Determinative and Confirmatory Procedures for the Detection of Fenbendazole in Liver Tissues of Swine Using LC-MS/MS v. 6.0	MERCK Animal Health

METHOD TITLE: Determinative and Confirmatory Procedures for the Detection of Fenbendazole in Liver Tissues of Swine Using LC-MS/MS v. 6.0

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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
ALOQ	Above Limit of Quantitation
amu	Atomic Mass Unit
BA	Bioanalytical
BLOQ	Below Limit of Quantitation
Control Blank	Blank matrix sample, fortified with IS only
CV	Coefficient of Variation
DMSO	Dimethyl Sulfoxide
Double Blank	Double Blank matrix sample, not fortified with IS or analyte
FBZ	Fenbendazole
FBZ-d3	Deuterated Fenbendazole used for internal standard
HDPE	High-Density Polyethylene
HPLC	High Performance Liquid Chromatography
LC-MS/MS	High Performance Liquid Chromatography – Tandem Mass Spectrometry
IS	Internal Standard
LC-MS	Liquid Chromatography – Mass Spectrometry
LOQ	Limit of Quantitation
LLOQ	Lower Limit of Quantitation
MAH	Merck Animal Health
MilliQ water	Water purified by a Millipore Synthesis A10
MSDS	Material Safety Data Sheet
n	Number of Samples
NA	Not Applicable
ppm	Parts per Million (µg/g)
psi	Pounds per Square Inch
pw	Peak Width
QC	Quality Control
PAR	Peak Area Ratio
RCF	Relative Centrifugal Force (x g)
rpm	Rotations per Minute
s	second
SL	Solvent Level
Solvent Blank	Methanol (MeOH) Sample
SST	System Suitability Test
SSTL	Standard 1
STD	Standard Calibrator
ULOQ	Upper Limit of Quantitation
v/v	Volume per Volume
v/v/v	Volume per Volume per Volume
WS	Working Solution

2 SCOPE AND FIELD OF APPLICATION

Fenbendazole (FBZ) is a broad spectrum benzimidazole anthelmintic used against gastrointestinal parasites and intended for use as a veterinary drug in swine. The marker residue for liver, the target tissue in swine in the U.S., is fenbendazole and the presumptive US tolerance is 3.2 ppm. This procedure describes the determinative and confirmatory SOP for the quantitation and identification of FBZ in swine liver. The determinative and confirmatory procedures, which are performed simultaneously, consist of a sample solvent extraction followed by LC-MS/MS detection.

The current method was validated in compliance with the following regulations and guidance documents:

- Food and Drug Administration/Center for Veterinary Medicine's (FDA/CVM's) Guidance for Industry 3, 2006: General Principles for Evaluating the Safety of Compounds Used in Food-producing Animals; V. Guidance for Approval of a Method of Analysis for Residues.
- FDA/CVM's Guidance for Industry 118, 2003: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues.
- FDA/CVM's Guidance for Industry 208, 2011**:** Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Validation of Analytical Methods Used in Residue Depletion Studies.

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

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

Virginiamycin	Tylosin	Chlorotetracycline
Bacitracin Zinc	Tiamulin	Tulathromycin
Doramectin	Dichlorovas	Oxytetracycline
Ivermectin	Oxfendazole	Tilmicosin
Ractopamine	Fenbendazole Sulfone	
Lincomycin	Pyrantel Tartrate	
Carbadox	Neomycin	

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

3 PRINCIPLE

One gram (± 0.05) of homogenized swine liver is fortified with deuterated fenbendazole internal standard (FBZ-d_3) and then extracted twice with methanol in two extraction steps. The sample extract is diluted to 20 mL with methanol. An aliquot of the methanol extract is diluted with methanol/purified water (60/40, v/v). The resulting solution is quantitatively analyzed using gradient reversed phase liquid chromatography with mass spectrometric detection (LC-MS/MS) using a positive ion multiple-reaction monitoring (MRM) with ion transition of *m/z* 300 → *m/z* 268 for fenbendazole (FBZ) and *m/z* 303

→*m/z* 268 for FBZ-d_3. Additional ion transitions from FBZ, m/z 300 → *m/z* 159 as qualifier 1 and *m/z* 300 → *m/z* 131 as qualifier 2 were monitored for the confirmatory method. See Figure 18.1 for the fenbendazole fragmentation scheme. Method limits of detection and quantitation calculated from solvent calibration curves generated during the method trial were 0.602 ng/mL (tissue equivalent 0.241 ppm) and 1.82 ng/mL (tissue equivalent 0.729 ppm), respectively.

4 WARNINGS AND SAFETY PRECAUTIONS

Take safety precautions common in the laboratory, *e.g.* wear lab coat, goggles and gloves if necessary. The MSDS of fenbendazole and fenbendazole-d_3 is attached as an appendix (Section 21) to this test procedure.

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. Common 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 / Catalog Number
Methanol (MeOH)	HPLC	Fisher / A454-4
Acetonitrile (ACN)	Optima	Fisher / A996-4
Acetonitrile, 0.1% formic acid	HPLC	Fisher / HB9823-4
Dimethyl sulfoxide (DMSO)	>99.5%	Fisher / D128-4
0.1% formic acid in water	HPLC	Fisher / LS118-4
Water (H_2O, purified)	18 MΩ/cm or HPLC	Millipore or equivalent or Fisher
Formic Acid	Min. 88%, ACS grade	Fisher / A118P-500

5.2 Solutions

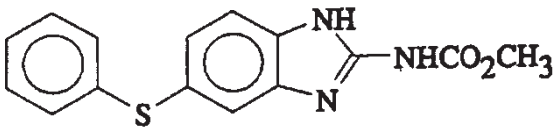
The following solutions may be prepared (by volume-to-volume equivalence or by dilution) in different quantities. Measure volume using a suitably sized graduated cylinder or graduated pipette.

Table 5-2: Reagents to be Used in this Test Procedure	
Reagent	**Preparation and Storage**
HPLC – Mobile Phase A Mobile Phase A: 0.1% Formic Acid in Water, v/v	Use commercially available pre-made 0.1% formic acid in water. Mobile phase is stable for 2 weeks at room temperature once transferred into reagent reservoir. Alternatively, mix 1 mL of formic acid in 1 L of purified water.
HPLC – Mobile Phase B Mobile Phase B: 0.1% Formic Acid in Acetonitrile, v/v	Use commercially available pre-made 0.1% formic acid in acetonitrile. Mobile phase is stable for 2 weeks at room temperature once transferred into reagent reservoir. Alternatively, mix 1 mL of formic acid in 1 L of acetonitrile.
Dilution solution (methanol/purified water, 60/40, v/v)	Mix 600 mL methanol with 400 mL purified water. (Volume contraction is not considered). Stable for 1 month at room temperature.
Injector wash solution for autosampler (Methanol/acetonitrile/purified water, 30/30/40, v/v/v)	Mix 300 mL methanol with 300 mL acetonitrile and 400 mL purified water. (Volume contraction is not considered). Stable for 1 month at room temperature.

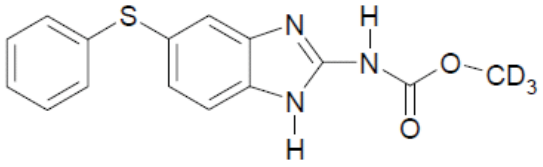
5.3 Reference Compound

The reference compound, fenbendazole (FBZ), and the internal standard, FBZ-d_3, are retested periodically and the actual content from these retests are used for the relevant calculations. The two standards are currently available from commercial vendors and these may be used but must have a certificate of analysis and be of known isotopic and/or chemical purity.

5.3.1 Reference Compound FBZ

Name:	FBZ (Fenbendazole)
Active ingredient:	FBZ
CAS-No.:	43210-67-9
Chemical name:	Carbamic acid, N-[6-(phenylthio)-1H-benzimidazol-2-yl]-, methyl ester
Formula:	$C_{15}H_{13}N_3O_2S$
Molecular weight:	299.349 g/mol
Appearance / colour:	almost white powder, odourless
Storage conditions:	Room temperature
Supplier:	Intervet, Mexico
Structural formula:	

5.3.2 FBZ-d_3 (Used as Internal Standard)

Name:	Fenbendazole-d_3
CAS-No.:	NA
Chemical Name:	5-Pheylsulfanyl-1-H-benzoimidazol-2-yl)-carbamic acid methyl-d_3 ester
Formula:	$C_{15}H_{10}D_3N_3O_2S$
Molecular Weight:	302.35 g/mol
Appearance/Color:	white powder
Storage Conditions:	2-8 °C
Supplier	Witega (Berlin, Germany)
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 3,300 RCF (x *g*) with appropriate rotor
Cylinders - graduated – 100, 250, 500, 1000 and 2000 mL
Flasks - volumetric with glass stopper – 10, 20, 25, 50, and 100 mL
Freezers - capable of maintaining temperatures ≤ -65°C and ≤ -10°C
Refrigerator - capable of maintaining temperatures 2-8°C
Millipore Water System
Rainin EDP3 Pipets and tips
Robot Coupe®, commercially available cryogenic meat grinder or food blender such as Waring Commercial Laboratory Blender
Vortex mixer – Vortex-Genie 2
Vortex multi-tube mixer / Eberbach shaker

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
500 mL or 1 L polypropylene sample bottles – Fisher brand
20 mL scintillation vials
2 mL 96-well plates and cap mats - Analytical Sales and Services
2 mL glass or polypropylene autosampler vials

6.3 LC-MS Equipment

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

Table 6-3: LC-MS list
Thermo Transcend Allegros UPLC pumps, Thermo PAL autosampler or Thermo Open Access LC System
Primary HPLC Column: MacMod Ace 3 C18, 2.1 x 50 mm, Part Number ACE-111-0502 Alternate HPLC Column (used for ruggedness test): Acclaim 120 C18, 3 µm, 2.1 x 50 mm, product # 059128
MS spectrometer– Applied Biosystems, API 4000 Triple Quadrupole and API4000 Qtrap, or Thermo Vantage Triple Quadrupole
LC/MS Data acquisition system – Applied Biosystems, Analyst, Version 1.4 or Thermo LCQuan v. 2.6
Data calculation software – Analyst v. 1.4.2 or LCQuan v. 2.6 with Microsoft Excel or Watson v. 7.3

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.

7.1 FBZ and FBZ-d_3 DMSO Stock Solution

All stock solutions of FBZ and FBZ-d_3 are prepared in dimethyl sulfoxide (DMSO).

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

Weigh approximately 20 mg of reference standard directly into an appropriate container and record the exact weight to the nearest 0.1 mg. Using a calibrated pipette, add an appropriate amount of DMSO to yield a concentration of 2000 µg/mL, after correction

for purity, and dissolve (vortex to mix) the standard. The stability of this stock solution is 2 months in a freezer set to -20°C.

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

This solution is prepared from a second independent weighing procedure (according to Section 7.1.1). It is applied for preparation of the quality control (QC) solutions and spiking of the QC samples. The stability of this stock solution is 2 months in a freezer set to -20°C.

7.1.3 Preparation of FBZ-d_3 DMSO Internal Standard Stock Solution at 1,000 µg/mL (FBZ-D_3 DMSO Stock 1)

Weigh approximately 20 mg of FBZ-d_3 reference standard; record the exact weight to the nearest 0.1 mg. Dissolve the standard with an appropriate amount of DMSO to yield a concentration of 1000 µg/mL after corrected for purity. The stability of this stock solution is 2 months in a freezer set to -20°C.

7.1.4 FBZ-d3 Internal Standard Fortification Solutions for Liver

Transfer aliquots of the FBZ-d_3 DMSO stock solution (Section 7.1.3) into an appropriate bottle or container and dilute with methanol according to the following scheme (Table 7-1-4-1). The IS fortification solution is stable for 2 months in a freezer set to -20°C.

Table 7-1-4-1 FBZ-d_3 Internal Standard Fortification Solution for Liver – Scheme for Aliquot Transfers of Solutions

Working Solution ID	Concentration [µg/mL]	Volume of Solution Taken	Final Volume [mL]
Liver FBZ-d_3 fortification	30	1500 µL of FBZ-d_3 DMSO stock solution	50

7.1.5 Comparison of Stock Solutions

A stock solution comparison is required when new stock solutions are prepared in order to demonstrate equivalence. Dilute each of the two stock solutions (Sections 7.1.1 and 7.1.2) to an appropriate concentration and add an appropriate concentration of IS fortification solution (Section 7.1.4).

Each of the two stock solutions needs to be properly diluted with dilution solution according to following schemes. The suggested concentrations are 7.5 ng/mL (FBZ) and 7.5 ng/mL (IS).

Table 7-1-5-1: Preparation of Intermediate Solutions for Stock Comparison				
Intermediate Solution ID	**Final Concentration [ng/mL]**	**FBZ DMSO Stock Solution**		**Final Volume [mL]**
		Starting Concentration (µg/mL)	**Volume Taken (µL)**	
FBZ STD Stock Intermediate Solution	5,000	2,000 (Stock Solution)	125	50
FBZ QC Stock Intermediate Solution	5,000	2,000 (QC Stock Solution)	125	50

Table 7-1-5-2: Preparation of Final Dilutions for Stock Comparison						
Final Dilution Solution ID	**Final Concentration (ng/mL) [FBZ / IS]**	**FBZ Stock Intermediate Solution**		**IS Fort. Solution**		**Final Volume [mL]**
		Starting Concentration [ng/mL]	**Volume Taken [µL]**	**Starting Concentration [µg/mL]**	**Volume Taken [µL]**	
FBZ STD Stock Final Dilution	7.5 / 7.5	5,000	150	30	25	100
FBZ QC Stock Final Dilution	7.5 / 7.5	5,000	150	30	25	100

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

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

Stock comparison solutions are to be prepared on the day they are used and discarded after use. If the mean percent difference and/or precision are not within ± 5%, the solutions are not considered equivalent and fresh solutions (stock and/or intermediate) will be prepared and compared.

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

Transfer aliquots of the FBZ DMSO stock solution 1 (Section 7.1.1) into appropriate volumetric flasks and bring to volume with methanol according to the following scheme (Table 7-2-1). All working solutions are stored in a -20°C freezer and stable for 2 months.

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

Working Solution ID	Final Concentration [µg/mL]	Volume of Solution Taken	Final Volume [mL]
SL 8 liver	1000	5000 µL of stock solution	10
SL 7 liver	100	1000 µL of SL 8 liver	10
SL 6 liver	75	750 µL of SL 8 liver	10
SL 5 liver	60	600 µL of SL 8 liver	10
SL 4 liver	50	500 µL of SL 8 liver	10
SL 3 liver	30	300 µL of SL 8 liver	10
SL 2 liver	15	150 µL of SL 8 liver	10
SL 1 liver	10	100 µL of SL 8 liver	10

7.3 FBZ Quality Control Fortification Solutions for Liver

Transfer aliquots of the FBZ DMSO stock solution 2 (Section 7.1.2) into appropriate volumetric flasks and bring to volume with methanol according to the following scheme (Table 7-3-1). All QC fortification solutions are stored in a freezer set to -20°C and stable for 2 months.

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

Working Solution ID	Final Concentration [µg/mL]	Volume of Solution Taken	Final Volume [mL]
QC SL 5 liver	500	5000 µL of QC stock solution	20
QC SL 4 liver	80	4000 µL of QC SL 5 liver	25
QC SL 3 liver	64	3200 µL of QC SL 5 liver	25
QC SL 2 liver	32	1600 µL of QC SL 5 liver	25
QC SL 1 liver	16	800 µL of QC SL 5 liver	25

7.4 Solvent Calibration Curve for Liver

For preparation of the solvent calibration curve; add 100 µL of the respective working solutions (Section 7.2), 100 µL of the IS (FBZ-d_3) fortification solution (Section 7.1.4) (30 µg/mL) to a 20 mL vial or 20 mL volumetric flask. Pipette 19.8 mL methanol to the vial or fill the volumetric flask to volume with methanol and them mix well to give W-Mix-Stds (see Table 7-4-1). Store all W-Mix-Stds in a freezer set at -20°C. The W-Mix-Stds methanolic solution is stable for 1 month.

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

W-Mix-Stds: Mix 100 µL of FBZ- -D_3 fortification solution (Section 7.1.4) with 100 µL of SL-1-7 liver (Section 7.2) and add 19.8 mL of methanol with a calibrated pipette

Solution ID	Volume of SL 1 - 7 Liver Taken / Concentration [µg/mL]	Volume of IS Solution Taken	Final Volume [mL]	Final Concentration [ng/mL]
W-Mix-Std-7	100 µL of SL 7 Liver / 100	100 µL	20	500
W-Mix-Std-6	100 µL of SL 6 Liver / 75	100 µL	20	375
W-Mix-Std-5	100 µL of SL 5 liver 60	100 µL	20	300
W-Mix-Std-4	100 µL of SL 4 liver / 50	100 µL	20	250
W-Mix-Std-3	100 µL of SL 3 liver /30	100 µL	20	150
W-Mix-Std-2	100 µL of SL 2 liver / 15	100 µL	20	75
W-Mix-Std-1	100 µL of SL 1 liver / 10	100 µL	20	50

Combine 0.5 mL of each W-Mix-Stds solution with 9.5 mL of methanol/purified water (60/40, v/v) and vortex well to give Liver-Stds (see Table 7-4-2). Transfer an appropriate volume of Liver-Stds to a 96-well plate or to autosampler vials for LC-MS/MS analysis. The standard solution concentrations (ng/mL) and the corresponding tissue concentrations (ppm) are specified in Table 7-4-2. The calibration standards are prepared fresh daily.

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

Calibration Curve: Mix 0.5 mL of W-Mix-Stds with 9.5 mL dilution solution (methanol:water, 60/40, v/v) in 20 mL vial

Standard-ID	W-Mix-STD Solution ID	Final Concentration [ng/mL]	Liver Tissue Equivalent Concentration [ppm][a]
Liver-Std-7	W-Mix-Std-7	25.0	10
Liver-Std-6	W-Mix-Std-6	18.75	7.5
Liver-Std-5	W-Mix-Std-5	15.0	6.0
Liver-Std-4	W-Mix-Std-4	12.5	5.0
Liver-Std-3	W-Mix-Std-3	7.50	3.0
Liver-Std-2	W-Mix-Std-2	3.75	1.5
Liver-Std-1	W-Mix-Std-1	2.5	1.0

Note: the nominal concentration of internal standard in Liver-Stds is 7.5 ng/mL, equivalent to 3 ppm in tissue.

[a]Extraction process is a 400x dilution of residues (1 g tissue extracted in 20 mL of Methanol; 50 µL of extract diluted to 1 mL with dilution solution). Therefore, conversion factor from ppm tissue equivalents to solvent concentration (ng/mL) is 2.5 (*i.e* 1000 / 400).

7.5 Quality Control Samples for Liver

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

For preparation of the QC samples, 100 µL of the respective QC fortification solutions (Section 7.3) and 100 µL of the IS fortification solution (Section 7.1.4) (30 µg/mL) are spiked into 1 ± 0.05 g of blank liver (see Table 7-5-1). For routine sample analysis, QC samples are prepared fresh daily.

Table 7-5-1:Quality Control Samples (Liver)

Concentration of Quality Control Samples [ppm][a]	Spiking Volume of Internal Standard Solution	Spike Volume of Working Solution
8.00	100 µL	100 µL of QC SL 4 liver
6.40	100 µL	100 µL of QC SL 3 liver
3.20	100 µL	100 µL of QC SL 2 liver
1.60	100 µL	100 µL of QC SL 1 liver

a. Internal standard = 3 ppm.

Further sample preparation is described in Section 9.1.

8 SAMPLE HANDLING AND SAMPLING

8.1 Homogenize Tissue Sample

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

Note: the liver should be chopped into approximately 1" cubes and these should be thoroughly mixed prior to homogenization to insure complete homogeneity is achieved.

Chopped tissue is mixed with dry ice and ground with a Robot Coupe® or other food processor until it becomes a uniform powder. The powdered tissue (containing dry ice) is transferred into a suitable container (*e.g.* a 500 mL or 1 L polypropylene sample container). The container is loosely sealed or capped and stored in freezer set to -20°C overnight or longer to allow the dry ice sublime. After all the dry ice has been sublimed, the container is sealed and stored at ≤ -65°C freezer for longer term storage.

8.2 Sample storage

Control and incurred samples are stored in suitable container in a freezer at ≤ -65°C. It is recommended to keep tissue in frozen powdered form until analysis. Fenbendazole is stable for 1 year in homogenized and intact liver at this temperature, the maximum interval tested.

8.3 Stability Results for Swine Liver Tissue

Fenbendazole is stable for four freeze thaw cycles in homogenized liver tissues. The methanolic extract of liver tissue is stable for 10 days at refrigeration storage. Fenbendazole is stable at room temperature in liver tissue for 24 hours.

9 PROCEDURE FOR DETERMINATION AND CONFIRMATION OF FBZ IN SWINE LIVER

Using this procedure, approximately 52 samples can be extracted in an 8-hour day by an individual analyst and the LC-MS/MS analysis initiated. All of the procedure (except weighing of aliquots) is performed at room temperature unless otherwise specified.

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

Note: The extraction method described below applies to both the determinative and confirmatory procedures,

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

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

Note: Tissue samples can be weighed out on a different day to facilitate the process. Samples should be preferably weighed on dry ice while frozen and still in powdery form The remaining tissue samples should be returned to storage at ≤ -65 ℃. Thawing has no adverse impact on the sample integrity for 4 freeze/thaw cycles. If the sample has thawed, a disposable transfer pipette can be used to weigh the aliquots and the tissue should be returned to storage afterwards.

9.1b Add 200 µL methanol to control liver sample aliquot(s) for the double blank sample. Add 100 µL methanol and 100 µL of internal standard fortification solution (Section 7.1.4) for control and incurred liver sample aliquots. Add 100 µL QC fortification solution (Section 7.3) and 100 µL of internal fortification standard (Section 7.1.4) to control liver aliquots for QC samples and briefly vortex after fortification. Leave the sample on the bench for approximately 10 min before extraction.

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

9.2 Extraction of tissue sample

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

9.2b Briefly vortex each sample individually to loosen the tissue and then vortex all samples for *ca.* 10 min. at high speed (setting at 7-9) using a multi-tube vortexer. Visually inspect all tubes to ensure tissue is swirling up and thoroughly mixed. If any sample did not swirl up during the initial vortex, re-vortex individually for up to 10 seconds so that the tissue solid can be mixed well with the extraction solvent, then put the individual sample back onto the multi-tube vortexer for 10 more minutes. **Note:** Alternatively, an Eberbach shaker can be used to agitate / extract the samples. The tubes are placed on their side in the shaker to assure sufficient mixing. The highest setting on the shaker should be used.

9.2c Centrifuge the sample at ~3300 rcf (~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 using a pipette or a bottletop dispenser.

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

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

9.2g Centrifuge the sample at ~3300x 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 (Section 9.2d).

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

9.2j Pipette 50 µL of the methanol liver tissue extract into the appropriate wells of a 2 mL 96-well plate or 2 mL autosampler vial and mix with 950 µL of dilution

solvent, methanol/purified water (60/40, v/v). Vortex and mix well for LC-MS/MS analysis. Store remaining methanol extract in refrigerator for possible re-assay. The methanol extract is stable for 10 days at refrigeration storage.

10 METHOD FLOW CHART

Transfer 1.00 ±0.05 g of the frozen homogenate into a 15 mL polypropylene centrifuge tube. Centrifuge the aliquots at 1000 rpm (200x g) for 1 minute. Completely thaw the samples prior to fortification.

↓

Add 200 µL methanol for the double blank sample. Add 100 µL methanol and 100 µL of internal standard fortification solution for control and incurred sample. Add 100 µL QC fortification solution and 100 µL of internal fortification standard for QC samples. Briefly vortex. Leave the sample on the bench for approximately 10 min before extraction.

↓

Add 8 mL methanol and vortex or shake for approximately 10 min.

↓

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

↓

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

↓

Add 8 mL methanol and vortex for approximately 10 min.

Critical Step: Pellet may be difficult to re-suspend. The pellet may be allowed to sit for *ca*. 10 minutes before vortexing to make resuspension easier. Vortex each sample individually prior to placing the samples on the multi-tube vortexer. If the pellet is difficult to re-suspend, a spatula may be used to break the pellet or the tube can be tapped against the bench top.

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

↓

Pipette 50 µL of the methanol liver tissue extract into the appropriate wells of a 2 mL 96-well plate or autosampler vial and mix with 950 µL of dilution solvent, methanol/purified water (60/40, v/v), for LC-MS/MS analysis.

11 LC-MS/MS ANALYSIS FOR THE DETERMINATIVE AND CONFIRMATORY PROCEDURES

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

On occasions it may be necessary to adjust the HPLC and MS conditions slightly to achieve acceptable peak shape and sensitivity. The HPLC and MS conditions should be adjusted such that acceptable performance of the LC-MS/MS system is met (Section 13.1.1).

11.1 HPLC Conditions

Retention time may depend on the HPLC system used. Approximate retention time observed during validation is specified.

HPLC System:	Thermo Transcend Allegros UPLC pumps, Thermo PAL autosampler
Column:	MacMod Ace 3 C18, 2.1 x 50 mm PN. ACE-111-0502
Column Temperature:	Ambient
Autosampler Temperature:	Ambient
Mobile Phase A:	0.1% Formic Acid
Mobile Phase B:	0.1% Formic Acid in Acetonitrile (v/v)
Injection Volume:	3 µL (may vary)
Run Time:	5.2 min/inj. (may vary, see notes below)
Retention Time:	*ca.* 2.1 min

Gradient Table:

Time (min)	Flow (mL/min)	%A	%B
initial	0.4	70	30
0.3	0.4	70	30
2.0	0.4	25	75
2.1	0.4	0	100
3.1	0.4	0	100
3.2	0.4	70	30
5.2	0.4	70	30

Notes:

- Note: re-equilibration time may be extended at end of run if needed to achieve stable retention time of approximately 2.1 min.
- Alternate column (Thermo Acclaim 120, C-18; 3um; 2.1X50 mm) has also been tested and verified to produce acceptable results (see Section 20).
- Alternative LC-MS/MS platform (Thermo Vantage with Thermo Accela pump, and Open Access autosampler) were also tested and verified to produce acceptable results.
- Alternate mobile phase composition (A=90:10 0.09% formic acid in water:0.09% formic acid in acetonitrile and B=90:10 0.09% formic acid in acetonitrile: 0.09% formic acid in water) was also tested and verified to produce acceptable results

11.2 MS Conditions

11.2.1 Tuning of Mass Spectrometer and MS Full Scan

The MS response of FBZ and FBZ-d_3 can be tuned by infusion of FBZ solution and FBZ-d_3 solution (suggested concentration at ~500 ng/mL). Other methods of tuning are also acceptable. Typically, the tuning is done by infusing a solution of the analyte of interest diluted in mobile phase using a tee connector prior to introduction into the MS. The conditions should be optimized in full scan mode for adequate detection of FBZ and FBZ-d_3 parent ions (*m/z* 300, *m/z* 303, respectively). The MS conditions should then be optimized in MS/MS mode for adequate detection of determinative product ion at *m/z* 268 for both FBZ and FBZ-d_3. Additionally, the MS conditions should be optimized in the MS/MS mode for adequate detection of the confirmatory product ions at *m/z* 159 and 131. The resultant MS parameter should be used for all analyses, although the operator may vary conditions for adequate sensitivity. The structure and proposed fragmentation pattern of FBZ is shown in Figure 18.1.

11.2.2 MS Conditions

The MS should be tuned as in Section 11.2.1. The MS parameters and Q1/Q3 transitions used during validation are as follows. Settings may depend on the MS system used and are for example only. Determinative and confirmatory transitions are provided below and are monitored simultaneously.

Table 11-2-2-1: MS System Parameters

	PI 4000/ API 4000 QTRAP	Thermo Vantage
Ionization interface	Turbo Ion Spray	HESI
Ionization mode	Positive	Positive
MS run time [min]	5.2	5.2
Source (TEM) Temperature [°C]	500	N/A
Vaporizer Temperature	N/A	317
DP	71 (FBZ) and 41 (IS)	N/A
CXP	4 - 8	N/A
Capillary Temperature [°C]	N/A	370
Curtain (CUR) gas [psi]	20	N/A
Sheath Gas (units)	N/A	60
Collision (CAD) gas [psi]	9	N/A
Aux Gas (units)	N/A	55
Ion source gas (GS1) 1 [psi]	80	N/A
Ion source gas (GS2) 2 [psi]	70	N/A
Ion (IS) Spray [V]	5000	5000
Entrance (EP) potential [V]	7	N/A

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

Table 11-2-2-2: MS/MS Transition Parameter for API 4000 QTrap and API 4000

Reference Compound	Precursor Ion Q1 Mass [m/z]	Collision Energy [V]	Q3 Mass [m/z]	Dwell Time [ms][c]
FBZ [a]	300.05	35	268.1 (quantifier)	20 - 200
Qual1 [b]	300.05	49	159.1 (qualifier)	20 - 200
Qual2 [b]	300.05	65	131.0 (qualifier)	50 - 2000
FBZ-d_3 [a]	303.12	33	268.15	20 - 200

a: quantitation purposes

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

c. Dwell time can be optimized to obtain enough data points across each peak and to obtain sufficient signal for each transition. Qual2 transition dwell time is typically required to be 10x the dwell time of the other transitions.

Table 11-2.2-3: MS/MS Transition Parameter for Thermo Vantage MS			
Reference compound	**Precursor ion Q1 mass [m/z][c]**	**Collision energy [V]**	**Q3 mass [m/z]**
FBZ [a]	300.102	6	268.100 (quantifier)
Qual1 [b]	300.101	21	159.100 (qualifier)
Qual2 [b]	300.100	32	131.100 (qualifier)
FBZ-d_3 [a]	303.100	6	268.100

a: quantitation purposes
b: qualifier transition used with confirmatory method, not used for quantitative purposes
c: Q1 masses must be different to allow quantitation using a single transition

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

Example chromatograms of standards, QC, incurred, control (with IS), and double blank of liver samples are shown in Figures 18.2, 18.4, 18.6, 18.8, and 18.10, respectively

11.3 System Suitability Test and Sample Injection Sequence

The LC-MS system should be conditioned first with ≥5 injections of FBZ standard at lowest concentration (standard 1).

11.3.1 System Suitability Test (SST)

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

11.3.2 Carryover Test

System carryover is assessed by injecting standard 7 immediately followed by a solvent blank (methanol/water (6/4, v/v)).

