1. Purpose & Scope

The objective of this work instruction is to provide instruction on the routine multi-class, multi-residue LC-MS/MS screening of aquaculture samples using components of LIBs 4562 and 4614, and DEN-LAB validation package # 13-37, 15-12, 15-13, 15-15, 15-22, 15-32 & 16-16.

---

2. Procedure

2.1 Sample Preparation

2.1.1 Aquaculture sample matrices which are appropriate for analysis include but are not limited to: tilapia, eel, swai, pangasius, basa, pompano, red drum, bass, croaker, catfish, trout, salmon, sablefish, scallops, shrimp, and frog legs.

2.1.2 Preparation of homogeneous samples of fish depends on whether skin and/or bones are considered edible for the particular species and product. Skin is removed from species whose skin is considered inedible (*e.g.*, catfish), as are other inedible portions, such as heads, tails, scales, fins, viscera, and inedible bones.

2.1.3 Remove at least 50 g of edible tissue from each of 12 subs. Combine the 600 g tissue in robot coupe with dry ice to homogenize into a fine powder. Note: It is effective to pulse the dry ice block in the robot coupe prior to adding the tissue.

2.1.4 Shrimp sample preparation

2.1.4.1 Thoroughly remove any breading before analysis.

2.1.4.2 Prepare one composite by combining portions of all subsamples. If 12 subsamples are collected (3 lb. or less per unit), select at random approximately 100 grams of shrimp (chipped from block if frozen) from each subsample.

2.1.4.3 If 6 subsamples (>3 lb. units) were collected, select randomly two 100 g portions taken from opposite ends of the subsample for the composite.

2.1.4.4 Homogenize sample by grinding with dry ice. Loosely close bag to allow carbon dioxide to sublime.

2.1.5 Catfish/Basa and other Pangasius species

2.1.5.1 Homogenize muscle (i.e. no skin).

2.1.6 Transfer composite into whirl-pak bag, and identify using a <u>red colored</u> permanent marker. (Black markers are known to contain crystal violet, an analyte of interest).

2.1.7 Store composited samples at -20˚C until the time of analysis. Place composites in designated area so extracting analyst knows which samples to run in the next batch.

2.1.8 Note for Domestic Samples:

2.1.8.1 For domestic samples retain 225 g as the 702(b) portion from each of the 12 fish samples in the lot.

2.1.9 **DNA Sequencing**

2.1.9.1 When a matrix has not been validated by LIB 4562; the Denver laboratory has determined that a new matrix can be verified by accomplishing 2 additional procedures.

2.1.9.2 This procedure also requires the prepping analyst to perform an in-house sample split in FACTS.

2.1.10 **Microbiology**

2.1.10.1 The Microbiology department will perform DNA identification.

2.1.10.2 ~20g from each of the first four subsamples will be collected before compositing the sample for the screening procedure.

2.1.10.3 The raw tissue will be placed into 4 separate 50mL Falcon tubes. Note: Each sub sample must be prepared aseptically.

2.1.10.4 Each tube will be identified with the sample number, sub number, initials, and the date of preparation.

2.1.10.5 The prepping analyst will then notify the Aquaculture supervisor to inform Microbiology that a sample is ready for DNA identification.

2.1.11 **Chemistry**

2.1.11.1 The analyst performing the screening analysis will perform an analysis on this sample in triplicate.

2.1.11.2 Sub 1 will be treated as a negative control.

2.1.11.3 Subs 2 & 3 will be treated as a spike and a duplicate. See § 2.2.2.5 for spiking and IS procedure.

2.2 Preparation of Standards (from neat material)

2.2.1 Standards are prepared as indicated in LIB 4562. See LIB 4562 for catalog # for individual standards. Alternatively, prepared solutions of mixed standards may be purchased from manufacturers such as SPEX CertiPrep. See section 2.3 for preparation and use of these pre-mixed solutions.

2.2.1.1 Stock solutions are prepared at an approximate concentration of ~200 µg/mL for each residue.

2.2.1.2 This concentration should correspond to the active drug compound, so the amounts weighed are adjusted to take into account purity and any counter-ions that are present.

2.2.1.3 Stock standards expire 1 year from preparation date (sooner if neat material expires). Exceptions: Dye analytes (MG, CV, BG, LMG, LCV) which expire 3 months from preparation date. Stock standards are stored at -20˚C.

2.2.1.4 All stocks are prepared in methanol except OXO and LCV. OXO is prepared in DMSO. All Dyes are prepared in acetonitrile.

2.2.1.5 Vigorously shake and sonicate if needed to dissolve material.

2.2.1.6 CIP and NOR require additional heating in a 50˚C water bath and further sonication to dissolve.

2.2.1.7 OXO will be a solid when stored at -20˚C. Thaw and sonicate prior to use.

2.2.2 Intermediate standard mixes

2.2.2.1 Intermediate standards expire 6 months from preparation date (sooner if neat material expires). Exceptions: Dye analytes (MG, CV, BG, LMG, LCV) expire 3 months from preparation date. Intermediate standards are stored at -20˚C.

2.2.2.2 Utilizing the table below, determining the volume of the individual analytes to achieve the desired concentration. The volumes listed are approximate values. Exact volumes depend on concentration of stock standards.

2.2.2.3 Each class of analyte is combined into a single 10.0 mL volumetric and brought to volume with methanol.

2.2.2.4 Preparation of IMS (intermediate mixed standards)

Mix #	Analyte	Class	Level of Interest (ng/mL)	Approx. Volume of Inter Std (µg/mL) added to IMS (mL)	Conc. of analyte in IMS (ng/mL)	Final volume (mL) of IMS
IMS 1	CIP	Fluoroquinolone	5	0.250	5,000	10
	ENR	Fluoroquinolone	5	0.250	5,000	
	NOR	Fluoroquinolone	5	0.250	5,000	
	DIF	Fluoroquinolone	5	0.250	5,000	
	SAR	Fluoroquinolone	5	0.250	5,000	
	DAN	Fluoroquinolone	5	0.250	5,000	
	OXO	Quinolone	10	0.500	10,000	
	NAL	Quinolone	10	0.500	10,000	
	FLU	Quinolone	10	0.500	10,000	
IMS 2	SAA	Sulfonamide	10	0.500	10,000	10
	SDZ	Sulfonamide	10	0.500	10,000	
	SPD	Sulfonamide	10	0.500	10,000	
	STZ	Sulfonamide	10	0.500	10,000	
	SMR	Sulfonamide	10	0.500	10,000	
	SMP	Sulfonamide	10	0.500	10,000	
	SCP	Sulfonamide	10	0.500	10,000	
	SEP	Sulfonamide	10	0.500	10,000	
	SMX	Sulfonamide	10	0.500	10,000	
	SDM	Sulfonamide	10	0.500	10,000	
	SDX	Sulfonamide	10	0.500	10,000	
	SQX	Sulfonamide	10	0.500	10,000	
	SMN	Sulfonamide	10	0.500	10,000	
	TMP	Potentiator	10	0.500	10,000	
	MT	Hormone	0.8	0.040	800	
IMS 3	CV	Dyes	1	0.050	1,000	10
	MG	Dyes	1	0.050	1,000	
	BG	Dyes	1	0.050	1,000	
	LCV	Dyes	1	0.050	1,000	
	LMG	Dyes	1	0.050	1,000	
IMS 4	FFA	Amphenicol	1000	2.00	50,000	10
	CAP	Amphenicol	0.3	0.050	1,000	
	FF	Amphenicol	1	0.050	1,000	
	TAP	Amphenicol	5	0.250	5,000	
IMS 5	MBZ	Benzimidazole	5	0.250	5,000	10
	MBZ-nh2	Benzimidazole	5	0.250	5,000	
	MBZ-oh	Benzimidazole	5	0.250	5,000	
IMS 6	OTC	Tetracycline	2000	2.00	50,000	10
	TC	Tetracycline	2000	2.00	50,000	
	CTC	Tetracycline	2000	2.00	50,000	
IMS7	SMZ-$^{13}C_6$	IS for Sulfas	10	1.00	10,000	10
	CAP-d5	IS for Amp	1	1.00	1,000	
	CV-d6	IS for CV	1	0.05	1,000	
	MG-d5	IS for MG	1	0.05	1,000	
IMS7 (cont)	NOR-d5	IS for NOR	1	0.250	5,000	10
	LCV-d6	IS for LCV	1	0.05	1,000	
	LMG-d5	IS for LMG	1	0.05	1,000	

2.2.2.5 Preparation of spiking standard from IMS standards.

2.2.2.5.1 Spiking Standard-CCV: Aliquot 1.00 mL of each IMS1 through IMS6 into a 10.0 mL volumetric flask and bring to volume with methanol. Spiking standard concentrations: 500 ng/mL fluoroquinolones, 1,000 ng/mL quinolones, 1,000 ng/mL sulfonamides and TMP, 100 ng/mL triphenylmethane dyes, 80 ng/mL MT, 5000 ng/mL tetracyclines, 500 ng/mL benzimidazoles, 100 ng/ml chloramphenicol/florfenicol, 500 thiamphenicol, and 5000 ng/mL florfenicol amine. Repeat for ICV set.

