Target Analyte: Oxfendazole	MERCK Animal Health

METHOD TITLE: Determinative and Confirmatory Procedures for the Analysis of Oxfendazole in Cattle Milk Using LC-MS/MS, Version 6

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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
ALQ	Above Limit of Quantitation
amu	Atomic Mass Unit
BA	Bioanalytical
ca.	Circa
CV	Coefficient of Variation
DMSO	Dimethyl Sulfoxide
HPLC	High Performance Liquid Chromatography
LC-MS/MS	High Performance Liquid Chromatography – Tandem Mass Spectrometry
IS	Internal Standard
LOD	Limit of Detection
LOQ	Limit of Quantitation
MAH	Merck Animal Health
SDS	Safety Data Sheet
n	Number of Samples
NA	Not Applicable
ppb	Parts per billion
psi	Pounds per Square Inch
QC	Quality Control (fortified milk)
Control Blank	Blank matrix sample, fortified with IS only
Double Blank	Double Blank matrix sample, not fortified with IS or analyte
Oxfendazole	FBZ-SO
RAR	Relative Abundance Ratio
Rcf	Relative centrifugal force
$R_{S/N}$	Signal to Noise Ratio
rpm	Rotations per Minute
s	Second
SDS	Safety Data Sheet
SL	(Working) Standard Level
SST	System Suitability Test
STD	Standard Calibrator
ULOQ	Upper Limit of Quantitation
v/v	Volume per Volume

2 SCOPE AND FIELD OF APPLICATION

Oxfendazole is a metabolite of fenbendazole. Fenbendazole is a broad spectrum benzimidazole anthelmintic approved for use in dairy cattle. The presumptive tolerance is 220 ppb in cattle milk. This procedure describes the determinative and confirmatory method for the analysis of oxfendazole in cattle raw milk. The analytical range of the method is 60 – 550 ppb oxfendazole equivalents in milk (0.3 – 2.75 ng/mL).

3 PRINCIPLE

Approximately one gram of homogenized cattle raw milk is fortified with internal standard (oxfendazole-D_3) and then extracted twice with methanol in two extraction steps. The sample extract is combined and the final volume is adjusted to 20 mL with methanol. An aliquot of the methanol extract (0.1 mL) is diluted with water (0.9 mL). The resulting solution is quantitatively analyzed using gradient reverse phase liquid chromatography with mass-spectrometric detection (LC-MS/MS) using a positive ion multiple-reaction monitoring (MRM) with ion transition of *m/z* 316 → *m/z* 159 for oxfendazole and *m/z* 319 →*m/z* 159 for oxfendazole-D_3.

Additional ion transitions from oxfendazole, m/z 316 → *m/z* 191 as qualifier 1 and *m/z* 316 → *m/z* 284 as qualifier 2 are monitored along with m/z 316 →159 (used for determinative method), are used for the confirmatory method.

Refer to section 19 for fragmentation scheme.

4 WARNINGS AND SAFETY PRECAUTIONS

Take safety precautions common in the laboratory, *e.g.* wear lab coat, goggles and gloves if necessary.

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. Chemical formulas or abbreviations are in parenthesis. 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	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)	Optima, HPLC, or MilliQ	Fisher

5.2 Solutions

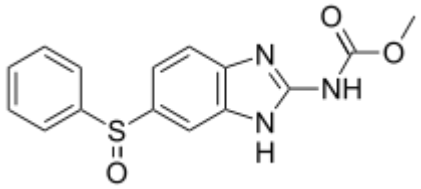
The following solutions may be prepared (by volume-to-volume equivalence or by dilution) in different quantities. Measure volume using a suitably sized graduated cylinder or graduated pipette.

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 commercially available 0.1% formic acid in water. Alternately, it can be made in the lab by adding 1000 mL of MilliQ water to a glass reagent bottle using a graduated cylinder and then adding 1 mL of formic acid (88%, certified ACS or HPLC grade) using a pipette, mix well. Mix well. Store at room temperature and stable for 1 month.
HPLC – Mobile Phase B: Mobile Phase B: 0.1% Formic Acid in Acetonitrile, v/v	Use commercially available 0.1% formic acid in acetonitrile. Alternately, it can be made by adding 1000 mL of acetonitrile to a glass reagent bottle using a graduated cylinder and adding 1 mL of formic acid (88%, certified ACS or HPLC grade) using a pipette, mix well. Mix well. Store at room temperature and stable for 1 month.
Autosampler Wash Solution (strong and/or weak wash): 100% Acetonitrile	Acetonitrile. Stable for 1 month at room temperature.
Solvent Blank: (Water:Methanol, 90:10, v/v)	Measure 900 mL water and 100 mL methanol in a graduated cylinder. Mix well. Store at room temperature and stable for 1 month.

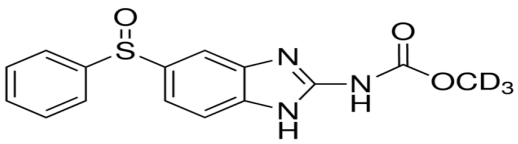
5.3 Reference Standard

The reference standard, oxfendazole, and the internal standard, oxfendazole-D_3, are commercially available and the purities of the currently available lots are used for the relevant calculations.

5.3.1 Oxfendazole

Name:	Oxfendazole
CAS Number	53716-50-0
Chemical name:	Methyl *N*-[6-(benzenesulfinyl)-1*H*-1,3-benzodiazol-2-yl]carbamate
Formula:	$C_{15}H_{13}N_3O_3S$
Molecular weight:	315.35 g/mol
Appearance / colour:	Solid white powder
Storage conditions:	Room Temperature (20-25ºC), protect from light
Supplier:	Crescent Chemical (Islandia, NY) or USP or Equivalent
Structural formula:	

5.3.2 Oxfendazole-D_3 (Used as Internal Standard)

Name:	Oxfendazole-D_3
CAS-No.:	1228182-54-4
Chemical Name:	(5-(Phenylsulfinyl)-1H-benzimidazol-2-yl)-carbamic acid methyl- D_3
Formula:	$C_{15}H_{10}D_3N_3O_3S$
Molecular Weight:	318.37 g/mol
Appearance/Color:	Light brown crystals
Storage Conditions:	Refrigerator, 2 – 8 ºC
Supplier	Witega or Sigma-Aldrich
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 precision of at least ±0.1 mg
Balance - capable of weighing 1 g accurately (at least ±0.01 g)
Centrifuge, refrigerated – capable of attaining 4000 rpm with appropriate rotor (~3300 x g)
Cylinders - graduated – 100, 250, 500, 1000 and 2000 mL
Flasks - volumetric with glass stopper – 10, 25, 50, and 100 mL
Freezer - capable of maintaining temperatures set at ≤ -65ºC
Freezer - capable of maintaining temperatures set at -20ºC
Rainin EDP3 Pipettes and tips
Refrigerator - capable of maintaining temperatures 2-8°C
Sonicator
Vortex mixer – Vortex-Genie 2
Multi-tube vortexer

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 Or 2 mL HPLC vials - Fisher brand or equivalent

6.3 LC-MS Equipment

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

Table 6.3: LC-MS List
Primary HPLC System: Waters Acquity UPLC System including pump, autosampler and column manager
Primary HPLC Column: MacMod Ace 3 C18, 2.1 x 50 mm, Part Number ACE-111-0502
Primary MS spectrometer– Applied Biosystems, API 4000 or newer model, Triple Quadrupole
Primary LC-MS Data acquisition system – Applied Biosystems, Analyst, Version 1.4.2 or higher
Alternate HPLC System: Shimadzu HPLC system
Alternate MS spectrometer– Thermo TSQ Vantage, Triple Quadrupole
Alternate LC-MS Data acquisition system – LCQuan, version 2.6
Alternate HPLC Column: Thermo Acclaim 120, C-18; 3um; 2.1 x 50 mm
Data calculation software – Thermo Fisher Scientific, Watson LIMS, Version 7.4, and Microsoft Excel

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 for all calculations.

7.1 Oxfendazole and Oxfendazole-D_3 Stock Solution

All stock solutions of oxfendazole and oxfendazole-D_3 are prepared in DMSO.

7.1.1 Preparation of Oxfendazole STD Stock Solution at 1.00 mg/mL (Oxfendazole Stock 1)

Accurately weigh reference standard (target weight 20.0 ± 2 mg after correcting for purity) into a weighing boat (or equivalent); record the exact weight. Transfer and dissolve the standard in a 20 mL volumetric flask using DMSO. Fill to the mark with DMSO. Vortex to mix for approximately 1 minute. This solution is used for the preparation of working standard 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 stock solution is stored in a freezer set at -20°C and is stable for 92 days.

7.1.2 Preparation of Oxfendazole Quality Control Stock Solution at 1.00 mg/mL (Oxfendazole Stock 2)

This solution is prepared from a second independent weighing procedure (according to Section 7.1.1). It is applied for the preparation of quality control (QC) standards and spiking of the QC samples. The stock solution is stored in a freezer set at -20°C and is stable for 92 days.

7.1.3 Preparation of Oxfendazole-D3 Internal Standard Stock Solution at 1.00 mg/mL (Oxfendazole-D3 Stock)

Accurately weigh reference standard (target weight 10.0 ± 1 mg after correcting for purity) into a weighing boat (or equivalent) and record the exact weight. Transfer and dissolve the standard in a 10 mL volumetric flask. Fill to the mark with DMSO. Sonicate for a minimum of 1 minute, and vortex for approximately 1 minute. This solution is used for the preparation of internal standard working solutions. The actual concentration will be used to determine the required volume of stock standard solution needed when further dilutions are prepared (see Sections 7.1.4). The stock solution is stored in a freezer set at -20°C and is stable for 92 days.

7.1.4 Oxfendazole-D_3 Internal Standard Fortification Solution

Using calibrated pipettes, transfer a 200 µL[1] aliquot of the Oxfendazole-D_3 stock solution (7.1.3) into a 100 mL volumetric flask and dilute to volume with methanol (Table 7.1.4). The fortification solution is stored in a freezer set at -20°C and is stable for 65 days.

Table 7.1.4 Preparation of Oxfendazole-D_3 Internal Standard Fortification Solution for Milk

Working Solution ID	Concentration [µg/mL]	Volume of Solution	Final Volume [mL]
Oxfendazole-D_3 Fortification	2.00	[1]200 µL of oxfendazole-D_3 DMSO stock solution	100

[1]Volume should 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 200 µL nominal = 222.0 µL actual)

7.1.5 Comparison of Oxfendazole Stock Standard Solutions

A stock solution comparison is required when new oxfendazole stock solutions are prepared (Oxfendazole Stock 1 and Oxfendazole Stock 2). Two stock standard solutions are prepared as described in sections 7.1.1 and 7.1.2.

Using calibrated pipettes and 50 mL volumetric flasks, each of the two stock solutions should be properly diluted with solvent blank (Section 5.2) according to schemes in Table 7.1.5-1, to prepare two intermediate solutions.

Table 7.1.5-1: Preparation of Intermediate Solutions for Stock Comparison

Intermediate Solution ID	Conc. (ng/mL)	FBZ-SO Stock Solution		Final Volume (mL)
		Conc. (µg/mL)	Volume (µL)	
FBZ-SO STD Inter Solution	2,000	1000 (Stock Solution)	100[1]	50
FBZ-SO QC Inter Solution	2,000	1000 (QC Stock Solution)	100[1]	50

[1]Volume should 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 100 µL nominal = 111.0 µL actual)

Each of the two intermediate solutions in Table 7.1.5-1 should be properly diluted with solvent blank (Section 5.2) using calibrated pipettes and 100 mL volumetric flasks according to schemes in Table 7.1.5-2.

Table 7.1.5-2: Preparation of Final Dilutions for Stock Comparison

Final Dilution Solution ID	Conc. (ng/mL) (FBZ-SO/IS)	FBZ-SO Intermediate Solution		IS Fortification Solution (Section 7.1.4)		Final Volume (mL)
		Conc. (ng/mL)	Volume (mL)	Conc. (µg/mL)	Volume (mL)	
FBZ-SO-STD-Final Dilution	2/2	2,000	0.100	2.0	0.100	100
FBZ-SO-QC- Final Dilution	2/2	2,000	0.100	2.0	0.100	100

The two final dilution solutions are analyzed using LC-MS/MS (n=6 injections of each FBZ-SO Final Dilution solution alternating) and the results are compared for equivalence. If the mean percent difference of the peak area ratio (PAR) of Oxfendazole/ Oxfendazole-D_3 is ± 5.0% and precision (%CV) of the replicates is ≤5.0%, the stock solutions are considered equivalent.

$$\textbf{\% Difference} = 100 \times \frac{\textbf{(mean of PAR of stock 1 – mean of PAR of stock 2)}}{\textbf{((mean of PAR of stock 1 + mean of PAR of stock 2)/2)}}$$

The final dilution solutions should be used fresh and discarded after use.

7.2 Working Standards for Oxfendazole Calibration Standards (SL 1 - 6)

Using calibrated pipettes, transfer aliquots of the Oxfendazole Stock 1 (7.1.1) into 100 mL volumetric flasks and dilute with methanol according to the following scheme (Table 7.2). Mix thoroughly. All working standards are stored in a freezer set at -20°C and are stable for 65 days.

Table 7.2: Preparation of Working Standards for Oxfendazole Calibration Standards

Working Standard ID	Concentration of Working Standards [µg/mL]	Volume of Oxfendazole Stock 1 (mL)	Final Volume [mL]
SL 6	5.50	[1]0.550	100
SL5	4.00	[1]0.400	100
SL 4	3.00	[1]0.300	100
SL 3	2.20	[1]0.220	100
SL 2	1.50	[1]0.150	100
SL 1	0.600	[1]0.0600	100

[1]Volume should 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 0.550 mL nominal = 0.610 mL actual)

7.3 Preparation of Oxfendazole Quality Control Fortification Standard

Using calibrated pipettes, transfer aliquots of the Oxfendazole Stock 2 (7.1.2) into 100 mL volumetric flasks and dilute with methanol according to the following scheme (Table 7.3). Mix thoroughly. All QC fortification standards are stored in a freezer set at -20°C are stable for 90 days.

Table 7.3: Oxfendazole QC Fortification Standard for Quality Control Samples

QC Fortification Standard ID	Concentration of QC Fortification Standard [µg/mL]	Volume of Oxfendazole Stock 2 (mL)	Final Volume of QC Fortification Standard [mL]
QC SL 3	4.40	[1]0.440	100
QC SL 2	2.20	[1]0.220	100
QC SL 1	1.10	[1]0.110	100

[1]Volume should 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 0.440 mL nominal = 0.488 mL actual).

7.4 Oxfendazole Calibration Standards

Using calibrated pipettes, transfer 100 µL of the respective FBZ-SO working standards (SL 1 through SL 6, 7.2) and 100 µL of the IS (oxfendazole-D_3) fortification standard (2.0 µg/mL, 7.1.4) into 20 mL volumetric flasks. Fill to the mark with methanol and mix well to give W-Mix-Stds (See Table 7.4-1). All W-Mix-Stds solutions are stored in a freezer set at -20°C and are stable for 65 days.

Transfer 100 µL of each W-Mix-Stds solution into respective 2 mL HPLC vial or 2 mL 96 well plate. Add 900 µL of water and thoroughly mixto give the solvent calibration standard (see Table 7.4-2). The solvent calibration standards should be prepared daily prior to use.

The correlation between oxfendazole solvent calibration standard concentrations and milk equivalent concentrations is presented in Table 7.4-2. Based on the extraction procedure (extraction step 9.2a - 9.2h), the conversion factor between solvent calibration (ng/mL) standard and respective milk equivalent concentration (ppb) is:

Concentration in milk (ppb) = solvent standard concentration (ng/mL) ×200 (conversion factor)

Table 7.4-1: Preparation of W-Mix STD Solutions

Mix 100 µL of Oxfendazole-D_3 fortification standard (7.1.4) with 100 µL of SL 1-6 (7.2) and dilute to volume with methanol

Calibration Standard ID	Vol. of SL 1-6	Vol. of IS Fortification solution	Final Vol (mL)	Conc. (ng/mL)
W-Mix-Std-6	100 µL of SL 6	100 µL	20	27.5
W-Mix-Std-5	100 µL of SL 5	100 µL	20	20.0
W-Mix-Std-4	100 µL of SL 4	100 µL	20	15.0
W-Mix-Std-3	100 µL of SL 3	100 µL	20	11.0
W-Mix-Std-2	100 µL of SL 2	100 µL	20	7.50
W-Mix-Std-1	100 µL of SL 1	100 µL	20	3.00

Note: the nominal concentration of internal standard in W-Mix-Stds is 10.0 ng/mL.

Table 7.4-2: Preparation of Oxfendazole Calibration Standards			
Mix 100 µL of W-Mix-Stds with 900 µL water			
Calibration Std-ID	**W-Mix-STD Solution ID**	**Calibration Standard Conc. (ng/mL)**	**Milk Equivalent Std Conc. (ppb)**
Std-6	W-Mix-Std-6	2.75	550
Std-5	W-Mix-Std-5	2.00	400
Std-4	W-Mix-Std-4	1.50	300
Std-3	W-Mix-Std-3	1.10	220
Std-2	W-Mix-Std-2	0.750	150
Std-1	W-Mix-Std-1	0.300	60.0

Note: the nominal concentration of internal standard in Stds is 1.00 ng/mL (200 ng/g milk equivalent concentration).

7.5 Quality Control Samples for Milk

For routine analysis, a minimum of one Double Blank, one Control Blank, and two milk QC samples fortified at tolerance (duplicate of QC2, 220 ppb milk equivalent concentration) are required for each sample analysis set.

