Determinative and Confirmatory Procedures for the Analysis of Fenbendazole Sulfone in Turkey Liver Tissue Using LC-MS/MS, Version 9.0	MERCK Animal Health

METHOD TITLE: Determinative and Confirmatory Procedures for the Analysis of Fenbendazole Sulfone in Turkey Liver Tissue Using LC-MS/MS, Version 9.0

APPROVAL SIGNATURES:

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1 GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

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

ACN	Acetonitrile
amu	Atomic Mass Unit
BA	Bioanalytical
CV	Coefficient of Variation
DMSO	Dimethyl Sulfoxide
FBZ-SO_2	Fenbendazole Sulfone
FBZ-SO_2-D_3	Fenbendazole Sulfone-D_3
HPLC	High Performance Liquid Chromatography
LC-MS/MS	High Performance Liquid Chromatography – Tandem Mass Spectrometry
IS	Internal Standard
LC-MS	Liquid Chromatography – Mass Spectrometry
LOQ	Limit of Quantitation
MAH	Merck Animal Health
MSDS	Material Safety Data Sheet
n	Number of Samples
NA	Not Applicable
psi	Pounds per Square Inch
MilliQ water	Water purified by a Millipore Synthesis A10
QC	Quality Control (fortified tissue)
Control Blank	Blank matrix sample, fortified with IS only
Double Blank	Double Blank matrix sample, not fortified with IS or analyte
Qual1 and Qual2	Qualifier 1 and Qualifier 2
rcf	Relative Centrifugal Force
rpm	Rotations per Minute
s	Second
Solvent Blank	Methanol (MeOH) Sample
SL	Solvent Level
S/N	Signal to Noise
SST	System Suitability Test
STD	Standard Calibrator
ULOQ	Upper Limit of Quantitation
v/v	Volume per Volume
v/v/v	Volume per Volume per Volume
WS	Working Solution

2 SCOPE AND FIELD OF APPLICATION

Fenbendazole sulfone is a metabolite of fenbendazole. Fenbendazole is a broad spectrum benzimidazole anthelmintic used against gastrointestinal parasites and intended for use as a veterinary drug in turkey. This procedure describes the method for the analysis and identification of fenbendazole sulfone (FBZ-SO_2) in turkey liver tissue. This method describes analytical procedures for both quantitative determination and confirmation of fenbendazole sulfone in turkey liver. The analytical range of the method is 2.00 – 15.0 µg/g (equivalent to 5.0 – 37.5 ng/mL in solvent standard solution). The US tolerance for fenbendazole sulfone in turkey liver is 6.0 µg/g or ppm.

The compounds listed in Table 2-1 are other veterinary drugs registered for use in turkey in the U.S. They have been tested and shown not to significantly interfere with the method.

Table 2-1: Compounds (drugs) Tested for Interferences

Bacitracin Zinc Salt	Narasin
Lasalocid	Salinomycin
Tylosin	Virginiamycin
Robenidine Hydrochloride	Fenbendazole
Bambermycin	Nicarbazin
Monensin	Diclazuril

The current method was validated in accordance with the Food and Drug Administration's Good Laboratory Practices for Nonclinical Laboratory Studies, 21CFR58, which is also accepted by the OECD Commission Directive 1999/11/EC of March 8, 1999.

3 PRINCIPLE

Approximately one gram of homogenized turkey liver is fortified with internal standard (FBZ-SO_2-D_3) and then extracted twice with methanol in two extraction steps. The sample extract is diluted to 20 mL with methanol. An aliquot of the methanol extract is diluted with MilliQ Water/Acetonitrile (70/30, v/v). The resulting solution is quantitatively analyzed using gradient reversed phase liquid chromatography with mass-spectrometric detection (LC-MS/MS) using a positive ion multiple-reaction monitoring (MRM) with ion transition of *m/z* 332 → *m/z* 300 for fenbendazole sulfone (FBZ-SO_2) and *m/z* 335 →*m/z* 300 for FBZ-SO_2-D_3. Additional ion transitions of FBZ-SO_2, 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.

4 WARNINGS AND SAFETY PRECAUTIONS

Take safety precautions common in the laboratory, *e.g.* wear lab coat, goggles and gloves if necessary. The MSDS for fenbendazole sulfone and fenbendazole sulfone-D_3 are presented in Section 23 in this method.

5 REAGENTS AND MATERIALS

5.1 Reagent/Chemical

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: Reagent/Chemicals to be used in this test procedure

Chemical	Quality or purity	Supplier
Dry Ice	NA	NA
Methanol (MeOH)	Optima or HPLC	Fisher
Acetonitrile (ACN)	Optima or HPLC	Fisher
Acetonitrile + 0.1% formic acid	HPLC	Fisher
Formic Acid	Certified ACS or HPLC	Fisher
Dimethyl sulfoxide (DMSO)	HPLC or Certified ACS	Fisher
0.1% formic acid in Water	HPLC	Fisher
Water (H_2O)	>18 MΩ·cm	Millipore or equivalent

5.2 Solutions

The following solutions may be prepared (by volume-to-volume equivalence or by dilution) in different quantities. Measure volume using a suitably sized graduated cylinder or graduated pipette.

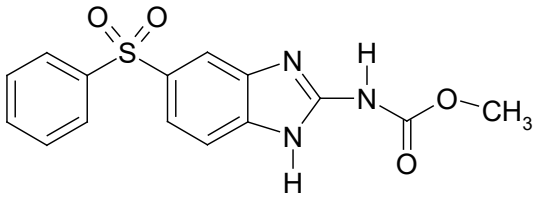
Table 5-2: Reagents to be Used in this Test Procedure

Solution	Preparation and Storage
HPLC – Mobile Phase A Mobile Phase A: 0.1% Formic Acid in Water, v/v	Use pre-made 0.1% formic acid in water. Alternatively, add 2000 mL of water to a glass reagent bottle using a graduated cylinder and add 2 mL of formic acid using a disposable pipette. Mix well. Store at room temperature and stable for 2 weeks.
HPLC – Mobile Phase B Mobile Phase B: 0.1% Formic Acid in Acetonitrile, v/v	Use pre-made 0.1% formic acid in acetonitrile. Alternatively, add 2000 mL of acetonitrile to a glass reagent bottle using a graduated cylinder and add 2 mL of formic acid using a disposable pipette. Mix well. Store at room temperature and stable for 2 weeks.
Dilution Solution: MilliQ Water/Acetonitrile, 70/30, v/v	Add 700 mL of water using a graduated cylinder and 300 mL of acetonitrile using a graduated cylinder into a glass reagent bottle. Mix well. Store at room temperature and stable for 1 month.
Autosampler Wash 1 Solution: Mobile Phase A/Mobile Phase B 70/30, v/v	Add 700 mL of mobile phase A (water +0.1% formic acid) and Add 300 mL of mobile phase B (acetonitrile +0.1% formic acid) using a graduated cylinder into a glass reagent bottle. Mix well. Store at room temperature. Store at room temperature and stable for 2 weeks.
Autosampler Wash 2 Solution: 100% Acetonitrile	Acetonitrile. Stable for 1 month at room temperature.
Autosampler Wash 2 Solution: **100% Acetonitrile** **(Thermo PAL Open Access Autosampler)**	Acetonitrile. Stable for 1 month at room temperature. **NOTE:** Only one autosampler wash is used for the Accela Autosampler.

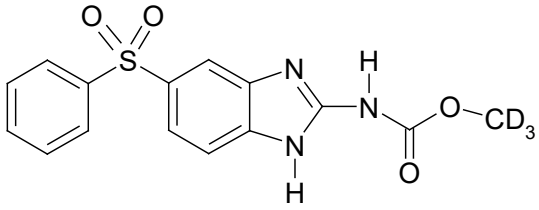
5.3 Reference Compound

The reference compound, fenbendazole Sulfone (FBZ-SO_2), and the internal standard, FBZ-SO_2-D_3, are retested periodically and the actual content from these retests are used for the relevant calculations.

5.3.1 Reference Compound FBZ-SO_2

Name:	FBZ-SO_2 (Fenbendazole Sulfone)
Compound Number	AH 247250
CAS Number	54029-20-8
Chemical name:	(5-Benzenesulfonyl-1*H*-benzoimidazol-2-yl)-carbamic acid methyl ester
Formula:	$C_{15}H_{13}N_3O_4S$
Molecular weight:	331.35 g/mol
Appearance / colour:	Solid white powder
Storage conditions:	≤ - 15 °C, dark
Supplier:	Australian Government National Measurement Institute (Pymble NSW, Australia) or other vendor
Structural formula:	

5.3.2 FBZ-SO_2-D_3 (Used as Internal Standard)

Name:	Fenbendazole Sulfone-D_3
CAS-No.:	1228182-49-7
Chemical Name:	(5- Benzenesulfonyl-1-H-benzoimidazol-2-yl)-carbamic acid methyl-D_3 ester
Formula:	$C_{15}H_{10}D_3N_3O_4S$
Molecular Weight:	334.35 g/mol
Appearance/Color:	solid white powder
Storage Conditions:	2-8 °C
Supplier	Witega (Berlin, Germany) or other vendor
Structural formula:	

6 APPARATUS AND EQUIPMENT

6.1 General Apparatus

Equivalent apparatus may be substituted if acceptable performance is demonstrated, except where indicated. 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 at least 0.01 g)
Centrifuge, refrigerated – capable of attaining 3300 x g with appropriate rotor
Cylinders - graduated – 100, 250, 500, 1000 and 2000 mL
Flasks - volumetric with glass stopper – 5,10, 20, 25, 50, and 100 mL
Freezer - capable of maintaining temperatures ≤ -65°C and ≤ -10°C
Refrigerator - capable of maintaining temperatures 2-8°C
Millipore Water System
Rainin EDP3 Pipets or other equivalent pipets

Robot Coupe®, commercially available cryogenic meat grinder or food blender such as Waring Commercial Laboratory Blender
Vortex mixer – Vortex-Genie 2
Vortex multitubes

6.2 Supplies

The following supplies are listed as examples, unless otherwise stated. Other supplies of equivalent quality and abilities provided by other vendors may be used.