2.2.2.5.2 Internal Standard: Aliquot 1.00 mL of IMS 7 into a 10.0 mL volumetric flask; add 1 mL of 10,000 ng/mL SMZc13 and bring to volume with methanol. Internal standard concentrations: 1,000 ng/mL SMZc13, 100 ng/mL Cap-d5, 100 ng/mL CV-d6, MG-d5 100 ng/mL, NOR-d5 500 ng/mL, LCV-d6 100 ng/mL, and LMG-d5 100 ng/mL.

2.2.2.6 Spiking and Internal standards expires 3 months from preparation date and are stored at -20˚C. Note: These should be removed from freezer only to remove an aliquot for sample preparation.

2.2.2.7 Matrix standard/Spikes; 1.0x level: Fortify 1.0x level matrix standard (CCV & ICV) and spike/duplicate by adding 0.040 mL of Spiking standard (§2.2.2.3.1) + 0.040 mL of internal standard spiking solution (§2.2.2.3.2) to 4.00g (±0.03) portion of control tissue (assuming IMS concentrations are as indicated in Table 2.2.2.2.

2.2.2.7.1 Example spiking calculation:

0.040 mL	**1,000 ng**	**= 10.0 ng**
4.00 g tissue	**mL**	**g**

Equivalent amount (ng/mL) in vial due to 2x concentration factor (4 g tissue to 2 mL final volume):

10.0 ng	**4.00 g**	**= 20.0 ng**
g	**2.00 mL**	**mL**

2.2.2.8 Solvent Standard for LC-MS/MS: Prepare a solvent standard at the 1x level of interest to assess instrument suitability by adding 0.040 mL of spiking standard and 0.040 mL of internal standard spiking solution to an autosampler vial. Add 1.92 mL of dissolution solution to give a final volume of 2 mL.

2.3 **Preparation of Standards using SPEX CertiPrep custom made mixes**

2.3.1 When ordering these custom mixes, you must provide a quote to SPEX CertiPrep. Certificates of analysis are provided in appendices D, E and F and include CAS numbers necessary for ordering information.

2.3.1.1 Dyes mix (10 µg/mL), cat # LC-FDACO-21, expiration date is indicated on certificate of analysis. Store at 2-8˚C.

2.3.1.2 Fluoroquinolones (50 µg/mL)/Quinolones (100 µg/mL each) mix, cat #LC-FDACO-15, expiration date is indicated on certificate of analysis. Store at 2-8˚C.

2.3.1.3 Sulfonamides (100 µg/mL) / trimethoprim (100 µg/mL) / methyl testosterone (8 µg/mL) mix. Cat # GO-FDACO-16, expiration date is indicated on certificate of analysis. Store at 2-8˚C.

2.3.2 Preparation of intermediate mixed standard (IMS) dilution to equivalent levels in IMS 1-7 in table 2.2.2.2.

2.3.3 IMPORTANT: Sonicate all SPEX ampules for 15 minutes prior to taking an aliquot for dilution.

2.3.3.1 Three individual intermediates are prepared by aliquotting 1mL of each of the three custom mixes and diluting each to 10.0 mL with methanol in a volumetric flask (separate intermediates). Diluted mixed standards are stored at -20˚C and expire 6 months from the preparation date. Wrap the dye mix with aluminum foil to protect from light.

2.3.4 Spiking standard prepared from diluted SPEX mixes:

2.3.4.1 1mL of each of the three IMS solutions prepared in 2.3.4.1 are combined in a 10.0 mL volumetric flask and diluted to volume with methanol. Mixed spiking standard is stored at at -20˚C and expires 3 months from the preparation date. Wrap the dye mix with aluminum foil to protect from light.

2.3.5 Prepare a second set of solutions from a different lot or a different ampule of the same lot to serve as ICV IMS and spiking solutions.

2.4 Reagents (equivalent reagents may be substituted)

2.4.1 Acetonitrile, LC-MS grade (Burdick and Jackson, Muskegon, MI)

2.4.2 Methanol, LC-MS grade (Burdick and Jackson, Muskegon, MI)

2.4.3 Dimethyl sulfoxide (DMSO) (Burdick and Jackson, Muskegon, MI)

2.4.4 Citric acid monohydrate, cat #M-9605 (Fisher Scientific, Fair Lawn, NJ).

2.4.5 Sodium phosphate dibasic anhydrous (Na_2HPO_4) cat #E5513 (Fisher Scientific, Fair Lawn, NJ).

2.4.6 p-toluenesulfonic acid monohydrate (p-TSA), ACS reagent ≥98.5%, cat # 402885-500G (Sigma Aldrich, St. Louis, MO)

2.4.7 N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD) >95%, Cat # T3134-5G (Sigma Aldrich, St. Louis, MO)

2.4.8 Ethylenediamine tetra acetic acid disodium salt dihydrate (EDTA) Cat # BP120 (Fisher Scientific, Fair Lawn, NJ)

2.4.9 Potassium hydroxide (KOH), >85% cat # P1767 (Sigma Aldrich, St. Louis, MO)

2.4.9.1.1 To prepare 100 mL of 1 M KOH: dissolve 5.6 g of KOH in 100 mL DI water.

2.4.9.2 Extraction Buffer (EDTA-McIlvaine buffer)

2.4.9.2.1 To prepare 200 mL of McIlvaine buffer: Weigh 2.6 g citric acid monohydrate, 2.18 g Na_2HPO_4, 7.4 g Na_2EDTA dihydrate, and 5.8 g NaCl into a 250-mL mixing cylinder. To this, add 150 mL DI water with a stir bar and heat with stirring to dissolve the crystals. Allow to cool, then adjust the pH to 4.5 with 1.0 M potassium hydroxide and bring volume to 200 with DI water.

2.4.9.3 Dissolution solution: acetonitrile-formic acid-water (10+0.4+89.6) by volume

2.4.9.3.1 To prepare 250mL of dissolution solution: In a 250-mL mixing cylinder, combine 25 mL acetonitrile, 1 mL formic acid and fill to volume (224 mL) water. Invert to mix. Expiration date is 1 year from preparation date. Store this at room temperature.

2.4.9.4 ~1 mg/mL TMPD solution

2.4.9.4.1 To prepare 10 mL of ~1 mg/mL TMPD: Weigh ~10mg TMPD. Add 10 mL of 20:80 acetonitrile: methanol (v/v). Vigorously shake or sonicate for 15 minutes to dissolve. Store at -20˚C. Expiration is 1 month from preparation date. Protect from light. If solution turns a purple color, discard and prepare a new solution.

2.4.9.5 1 M p-TSA

2.4.9.5.1 To prepare 100 mL of 1 M p-TSA: Dissolve 19 g of p-TSA in 100 mL water. Expiration date is 1 year from preparation date. Store this reagent at room temperature.

2.4.10 Formic Acid, LC-MS grade

2.4.11 Water, LC-MS grade

2.4.12 Sodium Chloride

2.4.13 0.1% formic acid in water (Mobile Phase A):

2.4.13.1 To prepare 1 L of 0.1% formic acid in water: 1 mL formic acid into final volume of 1 L of water.

2.5 **Consumables (equivalent consumables may be substituted)**

2.5.1 Centrifuge tubes, Falcon® Blue Max™, 50 mL tubes cat # 352098, (VWR, International, Denver CO)

2.5.2 Ceramic homogenizers, Cat. #5982-9313, (Agilent Technologies, Santa Clara, CA.)

2.5.3 Syringes, disposable plastic, latex free, 1 mL (Cat. #309602, Becton-Dickinson, Rutherford, NJ),

2.5.4 Syringe Filters, Acrodisc® 13 mm with PTFE Membrane, 0.2 um, male slip Luer outlet, (cat # 4542, Pall Life Sciences, Ann Arbor, MI).

2.5.5 Low-volume polypropylene vials (0.6 mL volume, #69400-124 National Scientific through VWR, Denver, CO) with pre-scored snap caps (#242775, Wheaton, Millville, NJ).

