METHOD TITLE Determinative and Confirmatory Procedures for the Assay of Fenbendazole Sulfone in Chicken Egg using LC-MS/MS

APPROVAL SIGNATURES:

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TABLE OF CONTENTS

1. GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

This section provides abbreviations and definitions of terms and concepts commonly used throughout of this method.

ALQ	Above the Limit of Quantification
approx. +5°C	Storage between +2°C and +8°C - Set point +5°C
approx. -20°C	Storage under -15°C - Set point -20°C
approx. -80°C	Storage under -70°C - Set point -80°C
BLQ	Below the Limit of Quantification
CAS	Chemical Abstracts Service
cLOQ	Calculated Limit Of Quantification
Conc.	Concentration
CV	Coefficient of Variation
Diff.	Difference
DMSO	Dimethyl Sulfoxide
ESI	ElectroSpray Ionization
IntS	Intermediate Solution for calibration standard samples
IntSQC	Intermediate Solution for QC samples
IS	Internal Standard
LC	Liquid Chromatography
LOQ	Limit Of Quantification
LOD	Limit Of Detection
MixStd	Mix Standard
MRT	Mean Retention Time
MS	Mass Spectrometry
MSDS	Material Safety Data Sheet
PAR	Analyte / Internal Standard Peak Area Ratio
QC	Quality Control
RAR	Relative Abundance Ratio
RT	Retention Time
S/N	Signal-to-Noise
SOP	Standard Operating Procedure
SRM	Selected Reaction Monitoring
SS	Stock Solution for calibration standard samples
SSIS	Stock Solution of Internal Standard
SSQC	Stock Solution for QC samples
SST	System Suitability Testing

SSTL	Low System Suitability Testing sample
Std	Standard
ULOQ	Upper Limit of Quantification
v/v	Volume to volume
WS	Working Solution for calibration standard samples
WSIS	Working Solution of Internal Standard
WSQC	Working Solution for QC samples

2. SCOPE AND FIELD OF APPLICATION

Fenbendazole is a broad spectrum benzimidazole anthelmintic used in different animal species against gastrointestinal parasites. Fenbendazole sulfone is the major metabolite and the marker residue of fenbendazole in chicken eggs and the US tolerance is 1.8 ppm. This document describes the determinative and confirmatory analytical method using liquid chromatography coupled with tandem mass spectrometry detection (LC-MS/MS) for the assay of fenbendazole sulfone in chicken egg.

The determinative and confirmatory procedures consist of a sample solvent extraction followed by LC-MS/MS analysis. The estimated limit of detection (LOD) is 0.049 ppm (0.049 µg/g) and the estimated limit of quantitation (LOQ) is 0.148 ppm (0.148 µg/g).

The compounds listed in Table 2- are other veterinary drugs registered for use in chicken in the U.S., as well as parent drug of fenbendazole sulfone (fenbendazole) and its other potential metabolite (oxfendazole). They have been tested and shown not to significantly interfere with the method.

Table 2-1 Compounds (drugs) tested for interferences

Fenbendazole	Amprolium	Chlortetracycline	Tylosin
Oxfendazole	Bacitracin	Erythromycin	

3. PRINCIPLE

One gram of homogenized chicken egg is spiked with deuterated fenbendazole sulfone internal standard and then extracted twice with methanol. The sample extract is diluted to 20 mL with methanol. An aliquot of the methanol extract is diluted with methanol/purified water (60/40, v/v) mixture. The resulting solution is quantitatively analyzed using gradient reversed phase liquid chromatography with mass-spectrometric detection (LC-MS/MS) using a positive ion selected reaction monitoring (SRM) with ion transition of *m/z* 332 → *m/z* 300 for fenbendazole sulfone and *m/z* 335 → *m/z* 300 for fenbendazole sulfone-d3. Additional ion transitions from fenbendazole sulfone, *m/z* 332 → *m/z* 159 as qualifier 1 and *m/z* 332 → *m/z* 104 as qualifier 2 were monitored for the confirmatory method. See Figure 20.1 for the fenbendazole sulfone fragmentation scheme.

4. WARNINGS AND SAFETY PRECAUTIONS

4.1. Safety

Take safety precautions common in the laboratory, e.g. wear lab coat, goggles and gloves if necessary. The MSDS of fenbendazole sulfone and fenbendazole sulfone-d3 is attached as an appendix (see Section 22.1) to this test procedure.

Reference standard (fenbendazole sulfone) and internal standard (fenbendazole sulfone-d3) are harmful substances and must be carefully handled in well ventilated areas using appropriate personal protection.

All organic solvents must be treated as potentially hazardous and all procedures using them must be performed in a fume hood.

5. REAGENTS AND MATERIALS

5.1. Reagents and chemicals

Reagents and chemicals listed in Table 5-1 were used during the validation procedure.

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water of equivalent purity. Alternate suppliers may be used.

Table 5-1 Reagents and chemicals to be used in this test procedure

Reagent/chemical	Quality or purity	Supplier / Catalog Number
Acetonitrile	Optima	Fisher / A996-4
Dimethyl sulfoxide (DMSO)	>99.5%	Fisher / D128-4
Formic acid	Min. 88%, ACS grade	Fisher / A118P-500
Methanol	HPLC	Fisher / A454-4
Purified water	18 MΩ•cm or HPLC or LC-MS	Millipore or equivalent or Fisher

5.2. Solutions

The following solutions may be prepared in different quantities, as long as appropriate proportions are used. Measure volume using a suitably sized graduated cylinder or calibrated pipette.

Table 5-2 Reagents to be used in this test procedure

Reagent	Preparation	Storage
Dilution solvent: Methanol/purified water (60/40, v/v)	Mix 60 mL of methanol and 40 mL of purified water.	Stable for 1 month at room temperature.
Mobile phase A: Purified water acidified with 0.1 % formic acid	Add 500 µL of formic acid to 500 mL of purified water or use the equivalent commercially available solution.	Stable for 1 month at room temperature.
Mobile phase B: Acetonitrile acidified with 0.1 % formic acid	Add 500 µL of formic acid to 500 mL of acetonitrile or use the equivalent commercially available solution.	Stable for 1 month at room temperature.
Needle wash solvent: Purified water/ methanol/acetonitrile/formic acid (40/30/30/1, v/v/v/v)	Mix 400 mL of purified water, 300 mL of methanol, 300 mL of acetonitrile and 10 mL of formic acid.	Stable for 1 month at room temperature.
Needle wash solvent (Weak solvent): Purified water/acetonitrile (70/30, v/v)	Mix 700 mL of purified water and 300 mL of acetonitrile.	Stable for 1 month at room temperature.

5.3. Analytical standards

Details on analytical standards presented in Table 5-3-1 and Table 5-3-2 refer to analytical batches used during the validation. Alternate vendors and/or batches may be used but the standards must have a certificate of analysis and be of known purity.

Table 5-3-1 Reference standard: Fenbendazole sulfone

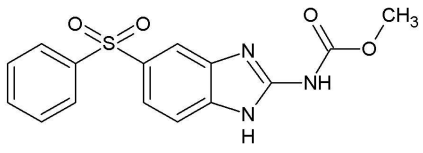
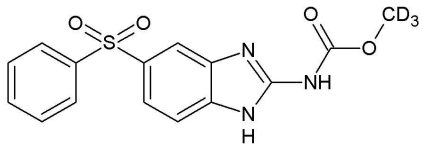
Name	Fenbendazole sulfone
CAS no.	54029-20-8
Chemical name	Methyl-5-Phenylsulfonyl-2-Benzimidazole-Carbamate
Formula	$C_{15}H_{13}N_3O_4S$
Molecular weight (g/mole)	331.35
Structural formula	
Appearance/color	White powder
Storage conditions	-25°C ±10°C, protected from light
Supplier	MSD Animal Health Innovation GmbH
Manufacturing site	Australian Government National Measurement Institute
Batch	08-AV-01
MSDS	Section 22.1.1

Table 5-3-2 Internal standard: Fenbendazole sulfone-d3

Name	Fenbendazole sulfone-d3
CAS no.	1228182-49-7
Chemical name	(5-Benzenesulfonyl-1H-benzoimidazol-2-yl)-carbamic acid methyl-D_3 ester
Formula	$C_{15}H_{10}D_3N_3O_4S$
Molecular weight (g/mole)	334.36
Structural formula	
Appearance/color	White powder
Storage conditions	+5°C ±3°C, protected from light
Supplier	MSD Animal Health Innovation GmbH
Manufacturing site	Witega Laboratorien Berlin
Batch	080310
MSDS	Section 22.1.2

6. APPARATUS AND EQUIPMENT

6.1. General apparatus

The devices listed in Table 6-1 refer to apparatus used during the validation and are given as examples. Equivalent apparatus may be substituted if acceptable performance is demonstrated. Manufacturers, model numbers, and part numbers specified here were used during method development and validation.

Table 6-1 Device list

Balance - analytical, with a readability of at least 0.1 mg
Balance - capable of weighing 1 g accurately (readability of at least ±0.001 g)
Genie 2 single unit vortex (Scientific Industries)
Centrifuge, refrigerated – capable of attaining ~ 2400 x g (4000 rpm for Sorvall Legend XTR)
Rainin EDP3 Pipettes and tips
Multitube Vortex
Volumetric flasks of 10, 20, 25 and 50 mL, with stopper
Volumetric pipettes of 20 mL
Kinematica Polytron Mixer with PT 1200 E 12 mm generator

6.2. Supplies

The supplies listed in Table 6-2 refer to those used during the validation and are given as examples, unless otherwise stated. Other supplies of equivalent quality and abilities provided by other vendors may be used.

Table 6-2 Supplies

Pasteur pipettes
Polypropylene conic tubes of 15 and 50 mL (Fisher Brand)
2 mL 96-well plates and cap mats - Analytical Sales and Services
2 mL autosampler vials

6.3. LC-MS/MS equipment

Manufacturers and model numbers specified in Table 6-3 were used during the validation. Equivalent apparatus and software may be substituted if acceptable performance is demonstrated.

Table 6-3 LC-MS/MS list

	LC-MS/MS equipment no.1	**LC-MS/MS equipment no.2**	**LC-MS/MS equipment no.3**	**LC-MS/MS equipment no.4**
Validation use	Core validation in spiked and incurred samples	Robustness test on spiked samples	Additional test of LOD and cLOQ assessments on spiked samples	Long-term stability assessment
LC pump	G1310A quaternary pump, G1322A degasser (Agilent)	2695 Alliance Separation Module (Waters)	Acquity UPLC (Waters)	Acquity FTN (Waters)
Autosampler	G1367B autosampler, G1330B thermostat (Agilent)	2695 Alliance Separation Module (Waters)	Acquity UPLC (Waters)	Acquity IClass (Waters)
Column oven	G1316A thermostated column compartment (Agilent)	Alliance column heater (Waters)	Acquity UPLC high temperature column heater (Waters)	Acquity UPLC high temperature column heater (Waters)
Mass spectrometer	$EP10^{+}$ $HSID^{++}$ (Ionics)	Quattro Micro™ API (Waters)	API 4000 (AB Sciex)	Xevo TQS (Waters)
Acquisition software	Analyst® Version 1.4.2 (AB Sciex)	MassLynx™ Security 4.1 (Waters)	Analyst® Version 1.6.1 (AB Sciex)	MassLynx™ Security 4.1 (Waters)
Data treatment software	Analyst® Version 1.4.2 (AB Sciex)	QuanLynx™ 4.1 (Waters)	Analyst® Version 1.6.1 (AB Sciex)	TargetLynx™ 4.1 (Waters)

7. PREPARATION OF STANDARD SOLUTIONS

Different volumes with the same concentrations can be prepared and it is not considered to be a method deviation. All solutions should be mixed well before transfer or use. The following solutions should be stored in a freezer (set to -20°C). Return solutions to freezer after use.

7.1. Stock solutions of fenbendazole sulfone and fenbendazole sulfone-d3 (IS)

All stock solutions of fenbendazole sulfone and fenbendazole sulfone-d3 (IS) are prepared in volumetric flasks in dimethyl sulfoxide (DMSO). The stock solutions of fenbendazole sulfone and fenbendazole sulfone-d3 (IS) are stored in glass containers in a freezer set to -20°C.

7.1.1. Stock solution of fenbendazole sulfone at 2,000 µg/mL (SS)

Accurately weigh fenbendazole sulfone reference standard (target weight 20.0 mg after correcting for purity); record the exact weight to the nearest 0.01 mg. Transfer and dissolve the standard with DMSO into a 10 mL volumetric flask and fill to mark with additional DMSO. Vortex to mix. The solution is used for the preparation of calibration standard working solutions. The actual concentration will be used to determine the required volume of stock solution needed when further dilutions are prepared (see Sections 7.1.5 and 7.2). Stock solutions of fenbendazole sulfone are stable for at least 83 days in a freezer set to -20°C.

Critical Note: fenbendazole sulfone tends to stick to a metal spatula and a flat spatula works better than one with a groove or indent. Also an anti-static gun can be used if there is still difficulty getting the standard off of the spatula.

Critical Note: Because of the stickiness of fenbendazole sulfone, the analyst may weigh into a 20 mL scintillation vial or weighing boat.

Critical Note: DMSO stock solutions stored in a freezer take several hours at room temperature to completely thaw. The stock solution can be subdivided into smaller volumes which will thaw more quickly at room temperature.

7.1.2. Stock solution of fenbendazole sulfone at 2,000 µg/mL (SSQC)

This solution (SSQC) is prepared from a second independent weighing procedure (according to section 7.1.1). It is applied for preparation of the quality control (QC) working solutions used for spiking the QC samples. Stock solutions of fenbendazole sulfone are stable for at least 83 days in a freezer set to -20°C.

7.1.3. Stock solution of fenbendazole sulfone-d3 at 1,000 µg/mL (SSIS)

Accurately weigh fenbendazole sulfone-d3 reference standard (target weight 10.0 mg after correcting for purity); record the exact weight to the nearest 0.01 mg. Transfer and dissolve the standard with DMSO into a 10 mL volumetric flask and fill to mark with additional DMSO. Vortex to mix. This solution is used for the preparation of internal standard fortification solution. The actual concentration will be used to determine the required volume of stock solution needed when further dilutions are prepared (see Section 7.1.4). Stock solutions of fenbendazole sulfone-d3 are stable for at least 83 days in a freezer set to -20°C.

Caution: The internal standard may take longer to dissolve than the analyte.

7.1.4. Working solutions of fenbendazole sulfone-d3 (WSIS, 20 µg/mL)

All working solutions of fenbendazole sulfone-d3 (IS) are prepared in volumetric flasks by dilution of stock solutions in methanol. The working solutions of fenbendazole sulfone-d3 are stored in glass containers in a freezer set to -20°C.

Using a calibrated pipette, transfer an aliquot of the stock solution of fenbendazole sulfone-d3 (SSIS, 1,000 µg/mL) (7.1.3) into a 50 mL volumetric flask and dilute with methanol to prepare the internal standard fortification solution of 20 µg/mL. Nominal aliquot volume is 1.0 mL but the

volume needs to be adjusted accordingly if the stock solution concentration is different from the nominal concentration, 1000 µg/mL (*e.g.* 1000 µg/mL nominal / 901 µg/mL actual x 1.0 mL nominal = 1.11 mL actual). Working solution of fenbendazole sulfone-d3 is stable for at least 78 days in a freezer set to -20°C.

7.1.5. Stock solution comparison

A stock solution comparison is required when new stock solutions are prepared in order to demonstrate equivalence. Each of the two stock solutions needs to be properly diluted with dilution solvent (5.2) according to the scheme in Table 7-1-5-1 using a calibrated pipette and 50 mL volumetric flasks, to prepare two intermediate solutions.

Table 7-1-5-1: Preparation of Intermediate Solutions for Stock Comparison

Intermediate Solution ID	Final Concentration [ng/mL]	Nominal Starting Concentration (µg/mL)	Volume Taken (µL)[1]	Final Volume [mL]
SS STD Stock Intermediate Solution	5,000	2,000 (SS Stock Solution)	125	50
SSQC Stock Intermediate Solution	5,000	2,000 (SSQC Stock Solution)	125	50

[1]Volume needs to be adjusted accordingly if the stock solution concentration is different from the nominal concentration, 2000 µg/mL (*e.g.* 2000 µg/mL nominal/1901 µg/mL actual x 125 µL nominal = 131.5 µL actual).

The two intermediate solutions in Table 7-1-5-1 are then diluted with dilution solvent (5.2) using calibrated pipettes and 100 mL volumetric flasks according to the scheme in Table 7-1-5-2.

Table 7-1-5-2: Preparation of Final Dilutions for Stock Comparison

Final Dilution Solution ID	Final Concentration (ng/mL) [FBZ-SO_2 / IS]	Intermediate FBZ-SO2 Solution		IS Working Solution		Final Volume [mL]
		Starting Concentration [ng/mL]	Volume Taken [µL]	Starting Concentration [µg/mL]	Volume Taken [µL]	
SS Stock Final Dilution	7.5 / 5.0	5,000	150	20	25	100
SSQC Stock Final Dilution	7.5 / 5.0	5,000	150	20	25	100

Six replicates of each stock comparison solution should be analyzed by LC-MS/MS. The precision (%CV) for the peak area ratio (PAR) for each solution and the % Difference between the PAR averages for the 2 solutions should be ≤5%. The % Difference is calculated using the following equation:

$$\textbf{\% Difference} = 100 \times \frac{\textbf{(mean of PAR of SS stock} - \textbf{mean of PAR of SSQC stock)}}{\textbf{((mean of PAR of SS stock} + \textbf{mean of PAR of SSQC stock)/2)}}$$

Stock comparison solutions are to be prepared on the day they are used and discarded after use.