Table 6-2:Supplies
15-mL polypropylene graduated centrifuge tubes with screw cap - Fisher brand
50-mL polypropylene graduated centrifuge tubes with screw cap - Fisher brand
2 mL 96-well plates and cap mats - Analytical Sales and Services
2 mL glass autosampler vials

6.3 LC-MS Equipment

Equivalent apparatus and software may be substituted if acceptable performance is demonstrated as suggested in Section 11. Manufacturers and model numbers specified here were used during method development and validation.

Table 6-3: LC-MS list
Primary HPLC Column: Ace 3 C18, 2.1 x 50 mm, Part Number ACE-111-0502, Mac-Mod Analytical Inc. Alternate HPLC Column (used for ruggedness test): Acclaim 120 C18, 3 µm, 2.1 x 50 mm, Part Number 59128, Thermo Scientific.
Primary MS spectrometer– Applied Biosystems, API4000 Triple Quadrupole
Primary LC/MS Data acquisition system – Applied Biosystems, Analyst, Version 1.4.2
Primary HPLC Systems: a) Shimadzu pump modelLC-10ADvp, Autosampler model SIL-HTC, System Controller: SCL-10Avp. b) Waters Acquity I-Class UPLC
MS spectrometer (secondary) – Thermo TSQ Vantage, Triple Quadrupole
HPLC System (secondary): Thermo Accela 1250 pump
LC/MS Data acquisition system – LC Quan, version 2.6
Data calculation software – Microsoft Excel or Watson LIMS

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 exact concentrations should be reported and used throughout all calculations. The following solutions should be stored in a freezer set to -20°C. Return solutions to freezer immediately after use.

7.1 FBZ-SO_2 and FBZ-SO_2-D_3 DMSO Stock Solution

All stock solutions of FBZ-SO_2 and FBZ-SO_2-D_3 are prepared in dimethyl sulfoxide (DMSO). The FBZ-SO_2 and FBZ-SO_2-D_3 DMSO stock solutions are stored in a -20°C freezer.

7.1.1 Preparation of FBZ-SO_2 STD DMSO Stock Solution at 2,000 µg/mL (FBZ-SO_2 DMSO Stock 1)

Accurately weigh reference standard, (target weight 10.0 mg after correcting for purity) into a weighing boat, record the exact weight to at least the nearest 0.1 mg, transfer and dissolve the standard into a 5 mL volumetric flask, and fill to the mark with DMSO. Vortex to mix. This 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). The stability of this stock solution is 83 days.

7.1.2 Preparation of FBZ-SO_2 Quality Control DMSO Stock Solution at 2,000 µg/mL (FBZ-SO_2 DMSO Stock 2)

This solution is prepared from a second independent weighing procedure (according to Section 7.1.1). It is used for preparation of the quality control (QC) fortification solution.

7.1.3 **P**reparation of FBZ-SO_2-D_3 DMSO Internal Standard Stock Solution at 1,000 µg/mL (FBZ-SO_2-D_3 DMSO Stock)

Accurately weigh FBZ-SO_2-D_3 reference standard (target weight 10.0 mg after correcting for purity) into a weighing boat, record the exact weight to at least the nearest 0.1 mg, transfer and dissolve the standard into a 10 mL volumetric flask, and fill to the mark with 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 Sections 7.1.4). The stability of this stock solution is 83 days.

7.1.4 FBZ-SO_2-D_3 Internal Standard Fortification Solution for Liver

Using a calibrated pipette, transfer an aliquot of FBZ-SO2-D_3 internal standard stock solution (7.1.3) into a 50 mL volumetric flasks and dilute with methanol to prepare the

internal standard fortification solution of 30 µg/mL. Nominal aliquot volume is 1.5 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.* an actual stock solution of concentration 901 µg/mL the required adjusted volume is: 1000 µg/mL nominal/901 µg/mL actual x 1.5 mL nominal = 1.66 mL). The IS fortification solution is stored in a -20°C freezer and stable for 83 days.

7.1.5 Comparison of Stock Solutions

A stock solution comparison is required when new stock solutions are prepared. Two stock solutions are prepared using calibrated pipettes and volumetric flasks. One is used for the preparation of standard curve working solutions. The other stock solution is used to prepare QC working solutions.

Each of the two stock solutions are diluted with dilution solution (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	FBZ-SO_2 DMSO Stock Solution		Intermediate Solution Conc. (ng/mL)
	Nominal Conc. (µg/mL)	Volume Taken (µL)	
FBZ-SO_2 STD Stock Inter Solution	2,000 (Stock Solution)	125[1]	5,000
FBZ-SO_2 QC Stock Inter Solution	2,000 (QC Stock Solution)	125[1]	5,000

[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 solution (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	**FBZ-SO_2 Stock Inter Solution**		**IS Fort. Solution (Section 7.1.4)**		**Conc. (ng/mL) (FBZ-SO_2/IS)**
	Conc. (ng/mL)	**Volume Taken (µL)**	**Conc. (µg/mL)**	**Volume Taken (µL)**	
FBZ-SO_2-STD-Stock Final Dilution	5,000	200	30	40	10/12
FBZ-SO_2-QC-Stock Final Dilution	5,000	200	30	40	10/12

The two final dilution solutions are analyzed using LC/MS-MS (n=6 injections of each stock solution alternating) and the results compared for equivalence. If the mean difference of the peak area ratio (PAR) are within ± 5% and precision of the replicates are ≤5%, they will be considered equivalent.

$$\textbf{\% Difference} = 100 \times \frac{\textbf{(mean of PAR of stock A – mean of PAR of stock B)}}{\textbf{((mean of PAR of stock A + mean of PAR of stock B)/2)}}$$

Prepare stock comparison solutions on the day of use and discard after use.

7.2 Working Solution for FBZ-SO_2 Calibration Standards for Liver (SL 8 Liver – SL 1 Liver)

Using a calibrated pipette, transfer an aliquot of the FBZ-SO_2 STD DMSO stock solution 1 (7.1.1) into a 5 mL volumetric flask and dilute with methanol according to the following scheme (Table 7-2-1) to prepare SL 8 Liver. Further dilutions of this solution and subsequent solutions are then prepared using calibrated pipettes and 10 mL volumetric flasks as per Table 7-2-1. The diluent is methanol. All working solutions are stored in a -20°C freezer and are stable for 78 days.

Table 7-2-1: Working solutions for FBZ-SO_2 calibration standards (liver) – scheme for aliquot transfers of solutions

Working Solution ID	Concentration of Working Solution [µg/mL]	Volume of Solution Used	Final Volume [mL]
SL 8 Liver	1000	2.5 mL of stock solution[1]	5
SL 7 Liver	150	1.50 mL of SL 8 Liver	10
SL 6 Liver	100	1.0 mL of SL 8 Liver	10
SL 5 Liver	75	750 µL of SL 8 Liver	10
SL 4 Liver	60	600 µL of SL 8 Liver	10
SL 3 Liver	45	3.0 mL of SL 7 Liver	10
SL 2 Liver	30	2.0 mL of SL 7 Liver	10
SL 1 Liver	20	2.0 mL of SL 6 Liver	10

[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 2.5 mL nominal = 2.63 mL actual).

7.3 FBZ-SO_2 Quality Control Fortification Solutions for Liver

Using a calibrated pipette, transfer an aliquot of the FBZ-SO_2 QC DMSO stock solution 2 (Section 7.1.2) into a 10 mL volumetric flask and dilute with methanol according to the following scheme (Table 7-3-1). Further dilutions of this solution are then prepared using calibrated pipettes and 10 mL volumetric flasks as per Table 7-3-1. The diluent is methanol. All QC fortification solutions are stored in a -20°C freezer and are stable for 78 days.

Table 7-3-1: Working Solutions for FBZ-SO_2 Quality Control Standards (Liver) – Scheme for Aliquot Transfers of Solutions

Working Solution ID	Concentration of Working Solution [µg/mL]	Volume of Solution Used	Final Volume [mL]
QC SL 4 Liver	500	2.5 mL of QC stock solution[1]	10
QC SL 3 Liver	120	2.40 mL of QC SL 4 Liver	10
QC SL 2 Liver	60	1.20 mL of QC SL 4 Liver	10
QC SL 1 Liver	30	0.60 mL of QC SL 4 Liver	10

[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 2.5 mL nominal = 2.63 mL actual).

7.4 Solvent Calibration Curve for Liver

Using a calibrated pipette, transfer 100 µL each of the respective working solutions (7.2) and 100 µL of the IS (FBZ-SO_2-D_3) fortification solution (7.1.4) (30 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). The W-Mix–Stds solutions are stable for 78 days at -20°C.

Table 7-4-1: Preparation of W-Mix STD Solutions

Solution ID	Volume of Working Solutions (Table 7.2-1) taken	Volume of IS solution taken (30 µg/mL)	Final Volume (mL)	Concentration (ng/mL)
W-Mix-Std-7	100 µL of SL 7	100 µL	20	750
W-Mix-Std-6	100 µL of SL 6	100 µL	20	500
W-Mix-Std-5	100 µL of SL 5	100 µL	20	375
W-Mix-Std-4	100 µL of SL 4	100 µL	20	300
W-Mix-Std-3	100 µL of SL 3	100 µL	20	225
W-Mix-Std-2	100 µL of SL 2	100 µL	20	150
W-Mix-Std-1	100 µL of SL 1	100 µL	20	100

Note: the nominal concentration of internal standard in W-Mix-Stds is 150 ng/mL.

Using a calibrated pipette, transfer 50 µL of each W-Mix-Stds solution into a 2 mL 96-well plate or 2 mL autosampler vial and mix with 950 µL of dilution solution to give Liver-Stds (see Table 7-4-2). Vortex and mix well for LC-MS/MS analysis. The Liver-Stds calibration solutions are stable for 15 days in the autosampler at room temperature.