2.5.6 National Scientific HPLC snap caps, cat # 66030-608 (VWR, International, Denver CO)

2.5.7 LC-MS Column – Waters XSelect HSS T3, 3 x 100 mm, 2.5 µm **(**Cat#: 186006155, Waters Corporation Milford, Mass)

2.6 **Equipment (equivalent equipment may be substituted)**

2.6.1 Vortex Mixers – Vortex Genie 2 (P/N: G-560, Scientific Industries, Inc., Bohemia, NY) and Multi-Tube Vortexer (P/N: 02-215-450, Fisher Scientific, Houston, TX)

2.6.2 Centrifuge – Sorvall™ RC 6+ (Cat. No. 46910), with Fiberlite™ F13-14 x 50cy Fixed Angle Rotor (Thermo Fisher Scientific, Waltham, MA), capable of operating at 4000 rpm (2730 rcf) for 5 min with refrigeration to 5 ˚C.

2.6.3 Pipettors; adjustable volume: 10-100 µL, 20-200 µL, 0.5-5 mL, and 1-10 mL (Sartorius Corp., Bohemia, New York)

2.6.4 Nitrogen evaporator: N-Evap, set at 50˚C with Nitrogen flow of 10-15 psi (Organomation Associates, Inc, Berlin, MA)

2.6.5 Sonicator - 8892 Ultrasonic Cleaner (Cole-Parmer, Vernon Hills, IL)

2.6.6 Balance – PA3102, capable of weighing 0.1 g Pioneer™ (Ohaus Corp., PineBrook, NJ)

2.6.7 Microbalance – XP26, capable of weighing 0.001 mg, (Mettler Toledo, Columbus, OH)

2.6.8 Hot plate with magnetic stirrer (Cat. #97042-714, VWR INT., Inc.)

2.6.9 LC-MS/MS systems:

2.6.9.1 Agilent 6490/6495 triple quadrupole (with i-Funnel technology) mass spectrometer coupled to an Agilent 1200 series liquid chromatograph

and auto sampler was used for analysis. The ion source was electrospray ionization with Agilent Jet Stream Technology (AJS-ESI) utilized in the positive ion mode for all analytes. The data system used was Mass Hunter software version B06/B08.

2.6.9.2 AB Sciex QTRAP 5500: LEAP HTC PAL Injection System with a 3-drawer cooling stack and a valve self-washing system (LEAP Technologies, Carrboro, NC); 20 µL loop and 100 µL syringe installed. Liquid Chromatograph Tandem Mass Spectrometer - Agilent 1200 Series LC (Avondale, PA) interfaced to an AB SCIEX QTRAP® 5500 MS/MS System (Framingham, MA) with Turbo V Ion Source ElectroSpray Ionization, Positive and Negative Mode. Analyst 1.6.2 software operated both instruments.

2.7 **Extraction**

2.7.1 Weigh 4.00 g (± 0.03 g) frozen, ground tissue into a 50 mL centrifuge tube. Include one empty tube to serve as reagent blank.

2.7.2 Add 0.040 mL Internal Spiking solution to all samples, matrix standards, and controls.

2.7.3 Add 0.040 mL spiking standard to 1.0x matrix standard (CCV), spike, and duplicate spike.

2.7.4 Add 0.040 mL ICV spiking standard to 1.0x ICV tube.

2.7.5 Add 2.0 mL EDTA-McIlvaine buffer to all tubes and mix using a vortex mixer for 10 sec.

2.7.6 Add 10 mL acetonitrile, 0.100 mL *p*-TSA (swirl tube to mix), 0.100 mL TMPD (swirl tube to mix), 2 g NaCl and a ceramic homogenizer pellet to each sample.

2.7.7 Apply screw cap and mechanically shake tube vigorously for 5 min.

2.7.8 Centrifuge tube at 6000 rpm (7600 rcf) at 5 ˚C for 5 min.

2.7.9 Using a Pasteur or transfer pipette, transfer upper organic layer into a clean 50 mL centrifuge tube.

2.7.10 Add an additional 10 mL acetonitrile to the original tissue and buffer mix.

2.7.11 Mechanically shake tube for 5 min and centrifuge, as above.

2.7.12 Combine acetonitrile layers and evaporate the acetonitrile phase to dryness using a water bath heated to 50-55˚C with nitrogen purge.

2.7.13 Reconstitute the residue with 2.0 mL of the dissolution solution.

2.7.14 Vigorously mix using a vortex mixer for 30 sec and place in sonicator for 5 min.

2.7.15 Centrifuge tube at 10,000 rpm (12,600 rcf) at 5˚C for 5 min.

2.7.16 Filter at least a 0.5 mL portion through a PTFE syringe filter into an LC vial for MS analysis. NOTE: discard the first 0.2 mL (2-3 drops) of extract that come through the filter. Aberrant recoveries have been observed, especially for the dye analytes if the very first portion of filtrate is collected in the vial and analyzed.

2.7.17 Analyze via LC-MS/MS.

2.7.17.1 Establish LC-MS/MS system suitability by injecting a solvent standard sufficient number of times until instrument is equilibrated, demonstrated by observation of all analytes present in correct retention time windows and stable analyte response.

2.8 **LC-MS/MS parameters – see appendices B and C**

2.8.1 Modified LIB 4562 can be analyzed on the Agilent 6490 & 6495. The ABI5500 has not validated the additional analytes for modified LIB 4562; it is currently in progress.

3. Quality Control

3.1 Use current QC limits (% Recovery for spikes, CCV, ICV, and spike RPD) from QC database, found at C:\QCDB\QC System.mdb. This is located as a shortcut on the user's windows desktop.

3.1.1 Current QC limits (analysis→multiresidue fish; method→LIB 4562 1x; matrix→tilapia). When chart appears; use arrows to find data for LIB 4562 1x analytes.

3.1.2 The limits in the QC database for the spike, ICV and CCV recoveries are based on average recoveries ±2 SD warning limits. In some cases; 2 SD made ranges narrower than 90-110% for CCVs (some sulfonamide analytes), so those ranges were widened to 90-110% based on ORA-LAB-5.4.5. The RPD acceptance range is 2.51 x the average relative percent different of the spike and duplicate spike.

3.2 **Positive confirmation of identity (for presumptive positive samples, and QC samples (spikes/ICV)).**

3.2.1 Signal to noise must be >3:1

3.2.2 Retention time (RT) must match the comparison standard(s) within 5%.

3.2.3 Ion ratios must match the comparison standard(s) by an absolute value of 20% for all analytes.

4. Presumptive Positive samples

4.1 Any regulatory sample that screen presumptively positive at levels indicated in the table below is subsequently analyzed by an additional quantitative method.

4.1.1 Presumptive Positive level (additional quantitative analysis required)

Matrix Class of Analyte	Tilapia	Salmon	Catfish	Crab	Swai	Shrimp	Pompano	Lobster	Frog	Scallops	Trout	Eel
Sulfas	7.2 [SDZ 72.2%]		8.0 [SPD 80.4%]	8.0 [SDZ 82.0%]		5.4 [SDM 53.7%]						6.7 [SDZ 67.4%]
Trimethoprim	8.0 [TMP 92.3%]		8.0 [TMP 95.0%]	7.4 [TMP 73.6%]		7.8 [TMP 77.9%]						8.0 [TMP 87.2%]
Fluoroquinolones	4.0 [CIP 85.8%]		4.0 [CIP 84.8%]	4.0 [NOR 91.6%]		2.7 [DAN 54.0%]						2.6 [DAN 51.3%]
Quinolones	8.0 [OXO 110.9%]		7.6 [OXO 76.3%]	8.0 [OXO 97.6%]		8.0 [OXO 87.1%]						8.0 [FLU 86.9%]
Triphenylmethane Dyes	0.8 [92.0 LCV]		0.8 [LMG 92.6%]	0.8 [LCV 96.5%]		0.8 [CV 101.2%]						0.8 [MG 91.8%]
Methyl Testosterone	0.64 [MT 100.9%]		0.43 [MT 53.9%]	0.44 [MT 55.0%]		0.64 [MT 220.0%]						0.28 [MT 35.9%]
Chloramphenicol	0.24 [CAP 109.4%]		0.24 [CAP 108.7%]	0.24 [CAP 89.6%]		0.24 [CAP 103.6%]						0.24 [CAP 93.1%]
Florfenicol	0.8 [FF 118.3%]		0.8 [FF 117.2%]	0.8 [FF 121.2%]		0.8 [FF 95.4%]						0.8 [FF 101.5%]
Florfenicol Amine	40.0 [FFA 95.9%]		40.0 [FFA 85.6%]	40.0 [FFA 121.1%]		40.0 [FFA 95.8%]						40.0 [FFA 94.6%]
Thiamphenicol	4.0 [TAP 107.3%]		4.0 [TAP 114.8%]	4.0 [TAP 100.3%]		4.0 [TAP 105.9%]						3.5 [TAP 70.9%]
Tetracyclines	40.0 [OTC 106.5%]		40.0 [OTC 90.6%]	35.0 [TC 69.9%]		28.2 [TC 56.3%]						40.0 [CTC 94.9%]
Benzimidazoles	4.0 [MBZ-NH2 96.7%]		3.3 [MBZ 66.1%]	4.0 [MBZ-OH 112.6%]		4.0 [MBZ-NH2 95.0%]						3.1 [MBZ 61.3%]
	15-32a1		15-32c3	15-32d3		15-32f3						15-32L3
	ppb [% recovery]											

Presumptive positive levels were calculated by using validation data from QMiS 15-32; taking each class of compounds and analyzing their spike recovery data. This data will be updated when further validation packets are approved. The poorest performer in each class was used to calculate this level. Samples below the TTL but above the confidence level will be assumed as a presumptive positive and ran under full quantitation. This table is a comparison for all validated matrices under QMiS 15-32. Additional matrices, not validated at this time, are assumed as presumptive positive of the area response is ≥ 70% for all analytes except for triphenylmethane dyes which are ≥ 40% when compared to the reference 1x level tilapia standard (LIB 4562; pg. 8). See LIB 4562 and LIB 4614 for further guidance on acceptance criteria.