For preparation of the QC samples, 100 µL of the respective QC fortification standard (7.3) and 100 µL of the IS fortification standard (2.0 µg/mL, 7.1.4) are spiked into 1 g of blank milk from untreated cow (see Table 7.5-1). For routine sample analysis, QC samples should be prepared fresh with each sample analysis set.

Table 7.5-1: Quality control samples (milk)		
Concentration of Quality Control Sample [ppb]	**Spiking Volume of Internal Standard Fortification Solution**	**Volume of QC Fortification Standard**
440	100 µL	100 µL of QC SL 3
220	100 µL	100 µL of QC SL 2
110	100 µL	100 µL of QC SL 1

8 SAMPLE HANDLING AND STORAGE

8.1 Sample handling and storage

Milk samples are stored in suitable containers in a freezer set at ≤-65°C. Milk samples should be thawed at room temperature (20 – 25°C) and vortexed prior to aliquoting. No

further homogenization is required. To reduce freeze/thaw cycles, bulk samples of milk should be subdivided into multiple smaller aliquots.

9 PROCEDURE FOR DETERMINATION OF OXFENDAZOLE IN CATTLE MILK

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

9.1a Thaw control and incurred milk samples at room temperature, or in water prior to aliquoting. Vortex the thawed samples and then, transfer 1.00 g (± 0.05 g) of control or incurred milk sample into a tared 15-mL polypropylene tube. Record or print the exact weight as shown on the balance.

Note: Usually transferring ~1 mL of milk with calibrated pipet gives the desired weight of milk.

9.1b Add 200 µL methanol to the double blank sample. Add 100 µL methanol and 100 µL of Oxfendazole-D_3 Internal Standard Fortification Solution (7.1.4) to the control and incurred/unknown sample. Add 100 µL Oxfendazole QC Fortification Standard (7.3) and 100 µL of Oxfendazole-D_3 Internal Standard Fortification Solution (7.1.4) for QC samples. Briefly vortex at moderate speed and leave the sample on the bench for approximately 10 min before extraction.

For fortified QC milk samples, a nominal weight of 1 g should be used for the determination of recovery (actual weight should be recorded).

For incurred and unknown samples, the exact sample weight is used for all calculations. The correction factor for sample weight = nominal weight / actual weight

1.0 g = nominal weight.

9.2 Extraction of milk sample

9.2a Add 8 mL of methanol into the 15-mL polypropylene tube containing the sample.

9.2b Vortex the sample for *ca.* 10 min. at high speed using a multitube vortexer. Visually inspect all tubes to ensure milk is swirling up and

thoroughly mixed. 9.2c. Centrifuge the sample at ~3300 rcf (e.g. 4000 rpm for Sorvall Legend XTR) for *ca.* 10 min. at *ca.* 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.

Critical Step: After addition of methanol, it is helpful to allow mixture to stand for approximately 5 minutes prior to proceeding. Vortex each sample to resuspend the pellet prior to placing the samples on the multitube vortexer. For better vortexing, it is recommended to use palm against the tube cap instead of holding the tube with fingers.

9.2f Vortex the sample for *ca.* 10 min. at high speed using a multitube vortexer. Visually inspect all tubes to ensure tissue is swirling up and thoroughly mixed.

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

9.2h Transfer the supernatant to the same pre-labeled 50 mL polypropylene tube (9.2d) and adjust the volume to 20 mL mark with methanol. Vortex and mix well.

9.2i Pipette 100 µL of the methanol milk extract into the appropriate wells of a 2 mL 96-well plate or autosampler vials and mix with 900 µL of water. Vortex and mix well for LC-MS/MS analysis (stable for 24 days at room temperature). Store remaining methanol extract in refrigerator for possible re-assay (stable for 26 days 2-8ºC).

10 METHOD FLOW CHART

Transfer 1.00 ± 0.05 g of the milk sample into a 15-mL polypropylene centrifuge tube.

↓

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 approximately 10 min before extraction.

↓

Add 8 mL methanol and vortex for approximately 10 min.

↓

Centrifuge at ~3300 rcf (e.g. 4000 rpm for Sorvall Legend XTR) for *ca.* 10 min. at *ca.* 10°C.

↓

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

↓

Add 8 mL methanol to the pellet and vortex for approximately 10 min.

See critical information under 9.2e in extraction procedure

↓

Centrifuge at ~3300 rcf (e.g. 4000 rpm for Sorvall Legend XTR) for *ca.* 10 min. at *ca.* 10°C.

↓

Combine the methanol extracts and adjust the volume to 20 mL mark with methanol. Vortex and mix well

↓

Pipette 100 µL of the methanol milk extract into the appropriate wells of a 2 mL 96-well plate or 2 mL HPLC vials and mix with 900 µL of water 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.

The LC and MS conditions should be adjusted such that acceptable performance of the LC-MS/MS system is achieved.

11.1 HPLC Conditions

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

HPLC System:	Primary: Waters Acquity UPLC with pump, autosampler and column manager Alternate: Shimadzu HPLC system
Column:	Primary: MacMod ACE 3 C18, 2.1 x 50 mm Part Number ACE-111-0502 Alternate : Thermo Acclaim 120, C18, 3 µm, 2.1 x 50 mm
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)
Autosampler Wash:	100% Acetonitrile
Injection Volume:	2-10 µL (may vary based on sensitivity)
Run Time:	5.2 min/inj.
Retention Time:	*ca.* 1.5 min

Gradient Table:

Time (min)	Flow (µL/min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	400	90	10
0.3	400	90	10
2.0	400	25	75
2.1	400	0	100
3.1	400	0	100
3.2	400	90	10
5.2	400	90	10

11.2 MS Conditions

11.2.1 Tuning of Mass Spectrometer and MS Full Scan

The MS response of oxfendazole and oxfendazole-D_3 should be tuned by infusion of appropriate standard solutions. For the determinative method, the MS conditions should be optimized in MS/MS mode for MRM transitions of *m/z* 316 → *m/z* 159 and *m/z* 319 → *m/z* 159 for both oxfendazole and oxfendazole-D_3, respectively. For the confirmatory method, additional ion transitions for oxfendazole, *m/z* 316 → *m/z* 191 as qualifier 1 and *m/z* 316 → *m/z* 284 as qualifier 2 are monitored simultaneously.

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 Parameters for API 4000/ API 4000 QTRAP	
Ionization interface	Turbo Ion Spray
Ionization mode	Positive
Approximate MS run time [min]	5.2
Source (TEM) Temperature [°C]	600
Curtain (CUR) gas [psi]	10
Collision (CAD) gas [psi]	4
Ion source gas (GS1) 1 [psi]	60
Ion source gas (GS2) 2 [psi]	60
Ion (IS) Spray [V]	5500
DP	75
Entrance (EP) potential [V]	10

Table 11.2.2-2: System Parameters for Thermo Vantage	
Ion Source Type	HESI
Ionization Mode	Positive
Spray Voltage (V)	3500
Vaporizer Temperature (°C)	30
Sheath Gas Pressure (psi)	20
Ion Sweep Gas Pressure (psi)	0.0
Aux Gas Pressure (V)	5
Capillary Temperature (°C)	270

MRM transition parameters are as follows

Table 11.2.2-3: MS/MS Transition Parameter					
Reference Standard	**Precursor ion Q1 mass [amu]**	**CE**	**Collision energy (V)**	**Q3 mass [amu]**	**Dwell time (ms)**
Oxfendazole [a]	316	47	11	159 (quantifier)	100
Oxfendazole-Qual_1 [b]	316	31	14	191(qualifier)	100
Oxfendazole-Qual_2 [b]	316	26	24	284 (qualifier)	100
Oxfendazole - d_3 [a]	319	38	11	159	100

a: quantitation purposes

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

Examples of chromatograms of standards, QC, incurred, control (with IS), and double blank of milk samples are displayed in section 20.

12 SYSTEM SUITABILITY TEST AND SAMPLE INJECTION SEQUENCE

12.1 System Suitability Test (SST)

System suitability should be performed by injection of the lowest standard (Std-1, 0.3 ng/mL) at least 5 times to assess reproducibility and sensitivity of MS response.

12.2 Analysis Sequence

All 6 calibration standards are analyzed before extracted samples including control samples, double blank, QC, and incurred samples. The extracted samples are followed (bracketed) by all 6 standards.

A possible sequence order consisting of system suitability test (SST) samples, solvent calibration, and QC samples within a series is presented below.

System Suitability Test SST (Std-1)	n ≥5 injections (SST reproducibility)
Solvent blank (water:methanol, 90:10)	1 injection
Std-1 to Std-6	1 injection each
Solvent blank	1 injections
Followed by milk samples, including double blank, control, QCs, and unknown samples.	1 injection each
Solvent blank (water:methanol, 90:10)	1 injections
Std-1 to Std-6	1 injection each

13 CALCULATION AND REPORTING OF RESULTS

13.1 Method of Calculation

A standard calibration curve is generated from non-weighted linear regression analysis of peak area ratio versus oxfendazole milk equivalent concentration (ppb). The standard curve plots peak area ratios of oxfendazole / oxfendazole-D_3 versus the milk equivalent concentrations of oxfendazole calibration standards. The extraction process results in a 200x dilution of residues (1 g milk extracted in 20 mL of Methanol; 100 µL of extract diluted to 1 mL with dilution solution). Therefore, the conversion factor from solvent concentration (ng/mL) to milk equivalents (ng/g or ppb) is 200. The calibration curves are calculated by simple linear regression:

$y=mx+b$

The concentration of each sample is calculated using the formula:

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

Where, y = peak area ratio of analyte to IS
x = sample concentration (ppb)
m = slope
b = y-intercept

The oxfendazole concentrations are expressed as ppb milk equivalent concentration. A typical calibration curve for solvent standards is displayed in section 19.

13.2 Calculation of Unknown Concentrations from Incurred milk samples and Fortified Samples

The following equation will calculate the concentration in ppb:

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

Where:
C_T is the concentration of oxfendazole in ppb in the sample,
C_I is the calculated concentration of oxfendazole in ppb from the standard curve where the nominal concentrations of standards are in ppb and are based on 1.0 g sample size.
S_W is the sample weight ratio of actual to nominal of the incurred or unknown sample (nominal weight of 1 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 = 100 ppb S_W = 1.06

$$C_T = \frac{100}{1.06} = 94.3 \text{ ppb}$$

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

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

Where:
C_T is the measured concentration of oxfendazole in ppb in the sample,
C_F is the milk fortification level in ppb.

An example of recovery calculation is given below:

C_T=100 ppb C_F=102 ppb

$$\%\text{Recovery} = \left(\frac{100}{102}\right) \times 100 = 98.0\%$$

13.3 Calculation and Expression of Results for Oxfendazole Confirmation

13.3.1 Calculation of Ion Ratio

Data acquisition for confirmatory analysis can be performed simultaneously with the determinative analysis. The ion ratio (expressed as Relative Abundance Ratio or RAR) of the product ions (*m/z* 191 or *m/z* 284) compared to the reference ion (*m/z* 159) is calculated for each sample according to the following equation:

$$\text{RAR} = \frac{\text{peak area of product ion}}{\text{peak area of reference ion}} \times 100\%$$

13.3.2 Comparison of Ion Retention Times

For each sample, the retention time of oxfendazole for each transition is compared with the average retention time of the corresponding transition for standards.

13.3.3 Calculation of Ion Signal to Noise Ratio

The signal to noise ($R_{S/N}$) ratio for each confirmatory transition should be determined for each sample.

14 ACCEPTANCE CRITERIA

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

14.1 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 (SST, Std-1, 0.300 ng/mL) should be performed prior to injection of a sample set for determinative ion only.

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 minimum signal-to-noise ratio of 10:1 and reproducible oxfendazole/oxfendazole-D_3 peak area ratio and oxfendazole retention times with CV ≤ 5.0% must be met for at least five consecutive injections of Std-1, 0.300 ng/mL.

14.2 Accuracy and Precision: Quality Control Sample Acceptance Criteria

. The acceptance criteria for the mean accuracy of QC samples should be 80% to 110% (-20% to +10% bias) and the precision expressed as coefficient of variation (% CV) should be ≤10%.

14.3 Standard Calibration Curve

The non-weighted linear regression should have a coefficient of determination (r^2) ≥0.99.

14.4 Confirmation Criteria

Analytical data must meet all of the following criteria in order to confirm the presence of oxfendazole in a cattle milk sample. The three monitored ion transitions are *m/z* 316→ *m/z* 159, *m/z* 316 → *m/z* 191, and *m/z* 316→ *m/z* 284 with *m/z* 316→ *m/z* 159 designated as the reference transition (quantitative transition).

Acceptance criteria for confirmatory analysis are listed as:

1. The relative abundance ratios (RAR) for *m/z* 191/159 and *m/z* 284/159 in QC and incurred samples should match the average RAR in solvent standards for the corresponding ions within ± 10% arithmetically.
2. The retention time for each product ion (*m/z* 159, 191 and 284) in QC and incurred samples should match the average retention time for the corresponding product ion in solvent standards within ± 5%.
3. Signal to noise ratio ($R_{S/N}$) should be > 50:1 for confirmatory ions *m/z* 191 and *m/z* 284.

The control cattle milk must fail to confirm.

15 LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The limit of detection (LOD) for the method is 7.2 ppb. The limit of quantitation (LOQ) for the method is 21.8 ppb.

16 DILUTION

When a quantitative result is above the standard curve range (milk equivalent concentration > 550 ppb), it should be marked (suggested "ALQ"). Aliquots of the methanol extract can be diluted with control blank extract and re-analyzed.

17 STABILITY

17.1 Stability of Oxfendazole and Oxfendazole-D_3 Stock and Working Standard Solutions

Oxfendazole-D_3 Internal Standard Fortification Solution stored at –20 °C is stable for 65 days. Oxfendazole and Oxfendazole-D_3 Stock Solutions stored at -20 °C are stable for 92 days. QC Fortification Standard Solutions stored at –20 °C are stable for 90 days. Working Standard Solutions for oxfendazole calibration standards stored at –20 °C are stable for 65 days.

17.2 Stability of Cattle Milk Extract

Cattle milk methanol extract is stable for 26 days at 2-8ºC storage.

17.3 Stability of Samples in Mobile Phase and in Autosampler

Extracted samples in 96-well plates or autosampler vials, stored in the autosampler at room temperature, are stable for 24 days.

17.4 Stability for Cattle Milk Samples

Oxfendazole is stable in cattle milk: 1) for 24 hours at room temperature 2) after 2 freeze/thaw cycles from temperatures ≤-65°C to room temperature 3) for 3 months in a freezer with temperature ≤-65°C.