Table 7-4-2: Preparation of Solvent Calibration Curve (Liver-Stds)

Std-ID	W-Mix-STD Solution ID	Conc. (ng/mL)	Final Vol (mL)	Liver Equivalent Conc. (ppm)
Liver-Std-7	W-Mix-Std-7	37.5	1.00	15.0
Liver-Std-6	W-Mix-Std-6	25.0	1.00	10.0
Liver-Std-5	W-Mix-Std-5	18.75	1.00	7.5
Liver-Std-4	W-Mix-Std-4	15.0	1.00	6.0
Liver-Std-3	W-Mix-Std-3	11.25	1.00	4.5
Liver-Std-2	W-Mix-Std-2	7.5	1.00	3.0
Liver-Std-1	W-Mix-Std-1	5.0	1.00	2.0

Note: the nominal concentration of internal standard in Liver-Stds is 7.5 ng/mL.

The correlation between solvent calibration standard concentrations and tissue equivalent concentrations are presented in Table 7-4-2. The extraction process is 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 ppm tissue equivalents (µg/g) to solvent concentration (ng/mL) is 2.5 (*i.e* 1000 / 400).

Refer to extraction steps 9.2i, 9.2j, and 9.1a for extraction volume, dilution factor, and liver sample weight, respectively.

7.5 Quality Control Samples for Liver

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 preparation of the QC samples, 100 µL of the respective QC fortification solutions (7.3) and 100 µL of the IS fortification solution (7.1.4) (30 µg/mL) are spiked into 1 g of blank liver (see Table 7-5-1). For routine sample analysis, QC samples are prepared fresh daily.

Table 7-5-1:Quality control samples

Concentration of Quality Control Samples [ppm]	Spiking Volume of 30 µg/mL IS Solution	Spike Volume of Working Solution
12.0	100 µL	100 µL of QC SL 3 Liver
6.0	100 µL	100 µL of QC SL 2 Liver
3.0	100 µL	100 µL of QC SL 1 Liver

Further sample preparation is described in Section 9.1.

8 SAMPLE HANDLING AND SAMPLING

8.1 Homogenize Tissue Sample

A Robot Coupe®, a meat grinder, or a food blender may be used to process tissues. Tissue sample is chopped into small pieces to facilitate the grinding process. If it is frozen intact, the tissue may need to be partially thawed before chopping into the small pieces that will fit into the grinding apparatus.

Chopped tissue is mixed with dry ice and ground with a Robot Coupe® or other food processor until it becomes a uniform powder. The powdered tissue (containing dry ice) is transferred into a suitable container. The container is loosely sealed or capped and stored in freezer set at -20°C overnight or longer to allow the dry ice sublime. After all the dry ice has been sublimed, the container is sealed and stored in a freezer set at -80°C freezer for longer term storage.

8.2 Sample storage

Control and incurred samples are stored in suitable container in a freezer set at -80°C. It is recommended to keep tissue in frozen powdered form until analysis. Refer to Section 17 in this method SOP for additional sample handling and storage stability of fenbendazole sulfone in turkey liver.

9 EXTRACTION PROCEDURE FOR FBZ-SO_2 IN TURKEY LIVER

It is suggested to have sample labels (four sets of labels per sample) and necessary containers ready before performing the procedure.

9.1 Preparation of incurred, quality control, control, and double blank samples

Note: Use a spatula pre-chilled in dry ice and keep spatula chilled between sample weighings.

Tissue samples can be weighed out on a different day to facilitate the process. Samples should be weighed on dry ice, and stored in a -80℃ freezer . The remaining tissue samples should be returned to storage in a -80℃ freezer.

Thaw tissues prior to fortification step.

9.1a Accurately weigh 1.00 g (±0.05 g) of control or incurred sample into a 15-mL polypropylene tube on dry ice. Record or print the exact weight as shown on the balance. Centrifuge the sample at 1000 rpm (200x g) for approximately 1 min. Completely thaw tissues prior to the fortification step.

9.1b Add 200 µL methanol for the double blank sample. Add 100 µL methanol and 100 µL of internal standard fortification solution (7.1.4) for control and incurred sample. Add 100 µL QC fortification solution (7.3) and 100 µL of internal fortification standard (7.1.4) for QC samples. Briefly vortex and leave the sample on the bench for approximately 10 min before extraction.

For fortified QC tissue samples, a nominal tissue weight of 1 g should be used for the determination of recovery (actual weight should be recorded). For study samples, correction of weight is required. A weight correction factor will be applied. Weight Correction Factor = nominal weight / actual weight (1.0 grams = nominal weight).

9.2 Extraction of tissue sample

9.2a Add 8 mL of methanol into the 15-mL polypropylene tube containing the sample using a pipette or a bottletop dispenser.

9.2b Briefly vortex each sample individually to loosen the tissue and then vortex the samples for approximately 10 min. at high speed (setting at 7-9) using a multitube vortexer. Visually inspect all tubes to ensure tissue is swirling up and thoroughly mixed. If any sample did not swirl up during the initial vortex, vortex the individual tube on a regular vortex mixer for up to 10 seconds so that the tissue solid can be mixed well with the extraction solvent, then put the individual sample back onto the multitube vortexer for 10 more minutes.

9.2c Centrifuge the sample at approximately 3300 rcf (4000 rpm for Sorvall Legend XTR) for approximately 10 min. at approximately 10°C.

9.2d Transfer the supernatant to a clean pre-labeled 50-mL polypropylene tube.

9.2e Add 8 mL of methanol into the 15 mL polypropylene tube containing the pellet using a pipette or a bottletop dispenser.

Critical Step: Pellet may be difficult to re-suspend. The pellet may be allowed to sit for approximately 10 minutes before vortexing in order to make resuspension easier. Vortex each sample individually prior to placing the samples on the multi-tube vortexer (Section 9.2f). If the pellet is difficult to re-suspend, a clean spatula or similar implement may be used to break the pellet or the tube can be tapped against the bench top.

9.2f Vortex the sample for approximately 10 min. at high speed (setting at 7-9) using a multi-tube vortexer. Visually inspect all tubes to ensure tissue is swirling up and thoroughly mixed. If any sample did not swirl up during the initial vortex, vortex the individual tube on a regular vortex mixer for up to 10 seconds so that the tissue solid can be mixed well with the extraction solvent, then put the individual sample back onto the multi-tube vortexer for 10 more minutes.

9.2g Centrifuge the sample at ~3300 rcf (4000 rpm for Sorvall Legend XTR) for approximately 10 min. at approximately 10°C.

9.2h Transfer the supernatant to the same pre-labeled 50 mL polypropylene tube (Section 9.2d).

9.2i Adjust the volume to 20 mL mark with methanol. Vortex and mix well. Centrifuge at ~3300 rcf (4000 rpm for Sorvall Legend XTR) for approximately 10 min. at approximately 10°C.

9.2j Pipette 50 µL of the methanol liver tissue extract into the appropriate wells of a 2 mL 96-well plate or 2 mL autosampler vial and mix with 950 µL of dilution solvent, MilliQ Water / Acetonitrile (70/30, v/v). Vortex and mix well for LC-MS/MS analysis. Store remaining methanol extract in refrigerator for possible re-assay. The methanol extract is stable for 23 days at refrigeration storage. Extracted samples in

96-well plates or autosampler vials, stored at room temperature , are stable for up to 15 days.

10 METHOD FLOW CHART

Transfer 1.00 ±0.05 g of the frozen homogenate into a 15-mL polypropylene centrifuge tube. Completely thaw prior to fortification.

↓

Add 200 µL methanol for the double blank sample. Add 100 µL methanol and 100 µL of internal standard fortification solution for control and incurred sample. Add 100 µL QC fortification solution and 100 µL of internal fortification standard for QC samples. Briefly vortex. Leave the sample on the bench for no more than 10 min before extraction.

↓

Add 8 mL methanol and vortex for approximately 10 min.

↓

Centrifuge at ~3300 rcf (4000 rpm for Sorvall Legend XTR) for approximately 10 min at approximately 10 °C.

↓

Transfer the supernatant to a clean pre-labeled 50-mL polypropylene tube

↓

Add 8 mL methanol and vortex for approximately 10 min.

↓

Centrifuge at ~3300 rcf (4000 rpm for Sorvall Legend XTR) for approximately 10 min at approximately 10 C.

↓

Combine the methanol extracts and adjust the volume to 20 mL mark with methanol. Vortex and mix well. Centrifuge at ~3300 rcf (4000 rpm for Sorvall Legend XTR) for approximately 10 min. at approximately 10°C.

↓

Pipette 50 µL of the methanol liver tissue extract into the appropriate wells of a 2 mL 96-well plate or 2 mL LC vial and mix with 950 µL of dilution solvent, MilliQ Water/Acetonitrile (70/30, 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 HPLC Conditions

Settings may depend on the HPLC system used and are for example only.

HPLC System:	HPLC System: Shimadzu pump model LC-10ADvp, Autosampler model SIL-HTC, System Controller: SCL-10Avp or Waters Acquity I-Class UPLC
Alternate HPLC System:	Thermo Accela 1250 Pump, Thermo PAL Open Access Autosampler
Column:	MacMod Ace 3 C18, 2.1 x 50 mm Part Number ACE-111-0502
Alternate Column :	Acclaim 120 C18 3µm 120Å 50×2.1 mm, Thermo Scientific. Part Number 59128.
Column Temperature:	Ambient
Autosampler Temperature:	Ambient
Mobile Phase A:	0.1% Formic Acid in Water (v/v)
Mobile Phase B:	0.1% Formic Acid in Acetonitrile (v/v)
Alternate Mobile Phase A:	Water: Acetonitrile: Formic Acid (90:10: 0.09, v:v:v)
Alternate Mobile Phase B:	Water: Acetonitrile: Formic Acid (10:90:0.09, v:v:v)
Injection Volume:	10 µL (may vary)
Run Time:	5.2 min/inj.
Retention Time:	approximately 1.5 min

Gradient Table:

Time (min)	Flow (µL/min)	Mobile Phase A (%)	Mobile Phase B (%)
Initial (0.1)*	400	70	30
0.3	400	70	30
2.0	400	25	75
2.1	400	0	100
3.1	400	0	100
3.2	400	70	30
5.2**	400	70	30

*Shimadzu System Controller starts from 0.1, Thermo Pump starts from 0.0
**Shimadzu System Controller stop

11.2 MS Conditions

11.2.1 Tuning of Mass Spectrometer

The MS response of FBZ-SO_2 and FBZ-SO_2-D_3 can be tuned by infusion of appropriate solutions. Other methods of tuning are also acceptable. 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 FBZ-SO_2 and FBZ-SO_2-D_3 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 FBZ-SO_2 and FBZ-SO_2-D_3 for determination and for the adequate detection of product ions at m/z 159 and 104 for FBZ-SO_2 for confirmation. The resultant MS parameter should be used for all analyses, although the operator may vary conditions for adequate sensitivity. The structure and proposed fragmentation pattern of FBZ-SO_2 is shown in section 22. The MS spectra of FBZ-SO_2 at various collision energy will be listed after relevant tests.