11.3.3 Bracketing of Calibration Curve Standards

A set of 7 calibration curve standards is run before extracted samples including control samples, double blank, QC, and incurred samples. The extracted samples are followed (bracketed) by re-injection of the set of 7 calibration curve standards. Both sets of calibration curve standards are used to construct the calibration curve.

11.3.4 Analysis Sequence

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

System Suitability Test SSTL (Std-1)	n ≥5 injections (SSTL reproducibility)
System Suitability Test SSTH (Std-7)	1 injection (SSTH)
Solvent blank (methanol/water (6/4, v/v)	2 injections (SST carry over)
Std-1 to Std-7	1 injection each
Solvent blank	1 injections
Followed by tissue samples, including double blank, control, QCs, and study sample.	
Solvent blank	1 injections
Std-1 to Std-7	1 injection each

11.4 HPLC Column Maintenance (Optional)

The HPLC column may be flushed with water followed by acetonitrile after each analysis sequence. The column flushing will help maintain the HPLC column performance.

12 CALCULATION AND REPORTING OF RESULTS

12.1 Method of Calculation (Determinative Analysis)

Quantitation of FBZ is accomplished using an internal standard calibration method with a FBZ standard concentration range of 2.5 to 25.0 ng/mL (1.00 ppm to 10.0 ppm tissue equivalents). The concentration of the internal standard is 7.5 ng/mL (3.00 ppm tissue equivalents). A standard calibration curve is generated from non-weighted linear regression analysis of peak area ratio versus concentration (ppm) of FBZ.

A typical standard calibration curve for liver is shown in Figure 18.12.

The point of origin is excluded when fitting the calibration curve. The regression equation is then used to calculate the concentration of FBZ in the samples. If the regression obtained in an analytical set yields an acceptable coefficient of determination and meets the stated criteria (Section 13.1.3), the regression equation can be used to determine the concentration of each sample in the set. If the regression does not meet acceptability criteria, the set is deemed not acceptable and has to be repeated by re-injecting the standards and samples or by preparing new standards and/or new sample extracts for re-analysis.

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

$$y = mx + b$$

Where, y = analyte:IS peak area ratio
x = tissue equivalent concentration (ppm) of the standards
m = slope
b = y-intercept

The concentration (ppm) of FBZ in each sample is calculated using the formula:

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

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

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

The following equation will calculate the concentration in ppm:

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

Where:
C_T is the concentration of FBZ in ppm in the sample,
C_I is the calculated concentration of FBZ in ppm from the standard curve where the nominal concentrations of standards are in ppm.
S_W is the weight in g of the initial samples (nominal weight of 1 g is used for fortified samples and exact weight is used for control and/or incurred samples).

An example of a concentration calculation is given below:

$C_I = 12.1$ ppm $S_W = 1.05$ g

$$C_T = \frac{12.1}{1.05} = 11.5 \text{ ppm}$$

Accuracy is calculated from fortified QC samples using the equation:

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

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

An example calculation for accuracy is given below:

C_T=0.502 ppm C_F=0.508 ppm

$$\%\text{Accuracy} = \left(\frac{0.502}{0.508}\right) \times 100 = 98.8\%$$

12.3 Automation of Calculations (Determinative Analysis)

The chromatographic software may be used to integrate chromatograms, calculate results, and print and save chromatographic reports. A processing method may be prepared to automate integration and the calculation of results. The same integration parameters should be used to integrate all chromatograms within an entire batch. If it is necessary to use different integration parameters for one or more samples in a batch, justification should be documented. Verify that all chromatograms are correctly integrated. The integration parameters need to be carefully applied to minimize artifacts. Resultant reports may then be generated and printed..

12.4 Identification Criteria (Confirmatory Analysis)

Identification is based on the relative abundance of *m/z* 159 and *m/z* 131 to the base peak, *m/z* 268 and the relative retention time. The relative abundance of each ion is calculated as described below:

$$\text{Relative Abundance} = \frac{\text{Area of Product Ion Peak}}{\text{Area of Base Peak}}$$

For positive identification, (1) the relative abundance of the two ions (*m/z* 159 and 131) to the base peak, *m/z* 268, must be within ±10% of the average relative abundance for each product ion in the solvent standards; (2) the retention time must be within ±5% of the average retention time for the solvent standards; and (3) the signal-to-noise ratio of the confirmatory peaks, *m/z* 159 and *m/z* 131, must be >50.

A sample must meet all three of the above identification criteria to confirm; if any of the three criteria are not met, the sample fails confirmation.

12.5 Automation of Calculations (Confirmatory Analysis)

The chromatographic software may be used to integrate chromatograms, calculate results, and print and save reports. A processing method may be prepared to automate integration and the calculation of results. Resultant reports may then be generated and printed. The same integration parameters should be used to integrate all chromatograms within an entire batch. If it is necessary to use different integration parameters for one or more samples in a batch, justification should be documented. The generated results can be imported to Microsoft® Excel for further data calculation and summary.

13 ACCEPTABILITY CRITERIA

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

13.1 Determinative Procedure

13.1.1 System Suitability Test: Reproducibility and System Carry-over

To demonstrate acceptable performance of the LC-MS/MS system, the system suitability injections of a standard at the lowest calibration level (SSTL, standard 1) should be performed prior to injection of a sample set (Section 11.3.1).

It is advised that the analyst check the chromatograms of the system suitability injections to ensure that all the monitored ions are detected. In addition, a minimum signal-to-noise ratio of 50:1 and reproducible FBZ/FBZ-d_3 peak area ratio and FBZ retention times with CV $\leq$ 5% must be met for the five consecutive injections of standard 1.

The system carry over (solvent blank) after injection of SSTH (standard 7) has to be $\leq$20.0% of the average FBZ area of SSTL (standard 1).

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

If the MS detector sensitivity is low and gives poor precision at the LOQ, tuning the instrument may improve the sensitivity. If the sensitivity remains low, instrument calibration, cleaning, and/or repair should be performed. Alternatively, the injection volume can be increased.

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

13.1.2 Accuracy and Precision: Quality Control Sample Acceptance Criteria

For routine analysis, the results of the QC samples will provide the basis for accepting or rejecting the analytical run. The acceptance criteria for accuracy of QC samples is 80% to 110%.

13.1.3 Standard Calibration Curve

The non-weighted linear regression should have a coefficient of determination (r^2) $\geq$0.990 or correlation coefficient (r) $\geq$0.995 for a standard curve of FBZ ranging from 1.00 ppm to 10.0 ppm for liver tissue equivalent.

Back-calculated accuracy should be within ±10% of the nominal, except the lowest standard, which should be within ±15% of the nominal. A maximum of two standards (cannot be the same level) can be excluded if they cannot meet the above accuracy criteria. A standard can also be excluded if an instrument problem or injection error

occurs during the analysis of that standard. At least 12 data points should be used to calculate the results. If less than six concentration levels remain, or if the remaining data points levels yield a regression coefficient of determination of less than 0.990, then the set is deemed not acceptable and has to be repeated (either re-injected or re-extracted, depending upon the suspected source of failure).

13.1.4 Selectivity

Control tissues should not contain endogenous or exogenous substances that may interfere at the retention time of FBZ. Typically, any interference should be ≤20% of FBZ peak area for the LOQ standard.

13.2 Confirmatory Procedure

13.2.1 System Suitability Test, System Carry-over and Signal to Noise

The coefficient of variation (CV; n≥5) of the peak area ratio of each fragment ion (*m/z* 159 and *m/z* 131) to base ion (*m/z* 268) for the SSTL (standard 1) must be ≤5%.

The system carry over (solvent blank) after injection of SSTH (standard 7) has to be <20.0% of the average FBZ area of SSTL (standard 1) for each of the confirmatory transitions.

The signal-to-noise ratio of the confirmatory peaks, *m/z* 159 and *m/z* 131, must be >50. If sensitivity of the instrument is too high, the signal-to-noise criteria may be set to a percentage of the LOQ standard signal-to-noise ratio.

13.2.2 Quality Control

For routine analysis, one control liver sample and one quality control (QC) liver sample fortified with fenbendazole at the tolerance (3.2 ppm) must be assayed along with a solvent standard with concentration equivalent to tolerance and unknown samples. The control sample must fail to confirm. The QC sample must meet the identification criteria (Section 12.4).

14 LIMIT OF QUANTITATION

The theoretical limit of quantitation (LOQ) calculated from calibration curve data generated during the method trial was 0.729 ppm.

Quantitative information below the LOQ should be reported and footnoted as BLOQ. The analyst should note this result with appropriate annotations and footnotes in the analytical results.

The upper limit of quantitation (ULOQ) is the highest concentration of the calibration curve (10.0 ppm).

15 DILUTION

Quantitative results for incurred samples and fortified QC samples should only be reported within the concentration range for which the standard curve demonstrates acceptable linear regression. When a quantitative result is above the standard curve range, it should be marked (suggested "ALQ"). If dilutions are needed, dilute an appropriate amount of extract from the test sample with control extract. Alternatively, an aliquot of the tissue sample may be diluted with control tissue and the extraction repeated. The reported result should take into account the dilution factor used.

16 STABILITY

16.1 Stability of FBZ and FBZ-d_3 Stock Standard Solutions or Working Standard Solutions

All standard solutions (Section 7) stored in a freezer set at -20 °C are stable for 2 months.

16.2 Stability of Tissue Extract

Tissue methanol extract is stable for at least 10 days at refrigeration storage.

16.3 Stability of Samples in Final Injection Solvent (Dilution Solution)

Extracted samples stored at room temperature are stable for 10 days.

16.4 Long Term Storage Stability

Swine liver samples containing fenbendazole residues are stable in freezers set at temperatures < -20ºC or < -80ºC for 12 months.

17 NOTES TO ANALYSTS

17.1 Minimization of Carryover

To minimize possible carryover of FBZ, it is recommended to inject solvent (methanol/water, 6/4, v/v) after injection of a high concentration calibration standard.

17.2 Data Not Used

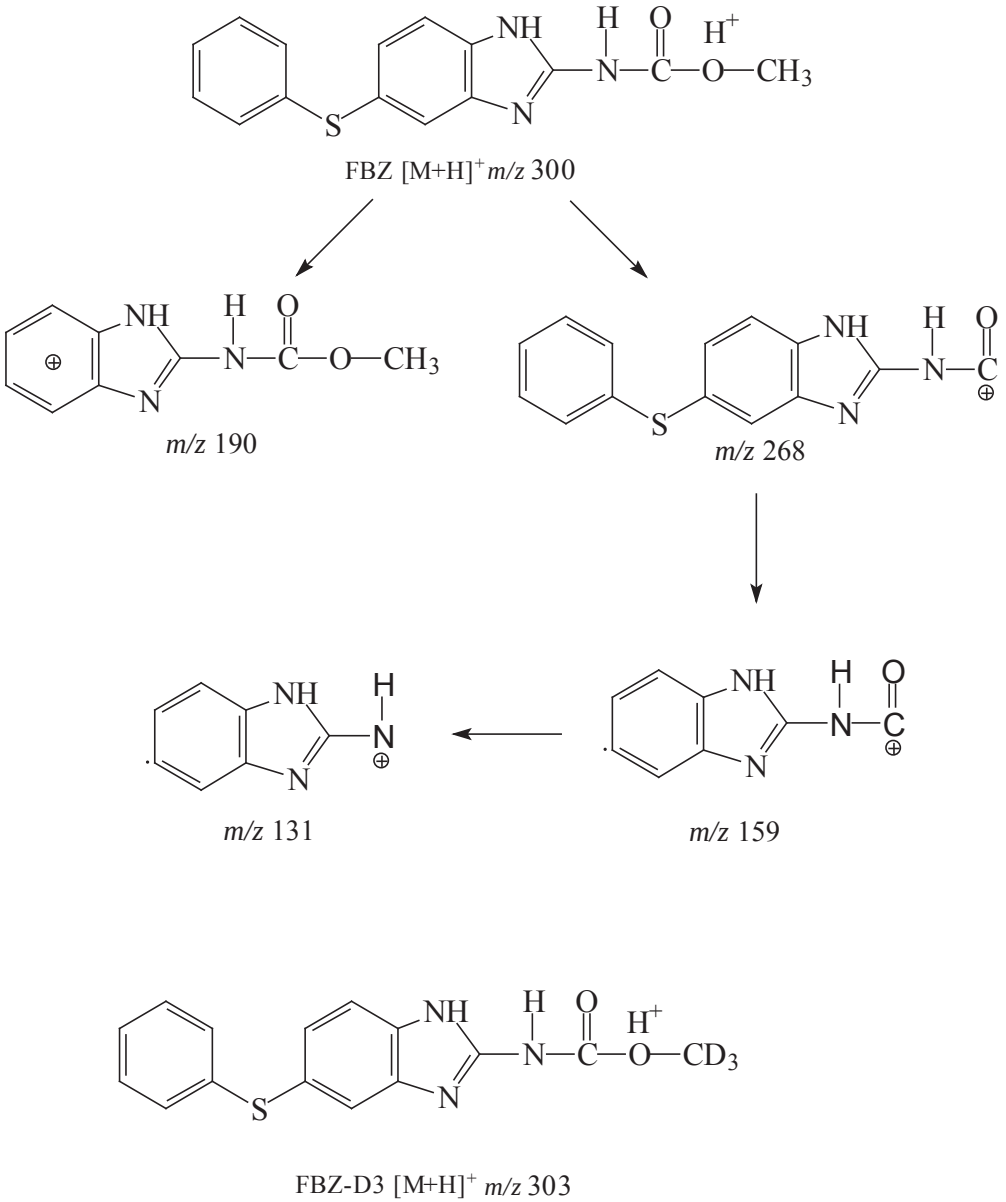
Data from blank solvent injections and conditioning samples are not used, neither are the above-range samples (calculated concentration above highest calibration standard) and over-diluted samples (calculated concentration below LOQ). The data not used should be identified, and the reason for rejection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

17.3 IS Monitoring and LC-MS/MS System Maintenance

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.

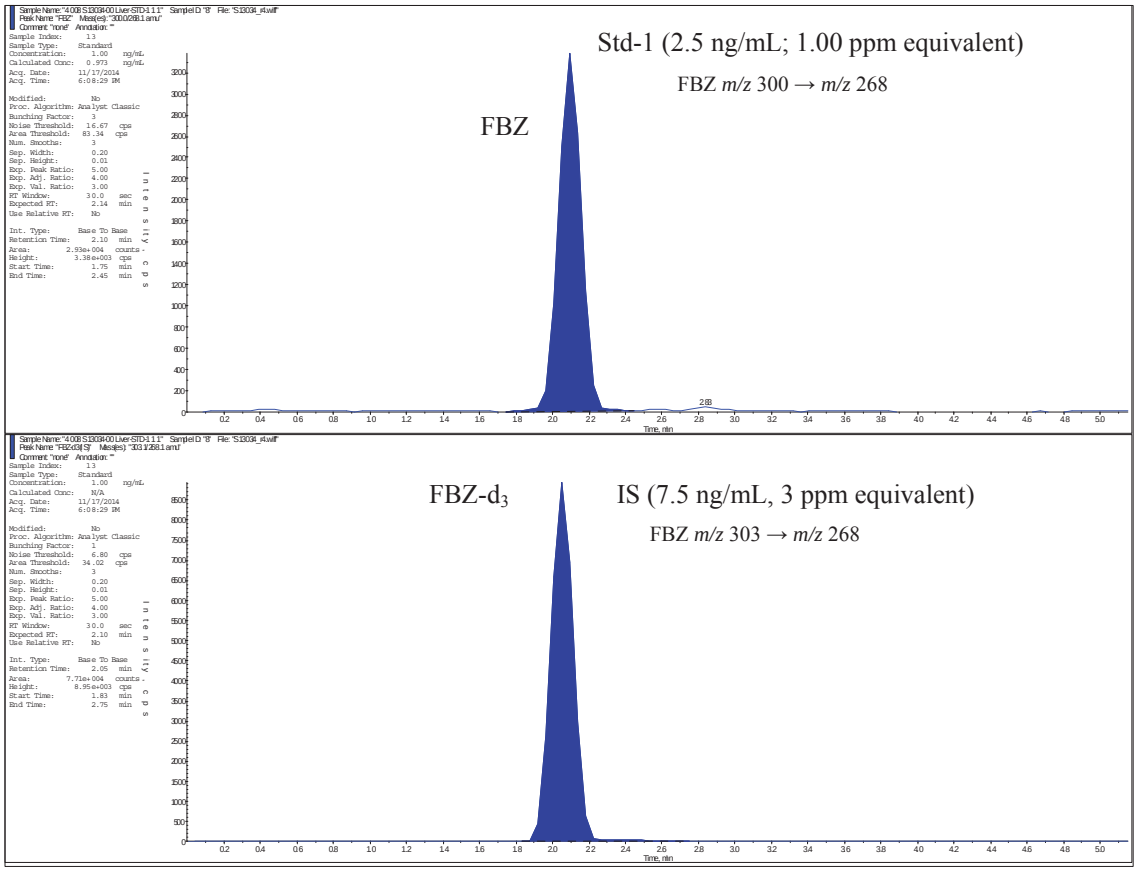
18 FIGURES

18.1 Structure and Proposed Fragmentation of FBZ

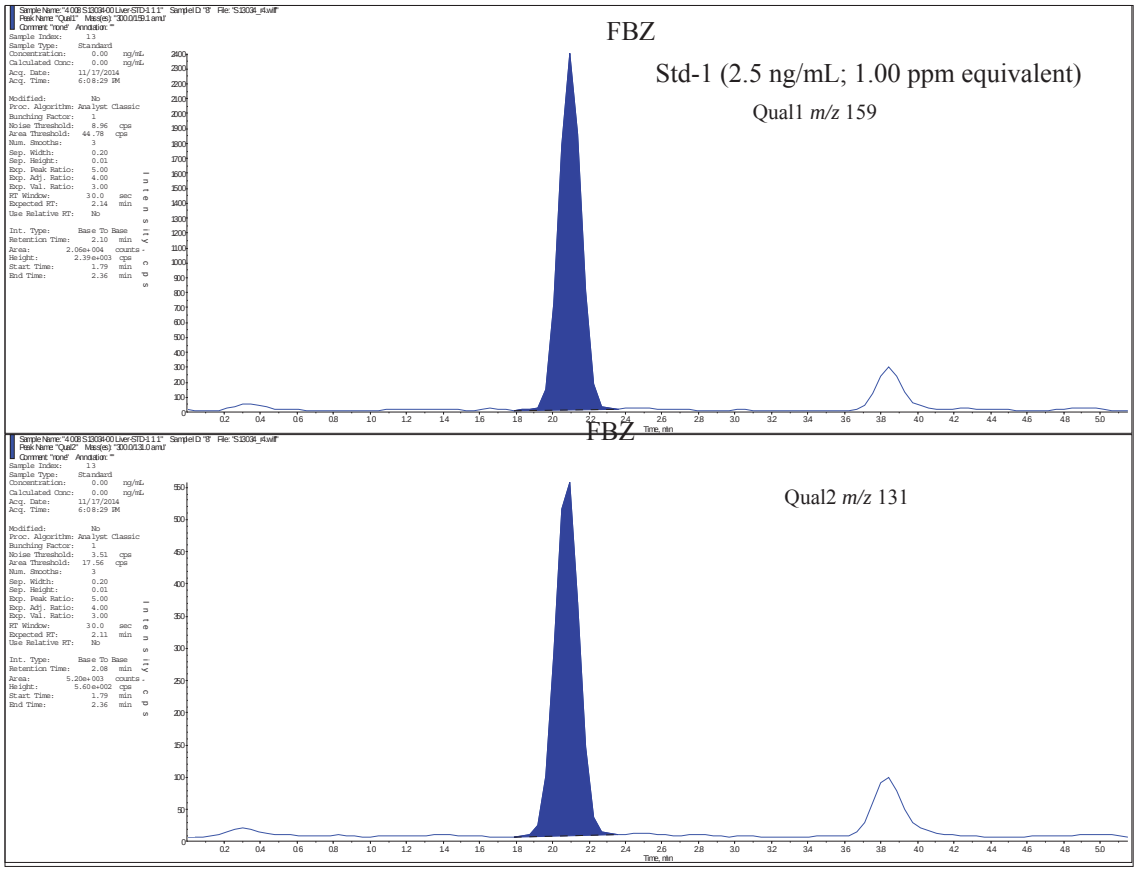


FBZ-D3 $[M+H]^+$ *m/z* 303

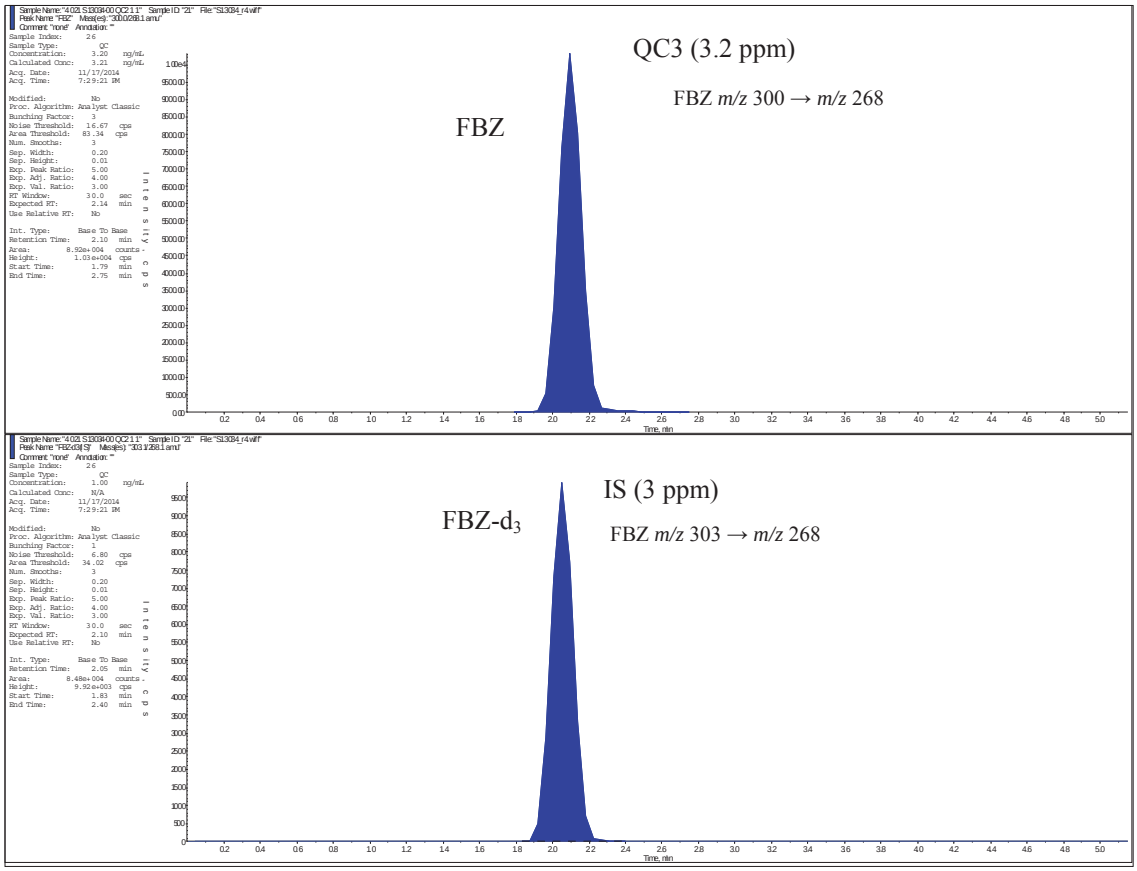
18.2 Typical Ion Chromatograms of FBZ Liver Standards (Determinative Transitions)



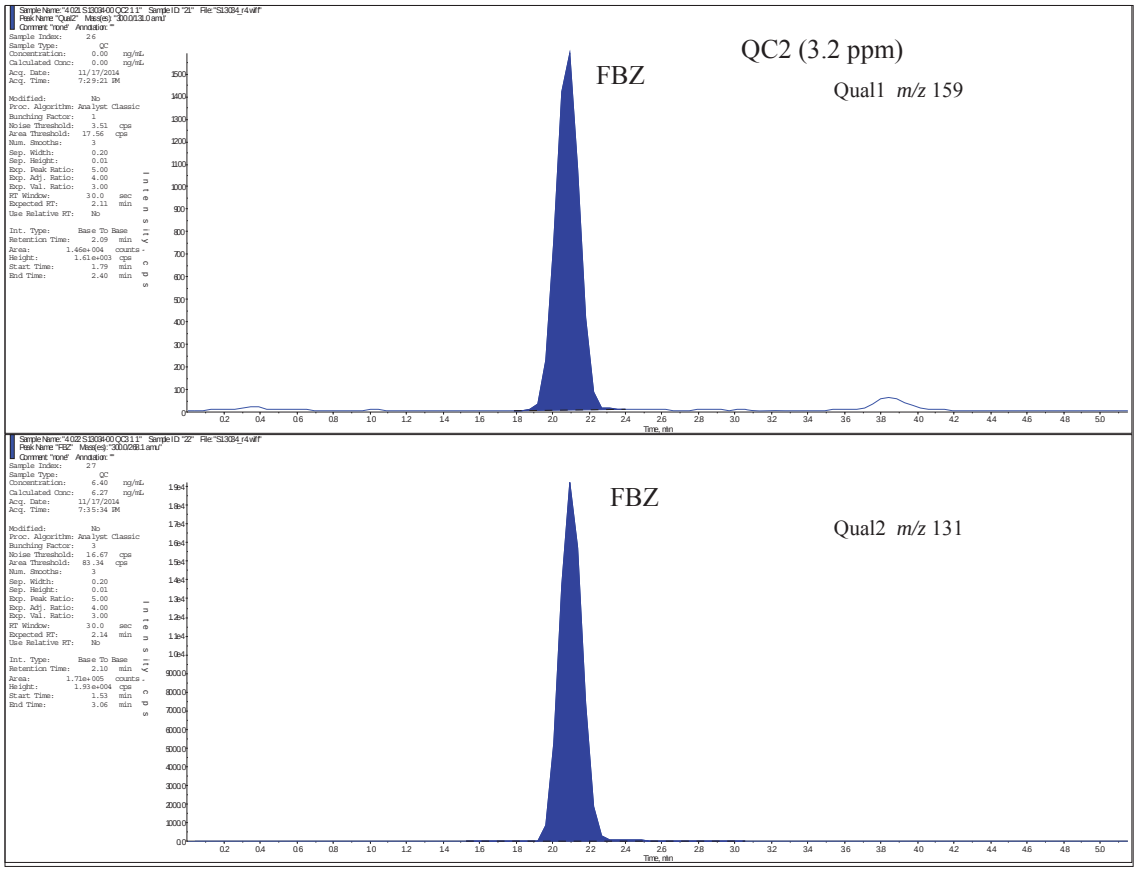
18.3 Typical Ion Chromatograms of FBZ Liver Standards (Confirmatory Transitions)



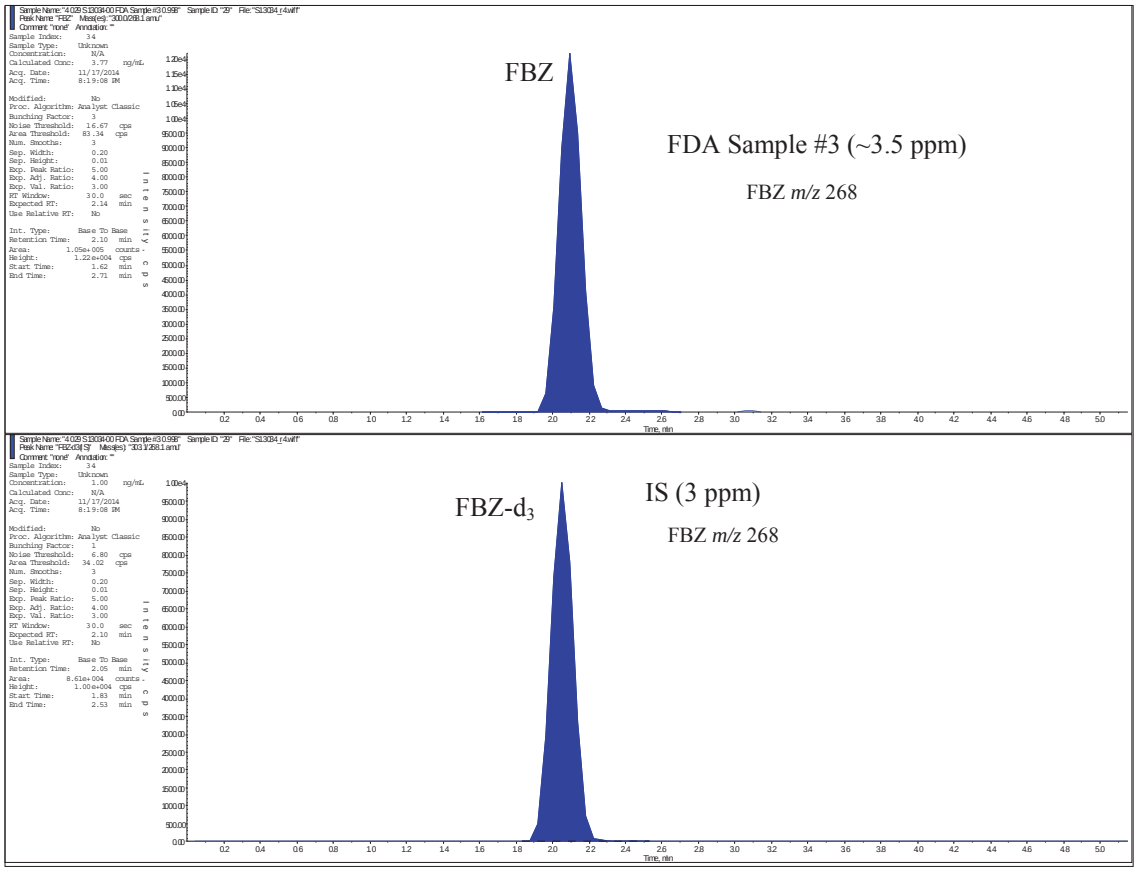
18.4 Typical Ion Chromatograms of Liver FBZ Quality Control Sample (Determinative Transitions)