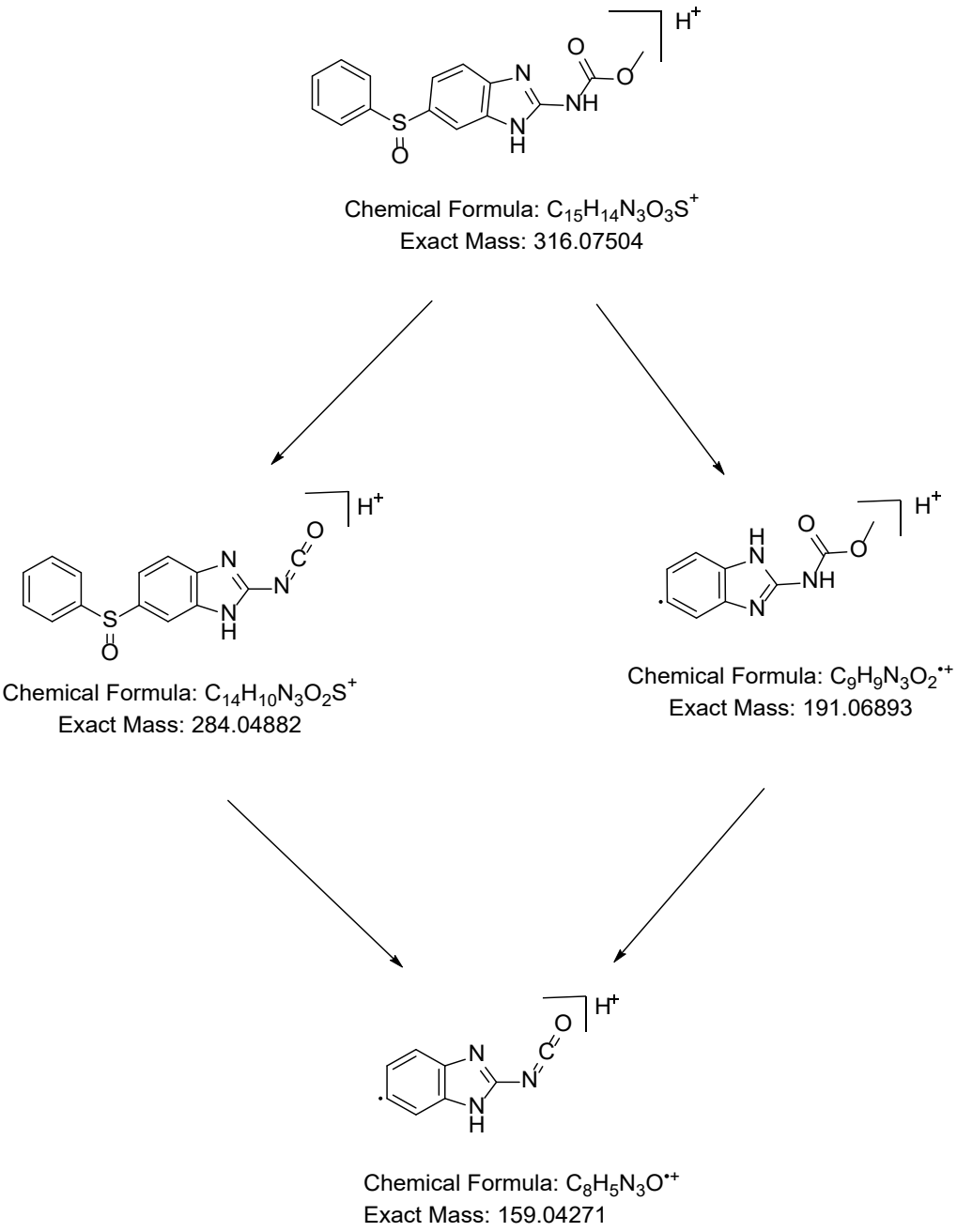
18 NOTES TO ANALYSTS

18.1 IS Monitoring and LC-MS/MS System Cleanness

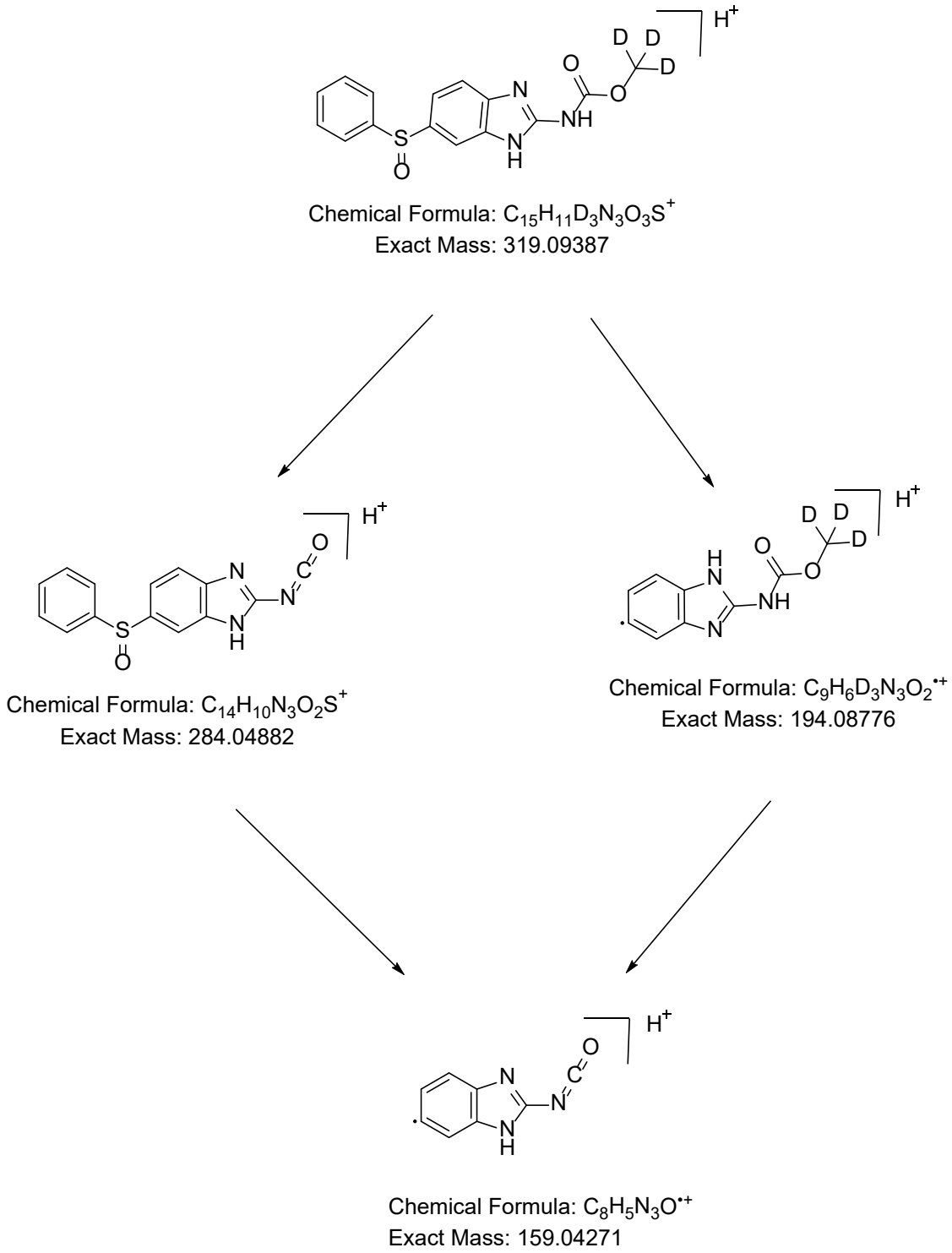
Monitor IS performance by matric plot to ensure there is no major variability. Otherwise, troubleshoot the system. When instrument responses are decreased overtime, the analytical HPLC column may be changed or the Mass Spec ion source may be cleaned.

19 STRUCTURE, PROPOSED FRAGMENTATION OF OXFENDAZOLE AND OXFENDAZOLE-D_3 AND DATA FROM METHOD TRIAL

19.1 Structure and Proposed Fragmentation of Oxfendazole



19.2 Structure and Proposed Fragmentation of Oxfendazole-D_3



19.3 Data from Method Trial

19.3.1 Determinative Data from Reference Laboratory

- Phase III, Day 1 Analytical Results

Standards

Run ID	Sample ID	Type	Nominal Conc. (ppb)	FBZ-SO RT (min)	FBZ-SO Peak Area	FBZ-SO-d3 Peak Area	Peak Area Ratio FBZ-SO/ FBZ-SO-d3	Calc. Conc (ppb)	% Rec
3	S18001-00 Std-1 1	Standard	60	1.43	25056	84565	0.296293	60.5	101
	S18001-00 Std-2 1	Standard	150	1.43	57595	79473	0.724712	155	103
	S18001-00 Std-3 1	Standard	220	1.43	90869	88768	1.023668	220	100
	S18001-00 Std-4 1	Standard	300	1.43	118980	88485	1.344635	291	97.0
	S18001-00 Std-5 1	Standard	400	1.42	159740	88059	1.814011	394	98.5
	S18001-00 Std-6 1	Standard	550	1.43	219901	87560	2.511432	548	99.6
	S18001-00 Std-1 2	Standard	60	1.43	24933	88645	0.281268	57.2	95.3
	S18001-00 Std-2 2	Standard	150	1.43	61367	85564	0.717206	153	102
	S18001-00 Std-3 2	Standard	220	1.43	87706	84316	1.040206	224	102
	S18001-00 Std-4 2	Standard	300	1.43	123232	87343	1.410897	306	102
	S18001-00 Std-5 2	Standard	400	1.43	154850	86837	1.783226	388	97.0
	S18001-00 Std-6 2	Standard	550	1.43	206881	80110	2.582462	563	102

Slope	Intercept	R-Squared
0.004546	0.02131	0.9983

Control and Fortified QC Samples, Blinded Samples

Run ID	Sample ID	Type	Nominal Conc. (ppb)	FBZ-SO RT (min)	FBZ-SO Peak Area	FBZ-SO-d3 Peak Area	Peak Area Ratio FBZ-SO/ FBZ-SO-d3	Calc. Conc (ppb)	% Rec	Average
3	S18001-00 QC 1 1	Quality Control	110	1.43	37009	77566	0.477129	100	90.9	
	S18001-00 QC 1 2	Quality Control	110	1.43	40240	79177	0.508228	107	97.3	94.1
	S18001-00 QC 2 1	Quality Control	220	1.42	78501	80213	0.978657	211	95.9	
	S18001-00 QC 2 2	Quality Control	220	1.43	79240	79272	0.999596	215	97.7	96.8
	S18001-00 QC 3 1	Quality Control	440	1.43	156622	78669	1.990899	433	98.4	
	S18001-00 QC 3 2	Quality Control	440	1.43	178606	89907	1.986564	432	98.2	98.3
	S18001-00 Double Blank	Control	0	1.41	1080	0	N/A	0		
	S18001-00 Double Blank	Control	0	1.41	591	42	N/A[1]	0		
	S18001-00 CONTROL_BLANK	Control	0	1.41	1192	79077	0.015074	BLQ (-1.37)		
	S18001-00 CONTROL_BLANK	Control	0	1.41	977	52813	0.018499	BLQ (-0.618)		
	S18001-00 8927P	Unknown	N/A	1.43	67050	79964	0.838502	174		
	S18001-00 4158P	Unknown	N/A	1.43	64589	78419	0.823640	172		
	S18001-00 9012P	Unknown	N/A	1.42	790	76529	0.010323	BLQ (-2.32)		
	S18001-00 4458P	Unknown	N/A	1.43	134046	79962	1.676371	350		
	S18001-00 1449P	Unknown	N/A	1.42	982	82729	0.011870	BLQ (-2.00)		

[1]Measured IS peak which is background noise is significantly lower than IS peak for all other samples.

- Phase III, Day 2 Analytical Results

Standards

Run ID	Sample ID	Type	Nominal Conc. (ppb)	FBZ-SO RT (min)	FBZ-SO Peak Area	FBZ-SO-d3 Peak Area	Peak Area Ratio FBZ-SO/ FBZ-SO-d3	Calc. Conc (ppb)	% Rec
4	S18001-00 Std-1 1	Standard	60	1.43	4884	18273	0.267280	56.9	94.8
	S18001-00 Std-2 1	Standard	150	1.43	12453	16700	0.745689	161	107
	S18001-00 Std-3 1	Standard	220	1.43	18640	18242	1.021818	221	101
	S18001-00 Std-4 1	Standard	300	1.43	26633	19300	1.379948	298	99.3
	S18001-00 Std-5 1	Standard	400	1.43	35936	19555	1.837689	398	99.5
	S18001-00 Std-6 1	Standard	550	1.43	47344	18328	2.583151	559	102
	S18001-00 Std-1 2	Standard	60	1.43	5088	18403	0.276477	58.9	98.2
	S18001-00 Std-2 2	Standard	150	1.43	12765	17822	0.716250	154	103
	S18001-00 Std-3 2	Standard	220	1.43	19597	20026	0.978578	211	95.9
	S18001-00 Std-4 2	Standard	300	1.43	25717	18903	1.360472	294	98.0
	S18001-00 Std-5 2	Standard	400	1.43	35459	18798	1.886318	408	102
	S18001-00 Std-6 2	Standard	550	1.43	46917	18781	2.498110	541	98.4

Slope	Intercept	R-Squared
0.004610	0.005114	0.9984

Control and Fortified QC Samples, Blinded Samples

Run ID	Sample ID	Type	Nominal Conc. (ppb)	FBZ-SO RT (min)	FBZ-SO Peak Area	FBZ-SO-d3 Peak Area	Peak Area Ratio FBZ-SO/ FBZ-SO-d3	Calc. Conc (ppb)	% Rec	Average
4	S18001-00 QC 1 1	Quality Control	110	1.43	10268	20568	0.499222	107	97.3	
	S18001-00 QC 1 2	Quality Control	110	1.43	12304	23919	0.514403	110	100.0	98.7
	S18001-00 QC 2 1	Quality Control	220	1.43	17082	16864	1.012927	219	99.5	
	S18001-00 QC 2 2	Quality Control	220	1.43	24985	24773	1.008558	218	99.1	99.3
	S18001-00 QC 3 1	Quality Control	440	1.43	37045	18241	2.030865	439	99.8	
	S18001-00 QC 3 2	Quality Control	440	1.43	40510	20118	2.013620	436	99.1	99.5
	S18001-00 Double Blank	Control	0	0.00	0	20	N/A	0		
	S18001-00 Double Blank	Control	0	0.00	0	0	N/A	0		
	S18001-00 CONTROL_BLANK	Control	0	0.00	0	18587	0	BLQ (-1.11)		
	S18001-00 CONTROL_BLANK	Control	0	1.42	184	17774	0.010352	BLQ (1.14)		
	S18001-00 3983P	Unknown	N/A	1.43	27202	16014	1.698639	355		
	S18001-00 3908P	Unknown	N/A	1.43	15372	18599	0.826496	173		
	S18001-00 6767P	Unknown	N/A	1.43	40740	22791	1.787548	372		
	S18001-00 2566P	Unknown	N/A	1.43	21268	24688	0.861471	180		
	S18001-00 7450P	Unknown	N/A	0.00	0	26522	0	BLQ (-1.07)		

- Phase III, Day 3 Analytical Results

Standards

Run ID	Sample ID	Type	Nominal Conc. (ppb)	FBZ-SO RT (min)	FBZ-SO Peak Area	FBZ-SO-d3 Peak Area	Peak Area Ratio FBZ-SO/ FBZ-SO-d3	Calc. Conc (ppb)	% Rec
5	S18001-00 Std-1 1	Standard	60	1.43	25775	87865	0.293348	59.3	98.8
	S18001-00 Std-2 1	Standard	150	1.43	63416	86261	0.735164	157	105
	S18001-00 Std-3 1	Standard	220	1.43	87474	83036	1.053447	228	104
	S18001-00 Std-4 1	Standard	300	1.43	115332	83146	1.387102	302	101
	S18001-00 Std-5 1	Standard	400	1.43	154887	84319	1.836917	401	100
	S18001-00 Std-6 1	Standard	550	1.43	200328	79034	2.534707	556	101
	S18001-00 Std-1 2	Standard	60	1.43	24499	89603	0.273417	54.9	91.5
	S18001-00 Std-2 2	Standard	150	1.43	61357	87470	0.701463	150	100
	S18001-00 Std-3 2	Standard	220	1.43	87374	85857	1.017669	220	100
	S18001-00 Std-4 2	Standard	300	1.43	113661	84377	1.347061	293	97.7
	S18001-00 Std-5 2	Standard	400	1.43	153614	85867	1.788976	391	97.7
	S18001-00 Std-6 2	Standard	550	1.43	206011	82446	2.498739	548	99.6

Slope	Intercept	R-Squared
0.004512	0.02569	0.9990

Control and Fortified QC Samples, Blinded Samples

Run ID	Sample ID	Type	Nominal Conc. (ppb)	FBZ-SO RT (min)	FBZ-SO Peak Area	FBZ-SO-d3 Peak Area	Peak Area Ratio FBZ-SO/ FBZ-SO-d3	Calc. Conc (ppb)	% Rec	Average
5	S18001-00 QC 1 1	Quality Control	110	1.43	39769	81451	0.488257	103	93.6	
	S18001-00 QC 1 2	Quality Control	110	1.43	41298	81956	0.503905	106	96.4	95.0
	S18001-00 QC 2 1	Quality Control	220	1.43	84814	84788	1.000307	216	98.2	
	S18001-00 QC 2 2	Quality Control	220	1.43	80779	81264	0.994032	215	97.7	98.0
	S18001-00 QC 3 1	Quality Control	440	1.43	163678	83765	1.954014	427	97.0	
	S18001-00 QC 3 2	Quality Control	440	1.43	171486	86958	1.972055	431	98.0	97.5
	S18001-00 Double Blank	Control	0	1.42	146	0	N/A	0		
	S18001-00 Double Blank	Control	0	1.42	92	96	N/A[1]	0		
	S18001-00 CONTROL_BLANK	Control	0	1.43	735	84450	0.008703	BLQ (-3.76)		
	S18001-00 CONTROL_BLANK	Control	0	1.42	487	80569	0.006045	BLQ (-4.35)		
	S18001-00 2896P	Unknown	N/A	1.43	138307	85240	1.62256	344		
	S18001-00 9878P	Unknown	N/A	1.43	69479	84908	0.818286	171		
	S18001-00 7534P	Unknown	N/A	1.42	286	80880	0.003536	BLQ (-4.78)		
	S18001-00 3459P	Unknown	N/A	1.43	137539	83034	1.656418	351		
	S18001-00 8281P	Unknown	N/A	1.42	249	78941	0.003154	BLQ (-4.91)		

[1] Measured IS peak which is background noise is significantly lower than IS peak for all other samples.

- Summary of Determinative Results for Fortified and Control Samples

Summary of Determinative Results for Fortified and Control Samples

Run ID	Sample ID	Nominal Conc. (ppb)	Ret Time (min)	Area	IS Area	Peak Area Ratio	Conc. Found (ppb)	%Rec
3	Double Blank	0	1.41	1080	0	N/A	0	N/A
3	Double Blank	0	1.41	591	42	N/A[1]	0	N/A
4	Double Blank	0	N/A	0	20	0	0	N/A
4	Double Blank	0	N/A	0	0	N/A	0	N/A
5	Double Blank	0	1.42	146	0	N/A	0	N/A
5	Double Blank	0	1.42	92	96	N/A[1]	0	N/A
3	Control Blank	0	1.41	1192	79077	0.015074	BLQ (-1.37)	N/A
3	Control Blank	0	1.41	977	52813	0.018499	BLQ (-0.618)	N/A
4	Control Blank	0	0	0	18587	0	BLQ (-1.11)	N/A
4	Control Blank	0	1.42	184	17774	0.010352	BLQ (1.14)	N/A
5	Control Blank	0	1.43	735	84450	0.008703	BLQ (-3.76)	N/A
5	Control Blank	0	1.42	487	80569	0.006045	BLQ (-4.35)	N/A
3	QC1	110	1.43	37009	77566	0.477129	100	90.9
3	QC1	110	1.43	40240	79177	0.508228	107	97.3
4	QC1	110	1.43	10268	20568	0.499222	107	97.3
4	QC1	110	1.43	12304	23919	0.514403	110	100
5	QC1	110	1.43	39769	81451	0.488257	103	93.6
5	QC1	110	1.43	41298	81956	0.503905	106	96.4
							Mean:	96.4
							%CV:	3.3
3	QC2	220	1.42	78501	80213	0.978657	211	95.9
3	QC2	220	1.43	79240	79272	0.999596	215	97.7
4	QC2	220	1.43	17082	16864	1.012927	219	99.5
4	QC2	220	1.43	24985	24773	1.008558	218	99.1
5	QC2	220	1.43	84814	84788	1.000307	216	98.2
5	QC2	220	1.43	80779	81264	0.994032	215	97.7
							Mean:	98.2
							%CV:	1.3
3	QC3	440	1.43	156622	78669	1.990899	433	98.4
3	QC3	440	1.43	178606	89907	1.986564	432	98.2
4	QC3	440	1.43	37045	18241	2.030865	439	99.8
4	QC3	440	1.43	40510	20118	2.01362	436	99.1
5	QC3	440	1.43	163678	83765	1.954014	427	97.0
5	QC3	440	1.43	171486	86958	1.972055	431	98.0
							Mean:	98.4
							%CV:	1.0

BLQ: < 60 ppb

[1]Measured IS peak which is background noise is significantly lower than IS peak for all other samples.

