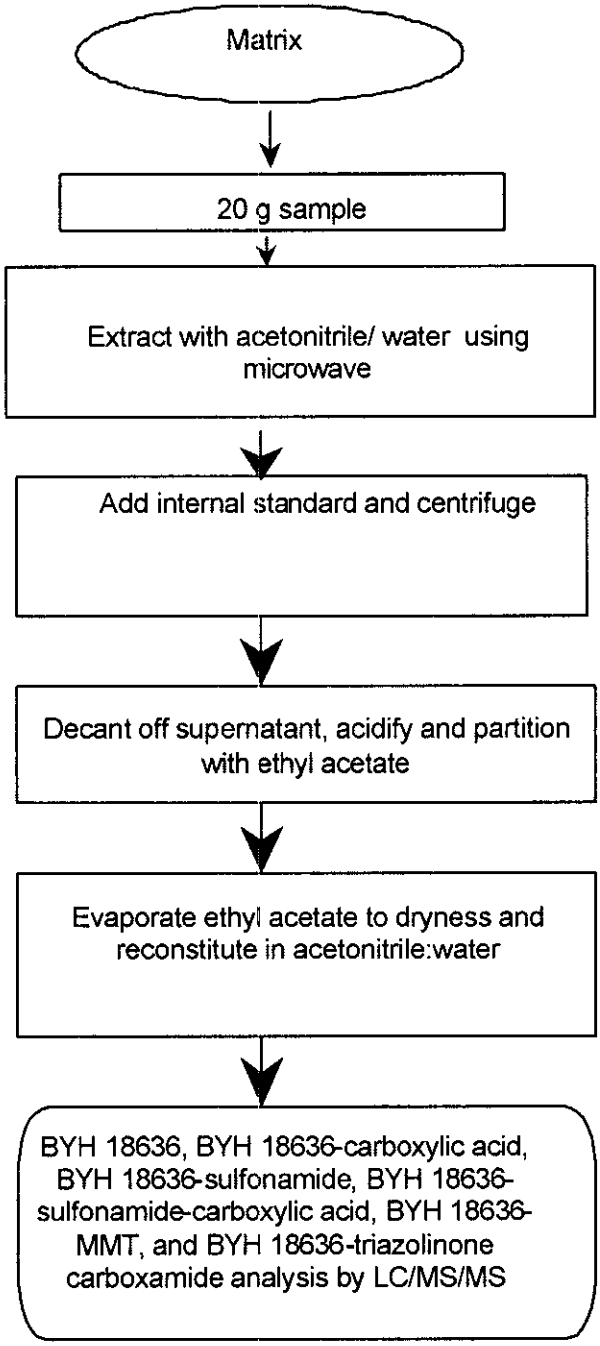
Therefore, for sample 06GS-NE-SLLOQ-003, which was fortified with 2.0ppb BYH 18636:

R = 1.963 ppb
S = 0.011 ppb
T = 2.0 ppb

$$\text{\% BYH 18636 Recovery} = \frac{(1.963 - 0.011)}{2.0} \times 100 = 98\%$$

Note: The above calculations were performed using rounded numbers and may vary slightly from the results presented in the raw data.

Appendix 6 Method Flow Chart



Appendix 7 Revision History

Method #	Revision	Description
GS-003-S06-01	01	Method prepared on completion of validation study[1]
GS-003-S06-02	02	Method updated on completion of ILV study[2]