18.5 Typical Ion Chromatograms of Liver FBZ Quality Control Sample (Confirmatory Transitions)

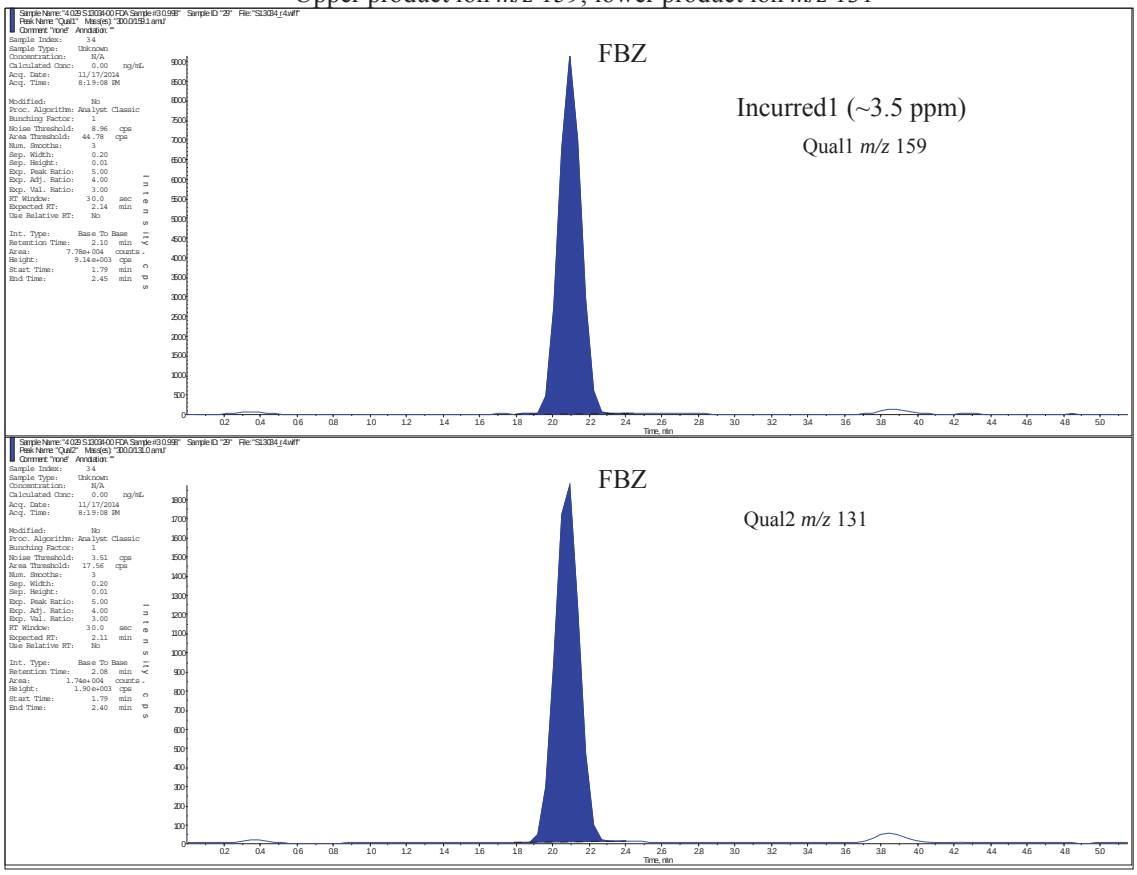


18.6 Typical Ion Chromatograms of Liver Incurred Sample (Determinative Transitions)

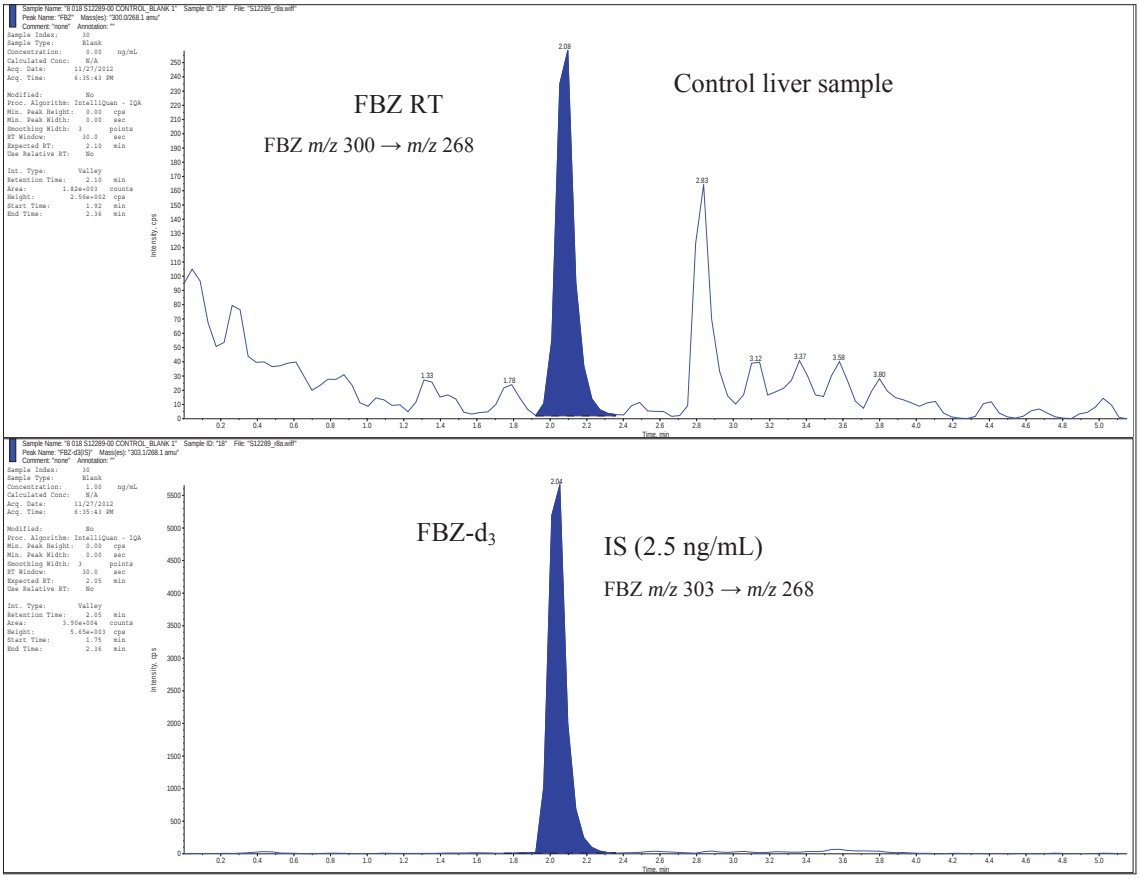


18.8 Typical Ion Chromatograms of Liver Incurred Sample (Confirmatory Transitions)

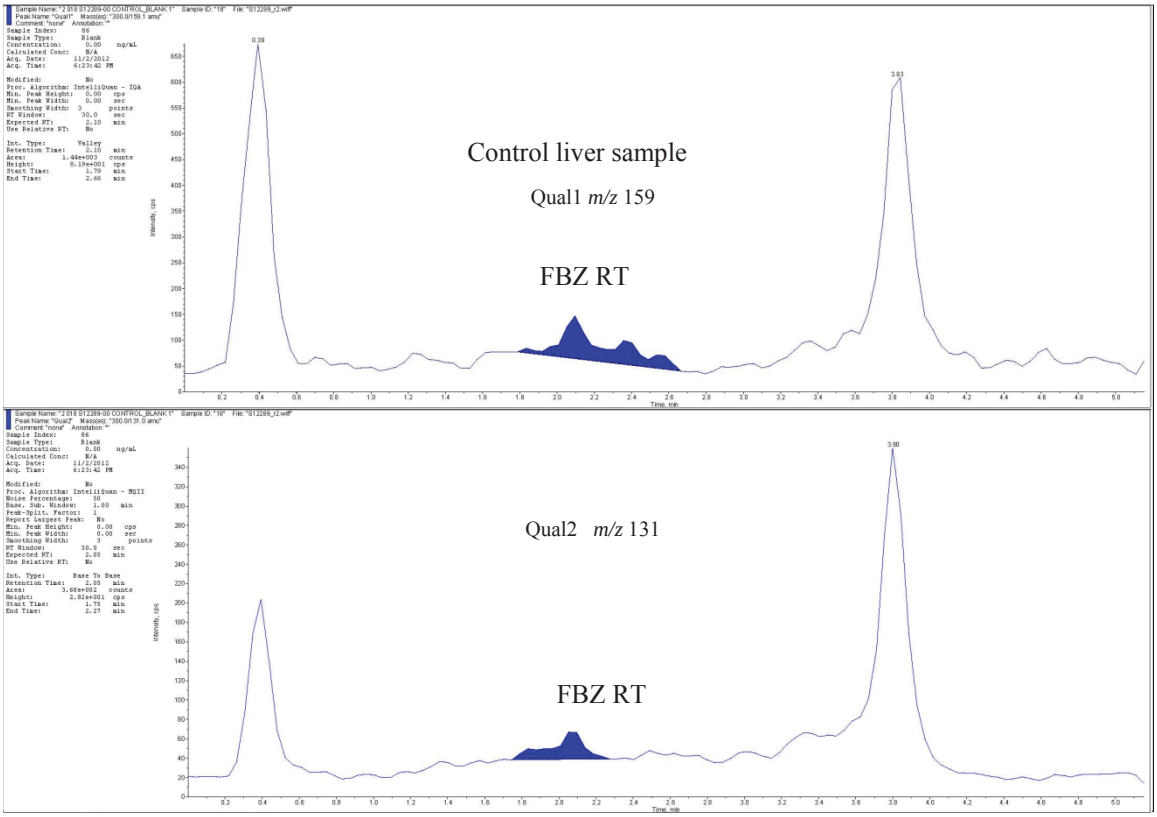
Upper product ion *m/z* 159; lower product ion *m/z* 131



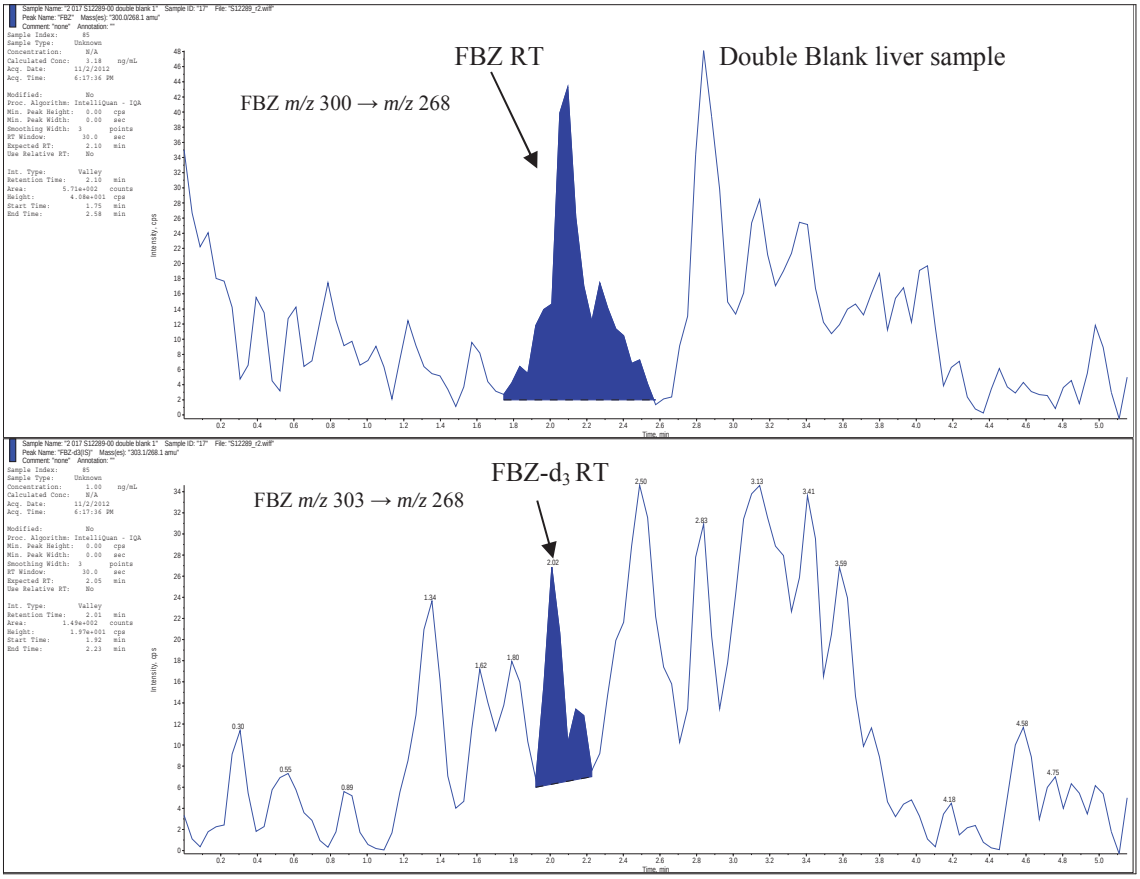
18.9 Typical Ion Chromatograms of Liver Control Sample (Determinative Transitions)



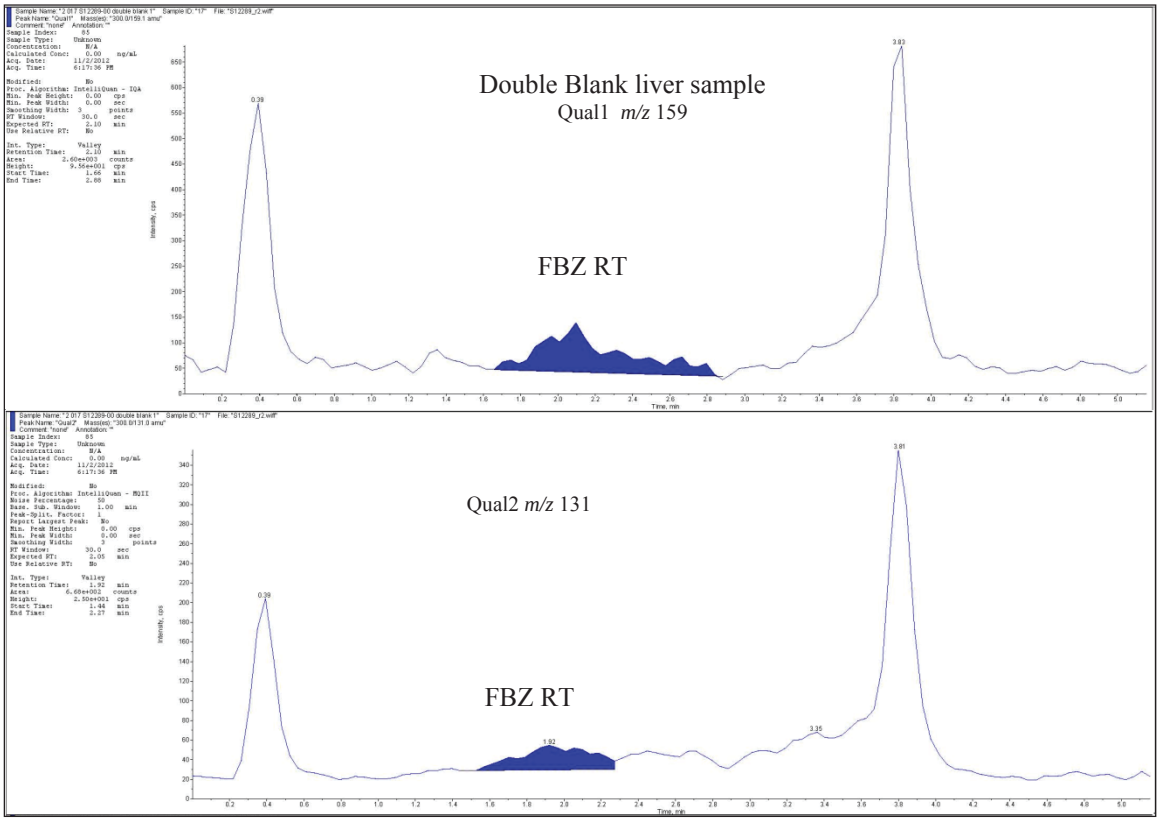
18.10 Typical Ion Chromatograms of Liver Control Sample (Confirmatory Transitions)



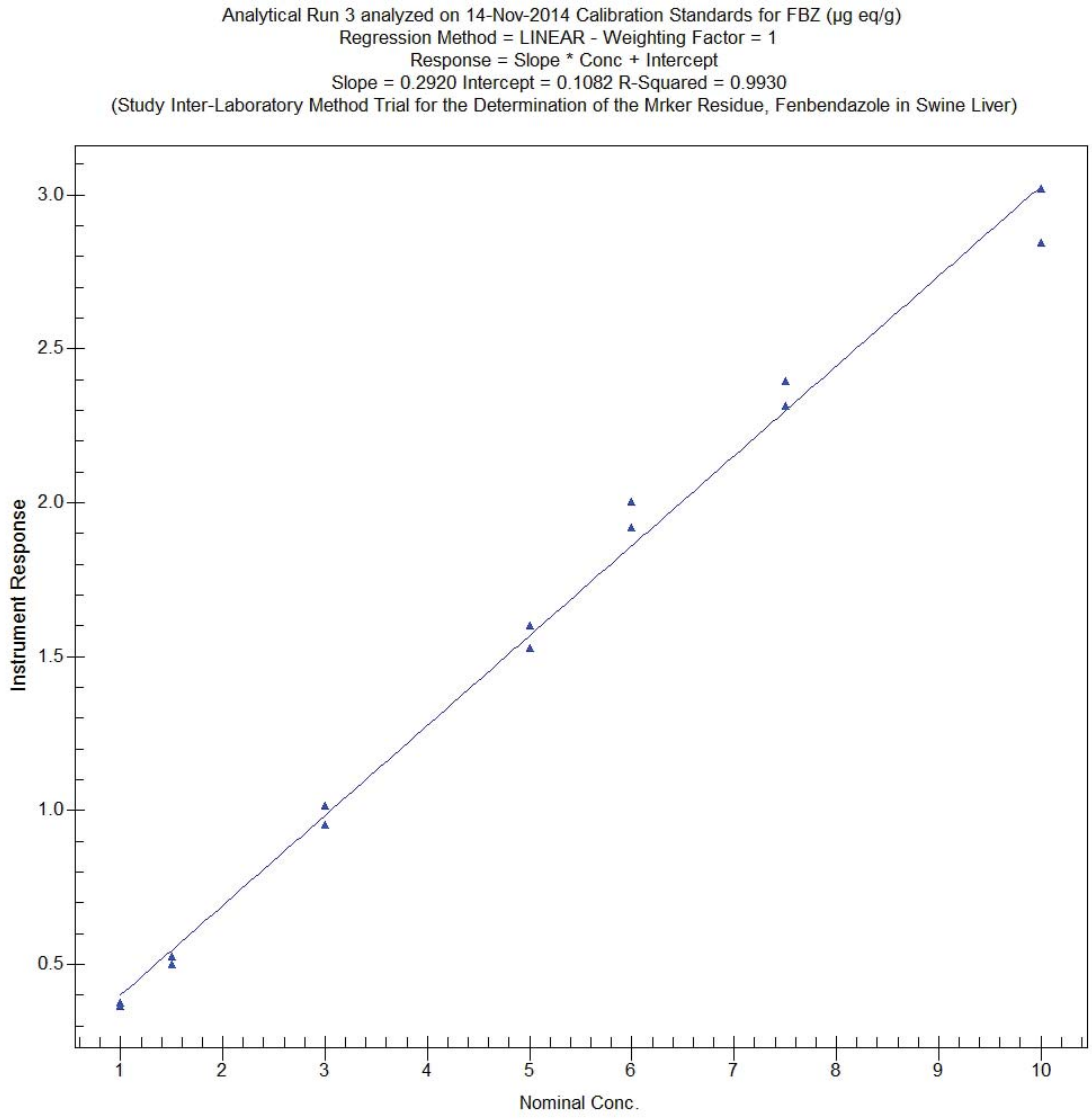
18.11 Typical Ion Chromatograms of Liver Double Blank Sample (Determinative Transitions)



18.12 Typical Ion Chromatograms of Liver Double Blank Sample (Confirmatory Transitions)



18.12 Typical Calibration Curve of FBZ Liver Standard



19 VALIDATION DATA SUMMARY

19.1 Determinative Procedure

Standard Curve Linearity (Liver)			
Std. Curve Range	0.25 to 7.5 ppm (nominal concentration 0.625 to 18.75 ng/mL)		
Watson Run ID	Slope	Intercept	Correlation Coefficient
2	1.19	-0.00601	0.9938
5	1.16	0.05201	0.9968
7	1.22	0.05543	0.9985

Precision & Accuracy (Liver)							
	QC	Conc. (ppm)	%CV	Mean %Recovery	Incur Animal#	Conc. (ppm)	%CV
Inter-Batch (n = 18)	Low	0.500	5.3	96.8	Mean Assay Conc. (n=18)		
	Medium	1.00	4.6	96.4	Incurred1	0.534	9.7
	High	2.00	4.7	95.5	Incurred2	1.66	5.4
Intra-Batch (n = 6)	LOQ	0.250	6.8	91.2			
	Low	0.500	2.6 – 7.5	94.4 – 99.0	mean assay conc. (n=6)		
	Medium	1.00	2.6 – 5.9	94.8 – 98.0	Incurred1	0.511 – 0.571	7.7 - 9.4
	High	2.00	3.6 – 6.2	94.5 – 96.5	Incurred2	1.58 – 1.73	2.2 – 5.9

Limit of Detection (LOD) [Limit of detection was estimated to be 0.0021 ppm using the calibration curve and the response value calculated by adding 3x the standard deviation of the background response to the average response at the retention time of analyte after analyses of blank matrix samples from 6 different sources (20 reps).
Specificity/Selectivity: No significant interference from 6 lots of control liver from different regions of the country was observed. The presence of 18 veterinary drugs does not affect the recovery of FBZ in QC samples. An interference in control tissue fortified with some of the veterinary drugs fortified at 1 ppm was slightly greater than 10% of the tolerance peak. This was believed to be caused by the presence of fenbendazole in the oxfendazole standard (one of the specificity compounds). For control tissue fortified with some of the veterinary drugs at 10 ppm, an interference peak in one of the confirmatory transitions slightly greater than 10% of the tolerance was observed.

Matrix Effect/method recovery: Results from matrix effects analysis indicate no consistent or significant matrix effects in either the analyte or the internal standard. Additionally, the degree of matrix effects in analyte and internal standard were comparable. Method recovery averaged >90% for both analyte and internal standard..

Ruggedness: the accuracy and precision data of liver sample analysis obtained from both LC/column systems are in good agreement acceptable accuracy and precision. The method was also shown to provide acceptable accuracy and precision when minor changes were made to the mobile phase composition and when an alternate Thermo Vantage LC-MS/MS platform was used..

Stability of Liver Tissue: The FBZ fortified quality control samples and incurred swine liver samples are stable on the bench at ambient temperature for at least 24 hours and are stable after subjected to at least 4 cycles of freeze (>12 hrs) and thaw (~1 hr).

Stability of FBZ and FBZ-d_3 Solutions:

The DMSO stock solution of FBZ and FBZ-d_3, the methanolic standard solutions of FBZ and FBZ-d_3, including QC fortification solution, FBZ-d_3 IS spiking solution, and standard curve working solutions were stable for ca. 2 months when stored in a freezer (ca. -20 °C)

Stability of methanolic tissue extract: the methanolic tissue extract is stable for at least 10 days when stored in refrigerator (ca. 4 °C). On month stability of these same extracts was demonstrated in the previous validation of this method at fortified concentrations based upon the original 3 ppm tolerance.

Stability of FBZa and FBZ-d3 in the final injection solution (dilution solution): Re-assay of standard and sample solutions that were left at autosampler ambient temperature for 10 days demonstrated that the assay result was reproducible as compare to the initial assay data.

19.2 Confirmatory Procedure

19.2.1 Mass Spectral Matching for Core Run 1

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
2 008 S12289-00 Liver-Std-1 1 1	1.46E+04	1.08E+04	3.50E+03	74.0		24.0	
2 009 S12289-00 Liver-Std-2 1 1	4.17E+04	2.97E+04	8.90E+03	71.2		21.3	
2 010 S12289-00 Liver-Std-3 1 1	8.08E+04	5.53E+04	1.84E+04	68.4		22.8	
2 011 S12289-00 Liver-Std-4 1 1	1.60E+05	1.20E+05	3.55E+04	75.0		22.2	
2 012 S12289-00 Liver-Std-5 1 1	2.64E+05	1.81E+05	5.76E+04	68.6		21.8	
2 013 S12289-00 Liver-Std-6 1 1	3.22E+05	2.35E+05	6.90E+04	73.0		21.4	
2 014 S12289-00 Liver-Std-7 1 1	3.79E+05	2.76E+05	8.47E+04	72.8		22.3	
2 015 S12289-00 Liver-Std-8 1 1	4.95E+05	3.64E+05	1.13E+05	73.5		22.8	
2 050 S12289-00 Liver-Std-1 2 1	1.32E+04	8.64E+03	3.23E+03	65.5		24.5	
2 051 S12289-00 Liver-Std-2 2 1	3.95E+04	2.94E+04	9.14E+03	74.4		23.1	
2 052 S12289-00 Liver-Std-3 2 1	7.63E+04	5.31E+04	1.66E+04	69.6		21.8	
2 053 S12289-00 Liver-Std-4 2 1	1.56E+05	1.02E+05	3.32E+04	65.4		21.3	
2 054 S12289-00 Liver-Std-5 2 1	2.44E+05	1.66E+05	5.53E+04	68.0		22.7	
2 055 S12289-00 Liver-Std-6 2 1	2.96E+05	1.90E+05	6.44E+04	64.2		21.8	
2 056 S12289-00 Liver-Std-7 2 1	3.58E+05	2.31E+05	8.02E+04	64.5		22.4	
2 057 S12289-00 Liver-Std-8 2 1	4.87E+05	3.06E+05	1.05E+05	62.8		21.6	
Average	**2.14E+05**	**1.47E+05**	**4.74E+04**	**69.4**		**22.4**	

19.2.1 Mass Spectral Matching for Core Run 1

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
2 019 S12289-00 1/2x new tolerance 1 1	2.39E+04	1.68E+04	5.46E+03	70.3		22.8	
2 024 S12289-00 1/2x new tolerance 2 1	2.44E+04	1.67E+04	5.34E+03	68.4		21.9	
2 029 S12289-00 1/2x new tolerance 3 1	2.56E+04	1.81E+04	5.70E+03	70.7		22.3	
2 034 S12289-00 1/2x new tolerance 4 1	2.44E+04	1.97E+04	5.66E+03	80.7		23.2	
2 039 S12289-00 1/2x new tolerance 5 1	2.43E+04	1.74E+04	5.59E+03	71.6		23.0	
2 044 S12289-00 1/2x new tolerance 6 1	2.71E+04	1.87E+04	5.84E+03	69.0		21.5	
Average	**2.50E+04**	**1.79E+04**	**5.60E+03**	**71.8**		**22.5**	
% CV	**4.80**	**6.52**	**3.18**	**6.31**	**Y**	**2.9**	**Y**
2 020 S12289-00 1x new tolerance 1 1	4.83E+04	3.39E+04	1.07E+04	70.2		22.2	
2 025 S12289-00 1x new tolerance 2 1	4.80E+04	3.50E+04	1.04E+04	72.9		21.7	
2 030 S12289-00 1x new tolerance 3 1	4.94E+04	3.40E+04	1.06E+04	68.8		21.5	
2 035 S12289-00 1x new tolerance 4 1	4.90E+04	3.28E+04	1.08E+04	66.9		22.0	
2 040 S12289-00 1x new tolerance 5 1	4.98E+04	3.35E+04	1.08E+04	67.3		21.7	
2 044 S12289-00 1/2x new tolerance 6 1	5.11E+04	3.39E+04	1.11E+04	66.3		21.7	
Average	**4.93E+04**	**3.39E+04**	**1.07E+04**	**68.7**		**21.8**	
% CV	**2.3**	**2.1**	**2.2**	**3.60**	**Y**	**1.2**	**Y**

19.2.1 Mass Spectral Matching for Core Run 1

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
2 021 S12289-00 2x new tolerance 1 1	9.21E+04	6.27E+04	1.93E+04	68.1		21.0	
2 026 S12289-00 2x new tolerance 2 1	9.43E+04	6.50E+04	2.01E+04	68.9		21.3	
2 031 S12289-00 2x new tolerance 3 1	9.35E+04	6.27E+04	2.14E+04	67.1		22.9	
2 036 S12289-00 2x new tolerance 4 1	1.04E+05	7.34E+04	2.14E+04	70.6		20.6	
2 041 S12289-00 2x new tolerance 5 1	1.08E+05	7.62E+04	2.39E+04	70.6		22.1	
2 046 S12289-00 2x new tolerance 6 1	9.55E+04	6.55E+04	2.09E+04	68.6		21.9	
Average	**9.79E+04**	**6.76E+04**	**2.12E+04**	**69.0**		**21.6**	
% CV	**6.6**	**8.5**	**7.4**	**2.02**	**Y**	**3.9**	**Y**
2 022 S12289-00 Incurred1 1 1	3.43E+04	2.57E+04	7.65E+03	74.9		22.3	
2 027 S12289-00 Incurred1 2 1	3.17E+04	2.24E+04	7.09E+03	70.7		22.4	
2 032 S12289-00 Incurred1 3 1	2.69E+04	1.88E+04	6.08E+03	69.9		22.6	
2 037 S12289-00 Incurred1 4 1	2.78E+04	2.14E+04	6.43E+03	77.0		23.1	
2 042 S12289-00 Incurred1 5 1	2.98E+04	2.05E+04	6.44E+03	68.8		21.6	
2 047 S12289-00 Incurred1 6 1	2.83E+04	1.92E+04	6.19E+03	67.8		21.9	
Average	**2.98E+04**	**2.13E+04**	**6.65E+03**	**71.5**		**22.3**	
% CV	**9.3**	**11.8**	**9.1**	**5.07**	**Y**	**2.4**	**Y**