19.3.2 Confirmatory Data from Reference Laboratory

- Summary Confirmatory Results for Standards

Summary Confirmatory Results for Standards

Run ID	Sample ID	m/z 159 Peak Area	m/z 191 Peak Area	m/z 284 Peak Area	RAPAR* m/z 191 Individual	RAPAR* m/z 284 Individual	Retention Time (min) m/z 159 Individual	Retention Time (min) m/z 191 Individual	Retention Time (min) m/z 284 Individual	S/N Ratio (> 50) m/z 191 Individual	S/N Ratio (> 50) m/z 284 Individual
3	3 007 S18001-00 Std-1 1 1	25056	18781	20130	75.0	80.3	1.43	1.42	1.42	658	693
	3 008 S18001-00 Std-2 1 1	57595	44962	50063	78.1	86.9	1.43	1.42	1.42	1681	1210
	3 009 S18001-00 Std-3 1 1	90869	66019	75059	72.7	82.6	1.43	1.42	1.42	2257	1640
	3 010 S18001-00 Std-4 1 1	118980	90791	102788	76.3	86.4	1.43	1.42	1.42	2465	2707
	3 011 S18001-00 Std-5 1 1	159740	118796	128989	74.4	80.7	1.42	1.42	1.42	3827	2323
	3 012 S18001-00 Std-6 1 1	219901	164193	188364	74.7	85.7	1.43	1.42	1.42	3372	3070
	3 029 S18001-00 Std-1 2 1	24933	20331	21581	81.5	86.6	1.43	1.42	1.42	802	985
	3 030 S18001-00 Std-2 2 1	61367	45778	50125	74.6	81.7	1.43	1.42	1.42	1546	1817
	3 031 S18001-00 Std-3 2 1	87706	64947	70372	74.1	80.2	1.43	1.42	1.42	2629	2182
	3 032 S18001-00 Std-4 2 1	123232	90163	97643	73.2	79.2	1.43	1.43	1.42	2701	1406
	3 033 S18001-00 Std-5 2 1	154850	120727	135113	78.0	87.3	1.43	1.42	1.42	3637	2650
	3 034 S18001-00 Std-6 2 1	206881	164198	180192	79.4	87.1	1.43	1.42	1.42	3190	3156
				Average:	**76.0**	**83.7**	**1.43**	**1.42**	**1.42**	NA	
4	4 007 S18001-00 Std-1 1 1	4884	4083	4670	83.6	95.6	1.43	1.42	1.42	148	127
	4 008 S18001-00 Std-2 1 1	12453	10078	10933	80.9	87.8	1.43	1.43	1.42	283	412
	4 009 S18001-00 Std-3 1 1	18640	14227	16338	76.3	87.7	1.43	1.42	1.42	509	463
	4 010 S18001-00 Std-4 1 1	26633	19857	22240	74.6	83.5	1.43	1.42	1.42	647	609
	4 011 S18001-00 Std-5 1 1	35936	27550	31151	76.7	86.7	1.43	1.42	1.42	707	668
	4 012 S18001-00 Std-6 1 1	47344	35847	40397	75.7	85.3	1.43	1.42	1.42	811	891
	4 029 S18001-00 Std-1 2 1	5088	4215	4551	82.8	89.4	1.43	1.42	1.42	161	189
	4 030 S18001-00 Std-2 2 1	12765	9256	10758	72.5	84.3	1.43	1.42	1.42	374	345
	4 031 S18001-00 Std-3 2 1	19597	15162	16888	77.4	86.2	1.43	1.42	1.42	389	497
	4 032 S18001-00 Std-4 2 1	25717	19818	22283	77.1	86.6	1.43	1.42	1.42	318	487
	4 033 S18001-00 Std-5 2 1	35459	27851	30944	78.5	87.3	1.43	1.42	1.42	1236	1417
	4 034 S18001-00 Std-6 2 1	46917	35487	43482	75.6	92.7	1.43	1.43	1.42	819	1489
				Average:	**77.6**	**87.8**	**1.43**	**1.42**	**1.42**	NA	
5	5 007 S18001-00 Std-1 1 1	25775	17683	20163	68.6	78.2	1.43	1.42	1.42	565	871
	5 008 S18001-00 Std-2 1 1	63416	44876	48441	70.8	76.4	1.43	1.43	1.42	1144	1319
	5 009 S18001-00 Std-3 1 1	87474	61335	70694	70.1	80.8	1.43	1.42	1.42	1634	2880
	5 010 S18001-00 Std-4 1 1	115332	86415	95243	74.9	82.6	1.43	1.42	1.42	3743	2782
	5 011 S18001-00 Std-5 1 1	154887	118026	133698	76.2	86.3	1.43	1.42	1.42	3076	4115
	5 012 S18001-00 Std-6 1 1	200328	153486	166218	76.6	83.0	1.43	1.42	1.42	2745	2889
	5 029 S18001-00 Std-1 2 1	24499	18512	20282	75.6	82.8	1.43	1.42	1.42	1168	879
	5 030 S18001-00 Std-2 2 1	61357	46454	52067	75.7	84.9	1.43	1.43	1.42	2288	2363
	5 031 S18001-00 Std-3 2 1	87374	62935	75759	72.0	86.7	1.43	1.43	1.42	2624	2252
	5 032 S18001-00 Std-4 2 1	113661	86073	95818	75.7	84.3	1.43	1.43	1.42	3030	2087
	5 033 S18001-00 Std-5 2 1	153614	115623	129568	75.3	84.3	1.43	1.42	1.42	2306	3294
	5 034 S18001-00 Std-6 2 1	206011	152722	170229	74.1	82.6	1.43	1.42	1.42	3060	5503
				Average:	**73.8**	**82.7**	**1.43**	**1.42**	**1.42**	NA	

*RAPAR: Relative Abundance Peak Area Ratio to m/z 159

- Summary Confirmatory Results for QCs and Confirming Blinded Samples

Summary Confirmatory Results for QCs and Confirming Blinded Samples													
Run ID	Sample ID	Relative Abundance Peak Area Ratio to m/z 159				Retention Time (min)						S/N Ratio (> 50)	
		m/z 191		m/z 284		m/z 159		m/z 191		m/z 284		m/z 191	m/z 284
		Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Individual
3	3 015 S18001-00 QC 1 1 1	71.7	66.0-86.0	81.2	73.7-93.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	1089	832
	3 025 S18001-00 QC 1 2 1	75.2	66.0-86.0	80.6	73.7-93.7	1.43	1.36-1.50	1.43	1.35-1.49	1.42	1.35-1.49	765	1231
4	4 015 S18001-00 QC 1 1 1	76.1	67.6-87.6	83.0	77.8-97.8	1.43	1.36-1.50	1.43	1.35-1.49	1.42	1.35-1.49	255	388
	4 025 S18001-00 QC 1 2 1	71.9	67.6-87.6	79.9	77.8-97.8	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	324	390
5	5 015 S18001-00 QC 1 1 1	75.0	63.8-83.8	83.9	72.7-92.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	1165	1083
	5 025 S18001-00 QC 1 2 1	71.7	63.8-83.8	77.6	72.7-92.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	1176	1297
3	3 016 S18001-00 QC 2 1 1	74.2	66.0-86.0	82.2	73.7-93.7	1.42	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	3008	2265
	3 026 S18001-00 QC 2 2 1	74.9	66.0-86.0	84.1	73.7-93.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	1764	2108
4	4 016 S18001-00 QC 2 1 1	73.5	67.6-87.6	84.4	77.8-97.8	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	423	512
	4 026 S18001-00 QC 2 2 1	74.8	67.6-87.6	82.7	77.8-97.8	1.43	1.36-1.50	1.43	1.35-1.49	1.42	1.35-1.49	681	796
5	5 016 S18001-00 QC 2 1 1	70.4	63.8-83.8	80.5	72.7-92.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	1468	2095
	5 026 S18001-00 QC 2 2 1	69.7	63.8-83.8	80.9	72.7-92.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	1832	2442
3	3 017 S18001-00 QC 3 1 1	73.4	66.0-86.0	79.7	73.7-93.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	2526	3366
	3 027 S18001-00 QC 3 2 1	71.9	66.0-86.0	81.2	73.7-93.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	2416	3413
4	4 017 S18001-00 QC 3 1 1	72.6	67.6-87.6	82.5	77.8-97.8	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	860	1225
	4 027 S18001-00 QC 3 2 1	74.8	67.6-87.6	84.3	77.8-97.8	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	964	1383
5	5 017 S18001-00 QC 3 1 1	72.9	63.8-83.8	81.9	72.7-92.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	2322	2650
	5 027 S18001-00 QC 3 2 1	70.5	63.8-83.8	79.0	72.7-92.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	3696	2244
3	3 019 S18001-00 8927P 1.031	75.4	66.0-86.0	80.6	73.7-93.7	1.43	1.36-1.50	1.43	1.35-1.49	1.42	1.35-1.49	1764	1519
	3 020 S18001-00 4158P 1.024	73.7	66.0-86.0	86.7	73.7-93.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	2008	1309
	3 022 S18001-00 4458P 1.041	72.8	66.0-86.0	82.3	73.7-93.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	2525	1711
4	4 019 S18001-00 3983P 0.967	74.1	67.6-87.6	85.2	77.8-97.8	1.43	1.36-1.50	1.43	1.35-1.49	1.42	1.35-1.49	708	882
	4 020 S18001-00 3908P 0.972	80.5	67.6-87.6	88.3	77.8-97.8	1.43	1.36-1.50	1.43	1.35-1.49	1.42	1.35-1.49	522	657
	4 021 S18001-00 6767P 0.962	74.8	67.6-87.6	80.9	77.8-97.8	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	813	1207
	4 022 S18001-00 2566P 0.97	74.5	67.6-87.6	83.7	77.8-97.8	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	769	524
5	5 019 S18001-00 2896P 0.973	72.8	63.8-83.8	81.3	72.7-92.7	1.43	1.36-1.50	1.43	1.35-1.49	1.42	1.35-1.49	3781	1844
	5 020 S18001-00 9878P 0.973	72.1	63.8-83.8	78.2	72.7-92.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	1353	1211
	5 022 S18001-00 3459P 0.97	72.7	63.8-83.8	82.8	72.7-92.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	3408	1491

- Summary Confirmatory Results for Control Blank and Nonconfirmed Blinded Samples

Summary Confirmatory Results for Control Blank and Nonconfirming Blinded Samples													
Run ID	Sample ID	Relative Abundance Peak Area Ratio to m/z 159				Retention Time (min)						S/N Ratio (> 50)	
		m/z 191		m/z 284		m/z 159		m/z 191		m/z 284		m/z 191	m/z 284
		Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Acc. Range	Individual	Individual
3	3 014 S18001-00 Double Blank 1	76.5	66.0-86.0	76.0	73.7-93.7	1.41	1.36-1.50	1.41	1.35-1.49	1.41	1.35-1.49	34	17
	3 036 S18001-00 Double Blank 1	81.7	66.0-86.0	88.7	73.7-93.7	1.41	1.36-1.50	1.42	1.35-1.49	1.41	1.35-1.49	24	21
4	4 014 S18001-00 Double Blank 1	N/A	67.6-87.6	N/A	77.8-97.8	N/A	1.36-1.50	N/A	1.35-1.49	1.42	1.35-1.49	N/A	12
	4 036 S18001-00 Double Blank 1	N/A	67.6-87.6	N/A	77.8-97.8	N/A	1.36-1.50	1.43	1.35-1.49	1.43	1.35-1.49	6	5
5	5 014 S18001-00 Double Blank 1	218	63.8-83.8	52.1	72.7-92.7	1.42	1.36-1.50	1.43	1.35-1.49	1.42	1.35-1.49	7	5
	5 036 S18001-00 Double Blank 1	138	63.8-83.8	111	72.7-92.7	1.42	1.36-1.50	1.41	1.35-1.49	1.43	1.35-1.49	4	5
3	3 013 S18001-00 CONTROL_BLANK 1	80.6	66.0-86.0	105	73.7-93.7	1.41	1.36-1.50	1.41	1.35-1.49	1.41	1.35-1.49	30	34
	3 035 S18001-00 CONTROL_BLANK 1	60.5	66.0-86.0	52.3	73.7-93.7	1.41	1.36-1.50	1.41	1.35-1.49	1.41	1.35-1.49	21	21
4	4 013 S18001-00 CONTROL_BLANK 1	N/A	67.6-87.6	N/A	77.8-97.8	N/A	1.36-1.50	1.41	1.35-1.49	1.42	1.35-1.49	13	5
	4 035 S18001-00 CONTROL_BLANK 1	176	67.6-87.6	103	77.8-97.8	1.42	1.36-1.50	1.41	1.35-1.49	1.41	1.35-1.49	8	7
5	5 013 S18001-00 CONTROL_BLANK 1	28.2	63.8-83.8	63.8	72.7-92.7	1.43	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	11	22
	5 035 S18001-00 CONTROL_BLANK 1	120	63.8-83.8	110	72.7-92.7	1.42	1.36-1.50	1.42	1.35-1.49	1.42	1.35-1.49	12	14
3	3 021 S18001-00 9012P 1.039	94.3	66.0-86.0	116	73.7-93.7	1.42	1.36-1.50	1.42	1.35-1.49	1.40	1.35-1.49	29	16
	3 023 S18001-00 1449P 1.04	74.8	66.0-86.0	93.1	73.7-93.7	1.42	1.36-1.50	1.42	1.35-1.49	1.41	1.35-1.49	31	19
4	4 023 S18001-00 7450P 0.961	N/A	67.6-87.6	N/A	77.8-97.8	N/A	1.36-1.50	N/A	1.35-1.49	1.39	1.35-1.49	N/A	8
5	5 021 S18001-00 7534P 0.973	81.8	63.8-83.8	122	72.7-92.7	1.42	1.36-1.50	1.43	1.35-1.49	1.42	1.35-1.49	6	13
	5 023 S18001-00 8281P 0.984	102	63.8-83.8	105	72.7-92.7	1.42	1.36-1.50	1.43	1.35-1.49	1.42	1.35-1.49	10	10

19.3.3 Results for the Analysis of Blinded Milk Samples (MAH GPD)

Blinded Sample	Sample ID	Oxfendazole RT (min)	Oxfendazole Peak Area	Oxfendazole-D_3 Peak Area	Peak Area Ratio	Measured Conc. (ppb)	Sample Weight (g)	Dilution Factor	Calculated Conc. (ppb)
FDA-1	9012P	1.42	790	76529	0.0103	-2.42	1.039	0.962	BLQ (-2.32)
	1449P	1.42	982	82729	0.0119	-2.08	1.040	0.962	BLQ (-2.00)
	7450P	0	0	26522	0	-1.11	1.041	0.961	BLQ (-1.07)
	7534P	1.42	286	80880	0.00354	-4.91	1.028	0.973	BLQ (-4.78)
	8281P	1.42	249	78941	0.00316	-4.99	1.016	0.984	BLQ (-4.91)
								Average	**NA**
								%CV	**NA**
FDA-3	8927P	1.43	67050	79964	0.838	180	1.031	0.970	174
	4158P	1.43	64589	78419	0.824	176	1.024	0.977	172
	3908P	1.43	15372	18599	0.827	178	1.029	0.972	173
	2566P	1.43	21268	24688	0.861	186	1.031	0.970	180
	9878P	1.43	69479	84908	0.818	176	1.028	0.973	171
								Average	**174**
								%CV	**2.03**
FDA-2	4458P	1.43	134046	79962	1.68	364	1.041	0.961	350
	3983P	1.43	27202	16014	1.70	367	1.034	0.967	355
	6767P	1.43	40740	22791	1.79	387	1.039	0.962	372
	2896P	1.43	138307	85240	1.62	354	1.028	0.973	344
	3459P	1.43	137539	83034	1.66	361	1.031	0.970	351
								Average	**354**
								%CV	**2.99**

NA Not Applicable

BLQ Below the lowest standard on the calibration curve (60 ppb oxfendazole milk equivalent concentration)

19.3.4 Results for the Analysis of Blinded Milk Samples (Primera)

Blinded Sample ID	Sample ID	RT (min)	Oxfendazole Peak Area	Oxfendazole-D_3 Peak Area	Peak Area Ratio	Measured Concentration (ppb)	Sample Weight (g)	Calculated Concentration (ppb)
FDA-1	3097P	0	0	208769	0	No Peak	1.030	No Peak
	7074P	0	0	208661	0	No Peak	1.027	No Peak
	1568P	0	0	191965	0	No Peak	1.031	No Peak
	6621P	0	0	201086	0	No Peak	1.029	No Peak
	3711P	0	0	266770	0	No Peak	1.028	No Peak
							Average	**NA**
							%CV	**NA**
FDA-3	2412P	1.88	185099	207365	0.893	180	1.021	176
	2846P	1.88	181447	204715	0.886	178	1.028	173
	4783P	1.86	178750	200679	0.891	177	1.028	172
	9458P	1.87	226979	246407	0.921	184	1.023	180
	9798P	1.87	223629	251649	0.889	177	1.013	175
							Average	**175**
							%CV	**1.73**
FDA-2	4765P	1.88	367065	201982	1.82	366	1.026	357
	6780P	1.86	360457	200027	1.80	363	1.025	354
	8547P	1.87	348622	193339	1.80	363	1.035	351
	8158P	1.87	450675	251301	1.79	362	1.024	354
	8797P	1.87	454551	254417	1.79	361	1.026	352
							Average	**353**
							%CV	**0.65**