7.2. Intermediate and working solutions of fenbendazole sulfone for the preparation of calibration standards

Using a calibrated pipette, transfer an aliquot (5 mL nominal, actual volume corrected for actual stock concentration) of the fenbendazole sulfone stock solution (SS) (7.1.1) into a 10 mL volumetric flask and dilute with methanol according to the following scheme (Table 7-2) to prepare IntS1 working solution. Further dilutions of this solution and subsequent solutions are then prepared using calibrated pipettes and 10 mL volumetric flasks as per Table 7-2. Working solutions of fenbendazole sulfone are stable for at least 78 days in a freezer set to -20°C and for at least 9 days at room temperature.

Table 7-2 Preparation of intermediate (IntS) and working (WS) solutions of fenbendazole sulfone

Working Solution ID	Concentration of Working Solution (µg/mL)	Volume of solution	Final Volume (mL)
IntS1	1,000	5,000 µL of SS[1] (volume is dependent upon SS concentration)	10.0
IntS2	80	800 µL of IntS1	10.0
WS6	50	500 µL of IntS1	10.0
WS5	40	400 µL of IntS1	10.0
WS4	30	300 µL of IntS1	10.0
WS3	20	200 µL of IntS1	10.0
WS2	10	100 µL of IntS1	10.0
WS1	6	750 µL of IntS2	10.0

[1]Volume needs to be adjusted accordingly if the stock solution concentration is different from the nominal concentration, 2000 µg/mL (*e.g.* 2000 µg/mL nominal/1901 µg/mL actual x 5.0 mL nominal = 5.26 mL actual).

7.3. Intermediate and working solutions of fenbendazole sulfone for the preparation of QC samples

Using a calibrated pipette, transfer an aliquot (5 mL nominal, actual volume corrected for actual stock concentration) of the fenbendazole sulfone QC stock solution (SSQC) (7.1.2) into a 10 mL volumetric flask and dilute with methanol to prepare IntSQC solution according to the following scheme (Table 7-3). Further dilutions of this solution are then prepared using calibrated pipettes and 10 mL volumetric flasks as per Table 7-3. Working solutions of fenbendazole sulfone are stable for at least 78 days in a freezer set to -20°C and for at least 9 days at room temperature.

Table 7-3 Preparation of intermediate (IntS) and working (WS) solutions of fenbendazole sulfone for QC samples

Solution identification	Concentration (µg/mL)	Volume Taken	Final Volume (mL)
IntSQC	1,000	5,000 µL of SSQC[1] (volume is dependent upon SSQC concentration)	10.0
WSQC High	36	360 µL of IntSQC	10.0
WSQC Mid	18	180 µL of IntSQC	10.0
WSQC Low	9	90 µL of IntSQC	10.0

[1]Volume needs to be adjusted accordingly if the stock solution concentration is different from the nominal concentration, 2000 µg/mL (*e.g.* 2000 µg/mL nominal/1901 µg/mL actual x 5.0 mL nominal = 5.26 mL actual).

7.4. Solvent calibration standards

For the preparation of the solvent calibration curve:

Using a calibrated pipette, transfer 100 µL each of the respective working solutions WS (7.2) and 100 µL of the WSIS (FBZ-SO2-D3) fortification solution (7.1.4) (20 µg/mL) to 20 mL volumetric flasks, fill to the mark with methanol and mix well to give W-Mix-Stds (see Table 7-4-1). Mix standard solutions of fenbendazole sulfone are stable for at least 78 days in a freezer set to -20°C and for at least 9 days at room temperature.

Table 7-4-1: Preparation of Mix Standard Solutions

Solution ID	Volume of WS1 - 6 Taken / Concentration [µg/mL]	Volume of IS Solution Taken	Final Volume [mL]	Final Concentration [ng/mL]
W-Mix-Std-6	100 µL of WS6 / 50	100 µL	20	250
W-Mix-Std-5	100 µL of WS5 40	100 µL	20	200
W-Mix-Std-4	100 µL of WS4 / 30	100 µL	20	150
W-Mix-Std-3	100 µL of WS3 /20	100 µL	20	100
W-Mix-Std-2	100 µL of WS2 / 10	100 µL	20	50
W-Mix-Std-1	100 µL of WS1 / 6	100 µL	20	30

Using a calibrated pipette, transfer 50 µL of each W-Mix-Stds solution into a LC vial or 96-well plate and dilute with 950 µL of dilution solvent (5.2) and mix well to give solvent calibration standards (see Table 7 4). The calibration standards (final dilution) are prepared fresh for each batch extraction.

Table 7-4 Preparation of solvent calibration standards

Standard-ID	W-Mix-Std Solution ID / Concentration (ng/mL)	Final Concentration [ng/mL]	Egg Equivalent Concentration [µg/g]
Std6	W-Mix-Std-6 / 250	12.5	5.00
Std5	W-Mix-Std-5 / 200	10.0	4.00
Std4	W-Mix-Std-4 / 150	7.5	3.00
Std3	W-Mix-Std-3 /100	5.0	2.00
Std2	W-Mix-Std-2 / 50	2.5	1.00
Std1	W-Mix-Std1 / 30	1.5	0.600

Note: the nominal concentration of internal standard in solvent standards is 5.0 ng/mL, equivalent to 2 ppm in egg.

The tissue equivalent concentrations of the solvent calibration standards are presented in Table 7-4. The extraction process results in a 400x dilution of residues (1 g tissue extracted in 20 mL of Methanol; 50 µL of extract diluted to 1 mL with dilution solution). Therefore, the conversion factor from solvent concentration (ng/mL) to tissue equivalents (µg/g or ppm) is 0.4 (*i.e.* 400 / 1000).

Refer to extraction steps 9.2.6, 9.2.8, and 9.1.1 for extraction volume, dilution factor, and egg sample weight, respectively.

7.5. Quality control (QC) samples

For routine use, a minimum of one Double Blank, one Control Blank, and two QC samples at tolerance are required for each sample analysis set.

For the preparation of the QC samples, spike 100 µL of the respective WSQC (7.3) and 100 µL of WSIS (7.1.4) on 1.000 ±0.050 g of control egg (see Table 7-5). For routine sample analysis, QC samples are prepared fresh for each batch extraction.

Table 7-5 Preparation of QC samples

QC sample identification	Weight of control egg	Spiking volume of WSIS	Spiking volume of WSQC	Egg conc. of fenbendazole sulfone [µg/g]	Egg conc. of IS [µg/g]
QC High	1.000 ±0.050 g	100 µL	100 µL of WSQC High	3.60	2.00
QC Mid	1.000 ±0.050 g	100 µL	100 µL of WSQC Mid	1.80	2.00
QC Low	1.000 ±0.050 g	100 µL	100 µL of WSQC Low	0.900	2.00

Further sample preparation is described in Section 9.1.

8. SAMPLING AND SAMPLE HANDLING

8.1. Egg sample homogenization and storage

8.1.1. Crack an egg in a suitable container.

8.1.2. Homogenize entire yolk and albumen together, using an Polytron mixer or comparable type mixer.

8.1.3. Between samples, rinse the dispersing element of the mixer into a bath of water, and then into a bath of ethanol.

8.1.4. Dry the dispersing element with absorbent paper.

From collection to homogenization, eggs are stored at room temperature. Egg homogenate (control or incurred samples) are aliquoted and stored in polypropylene containers. Sealed polypropylene containers may be stored in a freezer set to -20°C or <-65°C.

Refer to Section 17 for stability information.

9. PROCEDURE FOR DETERMINATION AND CONFIRMATION OF FENBENDAZOLE SULFONE IN CHICKEN EGG

Using this method, approximately 50 samples can be analyzed in an 8-hour day by an individual analyst. All of the procedure is performed at room temperature (20-25°C) unless otherwise specified.

9.1. Preparation of blank matrix, zero matrix, quality control and incurred samples

9.1.1. Accurately weigh 1.000 g (±0.050 g) of control or incurred sample, previously thawed at room temperature, into a 15 mL polypropylene conic tube. Record or print the exact weight as shown on the balance.

9.1.2. Add 200 µL of methanol for double blank matrix sample (no analyte, no internal standard), or,

Add 100 µL of methanol for control blank (internal standard only) and incurred sample, or,

Add 100 µL of WSQC (7.3) for QC samples.

9.1.3. Briefly vortex.

9.1.4. Add 100 µL of WSIS (7.1.4) for control blank, QC and incurred samples.

9.1.5. Briefly vortex and leave the samples at room temperature for approximately 10 minutes before applying the extraction procedure (9.2).

For fortified QC samples, a nominal tissue weight of 1.0 g should be used for the determination of recovery (actual weight should be recorded). For incurred samples, correction of weight is required. A dilution factor will be applied. Dilution Factor = nominal weight / actual weight (1.0 grams = nominal weight).

9.2. Extraction procedure

9.2.1. Add 8 mL of methanol into the 15 mL polypropylene conic tube containing the sample using a pipette. Cap the polypropylene tube.

9.2.2. Mix the sample on a multi-tube vortex set at high speed for 10 minutes.

9.2.3. Centrifuge the sample for 10 minutes at approximately 3,500 g and set at +10°C.

9.2.4. Transfer the supernatant to a 50 mL polypropylene graduated conical tube.

9.2.5. Add 8 mL of methanol. Disperse the pellet with a disposable pipette or spatula.

9.2.6. Repeat the extraction steps 9.2.2 to 9.2.4 on the dispersed pellet. Combine the methanol extracts and adjust the volume to 20 mL mark with methanol. Mix on a vortex.

9.2.7. Centrifuge the combined extracts for 5 minutes at approximately 3500 g and set at +10°C.

9.2.8. Pipette 50 µL of the methanol extract into a 2 mL glass vial or 2 mL 96-well plate and mix with 950 µL of methanol/purified water (60/40, v/v). Cap the vial or seal the plate with a cap mat. Briefly mix on a vortex.

Final matrix extracts are stable for at least 98 hours in the autosampler at room temperature and an analytical sequence can be totally re-injected within 96 hours if extracts are stored at room temperature

10. METHOD FLOW CHART

Transfer 1.000 ±0.050 g of control egg homogenate or incurred egg homogenate into a 15 mL polypropylene conic tube.

↓

Add 200 µL of methanol for double blank matrix sample. Add 100 µL of methanol and 100 µL of WSIS for control blank and incurred samples. Add 100 µL of WSQC and 100 µL of WSIS for QC samples. Briefly mix on a vortex. Leave the sample at room temperature for 10 minutes before extraction.

↓

Add 8 mL of methanol and mix on a multi-tube vortex for 10 minutes.

↓

Centrifuge for 10 minutes at approximately 3,500 g and set at +10°C.

↓

Transfer the supernatant to a 50 mL polypropylene graduated conical tube.

↓

Repeat the extraction steps. Combine the methanol extracts and adjust the volume to 20 mL mark with methanol. Mix on a vortex.

↓

Centrifuge the combined extracts for 5 minutes at approximately 3,500 g and set at +10°C.

↓

Pipette 50 µL of methanol extract into a 2 mL glass vial or 2 mL 96-well plate and mix with 950 µL of methanol/purified water (60/40, v/v) for LC-MS/MS analysis.

11. LC-MS/MS ANALYSIS

Equivalent apparatus may be substituted if acceptable performance is demonstrated. Manufacturers and model numbers specified here were used during method development and validation.

On occasions it may be necessary to adjust the LC and MS conditions slightly to achieve acceptable peak shape and sensitivity. The LC and MS conditions should be adjusted such that acceptable performance of the LC-MS/MS system is met.

11.1. LC conditions for determinative procedure

Approximate retention times observed during validation are specified.

LC column	MacMod Ace 3 C18, 2.1 x 50 mm; 3.0 µm
Column temperature	Ambient (not controlled)
Injection volume	5 µL (may vary)
Autosampler temperature	Ambient (not controlled)
Mobile phase A	0.1% Formic Acid
Mobile phase B	0.1% Formic Acid in Acetonitrile (v/v)
Gradient table	See Table 11-1
Run time	8.2 min/inj.
Retention times	approx. 1.4 min, for both fenbendazole sulfone and IS

Table 11-1 Gradient table

Time (minutes)	Flow rate (mL/min)	% of mobile phase A	% of mobile phase B
0.0	0.4	**70**	**30**
0.3	0.4	**70**	**30**
2.0	0.4	**25**	**75**
2.1	0.4	**0**	**100**
3.1	0.4	**0**	**100**
3.2	0.4	**70**	**30**
8.2	0.4	**70**	**30**

11.2. LC system care

LC system care may depend on the LC-MS/MS system used. Following settings correspond to those applied on the different systems used during the validation and are for example only.

11.2.1. LC-MS/MS system no.1

Needle wash solvent	Purified water/methanol/acetonitrile/formic acid (40/30/ 30/1, v/v/v/v)
Column wash solvent	Acetonitrile/purified water (80/20, v/v) at 0.2 mL/min for 60 minutes
Needle wash programming	See Table 11-2

Table 11-2 Needle wash programming

Step	Action
1	Wash needle in flush port for 15 sec
2	Draw def amount from sample, speed 200 µL/min, offset 1 mm
3	Wash needle in flush port for 15 sec
4	Inject
5	Remote start pulse, duration 10 x 12.5 msec

11.2.2. LC-MS/MS system no.2

Plunger seal wash solvent	Purified water/methanol (80/20, v/v)
Needle wash solvent	Purified water/methanol/acetonitrile/formic acid (40/30/ 30/1, v/v/v/v)
Column wash solvent	Acetonitrile/purified water (80/20, v/v) at 0.2 mL/min for 60 minutes

11.2.3. LC-MS/MS system no.3

Plunger seal wash solvent	Purified water/methanol (80/20, v/v)
Needle weak wash solvent	Purified water/acetonitrile (70/30, v/v) - 1500 µL
Needle strong wash solvent	Purified water/methanol/acetonitrile/formic acid (40/30/ 30/1, v/v/v/v) - 500 µL
Column wash solvent	Acetonitrile/purified water (80/20, v/v) at 0.2 mL/min for 60 minutes

11.2.4. LC-MS/MS system no.4

Plunger seal wash solvent	Purified water/methanol (80/20, v/v)
Needle wash solvents	
Exterior (Wash)	Purified water/methanol/acetonitrile/formic acid (40/30/ 30/1, v/v/v/v)
Exterior (Purge)	Purified water/acetonitrile (70/30, v/v)
Needle rinsing programming	Pre and post injection wash duration: 6 seconds
Column wash solvent	Acetonitrile/purified water (80/20, v/v) at 0.2 mL/min for 60 minutes

11.3. MS conditions for determinative procedure

11.3.1. Tuning of mass spectrometer

The MS response of fenbendazole sulfone and fenbendazole sulfone-d3 can be tuned by infusion of fenbendazole sulfone and fenbendazole sulfone-d3 solutions (suggested concentration at about 1 µg/mL). Typically, the tuning is done by infusing a solution of the analyte of interest diluted in

mobile phase using a tee connector prior to introduction into the MS. The conditions should be optimized in full scan mode for adequate detection of fenbendazole sulfone and fenbendazole sulfone-d3 parent ions (*m/z* 332, *m/z* 335, respectively). The MS conditions should then be optimized in MS/MS mode for adequate detection of product ion at *m/z* 300 for both fenbendazole sulfone and fenbendazole sulfone-d3. The resultant MS parameter should be used for all determinative analyses, although the operator may vary conditions for adequate sensitivity. The structure and proposed fragmentation pattern of fenbendazole sulfone (and thus fenbendazole sulfone-d3) is shown in Figure 20.1.

11.3.2. MS conditions

The MS should be tuned as described under Section 11.3.1. The MS parameters and SRM transitions used during validation are presented in Table 11-3, Table 11-4, Table 11-5 and Table 11-6. Settings may depend on the MS system used and are for example only.