5. Quantitative Analysis

5.1 A previously analyzed presumptive positive may be analyzed using this extraction with a matrix matched multi-point extracted standard curve.

5.1.1 Range of standard curve will be dependent on estimated amount found in screening analysis.

5.1.2 A starting point for an extracted curve would be 0.25x, 0.5x, 1x, 2x, 4x. More or less than five extracted standards may be used if necessary; as long as linearity is not compromised.

5.1.3 If a residue is detected at a very high level, it may be appropriate to weigh a smaller amount of tissue to reduce the concentration factor, thus reducing the in-vial concentration of analyte to prevent saturation of the instrument detector.

5.1.4 Weighing out less tissue would be appropriate for the Sulfonamides/Norfloxacin/Triphenylmethane Dyes analytes which use the internal standard for quantification, which cannot be diluted in-vial due to internal standard interference corrections.

5.1.5 At higher concentrations (above 10x), it may be necessary to quantitate without the use of an internal standard. This practice typically results in variability in final concentrations due to the elimination of the inherent correction factor of the internal standard.

6. FACTS Data reporting

6.1 Be sure to set the sample to 'In Progress' in FACTS. Complete the Sample Transfer screen after obtaining sample from sample custodian.

6.2 The regulatory enforcement action would be considered at and above 5 ppb level for the sum of enrofloxacin and ciprofloxacin.

6.3 The regulatory enforcement action would be considered at and above 1 ppb level for the sum of malachite green and leucomalachite green.

6.4 The regulatory enforcement action would be considered at and above 1 ppb level for the sum of crystal violet and leucocrystal violet.

6.5 It is the responsibility of the owner of the sample to input the data into FACTS.

6.6 FACTS ANT codes

6.6.1.1 L150: Sulfonamides Group (LIB 4562)

6.6.1.2 N215: Quinolones Group (LIB 4562)

6.6.1.3 N216: Fluoroquinolones Group

6.6.1.4 P260: Triphenylmethane Dyes Group (LIB 4562)

6.6.1.5 L147: Trimethoprim

6.6.1.6 G109: Methyl testosterone

6.6.1.7 M140: Tetracycline Group

6.6.1.8 D150: Amphenicol Group

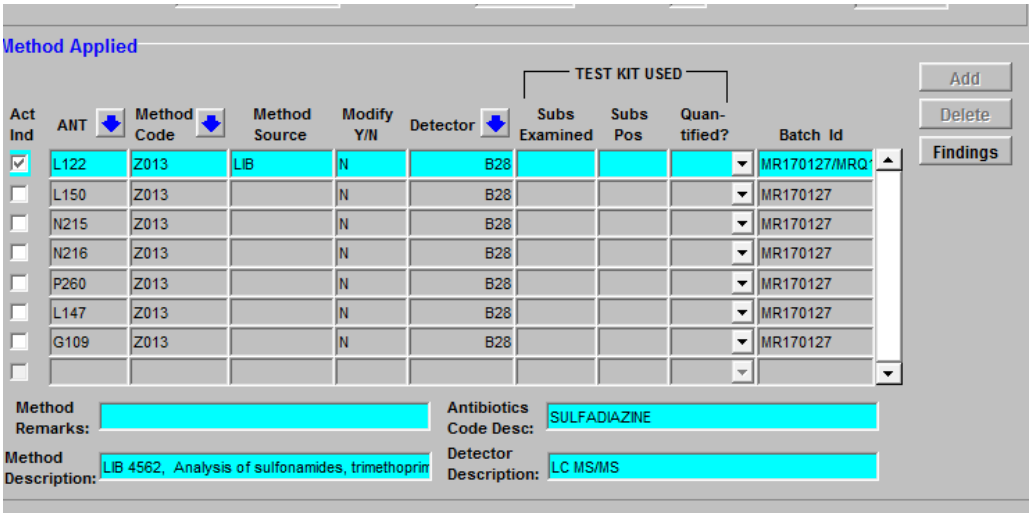
6.6.1.9 B020: Benzimidazole Group

6.7 Method code=Z013 “LIB 4562, Analysis of sulfonamides, trimethoprim, fluoroquinolones, quinolones, triphenylmethane dyes (and their leuco metabolites) and methyl testosterone in fish and shrimp using liquid chromatography mass spectrometry.”

6.8 Detector Code: B28 (LC MS/MS)

6.9 Batch ID: type in the batch name in the format “MRYYMMDD” (MR is the prefix for a multi-residue screening batch. MRQ is the prefix for a quantitative multi-residue additional analysis batch.

6.10 If one analyte within a group code is violative (Class 2 or 3), add a line with the individual code for that analyte (in the example below, L122 (sulfadiazine) was added).



6.11 For all samples; comments must be added on the “Findings” screen, you will have to enter the additional analytes introduced in the modification of LIB 4562.

6.11.1 L150 group: “Sulfamonomethoxine also not detected by modified LIB 4562”.

6.11.2 N216 group: “Difloxacin, sarafloxacin, and danofloxacin also not detected by modified LIB 4562”.

6.11.3 M140 group: “Tetracycline, oxytetracycline, and chlortetracycline not detected by modified LIB 4562”.

6.11.4 D150 group: “Chloramphenicol, thiamphenicol, florfenicol, and florfenicol amine not detected by modified LIB 4562”.

6.11.5 B020 group: “Mebendazole, mebendazole amine, and hydroxymebendazole not detected by modified LIB 4562”.

6.12 For nonviolative analytes (Class 1), on the “findings” screen, enter “O” for original analysis and check the box “Not Det” for each class of analytes.

6.13 For the violative analytes (Class 2 and 3), on the "Findings" screen, you will have to enter the semi-quantitative screening value (is a limitation of FACTS) for the Original analysis. Enter the QC data for the reagent blank, and the spike and duplicate. Enter the quantitative value from the matrix matched additional analysis, along with the QC data for the reagent blank, spike and duplicate for the additional analysis batch. Add a statement in the remarks section such as "sulfadiazine was detected by LIB 4562 screen and was confirmed and quantified using a matrix matched calibration curve".

6.14 For violative analysis, you must also enter data for the confirmation batch. On the "findings" screen enter data similar to §6.13 but add the following:

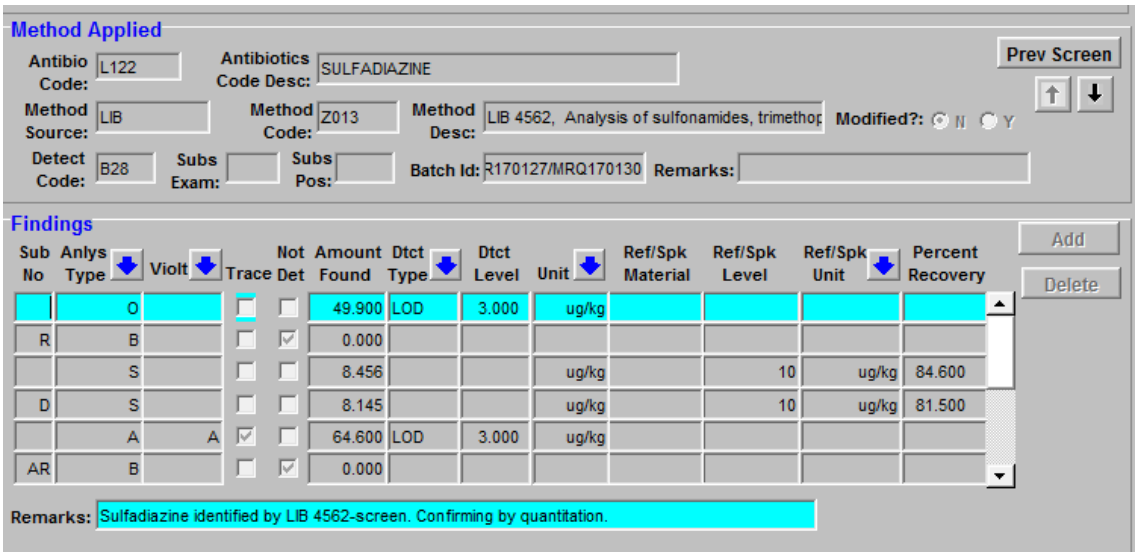
6.14.1 Analysis type is "A". Under comments field (for example): "Sulfadiazine (L122) confirmed by full quantitation using modified LIB 4562. All QC passed."