NA Not Applicable

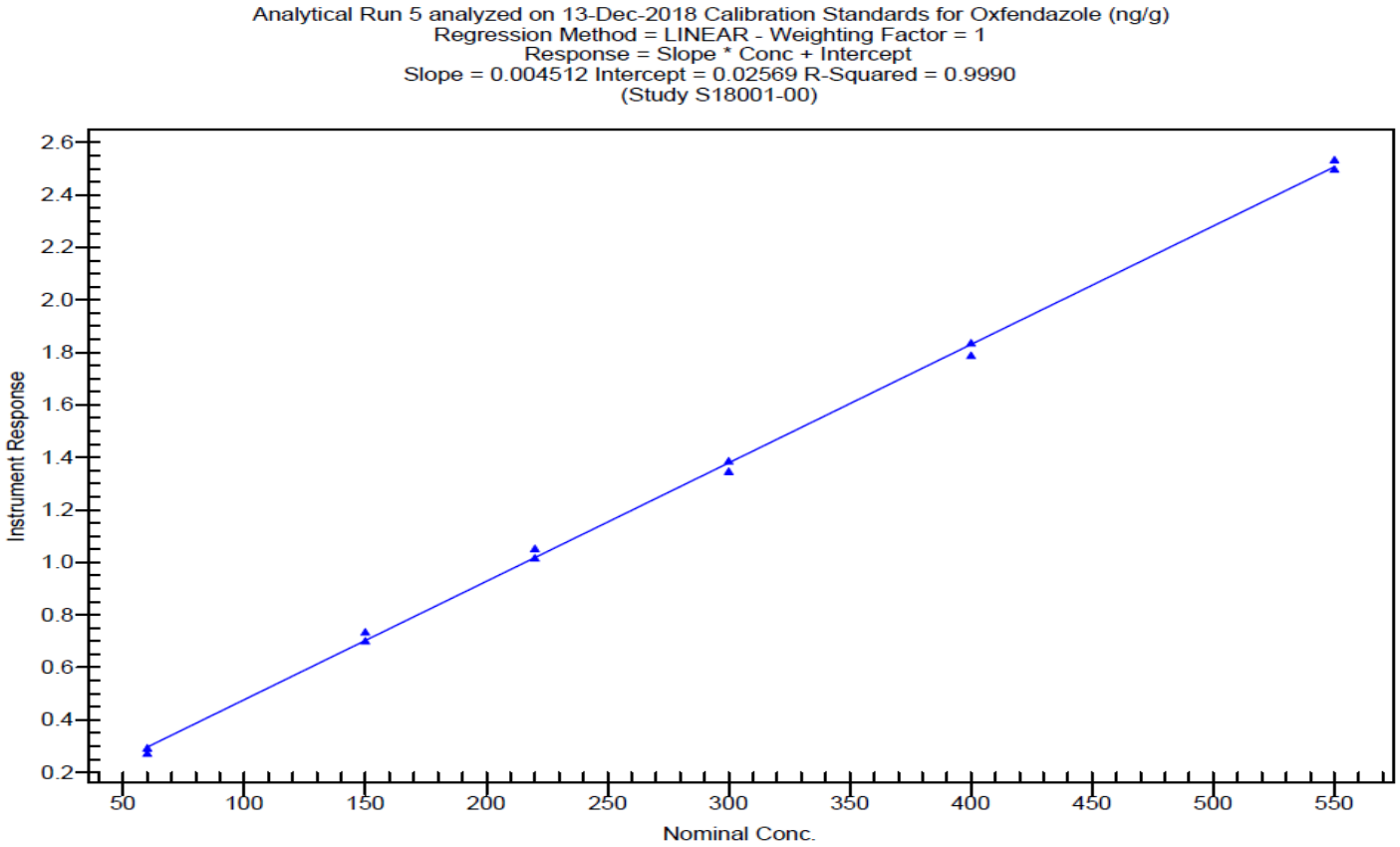
19.3.5 Results for the Analysis of Blinded Milk Samples (KCAS)

Blinded Sample ID	Sample ID	Oxfendazole RT (min)	Oxfendazole Peak Area	Oxfendazole-D_3 Peak Area	Peak Area Ratio	Measured Concentration (ppb)	Sample Weight (g)	Calculated Concentration (ppb)
FDA-1	5319P	0	0	41142	0	0	1.037	0
	5360P	0	0	21086	0	0	1.031	0
	8009P	0	0	19157	0	0	1.032	0
	1329P	0	0	25899	0	0	1.037	0
	6987P	0	0	26437	0	0	1.035	0
							Average	**0**
							%CV	**NA**
FDA-3	2079P	1.75	39773	42442	0.937	182	1.016	179
	7710P	1.75	36342	40231	0.903	176	1.018	173
	4611P	1.74	19608	19662	0.997	192	1.008	190
	2189P	1.75	23860	25922	0.920	177	1.006	176
	7243P	1.74	24197	25895	0.934	179	1.009	177
							Average	**179**
							%CV	**3.76**
FDA-2	2580P	1.74	75105	38864	1.93	376	1.042	361
	8550P	1.75	70445	38359	1.84	357	1.020	350
	7616P	1.74	38374	20276	1.89	365	1.020	358
	9337P	1.75	36373	19042	1.91	368	1.014	363
	9543P	1.75	49362	25871	1.91	366	1.014	361
							Average	**359**
							%CV	**1.42**

NA Not Applicable

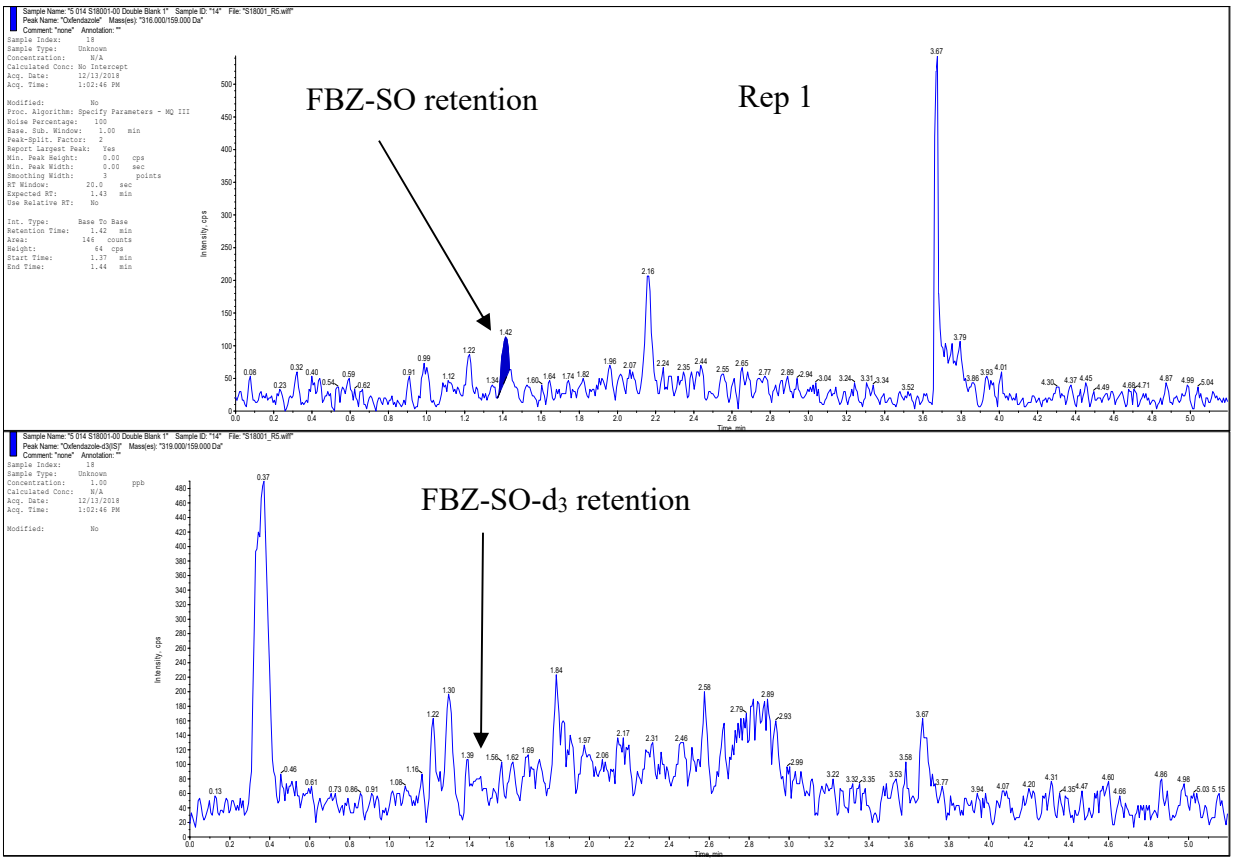
20 EXAMPLE STANDARD CURVE AND CHROMATOGRAMS FROM METHOD TRIAL (MAH GPD)

20.1 Example Standard Curve

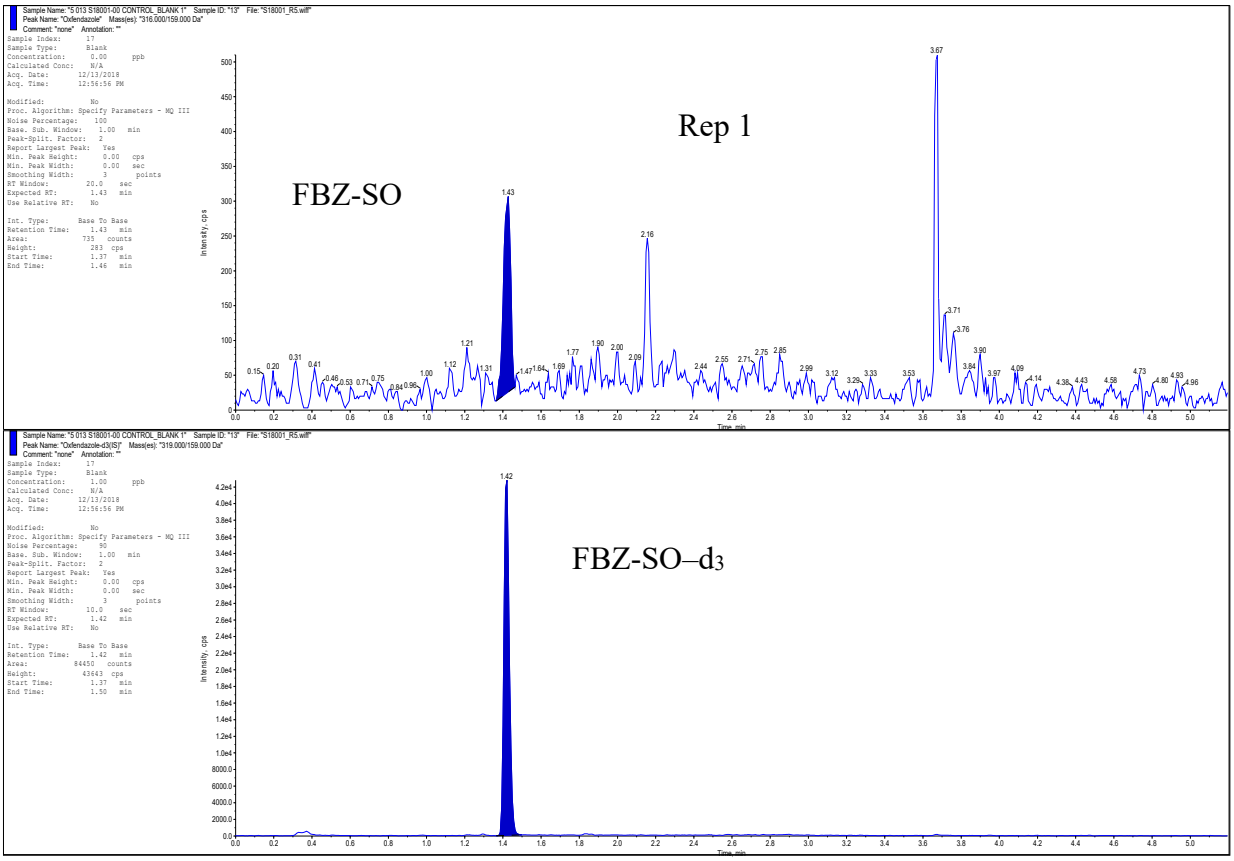


Standard curve plot is for Run 5 and from Watson

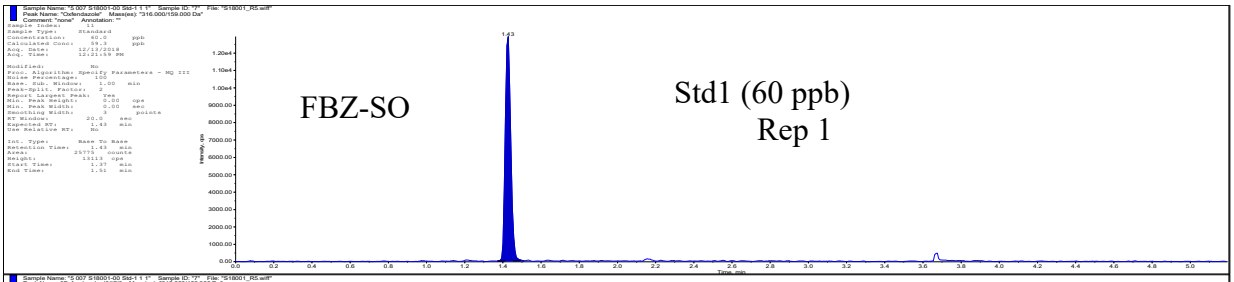
20.2 LC-MS/MS Chromatograms of Double Blank Sample in Determinative Analysis (Source File: S18001_R5.rdb)

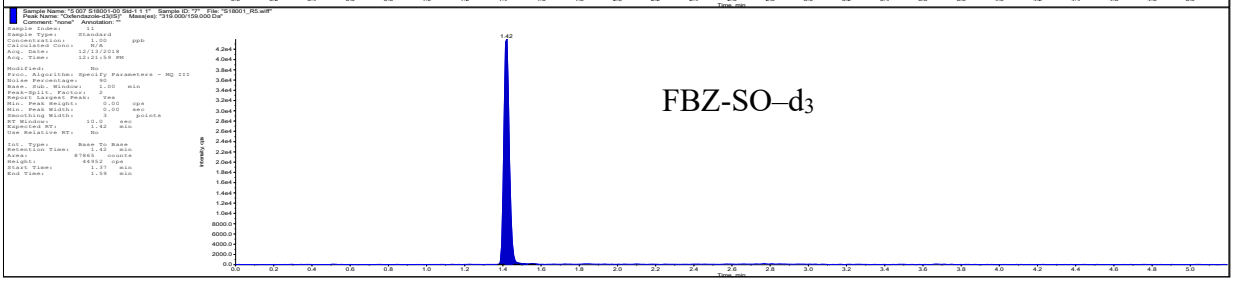


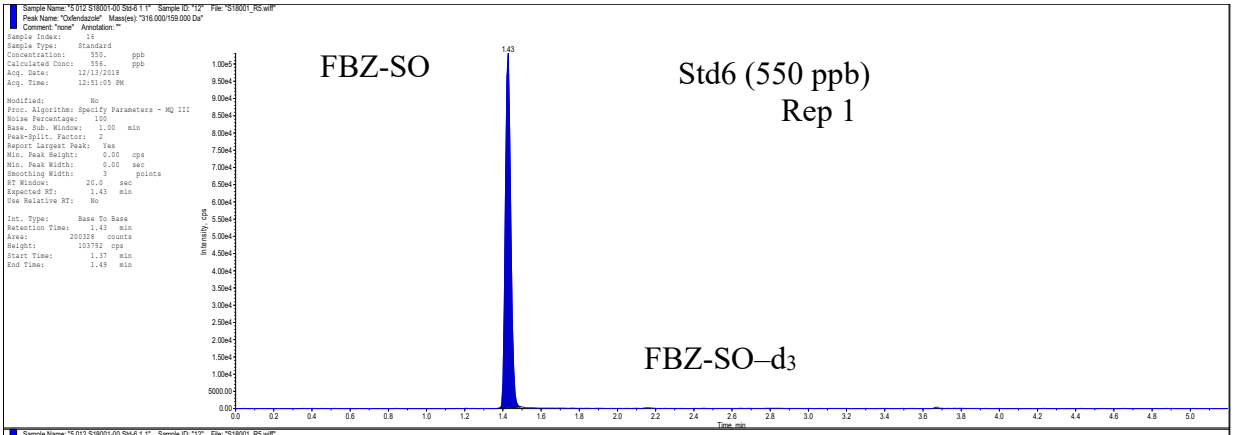
20.3 LC-MS/MS Chromatograms of Control Blank Sample in Determinative Analysis (Source File: S18001_R5.rdb)