19.2.1 Mass Spectral Matching for Core Run 1

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
2 023 S12289-00 Incurred2 1 1	8.90E+04	5.96E+04	1.83E+04	67.0		20.6	
2 028 S12289-00 Incurred2 2 1	9.94E+04	6.71E+04	2.13E+04	67.5		21.4	
2 033 S12289-00 Incurred2 3 1	8.30E+04	5.76E+04	1.76E+04	69.4		21.2	
2 038 S12289-00 Incurred2 4 1	8.86E+04	5.71E+04	1.82E+04	64.4		20.5	
2 043 S12289-00 Incurred2 5 1	9.04E+04	6.05E+04	1.88E+04	66.9		20.8	
2 048 S12289-00 Incurred2 6 1	8.84E+04	5.65E+04	1.92E+04	63.9		21.7	
Average	**8.98E+04**	**5.97E+04**	**1.89E+04**	**66.5**		**21.0**	
% CV	**6.0**	**6.6**	**6.9**	**3.06**	**Y**	**2.3**	**Y**
2 018 S12289-00 CONTROL_BLANK 1	5.27E+02	1.44E+03	3.68E+02	273.2	**N**	69.8	**N**

19.2.1 Mass Spectra Matching for Core Run 1

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
2 008 S12289-00 Liver-Std-1 1 1	2.10	2.10	Y	2.10	Y	129	Y	99	Y
2 009 S12289-00 Liver-Std-2 1 1	2.10	2.10	Y	2.10	Y	586	Y	369	Y
2 010 S12289-00 Liver-Std-3 1 1	2.10	2.10	Y	2.10	Y	930	Y	529	Y
2 011 S12289-00 Liver-Std-4 1 1	2.10	2.10	Y	2.10	Y	1510	Y	845	Y
2 012 S12289-00 Liver-Std-5 1 1	2.10	2.10	Y	2.10	Y	4990	Y	1480	Y
2 013 S12289-00 Liver-Std-6 1 1	2.10	2.10	Y	2.10	Y	3160	Y	2750	Y
2 014 S12289-00 Liver-Std-7 1 1	2.10	2.10	Y	2.10	Y	4240	Y	2000	Y
2 015 S12289-00 Liver-Std-8 1 1	2.10	2.10	Y	2.10	Y	3320	Y	4840	Y
2 050 S12289-00 Liver-Std-1 2 1	2.10	2.10	Y	2.05	Y	138	Y	223	Y
2 051 S12289-00 Liver-Std-2 2 1	2.10	2.10	Y	2.05	Y	485	Y	566	Y
2 052 S12289-00 Liver-Std-3 2 1	2.10	2.10	Y	2.05	Y	749	Y	1380	Y
2 053 S12289-00 Liver-Std-4 2 1	2.10	2.10	Y	2.05	Y	1490	Y	2540	Y
2 054 S12289-00 Liver-Std-5 2 1	2.10	2.10	Y	2.05	Y	2010	Y	4850	Y
2 055 S12289-00 Liver-Std-6 2 1	2.10	2.10	Y	2.05	Y	2800	Y	6040	Y
2 056 S12289-00 Liver-Std-7 2 1	2.10	2.10	Y	2.05	Y	2780	Y	5650	Y
2 057 S12289-00 Liver-Std-8 2 1	2.10	2.10	Y	2.05	Y	6850	Y	5460	Y
Average	**2.10**	**2.10**	**Y**	**2.08**	**Y**				

19.2.1 Mass Spectra Matching for Core Run 1

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
2 019 S12289-00 1/2x new tolerance 1 1	2.10	2.10	Y	2.10	Y	323	Y	194	Y
2 024 S12289-00 1/2x new tolerance 2 1	2.10	2.10	Y	2.05	Y	212	Y	259	Y
2 029 S12289-00 1/2x new tolerance 3 1	2.10	2.10	Y	2.05	Y	390	Y	243	Y
2 034 S12289-00 1/2x new tolerance 4 1	2.10	2.10	Y	2.05	Y	306	Y	269	Y
2 039 S12289-00 1/2x new tolerance 5 1	2.10	2.10	Y	2.05	Y	167	Y	450	Y
2 044 S12289-00 1/2x new tolerance 6 1	2.10	2.10	Y	2.05	Y	205	Y	255	Y
Average	**2.10**	**2.10**	**Y**	**2.06**	**Y**				
2 020 S12289-00 1x new tolerance 1 1	2.10	2.10	Y	2.05	Y	669	Y	754	Y
2 025 S12289-00 1x new tolerance 2 1	2.10	2.10	Y	2.05	Y	437	Y	517	Y
2 030 S12289-00 1x new tolerance 3 1	2.10	2.10	Y	2.05	Y	536	Y	470	Y
2 035 S12289-00 1x new tolerance 4 1	2.10	2.10	Y	2.05	Y	384	Y	642	Y
2 040 S12289-00 1x new tolerance 5 1	2.10	2.10	Y	2.05	Y	669	Y	621	Y
2 045 S12289-00 1x new tolerance 6 1	2.10	2.10	Y	2.05	Y	687	Y	794	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
2 021 S12289-00 2x new tolerance 1 1	2.10	2.10	Y	2.05	Y	1120	Y	852	Y
2 026 S12289-00 2x new tolerance 2 1	2.10	2.10	Y	2.05	Y	1110	Y	1100	Y
2 031 S12289-00 2x new tolerance 3 1	2.10	2.10	Y	2.05	Y	724	Y	2100	Y
2 036 S12289-00 2x new tolerance 4 1	2.10	2.10	Y	2.10	Y	943	Y	3630	Y
2 041 S12289-00 2x new tolerance 5 1	2.10	2.10	Y	2.05	Y	1250	Y	2150	Y
2 046 S12289-00 2x new tolerance 6 1	2.10	2.10	Y	2.05	Y	913	Y	2890	Y
Average	**2.10**	**2.10**	**Y**	**2.06**	**Y**				

19.2.1 Mass Spectra Matching for Core Run 1

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	**retention time[a]**		**Y/N**	**retention time[a]**	**Y/N**	**S/N**	**Y/N**	**S/N**	**Y/N**
2 022 S12289-00 Incurred1 1 1	2.10	2.10	Y	2.10	Y	566	Y	637	Y
2 027 S12289-00 Incurred1 2 1	2.10	2.10	Y	2.05	Y	428	Y	467	Y
2 032 S12289-00 Incurred1 3 1	2.10	2.10	Y	2.05	Y	285	Y	249	Y
2 037 S12289-00 Incurred1 4 1	2.10	2.10	Y	2.05	Y	560	Y	393	Y
2 042 S12289-00 Incurred1 5 1	2.10	2.10	Y	2.05	Y	268	Y	589	Y
2 047 S12289-00 Incurred1 6 1	2.10	2.10	Y	2.05	Y	277	Y	311	Y
Average	**2.10**	**2.10**	**Y**	**2.06**	**Y**				
2 023 S12289-00 Incurred2 1 1	2.10	2.10	Y	2.05	Y	771	Y	1630	Y
2 028 S12289-00 Incurred2 2 1	2.10	2.10	Y	2.05	Y	855	Y	1430	Y
2 033 S12289-00 Incurred2 3 1	2.10	2.10	Y	2.05	Y	1380	Y	1330	Y
2 038 S12289-00 Incurred2 4 1	2.10	2.10	Y	2.05	Y	1320	Y	1580	Y
2 043 S12289-00 Incurred2 5 1	2.10	2.10	Y	2.05	Y	1190	Y	612	Y
2 048 S12289-00 Incurred2 6 1	2.10	2.10	Y	2.05	Y	814	Y	1720	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
2 018 S12289-00 CONTROL_BLANK 1	2.10	2.10	Y	2.05	Y	7	N	9	N

[a]retention time in minutes

19.2.2 Mass Spectral Matching for Core Run 2

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
5 008 S12289-00 Liver-Std-1 1 1	2.11E+04	1.43E+04	4.07E+03	67.8		19.3	
5 009 S12289-00 Liver-Std-2 1 1	5.86E+04	4.20E+04	1.23E+04	71.7		21.0	
5 010 S12289-00 Liver-Std-3 1 1	1.16E+05	8.02E+04	2.35E+04	69.1		20.3	
5 011 S12289-00 Liver-Std-4 1 1	2.29E+05	1.60E+05	4.70E+04	69.9		20.5	
5 012 S12289-00 Liver-Std-5 1 1	3.58E+05	2.34E+05	7.04E+04	65.4		19.7	
5 013 S12289-00 Liver-Std-6 1 1	4.37E+05	2.97E+05	9.35E+04	68.0		21.4	
5 014 S12289-00 Liver-Std-7 1 1	5.43E+05	3.60E+05	1.11E+05	66.3		20.4	
5 015 S12289-00 Liver-Std-8 1 1	7.20E+05	4.76E+05	1.49E+05	66.1		20.7	
5 050 S12289-00 Liver-Std-1 2 1	1.97E+04	1.34E+04	4.74E+03	68.0		24.1	
5 051 S12289-00 Liver-Std-2 2 1	5.80E+04	3.86E+04	1.42E+04	66.6		24.5	
5 052 S12289-00 Liver-Std-3 2 1	1.11E+05	7.31E+04	2.79E+04	65.9		25.1	
5 053 S12289-00 Liver-Std-4 2 1	2.33E+05	1.48E+05	5.41E+04	63.5		23.2	
5 054 S12289-00 Liver-Std-5 2 1	3.67E+05	2.43E+05	8.84E+04	66.2		24.1	
5 055 S12289-00 Liver-Std-6 2 1	4.31E+05	2.71E+05	1.09E+05	62.9		25.3	
5 056 S12289-00 Liver-Std-7 2 1	4.95E+05	3.23E+05	1.26E+05	65.3		25.5	
5 057 S12289-00 Liver-Std-8 2 1	7.13E+05	4.62E+05	1.67E+05	64.8		23.4	
Average	**3.07E+05**	**2.02E+05**	**6.89E+04**	**66.7**		**22.4**	

19.2.2 Mass Spectral Matching for Core Run 2

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
5 019 S12289-00 1/2x new tolerance 1 1	3.63E+04	2.50E+04	7.44E+03	68.9		20.5	
5 024 S12289-00 1/2x new tolerance 2 1	4.11E+04	2.70E+04	7.96E+03	65.7		19.4	
5 029 S12289-00 1/2x new tolerance 3 1	3.76E+04	2.70E+04	8.80E+03	71.8		23.4	
5 034 S12289-00 1/2x new tolerance 4 1	3.54E+04	2.36E+04	8.03E+03	66.7		22.7	
5 039 S12289-00 1/2x new tolerance 5 1	3.61E+04	2.27E+04	8.24E+03	62.9		22.8	
5 044 S12289-00 1/2x new tolerance 6 1	3.57E+04	2.41E+04	8.12E+03	67.5		22.7	
Average	**3.70E+04**	**2.49E+04**	**8.10E+03**	**67.2**		**21.9**	
% CV	**5.76**	**7.18**	**5.44**	**4.48**	**Y**	**7.3**	**Y**
5 020 S12289-00 1x new tolerance 1 1	7.00E+04	4.67E+04	1.61E+04	66.7		23.0	
5 025 S12289-00 1x new tolerance 2 1	7.53E+04	4.98E+04	1.58E+04	66.1		21.0	
5 030 S12289-00 1x new tolerance 3 1	7.13E+04	4.62E+04	1.67E+04	64.8		23.4	
5 035 S12289-00 1x new tolerance 4 1	6.84E+04	4.28E+04	1.54E+04	62.6		22.5	
5 040 S12289-00 1x new tolerance 5 1	6.35E+04	4.41E+04	1.54E+04	69.4		24.3	
5 045 S12289-00 1x new tolerance 6 1	6.74E+04	4.88E+04	1.62E+04	72.4		24.0	
Average	**6.93E+04**	**4.64E+04**	**1.59E+04**	**67.0**		**23.0**	
% CV	**5.7**	**5.8**	**3.2**	**5.19**	**Y**	**5.2**	**Y**

19.2.2 Mass Spectral Matching for Core Run 2

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
5 021 S12289-00 2x new tolerance 1 1	1.43E+05	9.83E+04	3.11E+04	68.7		21.7	
5 026 S12289-00 2x new tolerance 2 1	1.48E+05	9.70E+04	3.21E+04	65.5		21.7	
5 031 S12289-00 2x new tolerance 3 1	1.43E+05	1.02E+05	3.16E+04	71.3		22.1	
5 036 S12289-00 2x new tolerance 4 1	1.37E+05	8.94E+04	3.18E+04	65.3		23.2	
5 041 S12289-00 2x new tolerance 5 1	1.19E+05	7.69E+04	2.68E+04	64.6		22.5	
5 046 S12289-00 2x new tolerance 6 1	1.39E+05	8.87E+04	3.11E+04	63.8		22.4	
Average	**1.38E+05**	**9.21E+04**	**3.08E+04**	**66.6**		**22.3**	
% CV	**7.3**	**9.8**	**6.4**	**4.33**	**Y**	**2.5**	**Y**
5 022 S12289-00 Incurred1 1 0.963	4.35E+04	2.90E+04	9.66E+03	66.7		22.2	
5 027 S12289-00 Incurred1 2 0.986	4.09E+04	2.71E+04	8.61E+03	66.3		21.1	
5 032 S12289-00 Incurred1 3 1.034	3.60E+04	2.24E+04	8.58E+03	62.2		23.8	
5 037 S12289-00 Incurred1 4 1.035	4.36E+04	2.86E+04	1.00E+04	65.6		22.9	
5 042 S12289-00 Incurred1 5 0.978	4.09E+04	2.36E+04	8.84E+03	57.7		21.6	
5 047 S12289-00 Incurred1 6 1	3.87E+04	2.78E+04	9.52E+03	71.8		24.6	
Average	**4.06E+04**	**2.64E+04**	**9.20E+03**	**65.0**		**22.7**	
% CV	**7.2**	**10.4**	**6.5**	**7.29**	**Y**	**5.9**	**Y**

19.2.2 Mass Spectral Matching for Core Run 2

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
5 023 S12289-00 Incurred2 1 1.033	1.42E+05	9.19E+04	3.03E+04	64.7		21.3	
5 028 S12289-00 Incurred2 2 0.964	1.24E+05	8.37E+04	2.76E+04	67.5		22.3	
5 033 S12289-00 Incurred2 3 1.037	1.25E+05	8.24E+04	2.84E+04	65.9		22.7	
5 038 S12289-00 Incurred2 4 1.03	1.20E+05	7.49E+04	2.59E+04	62.4		21.6	
5 043 S12289-00 Incurred2 5 1.01	1.11E+05	6.99E+04	2.48E+04	63.0		22.3	
5 048 S12289-00 Incurred2 6 1.006	1.09E+05	7.19E+04	2.76E+04	66.0		25.3	
Average	**1.22E+05**	**7.91E+04**	**2.74E+04**	**64.9**		**22.6**	
% CV	**9.8**	**10.6**	**7.0**	**2.99**	**Y**	**6.3**	**Y**
5 018 S12289-00 CONTROL_BLANK 1	2.51E+03	1.69E+03	7.50E+02	67.3	**Y**	29.9	**Y**

19.2.2 Mass Spectra Matching for Core Run 2

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
5 008 S12289-00 Liver-Std-1 1 1	2.10	2.10	Y	2.05	Y	214	Y	122	Y
5 009 S12289-00 Liver-Std-2 1 1	2.10	2.10	Y	2.05	Y	506	Y	785	Y
5 010 S12289-00 Liver-Std-3 1 1	2.10	2.10	Y	2.05	Y	1340	Y	1170	Y
5 011 S12289-00 Liver-Std-4 1 1	2.10	2.10	Y	2.05	Y	3140	Y	3460	Y
5 012 S12289-00 Liver-Std-5 1 1	2.10	2.10	Y	2.05	Y	4060	Y	3480	Y
5 013 S12289-00 Liver-Std-6 1 1	2.10	2.10	Y	2.05	Y	6310	Y	6780	Y
5 014 S12289-00 Liver-Std-7 1 1	2.10	2.10	Y	2.05	Y	3460	Y	6130	Y
5 015 S12289-00 Liver-Std-8 1 1	2.10	2.10	Y	2.05	Y	11700	Y	11000	Y
5 050 S12289-00 Liver-Std-1 2 1	2.10	2.10	Y	2.05	Y	143	Y	288	Y
5 051 S12289-00 Liver-Std-2 2 1	2.10	2.10	Y	2.05	Y	487	Y	1060	Y
5 052 S12289-00 Liver-Std-3 2 1	2.10	2.10	Y	2.05	Y	785	Y	1570	Y
5 053 S12289-00 Liver-Std-4 2 1	2.10	2.10	Y	2.05	Y	1670	Y	4370	Y
5 054 S12289-00 Liver-Std-5 2 1	2.10	2.10	Y	2.05	Y	3450	Y	11000	Y
5 055 S12289-00 Liver-Std-6 2 1	2.10	2.10	Y	2.05	Y	2580	Y	11300	Y
5 056 S12289-00 Liver-Std-7 2 1	2.10	2.10	Y	2.05	Y	4880	Y	19000	Y
5 057 S12289-00 Liver-Std-8 2 1	2.10	2.10	Y	2.05	Y	3650	Y	9860	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				

19.2.2 Mass Spectra Matching for Core Run 2

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
5 019 S12289-00 1/2x new tolerance 1 1	2.10	2.10	Y	2.05	Y	381	Y	470	Y
5 024 S12289-00 1/2x new tolerance 2 1	2.10	2.10	Y	2.05	Y	339	Y	614	Y
5 029 S12289-00 1/2x new tolerance 3 1	2.10	2.10	Y	2.05	Y	406	Y	1560	Y
5 034 S12289-00 1/2x new tolerance 4 1	2.10	2.10	Y	2.05	Y	274	Y	591	Y
5 039 S12289-00 1/2x new tolerance 5 1	2.10	2.10	Y	2.05	Y	320	Y	590	Y
5 044 S12289-00 1/2x new tolerance 6 1	2.10	2.10	Y	2.05	Y	245	Y	739	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
5 020 S12289-00 1x new tolerance 1 1	2.10	2.10	Y	2.05	Y	676	Y	1490	Y
5 025 S12289-00 1x new tolerance 2 1	2.10	2.10	Y	2.05	Y	510	Y	1360	Y
5 030 S12289-00 1x new tolerance 3 1	2.10	2.10	Y	2.05	Y	390	Y	1240	Y
5 035 S12289-00 1x new tolerance 4 1	2.10	2.10	Y	2.05	Y	398	Y	1110	Y
5 040 S12289-00 1x new tolerance 5 1	2.10	2.10	Y	2.05	Y	506	Y	848	Y
5 045 S12289-00 1x new tolerance 6 1	2.10	2.10	Y	2.05	Y	704	Y	3610	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				

19.2.2 Mass Spectra Matching for Core Run 2

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
5 021 S12289-00 2x new tolerance 1 1	2.10	2.10	Y	2.05	Y	996	Y	1620	Y
5 026 S12289-00 2x new tolerance 2 1	2.10	2.10	Y	2.05	Y	2130	Y	2440	Y
5 031 S12289-00 2x new tolerance 3 1	2.10	2.10	Y	2.05	Y	3090	Y	3730	Y
5 036 S12289-00 2x new tolerance 4 1	2.10	2.10	Y	2.05	Y	1240	Y	2390	Y
5 041 S12289-00 2x new tolerance 5 1	2.10	2.10	Y	2.05	Y	589	Y	2710	Y
5 046 S12289-00 2x new tolerance 6 1	2.10	2.10	Y	2.05	Y	1750	Y	2410	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
5 022 S12289-00 Incurred1 1 0.963	2.10	2.10	Y	2.05	Y	347	Y	925	Y
5 027 S12289-00 Incurred1 2 0.986	2.10	2.10	Y	2.05	Y	219	Y	754	Y
5 032 S12289-00 Incurred1 3 1.034	2.10	2.10	Y	2.05	Y	423	Y	1030	Y
5 037 S12289-00 Incurred1 4 1.035	2.10	2.10	Y	2.05	Y	507	Y	938	Y
5 042 S12289-00 Incurred1 5 0.978	2.10	2.10	Y	2.05	Y	317	Y	568	Y
5 047 S12289-00 Incurred1 6 1	2.10	2.10	Y	2.05	Y	272	Y	985	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
5 023 S12289-00 Incurred2 1 1.033	2.10	2.10	Y	2.05	Y	1210	Y	1940	2.10
5 028 S12289-00 Incurred2 2 0.964	2.10	2.10	Y	2.05	Y	900	Y	2720	2.10
5 033 S12289-00 Incurred2 3 1.037	2.10	2.10	Y	2.05	Y	1680	Y	2280	2.10
5 038 S12289-00 Incurred2 4 1.03	2.10	2.10	Y	2.05	Y	1240	Y	1490	2.10
5 043 S12289-00 Incurred2 5 1.01	2.10	2.10	Y	2.05	Y	908	Y	1950	2.10
5 048 S12289-00 Incurred2 6 1.006	2.10	2.10	Y	2.05	Y	1280	Y	1840	2.10
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				

19.2.2 Mass Spectra Matching for Core Run 2

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	**retention time[a]**		**Y/N**	**retention time[a]**	**Y/N**	**S/N**	**Y/N**	**S/N**	**Y/N**
5 018 S12289-00 CONTROL_BLANK 1	2.10	2.10	Y	2.05	Y	29	N	32	N

[a]retention time in minutes

19.2.3 Mass Spectral Matching for Core Run 3

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
7 008 S12289-00 Liver-Std-1 1 1	2.01E+04	1.21E+04	4.04E+03	60.2		20.1	
7 009 S12289-00 Liver-Std-2 1 1	4.30E+04	2.87E+04	1.17E+04	66.7		27.2	
7 010 S12289-00 Liver-Std-3 1 1	9.99E+04	5.89E+04	2.22E+04	59.0		22.2	
7 011 S12289-00 Liver-Std-4 1 1	1.85E+05	1.22E+05	4.48E+04	65.9		24.2	
7 012 S12289-00 Liver-Std-5 1 1	2.87E+05	1.74E+05	7.20E+04	60.6		25.1	
7 013 S12289-00 Liver-Std-6 1 1	3.34E+05	2.15E+05	8.82E+04	64.4		26.4	
7 014 S12289-00 Liver-Std-7 1 1	4.16E+05	2.73E+05	1.07E+05	65.6		25.7	
7 015 S12289-00 Liver-Std-8 1 1	6.07E+05	3.99E+05	1.45E+05	65.7		23.9	
7 056 S12289-00 Liver-Std-1 2 1	1.60E+04	9.76E+03	3.87E+03	61.0		24.2	
7 057 S12289-00 Liver-Std-2 2 1	4.35E+04	2.84E+04	1.11E+04	65.3		25.5	
7 058 S12289-00 Liver-Std-3 2 1	9.93E+04	6.30E+04	1.98E+04	63.4		19.9	
7 059 S12289-00 Liver-Std-4 2 1	1.61E+05	1.06E+05	4.04E+04	65.8		25.1	
7 060 S12289-00 Liver-Std-5 2 1	2.61E+05	1.71E+05	6.76E+04	65.5		25.9	
7 061 S12289-00 Liver-Std-6 2 1	3.20E+05	1.97E+05	8.16E+04	61.6		25.5	
7 062 S12289-00 Liver-Std-7 2 1	4.29E+05	2.69E+05	1.00E+05	62.7		23.3	
7 063 S12289-00 Liver-Std-8 2 1	5.71E+05	3.62E+05	1.38E+05	63.4		24.2	
Average	**2.43E+05**	**1.56E+05**	**5.98E+04**	**63.6**		**24.3**	

19.2.3 Mass Spectral Matching for Core Run 3

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
7 025 S12289-00 1/2x new tolerance 1 1	3.35E+04	2.22E+04	7.77E+03	66.3		23.2	
7 030 S12289-00 1/2x new tolerance 2 1	3.50E+04	2.23E+04	8.46E+03	63.7		24.2	
7 035 S12289-00 1/2x new tolerance 3 1	3.53E+04	2.15E+04	8.22E+03	60.9		23.3	
7 040 S12289-00 1/2x new tolerance 4 1	3.63E+04	2.35E+04	8.83E+03	64.7		24.3	
7 045 S12289-00 1/2x new tolerance 5 1	3.65E+04	2.09E+04	8.66E+03	57.3		23.7	
7 050 S12289-00 1/2x new tolerance 6 1	3.02E+04	1.91E+04	7.82E+03	63.2		25.9	
Average	**3.45E+04**	**2.16E+04**	**8.29E+03**	**62.7**		**24.1**	
% CV	**6.82**	**6.94**	**5.27**	**5.10**	**Y**	**4.1**	**Y**
7 026 S12289-00 1x new tolerance 1 1	5.67E+04	3.74E+04	1.41E+04	66.0		24.9	
7 031 S12289-00 1x new tolerance 2 1	6.71E+04	4.32E+04	1.67E+04	64.4		24.9	
7 036 S12289-00 1x new tolerance 3 1	5.99E+04	3.87E+04	1.61E+04	64.6		26.9	
7 041 S12289-00 1x new tolerance 4 1	6.78E+04	4.33E+04	1.70E+04	63.9		25.1	
7 046 S12289-00 1x new tolerance 5 1	5.61E+04	3.49E+04	1.39E+04	62.2		24.8	
7 051 S12289-00 1x new tolerance 6 1	5.41E+04	3.54E+04	1.40E+04	65.4		25.9	
Average	**6.03E+04**	**3.88E+04**	**1.53E+04**	**64.4**		**25.4**	
% CV	**9.7**	**9.5**	**9.5**	**2.04**	**Y**	**3.3**	**Y**

19.2.3 Mass Spectral Matching for Core Run 3

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
7 027 S12289-00 2x new tolerance 1 1	1.04E+05	6.36E+04	2.66E+04	61.2		25.6	
7 032 S12289-00 2x new tolerance 2 1	1.36E+05	8.10E+04	3.04E+04	59.6		22.4	
7 037 S12289-00 2x new tolerance 3 1	1.23E+05	7.85E+04	3.27E+04	63.8		26.6	
7 042 S12289-00 2x new tolerance 4 1	1.06E+05	6.64E+04	3.01E+04	62.6		28.4	
7 047 S12289-00 2x new tolerance 5 1	1.06E+05	6.75E+04	2.82E+04	63.7		26.6	
7 052 S12289-00 2x new tolerance 6 1	1.04E+05	6.75E+04	2.93E+04	64.9		28.2	
Average	**1.13E+05**	**7.08E+04**	**2.96E+04**	**62.6**		**26.3**	
% CV	**11.8**	**10.1**	**7.0**	**3.14**	**Y**	**8.4**	**Y**
7 028 S12289-00 Incurred1 1 1.034	4.02E+04	2.85E+04	8.45E+03	70.9		21.0	
7 033 S12289-00 Incurred1 2 1.003	3.44E+04	2.21E+04	8.03E+03	64.2		23.3	
7 038 S12289-00 Incurred1 3 1.013	3.96E+04	2.45E+04	9.92E+03	61.9		25.1	
7 043 S12289-00 Incurred1 4 1.028	3.08E+04	1.99E+04	8.64E+03	64.6		28.1	
7 048 S12289-00 Incurred1 5 1.001	3.67E+04	2.54E+04	8.45E+03	69.2		23.0	
7 053 S12289-00 Incurred1 6 0.978	2.62E+04	1.72E+04	7.57E+03	65.6		28.9	
Average	**3.47E+04**	**2.29E+04**	**8.51E+03**	**66.1**		**24.9**	
% CV	**15.6**	**17.7**	**9.3**	**5.08**	**Y**	**12.3**	**Y**

19.2.3 Mass Spectral Matching for Core Run 3

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
7 029 S12289-00 Incurred2 1 0.968	1.14E+05	7.26E+04	2.67E+04	63.7		23.4	
7 034 S12289-00 Incurred2 2 1.007	1.08E+05	7.32E+04	2.90E+04	67.8		26.9	
7 039 S12289-00 Incurred2 3 0.957	1.10E+05	7.17E+04	2.80E+04	65.2		25.5	
7 044 S12289-00 Incurred2 4 0.966	9.95E+04	6.28E+04	2.40E+04	63.1		24.1	
7 049 S12289-00 Incurred2 5 0.966	no data	no data	no data	N/A		N/A	
7 054 S12289-00 Incurred2 6 0.981	1.54E+05	9.67E+04	3.95E+04	62.8		25.6	
Average	**1.17E+05**	**7.54E+04**	**2.94E+04**	**64.5**		**25.1**	
% CV	**18.2**	**16.8**	**20.1**	**3.17**	**Y**	**5.4**	**Y**
7 019 S12289-00 LLOQ 1 1	1.68E+04	9.90E+03	4.09E+03	58.9		24.3	
7 020 S12289-00 LLOQ 2 1	1.61E+04	1.07E+04	3.50E+03	66.5		21.7	
7 021 S12289-00 LLOQ 3 1	1.75E+04	1.10E+04	4.21E+03	62.9		24.1	
7 022 S12289-00 LLOQ 4 1	1.55E+04	9.52E+03	4.07E+03	61.4		26.3	
7 023 S12289-00 LLOQ 5 1	1.75E+04	1.24E+04	4.48E+03	70.9		25.6	
7 024 S12289-00 LLOQ 6 1	1.74E+04	1.19E+04	4.27E+03	68.4		24.5	
Average	**1.68E+04**	**1.09E+04**	**4.10E+03**	**64.8**	**Y**	**24.4**	**Y**
% CV	**5.0**	**10.2**	**8.1**	**6.97**		**6.4**	
7 018 S12289-00 CONTROL_BLANK 1	2.51E+03	1.69E+03	7.50E+02	67.3	**Y**	29.9	**N**