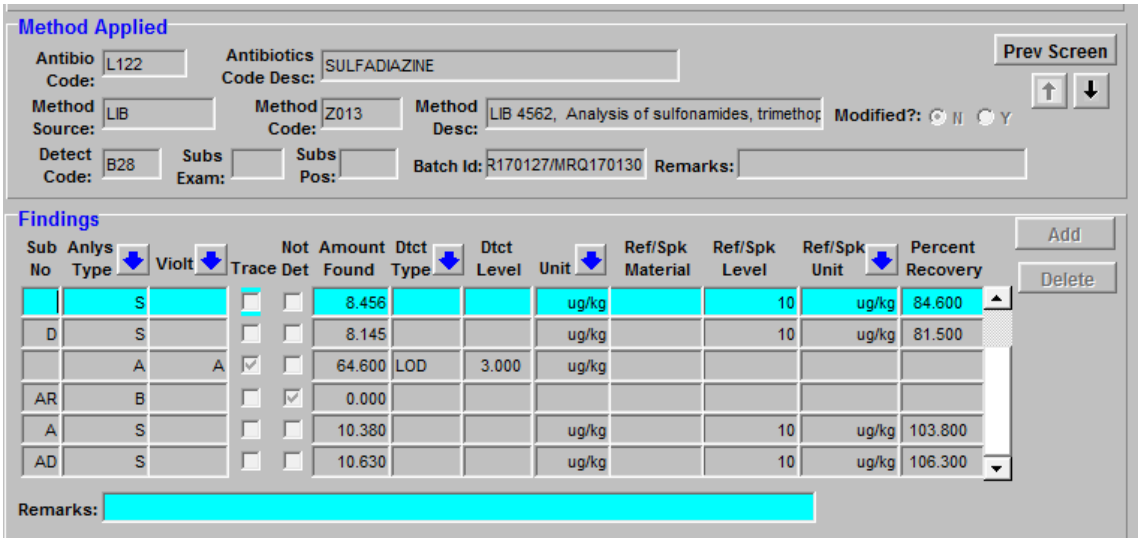
6.14.2 Under Violation line: Enter "A" for Class 3 and "X" for Class 2.

6.14.3 Reagent Blank is "AR" and "B" respectively. Enter corresponding data.

6.14.4 Spike is "A" and "S" respectively. Enter corresponding data.

6.14.5 Spike Duplicate is "AD" and "S" respectively. Enter corresponding data.





6.15 Any analytes not in the FACTS system will be entered as a “Z999” and the corresponding analytes will be defined in the “Antibiotics Code Desc” box.

6.16 Accomplishment hours: enter your accomplishment hours for sample preparation and worksheet assembly. Enter the extracting/instrument analyst’s hours. If the sample is violative, enter the check/additional analyst’s hours. Set all analysts’ status to ‘Complete’.

7. Worksheet preparation and assembly

7.1 Always use the current DEN-LB-CANT-431x AQUACULTURE pdf template(s) from QMiS.

7.2 Enter data on front page of worksheet, then save it in a folder identified as the sample number in the ‘Sample In Progress’ directory (typically the supervisor creates the folder, along with a blank 431 template saved as the sample number matrix 431, such as “123456 tilapia 431”): H:\ALL_LAB\3_Documentation\ScannedWorksheets\Chem\Samples_In_Progress\

7.3 The supervisor will also save a copy of the collection report in the same folder.

7.4 Enter sample preparation information on page 3 of the 431x document.

7.5 Save any photographs or photocopies of labeling in the folder as well.

7.6 After samples have been analyzed, the analyst who extracted/processed data prints off instrument summary report and acquisition method. Combine with raw data batch record (sample weights, etc) standards and reagent sheets. Give batch record to supervisor for review. Notify original analysts of results along with batch ID.

7.7 Scan or save pdf batch record and save as Batch "MR(Q)xxxxxx" to the following directory: H:\ALL_LAB\3_Documentation\Scanned Worksheets\Chem\Batch Records

7.8 Class 1 worksheet assembly:

7.8.1 Close out 431 by entering all required data: Box 11 for the reserve, batch number (on page 3), and on results section click on "All Negative" icon.

7.8.2 Electronically sign 431 on page 2 and 3 using your PIV and PIN. Enter date completed in box 12a.

7.8.3 Save the file.

7.8.4 Once everything is filled out, go to page 1 and click the "Analyst finished" box. This will bring up a print window, selected "Adobe PDF" as the printer and save a new copy as "123456 Tilapia 431 final". Note: Do not try to save this file as a regular PDF as it will not delete the instructions (page 1), you only want to save page 2 and 3.

7.8.5 Label and identify all pictures; these must be saved as "PDF's".

7.8.6 Close all windows and files.

7.8.7 Reopen your sample folder, right click, and combine all files using the "combine supported files in Acrobat" function. Include the 431 final, all photos, and the collection report.

7.8.8 Save this new file as "123456 Tilapia 431 final combined".

7.8.9 Notify supervisor or their actor that sample is ready for classification and close out.

7.9 Class 2 worksheet assembly:

7.9.1.1 Follow steps for class 1 worksheet assembly with the following additions.

7.9.1.2 Click the "check analysis" box on page one to create a second 431a results page.

7.9.1.3 Do not modify screening results. A Class 2 sample is classified between the MDL and the TTL.

7.9.1.4 On the second result page, enter the batch ID (MRQYYMMDD) and the quantitative amount found for the corresponding analyte. Use the correct number of significant figures. Update the action level column from 'no' to 'yes' for the detected analyte.

7.9.1.5 The class 2 worksheet is organized as follows:

7.9.1.5.1 431 front page

7.9.1.5.2 431a sample prep/result page for screening batch

7.9.1.5.3 431a result page for quantitative batch

7.9.1.5.4 431a pages for screening batch record

7.9.1.5.5 431a pages for quantitative batch record

7.9.1.5.6 Attachment: Standards preparation for screening batch

7.9.1.5.7 Attachment: Worklist/Sequence, Acquisition method, Instrument Configuration report (Agilent only)

7.9.1.5.8 Attachment: 'Quantitative' Analysis Summary Report for screening batch.

7.9.1.5.9 Attachment: Standards preparation for quantitative batch

7.9.1.5.10 Attachment: Worklist/Sequence, Acquisition method, Instrument Configuration report (configuration report is for Agilent systems only)

7.9.1.5.11 Attachment: 'Quantitative' Analysis Summary Report for quantitative batch (on AB SCIEX 5500, use 'Short' report for summary. For Class 2 samples, chromatographs and ion ratios are not included.

7.9.1.5.12 Labeling photos/photocopies, if applicable.

7.9.1.5.13 Collection report

7.10 Class 3 worksheet assembly:

7.10.1.1 Follow steps for class 1 worksheet assembly with the following additions.

7.10.1.2 Click the "check analysis" box on page one to create a second 431a results page.

7.10.1.3 Modify screening results by utilizing a ">" symbol for the corresponding analyte; i.e. for sulfadiazine ">10.0" in the results column and change the action level column from 'no' to 'yes'. Do <u>not</u> enter the semi-quantitative value on the result sheet. Do this for any additional analytes detected at or above the presumptive positive level in the screening batch.

7.10.1.4 On the second result page, enter the batch ID (MRQYYMMDD) and the quantitative amount found for the corresponding analyte. Use the correct number of significant figures. Also update the action level column from 'no' to 'yes' for the detected analyte.

7.10.1.5 The class 3 worksheet is organized as follows:

7.10.1.5.1 431 front page

7.10.1.5.2 431a sample prep/result page for screening batch

7.10.1.5.3 431a result page for quantitative batch

7.10.1.5.4 431a pages for screening batch record

7.10.1.5.5 431a pages for quantitative batch record

7.10.1.5.6 Attachment: Standards preparation for screening batch

7.10.1.5.7 Attachment: Reagent preparation for screening batch

7.10.1.5.8 Attachment: Worklist/Sequence, Acquisition method, Instrument Configuration report (Agilent only)

7.10.1.5.9 Attachment: 'Quantitative' Analysis Full Summary Report for screening batch, including all relevant chromatographs, ion ratios, and calibration curves.