Table 11-3 Determinative MS settings for LC-MS/MS system no.1 (Ionics EP10^{+} HSID^{++})

Ionization interface & mode	**Turbo Ion Spray (TIS) – Positive mode**
Source temperature (TEM)	450°C
Nebulizer gas (NEB)	13 psi
Curtain gas (CUR)	15 psi
Collision gas (CAD)	5 psi
Ion spray (IS)	4,500 V
Declustering potential (DP)	55 V
Focusing potential (FP)	60 V
Q1 & Q3 resolutions	Unit
Ion energy 1	0.5 V
Ion energy 3	2.7 V
Deflector (DF)	-50 V
Multiplier (CEM)	2,600 V
Vacuum gauge pressure	*approx.* 3.4e^{-5} Torr

Analyte ion	**SRM transition**	**Dwell time**	**Entrance potential (EP)**	**Collision energy (CE)**	**Exit Potential (EP)**
Fenbendazole sulfone (Quantifier)	*m/z* 332 ⟶ *m/z* 300	200 ms	10 V	32 eV	14 V
Fenbendazole sulfone-d3 (IS)	*m/z* 335 ⟶ *m/z* 300	200 ms	10 V	32 eV	14 V

Table 11-4 Determinative MS settings for LC-MS/MS system no.2 (Waters Quattro Micro)

Ionization interface & mode	**ElectoSpray Ionization (ESI) – Positive mode**			
Source temperature	130°C			
Desolvatation temperature	450°C			
Cone gas flow	20 L/h			
Desolvatation gas flow	650 L/h			
Capillary potential	3 kV			
Extractor potential	2 V			
RF Lens	0.2 V			
LM/HM Resolution 1	12.0/12.0			
Ion energy 1	0.4 V			
Entrance/exit potentials	0 V / 1 V			
LM/HM Resolution 1	14.0/14.0			
Ion energy 2	0.5 V			
Multiplier	650 V			
Gas cell Pirani pressure	*approx.* $4.0e^{-3}$ mbar			
Analyte ion	**SRM transition**	**Dwell time**	**Cone voltage**	**Collision energy**
Fenbendazole sulfone (Quantifier)	*m/z* 332 ⟶ *m/z* 300	0.1 s	28 V	22 eV
Fenbendazole sulfone-d3 (IS)	*m/z* 335 ⟶ *m/z* 300	0.1 s	28 V	20 eV

Table 11-5 Determinative MS settings for LC-MS/MS system no.3 (AB Sciex API 4000)

Ionization interface & mode	**Turbo Ion Spray (TIS) – Positive mode**
Source temperature (TEM)	450°C
Curtain gas (CUR)	25 psi
Collision gas (CAD)	10 psi
Ion source gas 1 (GS1)	50 psi
Ion source gas 2 (GS2)	40 psi
Ion spray (IS)	4,500 V
Declustering potential (DP)	85 V
Q1 & Q3 resolutions	Unit
Ion energy 1	0.5 V
Ion energy 3	-0.5 V
Deflector (DF)	0 V
Multiplier (CEM)	2,000 V
Vacuum gauge pressure	*approx.* $3.7e^{-5}$ Torr

Analyte ion	SRM transition	Dwell time	Entrance potential (EP)	Collision energy (CE)	Exit Potential (CXP)
Fenbendazole sulfone (Quantifier)	*m/z* 332 ⟶ *m/z* 300	200 ms	8 V	33 eV	18 V
Fenbendazole sulfone-d3 (IS)	*m/z* 335 ⟶ *m/z* 300	200 ms	8 V	33 eV	18 V

Table 11-6 Determinative MS settings for LC-MS/MS system no.4 (Waters Xevo TQS)

Ionization interface & mode	**ElectoSpray Ionization (ESI) – Positive mode**
Source temperature	150°C
Desolvatation temperature	500°C
Cone gas flow	150 L/h
Desolvatation gas flow	1000 L/h
Capillary potential	1.4 kV
Source offset	50 V
LM/HM Resolution 1	2.7/15.0
Ion energy 1	-0.1 V
Entrance/exit potentials	1 V / 1 V
LM/HM Resolution 1	2.7/14.9
Ion energy 2	0.8 V
Gain	1
Nebulizer pressure	*approx.* 7.0 bar
Collision gas flow	*approx.* 0.18 mL/min

Analyte ion	SRM transition	Dwell time	Cone voltage	Collision energy
Fenbendazole sulfone (Quantifier)	*m/z* 332 ⟶ *m/z* 300	0.15 s	50 V	22 eV
Fenbendazole sulfone-d3 (IS)	*m/z* 335 ⟶ *m/z* 300	0.15 s	30 V	36 eV

The MS parameters should be established by tuning of the instrument to be used and its calibration. Differences from the above parameters are not considered a method deviation.

Representative LC-MS/MS chromatograms obtained using determinative procedure for double blank matrix sample, control sample, solvent calibrations standards at the lowest standard level (*i.e.* 0.600 ppm), control matrix samples fortified at the 0.600 ppm and incurred sample at about 2.000 ppm are shown in Figures 20.2.1, 20.2.2, 0, 0, 20.2.4, respectively.

11.4. MS conditions for confirmatory procedure

11.4.1. Tuning of mass spectrometer

The MS response of fenbendazole sulfone can be tuned by infusion of fenbendazole sulfone solution (suggested concentration at about 1 µg/mL). Typically, the tuning is done by infusing a solution of the analyte of interest diluted in mobile phase using a tee connector prior to introduction into the MS. The conditions should be optimized in full scan mode for adequate detection of fenbendazole sulfone parent ion (*m/z* 332). The MS conditions should then be optimized in MS/MS mode for adequate detection of product ions at *m/z* 300, *m/z* 159 and *m/z* 104. The resultant MS parameter should be used for all confirmatory analyses, although the operator may vary conditions for adequate sensitivity. The structure and proposed fragmentation pattern of fenbendazole sulfone is shown in Figure 20.1.

11.4.2. MS conditions

The MS should be tuned as described under Section 11.4.1. The MS parameters and SRM transitions used during validation are presented in Table 11-7. Settings may depend on the MS system used and are for example only.

Table 11-7 Confirmatory MS settings for LC-MS/MS system no.1 (Ionics EP10^{+} HSID^{++})

Ionization interface & mode	**Turbo Ion Spray (TIS) – Positive mode**
Source temperature (TEM)	450°C
Nebulizer gas (NEB)	13 psi
Curtain gas (CUR)	15 psi
Collision gas (CAD)	5 psi
Ion spray (IS)	4,500 V
Declustering potential (DP)	55 V
Focusing potential (FP)	55 V
Q1 & Q3 resolutions	Unit
Ion energy 1	1.4 V
Ion energy 3	2.4 V
Deflector (DF)	-50 V
Multiplier (CEM)	2,600 V
Vacuum gauge pressure	*approx.* 3.8e^{-5} Torr

Analyte ion	**SRM transition**	**Dwell time**	**Entrance potential (EP)**	**Collision energy (CE)**	**Exit Potential (CXP)**
Fenbendazole sulfone (Quantifier)	*m/z* 332 ⟶ *m/z* 300	200 ms	11 V	31 eV	13 V
Fenbendazole sulfone (Qualifier 1)	*m/z* 332 ⟶ *m/z* 159	200 ms	12 V	53 eV	9 V
Fenbendazole sulfone (Qualifier 2)	*m/z* 332 ⟶ *m/z* 104	200 ms	12 V	82 eV	3 V

The MS parameters should be established by tuning of the instrument to be used and its calibration. Differences from the above parameters are not considered a method deviation.

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Representative LC-MS/MS chromatograms obtained using confirmatory procedure for double blank matrix sample, control blank sample, solvent calibrations standards at 2.00 ppm and 4.00 ppm, control matrix sample fortified at low concentration level (*i.e.* 1.60 ppm) and incurred sample at about 2.00 ppm are shown in Figures 20.2.6, 0, 0, 0, 20.2.10 and 20.2.11, respectively.

11.5. System Suitability Test and Sample Injection Sequence

11.5.1. System Suitability Test (SST)

Once the system is stabilized, system suitability should be performed by injection of the lowest standard 1 for at least 5 times to assess reproducibility and sensitivity of MS response. Refer to Section 13.1.1 for system suitability acceptance criteria.

11.5.2. Standards

All 6 standards are run before a maximum of 50 extracted samples including QC and incurred samples. The extracted samples are followed (bracketed) by all 6 standards. Double blank and control blank samples are injected after the first injection of the standards.

11.5.3. Analysis sequence

A possible sequence order consisting of system suitability test (SST) samples, solvent calibration, and QC samples within a series is presented in Table 11-8. The SST solutions (Section 11.5.1) are used to check the LC-MS system.

Table 11-8 Example of an analytical sequence

Std1 System suitability test solution	≥ 5 injections
Dilution Solvent (methanol/purified water (60/40, v/v))	1 injection
Std1 to Std6	1 injection each
Double Blank and control blank samples	1 injection each
Maximum of 50 tissue samples, including QC and study samples	1 injection each
Std1 to Std6	1 re-injection each

12. CALCULATION AND REPORTING OF RESULTS

12.1. Method of calculation (determinative analysis)

Quantitation of fenbendazole sulfone is accomplished using an internal standard calibration method with a fenbendazole sulfone standard concentration range of 0.600 ppm to 5.00 ppm egg equivalents for chicken egg. A standard calibration curve is generated from non-weighted linear regression analysis of fenbendazole sulfone / fenbendazole sulfone-d3 peak area ratio versus egg equivalent concentration (ppm) of fenbendazole sulfone.

A typical standard calibration curve for chicken egg is shown in Figure 20.3.

If the regression obtained in an analytical set yields an acceptable coefficient of determination and meets the stated criteria (refer to Section 13), the regression equation can be used to determine the

concentration of each sample in the set. If the regression does not meet acceptability criteria, the set is deemed not acceptable and has to be repeated by re-injecting the standards and samples or by preparing new standards and/or new sample extracts for re-analysis.

A linear regression curve fit equation for the standard curve will determine the concentration of the sample injected using the following equation:

$$Y = AX + B$$

The concentration of each sample is calculated using the formula:

$$X = \frac{Y - B}{A}$$

Where, Y is MS detector calculated response using the analyte/IS area ratio,
X is sample concentration (ppm),
A is slope,
B is y-intercept.

12.2. Calculation of unknown concentrations from incurred-residue tissues and fortified samples

The exact concentration, rounded with 3 significant figures, should be reported and used throughout all of the calculations.

Recoveries (a measure of accuracy) are calculated from fortified QC samples using the equation:

$$\% \text{ Recovery} = \frac{C_T}{C_F} \times 100$$

Where, C_T is the calculated concentration of fenbendazole sulfone in ppm in the QC sample,
C_F is the tissue fortification level in ppm.

The following equation will calculate the concentration in ppm incurred-residue tissue samples:

$$C_T = \frac{C_I}{S_W}$$

Where, C_T is the concentration of fenbendazole sulfone in ppm in the sample,
C_I is the calculated concentration of fenbendazole sulfone in ppm from the standard curve where the nominal concentration of standards are in ppm and are based on 1.00 ±0.01 g sample size,
S_W is the weight in g of the initial sample (nominal weight of 1.00 ±0.01 g is used for fortified samples and exact weight is used for incurred samples).

13. ACCEPTABILITY CRITERIA FOR DETERMINATIVE PROCEDURE

Analytical data must meet the following criteria to establish adequate performance of the method.

13.1. Determinative Procedure

13.1.1. System suitability test: Reproducibility

To demonstrate acceptable performance of the LC-MS/MS system, the system suitability injections of a standard at the lowest calibration level (Std1 - SSTL) should be performed prior to injection of a sample set (refer to Section 11.5.1).

A signal-to-noise ratio higher than 10:1, a reproducible fenbendazole sulfone/fenbendazole sulfone-d3 peak area ratio with CV ≤ 5 % and a reproducible fenbendazole sulfone retention time with CV ≤ 5 % must be met for the (at least) five consecutive injections of SSTL (Std1).

If the MS detector sensitivity is low and gives poor precision at Std1, tuning the instrument may improve the sensitivity. If the sensitivity remains low, instrument calibration, cleaning, and/or repair should be performed.

If the MS detector sensitivity is too high and gives a non-linear standard curve, the instrument parameters may be changed to decrease the response.

13.1.2. Standard calibration curve

The non-weighted linear regression should have a coefficient of determination (r^2) ≥ 0.990 for a standard curve of fenbendazole sulfone ranging from 0.600 ppm to 5.00 ppm (egg equivalents) for chicken egg.

13.1.3. Accuracy: QC sample acceptance criteria

For routine analysis, the results of the QC samples will provide the basis for accepting or rejecting the analytical run. The acceptance criterion for accuracy of QC samples is 80% to 110%. The precision of the method as measured by the coefficient of variation (CV %) of replicates at different tissue fortifications should be ≤10%.

14. LIMIT OF QUANTITATION

The limit of quantitation (LOQ) calculated from calibration curve data generated during the method trial was 0.148 ppm egg equivalent. The lowest standard on the calibration curve is 0.600 ppm egg equivalents. Quantitative information below the lowest standard should be reported and footnoted as BLOQ. The analyst should note this result with appropriate annotations and footnotes in the analytical results.

The upper limit of quantitation (ULOQ) is set at the highest concentration of FBZ-SO_2 in the calibration standard curve, 5.00 ppm egg equivalents.

15. LIMIT OF DETECTION

An estimated limit of detection (LOD) based upon analysis of control egg samples and calculated from calibration curve data generated during the method trial was 0.049 ppm egg equivalent.

16. DILUTION

After analysis, samples found to have concentrations above the method calibration range should be diluted with control egg as appropriate, and the diluted sample will be re-analyzed. The sample concentration should then be calculated by application of the appropriate dilution factor to the result of the re-analysis.

17. STABILITY

17.1. Stability of fenbendazole sulfone and fenbendazole sulfone-d3 in stock and working solutions

Fenbendazole sulfone is stable in DMSO (2,000 µg/mL nominal) for at least 83 days in a freezer set to -20°C and for at least 5 days at room temperature, when stored in glass container.

Fenbendazole sulfone is stable in methanol (6.00 µg/mL to 64.0 µg/mL) for at least 78 days in a freezer set to -20°C and for at least 9 days at room temperature, when stored in glass container.

Fenbendazole sulfone-d3 (IS) is stable in DMSO (1,000 µg/mL nominal) for at least 83 days in a freezer set to -20°C and for at least 28 days at room temperature, when stored in glass container.

Fenbendazole sulfone-d3 (IS) is stable in methanol (20.0 µg/mL) for at least 78 days in a freezer set to -20°C and for at least 28 days at room temperature, when stored in glass container.

17.2. Stability of fenbendazole sulfone and fenbendazole sulfone-d3 in solvent calibration standards

Solvent calibration standards (see Section 7.4) are stable for at least 9 days in a refrigerator.

17.3. Stability of fenbendazole sulfone in final extract

Fenbendazole sulfone and fenbendazole sulfone-d3 are stable in final matrix extract for at least 98 hours in the autosampler at room temperature and an analytical sequence can be totally re-injected within 96 hours if final extracts are stored at room temperature.

17.4. Stability of fenbendazole sulfone in chicken egg homogenate

Fenbendazole sulfone is stable in egg homogenate:

- For at least 40 hours at room temperature.
- For at least 3 successive freeze (in a freezer set to -20°C or -80°C)/thaw (at room temperature) cycles
- For at least 371 days in a freezer set to -20°C
- For at least 376 days in a freezer set to -80°C.

18. NOTES TO ANALYSTS

The robustness of the method (chromatographic conditions) was validated by using different batches of the column Ace 3 C18 (MacMod).

The Acclaim C18 (Thermo Electron Corporation - 50 x 2.1 mm, 3.0 µm) was tested and found not satisfactory as an alternative column for analysis.

The injection volume must be adapted according to the sensitivity of the LC-MS/MS system, within a range from 2 µL to 20 µL.

Re-equilibration time used by reference lab was 2 minutes during method trial (final time 5.2 minutes rather than 8.2 minutes) with no apparent impact on chromatography or results.

19. CONFIRMATORY METHOD

19.1. Confirmatory Analysis

For confirmatory analysis, additional ion transitions from FBZ-SO2, *m/z* 332 → *m/z* 159 as qualifier 1 and *m/z* 332 → *m/z* 104 as qualifier 2 are monitored along with *m/z* 332 → *m/z* 300 used for determinative method. The instrument should be optimized with the comparison standard to obtain an S/N ≥ 50 for the qualifying ions.

Identification is based on the relative abundances of *m/z* 159 and *m/z* 104 to the base peak, *m/z* 300 and the relative retention time and the signal to noise ratio (S/N) of the qualifier ions. The relative abundance of each ion is calculated as described below:

$$\text{Relative Abundance} = \frac{\text{Area of Qualifier Ion Peak}}{\text{Area of Derterminative Ion Peak}} \text{ x } 100$$

19.2. Confirmation Criteria

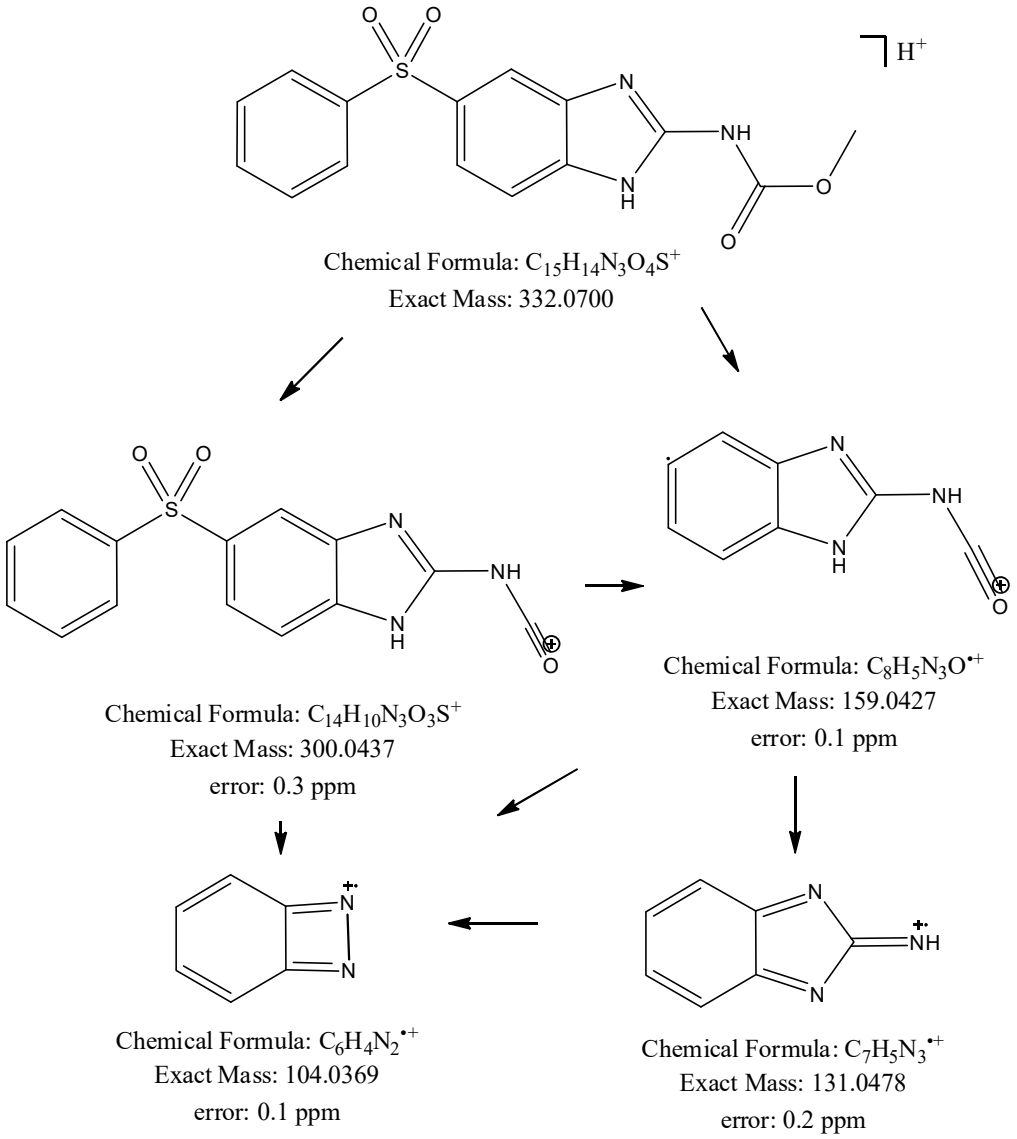
Acceptance criteria for confirmatory analysis are as follows:

- Relative abundance ratio of the confirmatory transitions *m/z* 332 → *m/z* 159 and *m/z* 332 → *m/z* 104 to the base peak, *m/z* 332 → *m/z* 300 in the QC and incurred samples should match the average relative abundance ratio in solvent standards within ±10% arithmetically.
- Signal to noise ratio (S/N) greater than 50 is required for all confirmatory peaks.
- The retention time of the confirmatory peaks in QC and incurred samples should match the retention time of the quantitative peak in solvent standards within ±5%.