19.2.3 Mass Spectra Matching for Core Run 3

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
7 008 S12289-00 Liver-Std-1 1 1	2.10	2.10	Y	2.05	Y	172	Y	320	Y
7 009 S12289-00 Liver-Std-2 1 1	2.10	2.10	Y	2.05	Y	388	Y	367	Y
7 010 S12289-00 Liver-Std-3 1 1	2.10	2.10	Y	2.05	Y	697	Y	1500	Y
7 011 S12289-00 Liver-Std-4 1 1	2.10	2.10	Y	2.05	Y	1600	Y	3100	Y
7 012 S12289-00 Liver-Std-5 1 1	2.10	2.10	Y	2.05	Y	3570	Y	6540	Y
7 013 S12289-00 Liver-Std-6 1 1	2.10	2.10	Y	2.05	Y	3210	Y	7000	Y
7 014 S12289-00 Liver-Std-7 1 1	2.10	2.10	Y	2.05	Y	3750	Y	5670	Y
7 015 S12289-00 Liver-Std-8 1 1	2.10	2.10	Y	2.05	Y	4520	Y	6890	Y
7 056 S12289-00 Liver-Std-1 2 1	2.10	2.10	Y	2.05	Y	149	Y	239	Y
7 057 S12289-00 Liver-Std-2 2 1	2.10	2.10	Y	2.05	Y	573	Y	812	Y
7 058 S12289-00 Liver-Std-3 2 1	2.10	2.10	Y	2.05	Y	1050	Y	932	Y
7 059 S12289-00 Liver-Std-4 2 1	2.10	2.10	Y	2.05	Y	1510	Y	2970	Y
7 060 S12289-00 Liver-Std-5 2 1	2.10	2.10	Y	2.05	Y	2370	Y	5340	Y
7 061 S12289-00 Liver-Std-6 2 1	2.10	2.10	Y	2.05	Y	2020	Y	4330	Y
7 062 S12289-00 Liver-Std-7 2 1	2.10	2.10	Y	2.05	Y	7270	Y	10900	Y
7 063 S12289-00 Liver-Std-8 2 1	2.10	2.10	Y	2.05	Y	10700	Y	12200	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
7 025 S12289-00 1/2x new tolerance 1 1	2.10	2.10	Y	2.05	Y	384	Y	536	Y
7 030 S12289-00 1/2x new tolerance 2 1	2.10	2.10	Y	2.05	Y	203	Y	858	Y
7 035 S12289-00 1/2x new tolerance 3 1	2.10	2.10	Y	2.05	Y	441	Y	408	Y
7 040 S12289-00 1/2x new tolerance 4 1	2.10	2.10	Y	2.05	Y	277	Y	562	Y
7 045 S12289-00 1/2x new tolerance 5 1	2.10	2.10	Y	2.05	Y	171	Y	454	Y

19.2.3 Mass Spectra Matching for Core Run 3

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
7 050 S12289-00 1/2x new tolerance 6 1	2.10	2.10	Y	2.05	Y	215	Y	512	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
7 026 S12289-00 1x new tolerance 1 1	2.10	2.10	Y	2.05	Y	451	Y	732	Y
7 031 S12289-00 1x new tolerance 2 1	2.10	2.10	Y	2.05	Y	781	Y	1310	Y
7 036 S12289-00 1x new tolerance 3 1	2.10	2.10	Y	2.05	Y	515	Y	1120	Y
7 041 S12289-00 1x new tolerance 4 1	2.10	2.10	Y	2.05	Y	409	Y	1140	Y
7 046 S12289-00 1x new tolerance 5 1	2.10	2.10	Y	2.05	Y	407	Y	755	Y
7 051 S12289-00 1x new tolerance 6 1	2.10	2.10	Y	2.05	Y	694	Y	1140	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
7 027 S12289-00 2x new tolerance 1 1	2.10	2.10	Y	2.05	Y	1470	Y	1050	Y
7 032 S12289-00 2x new tolerance 2 1	2.10	2.10	Y	2.05	Y	876	Y	2090	Y
7 037 S12289-00 2x new tolerance 3 1	2.10	2.10	Y	2.05	Y	969	Y	1970	Y
7 042 S12289-00 2x new tolerance 4 1	2.10	2.10	Y	2.05	Y	1110	Y	2250	Y
7 047 S12289-00 2x new tolerance 5 1	2.10	2.10	Y	2.05	Y	1170	Y	1960	Y
7 052 S12289-00 2x new tolerance 6 1	2.10	2.10	Y	2.05	Y	761	Y	1710	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
7 028 S12289-00 Incurred1 1 1.034	2.10	2.10	Y	2.05	Y	368	Y	609	Y
7 033 S12289-00 Incurred1 2 1.003	2.10	2.10	Y	2.05	Y	364	Y	1100	Y
7 038 S12289-00 Incurred1 3 1.013	2.10	2.10	Y	2.05	Y	408	Y	414	Y
7 043 S12289-00 Incurred1 4 1.028	2.10	2.10	Y	2.05	Y	443	Y	607	Y

19.2.3 Mass Spectra Matching for Core Run 3

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
7 048 S12289-00 Incurred1 5 1.001	2.10	2.10	Y	2.05	Y	325	Y	369	Y
7 053 S12289-00 Incurred1 6 0.978	2.10	2.10	Y	2.05	Y	337	Y	544	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
7 029 S12289-00 Incurred2 1 0.968	2.10	2.10	Y	2.05	Y	802	Y	2300	Y
7 034 S12289-00 Incurred2 2 1.007	2.10	2.10	Y	2.05	Y	871	Y	1880	Y
7 039 S12289-00 Incurred2 3 0.957	2.10	2.10	Y	2.05	Y	1150	Y	1740	Y
7 044 S12289-00 Incurred2 4 0.966	2.10	2.10	Y	2.05	Y	859	Y	1020	Y
7 049 S12289-00 Incurred2 5 0.966	no data[b]	no data[b]	N/A	no data[b]	N/A	no data[b]	N/A	no data[b]	N/A
7 054 S12289-00 Incurred2 6 0.981	2.10	2.10	Y	2.05	Y	1660	Y	1710	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
7 019 S12289-00 LLOQ 1 1	2.10	2.10	Y	2.05	Y	109	Y	444	Y
7 020 S12289-00 LLOQ 2 1	2.10	2.10	Y	2.05	Y	84	Y	177	Y
7 021 S12289-00 LLOQ 3 1	2.10	2.10	Y	2.05	Y	162	Y	282	Y
7 022 S12289-00 LLOQ 4 1	2.10	2.10	Y	2.05	Y	150	Y	187	Y
7 023 S12289-00 LLOQ 5 1	2.10	2.10	Y	2.05	Y	154	Y	265	Y
7 024 S12289-00 LLOQ 6 1	2.10	2.10	Y	2.05	Y	146	Y	283	Y
Average	**2.10**	**2.10**	**Y**	**2.05**	**Y**				
7 018 S12289-00 CONTROL_BLANK 1	2.10	2.10	Y	2.05	Y	24	Y	46	Y

[a]retention time in minutes. [b]Autosampler injection error.

19.3 Method Trial Determinative Procedure Data Summary

19.3.1 Summary of Determinative Results for Untreated and Incurred Samples in the Reference Laboratory

Standard Curve Linearity (Liver)			
Std. Curve Range	1.00 to 10.0 ppm (nominal concentration 2.5 to 25.0 ng/mL)		
Run ID	Slope	Intercept	Correlation Coefficient
2	0.2898	0.1108	0.9933
3	0.2920	0.1082	0.9930
4	0.3001	0.07802	0.9975

Precision & Accuracy (Liver)							
	QC Level	Conc. (ppm)	%CV	Mean %Recovery	Incur Animal#	Conc (ppm)	%CV
Inter-Batch (n = 6)	QC1	1.60	13.9	94.6	Mean Assay Conc. (n=5)		
	QC2	3.20	1.9	98.4	FDA Sample1	BLQ	N/A
	QC3	6.40	2.6	101.9	FDA Sample2	6.06	15.9
	QC4	8.00	4.5	103.6	FDA Sample3	3.47	6.2

19.3.2 Summary of Determinative Results for Untreated and Incurred Samples in Testing Laboratory 1

Standard Curve Linearity (Liver)			
Std. Curve Range	1.00 to 10.0 ppm (nominal concentration 2.5 to 25.0 ng/mL)		
Run ID	Slope	Intercept	Correlation Coefficient
Day 1, 20141215	0.283	0.0511	0.9998
Day 2, 20141216	0.27	0.0395	0.9987
Day 3, 20141217	0.273	0.0475	0.9996

Precision & Accuracy (Liver)							
	QC Level	Conc. (ppm)	%CV	Mean %Recovery	Blinded Samples	Conc (ppm)	%CV
Inter-Batch (n = 6)	QC1	1.60	3.91	93.3	Mean Assay Conc. (n=5)		
	QC2	3.20	1.83	96.8	FDA Sample1	BLQ	N/A
	QC3	6.40	2.42	95.8	FDA Sample2	6.24	3.99
	QC4	8.00	2.41	96.6	FDA Sample3	3.11	4.11

19.3.3 Summary of Determinative Results for Untreated and Incurred Samples in Testing Laboratory 2

Standard Curve Linearity (Liver)			
Std. Curve Range	1.00 to 10.0 ppm (nominal concentration 2.5 to 25.0 ng/mL)		
Run ID	Slope	Intercept	Correlation Coefficient
Day 1, 112514A	0.363	0.0277	0.9995
Day 2, 120414A	0.364	0.0149	0.9997
Day 3, 120514AR	0.367	0.00935	0.9993

Precision & Accuracy (Liver)							
	QC Level	Conc. (ppm)	%CV	Mean %Recovery	Blinded Samples	Conc (ppm)	%CV
Inter-Batch (n = 6)	QC1	1.60	1.62	96.9	Mean Assay Conc. (n=5)		
	QC2	3.20	1.98	99.3	FDA Sample1	BLQ	N/A
	QC3	6.40	1.67	97.5	FDA Sample2	5.60	6.55
	QC4	8.00	1.45	97.9	FDA Sample3	2.79	10.4

19.4 Reference Lab Method Trial Confirmatory Procedure Data Summary

19.4.1 Mass Spectral Matching for Method Trial Sample Analysis Run 1

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
2 008 S13034-00 Liver-STD-1 1 1	3.19E+04	2.74E+04	6.47E+03	85.9		20.3	
2 009 S13034-00 Liver-STD-2 1 1	4.50E+04	3.40E+04	8.41E+03	75.6		18.7	
2 010 S13034-00 Liver-STD-3 1 1	8.23E+04	6.22E+04	1.45E+04	75.6		17.6	
2 011 S13034-00 Liver-STD-4 1 1	1.33E+05	1.10E+05	2.56E+04	82.7		19.2	
2 012 S13034-00 Liver-STD-5 1 1	2.00E+05	1.53E+05	3.43E+04	76.5		17.2	
2 013 S13034-00 Liver-STD-6 1 1	2.10E+05	1.59E+05	3.60E+04	75.7		17.1	
2 014 S13034-00 Liver-STD-7 1 1	2.41E+05	1.99E+05	5.62E+04	82.6		23.3	
2 036 S13034-00 Liver-STD-1 2 1	2.97E+04	2.52E+04	6.13E+03	84.8		20.6	
2 037 S13034-00 Liver-STD-2 2 1	4.56E+04	3.52E+04	8.20E+03	77.2		18.0	
2 038 S13034-00 Liver-STD-3 2 1	7.99E+04	5.96E+04	1.46E+04	74.6		18.3	
2 039 S13034-00 Liver-STD-4 2 1	1.33E+05	1.02E+05	2.39E+04	76.7		18.0	
2 040 S13034-00 Liver-STD-5 2 1	1.68E+05	1.30E+05	3.01E+04	77.4		17.9	
2 041 S13034-00 Liver-STD-6 2 1	1.98E+05	1.58E+05	3.63E+04	79.8		18.3	
2 042 S13034-00 Liver-STD-7 2 1	2.84E+05	2.27E+05	5.38E+04	79.9		18.9	
Average	1.34E+05	1.06E+05	2.53E+04	78.9		18.8	
2 020 S13034-00 QC1 1 1	3.67E+04	2.52E+04	1.16E+04	68.7	N	31.6	N
2 031 S13034-00 QC1 2 1	6.49E+04	4.93E+04	1.16E+04	76.0	Y	17.9	Y

19.4.1 Mass Spectral Matching for Method Trial Sample Analysis Run 1

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
2 021 S13034-00 QC2 1 1	8.71E+04	6.97E+04	1.79E+04	80.0	Y	20.6	Y
2 032 S13034-00 QC2 2 1	8.89E+04	7.06E+04	1.69E+04	79.4	Y	19.0	Y
2 022 S13034-00 QC3 1 1	1.79E+05	1.39E+05	3.16E+04	77.7	Y	17.7	Y
2 033 S13034-00 QC3 2 1	1.86E+05	1.38E+05	3.15E+04	74.2	Y	16.9	Y
2 023 S13034-00 QC4 1 1	2.23E+05	1.70E+05	4.03E+04	76.2	Y	18.1	Y
2 034 S13034-00 QC4 2 1	2.30E+05	1.73E+05	3.97E+04	75.2	Y	17.3	Y
2 025 S13034-00 FDA Sample #1 1	3.38E+03	2.64E+03	1.05E+03	78.1	Y	31.1	N
2 026 S13034-00 FDA Sample #2 1.024	1.29E+05	9.56E+04	2.21E+04	74.1	Y	17.1	Y
2 027 S13034-00 FDA Sample #2 0.989	1.30E+05	9.85E+04	2.44E+04	75.8	Y	18.8	Y
2 028 S13034-00 FDA Sample #3 1	7.64E+04	6.09E+04	1.70E+04	79.7	Y	22.3	Y
2 029 S13034-00 FDA Sample #3 1.016	8.70E+04	7.00E+04	1.63E+04	80.5	Y	18.7	Y
2 016 S13034-00 double blank 1	2.26E+03	2.79E+03	5.62E+02	123.5	N	24.9	Y
2 017 S13034-00 double blank 1	2.24E+03	2.63E+03	6.84E+02	117.4	N	30.5	N
2 018 S13034-00 CONTROL_BLANK 1	4.16E+03	3.82E+03	1.01E+03	91.8	N	24.3	Y
2 019 S13034-00 CONTROL_BLANK 1	4.04E+03	3.25E+03	1.08E+03	80.4	Y	26.7	Y

19.4.1 Mass Spectral Matching for Method Trial Sample Analysis Run 1

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
2 008 S13034-00 Liver-STD-1 1 1	2.10	2.10	Y	2.10	Y	622	Y	675	Y
2 009 S13034-00 Liver-STD-2 1 1	2.10	2.10	Y	2.10	Y	781	Y	619	Y
2 010 S13034-00 Liver-STD-3 1 1	2.10	2.10	Y	2.10	Y	1840	Y	1180	Y
2 011 S13034-00 Liver-STD-4 1 1	2.10	2.10	Y	2.10	Y	4340	Y	2830	Y
2 012 S13034-00 Liver-STD-5 1 1	2.10	2.10	Y	2.10	Y	2480	Y	3150	Y
2 013 S13034-00 Liver-STD-6 1 1	2.10	2.10	Y	2.10	Y	8300	Y	3280	Y
2 014 S13034-00 Liver-STD-7 1 1	2.10	2.10	Y	2.10	Y	3800	Y	4690	Y
2 036 S13034-00 Liver-STD-1 2 1	2.10	2.10	Y	2.10	Y	379	Y	789	Y
2 037 S13034-00 Liver-STD-2 2 1	2.10	2.10	Y	2.10	Y	1540	Y	1010	Y
2 038 S13034-00 Liver-STD-3 2 1	2.10	2.10	Y	2.10	Y	1230	Y	496	Y
2 039 S13034-00 Liver-STD-4 2 1	2.10	2.10	Y	2.10	Y	2850	Y	1830	Y
2 040 S13034-00 Liver-STD-5 2 1	2.10	2.10	Y	2.10	Y	2520	Y	2260	Y
2 041 S13034-00 Liver-STD-6 2 1	2.10	2.10	Y	2.10	Y	7280	Y	2680	Y
2 042 S13034-00 Liver-STD-7 2 1	2.10	2.10	Y	2.10	Y	9230	Y	4150	Y
Average	2.10	2.10	Y	2.10	Y				
2 020 S13034-00 QC1 1 1	2.14	2.14	Y	2.10	Y	111	Y	179	Y
2 031 S13034-00 QC1 2 1	2.10	2.10	Y	2.10	Y	328	Y	185	Y
2 021 S13034-00 QC2 1 1	2.10	2.10	Y	2.10	Y	401	Y	269	Y
2 032 S13034-00 QC2 2 1	2.10	2.10	Y	2.10	Y	574	Y	264	Y

19.4.1 Mass Spectral Matching for Method Trial Sample Analysis Run 1

	Relative Retention Time Criteria (≤5%)					**S/N Criteria (≥50:1)**			
	m/z 268 FBZ	**m/z 159 Qual1**		**m/z 131 Qual2**		**m/z 159 Qual1**		**m/z 131 Qual2**	
Sample Name	**retention time[a]**		**Y/N**	**retention time[a]**	**Y/N**	**S/N**	**Y/N**	**S/N**	**Y/N**
2 022 S13034-00 QC3 1 1	2.10	2.10	Y	2.10	Y	1410	Y	462	Y
2 033 S13034-00 QC3 2 1	2.10	2.10	Y	2.10	Y	988	Y	398	Y
2 023 S13034-00 QC4 1 1	Y	2.10	2.10	Y	2.10	Y	996	Y	Y
2 034 S13034-00 QC4 2 1	Y	2.10	2.10	Y	2.10	Y	981	Y	Y
2 025 S13034-00 FDA Sample #1 1	N	2.10	2.10	Y	2.10	Y	9	N	10
2 026 S13034-00 FDA Sample #2 1.024	Y	2.10	2.10	Y	2.10	Y	587	Y	310
2 027 S13034-00 FDA Sample #2 0.989	Y	2.10	2.10	Y	2.10	Y	782	Y	396
2 028 S13034-00 FDA Sample #3 1	Y	2.10	2.10	Y	2.10	Y	327	Y	196
2 029 S13034-00 FDA Sample #3 1.016	Y	2.10	2.10	Y	2.10	Y	463	Y	205
2 016 S13034-00 double blank 1	2.10	2.10	Y	2.10	Y	7	N	4	N
2 017 S13034-00 double blank 1	2.10	2.10	Y	2.05	Y	9	N	4	N
2 018 S13034-00 CONTROL_BLANK 1	2.10	2.10	Y	2.05	Y	22	N	12	N
2 019 S13034-00 CONTROL_BLANK 1	2.10	2.10	Y	2.05	Y	15	N	9	N

[a]retention time in minutes

19.4.2 Mass Spectral Matching for Method Trial Sample Analysis Run 2

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
3 008 S13034-00 Liver-STD-1 1 1	2.92E+04	2.44E+04	5.95E+03	83.6		20.4	
3 009 S13034-00 Liver-STD-2 1 1	4.45E+04	3.35E+04	8.30E+03	75.3		18.7	
3 010 S13034-00 Liver-STD-3 1 1	7.78E+04	6.09E+04	1.43E+04	78.3		18.4	
3 011 S13034-00 Liver-STD-4 1 1	1.45E+05	1.18E+05	2.63E+04	81.4		18.1	
3 012 S13034-00 Liver-STD-5 1 1	1.65E+05	1.28E+05	3.01E+04	77.6		18.2	
3 013 S13034-00 Liver-STD-6 1 1	2.20E+05	1.68E+05	4.00E+04	76.4		18.2	
3 014 S13034-00 Liver-STD-7 1 1	2.52E+05	1.98E+05	5.13E+04	78.6		20.4	
3 036 S13034-00 Liver-STD-1 2 1	2.95E+04	2.24E+04	5.96E+03	75.9		20.2	
3 037 S13034-00 Liver-STD-2 2 1	4.33E+04	3.39E+04	8.16E+03	78.3		18.8	
3 038 S13034-00 Liver-STD-3 2 1	9.34E+04	6.91E+04	1.68E+04	74.0		18.0	
3 039 S13034-00 Liver-STD-4 2 1	1.28E+05	9.88E+04	2.40E+04	77.2		18.8	
3 040 S13034-00 Liver-STD-5 2 1	1.67E+05	1.25E+05	3.02E+04	74.9		18.1	
3 041 S13034-00 Liver-STD-6 2 1	2.17E+05	1.77E+05	4.08E+04	81.6		18.8	
3 042 S13034-00 Liver-STD-7 2 1	2.90E+05	2.35E+05	5.83E+04	81.0		20.1	
Average	1.36E+05	1.07E+05	2.57E+04	78.1		18.9	
3 020 S13034-00 QC1 1 1	5.15E+04	3.98E+04	9.99E+03	77.3	Y	19.4	Y
3 031 S13034-00 QC1 2 1	6.84E+04	5.27E+04	1.27E+04	77.0	Y	18.6	Y
3 021 S13034-00 QC2 1 1	9.09E+04	7.45E+04	1.88E+04	82.0	Y	20.7	Y
3 032 S13034-00 QC2 2 1	9.19E+04	6.83E+04	1.68E+04	74.3	Y	18.3	Y

19.4.2 Mass Spectral Matching for Method Trial Sample Analysis Run 2

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
3 022 S13034-00 QC3 1 1	1.83E+05	1.37E+05	3.29E+04	74.9	Y	18.0	Y
3 033 S13034-00 QC3 2 1	1.56E+05	1.24E+05	3.06E+04	79.5	Y	19.6	Y
3 023 S13034-00 QC4 1 1	2.06E+05	1.68E+05	4.20E+04	81.6	Y	20.4	Y
3 034 S13034-00 QC4 2 1	2.36E+05	1.74E+05	4.05E+04	73.7	Y	17.2	Y
3 025 S13034-00 FDA Sample #1 1	4.19E+03	3.33E+03	1.03E+03	79.5	Y	24.6	Y
3 026 S13034-00 FDA Sample #1 1	5.52E+03	4.65E+03	1.40E+03	84.2	Y	25.4	Y
3 027 S13034-00 FDA Sample #2 1.024	1.32E+05	1.04E+05	2.40E+04	78.8	Y	18.2	Y
3 028 S13034-00 FDA Sample #3 1	9.14E+04	7.38E+04	1.67E+04	80.7	Y	18.3	Y
3 029 S13034-00 FDA Sample #3 1.016	8.81E+04	6.62E+04	1.60E+04	75.1	Y	18.2	Y
3 016 S13034-00 double blank 1	2.87E+03	3.19E+03	6.73E+02	111.1	N	23.4	Y
3 017 S13034-00 double blank 1	3.32E+03	1.74E+03	8.17E+02	52.4	N	24.6	Y
3 018 S13034-00 CONTROL_BLANK 1	5.60E+03	4.35E+03	1.35E+03	77.7	Y	24.1	Y
3 019 S13034-00 CONTROL_BLANK 1	4.89E+03	4.43E+03	1.28E+03	90.6	N	26.2	Y

19.4.2 Mass Spectral Matching for Method Trial Sample Analysis Run 2

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
3 008 S13034-00 Liver-STD-1 1 1	2.10	2.10	Y	2.10	Y	496	Y	427	Y
3 009 S13034-00 Liver-STD-2 1 1	2.10	2.10	Y	2.10	Y	1010	Y	390	Y
3 010 S13034-00 Liver-STD-3 1 1	2.10	2.10	Y	2.10	Y	1250	Y	529	Y
3 011 S13034-00 Liver-STD-4 1 1	2.10	2.10	Y	2.10	Y	4580	Y	2390	Y
3 012 S13034-00 Liver-STD-5 1 1	2.10	2.10	Y	2.10	Y	3000	Y	2800	Y
3 013 S13034-00 Liver-STD-6 1 1	2.10	2.10	Y	2.10	Y	4690	Y	3610	Y
3 014 S13034-00 Liver-STD-7 1 1	2.10	2.10	Y	2.10	Y	7430	Y	5280	Y
3 036 S13034-00 Liver-STD-1 2 1	2.10	2.10	Y	2.10	Y	297	Y	648	Y
3 037 S13034-00 Liver-STD-2 2 1	2.10	2.10	Y	2.10	Y	491	Y	615	Y
3 038 S13034-00 Liver-STD-3 2 1	2.10	2.10	Y	2.10	Y	994	Y	981	Y
3 039 S13034-00 Liver-STD-4 2 1	2.10	2.10	Y	2.10	Y	2270	Y	1430	Y
3 040 S13034-00 Liver-STD-5 2 1	2.10	2.10	Y	2.10	Y	1790	Y	1940	Y
3 041 S13034-00 Liver-STD-6 2 1	2.10	2.10	Y	2.10	Y	4150	Y	4230	Y
3 042 S13034-00 Liver-STD-7 2 1	2.10	2.10	Y	2.10	Y	6240	Y	5620	Y
Average	2.10	2.10	Y	2.10	Y				
3 020 S13034-00 QC1 1 1	2.10	2.10	Y	2.10	Y	234	Y	136	Y
3 031 S13034-00 QC1 2 1	2.10	2.10	Y	2.10	Y	306	Y	154	Y
3 021 S13034-00 QC2 1 1	2.10	2.10	Y	2.10	Y	498	Y	338	Y
3 032 S13034-00 QC2 2 1	2.10	2.10	Y	2.10	Y	593	Y	215	Y
3 022 S13034-00 QC3 1 1	2.10	2.10	Y	2.10	Y	910	Y	511	Y

19.4.2 Mass Spectral Matching for Method Trial Sample Analysis Run 2

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
3 033 S13034-00 QC3 2 1	2.10	2.10	Y	2.10	Y	932	Y	626	Y
3 023 S13034-00 QC4 1 1	2.10	2.10	Y	2.10	N	1190	Y	641	Y
3 034 S13034-00 QC4 2 1	2.10	2.10	Y	2.10	Y	1530	Y	589	Y
3 025 S13034-00 FDA Sample #1 1	2.10	2.10	Y	2.10	Y	11	N	12	N
3 026 S13034-00 FDA Sample #1 1	2.10	2.10	Y	2.05	Y	32	N	16	N
3 027 S13034-00 FDA Sample #2 1.024	2.10	2.10	Y	2.10	Y	565	Y	296	Y
3 028 S13034-00 FDA Sample #3 1	2.10	2.10	Y	2.10	Y	442	Y	213	Y
3 029 S13034-00 FDA Sample #3 1.016	2.10	2.10	Y	2.10	Y	305	Y	168	Y
3 016 S13034-00 double blank 1	2.10	2.10	Y	2.05	Y	9	N	5	N
3 017 S13034-00 double blank 1	2.10	2.10	Y	2.05	Y	7	N	5	N
3 018 S13034-00 CONTROL_BLANK 1	2.10	2.10	Y	2.10	Y	19	N	13	N
3 019 S13034-00 CONTROL_BLANK 1	2.10	2.10	Y	2.10	Y	18	N	10	N

[a]retention time in minutes

19.4.3 Mass Spectral Matching for Method Trial Sample Analysis Run 3

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
4 008 S13034-00 Liver-STD-1 1 1	2.93E+04	2.06E+04	5.20E+03	70.3		17.7	
4 009 S13034-00 Liver-STD-2 1 1	5.04E+04	3.86E+04	8.09E+03	76.6		16.1	
4 010 S13034-00 Liver-STD-3 1 1	9.25E+04	6.81E+04	1.55E+04	73.6		16.8	
4 011 S13034-00 Liver-STD-4 1 1	1.60E+05	1.22E+05	2.81E+04	76.3		17.6	
4 012 S13034-00 Liver-STD-5 1 1	1.92E+05	1.37E+05	3.17E+04	71.4		16.5	
4 013 S13034-00 Liver-STD-6 1 1	2.27E+05	1.74E+05	3.81E+04	76.7		16.8	
4 014 S13034-00 Liver-STD-7 1 1	3.43E+05	2.46E+05	5.68E+04	71.7		16.6	
4 036 S13034-00 Liver-STD-1 2 1	2.82E+04	2.35E+04	6.26E+03	83.3		22.2	
4 037 S13034-00 Liver-STD-2 2 1	5.27E+04	3.64E+04	8.39E+03	69.1		15.9	
4 038 S13034-00 Liver-STD-3 2 1	8.83E+04	6.64E+04	1.56E+04	75.2		17.7	
4 039 S13034-00 Liver-STD-4 2 1	1.47E+05	1.04E+05	2.36E+04	70.7		16.1	
4 040 S13034-00 Liver-STD-5 2 1	1.76E+05	1.31E+05	2.88E+04	74.4		16.4	
4 041 S13034-00 Liver-STD-6 2 1	2.26E+05	1.66E+05	3.72E+04	73.5		16.5	
4 042 S13034-00 Liver-STD-7 2 1	3.77E+05	2.65E+05	5.93E+04	70.3		15.7	
Average	1.56E+05	1.14E+05	2.59E+04	73.8		17.0	
4 020 S13034-00 QC1 1 1	4.18E+04	3.35E+04	9.24E+03	80.1	Y	22.1	Y
4 031 S13034-00 QC1 2 1	4.98E+04	3.37E+04	7.76E+03	67.7	Y	15.6	Y
4 021 S13034-00 QC2 1 1	8.92E+04	6.68E+04	1.46E+04	74.9	Y	16.4	Y

19.4.3 Mass Spectral Matching for Method Trial Sample Analysis Run 3

				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
4 032 S13034-00 QC2 2 1	9.53E+04	6.97E+04	1.57E+04	73.1	Y	16.5	Y
4 022 S13034-00 QC3 1 1	1.71E+05	1.39E+05	3.72E+04	81.3	Y	21.8	Y
4 033 S13034-00 QC3 2 1	1.76E+05	1.28E+05	2.83E+04	72.7	Y	16.1	Y
4 023 S13034-00 QC4 1 1	2.23E+05	1.67E+05	3.73E+04	74.9	Y	16.7	Y
4 034 S13034-00 QC4 2 1	1.81E+05	1.51E+05	3.82E+04	83.4	Y	21.1	Y
4 025 S13034-00 FDA Sample #1 1.022	4.50E+03	2.62E+03	6.23E+02	58.2	N	13.8	Y
4 026 S13034-00 FDA Sample #1 1.005	3.93E+03	2.89E+03	6.77E+02	73.5	Y	17.2	Y
4 027 S13034-00 FDA Sample #2 1.036	1.94E+05	1.38E+05	3.13E+04	71.1	Y	16.1	Y
4 028 S13034-00 FDA Sample #2 0.967	1.90E+05	1.44E+05	3.53E+04	75.8	Y	18.6	Y
4 029 S13034-00 FDA Sample #3 0.998	1.05E+05	7.78E+04	1.74E+04	74.1	Y	16.6	Y
4 016 S13034-00 double blank 1	1.66E+03	1.09E+03	0.00E+00	65.7	Y	0.0	N
4 017 S13034-00 double blank 1	3.15E+03	1.55E+03	5.08E+02	49.2	N	16.1	Y
4 018 S13034-00 CONTROL_BLANK 1	5.65E+03	3.44E+03	8.29E+02	60.9	N	14.7	Y
4 019 S13034-00 CONTROL_BLANK 1	4.02E+03	2.81E+03	6.05E+02	69.9	Y	15.0	Y