11.2.2 MS Conditions

The MS should be tuned as in Section 11.2.1. The suggested MS parameters and peak mass centers are as follows. Settings may depend on the MS system used and are for example only.

Table 11.2.2-1: System Parameter API 4000

Ionization interface	Turbo Ion Spray
Ionization mode	Positive
Approximate MS run time [min]	5
Source (TEM) Temperature [°C]	500
Curtain (CUR) gas [psi]	20
Collision (CAD) gas [psi]	11
Ion source gas (GS1) 1 [psi]	50
Ion source gas (GS2) 2 [psi]	50
Ion (IS) Spray [V]	5500
Entrance (EP) potential [V]	10

Table 11.2.2-2: System Parameter TSQ Vantage	
Ion Source Type	HESI
Ionization Mode	Positive
Spray Voltage (V)	3500
Vaporizer Temperature (°C)	380
Sheath Gas Pressure (psi)	10
Ion Sweep Gas Pressure (psi)	0.0
Aux Gas Pressure (V)	5
Capillary Temperature (°C)	270

MRM MS/MS transition parameters (API-4000) as follows

Table 11.2.2-3: MS/MS Transition Parameter

Reference compound	Precursor ion Q1 mass [amu]	Collision energy [V]	Q3 mass [amu]	Dwell time [ms]
FBZ-SO_2 [a]	332	31	300 (quantifier)	150
FBZ-SO_2-Qual_1 [b]	332	47	159 (qualifier)	150
FBZ-SO_2-Qual_2 [b]	332	75	104 (qualifier)	150
FBZ-SO_2-D_3 [a]	335	31	300	150

a: quantitation purposes

b: qualifier transition used with confirmatory method, not used for quantitative purposes

MRM MS/MS transition parameters (Thermo TSQVantage) as follows

Table 11.2.2-4: MS/MS Transition Parameter

Reference compound	Precursor ion Q1 mass [amu]	Collision energy [V]	Q3 mass [amu]	Dwell time [ms]
FBZ-SO_2 [a]	332	38	300 (quantifier)	150
FBZ-SO_2-Qual_1 [b]	332	55	159 (qualifier)	150
FBZ-SO_2-Qual_2 [b]	332	78	104(qualifier)	150
FBZ-SO_2-D_3 [a]	335	38	300	150

a: quantitation purposes

b: qualifier transition used with confirmatory method, not used for quantitative purposes

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.

11.3 System Suitability Test and Sample Injection Sequence

11.3.1 System Suitability Test (SST)

Once the system is conditioned, 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 for system suitability acceptance criteria.

11.3.2 Bracket Standard

All 7 standards are run before extracted samples including control samples, double blank, QC, and incurred samples. The extracted samples are followed (bracketed) by all 7 standards.

11.3.3 Analysis Sequence

A possible sequence order consisting of system suitability test (SST) samples, solvent calibration, and QC samples within a series is presented below. The SST solutions (Section 11.3.1) are used to check the LC-MS system.

System Suitability Test SSTL (Std-1)	n ≥5 injections (SSTL reproducibility)
Std-1 to Std-7	1 injection each
Solvent blank	1 injections
Followed by tissue samples, including double blank, control, QCs, and study samples.	1 injection each
Solvent blank	1 injections
Std-1 to Std-7	1 injection each

12 CALCULATION AND REPORTING OF RESULTS

12.1 Method of Calculation

Quantitation of FBZ-SO_2 is accomplished using an internal standard calibration method with a FBZ-SO_2 standard concentration range of 2.0 ppm to 15 ppm (tissue equivalent) for liver. A standard calibration curve is generated from non-weighted linear regression analysis of peak area ratio versus concentration (ppm) of FBZ-SO_2.

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

$y=mx+b$

The concentration of each sample is calculated using the formula:

$$x = \frac{y - b}{m}$$

Where, y = MS detector calculated response using the ratio of analyte to IS
x = sample concentration (ppm)
m = slope
b = y-intercept

Typically, FBZ-SO_2 concentrations of standard curve point are expressed as ppm tissue residue equivalent. A typical standard calibration curve for liver is displayed in section 21.5.

If the regression obtained in an analytical set yields an acceptable coefficient of determination and meets the stated criteria (Section 13.3), 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.

12.2 Calculation of Unknown Concentrations from Incurred-residue Tissues and Fortified Samples

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

The following equation will calculate the concentration (ppm) in the incurred samples:

$$C_T = \frac{(C_I)}{Sw}$$

Where:
C_T is the reported concentration of FBZ-SO_2 in ppm in the sample,
C_I is the calculated concentration of FBZ-SO_2 in ppm from the standard curve where the nominal concentrations of standards are in ppm and are based on 1.0 gram sample size.
S_W is the weight in g of the samples (nominal weight of 1 g is used for fortified samples and exact weight is used for control and incurred samples).

An example of a concentration calculation is given below:

C_I = 4.21 ppm S_W = 1.06 g

$$C_T = \frac{4.21}{1.06} = 3.97 \text{ ppm}$$

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

$$\%\text{Recovery} = \left(\frac{C_I}{C_F}\right) \times 100$$

Where:
C_I is the calculated concentration of FBZ-SO_2 in ppm in the sample,
C_F is the tissue fortification level in ppm.

An example of recovery calculation is given below:

C_I=6.51 ppm C_F=6.70 ppm

$$\%\text{Recovery} = \left(\frac{6.51}{6.70}\right) \times 100 = 97.2\%$$

13 ACCEPTABILITY CRITERIA

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

13.1 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 (SSTL, standard 1) should be performed prior to injection of a sample set (Section 11.3.1).

It is advised that the analyst check the chromatograms of the system suitability injections to ensure that all the monitored ions are detected. In addition, a reproducible FBZ-SO_2/FBZ-SO_2-D_3 peak area ratio and FBZ-SO_2 retention times with % CV ≤ 5% must be met for the at least five consecutive injections of standard 1.

The raw data and calculated results from the at least five consecutive injections are documented with each injection set.

13.2 Quality Control Sample Acceptance Criteria

The results of the QC samples will provide the basis for accepting or rejecting the analytical run. The acceptance criterion for the accuracy of QC samples is 80% to 110%.

13.3 Standard Calibration Curve

The non-weighted linear regression should have a coefficient of determination (r^2) $\geq$0.99 for a standard curve of FBZ-SO_2 ranging from 2.0 ppm to 15 ppm (tissue equivalent).

14 LIMIT OF QUANTITATION

Based on the approach recommended in VICH GL 49 (Annex 2), an estimated limit of quantitation (LOQ) calculated from calibration curve data generated during the validation was 0.710 ppm tissue equivalent.

The upper limit of quantitation (ULOQ) is set at the highest concentration of FBZ-SO_2 in the calibration standard curve, 15 ppm tissue equivalent.

15 LIMIT OF DETECTION

Based on the approach recommended in VICH GL 49 (Annex 2), an estimated limit of detection (LOD) calculated from calibration curve data generated during the validation was 0.234 ppm tissue equivalent.

16 DILUTION

After analysis, samples found to have concentrations above the method calibration range should be diluted with control turkey liver tissue as appropriate, and reanalyzed. 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 FBZ-SO_2 and FBZ-SO_2-D_3 Stock Standard Solutions or Working Standard Solutions

All stock standard solutions (Sections 7) stored at -20 °C are stable for 83 days. All working standard solutions (Sections 7) stored at -20 °C are stable for 78 days.

17.2 Stability of Tissue Extract

Tissue methanol extract is stable for at least 23 day time period at refrigeration storage.

17.3 Stability of Samples in Mobile Phase

Extracted samples in 96-well plates or autosampler vials stored in the autosampler at room temperature are stable for at least 15 days.

17.4 Stability of Samples after 4 freeze-thaw cycles

Samples are stable after 4 freeze-thaw cycles.

17.5 Stability in Matrix at Room Temperature

Samples are stable at room temperature for at least 24 hours.

17.6 Long Term Freezer Storage Stability

The long term freezer storage stability of FBZ-SO_2 in turkey liver tissue stored in a -80°C freezer was established for 6 months duration.

18 NOTES TO ANALYSTS

18.1 Minimization of Carryover

To minimize possible carryover of FBZ-SO_2, it is recommended to inject at least one solvent blank (MilliQ Water/Acetonitrile, 70/30, v/v) after injection of a high concentration calibration standards or sample.

18.2 Data Not Used

Data from blank solvent injections and conditioning samples are not used, neither are the over-ranged samples (calculated concentration above highest calibration standard) and over-diluted samples (calculated concentration below the lowest standard). The data not used should be identified, and the reason for rejection of data should be documented.

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 → 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 abundance of m/z 159 and m/z 104 to the base peak, m/z 300 and the relative retention time. The relative abundance of each ion is calculated as described below:

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

19.2 Acceptance Criteria

Acceptance criteria for confirmatory analysis are as follows:

- Relative abundance ratio of the confirmatory transitions to the determinative transition in 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-SO_2 in the turkey liver tissue extract. The control turkey liver tissue must fail to confirm. Failure to confirm is concluded if the extract does not meet one or more of the confirmation criteria outlined above in either/both qualifiers.