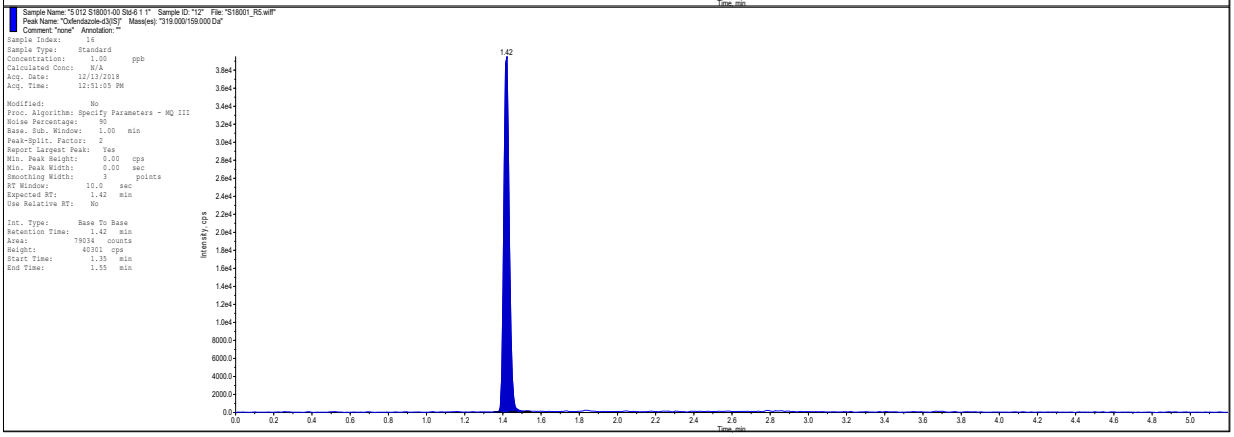


20.4 LC-MS/MS Chromatograms of FBZ-SO Solvent Standards in Determinative Analysis (Source File: S18001_R5.rdb)

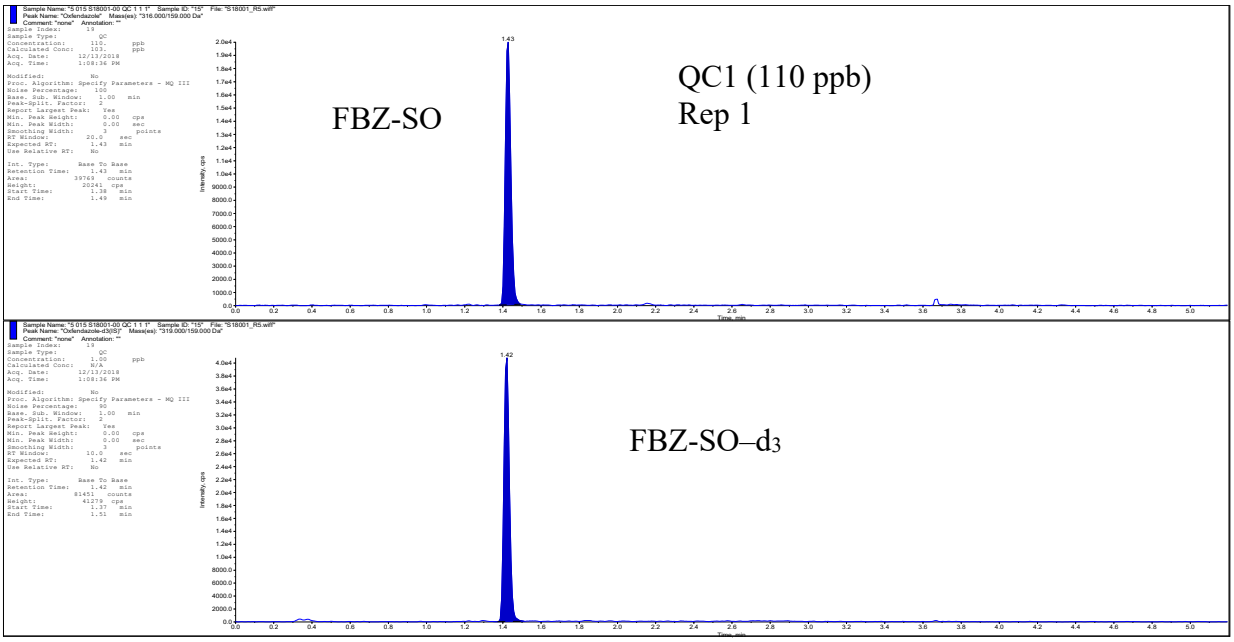


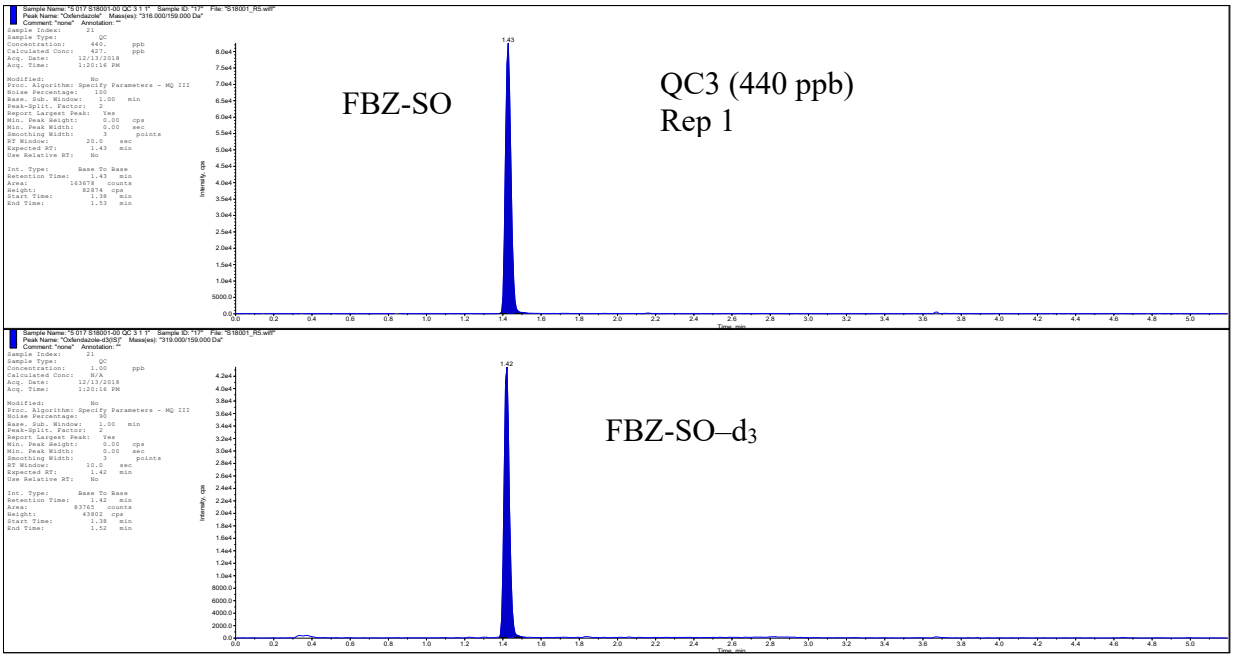




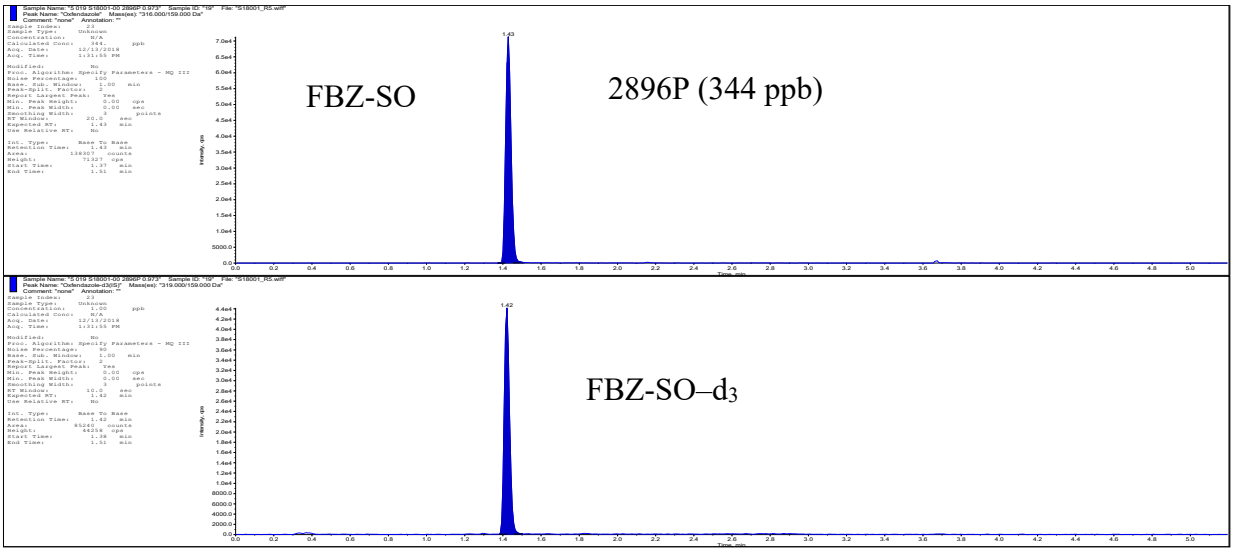


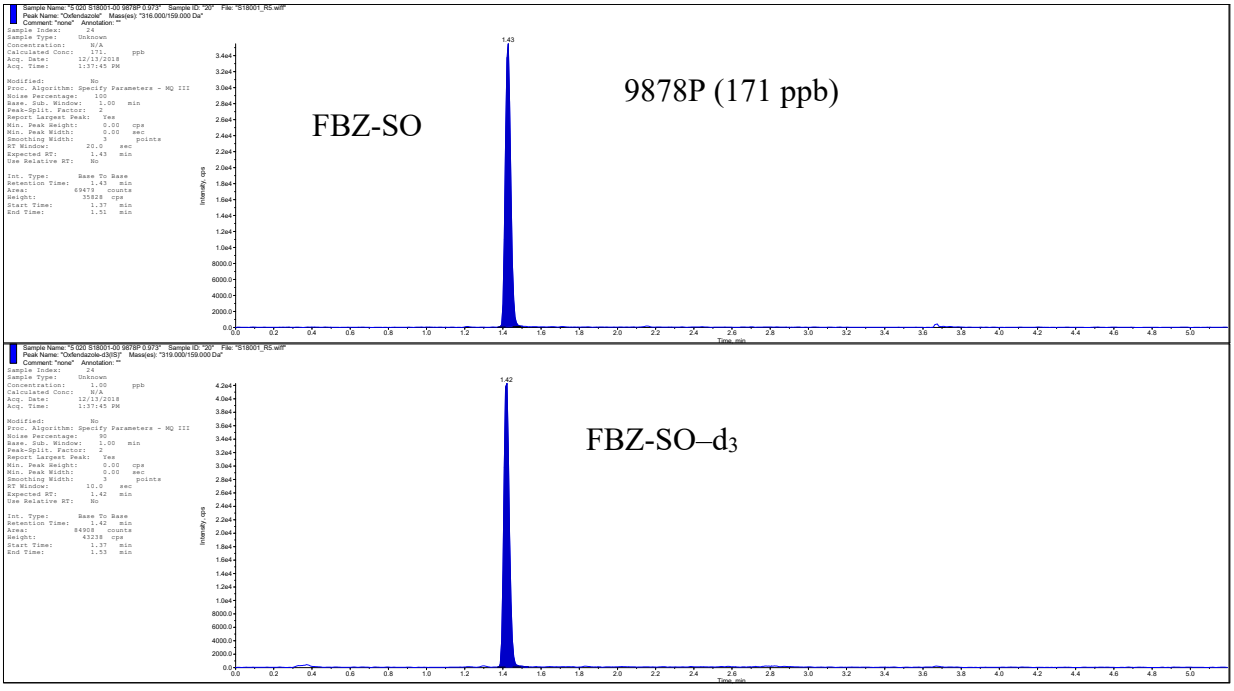
20.5 LC-MS/MS Chromatograms of FBZ-SO QC Sample in Determinative Analysis (Source File: S18001_R5.rdb)