All the three confirmation criteria listed in this section must be met for positive confirmation of FBZ-SO2 in the egg extract. The control egg samples must fail to confirm. Failure to confirm is concluded if the extract does not meet one or more of the confirmation criteria outlined above.

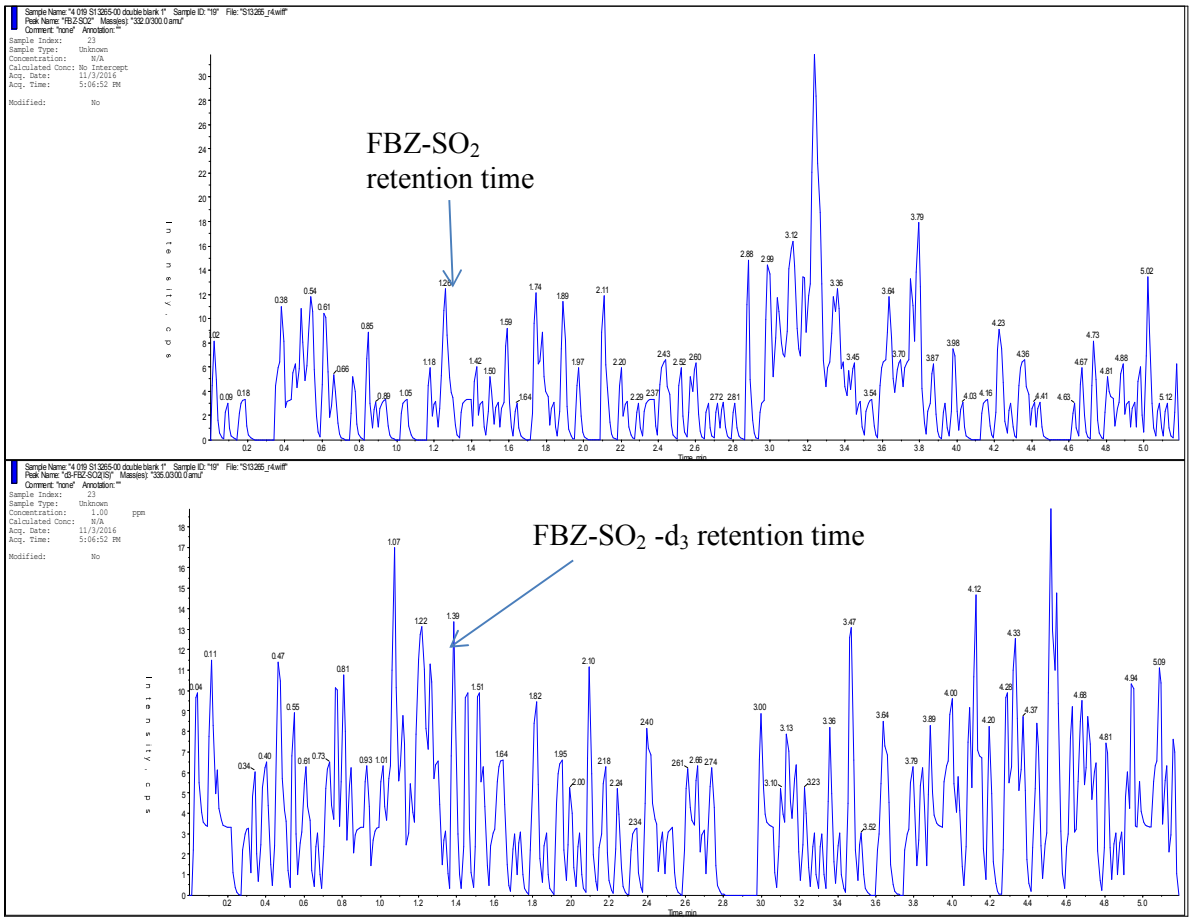
20. FIGURES

20.1. Proposed fragmentation pattern of fenbendazole sulfone and structures of monitored ions

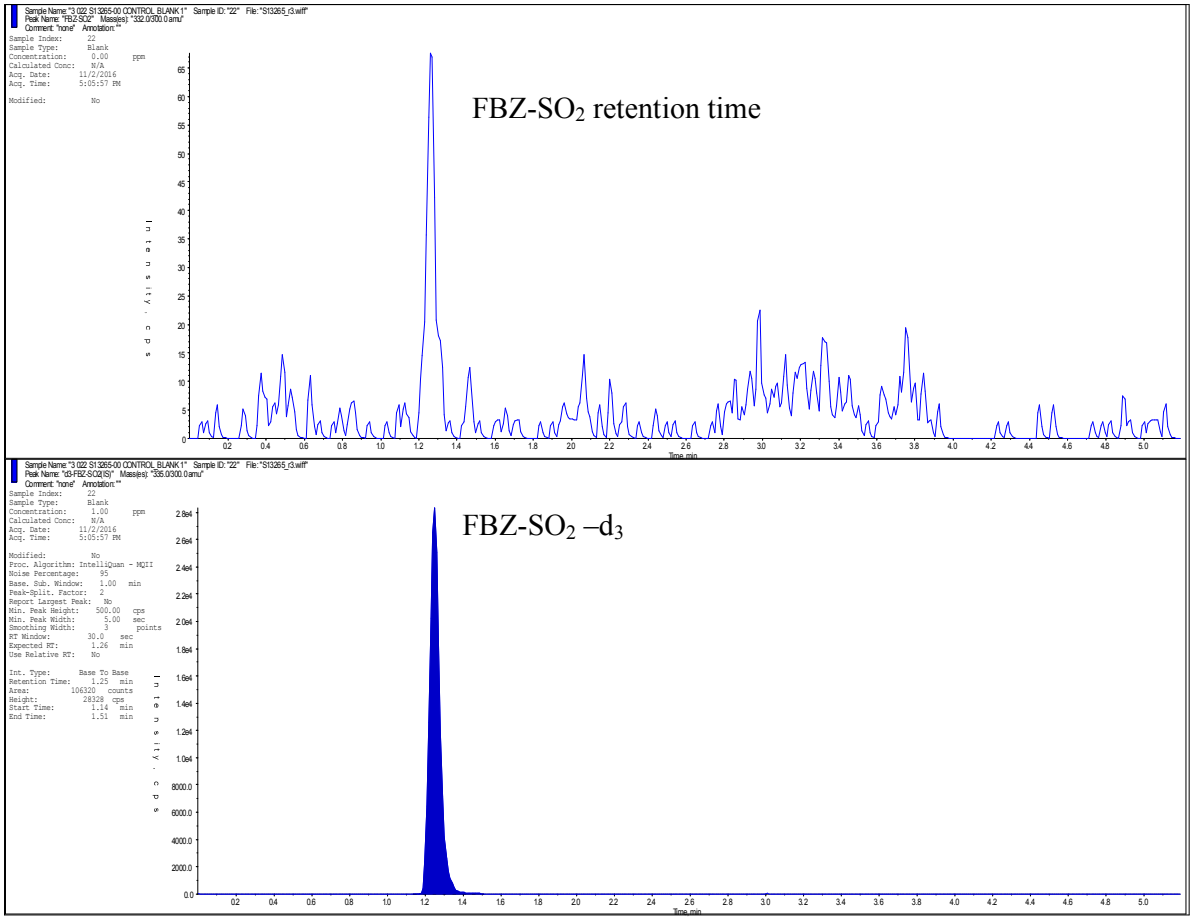


20.2. Chromatograms

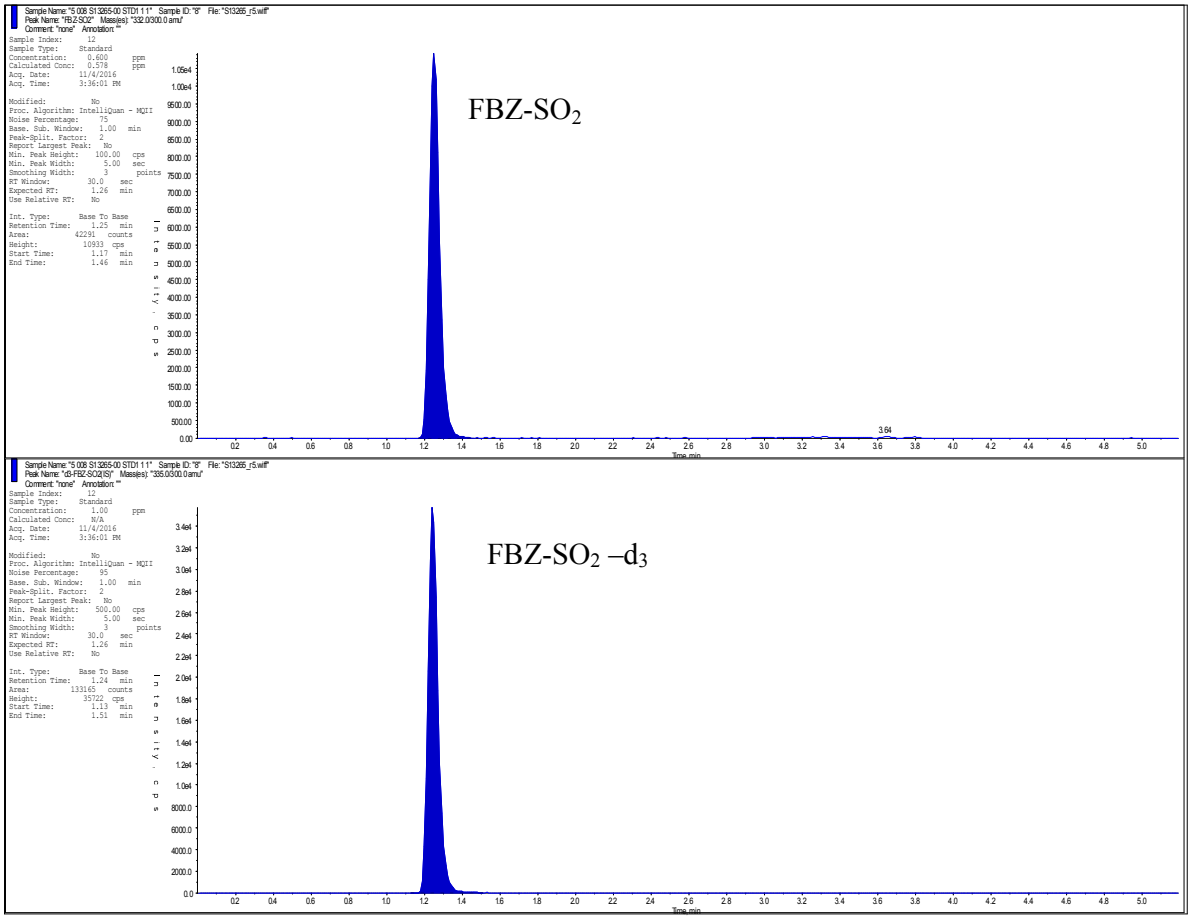
20.2.1. Representative LC-MS/MS chromatogram of double blank matrix sample



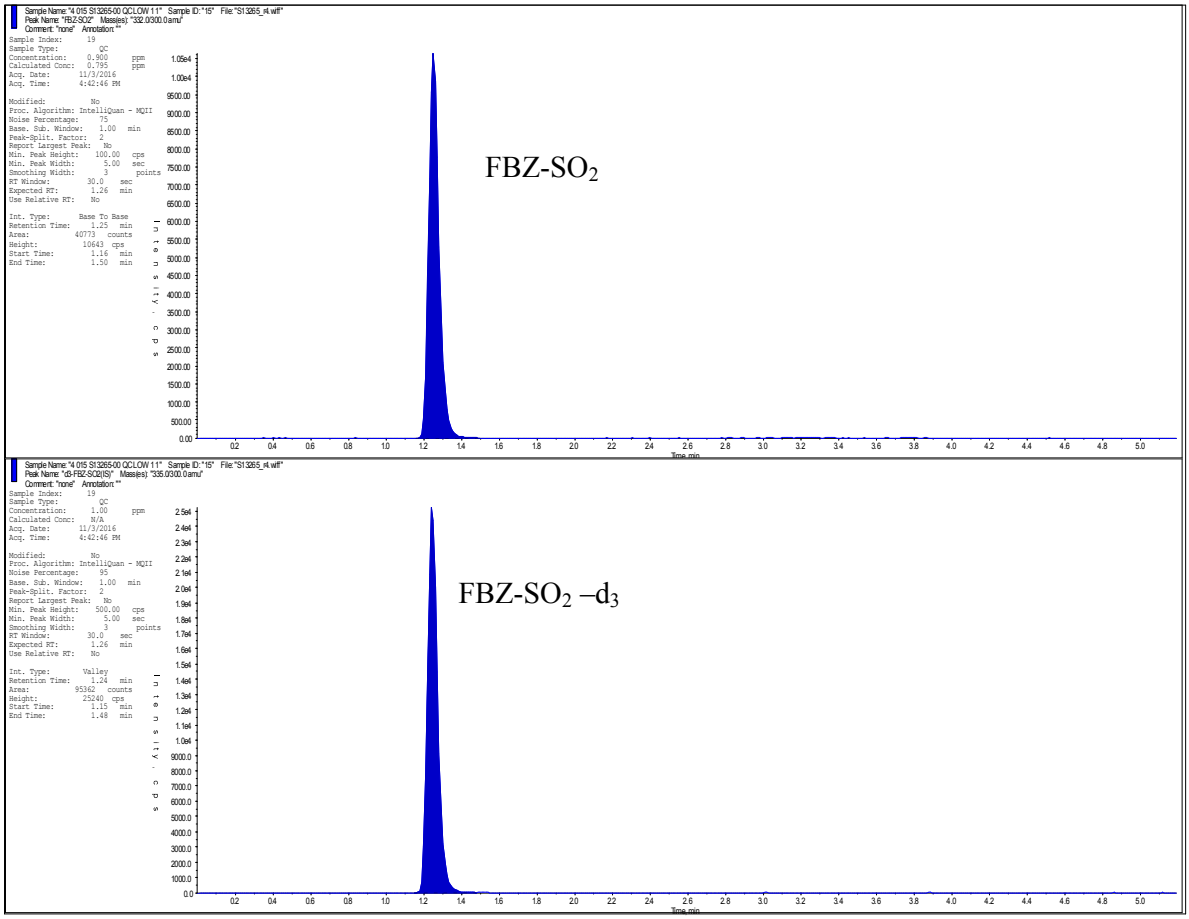
20.2.2. Representative LC-MS/MS chromatogram of control blank sample



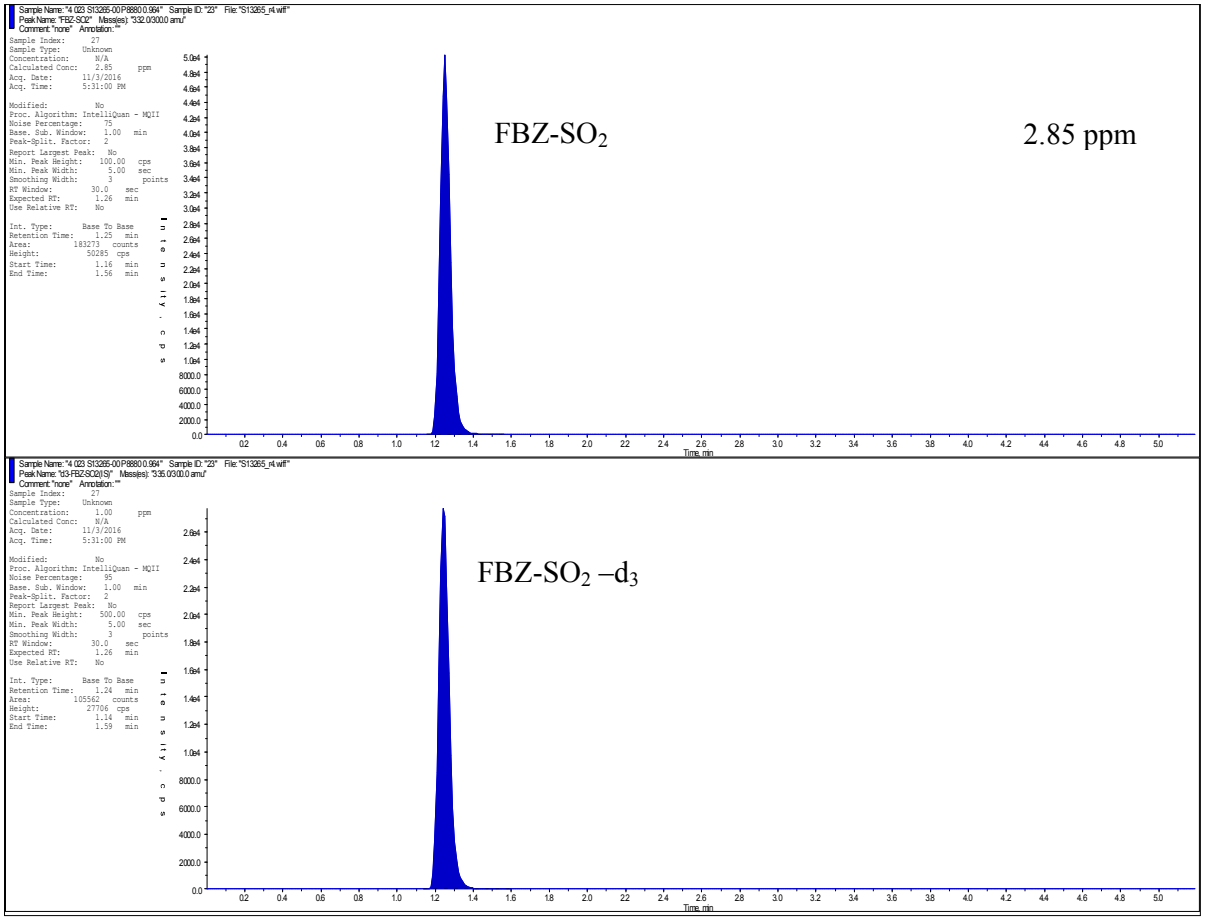
20.2.3. Representative LC-MS/MS chromatogram of solvent calibration standard of fenbendazole sulfone at 0.600 ppm eq or 1.5 ng/mL