20 SUMMARY OF DETERMINATIVE METHOD CONCENTRATION DATA FOR BLINDED UNTREATED AND INCURRED SAMPLES ANALYZED IN THE REFERENCE LABORATORY DURING A METHOD TRIAL STUDY OF THE METHOD

Randomly Assigned ID	Concentration (ppm)
1261P	BLQ
4566P	BLQ
6095P	BLQ
6181P	BLQ
6344P	BLQ

1943P	3.84
2356P	3.82
6091P	3.93
8220P	4.00
8881P	3.78
Average	3.87
%CV	2.3

1655P	7.38
6193P	6.91
6279P	7.14
6600P	7.02
6792P	6.91
Average	7.07
%CV	2.8

21 SUMMARY RESULTS AND EXAMPLE CHROMATOGRAMS FROM THE SINGLE-LABORATORY VALIDATION

This section contains results and data from Phase III runs in the single-laboratory validation.

21.1 Summary of Double Blank, Control Blank, and QC Results

Run ID	Sample ID	Nominal Conc. (ppm)	Ret Time (min)	Area	IS Area	Peak Area Ratio	Conc. Found (ppm)	%Rec.
6	Double Blank	0	1.26	573	546	1.049451	N/A	N/A
6	Double Blank	0	1.28	123	21	5.857143	N/A	N/A
7	Double Blank	0	1.25	264	130	2.030769	N/A	N/A
7	Double Blank	0	1.26	132	24	5.500000	N/A	N/A
8	Double Blank	0	1.24	122	34	3.588235	N/A	N/A
8	Double Blank	0	1.23	97	4	24.250000	N/A	N/A
6	Control Blank	0	1.26	1163	203321	0.005720	-1.98[a]	N/A
6	Control Blank	0	1.26	1225	195510	0.006266	-1.97[a]	N/A
7	Control Blank	0	1.25	1700	220799	0.007699	-0.0913[a]	N/A
7	Control Blank	0	1.25	1298	213996	0.006066	-0.0956[a]	N/A
8	Control Blank	0	1.24	1258	214474	0.005866	-1.41[a]	N/A
8	Control Blank	0	1.24	1252	210527	0.005947	-1.41[a]	N/A
6	QC1	3.00	1.26	229436	195245	1.175118	2.93	97.7
6	QC1	3.00	1.26	233071	197676	1.179056	2.94	98.0
7	QC1	3.00	1.25	249933	216167	1.156203	2.91	97.0
7	QC1	3.00	1.25	231581	200789	1.153355	2.91	97.0
8	QC1	3.00	1.24	264410	224482	1.177867	2.94	98.0
8	QC1	3.00	1.24	249771	217646	1.147602	2.86	95.3
							mean	97.3
							%CV	1.0
6	QC2	6.00	1.26	450488	195937	2.299147	5.94	99.0
6	QC2	6.00	1.26	434888	198783	2.187752	5.65	94.2
7	QC2	6.00	1.25	459290	209771	2.189483	5.62	93.7
7	QC2	6.00	1.25	510067	221494	2.302848	5.91	98.5
8	QC2	6.00	1.24	489050	218885	2.234278	5.72	95.3
8	QC2	6.00	1.24	481907	213589	2.256235	5.78	96.3
							mean	96.2
							%CV	2.3
6	QC3	12.0	1.26	887840	198190	4.479742	11.8	98.3
6	QC3	12.0	1.26	935624	195219	4.792689	12.6	105.0
7	QC3	12.0	1.24	979327	226111	4.331178	11.2	93.3
7	QC3	12.0	1.25	951804	210276	4.526451	11.7	97.5
8	QC3	12.0	1.24	1000145	221597	4.513351	11.7	97.5
8	QC3	12.0	1.24	1017951	222164	4.581980	11.9	99.2
							mean	98.3
	[a]BLQ<2.00 ppm						%CV	3.8

21.2 Summary Confirmatory Results

Summary of Confirmatory Results for Blank Samples and Blinded Blank Samples

Run ID	Sample ID	Relative Abundance Peak Area Ratio to *m/z* 300				Retention Time (min)						S/N>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
6	6 015 S16295-00 Double Blank	67.5	51.0-71.0	16.2	10.0-30.0	1.26	1.20-1.32	1.26	1.20-1.32	1.25	1.19-1.32	7	4
	6 016 S16295-00 Double Blank	9.8	51.0-71.0	0.0	10.0-30.0	1.28	1.20-1.32	1.28	1.20-1.32	NA	1.19-1.32	1	0
7	7 015 S16295-00 Double Blank	97.0	51.2-71.2	NA	10.1-30.1	1.25	1.18-1.31	1.25	1.18-1.31	NA	1.18-1.30	3	0
	7 016 S16295-00 Double Blank	90.2	51.2-71.2	NA	10.1-30.1	1.26	1.18-1.31	1.25	1.18-1.31	NA	1.18-1.30	2	0
8	8 020 S15231-00 Double Blank 1	79.5	50.7-70.7	NA	10.0-30.0	1.24	1.18-1.30	1.24	1.18-1.30	NA	1.18-1.30	3	0
	8 021 S15231-00 Double Blank 1	76.3	50.7-70.7	NA	10.0-30.0	1.23	1.18-1.30	1.24	1.18-1.30	NA	1.18-1.30	2	0
6	6 017 S16295-00 Control Blank	57.0	51.0-71.0	20.0	10.0-30.0	1.26	1.20-1.32	1.26	1.20-1.32	1.25	1.19-1.32	15	11
	6 018 S16295-00 Control Blank	63.8	51.0-71.0	19.4	10.0-30.0	1.26	1.20-1.32	1.26	1.20-1.32	1.27	1.19-1.32	14	5
7	7 017 S16295-00 Control Blank	64.5	51.2-71.2	22.7	10.1-30.1	1.25	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	29	22
	7 018 S16295-00 Control Blank	66.3	51.2-71.2	16.7	10.1-30.1	1.25	1.18-1.31	1.25	1.18-1.31	1.24	1.18-1.30	19	9
8	8 022 S15231-00 Control Blank 1	54.5	50.7-70.7	19.8	10.0-30.0	1.24	1.18-1.30	1.24	1.18-1.30	1.24	1.18-1.30	17	16
	8 023 S15231-00 Control Blank 1	70.5	50.7-70.7	25.0	10.0-30.0	1.24	1.18-1.30	1.23	1.18-1.30	1.23	1.18-1.30	16	21
6	6 023 S16295-00 1261P 1	63.7	51.0-71.0	17.6	10.0-30.0	1.26	1.20-1.32	1.25	1.20-1.32	1.25	1.19-1.32	12	6
	6 027 S16295-00 4566P1	63.2	51.0-71.0	16.5	10.0-30.0	1.26	1.20-1.32	1.26	1.20-1.32	1.25	1.19-1.32	13	7
7	7 024 S16295-00 6095P	56.7	51.2-71.2	23.8	10.1-30.1	1.25	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	20	18
	7 025 S16295-00 6181P	63.7	51.2-71.2	20.2	10.1-30.1	1.24	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	15	9
8	8 023 S16295-00 6344P1	60.3	50.7-70.7	16.4	10.0-30.0	1.25	1.18-1.30	1.23	1.18-1.30	1.24	1.18-1.30	20	8