19.4.3 Mass Spectral Matching for Method Trial Sample Analysis Run 3

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
4 008 S13034-00 Liver-STD-1 1 1	2.10	2.10	Y	2.08	Y	140	Y	127	Y
4 009 S13034-00 Liver-STD-2 1 1	2.10	2.10	Y	2.09	Y	1710	Y	764	Y
4 010 S13034-00 Liver-STD-3 1 1	2.10	2.10	Y	2.08	Y	1870	Y	2350	Y
4 011 S13034-00 Liver-STD-4 1 1	2.10	2.10	Y	2.09	Y	3260	Y	1720	Y
4 012 S13034-00 Liver-STD-5 1 1	2.10	2.10	Y	2.09	Y	4370	Y	8440	Y
4 013 S13034-00 Liver-STD-6 1 1	2.10	2.10	Y	2.09	Y	6630	Y	4560	Y
4 014 S13034-00 Liver-STD-7 1 1	2.10	2.10	Y	2.09	Y	7980	Y	9560	Y
4 036 S13034-00 Liver-STD-1 2 1	2.10	2.10	Y	2.10	Y	408	Y	202	Y
4 037 S13034-00 Liver-STD-2 2 1	2.10	2.10	Y	2.10	Y	1000	Y	973	Y
4 038 S13034-00 Liver-STD-3 2 1	2.10	2.10	Y	2.10	Y	1450	Y	2640	Y
4 039 S13034-00 Liver-STD-4 2 1	2.10	2.10	Y	2.10	Y	4100	Y	2010	Y
4 040 S13034-00 Liver-STD-5 2 1	2.10	2.10	Y	2.10	Y	2510	Y	4110	Y
4 041 S13034-00 Liver-STD-6 2 1	2.10	2.10	Y	2.10	Y	3470	Y	7530	Y
4 042 S13034-00 Liver-STD-7 2 1	2.10	2.10	Y	2.10	Y	4870	Y	7930	Y
Average	2.10	2.10	Y	2.09	Y				
4 020 S13034-00 QC1 1 1	2.10	2.10	Y	2.09	Y	258	Y	157	Y
4 031 S13034-00 QC1 2 1	2.10	2.10	Y	2.08	Y	272	Y	187	Y
4 021 S13034-00 QC2 1 1	2.10	2.10	Y	2.09	Y	351	Y	329	Y
4 032 S13034-00 QC2 2 1	2.10	2.10	Y	2.08	Y	432	Y	292	Y
4 022 S13034-00 QC3 1 1	2.10	2.10	Y	2.09	Y	839	Y	829	Y

19.4.3 Mass Spectral Matching for Method Trial Sample Analysis Run 3

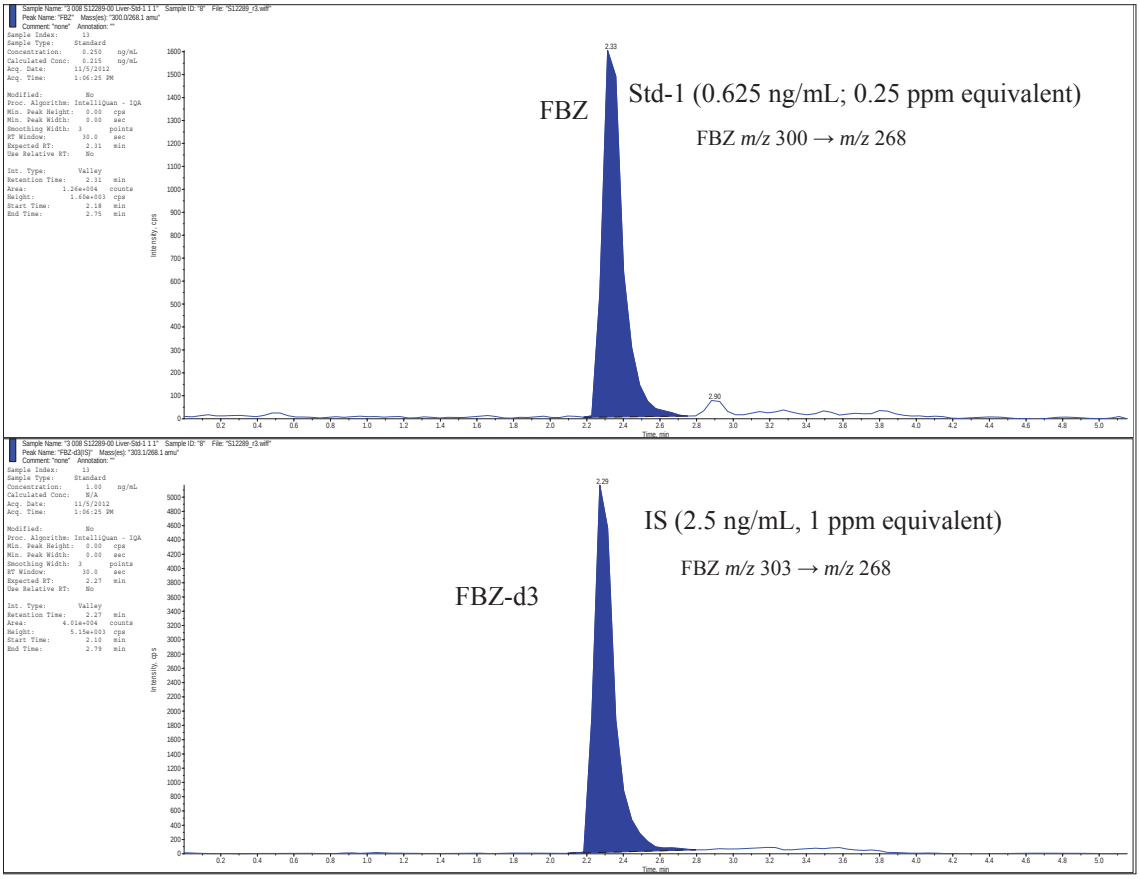
	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
4 033 S13034-00 QC3 2 1	2.10	2.10	Y	2.08	Y	764	Y	658	Y
4 023 S13034-00 QC4 1 1	2.10	2.10	Y	2.09	Y	1440	Y	732	Y
4 034 S13034-00 QC4 2 1	2.10	2.10	Y	2.09	Y	738	Y	585	Y
4 025 S13034-00 FDA Sample #1 1.022	2.10	2.09	Y	2.08	Y	21	N	10	N
4 026 S13034-00 FDA Sample #1 1.005	2.10	2.10	Y	2.08	Y	16	N	10	N
4 027 S13034-00 FDA Sample #2 1.036	2.10	2.10	Y	2.08	Y	842	Y	661	Y
4 028 S13034-00 FDA Sample #2 0.967	2.10	2.10	Y	2.09	Y	699	Y	761	Y
4 029 S13034-00 FDA Sample #3 0.998	2.10	2.10	Y	2.08	Y	430	Y	272	Y
4 016 S13034-00 double blank 1	2.11	2.10	Y	2.08	Y	4	N	3	N
4 017 S13034-00 double blank 1	2.10	2.10	Y	2.08	Y	11	N	8	N
4 018 S13034-00 CONTROL_BLANK 1	2.10	2.10	Y	2.09	Y	20	N	15	N
4 019 S13034-00 CONTROL_BLANK 1	2.10	2.10	Y	2.09	Y	16	N	13	N

[a]retention time in minutes

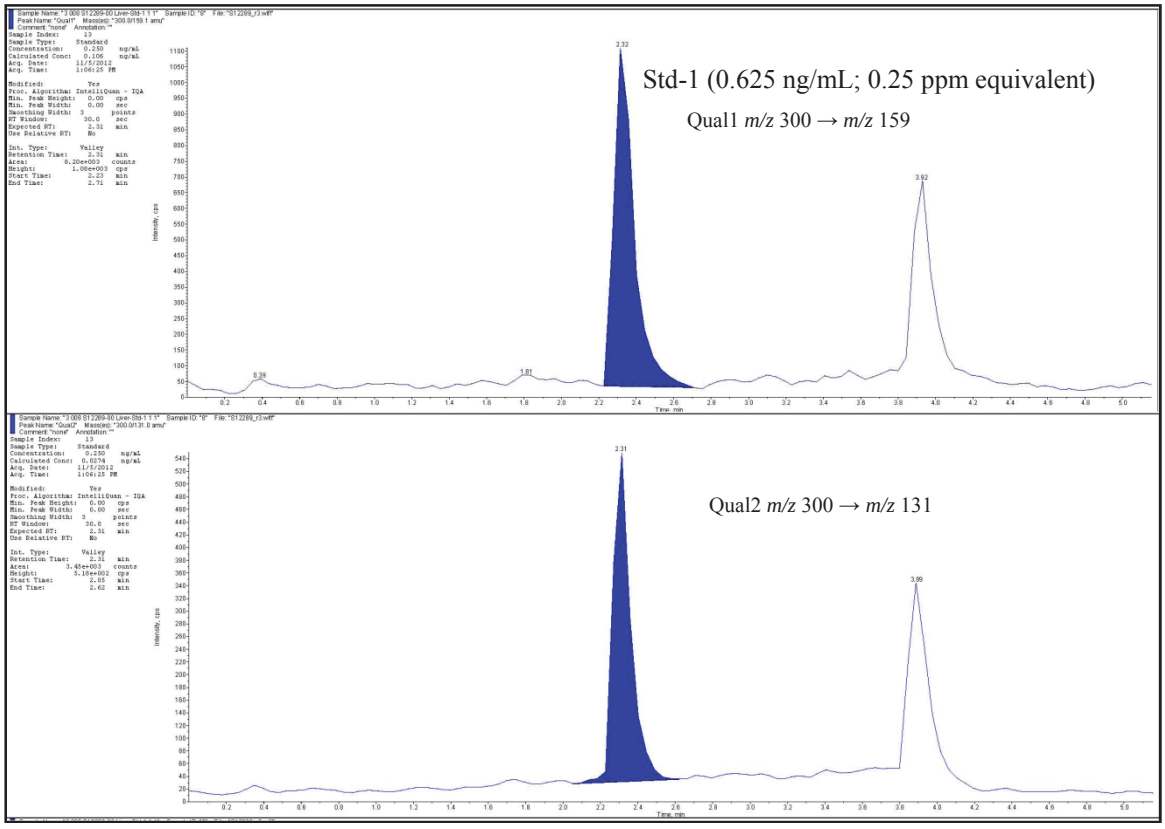
20 ALTERNATE HPLC CONDITIONS

The solutions prepared for method inter-batch and intra-batch accuracy and precision study were analyzed using an alternate C18 column. The accuracy and precision data obtained with the alternate column are in agreement with the data shown in Section 19, the validation data summary. The alternate column is the Acclaim 120 (C-18; 3um; 2.1X50 mm). All other LC conditions remain the same.

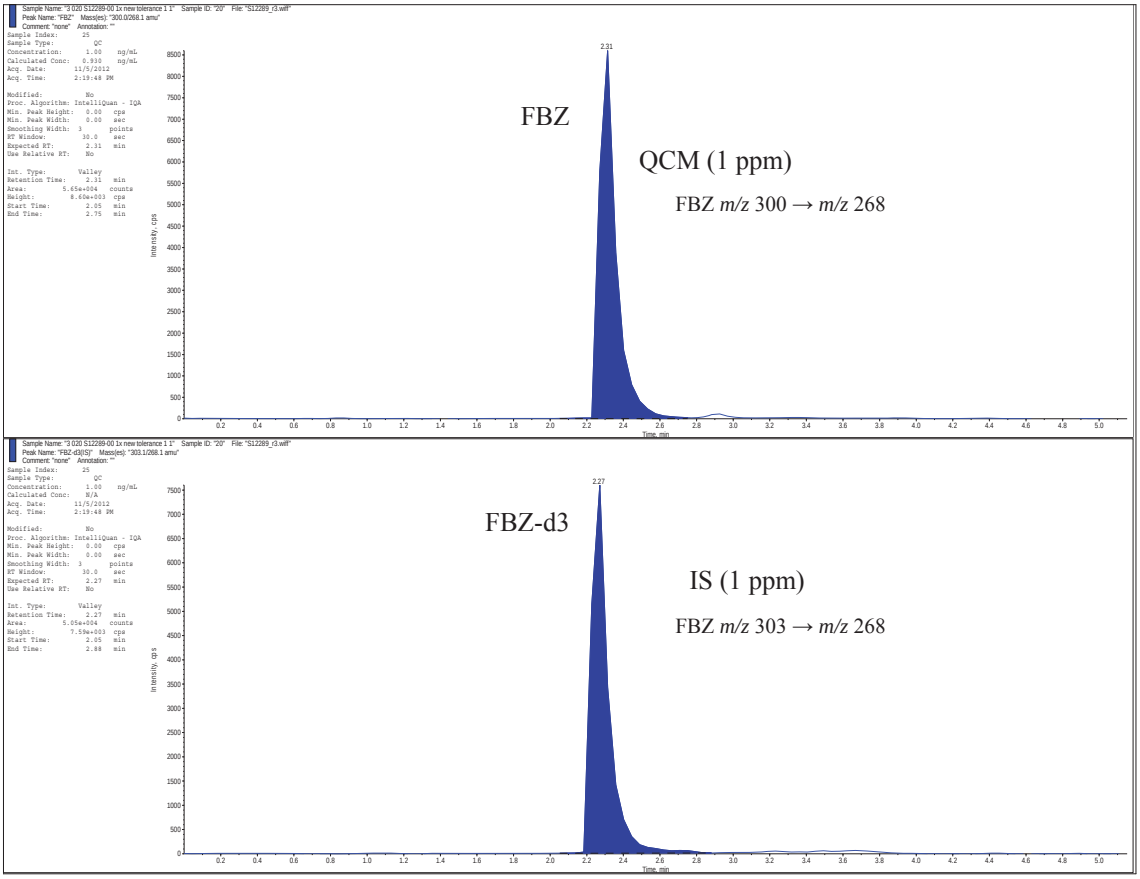
20.1 Typical Ion Chromatograms of FBZ Liver Standards (Alternate HPLC Column, Determinative Transitions)



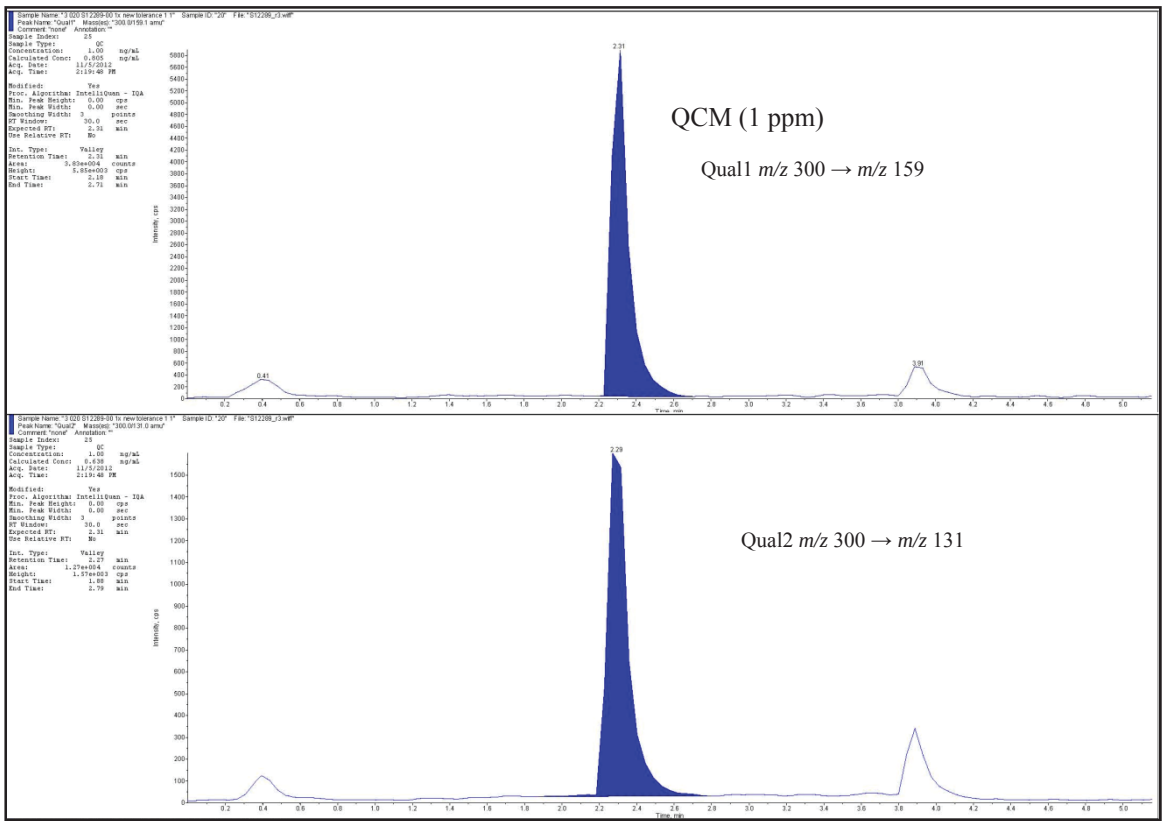
20.2 Typical Ion Chromatograms of FBZ Liver Standards (Alternate HPLC Column, Confirmatory Transitions)



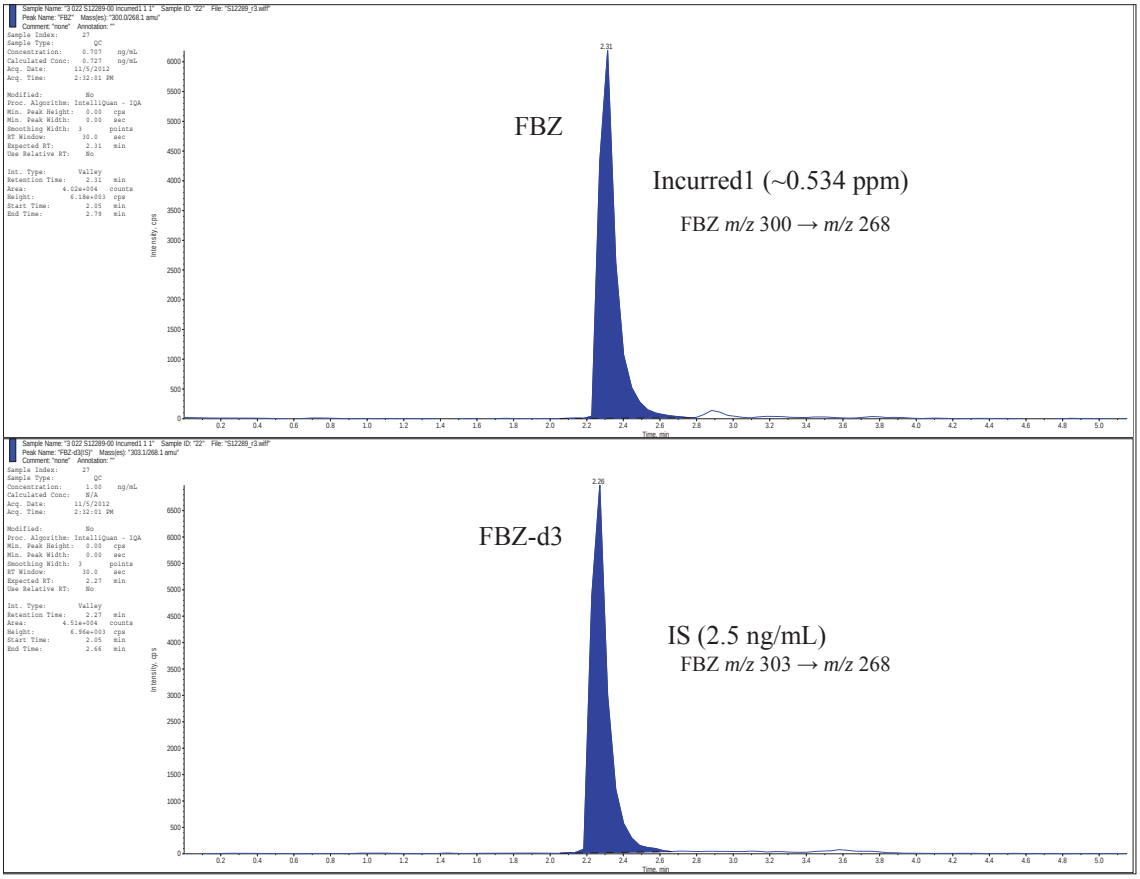
20.3 Typical Ion Chromatograms of Liver FBZ Quality Control Sample (Alternate HPLC Column, Determinative Transitions)



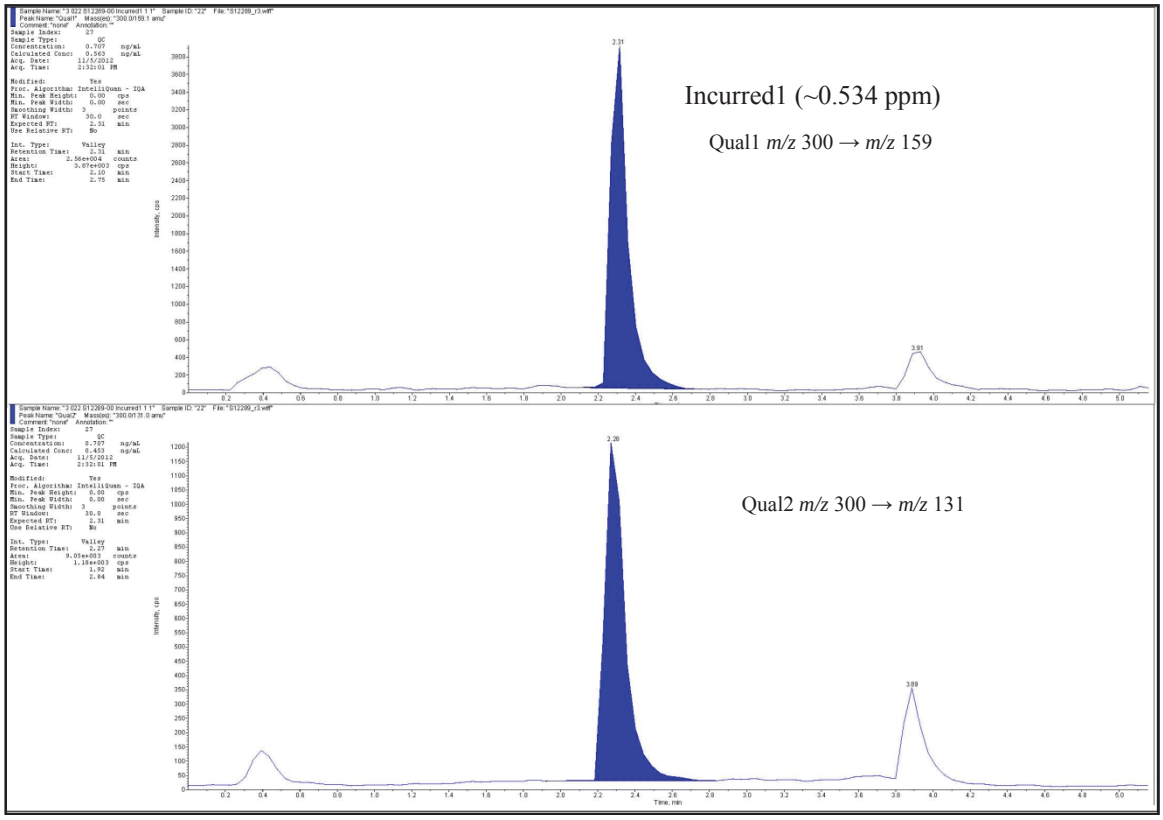
20.4 Typical Ion Chromatograms of Liver FBZ Quality Control Sample (Alternate HPLC Column, Confirmatory Transitions)



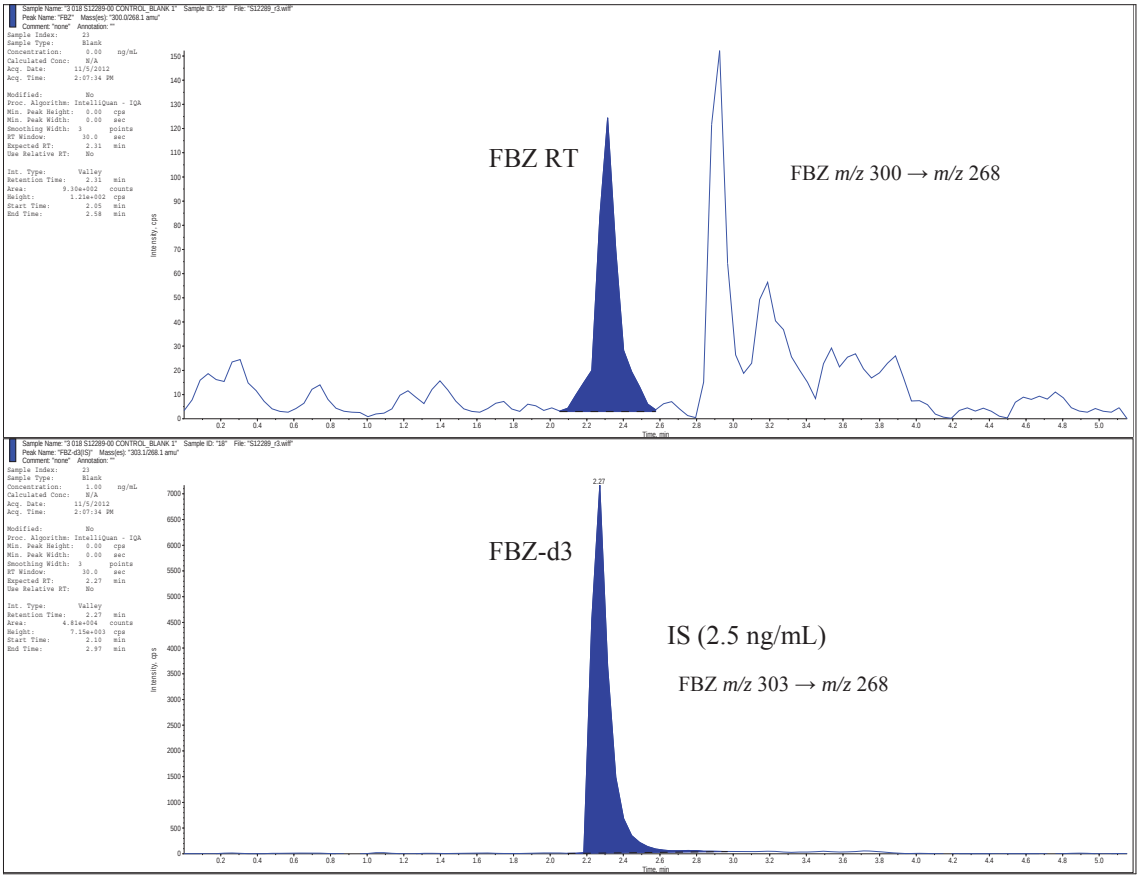
20.5 Typical Ion Chromatograms of Liver Incurred Sample (Alternate HPLC Column, Determinative Transitions)



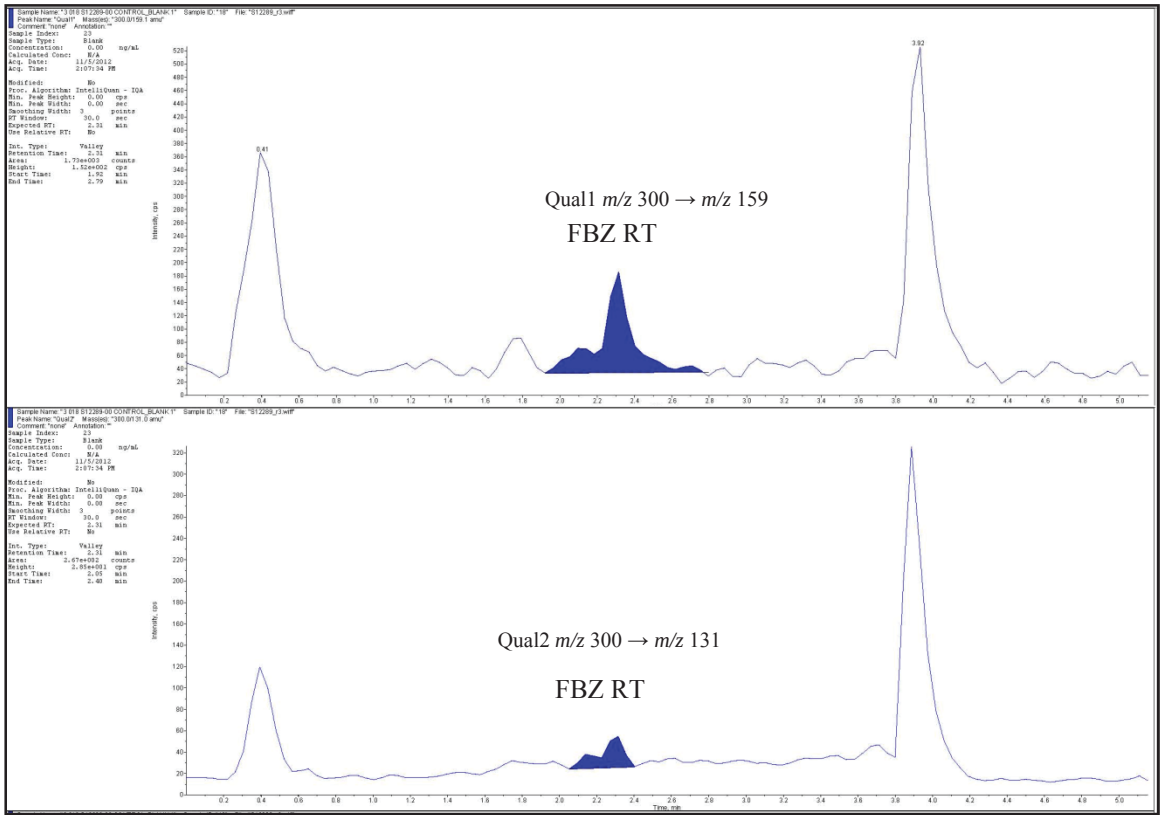
20.6 Typical Ion Chromatograms of Liver Incurred Sample (Alternate HPLC Column, Confirmatory Transitions)