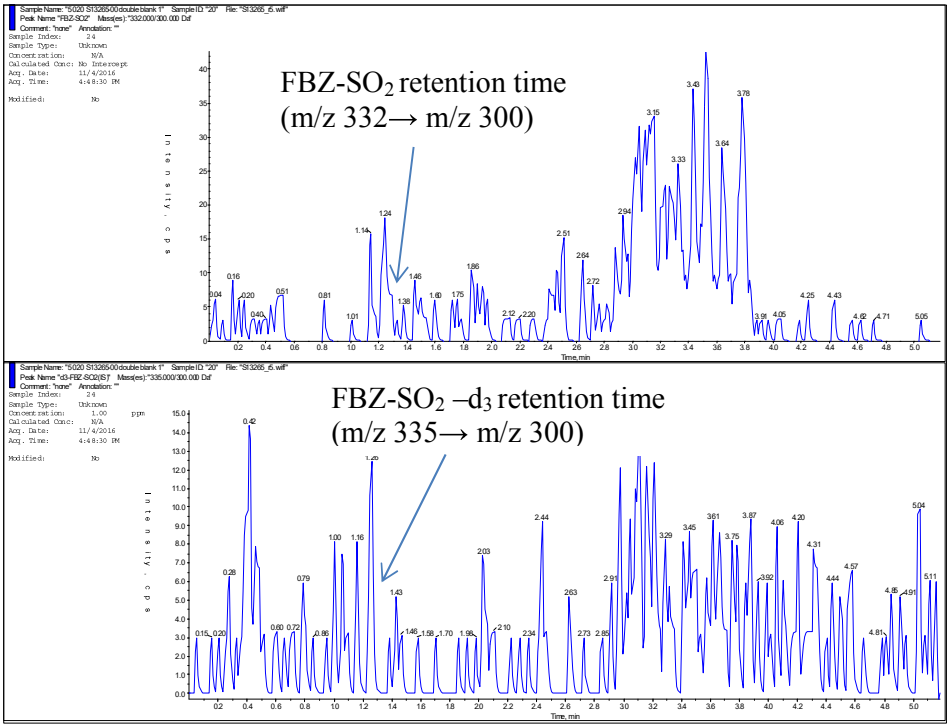
20.2.4. Representative LC-MS/MS chromatogram of control matrix sample fortified with fenbendazole sulfone at the ½ X tolerance (*i.e.* 0.900 ppm)

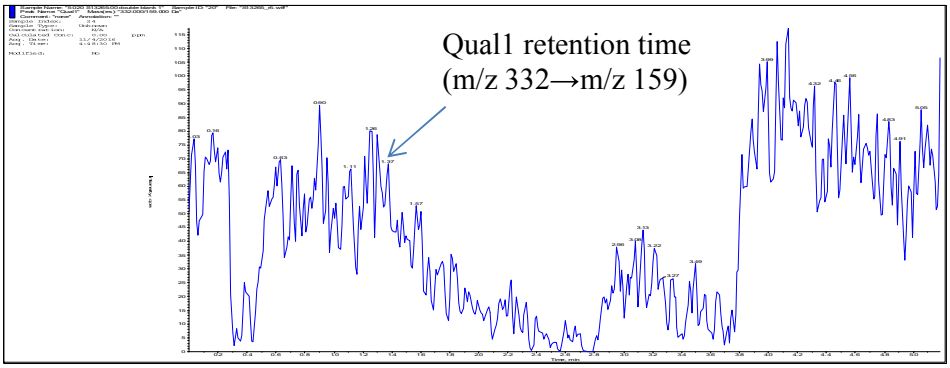


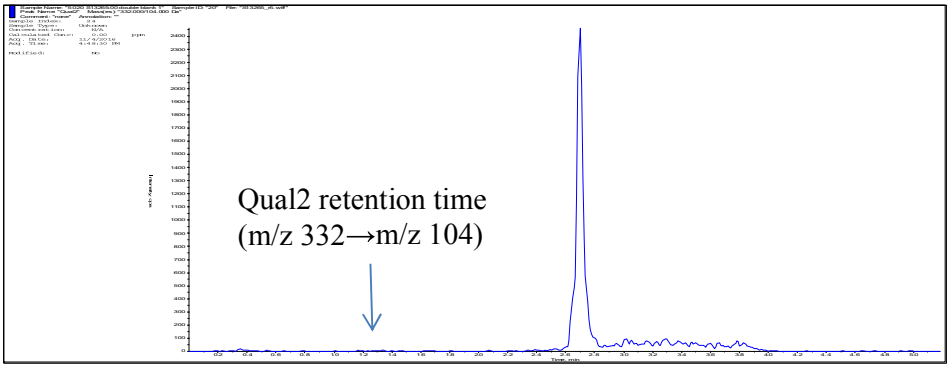
20.2.5. Representative LC-MS/MS chromatogram of incurred sample



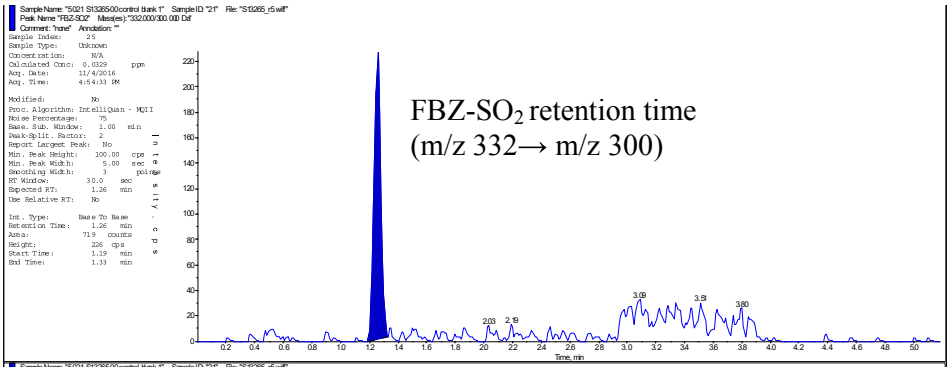
20.2.6. Representative LC-MS/MS chromatogram of double blank matrix sample obtained for confirmatory analysis

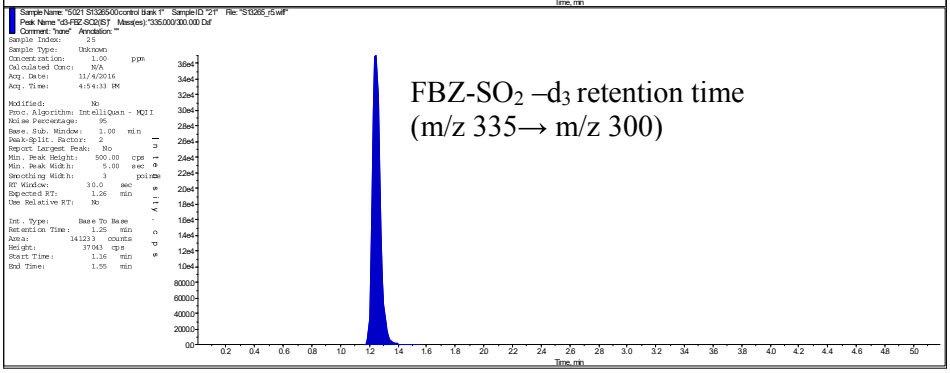


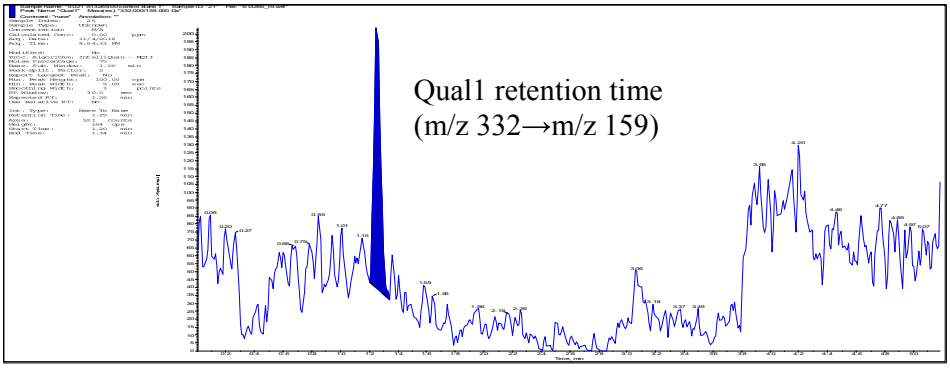


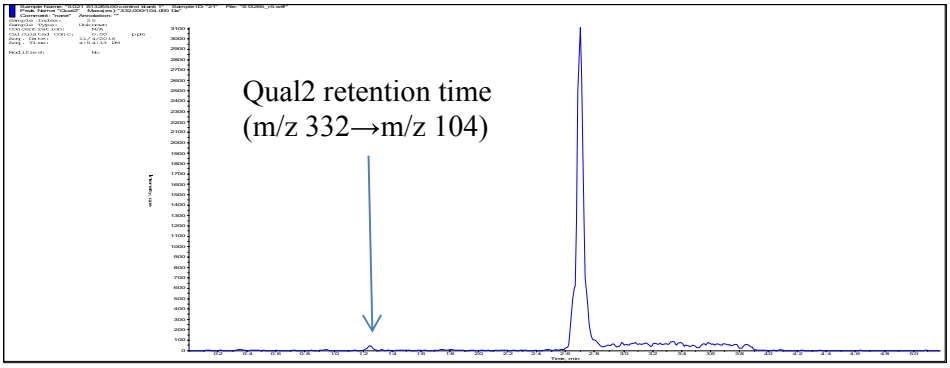


20.2.7. Representative LC-MS/MS chromatogram of control blank sample obtained for confirmatory analysis

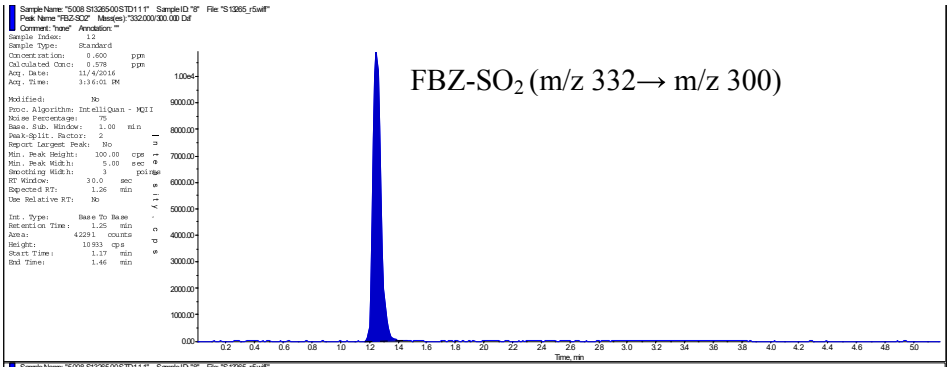


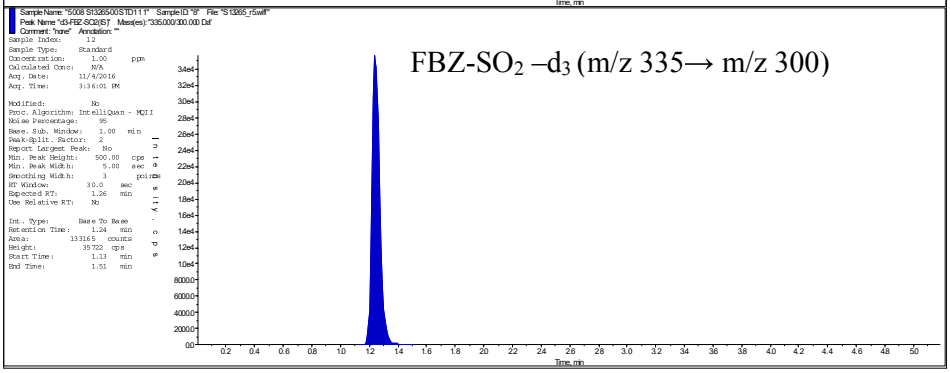


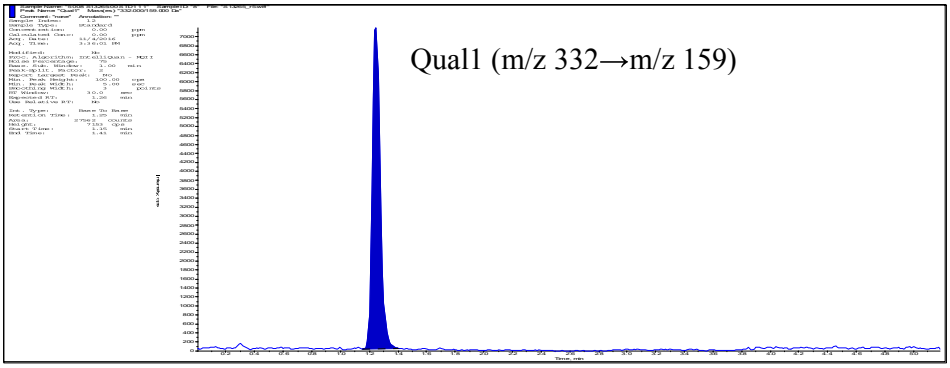


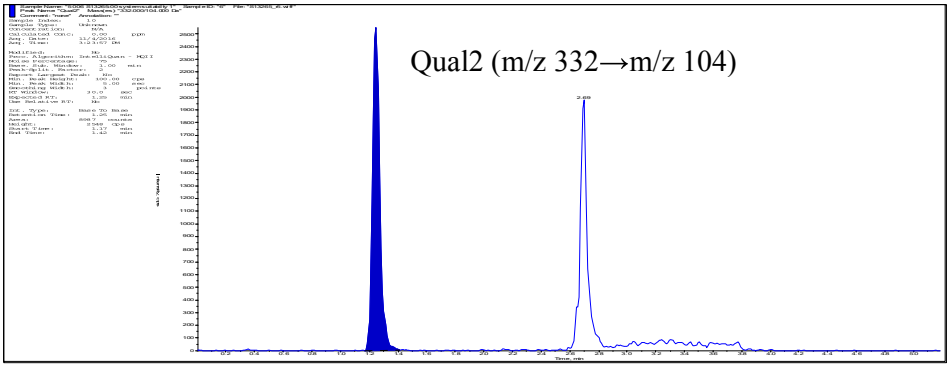


20.2.8. Representative LC-MS/MS chromatogram of solvent calibration standard of fenbendazole sulfone at 0.600 ppm eq. (1.5 ng/mL), obtained for confirmatory analysis