Summary Confirmatory Results for Standards											
					RAPAR*		Retention Time (min)			S/N Ratio (>50)	
Run ID	Sample ID	*m/z* 300 peak area	*m/z* 159 peak area	*m/z* 104 peak area	*m/z* 159	*m/z* 104	*m/z* 300	*m/z* 159	*m/z* 104	*m/z* 159	*m/z* 104
6	6 007 S16295-00 Liver-Std-1 1	327639	200917	66190	61.3	20.2	1.27	1.26	1.26	5020	2170
	6 008 S16295-00 Liver-Std-2 1	505608	304898	98319	60.3	19.4	1.27	1.26	1.26	6610	2760
	6 009 S16295-00 Liver-Std-3 1	737754	451961	151223	61.3	20.5	1.26	1.26	1.26	12900	3200
	6 010 S16295-00 Liver-Std-4 1	1011100	599931	199121	59.3	19.7	1.26	1.26	1.26	14700	4800
	6 011 S16295-00 Liver-Std-5 1	1244302	779102	253411	62.6	20.4	1.26	1.26	1.26	19000	7020
	6 012 S16295-00 Liver-Std-6 1	1767763	1059103	348479	59.9	19.7	1.26	1.26	1.26	16900	9140
	6 013 S16295-00 Liver-Std-7 1	2574061	1583596	520401	61.5	20.2	1.26	1.26	1.25	26500	13700
	6 033 S16295-00 Liver-Std-1 2	347453	212224	68485	61.1	19.7	1.26	1.25	1.25	5610	1570
	6 034 S16295-00 Liver-Std-2 2	537587	324954	106240	60.4	19.8	1.26	1.25	1.25	7120	2440
	6 035 S16295-00 Liver-Std-3 2	770974	473545	157281	61.4	20.4	1.26	1.25	1.25	11000	4070
	6 036 S16295-00 Liver-Std-4 2	1015792	620336	207689	61.1	20.4	1.26	1.25	1.25	16300	5290
	6 037 S16295-00 Liver-Std-5 2	1295288	801634	259197	61.9	20.0	1.26	1.25	1.25	13400	6850
	6 038 S16295-00 Liver-Std-6 2	1800251	1098244	362255	61.0	20.1	1.26	1.25	1.25	17800	14000
	6 039 S16295-00 Liver-Std-7 2	2674069	1640734	534024	61.4	20.0	1.26	1.25	1.25	43300	12800
				Average	61.0	20.0	1.26	1.26	1.25	N/A	
7	7 007 S16295-00 Liver-Std-1 1	363231	223005	74263	61.4	20.4	1.25	1.25	1.25	6389	1581
	7 008 S16295-00 Liver-Std-2 1	564381	335872	111366	59.5	19.7	1.25	1.25	1.24	4953	1862
	7 009 S16295-00 Liver-Std-3 1	822569	504901	162775	61.4	19.8	1.25	1.25	1.24	14540	2757
	7 010 S16295-00 Liver-Std-4 1	1116711	667444	219681	59.8	19.7	1.25	1.25	1.24	17358	3761
	7 011 S16295-00 Liver-Std-5 1	1359198	836285	277003	61.5	20.4	1.25	1.25	1.24	18119	4638
	7 012 S16295-00 Liver-Std-6 1	1879910	1170609	380037	62.3	20.2	1.25	1.25	1.24	24109	9062
	7 013 S16295-00 Liver-Std-7 1	2854444	1756232	562598	61.5	19.7	1.25	1.25	1.24	38125	15052
	7 007 S16295-00 Liver-Std-1 2	378022	229951	76179	60.8	20.2	1.25	1.24	1.24	5544	1827
	7 008 S16295-00 Liver-Std-2 2	572989	345651	114493	60.3	20.0	1.25	1.24	1.24	6592	2685
	7 009 S16295-00 Liver-Std-3 2	849932	522419	170618	61.5	20.1	1.25	1.24	1.24	12813	4134
	7 010 S16295-00 Liver-Std-4 2	1096404	699017	227560	63.8	20.8	1.24	1.24	1.24	10442	4235
	7 011 S16295-00 Liver-Std-5 2	1415211	869007	283420	61.4	20.0	1.24	1.24	1.24	18951	4870
	7 012 S16295-00 Liver-Std-6 2	1955159	1195604	399022	61.2	20.4	1.24	1.24	1.24	21638	11042
	7 013 S16295-00 Liver-Std-7 2	2964571	1794071	583048	60.5	19.7	1.24	1.24	1.24	32203	14300
				Average	61.2	20.1	1.25	1.25	1.24	N/A	
8	8 007 S16295-00 Liver-Std-1 1	372387	230417	72412	61.9	19.4	1.25	1.24	1.24	4946	4778
	8 008 S16295-00 Liver-Std-2 1	574532	344293	113366	59.9	19.7	1.25	1.24	1.24	8494	5812
	8 009 S16295-00 Liver-Std-3 1	841156	512032	167815	60.9	20.0	1.25	1.24	1.24	12931	6709
	8 010 S16295-00 Liver-Std-4 1	1115975	681641	224846	61.1	20.1	1.25	1.24	1.24	11833	13720
	8 011 S16295-00 Liver-Std-5 1	1434416	848496	282893	59.2	19.7	1.24	1.24	1.24	19692	15536
	8 012 S16295-00 Liver-Std-6 1	1979875	1188217	395661	60.0	20.0	1.25	1.24	1.24	22211	19376
	8 013 S16295-00 Liver-Std-7 1	2953002	1763505	593624	59.7	20.1	1.24	1.24	1.24	24631	25982
	8 033 S16295-00 Liver-Std-1 2	378972	228729	75295	60.4	19.9	1.24	1.24	1.24	5523	3560
	8 034 S16295-00 Liver-Std-2 2	572660	352582	116077	61.6	20.3	1.24	1.24	1.23	11166	5944
	8 035 S16295-00 Liver-Std-3 2	831316	503081	168523	60.5	20.3	1.24	1.24	1.24	13107	6406
	8 036 S16295-00 Liver-Std-4 2	1139935	691050	229539	60.6	20.1	1.24	1.24	1.23	17371	10154
	8 037 S16295-00 Liver-Std-5 2	1433043	861406	282147	60.1	19.7	1.24	1.24	1.23	20701	14509
	8 038 S16295-00 Liver-Std-6 2	1935258	1201073	397371	62.1	20.5	1.24	1.24	1.23	25195	14677
	8 039 S16295-00 Liver-Std-7 2	2889287	1784277	580759	61.8	20.1	1.24	1.24	1.23	29095	18447
				Average	60.7	20.0	1.24	1.24	1.24	N/A	

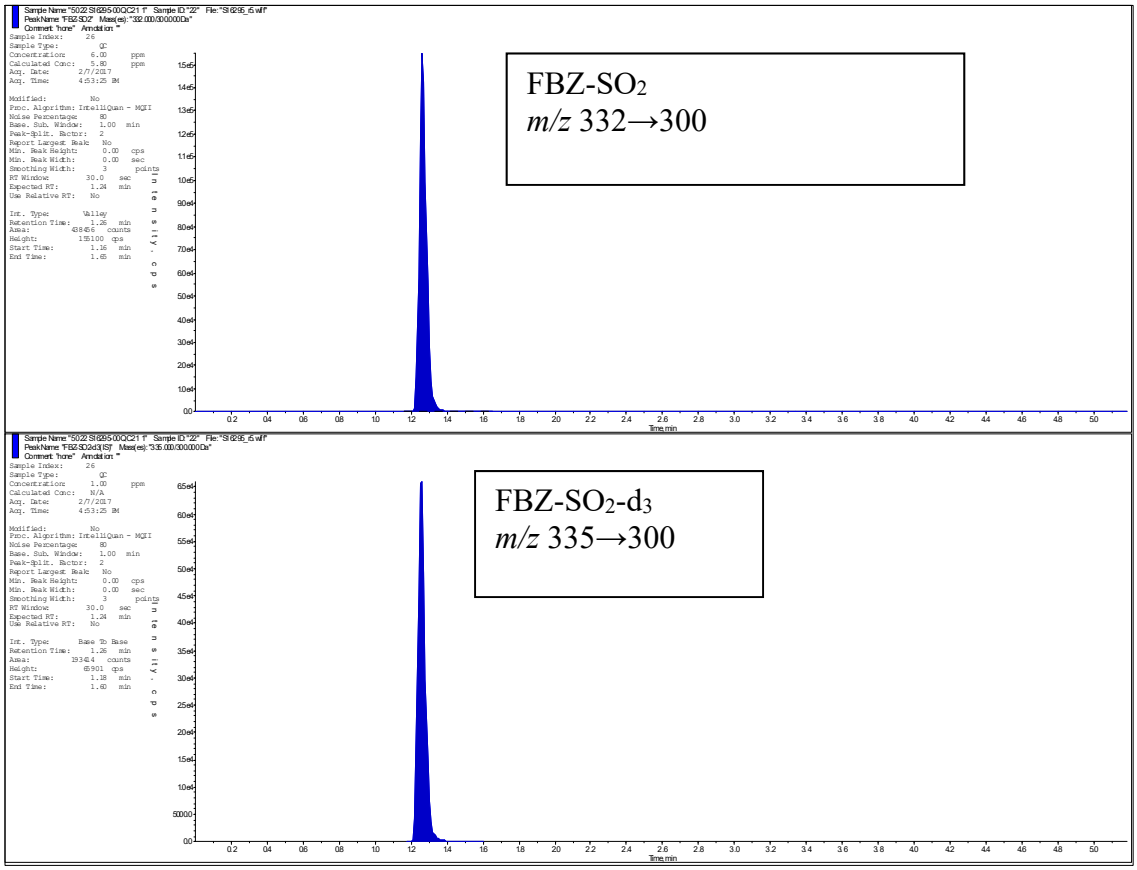
*RAPAR = relative abundance peak area ratio to *m/z* 300