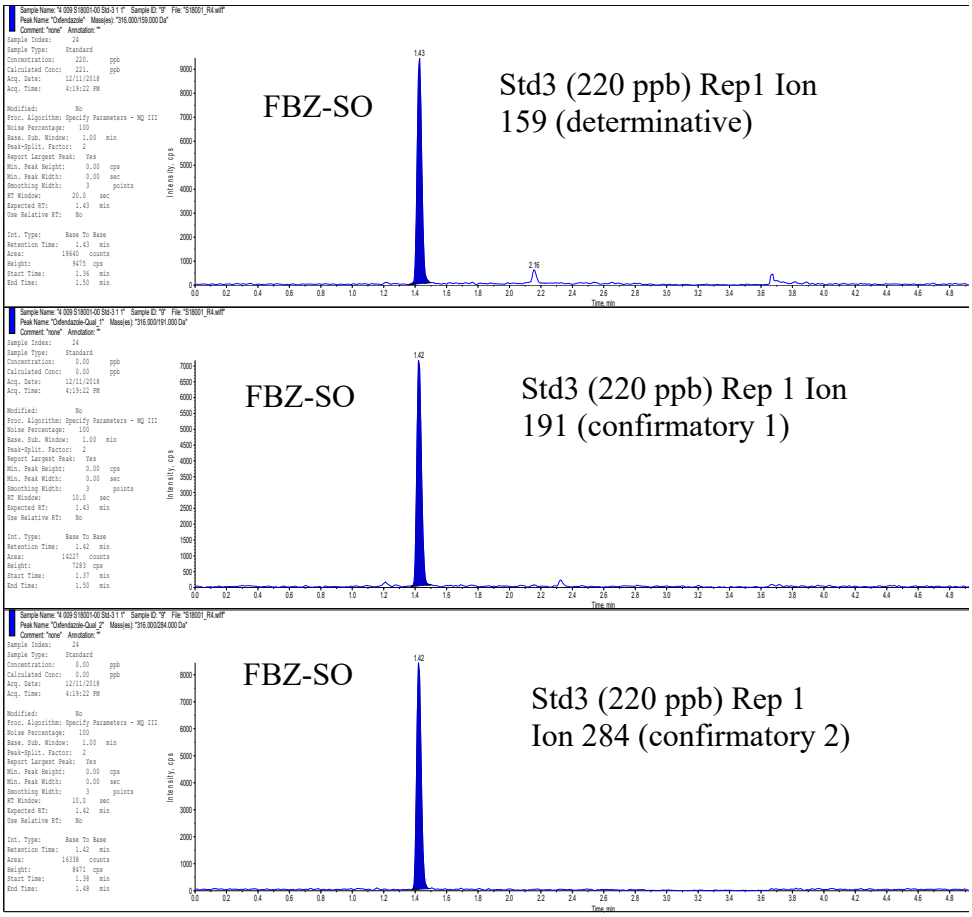


20.6 LC-MS/MS Chromatograms of Blinded Samples in Determinative Analysis (Source File: S18001_R5.rdb)

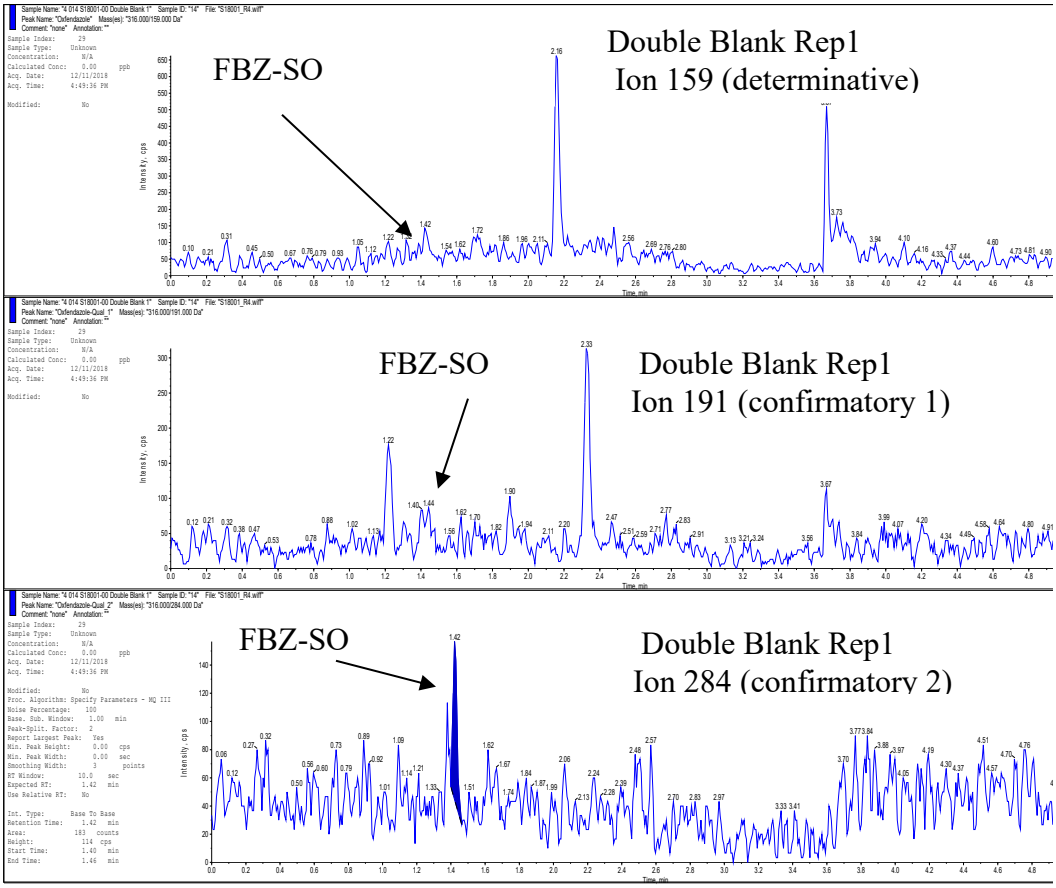




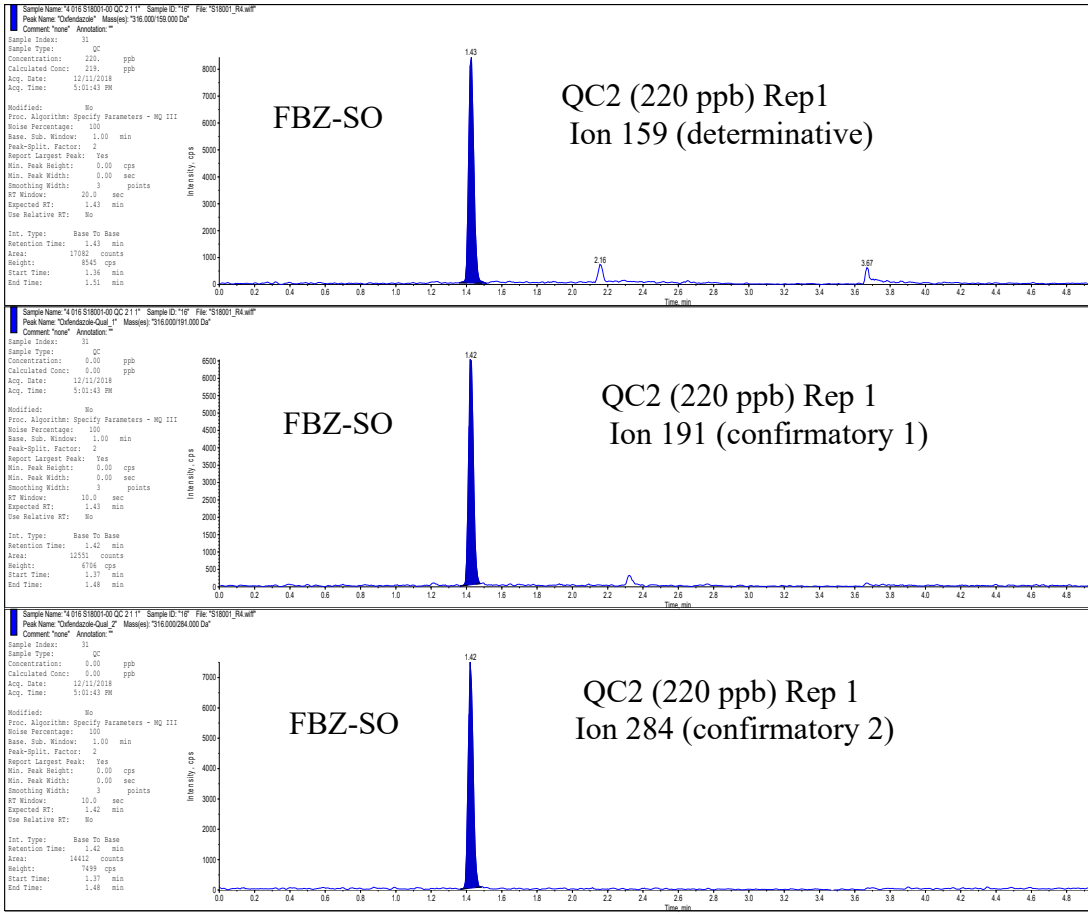
20.7 LC-MS/MS Chromatograms of FBZ-SO Solvent Standard in Confirmatory Analysis (Source File: S18001_R4.rdb)



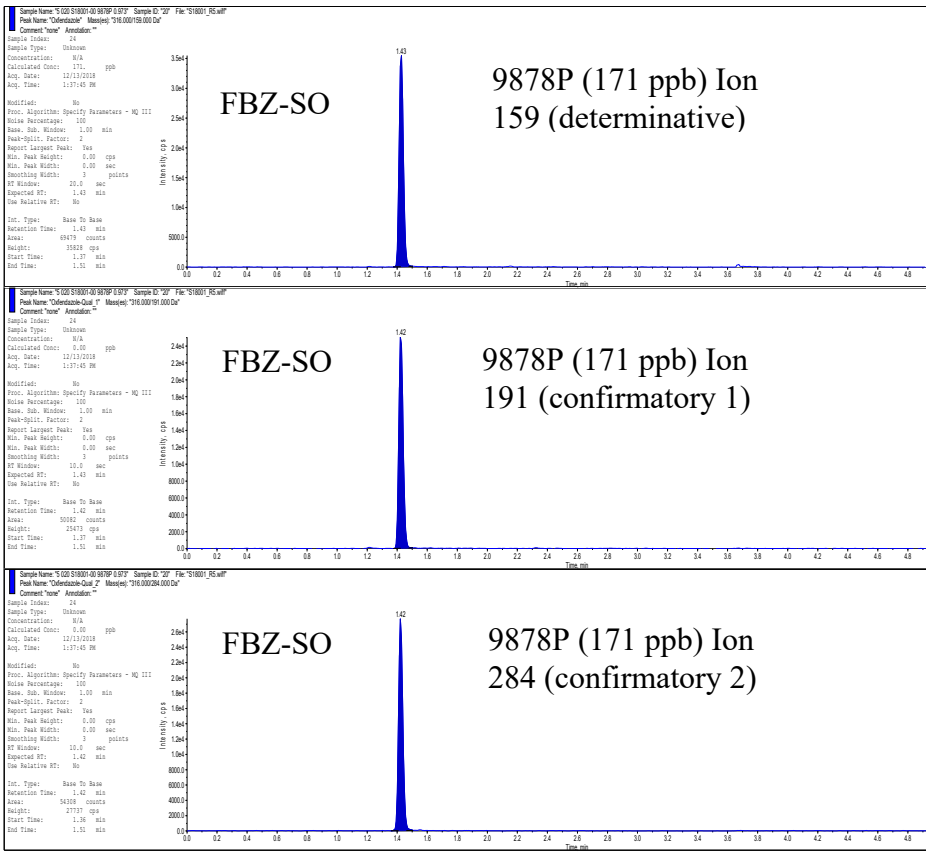
20.8 LC-MS/MS Chromatograms of Double Blank Milk Sample in Confirmatory Analysis (Source File: S18001_R4.rdb)



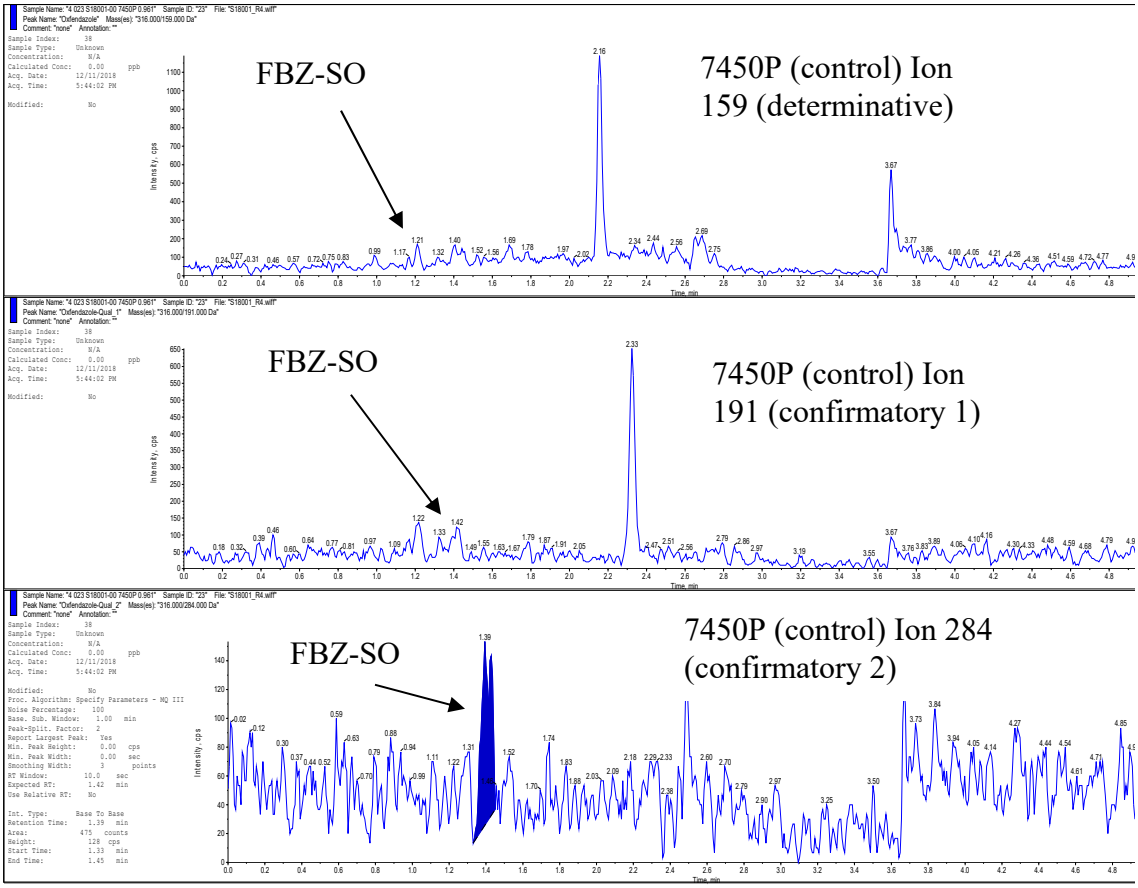
20.9 LC-MS/MS Chromatograms of FBZ-SO QC Sample in Confirmatory Analysis (Source File: S18001_R4.rdb)



20.10 LC-MS/MS Chromatograms of Blinded Milk Sample in Confirmatory Analysis (Source File: S18001_R5.rdb)



20.11 LC-MS/MS Chromatograms of Blinded Milk Sample Control in Confirmatory Analysis (Source File: S18001_R4.rdb)



21 SAFETY DATA SHEETS (SDS)

21.1 SDS for Oxfendazole

SAFETY DATA SHEET

1. Identification

Product identifier **Oxfendazole**

Other means of identification

Catalog number 1483301

Chemical name Carbamic acid, 5-(phenylsulfinyl)-1H-benzimidazol-2-yl-, methyl ester

Recommended use Specified quality tests and assay use only.

Recommended restrictions Not for use as a drug. Not for administration to humans or animals.

Manufacturer/Importer/Supplier/Distributor information

Company name U. S. Pharmacopeia

Address 12601 Twinbrook Parkway
Rockville
MD
20852-1790
US

Telephone RS Technical Services 301-816-8129

Website www.usp.org

E-mail RSTECH@usp.org

Emergency phone number CHEMTREC within US & Canada 1-800-424-9300
CHEMTREC outside US & Canada +1 703-527-3887

2. Hazard(s) identification

Physical hazards Not classified.

Health hazards Not classified.

OSHA hazard(s) Not classified.

Label elements

Hazard symbol No symbol.

Signal word Not available.

Hazard statement Not available.

Precautionary statement

Prevention Not available.

Response Not available.

Storage Not available.

Disposal Not available.

Hazard(s) not otherwise classified (HNOC) Not classified.

3. Composition/information on ingredients

Substance

Non-hazardous components

Chemical name	Common name and synonyms	CAS number	%
Oxfendazole		53716-50-0	100

4. First-aid measures

Inhalation Move to fresh air. Call a physician if symptoms develop or persist.

Skin contact Rinse skin with water/shower. Get medical attention if irritation develops and persists.

Eye contact Rinse with water. Get medical attention if irritation develops and persists.

Ingestion Rinse mouth. If ingestion of a large amount does occur, call a poison control center immediately.

Most important symptoms/effects, acute and delayed Gastrointestinal disturbances.

Indication of immediate medical attention and special treatment needed	Treat symptomatically.
General information	Remove from exposure. Remove contaminated clothing. For treatment advice, seek guidance from an occupational health physician or other licensed health-care provider familiar with workplace chemical exposures. In the United States, the national poison control center phone number is 1-800-222-1222. If person is not breathing, give artificial respiration. If breathing is difficult, give oxygen if available. Persons developing serious hypersensitivity (anaphylactic) reactions must receive immediate medical attention.

5. Fire-fighting measures

Suitable extinguishing media	Use fire-extinguishing media appropriate for surrounding materials. Water. Foam. Dry chemical or CO2.
Unsuitable extinguishing media	None known.
Specific hazards arising from the chemical	No unusual fire or explosion hazards noted.
Special protective equipment and precautions for firefighters	Wear suitable protective equipment.
Fire-fighting equipment/instructions	Use water spray to cool unopened containers. As with all fires, evacuate personnel to a safe area. Firefighters should use self-contained breathing equipment and protective clothing.
Specific methods	Use standard firefighting procedures and consider the hazards of other involved materials.

6. Accidental release measures

Personal precautions, protective equipment and emergency procedures	Keep unnecessary personnel away. Do not touch damaged containers or spilled material unless wearing appropriate protective clothing. Ensure adequate ventilation. Avoid inhalation of dust from the spilled material. Wear appropriate personal protective equipment.
Methods and materials for containment and cleaning up	Sweep up or vacuum up spillage and collect in suitable container for disposal. Avoid the generation of dusts during clean-up. For waste disposal, see section 13 of the SDS. Clean surface thoroughly to remove residual contamination.

7. Handling and storage

Precautions for safe handling	As a general rule, when handling USP Reference Standards, avoid all contact and inhalation of dust, mists, and/or vapors associated with the material. Clean equipment and work surfaces with suitable detergent or solvent after use. After removing gloves, wash hands and other exposed skin thoroughly.
Conditions for safe storage, including any incompatibilities	Store in tight container as defined in the USP-NF. This material should be handled and stored per label instructions to ensure product integrity.

8. Exposure controls/personal protection

Biological limit values	No biological exposure limits noted for the ingredient(s).
Appropriate engineering controls	Airborne exposure should be controlled primarily by engineering controls such as general dilution ventilation, local exhaust ventilation, or process enclosure. Local exhaust ventilation is generally preferred to general exhaust because it can control the contaminant at its source, preventing dispersion into the work area. An industrial hygiene survey involving air monitoring may be used to determine the effectiveness of engineering controls. Effectiveness of engineering controls intended for use with highly potent materials should be assessed by use of nontoxic surrogate materials.

Individual protection measures, such as personal protective equipment

Eye/face protection	Safety glasses with sideshields are recommended. Face shields or goggles may be required if splash potential exists or if corrosive materials are present. Approved eye protection (e.g., bearing the ANSI Z87 or CSA stamp) is preferred. Maintain eyewash facilities in the work area.
Skin protection	
Hand protection	Chemically compatible gloves. For handling solutions, ensure that the glove material is protective against the solvent being used. Use handling practices that minimize direct hand contact. Employees who are sensitive to natural rubber (latex) should use nitrile or other synthetic nonlatex gloves. Use of powdered latex gloves should be avoided due to the risk of latex allergy.
Other	For handling of laboratory scale quantities, a cloth lab coat is recommended. Where significant quantities are handled, work clothing may be necessary to prevent take-home contamination.
Respiratory protection	Where respirators are deemed necessary to reduce or control occupational exposures, use NIOSH-approved respiratory protection and have an effective respirator program in place (applicable U.S. regulation OSHA 29 CFR 1910.134).
Thermal hazards	Not available.
General hygiene considerations	Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Appearance	White or almost white powder.
Physical state	Solid.
Form	Powder.
Odor	Not available.
Odor threshold	Not available.
pH	Not available.
Melting point/freezing point	473 - 509 °F (245 - 265 °C) (decomposes)
Initial boiling point and boiling range	Not available.
Flash point	Not available.
Evaporation rate	Not available.
Flammability (solid, gas)	Not applicable.
Upper/lower flammability or explosive limits	
Flammability limit - lower (%)	Not available.
Flammability limit - upper (%)	Not available.
Explosive limit - lower (%)	Not available.
Explosive limit - upper (%)	Not available.
Vapor pressure	< 0.0000001 kPa at 25 °C
Vapor density	Not available.
Relative density	Not available.
Solubility in water	Practically insoluble.
Partition coefficient (n-octanol/water)	Not available.
Auto-ignition temperature	Not available.
Decomposition temperature	Not available.
Viscosity	Not available.
Other information	
Chemical family	Benzimidazole carbamate.
Molecular formula	C15H13N3O3S
Molecular weight	315.35

10. Stability and reactivity

Reactivity	No reactivity hazards known.
Chemical stability	Material is stable under normal conditions.
Possibility of hazardous reactions	No dangerous reaction known under conditions of normal use.
Conditions to avoid	None known.
Incompatible materials	Strong oxidizing agents.
Hazardous decomposition products	Irritating and/or toxic fumes or gases. Emits toxic fumes under fire conditions. SOx. NOx.

11. Toxicological information

Information on likely routes of exposure

Ingestion	Based on available data, the classification criteria are not met.
Inhalation	Due to lack of data the classification is not possible.
Skin contact	Due to lack of data the classification is not possible.
Eye contact	Based on available data, the classification criteria are not met.
Symptoms related to the physical, chemical, and toxicological characteristics	Abdominal pain. Diarrhea. Nausea. Vomiting. Dizziness. Headache. Sore throat. Fever. Tiredness. Weakness. Hair loss.

Acute toxicity

Product	Species	Test Results
Oxfendazole (CAS 53716-50-0)		
Acute		
Oral		
LD50	Rat	> 6400 mg/kg

Skin corrosion/irritation Based on available data, the classification criteria are not met.

Serious eye damage/eye irritation Based on available data, the classification criteria are not met.

Local effects
100 mg Eye irritancy
Result: Non-irritant.
Species: Rabbit
Skin irritancy
Result: Non-irritant.
Species: Rabbit

Respiratory sensitization Due to lack of data the classification is not possible.

Skin sensitization Based on available data, the classification criteria are not met.

Sensitization
Dermal sensitization study
Result: Non-sensitizing.
Species: Guinea pig

Germ cell mutagenicity Due to lack of data the classification is not possible. Data from germ cell mutagenicity tests were not found.