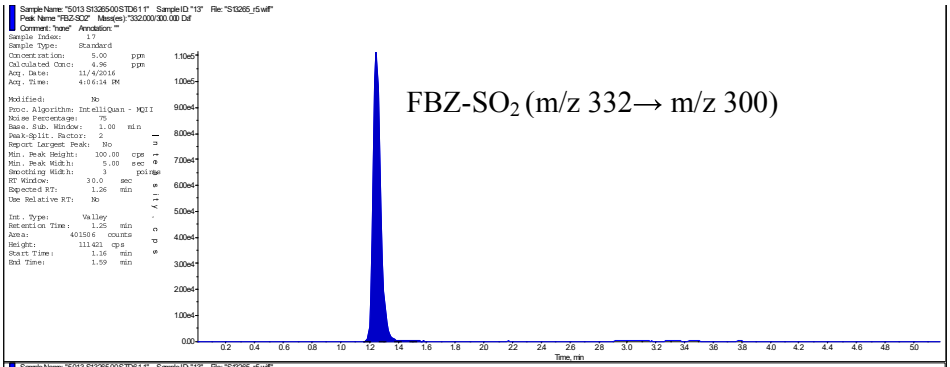


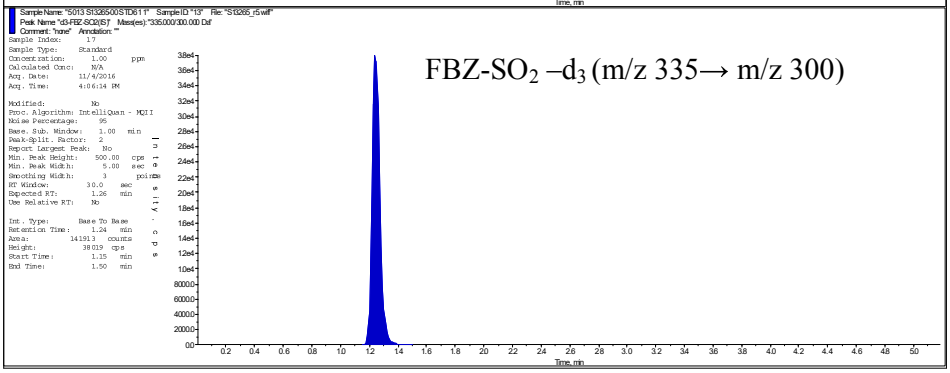


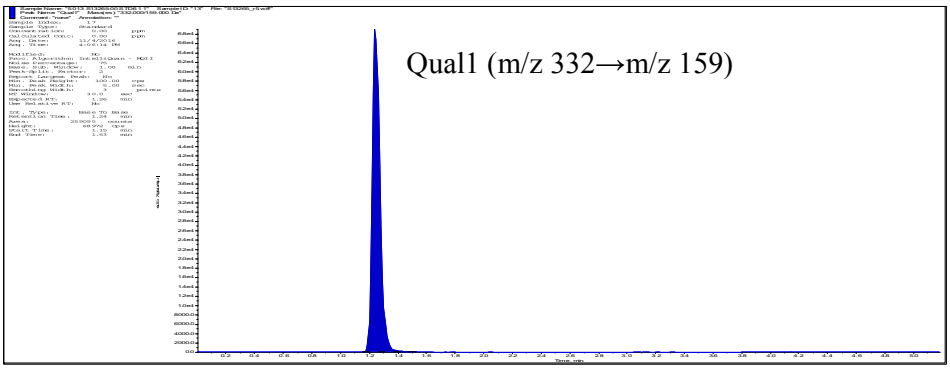


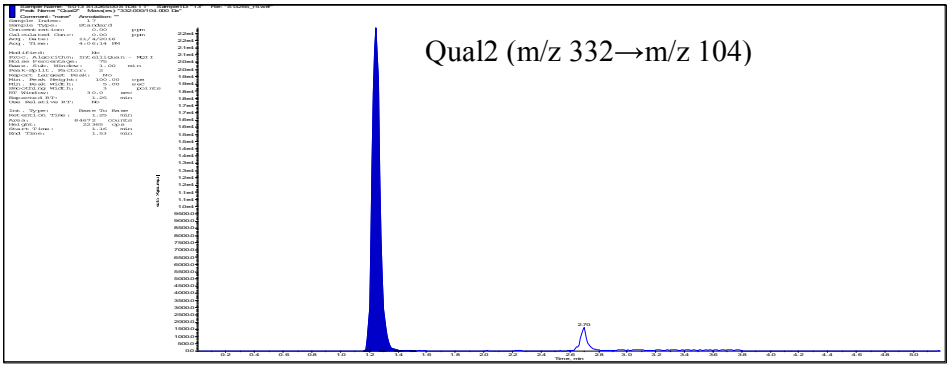


20.2.9. Representative LC-MS/MS chromatogram of solvent calibration standard of fenbendazole sulfone at 5.00 ppm eq. (12.5 ng/mL), obtained for confirmatory analysis

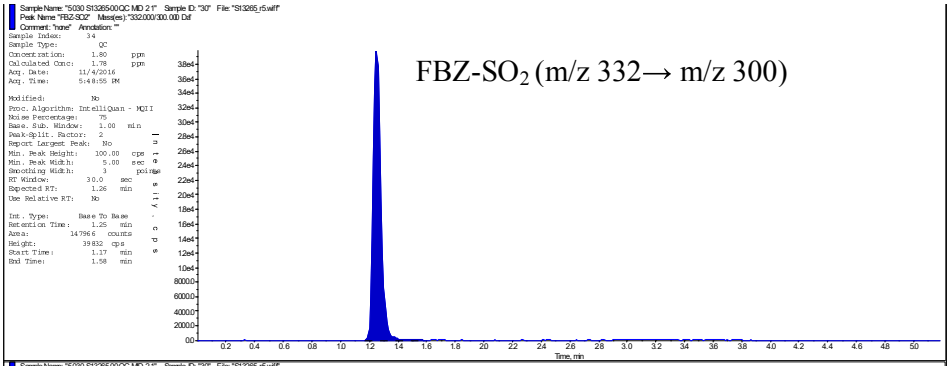


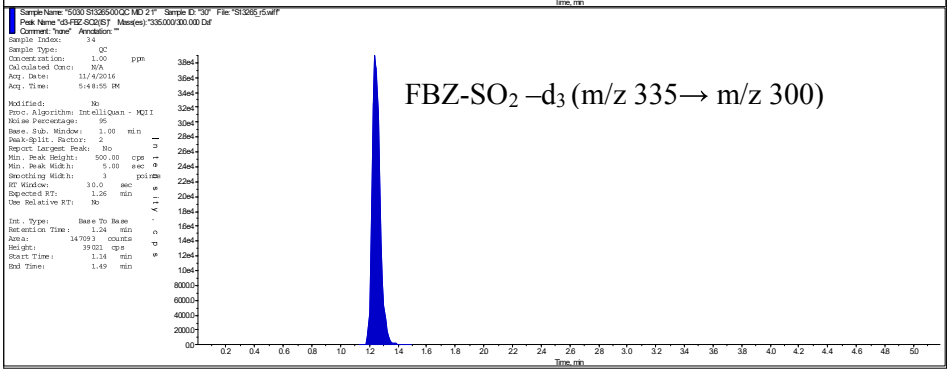


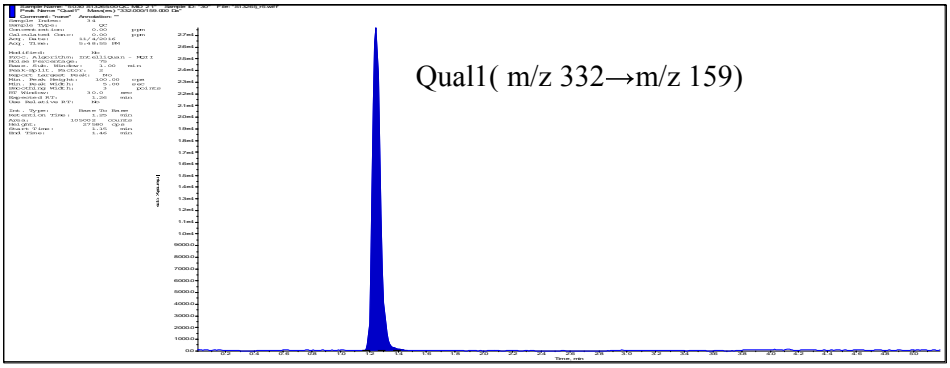


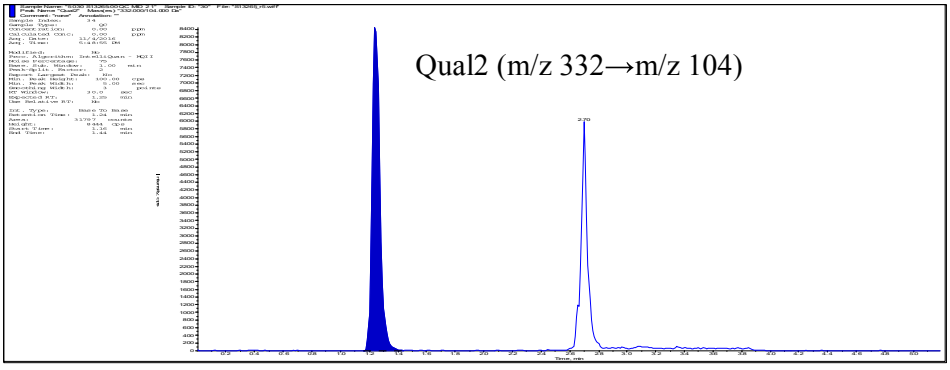


20.2.10. Representative LC-MS/MS chromatogram of control matrix sample fortified with fenbendazole sulfone at tolerance (*i.e.* 1.80 ppm), obtained for confirmatory analysis

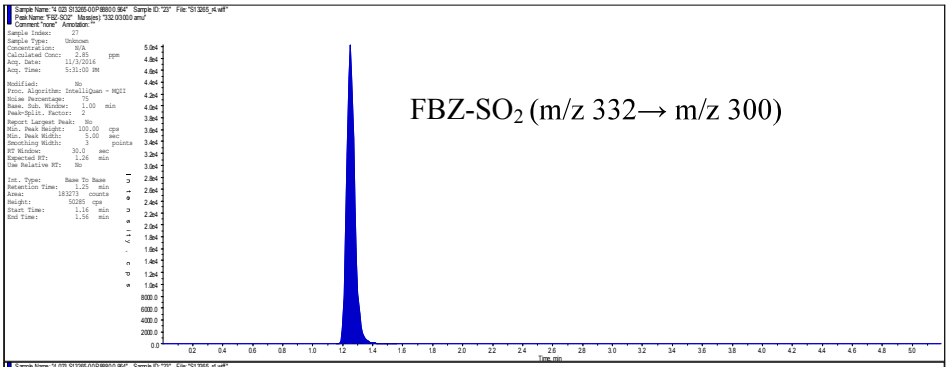


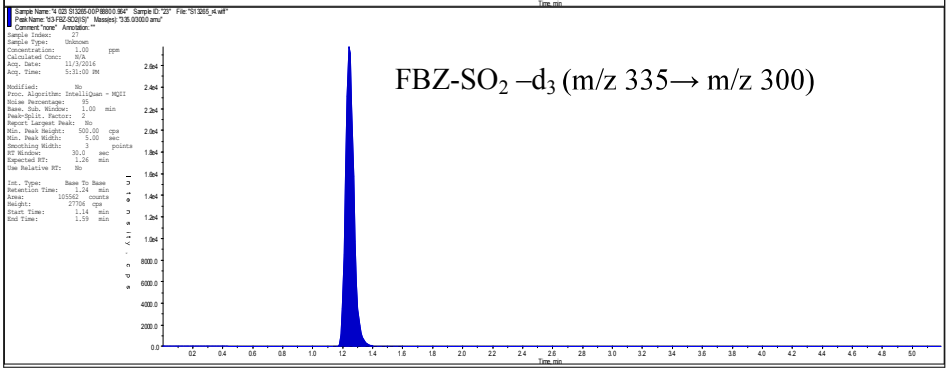


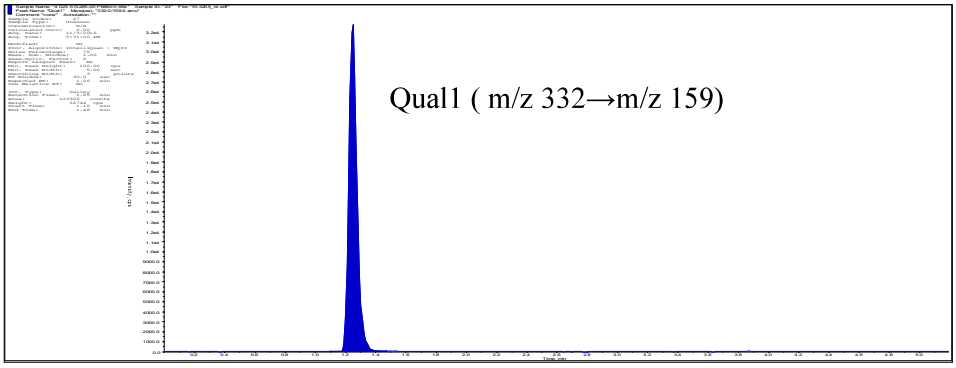


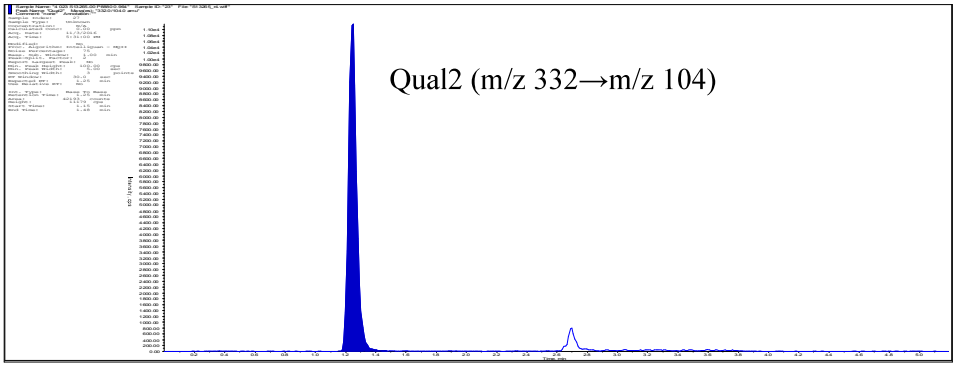


20.2.11. Representative LC-MS/MS chromatogram of incurred sample, obtained for confirmatory analysis

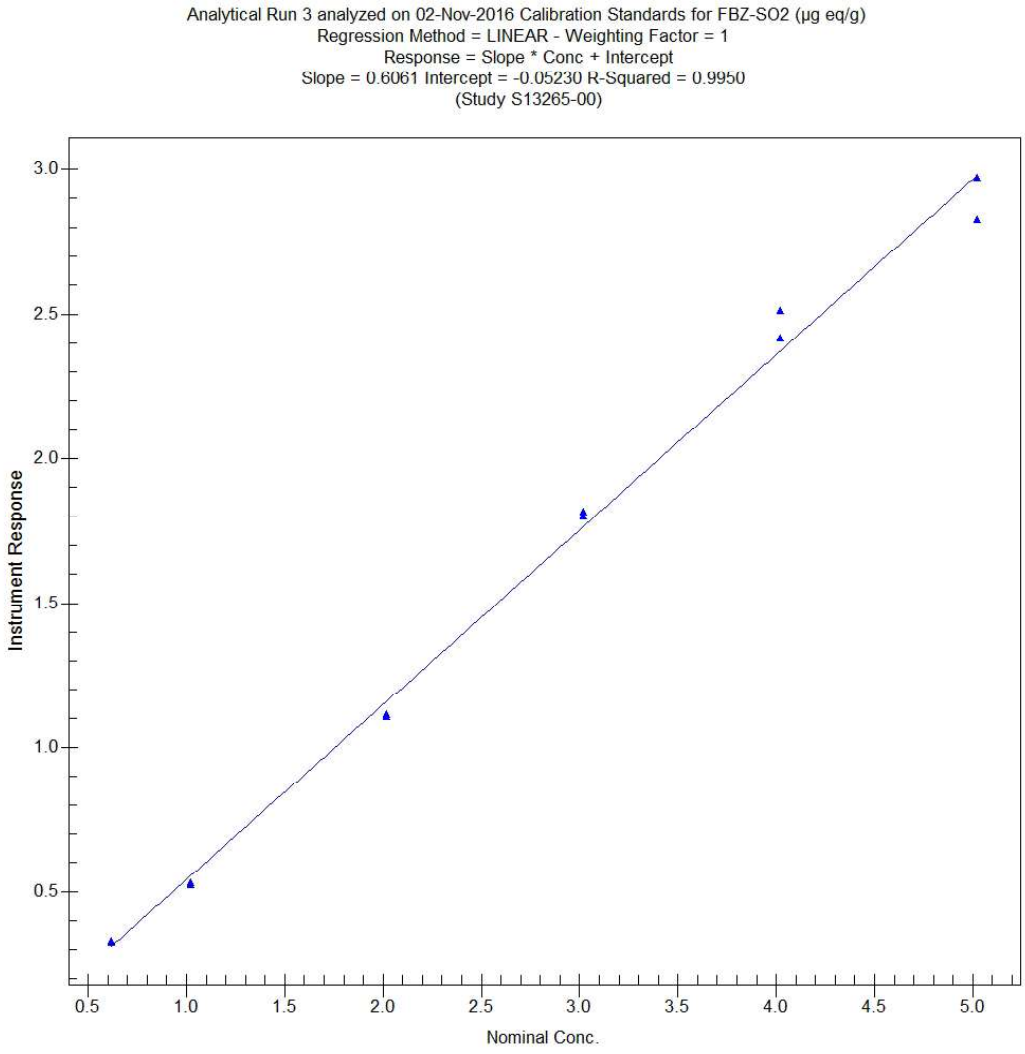








20.3. Representative calibration curve obtained for fenbendazole sulfone solvent calibration standards from 0.600 ppm to 5.00 ppm



21. VALIDATION DATA SUMMARY

21.1. Determinative Procedure

Standard curve linearity

Standard curve range	0.600 to 6.40 ppm (nominal concentration 1.50 to 16.0 ng/mL)		
Run	Slope	Intercept	Coefficient of correlation
1	0.702	0.0792	0.9938
2	0.641	0.0349	0.9970
3	0.632	-0.00918	0.9932

Accuracy and precision	Spiked level	Conc. (ppm)	%CV	Mean %Recovery	Incurred level	Conc. (ppm)	%CV
Between run (n=15)	Low Std	0.600	5.5	97.2	Low	1.480	4.9
	Low	1.600	5.9	97.4	High	2.861	3.0
	Mid	3.200	6.5	101.2			
	High	6.400	7.9	104.0			
Within run (n=5)	Low Std	0.600	4.1 – 5.5	93.5 – 99.5	Low	1.425 – 1.532	2.0 – 6.0
	Low	1.600	1.9 – 7.5	93.3 – 102.8	High	2.806 – 2.902	1.3 – 4.5
	Mid	3.200	4.9 – 6.6	96.9 – 104.8			
	High	6.400	4.9 – 7.3	95.8 – 108.3			
	Dil	32.000	7.1	101.5			
Acceptance criteria			≤ 10	80 - 110			≤ 10

Limit of Detection (LOD): 0.049 ppm

Limit of Quantification (LOQ): 0.148 ppm

Specificity, carry-over & selectivity: No significant interference from 6 different sources of chicken egg was observed. No carry-over was observed. No significant interference from tested veterinary drugs (fenbendazole, oxfendazole, amprolium, bacitracin, chlortetracycline, erythromycin, tylosin) and from IS was observed.