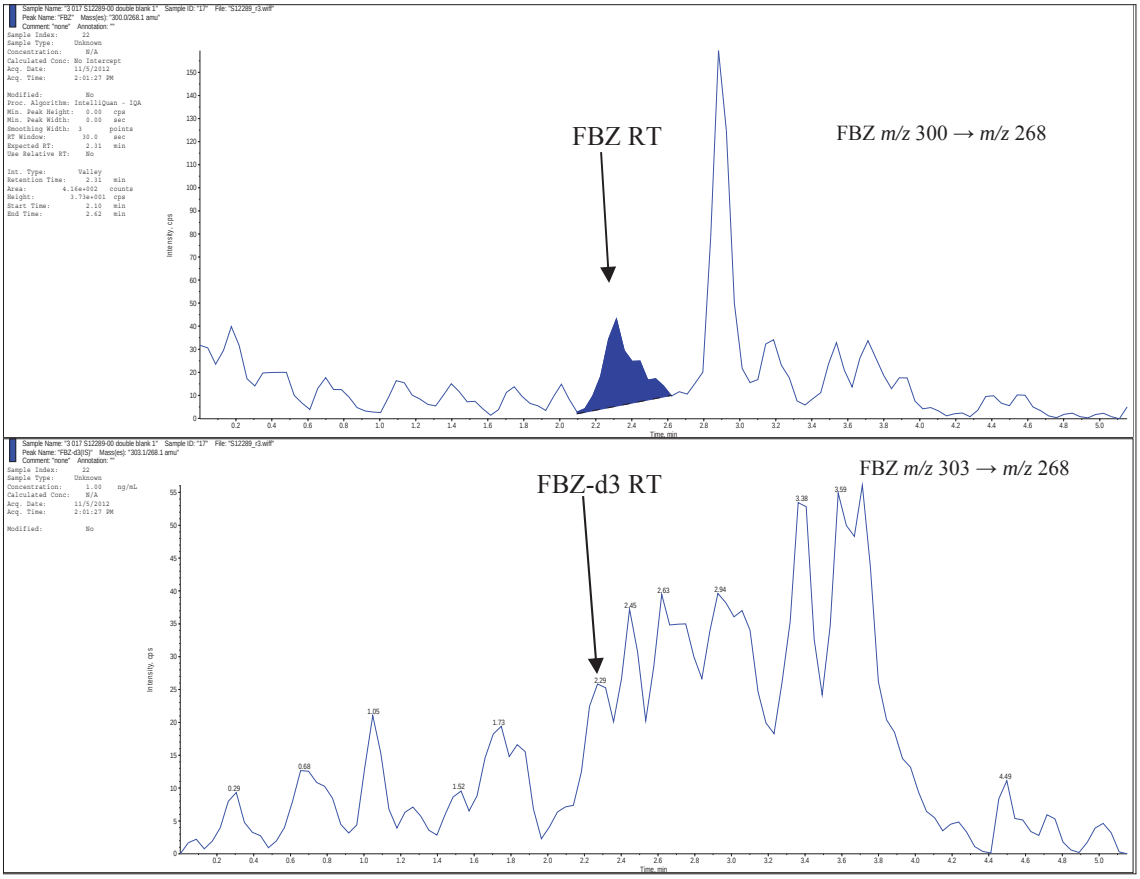
20.7 Typical Ion Chromatograms of Liver Control Sample (Alternate HPLC Column, Determinative Transitions)



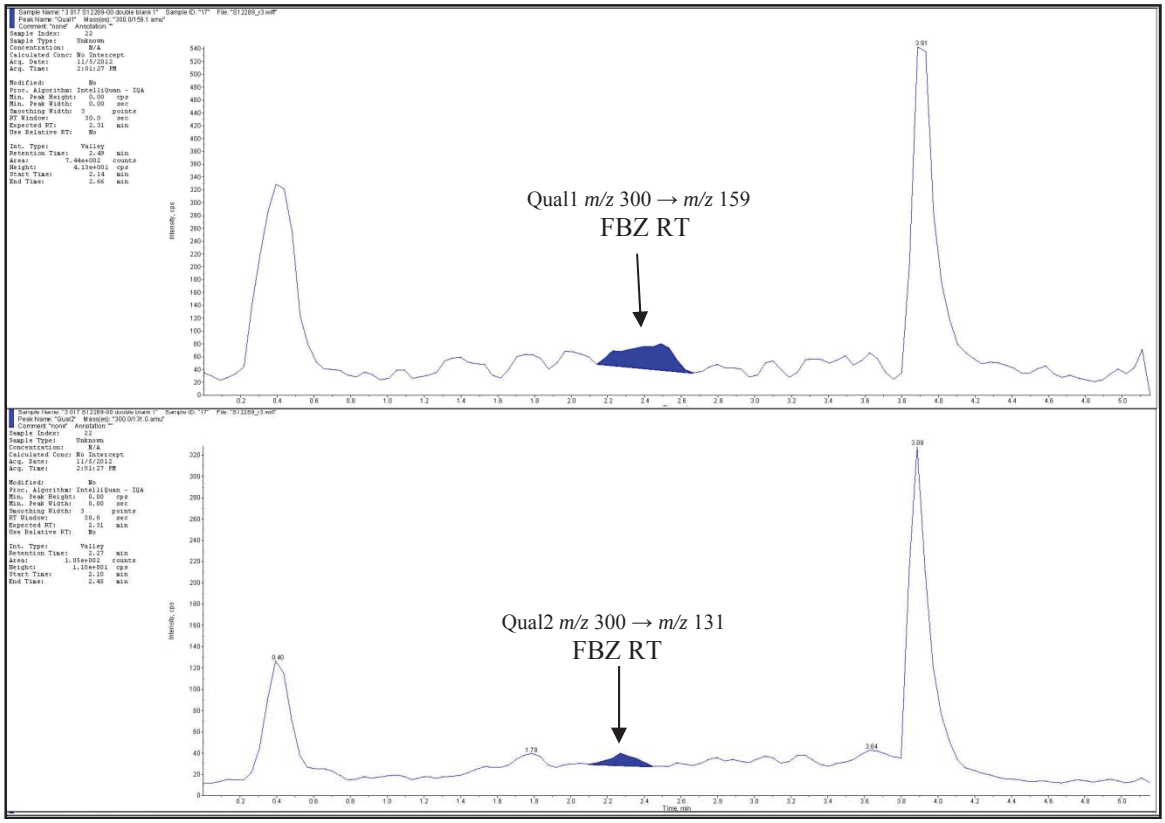
20.8 Typical Ion Chromatograms of Liver Control Sample (Alternate HPLC Column, Confirmatory Transitions)



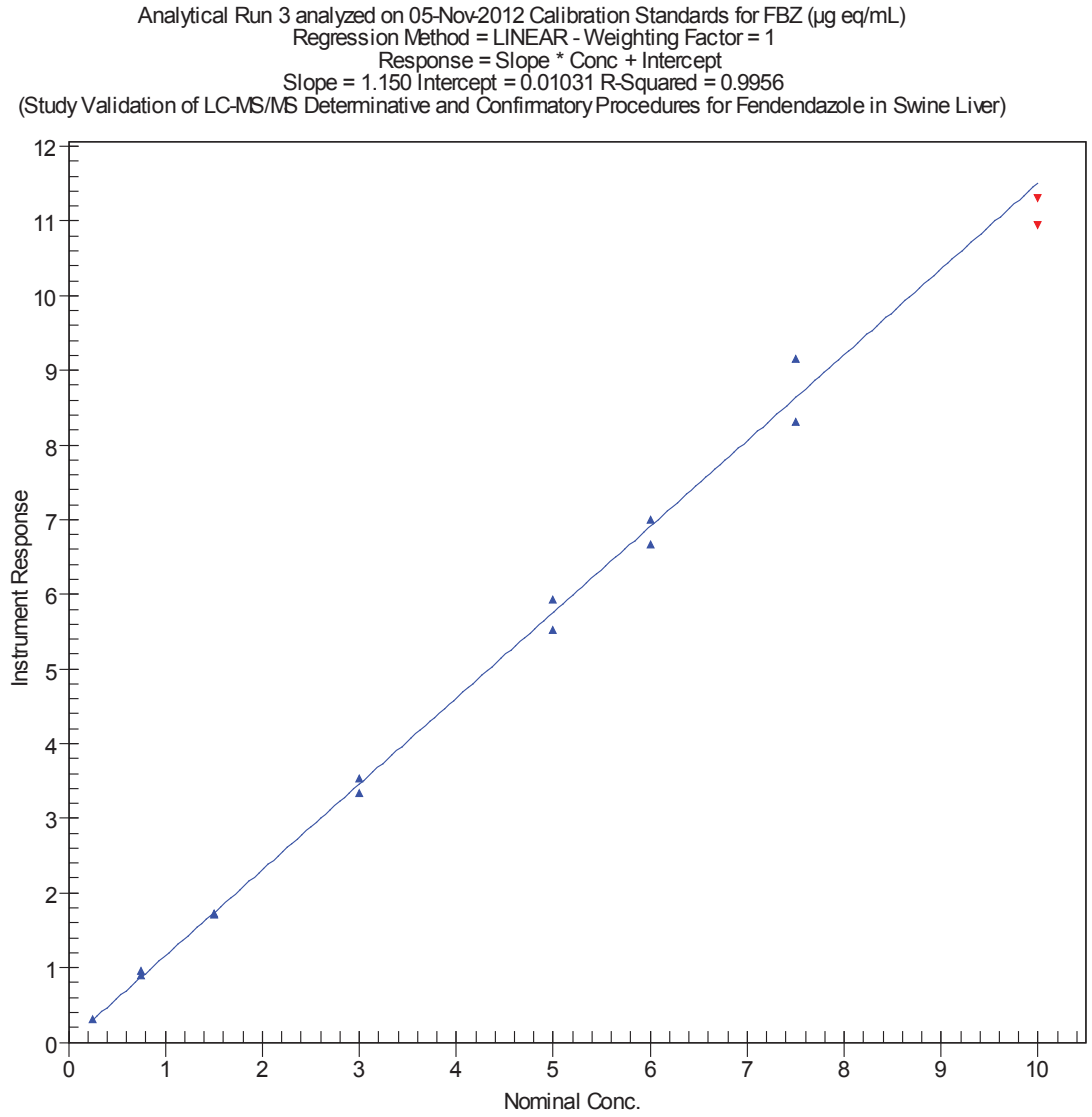
20.9 Typical Ion Chromatograms of Liver Double Blank Sample (Alternate HPLC Column, Determinative Transitions)



20.10 Typical Ion Chromatograms of Liver Double Blank Sample (Alternate HPLC Column, Confirmatory Transitions)



20.11 Typical Calibration Curve of FBZ Liver Standard (Alternate HPLC Column)



10 ppm equivalent standard level is excluded because the calibration curves generated with this level were not consistently linear over the course of validation

20.12 Validation Data Summary (Alternate Column) Determinative Method

Standard Curve Linearity (Liver)							
Std. Curve Range	0.25 to 7.5 ppm (nominal concentration 0.625 to 18.75 ng/mL)						
Curve (n=2)	Slope	Intercept	Correlation Coefficient				
	1.15	0.0103	0.9956				
Precision & Accuracy (Liver)							
	QC	Conc. (ppm)	%CV	Mean %Recovery	Incur Animal#	Conc. (ppm)	%CV
Intra-Batch (n = 6)	Low	0.500	3.1	100.2	mean assay conc. (n=6)		
	Medium	1.00	4.6	100.0	Incurred1	0.599	14.2
	High	2.00	2.1	97.5	Incurred2	1.70	4.4

20.13 Mass Spectra Matching for Alternate Column

	Relative Abundance Ratio Criteria						
				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	m/z 268 FBZ peak area (A)	m/z 159 FBZ-Qual1 peak area (B)	m/z 131 FBZ Qual2 peak area (C)	m/z 159 (B/A)*100	Meets Acceptance Criteria (within ±10%)? (Y or N)	m/z 131 (C/A)*100	Meets Acceptance Criteria (within ±10%)? (Y or N)
3 008 S12289-00 Liver-Std-1 1 1	1.26E+04	8.20E+03	3.45E+03	65.1		27.4	
3 009 S12289-00 Liver-Std-2 1 1	4.00E+04	2.92E+04	1.14E+04	73.0		28.5	
3 010 S12289-00 Liver-Std-3 1 1	7.47E+04	5.44E+04	2.03E+04	72.8		27.2	
3 011 S12289-00 Liver-Std-4 1 1	1.49E+05	1.12E+05	3.96E+04	75.2		26.6	
3 012 S12289-00 Liver-Std-5 1 1	2.70E+05	1.97E+05	6.56E+04	73.0		24.3	
3 013 S12289-00 Liver-Std-6 1 1	3.00E+05	2.17E+05	7.63E+04	72.3		25.4	
3 014 S12289-00 Liver-Std-7 1 1	4.13E+05	2.98E+05	9.40E+04	72.2		22.8	
3 015 S12289-00 Liver-Std-8 1 1	5.56E+05	3.85E+05	1.28E+05	69.2		23.0	
3 050 S12289-00 Liver-Std-1 2 1	1.13E+04	9.00E+03	3.37E+03	79.6		29.8	
3 051 S12289-00 Liver-Std-2 2 1	3.81E+04	2.85E+04	1.07E+04	74.8		28.1	
3 052 S12289-00 Liver-Std-3 2 1	6.53E+04	4.88E+04	1.93E+04	74.7		29.6	
3 053 S12289-00 Liver-Std-4 2 1	1.38E+05	1.01E+05	3.65E+04	73.2		26.4	
3 054 S12289-00 Liver-Std-5 2 1	2.23E+05	1.61E+05	6.02E+04	72.2		27.0	
3 055 S12289-00 Liver-Std-6 2 1	2.65E+05	1.99E+05	6.81E+04	75.1		25.7	
3 056 S12289-00 Liver-Std-7 2 1	3.33E+05	2.38E+05	8.90E+04	71.5		26.7	
3 057 S12289-00 Liver-Std-8 2 1	4.72E+05	3.36E+05	1.18E+05	71.2		25.0	
Average	**2.10E+05**	**1.51E+05**	**5.27E+04**	**72.8**		**26.5**	

20.13 Mass Spectra Matching for Alternate Column

	Relative Abundance Ratio Criteria						
				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	m/z 268 FBZ peak area (A)	m/z 159 FBZ-Qual1 peak area (B)	m/z 131 FBZ Qual2 peak area (C)	m/z 159 (B/A)*100	Meets Acceptance Criteria (within ±10%)? (Y or N)	m/z 131 (C/A)*100	Meets Acceptance Criteria (within ±10%)? (Y or N)
3 019 S12289-00 1/2x new tolerance 1 1	2.68E+04	1.93E+04	6.06E+03	72.0		22.6	
3 024 S12289-00 1/2x new tolerance 2 1	2.77E+04	1.80E+04	6.23E+03	65.0		22.5	
3 029 S12289-00 1/2x new tolerance 3 1	2.62E+04	1.73E+04	6.51E+03	66.0		24.8	
3 034 S12289-00 1/2x new tolerance 4 1	2.35E+04	1.58E+04	6.95E+03	67.2		29.6	
3 039 S12289-00 1/2x new tolerance 5 1	2.12E+04	1.57E+04	6.94E+03	74.1		32.7	
3 044 S12289-00 1/2x new tolerance 6 1	2.28E+04	1.53E+04	6.91E+03	67.1		30.3	
Average	**2.47E+04**	**1.69E+04**	**6.60E+03**	**68.6**	**Y**	**27.1**	**Y**
% CV	**10.39**	**9.30**	**5.95**	**5.27**		**16.1**	
3 020 S12289-00 1x new tolerance 1 1	5.65E+04	3.83E+04	1.27E+04	67.8		22.5	
3 025 S12289-00 1x new tolerance 2 1	5.20E+04	3.34E+04	1.19E+04	64.2		22.9	
3 030 S12289-00 1x new tolerance 3 1	5.29E+04	3.26E+04	1.29E+04	61.6		24.4	
3 035 S12289-00 1x new tolerance 4 1	4.33E+04	2.79E+04	1.25E+04	64.4		28.9	
3 040 S12289-00 1x new tolerance 5 1	4.16E+04	3.08E+04	1.32E+04	74.0		31.7	
3 045 S12289-00 1x new tolerance 6 1	4.26E+04	2.73E+04	1.25E+04	64.1		29.3	
Average	**4.82E+04**	**3.17E+04**	**1.26E+04**	**66.0**	**Y**	**26.6**	**Y**
% CV	**13.3**	**12.8**	**3.5**	**6.64**		**14.5**	

20.13 Mass Spectra Matching for Alternate Column

	Relative Abundance Ratio Criteria						
				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	m/z 268 FBZ peak area (A)	m/z 159 FBZ-Qual1 peak area (B)	m/z 131 FBZ Qual2 peak area (C)	m/z 159 (B/A)*100	Meets Acceptance Criteria (within ±10%)? (Y or N)	m/z 131 (C/A)*100	Meets Acceptance Criteria (within ±10%)? (Y or N)
3 021 S12289-00 2x new tolerance 1 1	1.06E+05	7.11E+04	2.29E+04	67.1		21.6	
3 026 S12289-00 2x new tolerance 2 1	9.63E+04	6.36E+04	2.43E+04	66.0		25.2	
3 031 S12289-00 2x new tolerance 3 1	9.45E+04	6.13E+04	2.60E+04	64.9		27.5	
3 036 S12289-00 2x new tolerance 4 1	8.64E+04	5.67E+04	2.61E+04	65.6		30.2	
3 041 S12289-00 2x new tolerance 5 1	9.06E+04	6.62E+04	2.81E+04	73.1		31.0	
3 046 S12289-00 2x new tolerance 6 1	8.31E+04	5.98E+04	2.49E+04	72.0		30.0	
Average	**9.28E+04**	**6.31E+04**	**2.54E+04**	**68.1**	**Y**	**27.6**	**Y**
% CV	**8.7**	**8.0**	**7.0**	**5.15**		**13.1**	
3 022 S12289-00 Incurred1 1 1	4.02E+04	2.56E+04	9.05E+03	63.7		22.5	
3 027 S12289-00 Incurred1 2 1	3.51E+04	2.14E+04	8.48E+03	61.0		24.2	
3 032 S12289-00 Incurred1 3 1	2.53E+04	1.55E+04	6.86E+03	61.3		27.1	
3 037 S12289-00 Incurred1 4 1	2.76E+04	1.68E+04	7.98E+03	60.9		28.9	
3 042 S12289-00 Incurred1 5 1	2.28E+04	1.68E+04	7.26E+03	73.7		31.8	
3 047 S12289-00 Incurred1 6 1	2.41E+04	1.75E+04	7.24E+03	72.6		30.0	
Average	**2.92E+04**	**1.89E+04**	**7.81E+03**	**65.5**	**Y**	**27.4**	**Y**
% CV	**23.8**	**20.2**	**10.8**	**9.18**		**13.0**	

20.13 Mass Spectra Matching for Alternate Column

	Relative Abundance Ratio Criteria						
				Peak Area Ratio Relative to m/z 268 (A)			
Sample Name	**m/z 268 FBZ peak area (A)**	**m/z 159 FBZ-Qual1 peak area (B)**	**m/z 131 FBZ Qual2 peak area (C)**	**m/z 159 (B/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**	**m/z 131 (C/A)*100**	**Meets Acceptance Criteria (within ±10%)? (Y or N)**
3 023 S12289-00 Incurred2 1 1	1.01E+05	6.79E+04	2.21E+04	67.2		24.2	
3 028 S12289-00 Incurred2 2 1	1.05E+05	6.89E+04	2.41E+04	65.6		23.0	
3 033 S12289-00 Incurred2 3 1	7.21E+04	4.92E+04	2.09E+04	68.2		29.0	
3 038 S12289-00 Incurred2 4 1	7.30E+04	5.18E+04	2.33E+04	71.0		31.9	
3 043 S12289-00 Incurred2 5 1	7.49E+04	5.22E+04	2.24E+04	69.7		29.9	
3 048 S12289-00 Incurred2 6 1	7.64E+04	5.66E+04	2.25E+04	74.1		29.5	
Average	**8.37E+04**	**5.78E+04**	**2.26E+04**	**69.3**	**Y**	**27.9**	**Y**
% CV	**18.0**	**14.9**	**4.8**	**4.32**		**12.6**	
3 018 S12289-00 CONTROL_BLANK 1	9.30E+02	1.73E+03	2.27E+02	186.0	**N**	24.4	**Y**

20.13 Mass Spectra Matching for Alternate Column

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
3 008 S12289-00 Liver-Std-1 1 1	2.31	2.31	Y	2.31	Y	75	Y	119	Y
3 009 S12289-00 Liver-Std-2 1 1	2.31	2.31	Y	2.31	Y	549	Y	418	Y
3 010 S12289-00 Liver-Std-3 1 1	2.31	2.31	Y	2.31	Y	567	Y	838	Y
3 011 S12289-00 Liver-Std-4 1 1	2.31	2.31	Y	2.31	Y	1970	Y	1240	Y
3 012 S12289-00 Liver-Std-5 1 1	2.31	2.31	Y	2.31	Y	2480	Y	2410	Y
3 013 S12289-00 Liver-Std-6 1 1	2.31	2.31	Y	2.31	Y	3600	Y	3240	Y
3 014 S12289-00 Liver-Std-7 1 1	2.31	2.31	Y	2.31	Y	8620	Y	5620	Y
3 015 S12289-00 Liver-Std-8 1 1	2.31	2.31	Y	2.31	Y	9130	Y	3980	Y
3 050 S12289-00 Liver-Std-1 2 1	2.27	2.27	Y	2.27	Y	111	Y	166	Y
3 051 S12289-00 Liver-Std-2 2 1	2.27	2.27	Y	2.27	Y	553	Y	494	Y
3 052 S12289-00 Liver-Std-3 2 1	2.27	2.27	Y	2.27	Y	750	Y	1170	Y
3 053 S12289-00 Liver-Std-4 2 1	2.27	2.27	Y	2.27	Y	2620	Y	1330	Y
3 054 S12289-00 Liver-Std-5 2 1	2.27	2.27	Y	2.27	Y	3470	Y	6910	Y
3 055 S12289-00 Liver-Std-6 2 1	2.27	2.27	Y	2.27	Y	3070	Y	7330	Y
3 056 S12289-00 Liver-Std-7 2 1	2.27	2.27	Y	2.27	Y	3620	Y	6950	Y
3 057 S12289-00 Liver-Std-8 2 1	2.27	2.27	Y	2.27	Y	4510	Y	4830	Y
Average	**2.29**	**2.29**	**Y**	**2.29**	**Y**				

20.13 Mass Spectra Matching for Alternate Column

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
3 019 S12289-00 1/2x new tolerance 1 1	2.31	2.31	Y	2.27	Y	469	Y	370	Y
3 024 S12289-00 1/2x new tolerance 2 1	2.31	2.31	Y	2.27	Y	230	Y	565	Y
3 029 S12289-00 1/2x new tolerance 3 1	2.31	2.31	Y	2.27	Y	398	Y	479	Y
3 034 S12289-00 1/2x new tolerance 4 1	2.31	2.31	Y	2.27	Y	262	Y	601	Y
3 039 S12289-00 1/2x new tolerance 5 1	2.31	2.31	Y	2.27	Y	281	Y	690	Y
3 044 S12289-00 1/2x new tolerance 6 1	2.27	2.27	Y	2.27	Y	342	Y	536	Y
Average	**2.30**	**2.30**	**Y**	**2.27**	**Y**				
3 020 S12289-00 1x new tolerance 1 1	2.31	2.31	Y	2.27	Y	531	Y	962	Y
3 025 S12289-00 1x new tolerance 2 1	2.31	2.31	Y	2.27	Y	966	Y	1090	Y
3 030 S12289-00 1x new tolerance 3 1	2.31	2.31	Y	2.27	Y	641	Y	1400	Y
3 035 S12289-00 1x new tolerance 4 1	2.31	2.31	Y	2.27	Y	418	Y	1060	Y
3 040 S12289-00 1x new tolerance 5 1	2.31	2.27	Y	2.27	Y	405	Y	1900	Y
3 045 S12289-00 1x new tolerance 6 1	2.31	2.27	Y	2.27	Y	416	Y	1380	Y
Average	**2.31**	**2.30**	**Y**	**2.27**	**Y**				
3 021 S12289-00 2x new tolerance 1 1	2.31	2.31	Y	2.27	Y	1510	Y	1440	Y
3 026 S12289-00 2x new tolerance 2 1	2.31	2.31	Y	2.27	Y	949	Y	4820	Y
3 031 S12289-00 2x new tolerance 3 1	2.31	2.31	Y	2.27	Y	1440	Y	2520	Y
3 036 S12289-00 2x new tolerance 4 1	2.31	2.31	Y	2.27	Y	1180	Y	1890	Y
3 041 S12289-00 2x new tolerance 5 1	2.27	2.27	Y	2.27	Y	1290	Y	3110	Y
3 046 S12289-00 2x new tolerance 6 1	2.27	2.27	Y	2.27	Y	1250	Y	2190	Y
Average	**2.30**	**2.30**	**Y**	**2.27**	**Y**				

20.13 Mass Spectra Matching for Alternate Column

	Relative Retention Time Criteria (≤5%)					S/N Criteria (≥50:1)			
	m/z 268 FBZ	m/z 159 Qual1		m/z 131 Qual2		m/z 159 Qual1		m/z 131 Qual2	
Sample Name	retention time[a]		Y/N	retention time[a]	Y/N	S/N	Y/N	S/N	Y/N
3 022 S12289-00 Incurred1 1 1	2.31	2.31	Y	2.27	Y	302	Y	751	Y
3 027 S12289-00 Incurred1 2 1	2.31	2.31	Y	2.27	Y	466	Y	1180	Y
3 032 S12289-00 Incurred1 3 1	2.31	2.31	Y	2.27	Y	148	Y	901	Y
3 037 S12289-00 Incurred1 4 1	2.31	2.31	Y	2.27	Y	278	Y	741	Y
3 042 S12289-00 Incurred1 5 1	2.27	2.27	Y	2.27	Y	368	Y	668	Y
3 047 S12289-00 Incurred1 6 1	2.27	2.27	Y	2.27	Y	363	Y	578	Y
Average	**2.30**	**2.30**	**Y**	**2.27**	**Y**				
3 023 S12289-00 Incurred2 1 1	2.31	2.31	Y	2.31	Y	1060	Y	1370	Y
3 028 S12289-00 Incurred2 2 1	2.31	2.31	Y	2.27	Y	1740	Y	1760	Y
3 033 S12289-00 Incurred2 3 1	2.31	2.31	Y	2.27	Y	262	Y	601	Y
3 038 S12289-00 Incurred2 4 1	2.31	2.27	Y	2.27	Y	821	Y	2360	Y
3 043 S12289-00 Incurred2 5 1	2.27	2.27	Y	2.27	Y	864	Y	2150	Y
3 048 S12289-00 Incurred2 6 1	2.27	2.27	Y	2.27	Y	771	Y	5350	Y
Average	**2.30**	**2.29**	**Y**	**2.28**	**Y**				
3 018 S12289-00 CONTROL_BLANK 1	2.31	2.31	Y	2.31	Y	18.8	N	24.7	N

[a]retention time in minutes

21 MATERIAL SAFETY DATA SHEET

21.1 Material Safety Data Sheet of FBZ

Intervet
Schering-Plough Animal Health

Societe INTERVET PRODUCTIONS S.A.
Rue de Lyons
27460 IGOVILLE France

SAFETY DATA SHEET

Schering-Plough urges each user or recipient of this SDS to read the entire data sheet to become aware of the hazards associated with this material.

SECTION 1. IDENTIFICATION OF SUBSTANCE AND CONTACT INFORMATION

SDS NAME:	**Fenbendazole**
SYNONYM(S):	20% Fenbendazole Suspension [Active Ingredient of] PANACUR AQUASOL [Active Ingredient of] SAFE-GUARD [Active Ingredient of]
MSDS NUMBER:	SP002081
EMERGENCY NUMBER(S):	Schering-Plough Security Control Center (908) 820-6921 (24 hours) EU Transportation Emergencies - Carechem24: +44 (0)208 762 8322 (24 hours/7 days/week)
INFORMATION:	+33 (0)2 32 98 92 70 (Societe INTERVET Productions S.A.)
SCHERING-PLOUGH SDS HELPLINE:	+1 (908) 473-3371 (Worldwide) Monday to Friday, 9am to 5pm (US Eastern Time)
SCHERING-PLOUGH SDS EMAIL:	spmsds@spcorp.com

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SECTION 2. HAZARDS IDENTIFICATION

EU CLASSIFICATION(S): R63, R50/53

EMERGENCY OVERVIEW

White to off-white
Powder
Odorless
May be irritating to eyes, skin or respiratory tract.
May cause developmental effects.
May cause effects to:
liver
gastrointestinal tract
immune system
blood
central nervous system
fetus
Very toxic to aquatic organisms.
May cause long-term adverse effects in the aquatic environment.

POTENTIAL HEALTH EFFECTS:

The active ingredient fenbendazole is a benzimidazole carbamate anthelmintic that is structurally related to mebendazole. Therapeutic use of mebendazole, a substance of the same chemical class as fenbendazole, has been reported to cause gastrointestinal disturbances (transient abdominal pain), diarrhea, headache, and dizziness. Frequent effects reported after treatment with high-doses of mebendazole have included allergic reactions (fever and skin reactions), raised liver enzyme values, alopecia, bone marrow depression, reduced leucocyte count and raised serum-transaminase values.

A number of oral subchronic and chronic animal studies have been conducted with fenbendazole and have demonstrated that the liver is the main target tissue. In addition, stomach, kidneys, blood, immune system, and central nervous system are also affected by treatment with fenbendazole. Devlopmental effects have been reported in rabbits following treatment with fenbendazole.

LISTED CARCINOGENS

No carcinogens or potential carcinogens listed by IARC or EU Directive 90/394 (Annex I) in this mixture.

SECTION 3. COMPOSITION AND INFORMATION ON INGREDIENTS

SUBSTANCE / PREPARATION NAME: (5-(Phenylthio)-1H-benzimidazol-2-yl) carbamic acid methyl ester

ALTERNATE CHEMICAL NAME: Methyl-5-phenylthio-2-benzimidazole-carbamate

CHEMICAL FAMILY: Anthelmintic

PRODUCT USE: Active pharmaceutical ingredient (API)

CHEMICAL FORMULA: C15H13N3O2S

MOLECULAR WEIGHT: 299.4

CHEMICAL COMPOSITION

INGREDIENT	CAS NUMBER	EU NUMBER	EU CLASSIFICATION	PERCENT
Fenbendazole	43210-67-9	256-145-7	Xn: R63; N: R50/53	100

See section 15 for EU hazard classification symbols and risk and safety phrases.

SECTION 4. FIRST AID MEASURES

INHALATION: Remove to fresh air. If any trouble breathing, get immediate medical attention. Administer artificial respiration if breathing has ceased. If irritation or symptoms occur or persist, consult a physician.