Summary of Confirmatory Results for QC's and Blinded Samples

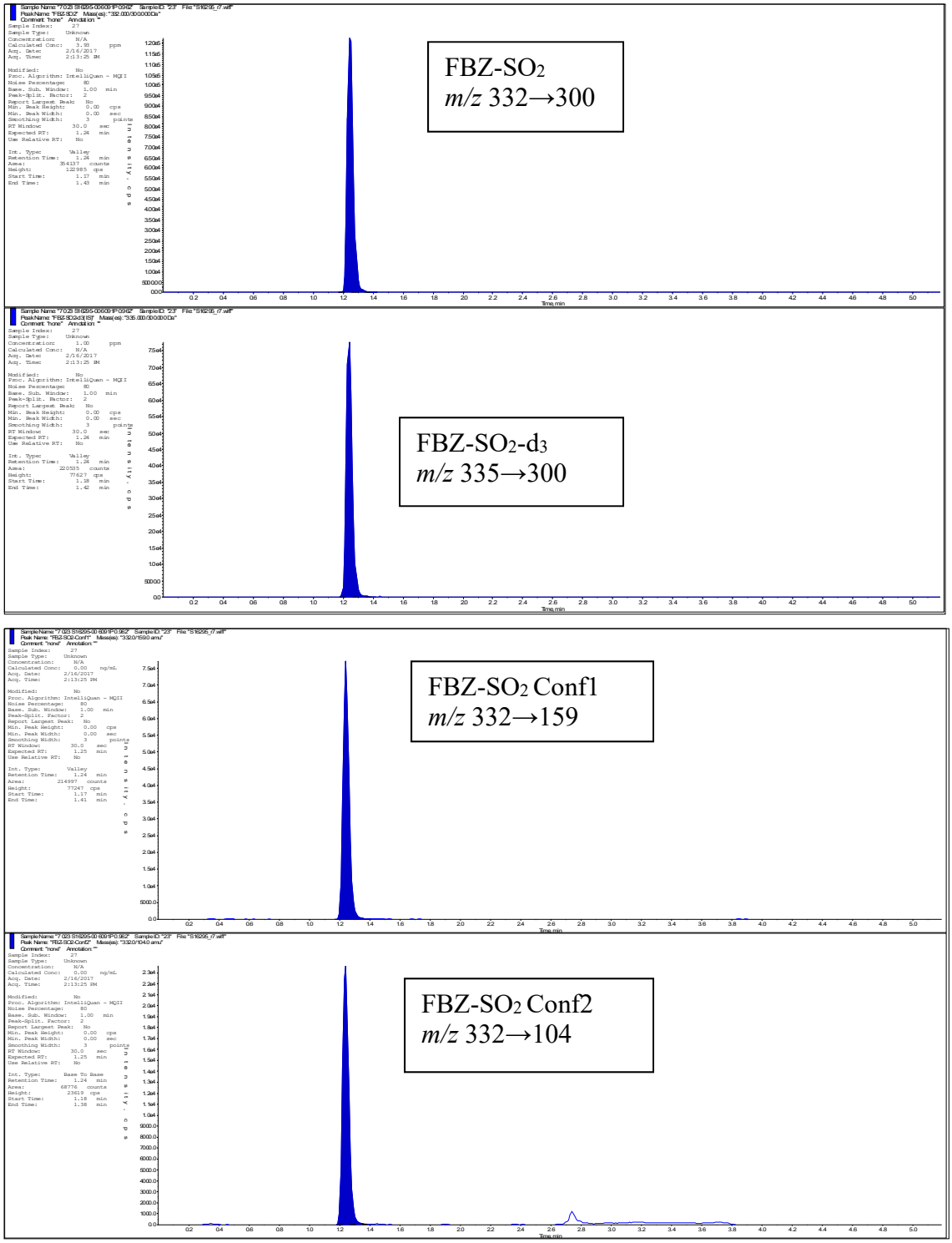
Run ID	Sample ID	Relative Abundance Peak Area Ratio to *m/z* 300				Retention Time (min)						S/N>50	
		m/z 159		*m/z* 104		*m/z* 300		*m/z* 159		*m/z 104*		m/z 159	m/z 104
		Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Individual
6	6 019 S16295-00 QC1 1	61.8	51.0-71.0	19.9	10.0-30.0	1.26	1.20-1.32	1.26	1.20-1.32	1.25	1.19-1.32	4710	1350
	6 020 S16295-00 QC1 2	59.6	51.0-71.0	20.0	10.0-30.0	1.26	1.20-1.32	1.25	1.20-1.32	1.25	1.19-1.32	2640	1410
7	7 019 S16295-00 QC1 1	60.6	51.2-71.2	20.0	10.1-30.1	1.25	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	2613	2662
	7 019 S16295-00 QC1 2	61.8	51.2-71.2	20.1	10.1-30.1	1.25	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	3781	2204
8	8 019 S16295-00 QC1 1	61.4	50.7-70.7	19.9	10.0-30.0	1.24	1.18-1.30	1.24	1.18-1.30	1.23	1.18-1.30	3967	2702
	8 029 S16295-00 QC1 2	60.7	50.7-70.7	20.0	10.0-30.0	1.24	1.18-1.30	1.24	1.18-1.30	1.23	1.18-1.30	3573	2343
6	6 020 S16295-00 QC2 1	58.5	51.0-71.0	19.3	10.0-30.0	1.26	1.20-1.32	1.26	1.20-1.32	1.25	1.19-1.32	7830	4510
	6 030 S16295-00 QC2 2	62.3	51.0-71.0	20.5	10.0-30.0	1.26	1.20-1.32	1.25	1.20-1.32	1.25	1.19-1.32	5850	2880
7	7 020 S16295-00 QC2 1	63.7	51.2-71.2	20.6	10.1-30.1	1.25	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	6098	4615
	7 020 S16295-00 QC2 2	60.1	51.2-71.2	20.3	10.1-30.1	1.25	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	5414	4054
8	8 020 S16295-00 QC2 1	61.3	50.7-70.7	20.1	10.0-30.0	1.24	1.18-1.30	1.23	1.18-1.30	1.23	1.18-1.30	6219	5630
	8 030 S16295-00 QC2 2	61.2	50.7-70.7	19.8	10.0-30.0	1.24	1.18-1.30	1.24	1.18-1.30	1.23	1.18-1.30	7295	7285
6	6 021 S16295-00 QC3 1	60.7	51.0-71.0	20.2	10.0-30.0	1.26	1.20-1.32	1.26	1.20-1.32	1.25	1.19-1.32	13100	6560
	6 031 S16295-00 QC3 2	58.7	51.0-71.0	19.7	10.0-30.0	1.26	2.96-3.28	1.26	1.20-1.32	1.25	1.19-1.32	17900	5620
7	7 021 S16295-00 QC3 1	61.5	51.2-71.2	21.1	10.1-30.1	1.24	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	20853	5655
	7 021 S16295-00 QC3 2	61.3	51.2-71.2	20.2	10.1-30.1	1.25	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	14675	6324
8	8 021 S16295-00 QC3 1	61.9	50.7-70.7	20.1	10.0-30.0	1.24	1.18-1.30	1.23	1.18-1.30	1.23	1.18-1.30	14291	12958
	8 031 S16295-00 QC3 2	61.6	50.7-70.7	20.0	10.0-30.0	1.24	1.18-1.30	1.24	1.18-1.30	1.23	1.18-1.30	14604	10628
6	6 024 S16295-00 1655P	59.2	51.0-71.0	19.9	10.0-30.0	1.26	1.20-1.32	1.26	1.20-1.32	1.25	1.19-1.32	8970	3800
	6 025 S16295-00 1943P	61.6	51.0-71.0	19.5	10.0-30.0	1.26	1.20-1.32	1.26	1.20-1.32	1.25	1.19-1.32	5370	1660
	6 026 S16295-00 2356P	60.0	51.0-71.0	19.9	10.0-30.0	1.26	1.20-1.32	1.26	1.20-1.32	1.25	1.19-1.32	5110	1940
7	7 023 S16295-00 6091P	60.7	51.2-71.2	19.4	10.1-30.1	1.24	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	4018	2347
	7 026 S16295-00 6193P	62.2	51.2-71.2	20.6	10.1-30.1	1.25	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	8621	5120
	7 027 S16295-00 6279P	60.8	51.2-71.2	19.7	10.1-30.1	1.25	1.18-1.31	1.24	1.18-1.31	1.24	1.18-1.30	6040	4716
8	8 024 S16295-00 6600P	60.7	50.7-70.7	20.3	10.0-30.0	1.24	1.18-1.30	1.24	1.18-1.30	1.23	1.18-1.30	7105	6928
	8 025 S16295-00 6792P	61.4	50.7-70.7	20.5	10.0-30.0	1.24	1.18-1.30	1.23	1.18-1.30	1.23	1.18-1.30	11638	5872
	8 026 S16295-00 8220P	59.5	50.7-70.7	19.2	10.0-30.0	1.24	1.18-1.30	1.23	1.18-1.30	1.23	1.18-1.30	6978	3225
	8 027 S16295-00 8881P	62.1	50.7-70.7	20.5	10.0-30.0	1.24	1.18-1.30	1.24	1.18-1.30	1.23	1.18-1.30	5099	2352

21.3 Example Chromatograms of QC and Blinded Samples

21.3.1 Example Chromatogram of QC Sample (6 ppm, Run 5, 1 of 2)



21.3.2 Example Chromatogram of Blinded Sample (3.93 ppm, Run 7, Sample ID 6091P)



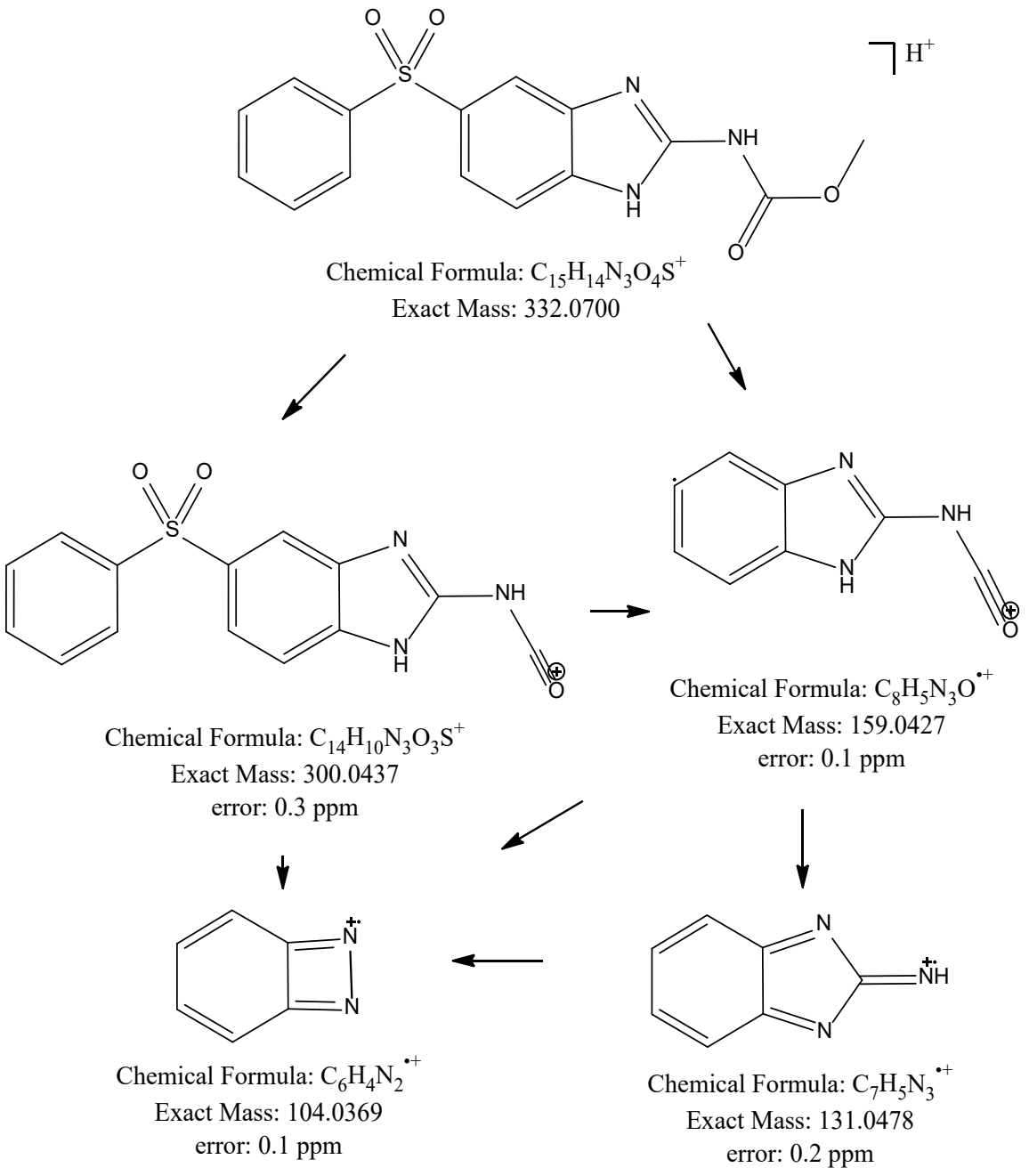
22 FRAGMENTATION REPORT FROM FT-ICR

A Bruker solariX™ 9.4-T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer with a quadrupole front end was used to conduct the experiments. The sample was introduced *via* Advion Triversa NanoMate™ robot and used as provided. A CAD (Collision-Activated Dissociation) experiment was performed in the collision cell portion of the Bruker FT-ICR instrument with argon as the collision gas, while the isolation occurred in the first quadrupole.