Matrix effect & recovery: Matrix effects analysis indicate no consistent or significant matrix effects in either the analyte or the internal standard. Additionally, the degree of matrix effects in analyte and internal standard were comparable (matrix effect ranging from -3.9 % to 6.9 %). Method recovery averaged >99 % for both analyte and IS (exhaustive extraction of the method was verified).

Ruggedness: The robustness of the method (chromatographic conditions) was proved by using different LC-MS/MS systems and by using different batches of the column Ace 3 C18 (MacMod) but not by switching to an equivalent column (Acclaim C18 - Thermo Electron Corporation - 50 x 2.1 mm, 3.0 µm). The injection volume must be adapted according to the sensitivity of the LC-MS/MS system, within a range from 2 µL to 20 µL.

Stability in solvent: The DMSO stock solutions of fenbendazole sulfone and IS are stable for at least 13 days at *approx.* -20°C and for at least 5 and 28 days, respectively, at room temperature. The methanolic working solutions of fenbendazole sulfone and IS are stable for at least 22 and 41 days, respectively, at *approx.* -20°C and for at least 9 and 28 days, respectively, at room temperature.

Stability in solvent calibration standard: Solvent calibration standards are stable for at least 9 days at *approx.* +5°C.

Stability in final matrix extract: Final matrix extracts are stable for at least 98 hours at room temp. and an analytical sequence can be totally re-injected within 96 hours if final extracts are stored at room temp.

Stability in chicken egg homogenate: Fenbendazole sulfone is stable in egg homogenate for at least 40 hours at room temp. for at least 3 successive freeze (at *approx.* -20°C or -80°C)/thaw (at room temperature) cycles, for at least 371 days at *approx.* -20°C and for at least 376 days at *approx.* -80°C.

21.2. Method Trial Determinative Procedure Data Summary

21.2.1. Summary of Determinative Results for Fortified and Control Samples in the Reference Laboratory

Run ID	Sample ID	Nominal Conc. (ppm)	Ret Time (min)	Area	IS Area	Peak Area Ratio	Conc. Found (ppm)	%Rec
3	Double Blank	0	N/A	0	0	N/A	N/A	N/A
3	Double Blank	0	N/A	0	0	N/A	N/A	N/A
4	Double Blank	0	N/A	0	0	N/A	N/A	N/A
4	Double Blank	0	N/A	0	0	N/A	N/A	N/A
5	Double Blank	0	N/A	0	0	N/A	N/A	N/A
5	Double Blank	0	N/A	0	0	N/A	N/A	N/A
3	Control Blank	0	1.26	456	93326	0.00489	BLQ (0.0943)	N/A
3	Control Blank	0	N/A	0	106320	0	BLQ (0.0863)	N/A
4	Control Blank	0	N/A	0	91990	0	BLQ (0.0885)	N/A
4	Control Blank	0	N/A	0	91621	0	BLQ (0.0885)	N/A
5	Control Blank	0	1.26	719	141233	0.00509	BLQ (0.0329)	N/A
5	Control Blank	0	1.25	600	133593	0.00449	BLQ (0.0319)	N/A
3	QC-Low	0.900	1.26	45120	95927	0.47036	0.862	95.8
3	QC-Low	0.900	1.25	44755	92280	0.48499	0.886	98.4
4	QC-Low	0.900	1.25	40773	95362	0.42756	0.795	88.3
4	QC-Low	0.900	1.25	41552	88466	0.46970	0.864	96.0
5	QC-Low	0.900	1.25	68370	140899	0.48524	0.871	96.8
5	QC-Low	0.900	1.25	74545	150165	0.49642	0.890	98.9
							mean	95.7
							%CV	4.0%
3	QC-Mid	1.80	1.26	101130	105016	0.96300	1.68	93.3
3	QC-Mid	1.80	1.25	98040	101432	0.96656	1.68	93.3
4	QC-Mid	1.80	1.25	100440	101672	0.98788	1.72	95.6
4	QC-Mid	1.80	1.25	97236	96974	1.00270	1.74	96.7
5	QC-Mid	1.80	1.25	139425	146710	0.95034	1.68	93.3
5	QC-Mid	1.80	1.25	147966	147093	1.00594	1.78	98.9
							mean	95.0
							%CV	2.4%
3	QC-High	3.60	1.26	203253	105513	1.92633	3.26	91.6
3	QC-High	3.60	1.25	178086	94234	1.88983	3.20	88.9
4	QC-High	3.60	1.25	183684	90082	2.03908	3.46	96.1
4	QC-High	3.60	1.25	194055	92430	2.09948	3.56	98.9
5	QC-High	3.60	1.25	287101	140206	2.04771	3.60	100.0
5	QC-High	3.60	1.25	299652	149372	2.00608	3.52	97.8
							mean	95.3
							%CV	4.8%

21.2.2. Results for the Analysis of Blinded Egg Samples in the Reference Laboratory

Sample ID	Sample Weight (g)	Retention Time (min)	FBZ-SO_2 Peak Area	FBZ-SO_2-d_3 Peak Area	Peak Area Ratio	FBZ-SO_2 Conc. (ppm)
P4272	1.04	0	0	95590	0	BLQ
P1177	0.97	1.25	419	91533	0.004578	BLQ
P7584	1.005	0	0	96887	0	BLQ
P1870	0.973	1.25	457	147818	0.003092	BLQ
P1661	1.032	1.24	660	141001	0.004681	BLQ
					Average	**NA**
					% CV	**NA**
P3694	0.986	1.25	62047	93646	0.662570	1.20
P6008	0.983	1.25	67283	99399	0.676898	1.22
P6530	0.99	1.25	62967	95943	0.656296	1.18
P1794	0.966	1.25	94806	153738	0.616673	1.14
P7010	1.033	1.25	98265	149322	0.658074	1.13
					Average	**1.17**
					% CV	**3.3**
P4663	1.007	1.26	170832	94083	1.815758	3.06
P6875	0.998	1.25	189486	106093	1.786037	3.04
P8880	1.037	1.25	183273	105562	1.736165	2.85
P5974	1.016	1.25	159072	90022	1.767035	2.96
P8075	0.99	1.25	248580	139999	1.775584	3.15
					Average	**3.01**
					% CV	**3.8**

21.2.3. Results for the Analysis of Blinded Egg Samples at Testing Laboratory 1

Sample ID	Sample Weight (g)	FBZ-SO_2 RT (min)	FBZ-SO_2 Peak Area	FBZ-SO_2-d_3 Peak Area	Peak Area Ratio	Calculated Concentration (ppm)	FBZ-SO_2 Concentration (ppm)	% Difference from MAH GPD Concentration
P-1803	0.996	NA	0	110665	NA	BLOQ	BLOQ	
P-7443	0.969	NA	0	94731	NA	BLOQ	BLOQ	
P-5558	0.986	NA	0	97117	NA	BLOQ	BLOQ	
P-9332	0.966	NA	0	104381	NA	BLOQ	BLOQ	
P-8206	1.008	NA	0	101342	NA	BLOQ	BLOQ	
FDA-1						**Average**	**NA**	**NA**
						%CV	**NA**	
P-3835	1.028	1.35	64644	109416	0.591	1.10	1.07	
P-3620	0.976	1.34	59984	110007	0.545	1.01	1.03	
P-4339	0.982	1.33	61329	112291	0.546	1.02	1.04	
P-9657	1.012	1.33	54766	94342	0.581	1.09	1.08	
P-7066	0.984	1.4	57369	103269	0.556	1.05	1.07	
FDA-2						**Average**	**1.06**	**-9.4%**
						%CV	**2.05**	
P-5687	1.017	1.29	162054	102928	1.57	2.99	2.94	
P-6662	0.999	1.32	144814	96454	1.5	2.90	2.90	
P-4326	1.002	1.33	146392	96176	1.52	2.94	2.93	
P-8355	1.008	1.39	156997	103717	1.51	2.92	2.90	
P-3921	0.993	1.38	152808	101352	1.51	2.91	2.93	
FDA-3						**Average**	**2.92**	**-3.0%**
						%CV	**0.64**	

21.2.4. Results for the Analysis of Blinded Egg Samples at Testing Laboratory 2

							$FBZ\text{-}SO_2$	% Difference
Sample ID	**Sample**	**RT**	**$FBZ\text{-}SO_2$**	**$FBZ\text{-}SO_2\text{-}d_3$**	**Peak**	**Calc. Conc.**	**Conc.**	**from MAH GPD**
	Weight (g)	**(min)**	**Area**	**Area**	**Area Ratio**	**(ppm)**	**(ppm)**	**Concentration**
P4575	1.033	0	0	91488	0	BLQ	BLQ	**NA**
P4374	0.988	0	0	93328	0	BLQ	BLQ	
P5616	0.999	0	0	97274	0	BLQ	BLQ	
P1460	1.021	0	0	92168	0	BLQ	BLQ	
P7180	0.972	0	0	90769	0	BLQ	BLQ	
						Average	**NA**	**NA**
			FDA-1			**% CV**	**NA**	
P5408	0.962	1.39	56264	96751	0.582	1.11	1.15	
P5082	0.978	1.39	56866	92682	0.614	1.17	1.20	
P2962	1.013	1.39	56987	95332	0.598	1.15	1.14	
P6823	1.022	1.39	60645	95138	0.637	1.22	1.19	
P4194	0.957	1.4	53952	96747	0.558	1.08	1.13	
						Average	**1.16**	**-0.85%**
			FDA-2			**% CV**	**2.68**	
P6947	0.957	1.39	151456	95517	1.59	3.06	3.20	
P1388	0.964	1.39	149210	94757	1.57	3.02	3.13	
P9268	1.038	1.39	160888	94422	1.7	3.26	3.14	
P7835	0.974	1.4	150480	94039	1.6	3.06	3.14	
P9813	0.957	1.4	150146	96634	1.55	2.97	3.10	
						Average	**3.14**	**4.3%**
			FDA-3			**% CV**	**1.16**	

21.3. Reference Lab Method Trial Confirmatory Procedure Data Summary

Summary Confirmatory Results for Standards											
Run ID	Sample ID	m/z 300 Peak Area	m/z 159 Peak Area	m/z 104 Peak Area	RAPAR*		Retention Time (min)			S/N Ratio (> 50)	
					m/z 159 Individual	m/z 104 Individual	m/z 300 Individual	m/z 159 Individual	m/z 104 Individual	m/z 159 Individual	m/z 104 Individual
3	3 008 S13265-00 STD1 1 1	31576	21216	7260	67.2	23.0	1.26	1.26	1.25	1020	705
	3 009 S13265-00 STD2 1 1	52926	37470	12960	70.8	24.5	1.26	1.26	1.25	1200	920
	3 010 S13265-00 STD3 1 1	101053	71761	23863	71.0	23.6	1.26	1.26	1.25	2370	2240
	3 011 S13265-00 STD4 1 1	184949	128019	43452	69.2	23.5	1.26	1.26	1.25	4110	4480
	3 012 S13265-00 STD5 1 1	217583	146368	49622	67.3	22.8	1.26	1.26	1.26	4780	2770
	3 013 S13265-00 STD6 1 1	416203	287828	98202	69.2	23.6	1.26	1.25	1.25	8320	3940
	3 033 S13265-00 STD1 2 1	31302	22151	7271	70.8	23.2	1.25	1.25	1.24	817	448
	3 034 S13265-00 STD2 2 1	53074	37581	13058	70.8	24.6	1.25	1.25	1.25	1150	840
	3 035 S13265-00 STD3 2 1	102189	70064	23962	68.6	23.4	1.26	1.25	1.25	3070	1100
	3 036 S13265-00 STD4 2 1	184201	124734	42941	67.7	23.3	1.25	1.25	1.25	4100	2890
	3 037 S13265-00 STD5 2 1	207088	148358	47480	71.6	22.9	1.25	1.25	1.25	4510	2560
	3 038 S13265-00 STD6 2 1	424928	286322	94636	67.4	22.3	1.25	1.25	1.25	9150	8750
				Average:	**69.3**	**23.4**	**1.26**	**1.25**	**1.25**	NA	
4	4 008 S13265-00 STD1 1 1	28974	20649	7094	71.3	24.5	1.25	1.25	1.24	585	888
	4 009 S13265-00 STD2 1 1	51175	35351	11912	69.1	23.3	1.25	1.25	1.24	1010	1080
	4 010 S13265-00 STD3 1 1	96644	68783	22929	71.2	23.7	1.25	1.25	1.24	2780	1790
	4 011 S13265-00 STD4 1 1	173247	120298	41286	69.4	23.8	1.25	1.25	1.24	3940	2320
	4 012 S13265-00 STD5 1 1	206606	141518	48438	68.5	23.4	1.25	1.25	1.25	6150	3320
	4 013 S13265-00 STD6 1 1	275150	188987	62340	68.7	22.7	1.25	1.25	1.25	8050	3610
	4 033 S13265-00 STD1 2 1	29814	20773	6897	69.7	23.1	1.25	1.24	1.24	933	650
	4 034 S13265-00 STD2 2 1	49433	34360	11826	69.5	23.9	1.25	1.24	1.24	860	601
	4 035 S13265-00 STD3 2 1	92900	65514	22474	70.5	24.2	1.25	1.24	1.24	3060	1350
	[illegible]13265-00 STD4 2 1	175376	120745	40181	68.8	22.9	1.25	1.24	1.24	4640	3250

Pr Proprietary

Summary Confirmatory Results for Standards											
Run ID	Sample ID	m/z 300 Peak Area	m/z 159 Peak Area	m/z 104 Peak Area	RAPAR*		Retention Time (min)			S/N Ratio (> 50)	
					m/z 159 Individual	m/z 104 Individual	m/z 300 Individual	m/z 159 Individual	m/z 104 Individual	m/z 159 Individual	m/z 104 Individual
	4 037 S13265-00 STD5 2 1	200944	140921	46584	70.1	23.2	1.25	1.25	1.24	4840	3680
	4 038 S13265-00 STD6 2 1	266475	185975	62423	69.8	23.4	1.25	1.24	1.24	3960	3450
				Average:	**69.7**	**23.5**	**1.25**	**1.25**	**1.24**	NA	
5	5 008 S13265-00 STD1 1 1	42291	27562	8805	65.2	20.8	1.25	1.25	1.24	651	2060
	5 009 S13265-00 STD2 1 1	74235	48819	15501	65.8	20.9	1.25	1.25	1.24	1380	2470
	5 010 S13265-00 STD3 1 1	152785	100119	32408	65.5	21.2	1.25	1.24	1.24	2110	10200
	5 011 S13265-00 STD4 1 1	242218	155241	53213	64.1	22.0	1.25	1.25	1.24	2590	7970
	5 012 S13265-00 STD5 1 1	318358	217783	69020	68.4	21.7	1.25	1.25	1.24	4490	15900
	5 013 S13265-00 STD6 1 1	401506	259095	84672	64.5	21.1	1.25	1.24	1.25	5610	9790
	5 033 S13265-00 STD1 2 1	46605	31157	9930	66.9	21.3	1.25	1.25	1.24	592	1600
	5 034 S13265-00 STD2 2 1	78475	51825	16963	66.0	21.6	1.25	1.25	1.25	1200	2550
	5 035 S13265-00 STD3 2 1	161623	105552	33789	65.3	20.9	1.25	1.25	1.24	2300	5190
	5 036 S13265-00 STD4 2 1	244904	172687	59354	70.5	24.2	1.25	1.25	1.25	3920	18900
	5 037 S13265-00 STD5 2 1	325218	221990	76956	68.3	23.7	1.25	1.25	1.25	5930	16400
	5 038 S13265-00 STD6 2 1	410799	275175	90014	67.0	21.9	1.25	1.25	1.25	6450	28000
				Average:	**66.5**	**21.8**	**1.25**	**1.25**	**1.24**	NA	