Mutagenicity
Ames test (Salmonella typhimurium)
Result: Negative.

Carcinogenicity Due to lack of data the classification is not possible. This material is not considered to be a carcinogen by IARC, NTP, or OSHA.

1000 ppm Carcinogenicity study
Result: No evidence of carcinogenicity.
Species: Mouse

Reproductive toxicity Based on available data, the classification criteria are not met.

Reproductivity
10 mg/kg/day Reproductive study
Result: No birth defects.
Species: Rat
108 mg/kg/day Reproductive study
Result: No birth defects.
Species: Mouse

Specific target organ toxicity - single exposure Due to lack of data the classification is not possible.

Specific target organ toxicity - repeated exposure Due to lack of data the classification is not possible.

Aspiration hazard Based on available data, the classification criteria are not met.

12. Ecological information

Ecotoxicity No ecotoxicity data noted for the ingredient(s).

Persistence and degradability No data is available on the degradability of this product.

Bioaccumulative potential Not available.

Mobility in soil Not available.

Other adverse effects Not available.

13. Disposal considerations

Disposal instructions Dispose in accordance with all applicable regulations. Under RCRA, it is the responsibility of the user of the product to determine, at the time of disposal, whether the product meets RCRA criteria for hazardous waste.

Local disposal regulations Not available.

Hazardous waste code Not available.

Waste from residues / unused products Dispose of in accordance with local regulations. Empty containers or liners may retain some product residues. This material and its container must be disposed of in a safe manner (see: Disposal instructions).

Contaminated packaging Empty containers should be taken to an approved waste handling site for recycling or disposal. Since emptied containers may retain product residue, follow label warnings even after container is emptied.

14. Transport information

DOT

Not regulated as a hazardous material by DOT.

IATA

Not regulated as a dangerous good.

Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code No information available.

15. Regulatory information

US federal regulations CERCLA/SARA Hazardous Substances - Not applicable.

One or more components are not listed on TSCA.

Superfund Amendments and Reauthorization Act of 1986 (SARA)

Hazard categories Immediate Hazard - No
Delayed Hazard - No
Fire Hazard - No
Pressure Hazard - No
Reactivity Hazard - No

SARA 302 Extremely hazardous substance No

SARA 311/312 Hazardous chemical No

Other federal regulations

Safe Drinking Water Act (SDWA) Not regulated.

Food and Drug Administration (FDA) Not regulated.

US state regulations California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65): This material is not known to contain any chemicals currently listed as carcinogens or reproductive toxins.

International Inventories

Country(s) or region	Inventory name	On inventory (yes/no)*
Australia	Australian Inventory of Chemical Substances (AICS)	Yes
Canada	Domestic Substances List (DSL)	Yes
Canada	Non-Domestic Substances List (NDSL)	No
China	Inventory of Existing Chemical Substances in China (IECSC)	No
Europe	European Inventory of Existing Commercial Chemical Substances (EINECS)	Yes
Europe	European List of Notified Chemical Substances (ELINCS)	No
Japan	Inventory of Existing and New Chemical Substances (ENCS)	No
Korea	Existing Chemicals List (ECL)	No
New Zealand	New Zealand Inventory	Yes
Philippines	Philippine Inventory of Chemicals and Chemical Substances (PICCS)	No
United States & Puerto Rico	Toxic Substances Control Act (TSCA) Inventory	No

*A "Yes" indicates that all components of this product comply with the inventory requirements administered by the governing country(s)

16. Other information, including date of preparation or last revision

Issue date 09-14-2004

Revision date 06-26-2015

Version # 02

Further information Not available.

Disclaimer

USP Reference Standards are sold for chemical test and assay purposes only, and NOT for human consumption. The information contained herein is applicable solely to the chemical substance when used as a USP Reference Standard and does not necessarily relate to any other use of the substance described, (i.e. at different concentrations, in drug dosage forms, or in bulk quantities). USP Reference Standards are intended for use by persons having technical skill and at their own discretion and risk. This information has been developed by USP staff from sources considered reliable but has not been independently verified by the USP. Therefore, the USP Convention cannot guarantee the accuracy of the information in these sources nor should the statements contained herein be considered an official expression. NO REPRESENTATION OR WARRANTY, EXPRESS OR IMPLIED, INCLUDING THE WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE is made with respect to the information contained herein.

Revision Information

This document has undergone significant changes and should be reviewed in its entirety.

21.2 SDS for Oxfendazole-D_3

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

1.1. Product identifier

Oxfendazole-D3

Further trade names

Fenbendazole sulfoxide-D3;
(5-Benzenesulfinyl-1(3)H-benzoimidazol-2-yl)-carbamic-acid-methyl-D3-ester

CAS No: 1228182-54-4

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

Use of the substance/mixture

Reference standard for analysis.

1.3. Details of the supplier of the safety data sheet

Company name:	WITEGA Laboratorien Berlin-Adlershof GmbH		
Street:	James-Franck-Strasse 4		
Place:	D-12489 Berlin		
Telephone:	+493063922001	Telefax:	+493063922007
e-mail:	witega@witega.de		
Contact person:	Dr. Daniel Aicher	Telephone:	+493063922002
e-mail:	aicher@witega.de		
Internet:	www.witega.de		

1.4. Emergency telephone number:

+493063922001

SECTION 2: Hazards identification

2.1. Classification of the substance or mixture

Regulation (EC) No. 1272/2008

This substance is not classified as hazardous in accordance with Regulation (EC) No. 1272/2008.

2.2. Label elements

Regulation (EC) No. 1272/2008

Precautionary statements

P281	Use personal protective equipment as required.
P262	Do not get in eyes, on skin, or on clothing.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

2.3. Other hazards

No data available

SECTION 3: Composition/information on ingredients

3.1. Substances

Sum formula:	C15H10D3N3O3S
Molecular weight:	318.35 g/mol

SECTION 4: First aid measures

4.1. Description of first aid measures

General information

In case of accident or unwellness, seek medical advice immediately (show directions for use or safety data sheet if possible).

After inhalation

Remove casualty to fresh air and keep warm and at rest. If breathing is irregular or stopped, administer artificial respiration.

After contact with skin

Remove contaminated, saturated clothing immediately. Subsequently wash off with: Water and soap

After contact with eyes

In case of contact with eyes flush immediately with plenty of flowing water for 10 to 15 minutes holding eyelids apart and consult an ophthalmologist.

After ingestion

Rinse mouth immediately and drink plenty of water. Get immediate medical advice/attention.
Do NOT induce vomiting. Never give anything by mouth to an unconscious person or a person with cramps.

4.2. Most important symptoms and effects, both acute and delayed

No data available

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

Water spray jet. Foam. Dry extinguishing powder. Carbon dioxide (CO2).

5.2. Special hazards arising from the substance or mixture

In case of fire may be liberated: Pyrolysis products, toxic. In case of fire and/or explosion do not breathe fumes.

5.3. Advice for firefighters

In case of fire: Wear self-contained breathing apparatus.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

Use personal protection equipment. Do not breathe gas/fumes/vapour/spray.

6.2. Environmental precautions

Do not allow to enter into surface water or drains.

6.3. Methods and material for containment and cleaning up

Take up dust-free and set down dust-free.

6.4. Reference to other sections

Disposal: see section 13

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Advice on safe handling

If handled uncovered, arrangements with local exhaust ventilation should be used if possible.

Advice on protection against fire and explosion

Keep away from sources of ignition - No smoking. Take precautionary measures against static discharges.

7.2. Conditions for safe storage, including any incompatibilities

Requirements for storage rooms and vessels

Keep container tightly closed in a cool, well-ventilated place. Avoid: UV-radiation/sunlight

Further information on storage conditions

storage temperature: 2-8°C

7.3. Specific end use(s)

none

SECTION 8: Exposure controls/personal protection

8.1. Control parameters

8.2. Exposure controls

Appropriate engineering controls

If handled uncovered, arrangements with local exhaust ventilation should be used if possible.If local exhaust ventilation is not possible or not sufficient, the entire working area should be ventilated by technical means.

Protective and hygiene measures

Use personal protection equipment.

Eye/face protection

Eye glasses with side protection

Hand protection

Wear suitable gloves. The quality of the protective gloves resistant to chemicals must be chosen as a function of the specific working place concentration and quantity of hazardous substances.

Skin protection

lab coat

Respiratory protection

In case of inadequate ventilation wear respiratory protection.

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Physical state:	solid
Colour:	white
Odour:	odourless
pH-Value:	No data available

Changes in the physical state

Melting point:	295-298 °C
Initial boiling point and boiling range:	No data available
Flash point:	No data available

Flammability

Solid:	No data available
Gas:	No data available

Explosive properties

No data available

Lower explosion limits:	No data available
Upper explosion limits:	No data available
Ignition temperature:	No data available

Auto-ignition temperature

Solid:	No data available
Gas:	No data available
Decomposition temperature:	No data available

Oxidizing properties

No data available

Vapour pressure:	No data available
Density:	No data available

Water solubility:	No data available

Solubility in other solvents

No data available

Partition coefficient:	No data available
Vapour density:	No data available
Evaporation rate:	No data available

9.2. Other information

none

SECTION 10: Stability and reactivity

10.1. Reactivity

Reacts with : Oxidising agent, Alkali (lye), Etchant and acids

10.2. Chemical stability

The product is chemically stable under recommended conditions of storage, use and temperature.

10.3. Possibility of hazardous reactions

No known hazardous reactions.

10.4. Conditions to avoid

Do not expose to temperatures exceeding 50 °C/122 °F.

10.5. Incompatible materials

Oxidising agent, Alkali (lye), Etchant and acids

10.6. Hazardous decomposition products

In case of fire may be liberated: Pyrolysis products, toxic.

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Acute toxicity

Based on available data, the classification criteria are not met.

Irritation and corrosivity

Based on available data, the classification criteria are not met.

Sensitising effects

Based on available data, the classification criteria are not met.

Carcinogenic/mutagenic/toxic effects for reproduction

Based on available data, the classification criteria are not met.

STOT-single exposure

Based on available data, the classification criteria are not met.

STOT-repeated exposure

Based on available data, the classification criteria are not met.

Aspiration hazard

Based on available data, the classification criteria are not met.

Specific effects in experiment on an animal

No data available

SECTION 12: Ecological information

12.1. Toxicity

No data available

12.2. Persistence and degradability

No data available

12.3. Bioaccumulative potential

No indication of bioaccumulation potential.

12.4. Mobility in soil

No data available

12.5. Results of PBT and vPvB assessment

This substance does not meet the PBT/vPvB criteria of REACH, Annex XIII.

12.6. Other adverse effects

No data available

SECTION 13: Disposal considerations

13.1. Waste treatment methods

Advice on disposal

Dispose of waste according to applicable legislation.
Do not allow to enter into surface water or drains. Do not allow to enter into soil/subsoil.

Contaminated packaging

This material and its container must be disposed of as hazardous waste.

SECTION 14: Transport information

Land transport (ADR/RID)

14.2. UN proper shipping name: No dangerous good in sense of this transport regulation.

Inland waterways transport (ADN)

14.2. UN proper shipping name: No dangerous good in sense of this transport regulation.

Marine transport (IMDG)

14.2. UN proper shipping name: No dangerous good in sense of this transport regulation.

Air transport (ICAO-TI/IATA-DGR)

14.2. UN proper shipping name: No dangerous good in sense of this transport regulation.

14.5. Environmental hazards

ENVIRONMENTALLY HAZARDOUS: no

14.6. Special precautions for user

No data available

14.7. Transport in bulk according to Annex II of Marpol and the IBC Code

No dangerous good in sense of this transport regulation.

SECTION 15: Regulatory information

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

National regulatory information

Employment restrictions: Observe restrictions to employment for juvenils according to the 'juvenile work protection guideline' (94/33/EC). Observe employment restrictions under the Maternity Protection Directive (92/85/EEC) for expectant or nursing mothers.

Water contaminating class (D): 3 - highly water contaminating

15.2. Chemical safety assessment

For this substance a chemical safety assessment has not been carried out.

SECTION 16: Other information

Further Information

This information is based on our present state of knowledge. However, it should not constitute a guarantee for any specific product properties and shall not establish a legally valid relationship. The substances are only for R&D. Do not use as a drug, in household or other applications.

22 METHOD CHANGE LOG

Version	Section	Change	Reason
Version 1 (effective 03-Mar-2017) to Version 4 (effective 09-Nov-2018)	1	Updated abbreviations	For clarification
	2	Removed “used against gastrointestinal parasites and this drug is”	For clarification
	5.2 (Table 5.1)	Addition of MilliQ to the water category	For clarification
	5.2 (Table 5.2)	Addition of “commercially available” for formic acid Addition strong and/or weak autosampler wash solution Removed “Solution” from “Solvent Blank Solution”	For clarification
	5.3	Replaced “Compound” with “Standard”	For clarification
	5.3.1	Updated the storage condition of oxfendazole	For clarification
	5.3.2	Oxfendazole-D_3 information has been changed	To reflect the IS used
	6.1	Changed freezer temperatures Added pipets and sonicator to the device list	For clarification
	7.1	Removed “DMSO” from all standard preparations Updated target weight for reference and IS standard Removed “and/or invert” for mixing of reference standard Added time requirement for vortexing of reference standard Changed the mixing technique and time for the IS from “vortex” to “sonication	For clarification

Version	Section	Change	Reason
		for approximately 1 minute” Added a footnote to the transfer volume Changed “needs to” to “should” Added 2.00 µg/mL in Section Title	
	Table 7.1.5-1	Updated the Table Reference Updated the peak area ratio (PAR) and Precision acceptance criteria	For clarification
	Table 7.1.5-2	Changed LC-MS-MS to LC-MS/MS	Per CVM recommendation at method trial protocol review
		Updated Intermediate and Fortification from the table	For clarification
	7.2	Removed two Working Standards for Oxfendazole Calibration Standards (SL 2 and SL 7) Added “mix thoroughly” for preparation of Working Standards Changed “needs to” to “should”	For clarification
	7.3	The lowest QC fortification standard (0.600 µg/mL) has been deleted	Not needed for future analysis

Version	Section	Change	Reason
		Added "mix thoroughly" for preparation of Quality Control Fortification Standards Changed "needs to" to "should"	For clarification
	7.4	Deleted the equation for calculation of dilution factor	Per CVM recommendation at method trial protocol review
		Decreased volumes for preparation of W-Mix-Stds solutions Removed two W-Mix Standard Solutions from Table 7.4-1 (Std-2 and Std-7) Changed "needs to" to "should"	For clarification
	7.5	Added "For routine analysis" Changed "needs to" to "should"	For clarification
	Table 7.5-1	QC sample at 60 ppb was deleted	Not needed for future analysis
	8.1	Changed freezer temperature from "-80ºC" to "≤-65ºC"	For clarification
	9	Added "water" to criterion of thawing samples. Updated amount of milk samples for transfer. Included a note for specifying volume of milk to obtain desired amount. Removed specific vortexing speeds and replaced with	For clarification

Version	Section	Change	Reason
		either “moderate” or “high” Updated the Critical Step with 5 minutes room temperature incubation time with methanol before resuspension Added temperature range for storage of methanol extract	
	10	Added an approximate time period for sample incubation prior to extraction	For clarification
	12	Removed 2 Calibration Standards from analysis sequence	For clarification
	13	Updated standard curve generation Updated S_w definition Added Section 13.3.2 “Comparison of Ion Retention Times” Added Section 13.3.3 “Calculation of Ion Signal to Noise Ratio	For clarification
		Included the calculation of the milk equivalent concentration of the sample extract (by the application of the dilution factor of 200	Per CVM recommendation at method trial protocol review
	14	Replaced “Replaced SSTL” with “SST” Updated Quality Control Sample Acceptance Criteria Updated one of the three Confirmatory Criteria ($R_{S/N}$)	

Version	Section	Change	Reason
	15	Changed LOD from 20.3 ppb to 18.0 ppb. Changed LOQ from 61.8 ppb to 53.0 ppb	Per CVM recommendation at method validation review
		In Section Title, changed the extra “AND” to “OF”	Per CVM recommendation at bridging study review and at method trial protocol review
	17.1	Oxfendazole and Oxfendazole-D3 stocks solutions stored at -20 ºC are stable for 92 days. QC spike solutions stored at -20 ºC are stable for 90 days	To correct the typo in the paragraph for clarification
	17.4	Changed stability of cattle milk samples stored at -80 ºC from 6 months to 3 months	Per CVM recommendation at method validation review
	18	Added a critical note for IS monitoring and LC/MS-MS system cleanness	For clarification
Version 4 (effective 09-Nov-2018) to Version 5 (effective 15-May-2019)	5	In table 5-1, added acetonitrile	Omitted
	9	In 9.2e, added note for better vortexing	For clarification
	19	Changed section title by replacing the data from validation with method trial	To reflect the updates from method trial study
	19.3	Changed section title and replaced validation data with method trial data	To reflect the updates from method trial study
	20	Changed section title and replaced standard curve and chromatograms from validation with standard curve and chromatograms from method trial	To reflect the updates from method trial study

Version	Section	Change	Reason
Version 5 (effective 15-May-2019) to Version 6 (effective 06-Jan-2020)	13.3.1	Replaced the incorrect sentence “Error! Objects cannot be created from editing field codes” with the correct equation for calculation of ion ratio	Correction made per CVM recommendation in I-001684-P-0229-HF
	14.4	Changed m/z 284with to m/z 284 with	Correction made per CVM recommendation in I-001684-P-0229-HF
	15	Changed LOD from 18.0 ppb to 7.2 ppb and LOQ from 53.0 ppb to 21.8 ppb	Updated the LOD and LOQ post-method trial per CVM recommendation in I-001684-P-0229-HF