7.10.1.5.10 Attachment: Standards preparation for quantitative batch

7.10.1.5.11 Attachment: Reagent preparation for quantitative batch

7.10.1.5.12 Attachment: Worklist/Sequence, Acquisition method, Instrument Configuration report (configuration report is for Agilent systems only)

7.10.1.5.13 Attachment: 'Quantitative' Analysis Summary Report for quantitative batch (on AB SCIEX 5500, use 'Short' report for summary, and 'long' report for ion ratios and chromatograms.

7.10.1.5.14 Labeling photos/photocopies if applicable

7.10.1.5.15 Collection report

7.10.2 A Class 2/Class 3 worksheet package must be printed or saved electronically and properly identified (all attachments labeled and numbered) before submitting to the supervisor. Do not use a highlighter on printed hard-copy – it makes the text below illegible when scanned).

---

8. Glossary/Definitions

A. RB: Reagent Blank. Used to verify reagents are uncontaminated by interfering components, the reagent blank is an extract that contains no sample matrix. Carried thorough the extraction as if it were a sample, one must be extracted with each batch and display no interference peaks at the reference times of interest at or above a ½ x level.

B. NC: Negative Control. Used to verify the lack of matrix effects, the control is an aliquot of matrix material known to contain no analytes of interest. One must be

extracted with each batch, and must display no interference peaks at reference times of interest at or above ½ x level.

C. SPK/DUP: Matrix spike/matrix spike duplicate. Used to demonstrate effective and reproducible extraction, the matrix spike and duplicate are two aliquots of negative control matrix material, each fortified at the 1x target level. A pair of matrix spikes must be extracted and analyzed with each batch.
D. ICV: Independent Calibration Verification. Used to assure the accuracy of the calibration curve, the ICV is an extracted 1x standard prepared from a secondary standard source.
E. CCV: Continuing Calibration Verification. Used to check the calibration during a run, the CCV is a re-injection of the 1x calibration standard. A CCV is analyzed after every ten extracts and at the end of the analytical sequence.

9. Records

9.1 DEN-LB-CANT-431x.001 1.0 - AQUACULTURE (check QMiS for current version)

9.2 DEN-LB-CANT-431a.001 2.0– AQUACULTURE SCREEN BATCH (check QMiS for current version)

10. References & Supporting Documents

10.1 LIB 4562: Analysis of sulfonamides, trimethoprim, fluoroquinolones, quinolones, triphenylmethane dyes (and their leuco metabolites) and methyltestosterone in fish and shrimp using liquid chromatography mass spectrometry

10.1.1 http://inside.fda.gov:9003/downloads/PolicyProcedures/Laboratories/LaboratoryInformationBulletins/UCM395603.pdf

10.2 LIB 4614: Method Transfer and Optimization of LIB 4562 (Multi-Residue LC-MS/MS Screening Method for Veterinary Drugs in Aquaculture Tissues) from the Agilent 6490 LC-MS/MS to a SCIEX 5500 QTRAP LC-MS/MS

10.2.1 http://inside.fda.gov:9003/downloads/PolicyProcedures/Laboratories/LaboratoryInformationBulletins/UCM521382.pdf

10.3 Storey, J.; Clark, S.; Johnson, A.; Andersen, W.; Turnipseed, S.; Lohne, J.; Burger, R.; Ayres, P.; Carr, J.; Madson, M. Analysis of sulfonamides, trimethoprim, fluoroquinolones, quinolones, triphenylmethane dyes and methyl testosterone in fish and shrimp using liquid chromatography-mass spectrometry. *J. Chromatogr. B*, 2014, 972, 328-47.

10.4 Compliance Program 7304.018, Chemotherapeutics in Seafood Compliance Program (FY 09/10/11)

10.4.1 http://www.fda.gov/downloads/Food/ComplianceEnforcement/ucm073192.pdf

10.5 DEN-LAB QMS #: 13-37, approved 6/10/14

10.6 DEN-LAB QMS #: 15-12, approved 7/5/16

10.7 DEN-LAB QMS #: 15-13, approved 7/5/16

10.8 DEN-LAB QMS #: 15-15, approved 8/12/16

10.9 DEN-LAB QMS #: 15-22, approved 7/31/15

10.10 DEN-LAB QMS#: 15-32a (tilapia), approved 8/1/17

10.11 DEN-LAB QMS#: 15-32c (catfish), approved 10/1/17

10.12 DEN-LAB QMS#: 15-32d (crab), approved 01/01/18

10.13 DEN-LAB QMS#: 15-32f (shrimp), approved 01/01/18

10.14 DEN-LAB QMS#: 15-32L (eel), approved 12/1/17

10.15 DEN-LAB QMS #: 16-16, approved 11/1/16

10.16 CVM # 118. Guidance for Industry Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues

10.16.1 http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052658.pdf

11. Document History

Version #	Status* (D,I, R, C)	Date	Author Name and Title	Approving Official Name and Title
1.0	I	3/9/17	T. Nickel, Chemist	Patrick Ayres, Supervisory Chemist
2.0	R	3/2/18	R. Burger, Chemist	Patrick R. Ayres, Supervisory Chemist
* - D: Draft, I: Initial, R: Revision, C: Cancel				

12. Change History

Version	Change
1.0	*Original. Documentation of parameters combined from LIBs 4562, 4614 and additional in house validations for additional matrices.*
02	*Major update of documentation to include the introduction of 20 new analytes for regulatory concern. These analytes were also posted with their corresponding QMiS documents and revised methodology to include presumptive positive check levels. A DNA sequencing prepping procedure with added for the assistance of microbiology in positively identifying new matrices in aquaculture. A set of revised FACTS instructions was included to address the procedure for Class 2 & 3 samples.*

Appendix A: Aquaculture multi-residue screen analytes, class, and method target level

Analyte	Class	"1x" method target level (ng/g)
Ciprofloxacin (CIP)	Fluoroquinolone	5
Enrofloxacin (ENR)	Fluoroquinolone	5
Norfloxacin (NOR)	Fluoroquinolone	5
Difloxacin (DIF)	Fluoroquinolone	5
Sarafloxacin (SAR)	Fluoroquinolone	5
Danofloxacin (DAN)	Fluoroquinolone	5
Flumequine (FLU)	Quinolone	10
Nalidixic Acid (NAL)	Quinolone	10
Oxolinic Acid (OXO)	Quinolone	10
Malachite Green (MG)	Triphenylmethane dye	1
Leucomalachite Green (LMG)	Triphenylmethane dye	1
Crystal Violet (CV)	Triphenylmethane dye	1
Leucocrystal Violet (LCV)	Triphenylmethane dye	1
Brilliant Green (BG)	Triphenylmethane dye	1
Sulfacetamide (SAA)	Sulfonamide	10
Sulfachloropyridazine (SCP)	Sulfonamide	10
Sulfadiazine (SDZ)	Sulfonamide	10
Sulfadimethoxine (SDM)	Sulfonamide	10
Sulfadoxine (SDZ)	Sulfonamide	10
Sulfaethoxypyridazine (SEP)	Sulfonamide	10
Sulfamerazine (SMR)	Sulfonamide	10
Sulfamethazine (SMZ)	Sulfonamide	10
Sulfamethoxazole (SMX)	Sulfonamide	10
Sulfamethoxypyridazine (SMP)	Sulfonamide	10
Sulfapyridine (SPD)	Sulfonamide	10
Sulfaquinoxaline (SQX)	Sulfonamide	10
Sulfathiazole (STZ)	Sulfonamide	10
Sulfamonomethoxine (SMN)	Sulfonamide	10
Trimethoprim (TMP)	Potentiator	10
Methyltestosterone (MT)	Hormone	0.8
Florfenicol Amine (FFA)	Amphenicol	1000
Florfenicol (FF)	Amphenicol	1
Chloramphenicol (CAP)	Amphenicol	0.3
Thiamphenicol (TAP)	Amphenicol	5
Mebendazole (MBZ)	Benzimidazole	5
Mebendazole Amine (MBZ-nh2)	Benzimidazole	5
Hydroxymebendazole (MBZ-oh)	Benzimidazole	5
Oxytetracycline (OTC)	Tetracycline	2000
Tetracycline (TC)	Tetracycline	2000
Chlortetracycline (CTC)	Tetracycline	2000

Appendix B: Agilent 6490/6495 Instrument method

Acquisition Method Report

Agilent Technologies

Acquisition Method Info

Method Name Waters T3 450 uL 49 cpds10132017.m

Method Path D:\MassHunter\Methods\multi residue fish\Waters T3 450 uL 49 cpds10132017.m

Method Description Modified LIB 4562

Device List

Multisampler
Binary Pump 1
Column Comp.
QQQ

MS QQQ Mass Spectrometer

Ion Source	AJS ESI	**Tune File**	atunes.tune.xml
Stop Mode	By StopTime	**Stop Time (min)**	14.5
Time Filter	On	**Time Filter Width (min)**	0.07
LC->Waste Pre Row	N/A	**LC->Waste Post Row**	11.5