SKIN CONTACT: In case of skin contact, while wearing protective gloves, carefully remove any contaminated clothing, including shoes, and wash skin thoroughly with soap and water. If irritation or symptoms occur or persist, consult a physician.

EYE CONTACT: In case of eye contact, immediately rinse eyes thoroughly with plenty of water. If wearing contact lenses, remove only after initial rinse, and continue rinsing eyes for at least 15 minutes. If irritation occurs or persists, consult a physician.

INGESTION: Rinse mouth and drink a glass of water. Do not induce vomiting unless under the direction of a qualified medical professional or Poison Control Center. If symptoms persist, consult a physician.

SECTION 5. FIRE FIGHTING MEASURES

FLAMMABILITY DATA:

Flash Point: Not determined (liquids) or not applicable (solids).

OTHER EXPLOSION HAZARDS:
Under normal conditions of use, this material does not present a significant fire or explosion hazard. However, like most organic compounds, this material may present a dust deflagration hazard if sufficient quantities are suspended in air. This hazard may exist where sufficient quantities of finely divided material are (or may become) suspended in air during typical process operations. An assessment of each operation should be conducted and suitable deflagration prevention and protection techniques employed. The sensitivity of this material to ignition by electrostatic discharges has not been determined. In the absence of testing data, all conductive plant items and operations personnel handling this material should be suitably grounded.

SPECIAL FIRE FIGHTING PROCEDURES:
Wear full protective clothing and self-contained breathing apparatus (SCBA).

SUITABLE EXTINGUISHING MEDIA:
Carbon dioxide (CO_2), extinguishing powder or water spray.

See Section 9 for Physical and Chemical Properties.

SECTION 6. ACCIDENTAL RELEASE MEASURES

PERSONAL PRECAUTIONS:
Avoid generation of dust during clean-up. Wear appropriate personal protective equipment as specified in Section 8. Keep personnel away from the clean-up area.

SPILL RESPONSE / CLEANUP:
All spills should be handled according to site requirements and based on precautions cited in the MSDS. In the case of liquids, use proper absorbent materials. For laboratories and small-scale operations, incidental spills within a hood or enclosure should be cleaned by using a HEPA filtered vacuum or wet cleaning methods as appropriate. For large dry or liquid spills or those spills outside enclosure or hood, appropriate emergency response personnel should be notified. In manufacturing and large-scale operations, HEPA vacuuming prior to wet mopping or cleaning is required.

See Sections 9 and 10 for additional physical, chemical, and hazard information.

SECTION 7. HANDLING AND STORAGE

HANDLING:
Avoid dust generation. Keep containers adequately sealed during material transfer, transport, or when not in use.

Appropriate handling of this material is dependent on many factors, including physical form, duration and frequency of process or task, and effectiveness of engineering controls. Site-specific risk assessments should be conducted to determine the feasibility and the appropriateness of all exposure control measures. See Section 8 (Exposure Controls) for additional guidance.

STORAGE:
Store in a cool, dry, well ventilated area.

See Section 8 for exposure controls and additional safe handling information.

SECTION 8. EXPOSURE CONTROLS AND PERSONAL PROTECTION

S-P HEALTH HAZARD CATEGORY (HHC):
The Schering-Plough Health Hazard Category (HHC) for this material is HHC2. Materials in this category are considered moderate health hazards. Health Hazard Categories are intended to be a component of workplace risk assessment. Consult your site safety and industrial hygiene staff for guidance on handling and control strategies.

S-P OCCUPATIONAL EXPOSURE GUIDELINE (OEG):
Schering-Plough Corporation has established an Occupational Exposure Guideline (OEG) of 100 mcg/m^3 (8-hr. TWA) for fenbendazole.

EXPOSURE CONTROLS:
The health hazard risks of handling this material are dependent on many factors, including physical form, duration and frequency of process or task, and effectiveness of engineering controls. Site-specific risk assessments should be conducted to determine the feasibility and the appropriateness of all exposure control measures. Exposure controls for normal operating or routine procedures follow a tiered strategy. Engineering controls are the preferred means of long-term or permanent exposure control. If engineering controls are not feasible, appropriate use of personal protective equipment (PPE) may be considered as alternative control measures. Exposure controls for non-routine operations must be evaluated and addressed as part of the site-specific risk assessment.

RECOMMENDED PERSONAL PROTECTIVE EQUIPMENT (PPE):

Respiratory Protection:	Respiratory protective equipment (RPE) may be required for certain laboratory and large-scale manufacturing tasks if potential airborne breathing zone concentrations of substances exceed the relevant exposure limit(s). Workplace risk assessment should be completed before specifying and implementing RPE usage. Potential exposure points and pathways, task duration and frequency, potential employee contact with the substance, and the ability of the substance to be rendered airborne during specific tasks should be evaluated. Initial and ongoing strategies of quantitative exposure measurement should be obtained as required by the workplace risk assessment. All RPE must conform to local and regional specifications for efficacy and performance. Consult your site or corporate health and safety professional for additional guidance.
Skin Protection:	Gloves that provide an appropriate barrier to the skin are recommended if there is potential for contact with this material. Consult your site safety staff for guidance.
Eye Protection:	Safety glasses with side shields. Use of goggles or full face protection may be required based on hazard, potential for contact, or level of exposure. Consult your site safety staff for guidance.
Body Protection:	In small-scale or laboratory operations, lab coats or equivalent protection is required. Disposable Tyvek or other dust impermeable suit should be considered based on procedure or level of exposure. Use of additional PPE such as shoe coverings, gauntlets, hood, or head covering may be necessary. Consult your site safety staff for guidance. In large-scale or manufacturing operations, disposable Tyvek or other dust impermeable suit is recommended and based on level of exposure. Use of additional PPE such as shoe coverings, gauntlets, hood, or head covering may be necessary. Consult your site safety staff for guidance.

EXPOSURE LIMIT VALUES

No exposure limits are available for this material.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

FORM:	Powder
COLOR:	White to off-white
ODOR:	Odorless
MOLECULAR WEIGHT:	299.4
pH:	7-7.5
MELTING POINT / RANGE:	233 deg C
SOLUBILITY:	
Water:	Insoluble
DMSO:	Soluble

See Section 5 for flammability/explosivity information.

SECTION 10. STABILITY AND REACTIVITY

STABILITY/ REACTIVITY:
Stable under normal conditions.

CONDITIONS AND MATERIALS TO AVOID:
None known.

HAZARDOUS DECOMPOSITION PRODUCTS / REACTIONS:
No dangerous decomposition is expected if used according to manufacturer's specifications.

SECTION 11. TOXICOLOGICAL INFORMATION

ACUTE TOXICITY DATA

SKIN:
Fenbendazole was not irritating to the skin of rabbits.

EYE:
Fenbendazole was not irritating to the eyes of rabbits.

ORAL:
Fenbendazole: Oral LD50: > 10 g/kg (rat)

REPEAT DOSE TOXICITY DATA

SUBCHRONIC / CHRONIC TOXICITY:
A number of oral subchronic and chronic animal studies have been conducted with fenbendazole and have demonstrated that the liver is the main target tissue. In addition, stomach, kidneys, blood, immune system, and central nervous system are also affected by treatment with fenbendazole.

Data in some animal species indicate that the ability of T and B lymphocytes to prcliferate in the secondary immune response may be suppressed during treatment with fenbendazole.

High oral dosages (500-3000 mg/kg/day) during 2-week dosing in rats caused reduced body weight gain, and severe renal and liver toxicity. Fenbendazole did not cause treatment-related effects when administered via stomach tube to immature rats at the rate of 0, 25, 250, and 2500 mg/kg b.w./day for 30 days. In a 90- day study, rats administered fenbendazole at 1600 to 2500 mg/kg /day showed tremors. No other treatment-related findings were reported.

Fenbendazole did not cause treatment-related effects in dogs administered oral dosages ranging from 50 to 250 mg/kg/day in a 6-day study, 20 to 125 mg/kg/day in a 90-day study, or 1 to 10 mg/kg/day in a 14-week study. At higher dosages, or in longer term studies, treatment-related effects were observed. Common effects observed in these additional studies include lymph follicle proliferation or nodules in the gastric mucosa. These effects were observed in dogs administered 250 mg/kg/day in a 30-day study, and in dogs given 8 to 20 mg/kg/day in one 6-month study and 20 to 125 mg/kg/day in another 6-month study. In addition to these effects, focal encephalomalacia, satellitosis, neuronophagia, perivascular inflammation or gliosis were observed in the cerebra of three dogs given 125 mg/kg/day for 6 months, and hyperplasia and congestion of the mesenteric lymph nodes were noted in dogs administered 8 to 20 mg/kg/day in the other 6-month study. [NOELS: 30-day Study: 25 mg/kg/day, 6-month Study (high-dose): none established, and 6-month Study (low-dose): 4 mg/kg/day]

REPRODUCTIVE / DEVELOPMENTAL TOXICITY:
Fenbendazole was found not to be teratogenic when tested in rats, dogs, or rabbits. Developmental effects (abortions, resorptions, and decreased fetal weights) were observed in the absence of maternal toxicity only in rabbits. When used in pigs, sheep, horses, and cattle, no relevant adverse effects on reproductive ability or offspring survival have been noted.

Fendbendazole was administered to rats at dietary dosages ranging from 5 to 135 mg/kg/day in a three-generation reproduction study. Reproductive and/or developmental effects observed in the 45 and 135 and 45 mg/kg/day dosage groups include reduced fertility indices, survival indices, pup weight, and pup growth, as well as diarrhea, yellow color, reduced activity, bloated stomach, and alopecia. These effects were more pronounced in the high-dose group. The NOEL for this study was 15 mg/kg/day for maternal and reproductive toxicity.

The potential embryotoxicity of fenbendazole was evaluated in pregnant rabbits, administered doses via stomach tube of 0, 10, 25, and 63 mg/kg/day on gestation days 7-19. Abortion or resorption of litters was observed in the 63 and 25 mg/kg/day dose groups. An increase in skeletal anomalies (13th rib) and delayed ossification of cranial bones also occurred in the high dose group. The NOEL for this study was 25 mg/kg/day.

Fenbendazole was administered to 2 groups of 12 female dogs at oral doses of 100 mg/kg/day, on gestation days 14-22 or 22-30. Developmental toxicity (stillborn pups and survival indices) were observed. About half the dogs in each group produced litters. No macroscopic abnormalities were observed in pups that died during the study.

MUTAGENICITY / GENOTOXICITY:
Fenbendazole was negative in a bacterial mutagenicity assay,a chromosomal aberration study, micronucleus, and DNA repair assay. It was weakly positive in the mouse lymphoma assay. Fenbendazole increased the mitotic index of HeLa cells in vitro, an effect that could be related to the ability of benzimidazoles to interfere with tubulin polymerization and thus inhibit spindle formation.

CARCINOGENICITY:
Fenbendazole was not carcinogenic in mice receiving 45 to 405 mg/kg fenbendazole in the diet for 2 years.

A two-year oral carcinogenicity study has been conducted in rats at dose levels of 0, 5, 15, 45, and 135 mg/kg/day. Treatment-related signs reported included diarrhea and red feces (45 mg/kg/day and 135 mg/kg/day) and reddish-brown urine (15, 45, and 135 mg/kg/day). Mortality was not statistically different from controls for any treatment group. Body weights and weight gains at study termination were significantly lower for the 45 and 135 mg/kg/day groups compared with controls. The alkaline phosphatase in all dose groups and SGOT in the high dose group were consistently elevated. Necropsy revealed enlargement or cyst formation in lymph nodes of rats in the two highest dose groups.liver mass and/or nodule formation, cyst formation in the liver of females, and testicular masses among males were reported at the 135 mg/kg/day dose-level.

Further treatment-related effects included sinus ectasia and hyperplasia of the mesenteric lymph nodes in all but the low dose group; Additionally, liver hypertrophy and hyperplasia, hepatocellular cytoplasmic vacuolation, bile duct proliferation, biliary cyst formation , and nodular hepatocellular hyperplasia were reported in female rats at the two highest dose levels. Testicular interstitial cell adenomas in the 135 mg/kg/day male rats were observed. The NOEL for this study was 5 mg/kg/day.

SECTION 12. ECOLOGICAL INFORMATION

ECOTOXICITY DATA

PRODUCT / CHEMICAL NAME	STUDY TYPE	RESULT
Fenbendazole	48-hr EC50 (Daphnia magna)	12 mcg/L
	96-hr LC50 (rainbow trout)	40 mcg/L
	96-hr LC50 (bluegill)	>19 mcg/L (21 days)

INGREDIENT ECOTOXICITY

Fenbendazole: 96-hr LC50 (trout): 40 mcg/L
48-hr LC50 (daphnia): 8.8-12 mcg/L
96-hr LC50 (zebra fish): >500 mg/kg
21-day LC50 (bluegill sunfish): >19 mcg/L
96-hr LC50 fish (Lepomis macrochirus): 1000 mg/L (highest concentration tested)
96-hr fish (Salmo gardneri): 7.5 mg/L (highest concentration tested)
Earthworm toxicity (LC50): 180 mg/kg (28 days)
Dung beetle toxicity (LD50): >770 mg/kg (7 days)

ENVIRONMENTAL DATA

PRODUCT / CHEMICAL NAME:	Fenbendazole
Partition Coefficient (log Pow) Results:	3.3
Aerobic Biodegradation(soil) Results:	DT50 between 4 and 12 days (for three types of soil)
Biodegradation Results:	Not readily biodegradable.
Environmental Data Comments:	Fenbendazole BCF: 240

SECTION 13. DISPOSAL CONSIDERATIONS

MATERIAL WASTE:
Disposal must be in accordance with applicable federal, state/provincial, and/or local regulations. Incineration is the preferred method of disposal, when appropriate. Operations that involve the crushing or shredding of waste materials or returned goods must be handled to meet the recommended exposure limit(s).

PACKAGING AND CONTAINERS:
Disposal must be in accordance with applicable federal, state/provincial, and/or local regulations.

SECTION 14. TRANSPORT INFORMATION

Refer to site-specific procedures and requirements for additional guidance.

IATA CLASSIFICATION:

Proper Shipping Name:	Environmentally hazardous substance, solid, n.o.s. (fenbendazole)
Hazard Class:	9
UN Number:	UN 3077
Packing Group:	III

ADR CLASSIFICATION:

Proper Shipping Name:	Environmentally hazardous substance, solid, n.o.s. (fenbendazole)
Hazard Class:	9
UN Number:	UN 3077
Packing Group:	III
Classification Code:	M7

IMDG CLASSIFICATION:

Proper Shipping Name:	Environmentally hazardous substance, solid, n.o.s. (fenbendazole)
Hazard Class:	9
UN Number:	UN 3077
Packing Group:	III

ADDITIONAL INFORMATION:
Shipment by ground under DOT is non-regulated, however, may be shipped per hazard classification above to facilitate multi-modal transport involving ICAO or IMO.

SECTION 15. REGULATORY INFORMATION

The following classification is based on available data and is in accordance with European Union criteria.

EUROPEAN UNION REGULATIONS:

Indication of Danger: N - Dangerous For The Environment
Xn - Harmful.

Risk Phrases:
R63 - Possible risk of harm to the unborn child.
R50/53 - Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety Phrases:
S46 - If swallowed, seek medical advice immediately and show this container or label.
S29 - Do not empty into drains.
S61 - Avoid release to the environment. Refer to special instructions/Safety data sheets.
S36/37 - Wear suitable protective clothing and gloves.

SECTION 16. OTHER INFORMATION

Although reasonable care has been taken in the preparation of this document, we extend no warranties and make no representations as to the accuracy or completeness of the information contained therein, and assume no responsibility regarding the suitability of this information for the user's intended purposes or for the consequence of its use. Each individual should make a determination as to the suitability of the information for their particular purpose(s).

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DEPARTMENT ISSUING MSDS:	Global Safety and Environmental Affairs Occupational and Environmental Toxicology Schering-Plough Corporation 556 Morris Avenue Summit, NJ 07901 USA
SCHERING-PLOUGH SDS HELPLINE:	+1 (908) 473-3371 (Worldwide) Monday to Friday, 9am to 5pm (US Eastern Time)
MSDS CREATION DATE:	03-Apr-2009
SECTIONS CHANGED (EU SUBFORMAT):	New SDS
SIGNIFICANT CHANGES (EU SUBFORMAT):	New regional format

21.2 Material Safety Data Sheet of FBZ-d_3

Trade name: Fenbendazole-D3 Page 1 of 3
Date of issue: 01.04.2008 **Revision Date:** 01.04.2008 **Date of printing:** 02.06.2008

Material Safety Data Sheet (according to EC Directive 2001/58/EEC)

1. Identification of the substance/preparation and of the company/untertaking

Trade name: **Fenbendazole-D3**

Use of the substance:	Reference substance for analytics
Date of issue:	01.04.2008
Revision date:	*01.04.2008*
Company:	Witega Laboratorien Berlin-Adlershof GmbH Magnusstraße 11 12489 Berlin
Phone:	+493063922001
Fax:	+493063922007
E-Mail/Internet:	witega@witega.de www.witega.de

2. Composition/information on ingredients

The described substance is pure: Yes

Chemical characterization:
Chemical name: (5-Phenylsulfanyl-1H-benzoimidazol-2yl)-carbamic acid methyl-D3 ester
Molecular Formula: C15H10D3N3O2S
Molecular Weight: 302.35 g/mole

3. Hazards identification

Critical hazards to man and environment:
Warning – substance not yet fully tested.

4. First-aid measures

After inhalation:
Move affected person into fresh air, keep warm and allow to rest.
After skin contact:
In case of contact with skin wash off immediately with plenty of water.
After eye contact:
In case of contact with eyes rinse thoroughly with water.
After swallowing:
Rinse out mouth and then drink plenty of water.
Notes for the doctor:
deuterated form of the anti-worm-drug Fenbendazole

5. Fire-fighting measures

Suitable extinguishing media:
Water. Foam. Dry powder. Carbon dioxide.
Special exposure hazards arising from the substance or preparation itself, combustion products, resulting gases, ...:
Substances potentially set free in case of fire: Nitrogen oxides (NOx). Carbon monoxide (CO). Carbon dioxide (CO2).
Special protective equipment for firefighters:
Use breathing apparatus with independent air supply (isolated).
Further information:
Co-ordinate fire-fighting measures to the fire surroundings.

Witega Laboratorien Berlin-Adlershof GmbH

6. Accidental release measures

Personal precautions:
Avoid dust formation. Use breathing apparatus if exposed to vapours/dust/aerosol.
Environmental precautions:
Do not allow to enter drains/surface waters/groundwater.
Methods for cleaning up:
Treat recovered material as prescribed in: Disposal considerations.

7. Handling and storage

Handling

Precautions for safe handling:
If handled uncovered, arrangements with local exhaust ventilation should be used if possible.
Measures to prevent fire/explosion::
No special measures necessary.

Storage

Design for storage rooms and vessels:
Brown glass.
Further advice on conditions of storage:
Storage temperature: 2-8°C
Storage class: 11

8. Exposure controls/personal protection

Ingredients with exposure limit values

Further information:
No national exposure limits have been set to date.

Personal protective equipment

Respiratory protection: Dust mask.
Hand protection: Disposable gloves.
Eye protection: Eye glasses with side protection.
Body protection: Laboratory smock.
Protection and hygiene measures:
See the national regulations for handling dangerous goods.

9. Physical and chemical properties

Form: Powder.
Colour: Colourless
Odour: Odourless

	Value/Range:		Unit:	at/for	Method
Melting point/melting range:	224	to 230	°C		*Leica Galen III*

10. Stability and reactivity

Materials to avoid:
Oxidizing agent, strong.
Hazardous decomposition products:
Stable up to melting point.
Further information:
No hazardous decomposition products if stored and handled as prescribed. No decomposition if used as prescribed.

11. Toxicological information

Toxicological tests

Acute toxicity
LD50: oral: Rat.
10000mg/kg
LD50: oral: Mouse.
10000mg/kg
Data relate to the unlabelled compound.
Carcinogenicity, mutagenicity and reproductive toxicity:
Experimental indication of toxicity of reproduction in animal studies.
Data relate to the unlabelled compound.

General comments

General comments:
No toxicological data are available.

12. Ecological information

Further ecological information

General information:
Ecological data are not available.

13. Disposal considerations

Product

Product Recommendation:
Send to a hazardous waste incinerator in compliance with official regulations.

Contaminated container

Recommendation:
Dispose of packages that cannot be cleaned.

14. Transport information

Further information

Further information:
No hazardous material as defined by the transport regulations (ADR/RID, IMDG-Code, ICAO-TI/IATA-DGR).

15. Regulatory information

National measures

Wassergefährdungsklasse: 2 **VwVwS:** self classification
Further information:
The product is classified and labelled in accordance with EC directives/GefStoffV:
"Warning - substance not yet fully tested".

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 OTHER VALIDATION DATA

22.1 Matrix Effect Data

At a 0.5 ppm fortification level, the mean matrix effects of the three replicates for the 6 individual lots of control chicken liver were from -12.4% to +16.3% for the analyte and -18.8% - +6.7% for the internal standard. Generally the nature (enhancement or suppression) and degree of matrix effects for the internal standard and analyte for each matrix lot corresponded well. The overall mean matrix effects at this level were +6.4% for the analyte and -0.6% for the internal standard. At the 2 ppm level, mean matrix effects in different lots of control swine liver ranged from -8.0% to +7.8% for the analyte and from -16.4% to +2.4% for the internal standard. The nature (enhancement or suppression) and degree of matrix effects for the internal standard and analyte for each matrix lot corresponded well. Overall mean matrix effects at this level were +1.4% for the analyte and -7.1% for the internal standard. Results from matrix effects analysis indicate no consistent or significant matrix effects on either the analyte or the internal standard.

22.2 Validation Experiments Conducted

The following experiments were conducted during method validation:

- Exhaustive extraction test
- System suitability test
- Limit of detection (LOD)
- Linearity (calibration curve) and range
- Precision and Accuracy (Core Runs)
- Lower limit of quantitation (LOQ) in Spiked Matrix
- Specificity / Selectivity
- Matrix effect & Interference compounds
- Extraction recovery
- Ruggedness/Robustness Testing
- Using alternate analytical column (Thermo Acclaim 120, C-18, 3 µm, 2.1 x 50 mm)
- Using alternate mobile phase (composition adjusted by 10% (Mobile Phase A 90:10 water, 0.09% formic acid and Mobile Phase B 90:10 Acetonitrile, 0.09% formic acid))
- Using alternate LC-MS/MS platform (Thermo Vantage LC-MS/MS system equipped with an API source, Thermo Accela pump, and Open Access autosampler)
- Stability Studies
- Confirmatory analysis

22.3 Previous Validation Studies

LC-MS/MS determinative and confirmatory procedures for fenbendazole in swine liver were validated as part of Intervet Study Number (SN) 08327. However, it was recognized that there were deficiencies and therefore additional validation was performed in Intervet SN S11097-00. For both previous studies, the tolerance was 6 ppm and fortification levels were 3, 6 and 12 ppm. The calibration curve range was 1.5 – 15 ppm. An identical

extraction procedure was used but the analytical column was a Waters µBondapak, 4.6 x 300 mm with isocratic elution and 1% acetic acid in water and in methanol for the mobile phases. For SN S11097-00, the Macmod Ace3 C18 column and mobile phase / gradient used in the current validation were tested as an alternate column and mobile phase / gradient as part of ruggedness testing.

23 METHOD CHANGE LOG

Version	Section	Change	Reason
Version 5 (effective 21-April 2015) to Version 6 (effective xx-January 2016)	2	Clarified that the presumptive tolerance is 3.2 ppm	Method updated to indicate a presumptive tolerance
	3	Method LOD/LOQ determined from method trial data added	Method updated to include method trial results
	8.1	Note to Analyst regarding homogenization added	Specified steps to take to insure sample homogeneity during processing
	19.1	Method of LOD calculation used in validation specified	Clarification
	19.3	Subsection titles updated	Clarification
	14	Method LOQ determined from method trial data added	Method updated to include method trial results
Version 4 (effective 30-Oct-2014 to Version 5 (effective 21-April 2015	7.4, 7.5. 9.1b	Hyperlink error to IS fortification solution corrected	Referenced section was moved between versions 3 and 4 and hyperlink was broken
	13.2.2	Updated confirmatory QC criteria.	Recommendation from Method Trial
	17.3	Added note for IS Monitoring and LC-MS/MS System Maintenance	Recommendation from Method Trial
	18.2 – 18.7, 18.12	Figures updated with method trial data	Proposed tolerance changed after validation completed
	19.1	LOD updated	Method of LOD calculation was changed
	19.3 – 19.4	Data tables added for method trial data	Proposed tolerance changed after validation completed
Version 3 (effective 14-Nov-2013 to	Section 2	Table 2-1 deleted, Table 2-2 re-numbered	Information in Table 2-1 duplicative

Version	Section	Change	Reason
Version 4 (effective 10-Oct-2014)	Section 7.1.5 (formerly Section 7.1.4)	Stock comparison procedure modified	Analyte and IS concentration changed due to change in proposed tolerance, more detail provided for preparation of stock comparison solutions
	Section 7.5	Moved to section 7.1.4	For clarity as solution used in this section required for stock comparison
	Sections 7.2 – 7.6	Standard and fortification levels modified	Analyte and IS concentration changed due to change in proposed tolerance.
	Section 9.1a	Added instructions for handling samples if they have been thawed	Clarification / additional information
	Section 9.2i	Added centrifuge step after combining extracts and adjusting volume to 20 mL	Step necessary to remove particulates prior to analysis
	Section 9.2 and 10	Additional detail about 2nd extraction added	Clarification
	Section 11.1	Added note about extending gradient if needed for re-equilibration between runs	Clarification
	Section 12.3	Added instructions for cases where all runs not integrated with same parameters	Clarification
	Section 13.1.1	Removed peak width at half height requirement for system suitability	Not necessary
	Section 13.2.3	Removed	Not needed
	Section 14	Clarified LOQ and ULOQ are highest and lowest points on calibration curve	Clarification
	Section 15	Modified dilution method	Method specified dilution procedure not acceptable
	Section 16.4	Added	Long term stability information added

Version	Section	Change	Reason
	Section 22	Changed to Additional Validation Data	More information from the validation added
	Section 23	Previously Section 22	Numbering changed due to insertion of new section 22.
Version 2 (effective 10-Jul-2013) to Version 3 (effective 14-Nov-2013)	various	Editorial changes made throughout.	Clarification.
	Table 2-1	Added method LOD	Missing from previous versions of method
	Section 7.1.4	Stock comparison description added.	Procedure for comparing stock required.
	Section 9.0	Added note to clarify that the extraction method applies to both the determinative and the confirmatory procedures.	Clarification.
	Section 9.1a	Clarifying that although freeze/thaw stability has been demonstrated, best practices for sample handling are to maintain samples in a frozen state at all times.	Clarification.
	Section 9.2a, b and d and 11.1	Added critical points.	To reflect ruggedness testing performed during validation.
	Section 11	Clarified in title that the LC-MS/MS analysis applies to both the determinative and confirmatory methods.	Clarification.
	Section 11.2	Added note to further explain that determinative and confirmatory transitions are monitored simultaneously.	Clarification.
	Section 11.2.2	Added MS parameters for Thermo Vantage MS detector	To reflect alternate MS detector tested during ruggedness testing.
	Sections 12.4 and 12.5	Added new sections with confirmatory criteria.	These criteria missing from original method.
	Section 13	Section divided into acceptability criteria for	Confirmatory criteria missing

Version	Section	Change	Reason
		determinative and confirmatory procedures.	from method.
	Subsections of Sections 18 and 20	Figures updated to include examples of confirmatory transitions.	Examples of confirmatory analysis missing from initial method version.
	Section 19.	Added confirmatory data for core runs.	Confirmatory data missing from initial method version.
	Section 20	Added confirmatory data for alternate column.	Confirmatory data missing from initial method version.
Version 1 (effective 17-Oct-2012) to Version 2 (effective 10-Jul-2013)	11.2.2	Requirement to include tune file with notebook removed.	Not necessary to include this in notebook.
	13.1	Increased acceptance criteria for S/N ratio from >10:1 to >50:1 for system suitability.	To more accurately reflect appropriate system suitability results.
	11.1, 11.2.1, Figure 20.2	Removed reference to divert valve.	Not used in current validation.
	Sections 9.0 11.3.3, 11.3.4 and 11.3.5	Changed maximum number of samples run in a set to 52 from approximately 30 or 32. Made column maintenance in 11.3.5 optional.	Changed to reflect maximum number of samples analyzed during validation. Column maintenance not necessary to maintain system.
	Table 6-1 and Sections 9.2b and 10	Added option of using Eberbach shaker.	Use of Eberbach shaker tested in validation.
	Section 9.2a and Section 10	Added option to extract with 2 x 10 mL of methanol rather than 2 x 8 mL.	This variation tested in validation.
	Table 7-5-1	Removed preparation of W-Mix-Std-8 and Liver-Std-8.	This level was not used during validation because of linearity issues with the solvent curve when this level was included.
	Tables 11.2.2-1, 2	Naming of qualifier ions and MS parameters updated.	Changes made to reflect naming and

Version	Section	Change	Reason
			parameters used during validation.
	Figure Sections 18.2 and 20.1	These figures removed and numbering of subsequent figures in these sections updated.	Not applicable to current validation.
	Figure Sections 18.3 – 18.8, 20.2 – 20.7, Table Sections 19 and 20.8	Updated with current validation data.	Updated with current validation data.
	Sections 8.3, 9.2f, 16.2 and 16.3.	Updated stability intervals.	Updated with current validation data.
	Table 6-3	Updated with alternate LC-MS/MS information and calculation software.	Updated with current validation data.
	Various	Removed wording from various sections regarding the use of the exact concentration of stock solutions	Stock preparation was changed in the previous method version.
	Various	Minor clarifications and grammatical corrections were made throughout.	Clarification.
XBL Method 11014 LIVER-D-M01 (effective 12-Jul-2012) to Version 1 (effective 17-Oct-2012)	Sections 7.2 – 7.6	The standard curve range was adjusted to cover the tolerance tested. The QC levels were likewise adjusted.	Validation was repeated using a 1 ppm tolerance in swine liver.
	Various	Minor grammatical and formatting errors and inconsistencies were corrected.	Clarification.
	Table 2-2	Changes in the interference compounds tested.	To better reflect the most commonly used swine veterinary drugs in the US.
	Section 6	Several minor changes were made to equipment and reagent suppliers.	To reflect the equipment and reagents used at the Intervet labs in Summit, NJ.