*RAPAR: Relative Abundance Peak Area Ratio to m/z 300

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Summary Confirmatory Results for QCs and Confirming Blinded Samples														
Run ID	Sample ID	Relative Abundance Peak Area Ratio to m/z 300				Retention Time (min)						S/N Ratio (> 50)	
		m/z 159		m/z 104		m/z 300		m/z 159		m/z 104		m/z 159	m/z 104
		Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Individual
3	3 015 S13265-00 QC LOW 1 1	66.7	59.3-79.3	23.6	13.4-33.4	1.26	1.20-1.32	1.25	1.19-1.31	1.25	1.19-1.31	751	737
	3 029 S13265-00 QC LOW 2 1	70.6	59.3-79.3	23.1	13.4-33.4	1.25	1.20-1.32	1.25	1.19-1.31	1.24	1.19-1.31	1170	1090
4	4 015 S13265-00 QC LOW 1 1	70.2	59.7-79.7	24.0	13.5-33.5	1.25	1.19-1.31	1.25	1.19-1.31	1.24	1.18-1.30	1210	530
	4 029 S13265-00 QC LOW 2 1	72.4	59.7-79.7	23.0	13.5-33.5	1.25	1.19-1.31	1.25	1.19-1.31	1.24	1.18-1.30	984	791
5	5 015 S13265-00 QC LOW 1 1	65.2	56.5-76.5	22.4	11.8-31.8	1.25	1.19-1.31	1.25	1.19-1.31	1.24	1.18-1.30	590	4010
	5 029 S13265-00 QC LOW 2 1	65.3	56.5-76.5	20.9	11.8-31.8	1.25	1.19-1.31	1.24	1.19-1.31	1.24	1.18-1.30	685	4860
3	3 016 S13265-00 QC MID 1 1	71.0	59.3-79.3	23.3	13.4-33.4	1.26	1.20-1.32	1.25	1.19-1.31	1.25	1.19-1.31	1890	1860
	3 030 S13265-00 QC MID 2 1	71.6	59.3-79.3	23.9	13.4-33.4	1.25	1.20-1.32	1.25	1.19-1.31	1.25	1.19-1.31	2600	1520
4	4 016 S13265-00 QC MID 1 1	69.1	59.7-79.7	24.2	13.5-33.5	1.25	1.19-1.31	1.25	1.19-1.31	1.24	1.18-1.30	2220	2180
	4 030 S13265-00 QC MID 2 1	70.7	59.7-79.7	24.0	13.5-33.5	1.25	1.19-1.31	1.25	1.19-1.31	1.25	1.18-1.30	2380	1560
5	5 016 S13265-00 QC MID 1 1	67.9	56.5-76.5	22.5	11.8-31.8	1.25	1.19-1.31	1.24	1.19-1.31	1.25	1.18-1.30	1710	5410
	5 030 S13265-00 QC MID 2 1	71.0	56.5-76.5	21.5	11.8-31.8	1.25	1.19-1.31	1.25	1.19-1.31	1.24	1.18-1.30	1680	3600
3	3 017 S13265-00 QC HIGH 1 1	68.9	59.3-79.3	23.3	13.4-33.4	1.26	1.20-1.32	1.25	1.19-1.31	1.25	1.19-1.31	5630	3220
	3 031 S13265-00 QC HIGH 2 1	69.3	59.3-79.3	23.2	13.4-33.4	1.25	1.20-1.32	1.25	1.19-1.31	1.25	1.19-1.31	4190	2280
4	4 017 S13265-00 QC HIGH 1 1	70.8	59.7-79.7	23.3	13.5-33.5	1.25	1.19-1.31	1.25	1.19-1.31	1.25	1.18-1.30	5000	3560
	4 031 S13265-00 QC HIGH 2 1	66.7	59.7-79.7	22.7	13.5-33.5	1.25	1.19-1.31	1.24	1.19-1.31	1.24	1.18-1.30	7360	3300
5	5 017 S13265-00 QC HIGH 1 1	65.1	56.5-76.5	21.5	11.8-31.8	1.25	1.19-1.31	1.25	1.19-1.31	1.24	1.18-1.30	2770	8590
	5 031 S13265-00 QC HIGH 2 1	67.1	56.5-76.5	22.4	11.8-31.8	1.25	1.19-1.31	1.25	1.19-1.31	1.24	1.18-1.30	3470	7660
3	3 023 S13265-00 P4663 0.993	69.4	59.3-79.3	23.1	13.4-33.4	1.26	1.20-1.32	1.25	1.19-1.31	1.25	1.19-1.31	5190	2480
	3 024 S13265-00 P6875 1.002	68.7	59.3-79.3	22.6	13.4-33.4	1.25	1.20-1.32	1.25	1.19-1.31	1.25	1.19-1.31	4990	3940
	3 025 S13265-00 P3694 1.014	70.2	59.3-79.3	22.4	13.4-33.4	1.25	1.20-1.32	1.25	1.19-1.31	1.25	1.19-1.31	1650	1640
	3 026 S13265-00 P6008 1.017	69.1	59.3-79.3	22.6	13.4-33.4	1.25	1.20-1.32	1.25	1.19-1.31	1.24	1.19-1.31	2450	815
4	4 023 S13265-00 P8880 0.964	67.6	59.7-79.7	23.0	13.5-33.5	1.25	1.19-1.31	1.25	1.19-1.31	1.25	1.18-1.30	3380	2380
	4 024 S13265-00 P5974 0.984	69.0	59.7-79.7	24.0	13.5-33.5	1.25	1.19-1.31	1.25	1.19-1.31	1.25	1.18-1.30	3480	3140
	4 025 S13265-00 P6530 1.01	68.7	59.7-79.7	24.5	13.5-33.5	1.25	1.19-1.31	1.25	1.19-1.31	1.25	1.18-1.30	1020	1020
5	5 023 S13265-00 P8075 1.001	67.1	56.5-76.5	21.1	11.8-31.8	1.25	1.19-1.31	1.24	1.19-1.31	1.24	1.18-1.30	2220	16300
	5 024 S13265-00 P1794 1.007	68.8	56.5-76.5	21.6	11.8-31.8	1.25	1.19-1.31	1.25	1.19-1.31	1.24	1.18-1.30	928	5190
	5 025 S13265-00 P7010 1.009	66.7	56.5-76.5	21.4	11.8-31.8	1.25	1.19-1.31	1.24	1.19-1.31	1.24	1.18-1.30	926	10900

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Summary Confirmatory Results for Control Blank and Nonconfirming Blinded Samples													
Run ID	Sample ID	Relative Abundance Peak Area Ratio to m/z 300				Retention Time (min)						S/N Ratio (> 50)	
		m/z 159		m/z 104		m/z 300		m/z 159		m/z 104		m/z 159	m/z 104
		Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Individual
3	3 019 S13265-00 double blank 1	N/A	59.3-79.3	N/A	13.4-33.4	0.00	1.20-1.32	0.00	1.19-1.31	0.00	1.19-1.31	N/A	N/A
	3 020 S13265-00 double blank 1	N/A	59.3-79.3	N/A	13.4-33.4	0.00	1.20-1.32	0.00	1.19-1.31	0.00	1.19-1.31	N/A	N/A
4	4 019 S13265-00 double blank 1	N/A	59.7-79.7	N/A	13.5-33.5	0.00	1.19-1.31	0.00	1.19-1.31	0.00	1.18-1.30	N/A	N/A
	4 020 S13265-00 double blank 1	N/A	59.7-79.7	N/A	13.5-33.5	0.00	1.19-1.31	0.00	1.19-1.31	0.00	1.18-1.30	N/A	N/A
5	5 019 S13265-00 double blank 1	N/A	56.5-76.5	N/A	11.8-31.8	0.00	1.19-1.31	0.00	1.19-1.31	0.00	1.18-1.30	N/A	N/A
	5 020 S13265-00 double blank 1	N/A	56.5-76.5	N/A	11.8-31.8	0.00	1.19-1.31	0.00	1.19-1.31	0.00	1.18-1.30	N/A	N/A
3	3 021 S13265-00 CONTROL_BLANK 1	107.2	59.3-79.3	0.0	13.4-33.4	1.26	1.20-1.32	1.25	1.19-1.31	0.00	1.19-1.31	17	N/A
	3 022 S13265-00 CONTROL_BLANK 1	N/A	59.3-79.3	N/A	13.4-33.4	0.00	1.20-1.32	0.00	1.19-1.31	0.00	1.19-1.31	N/A	N/A
4	4 021 S13265-00 control blank 1	N/A	59.7-79.7	N/A	13.5-33.5	0.00	1.19-1.31	0.00	1.19-1.31	0.00	1.18-1.30	N/A	N/A
	4 022 S13265-00 control blank 1	N/A	59.7-79.7	N/A	13.5-33.5	0.00	1.19-1.31	0.00	1.19-1.31	0.00	1.18-1.30	N/A	N/A
5	5 021 S13265-00 control blank 1	72.5	56.5-76.5	N/A	11.8-31.8	1.26	1.19-1.31	1.25	1.19-1.31	0.00	1.18-1.30	10	N/A
	5 022 S13265-00 control blank 1	87.0	56.5-76.5	N/A	11.8-31.8	1.25	1.19-1.31	1.24	1.19-1.31	0.00	1.18-1.30	8	N/A
3	3 027 S13265-00 P4272 0.962	N/A	59.3-79.3	N/A	13.4-33.4	0.00	1.20-1.32	0.00	1.19-1.31	0.00	1.19-1.31	N/A	N/A
4	4 026 S13265-00 P1177 1.031	0.0	59.7-79.7	0.0	13.5-33.5	1.25	1.19-1.31	0.00	1.19-1.31	0.00	1.18-1.30	N/A	N/A
	4 027 S13265-00 P7584 0.995	N/A	59.7-79.7	N/A	13.5-33.5	0.00	1.19-1.31	0.00	1.19-1.31	0.00	1.18-1.30	N/A	N/A
5	5 026 S13265-00 P1870 0.977	127.4	56.5-76.5	0.0	11.8-31.8	1.25	1.19-1.31	1.24	1.19-1.31	0.00	1.18-1.30	10	N/A
	5 027 S13265-00 P1661 1.004	86.8	56.5-76.5	0.0	11.8-31.8	1.24	1.19-1.31	1.25	1.19-1.31	0.00	1.18-1.30	5	N/A

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22. APPENDICES

22.1. Material safety data sheet (MSDS)

22.1.1. Fenbendazole sulfone

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.0 Revision Date 22.03.2013
Print Date 14.06.2013
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Fenbendazole sulfone
Product Number	:	32544
Brand	:	Fluka
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	54029-20-8

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich Chimie S.a.r.l L'Isle D'Abeau Chesnes F-38297 ST. QUENTIN FALLAVIER
Telephone	:	+33 (0)4 74 82 28 40
Fax	:	+33 (0)4 74 95 68 08
E-mail address	:	eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone #	:	I.N.R.S.:+33 (0)1 45 42 59 59

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Acute toxicity, Oral (Category 4), H302
Skin irritation (Category 2), H315
Skin sensitisation (Category 1), H317

For the full text of the H-Statements mentioned in this Section, see Section 16.

Classification according to EU Directives 67/548/EEC or 1999/45/EC
Xn Harmful R22, R38

For the full text of the R-phrases mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Warning

Hazard statement(s)
H302 Harmful if swallowed.
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.

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Precautionary statement(s) P280	Wear protective gloves.
Supplemental Hazard Statements	none

2.3 Other hazards - none

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms : (5-Benzenesulfonyl-1H-benzoimidazol-2-yl)-carbamic acid methyl ester

Formula : $C_{15}H_{13}N_3O_4S$

Molecular Weight : 331,35 g/mol

CAS-No. : 54029-20-8

Hazardous ingredients according to Regulation (EC) No 1272/2008

Component	Classification	Concentration
Fenbendazole sulfone		
	Acute Tox. 4; Skin Irrit. 2; Skin Sens. 1; H302, H315, H317	-

For the full text of the H-Statements and R-Phrases mentioned in this Section, see Section 16

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
no data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Carbon oxides, nitrogen oxides (NOx), Sulphur oxides

5.3 Advice for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

5.4 Further information
no data available

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SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust.
For personal protection see section 8.

6.2 Environmental precautions
Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.Normal measures for preventive fire protection.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

7.3 Specific end use(s)
A part from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Components with workplace control parameters

8.2 Exposure controls

Appropriate engineering controls
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Personal protective equipment

Eye/face protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.

Body Protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator.For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

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Control of environmental exposure
Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: solid Colour: colourless
b)	Odour	odourless
c)	Odour Threshold	no data available
d)	pH	no data available
e)	Melting point/freezing point	> 320 °C
f)	Initial boiling point and boiling range	no data available
g)	Flash point	no data available
h)	Evapouration rate	no data available
i)	Flammability (solid, gas)	no data available
j)	Upper/lower flammability or explosive limits	no data available
k)	Vapour pressure	no data available
l)	Vapour density	no data available
m)	Relative density	no data available
n)	Water solubility	no data available
o)	Partition coefficient: n-octanol/water	log Pow: 2,0
p)	Auto-ignition temperature	no data available
q)	Decomposition temperature	no data available
r)	Viscosity	no data available
s)	Explosive properties	no data available
t)	Oxidizing properties	no data available

9.2 Other safety information
no data available

SECTION 10: Stability and reactivity

10.1 Reactivity
no data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
no data available

10.4 Conditions to avoid
no data available

10.5 Incompatible materials
Strong acids and strong bases, Strong oxidizing agents

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10.6 Hazardous decomposition products
Other decomposition products - no data available
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitisation
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity
no data available

Specific target organ toxicity - single exposure
no data available

Specific target organ toxicity - repeated exposure
no data available

Aspiration hazard
no data available

Additional Information
RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity
no data available

12.2 Persistence and degradability
no data available

12.3 Bioaccumulative potential
no data available

12.4 Mobility in soil
no data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects

no data available

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SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

SECTION 14: Transport information

14.1 UN number
ADR/RID: - IMDG: - IATA: -

14.2 UN proper shipping name
ADR/RID: Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

14.3 Transport hazard class(es)
ADR/RID: - IMDG: - IATA: -

14.4 Packaging group
ADR/RID: - IMDG: - IATA: -

14.5 Environmental hazards
ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user
no data available

SECTION 15: Regulatory information

This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

no data available

15.2 Chemical Safety Assessment
For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
H302	Harmful if swallowed.
H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
Skin Irrit.	Skin irritation
Skin Sens.	Skin sensitisation

Full text of R-phrases referred to under sections 2 and 3

R22	Harmful if swallowed.
R38	Irritating to skin.

Further information
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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held

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22.1.2. Fenbendazole sulfone-d3

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.0 Revision Date 26.01.2011
Print Date 14.06.2013
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

1. IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1 Product identifiers

Product name : Fenbendazole sulfone-d_3

Product Number : 32545
Brand : Fluka
CAS-No. : 1228182-49-7

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Chimie S.a.r.l
L'Isle D'Abeau Chesnes
F-38297 ST. QUENTIN FALLAVIER

Telephone : +33 (0)4 74 82 28 40
Fax : +33 (0)4 74 95 68 08
E-mail address : eurtechserv@sial.com

1.4 Emergency telephone number

Emergency Phone # : I.N.R.S.:+33 (0)1 45 42 59 59

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 [EU-GHS/CLP]
Acute toxicity, Oral (Category 4)
Skin irritation (Category 2)
Skin sensitization (Category 1)

Classification according to EU Directives 67/548/EEC or 1999/45/EC
Harmful if swallowed. Irritating to skin.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008 [CLP]

Pictogram 

Signal word Warning

Hazard statement(s)
H302 Harmful if swallowed.
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.

Precautionary statement(s)
P280 Wear protective gloves.

Supplemental Hazard Statements none

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According to European Directive 67/548/EEC as amended.

Hazard symbol(s) 

R-phrase(s)

R22	Harmful if swallowed.
R38	Irritating to skin.

S-phrase(s) none

2.3 Other hazards - none

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Synonyms : (5-Benzenesulfonyl-1H-benzoimidazol-2-yl)-carbamic acid methyl-D3 ester

Formula : $C_{15}D_3H_{10}N_3O_4SC_{15}D_3H_{10}N_3O_4S$

Molecular Weight : 334,36 g/mol

Component		Concentration
Fenbendazole sulfone-d3 VETRANAL®		
CAS-No.	1228182-49-7	-

4. FIRST AID MEASURES

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

4.3 Indication of immediate medical attention and special treatment needed
no data available

5. FIRE-FIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Carbon oxides, nitrogen oxides (NOx), Sulphur oxides

5.3 Precautions for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

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5.4 Further information
no data available

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Avoid breathing dust.

6.2 Environmental precautions
Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.

7.2 Conditions for safe storage, including any incompatibilities
Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

7.3 Specific end uses
no data available

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

8.2 Exposure controls

Appropriate engineering controls
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Personal protective equipment

Eye/face protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.

Body Protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator.For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

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9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: solid Colour: colourless
b)	Odour	odourless
c)	Odour Threshold	no data available
d)	pH	no data available
e)	Melting/freezing point	> 310 °C
f)	Initial boiling point and boiling range	no data available
g)	Flash point	no data available
h)	Evaporation rate	no data available
i)	Flammability (solid, gas)	no data available
j)	Upper/lower flammability or explosive limits	no data available
k)	Vapour pressure	no data available
l)	Vapour density	no data available
m)	Relative density	no data available
n)	Water solubility	no data available
o)	Partition coefficient: n-octanol/water	no data available
p)	Autoignition temperature	no data available
q)	Decomposition temperature	no data available
r)	Viscosity	no data available
s)	Explosive properties	no data available
t)	Oxidizing properties	no data available

9.2 Other safety information

no data available

10. STABILITY AND REACTIVITY

10.1 Reactivity

no data available

10.2 Chemical stability

no data available

10.3 Possibility of hazardous reactions

no data available

10.4 Conditions to avoid

no data available

10.5 Incompatible materials

Strong acids and strong bases, Strong oxidizing agents

10.6 Hazardous decomposition products

Other decomposition products - no data available

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11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity
no data available

Specific target organ toxicity - single exposure
no data available

Specific target organ toxicity - repeated exposure
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Ingestion	Harmful if swallowed.
Skin	May be harmful if absorbed through skin. Causes skin irritation.

Signs and Symptoms of Exposure
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Additional Information
RTECS: Not available

12. ECOLOGICAL INFORMATION

12.1 Toxicity
no data available

12.2 Persistence and degradability
no data available

12.3 Bioaccumulative potential
no data available

12.4 Mobility in soil
no data available

12.5 Results of PBT and vPvB assessment
no data available

12.6 Other adverse effects
no data available

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13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

14.1 UN-Number
ADR/RID: - IMDG: - IATA: -

14.2 UN proper shipping name
ADR/RID: Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

14.3 Transport hazard class(es)
ADR/RID: - IMDG: - IATA: -

14.4 Packaging group
ADR/RID: - IMDG: - IATA: -

14.5 Environmental hazards
ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for users
no data available

15. REGULATORY INFORMATION

This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture
no data available

15.2 Chemical Safety Assessment
no data available

16. OTHER INFORMATION

Further information
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