Time Segments

Index	Start Time (min)	Scan Type	Ion Mode	Div Valve	Delta EMV	Store	Cycle Time (ms)	Triggered?	MRM Repeats
1	1	DynamicMRM	ESI+Agilent Jet Stream	To MS	300	Yes	500	No	3

Time Segment 1

Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Cell Acc (V)	Ret Time (min)	Ret Window	Polarity
brilliant green (BG)	No	385.01	Unit/Enh (6490)	340.9	Unit/Enh (6490)	380	50	4	9.15	1.1	Positive
brilliant green (BG)	No	385.01	Unit/Enh (6490)	296.9	Unit/Enh (6490)	380	62	4	9.15	1.1	Positive
brilliant green (BG)	No	385.01	Unit/Enh (6490)	240.8	Unit/Enh (6490)	380	70	4	9.15	1.1	Positive
cap d5 (IS)	Yes	325.9	Unit/Enh (6490)	157.1	Unit/Enh (6490)	380	14	4	6.8	1	Negative
chloramphenicol (CAP)	No	320.9	Unit/Enh (6490)	257.2	Unit/Enh (6490)	380	12	4	6.8	1	Negative
chloramphenicol (CAP)	No	320.9	Unit/Enh (6490)	193.8	Unit/Enh (6490)	380	10	4	6.8	1	Negative
chloramphenicol (CAP)	No	320.9	Unit/Enh (6490)	152.1	Unit/Enh (6490)	380	14	4	6.8	1	Negative
Chlorotetracycline (CTC)	No	479.9	Unit/Enh (6490)	463	Unit/Enh (6490)	380	14	4	5.75	1.1	Positive
Chlorotetracycline (CTC)	No	479.9	Unit/Enh (6490)	444.9	Unit/Enh (6490)	380	22	4	5.75	1.1	Positive
Chlorotetracycline (CTC)	No	479.9	Unit/Enh (6490)	155	Unit/Enh (6490)	380	38	4	5.75	1.1	Positive
ciprofloxacin HCl (CIP)	No	332.15	Unit/Enh (6490)	313.8	Unit/Enh (6490)	380	18	4	4.9	1	Positive
ciprofloxacin HCl (CIP)	No	332.15	Unit/Enh (6490)	244.9	Unit/Enh (6490)	380	26	4	4.9	1	Positive
ciprofloxacin HCl (CIP)	No	332.15	Unit/Enh (6490)	230.8	Unit/Enh (6490)	380	42	4	4.9	1	Positive
cv-D6 [IS]	Yes	378	Unit/Enh (6490)	362.1	Unit/Enh (6490)	380	46	4	9	2	Positive
danofloxacin (DAN)	No	358.39	Unit/Enh (6490)	340.2	Unit/Enh (6490)	380	30	4	5	1	Positive
danofloxacin (DAN)	No	358.39	Unit/Enh (6490)	255	Unit/Enh (6490)	380	46	4	5	1	Positive
danofloxacin (DAN)	No	358.39	Unit/Enh (6490)	82.2	Unit/Enh (6490)	380	46	4	5	1	Positive
difloxacin (DIF)	No	400	Unit/Enh (6490)	382.1	Unit/Enh (6490)	380	22	4	5.5	1	Positive
difloxacin (DIF)	No	400	Unit/Enh (6490)	356.1	Unit/Enh (6490)	380	22	4	5.5	1	Positive
difloxacin (DIF)	No	400	Unit/Enh (6490)	299.1	Unit/Enh (6490)	380	34	4	5.5	1	Positive

Report generation date: 02-Mar-2018 10:16:58 AM

Acquisition Method Report

Agilent Technologies

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Cell Acc (V)	Ret Time (min)	Ret Window	Polarity
enrofloxacin (ENR)	No	360.4	Unit/Enh (6490)	341.8	Unit/Enh (6490)	380	22	4	5.1	1	Positive
enrofloxacin (ENR)	No	360.4	Unit/Enh (6490)	315.9	Unit/Enh (6490)	380	18	4	5.1	1	Positive
enrofloxacin (ENR)	No	360.4	Unit/Enh (6490)	244.9	Unit/Enh (6490)	380	30	4	5.1	1	Positive
florfenicol (FF)	No	355.9	Unit/Enh (6490)	335.9	Unit/Enh (6490)	380	10	4	6.6	1.1	Negative
florfenicol (FF)	No	355.9	Unit/Enh (6490)	185	Unit/Enh (6490)	380	14	4	6.6	1.1	Negative
florfenicol (FF)	No	355.9	Unit/Enh (6490)	119.1	Unit/Enh (6490)	380	38	4	6.6	1.1	Negative
florfenicol amine (FFA)	No	248.3	Unit/Enh (6490)	230	Unit/Enh (6490)	380	10	4	2.9	3.5	Positive
florfenicol amine (FFA)	No	248.3	Unit/Enh (6490)	129.9	Unit/Enh (6490)	380	34	4	2.9	3.5	Positive
florfenicol amine (FFA)	No	248.3	Unit/Enh (6490)	90.9	Unit/Enh (6490)	380	58	4	2.9	3.5	Positive
flumequine (FLU)	No	262.26	Unit/Enh (6490)	243.8	Unit/Enh (6490)	380	22	4	8.1	1	Positive
flumequine (FLU)	No	262.26	Unit/Enh (6490)	201.7	Unit/Enh (6490)	380	34	4	8.1	1	Positive
flumequine (FLU)	No	262.26	Unit/Enh (6490)	125.8	Unit/Enh (6490)	380	54	4	8.1	1	Positive
gentian violet (GV/CV)	No	372.01	Unit/Enh (6490)	355.8	Unit/Enh (6490)	380	50	4	8.95	1.1	Positive
gentian violet (GV/CV)	No	372.01	Unit/Enh (6490)	339.9	Unit/Enh (6490)	380	62	4	8.95	1.1	Positive
gentian violet (GV/CV)	No	372.01	Unit/Enh (6490)	250.9	Unit/Enh (6490)	380	44	4	8.95	1.1	Positive
Hydroxymebendazole (MEB-oh)	No	298.3	Unit/Enh (6490)	266	Unit/Enh (6490)	380	30	4	5.6	1.1	Positive
Hydroxymebendazole (MEB-oh)	No	298.3	Unit/Enh (6490)	220	Unit/Enh (6490)	380	54	4	5.6	1.1	Positive
Hydroxymebendazole (MEB-oh)	No	298.3	Unit/Enh (6490)	79.1	Unit/Enh (6490)	380	50	4	5.6	1.1	Positive
leuco cv-D6 [IS]	Yes	380	Unit/Enh (6490)	364.2	Unit/Enh (6490)	380	28	4	5.95	1	Positive
leuco gv/cv (LGV/LCV)	No	374.51	Unit/Enh (6490)	358.4	Unit/Enh (6490)	380	26	4	6.3	1.1	Positive
leuco gv/cv (LGV/LCV)	No	374.51	Unit/Enh (6490)	253	Unit/Enh (6490)	380	42	4	6.3	1.1	Positive
leuco gv/cv (LGV/LCV)	No	374.51	Unit/Enh (6490)	239	Unit/Enh (6490)	380	30	4	6.3	1.1	Positive
leuco mg (LMG)	No	331.51	Unit/Enh (6490)	315.8	Unit/Enh (6490)	380	26	4	9.4	1.1	Positive
leuco mg (LMG)	No	331.51	Unit/Enh (6490)	238.9	Unit/Enh (6490)	380	36	4	9.4	1.1	Positive
leuco mg (LMG)	No	331.51	Unit/Enh (6490)	223	Unit/Enh (6490)	380	62	4	9.4	1.1	Positive
lmg-D5 [IS]	Yes	336	Unit/Enh (6490)	239	Unit/Enh (6490)	380	44	4	9.15	1	Positive
malachite green (MG)	No	329.01	Unit/Enh (6490)	312.9	Unit/Enh (6490)	380	42	4	8.2	1.1	Positive
malachite green (MG)	No	329.01	Unit/Enh (6490)	240.9	Unit/Enh (6490)	380	66	4	8.2	1.1	Positive
malachite green (MG)	No	329.01	Unit/Enh (6490)	207.8	Unit/Enh (6490)	380	54	4	8.2	1.1	Positive
Mebendazole (MEB)	No	296.3	Unit/Enh (6490)	263.9	Unit/Enh (6490)	380	26	4	7.35	1.1	Positive
Mebendazole (MEB)	No	296.3	Unit/Enh (6490)	105	Unit/Enh (6490)	380	38	4	7.35	1.1	Positive
Mebendazole (MEB)	No	296.3	Unit/Enh (6490)	77.1	Unit/Enh (6490)	380	50	4	7.35	1.1	Positive
Mebendazole Amine (MEB-nh2)	No	238.27	Unit/Enh (6490)	133	Unit/Enh (6490)	380	50	4	5.6	1.1	Positive
Mebendazole Amine (MEB-nh2)	No	238.27	Unit/Enh (6490)	105	Unit/Enh (6490)	380	30	4	5.6	1.1	Positive
Mebendazole Amine (MEB-nh2)	No	238.27	Unit/Enh (6490)	77.1	Unit/Enh (6490)	380	46	4	5.6	1.1	Positive