22.1 Proposed Fragmentation Pathways for Fenbendazole Sulfone at m/z 332

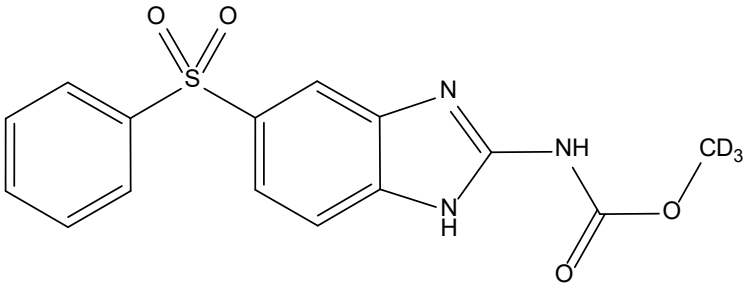
Proposed analyte product ions are 300, 159, and 104. The proposed product ion for determinative analysis is 300. The proposed product ions for confirmatory analysis are 159 and 104. The collision energy used was 40 eV.

See following figure.

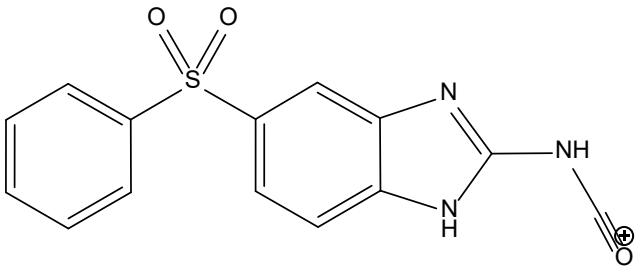

Chemical Formula: $C_{15}H_{14}N_3O_4S^+$
Exact Mass: 332.0700
Chemical Formula: $C_{14}H_{10}N_3O_3S^+$
Exact Mass: 300.0437
error: 0.3 ppm
Chemical Formula: $C_8H_5N_3O^{\bullet+}$
Exact Mass: 159.0427
error: 0.1 ppm
Chemical Formula: $C_6H_4N_2^{\bullet+}$
Exact Mass: 104.0369
error: 0.1 ppm
Chemical Formula: $C_7H_5N_3^{\bullet+}$
Exact Mass: 131.0478
error: 0.2 ppm

22.2 Proposed Fragmentation Pathways for Fenbendazole Sulfone-D_3 at m/z 335

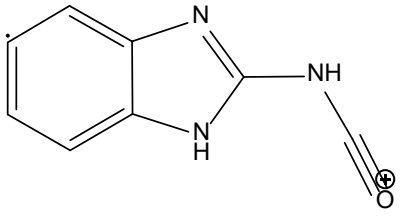
Proposed IS product ion is 300, for determinative analysis only. Internal standard is not used for confirmatory analysis. The collision energy used was 30 eV.



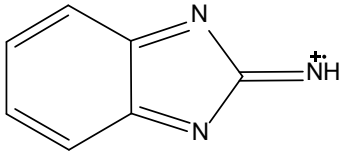
Chemical Formula: $C_{15}H_{11}D_3N_3O_4S^+$
Exact Mass: 335.0888



Chemical Formula: $C_{14}H_{10}N_3O_3S^+$
Exact Mass: 300.0437
error: 0.6 ppm



Chemical Formula: $C_8H_5N_3O^{\bullet+}$
Exact Mass: 159.0427
error: 0.3ppm



Chemical Formula: $C_7H_5N_3^{\bullet+}$
Exact Mass: 131.0478
error: 0.2 ppm

23 MATERIAL SAFETY DATA SHEETS (MSDS) FOR FENBENDAZOLE SULFONE AND FENBENDAZOLE SULFONE-D_3

23.1 MSDS for Fenbendazole Sulfone

SIGMA-ALDRICH sigma-aldrich.com

Material Safety Data Sheet

Version 5.0
Revision Date 03/22/2013
Print Date 11/26/2013

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Fenbendazole sulfone
Product Number	:	32544
Brand	:	Fluka
Supplier	:	Sigma-Aldrich 3050 Spruce Street SAINT LOUIS MO 63103 USA
Telephone	:	+1 800-325-5832
Fax	:	+1 800-325-5052
Emergency Phone # (For both supplier and manufacturer)	:	(314) 776-6555
Preparation Information	:	Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956

2. HAZARDS IDENTIFICATION

Emergency Overview

OSHA Hazards
Harmful by ingestion., Skin sensitiser, Irritant

GHS Classification
Acute toxicity, Oral (Category 4)
Skin irritation (Category 2)
Skin sensitisation (Category 1)

GHS Label elements, including precautionary statements

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)

P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
P301 + P312	IF SWALLOWED: Call a POISON CENTER or doctor/ physician if you feel unwell.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P321	Specific treatment (see supplemental first aid instructions on this label).
P330	Rinse mouth.
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362	Take off contaminated clothing and wash before reuse.
P501	Dispose of contents/ container to an approved waste disposal plant.

HMIS Classification

Health hazard:	2
Flammability:	0
Physical hazards:	0

NFPA Rating

Health hazard:	2
Fire:	0
Reactivity Hazard:	0

Potential Health Effects

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

3. COMPOSITION/INFORMATION ON INGREDIENTS

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

Component		Concentration
Fenbendazole sulfone		
CAS-No.	54029-20-8	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

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.

5. FIREFIGHTING MEASURES

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

Special protective equipment for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Sulphur oxides

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust.

Environmental precautions
Do not let product enter drains.

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.

7. HANDLING AND STORAGE

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.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

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).

Hand 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.

Eye 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 and 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.

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

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	colourless

Safety data

pH	no data available
Melting point/freezing point	> 320 °C (> 608 °F)
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Auto-ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available

Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	log Pow: 2.0
Relative vapour density	no data available
Odour	odourless
Odour Threshold	no data available
Evapouration rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Strong acids and strong bases, Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Sulphur oxides
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
no data available

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on 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.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	No component of this product present at levels greater than or equal to 0.1% is identified as a

known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

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

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

Synergistic effects
no data available

Additional Information
RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

OSHA Hazards
Harmful by ingestion., Skin sensitiser, Irritant

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Acute Health Hazard

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Fenbendazole sulfone	54029-20-8	

New Jersey Right To Know Components

	CAS-No.	Revision Date
Fenbendazole sulfone	54029-20-8	

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

Further information

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 liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

23.2 MSDS for Fenbendazole Sulfone-D_3

SIGMA-ALDRICH sigma-aldrich.com

Material Safety Data Sheet

Version 5.0
Revision Date 01/26/2011
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1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Fenbendazole sulfone-d_3		
Product Number	:	32545		
Brand	:	Fluka		
Product Use	:	For laboratory research purposes.		
Supplier	:	Sigma-Aldrich 3050 Spruce Street SAINT LOUIS MO 63103 USA	Manufacturer :	Sigma-Aldrich Corporation 3050 Spruce St. St. Louis, Missouri 63103 USA
Telephone	:	+1 800-325-5832		
Fax	:	+1 800-325-5052		
Emergency Phone # (For both supplier and manufacturer)	:	(314) 776-6555		
Preparation Information	:	Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956		

2. HAZARDS IDENTIFICATION

Emergency Overview

OSHA Hazards
Toxic by ingestion, Skin sensitiser, Irritant

GHS Classification
Acute toxicity, Oral (Category 4)
Skin irritation (Category 2)
Skin sensitization (Category 1)

GHS Label elements, including precautionary statements

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.

HMIS Classification

Health hazard:	2
Flammability:	0
Physical hazards:	0

NFPA Rating

Health hazard:	2
Fire:	0
Reactivity Hazard:	0

Potential Health Effects

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

3. COMPOSITION/INFORMATION ON INGREDIENTS

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

Formula : $C_{15}D_3H_{10}N_3O_4S$ $C_{15}D_3H_{10}N_3O_4S$
Molecular Weight : 334.36 g/mol

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

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

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.

5. FIRE-FIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

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

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Sulphur oxides

6. ACCIDENTAL RELEASE MEASURES

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

Environmental precautions
Do not let product enter drains.

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.

7. HANDLING AND STORAGE

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.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

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).

Hand 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.

Eye 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 and 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.

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

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	colourless

Safety data

pH	no data available
Melting/freezing point	> 310 °C (> 590 °F)
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	odourless

Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Strong acids and strong bases, Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Sulphur oxides
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
no data available

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on 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.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

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

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

Synergistic effects
no data available

Additional Information
RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

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.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

OSHA Hazards
Toxic by ingestion, Skin sensitiser, Irritant

DSL Status
This product contains the following components that are not on the Canadian DSL nor NDSL lists.

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

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Acute Health Hazard

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Fenbendazole sulfone-d3 VETRANAL®	1228182-49-7	

New Jersey Right To Know Components

	CAS-No.	Revision Date
Fenbendazole sulfone-d3 VETRANAL®	1228182-49-7	

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

Further information

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 Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.