Acquisition Method Report

Agilent Technologies

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Cell Acc (V)	Ret Time (min)	Ret Window	Polarity
methyl testosterone (MT)	No	303.46	Unit/Enh (6490)	109	Unit/Enh (6490)	380	42	4	9.3	1	Positive
methyl testosterone (MT)	No	303.46	Unit/Enh (6490)	97	Unit/Enh (6490)	380	26	4	9.3	1	Positive
methyl testosterone (MT)	No	303.46	Unit/Enh (6490)	78.9	Unit/Enh (6490)	380	58	4	9.3	1	Positive
mg-d5 [IS]	Yes	334.2	Unit/Enh (6490)	318.1	Unit/Enh (6490)	380	44	4	8.1	1	Positive
nalidixic acid (NAL)	No	233.25	Unit/Enh (6490)	214.8	Unit/Enh (6490)	380	14	4	7.9	1	Positive
nalidixic acid (NAL)	No	233.25	Unit/Enh (6490)	186.7	Unit/Enh (6490)	380	30	4	7.9	1	Positive
nalidixic acid (NAL)	No	233.25	Unit/Enh (6490)	103.8	Unit/Enh (6490)	380	54	4	7.9	1	Positive
nor-D5 [IS]	Yes	325.3	Unit/Enh (6490)	281.2	Unit/Enh (6490)	380	20	4	4.75	1	Positive
norfloxacin (NOR)	No	320.34	Unit/Enh (6490)	276.1	Unit/Enh (6490)	380	19	4	4.8	1	Positive
norfloxacin (NOR)	No	320.34	Unit/Enh (6490)	232.8	Unit/Enh (6490)	380	30	4	4.8	1	Positive
norfloxacin (NOR)	No	320.34	Unit/Enh (6490)	230.7	Unit/Enh (6490)	380	42	4	4.8	1	Positive
oxolinic acid (OXO)	No	262.24	Unit/Enh (6490)	243.7	Unit/Enh (6490)	380	18	4	6.9	1	Positive
oxolinic acid (OXO)	No	262.24	Unit/Enh (6490)	215.6	Unit/Enh (6490)	380	34	4	6.9	1	Positive
oxolinic acid (OXO)	No	262.24	Unit/Enh (6490)	159.8	Unit/Enh (6490)	380	46	4	6.9	1	Positive
Oxytetracycline (OTC)	No	461.4	Unit/Enh (6490)	443.2	Unit/Enh (6490)	380	10	4	4.9	1.1	Positive
Oxytetracycline (OTC)	No	461.4	Unit/Enh (6490)	426	Unit/Enh (6490)	380	18	4	4.9	1.1	Positive
Oxytetracycline (OTC)	No	461.4	Unit/Enh (6490)	337	Unit/Enh (6490)	380	34	4	4.9	1.1	Positive
sarafloxacin HCl (SAR)	No	386.13	Unit/Enh (6490)	368.1	Unit/Enh (6490)	380	26	4	5.45	1	Positive
sarafloxacin HCl (SAR)	No	386.13	Unit/Enh (6490)	342.2	Unit/Enh (6490)	380	18	4	5.45	1	Positive
sarafloxacin HCl (SAR)	No	386.13	Unit/Enh (6490)	299.2	Unit/Enh (6490)	380	30	4	5.45	1	Positive
smz 6c13 (IS)	Yes	285.11	Unit/Enh (6490)	186.1	Unit/Enh (6490)	380	17	4	5.5	1	Positive
sulfacetamide (SAA)	No	215.25	Unit/Enh (6490)	155.9	Unit/Enh (6490)	380	10	4	4.4	1	Positive
sulfacetamide (SAA)	No	215.25	Unit/Enh (6490)	108	Unit/Enh (6490)	380	18	4	4.4	1	Positive
sulfacetamide (SAA)	No	215.25	Unit/Enh (6490)	92	Unit/Enh (6490)	380	26	4	4.4	1	Positive
sulfachloropyridazine (SCP)	No	285.02	Unit/Enh (6490)	156.1	Unit/Enh (6490)	380	18	4	6.3	1	Positive
sulfachloropyridazine (SCP)	No	285.02	Unit/Enh (6490)	108.1	Unit/Enh (6490)	380	30	4	6.3	1	Positive
sulfachloropyridazine (SCP)	No	285.02	Unit/Enh (6490)	92.1	Unit/Enh (6490)	380	34	4	6.3	1	Positive
sulfadiazine (SDZ)	No	251.3	Unit/Enh (6490)	155.9	Unit/Enh (6490)	380	14	4	4.6	0.9	Positive
sulfadiazine (SDZ)	No	251.3	Unit/Enh (6490)	108	Unit/Enh (6490)	380	30	4	4.6	0.9	Positive
sulfadiazine (SDZ)	No	251.3	Unit/Enh (6490)	92	Unit/Enh (6490)	380	34	4	4.6	0.9	Positive
sulfadimethoxine (SDM)	No	311.35	Unit/Enh (6490)	155.9	Unit/Enh (6490)	380	22	4	7.2	1	Positive
sulfadimethoxine (SDM)	No	311.35	Unit/Enh (6490)	107.9	Unit/Enh (6490)	380	30	4	7.2	1	Positive
sulfadimethoxine (SDM)	No	311.35	Unit/Enh (6490)	92.1	Unit/Enh (6490)	380	42	4	7.2	1	Positive
sulfadoxine (SDX)	No	311.34	Unit/Enh (6490)	155.9	Unit/Enh (6490)	380	14	4	6.45	1	Positive
sulfadoxine (SDX)	No	311.34	Unit/Enh (6490)	107.9	Unit/Enh (6490)	380	22	4	6.45	1	Positive

Acquisition Method Report

Agilent Technologies

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Cell Acc (V)	Ret Time (min)	Ret Window	Polarity
sulfadoxine (SDX)	No	311.34	Unit/Enh (6490)	91.9	Unit/Enh (6490)	380	42	4	6.45	1	Positive
sulfaethoxy pyridazine (SEP)	No	295.34	Unit/Enh (6490)	156	Unit/Enh (6490)	380	30	4	6.5	1	Positive
sulfaethoxy pyridazine (SEP)	No	295.34	Unit/Enh (6490)	107.9	Unit/Enh (6490)	380	34	4	6.5	1	Positive
sulfaethoxy pyridazine (SEP)	No	295.34	Unit/Enh (6490)	91.9	Unit/Enh (6490)	380	38	4	6.5	1	Positive
sulfameraz ine (SMR)	No	265.31	Unit/Enh (6490)	156	Unit/Enh (6490)	380	18	4	5.15	1	Positive
sulfameraz ine (SMR)	No	265.31	Unit/Enh (6490)	108	Unit/Enh (6490)	380	30	4	5.15	1	Positive
sulfameraz ine (SMR)	No	265.31	Unit/Enh (6490)	91.9	Unit/Enh (6490)	380	34	4	5.15	1	Positive
sulfametha zine (SMZ)	No	279.34	Unit/Enh (6490)	185.9	Unit/Enh (6490)	380	22	4	5.5	1	Positive
sulfametha zine (SMZ)	No	279.34	Unit/Enh (6490)	123.9	Unit/Enh (6490)	380	30	4	5.5	1	Positive
sulfametha zine (SMZ)	No	279.34	Unit/Enh (6490)	92	Unit/Enh (6490)	380	30	4	5.5	1	Positive
sulfametho xazole (SMX)	No	254.29	Unit/Enh (6490)	155.9	Unit/Enh (6490)	380	14	4	6.6	1	Positive
sulfametho xazole (SMX)	No	254.29	Unit/Enh (6490)	107.9	Unit/Enh (6490)	380	22	4	6.6	1	Positive
sulfametho xazole (SMX)	No	254.29	Unit/Enh (6490)	92	Unit/Enh (6490)	380	26	4	6.6	1	Positive
sulfametho xypyridazin e (SMP)	No	281.31	Unit/Enh (6490)	156.1	Unit/Enh (6490)	380	18	4	5.6	0.8	Positive
sulfametho xypyridazin e (SMP)	No	281.31	Unit/Enh (6490)	107.9	Unit/Enh (6490)	380	20	4	5.6	0.8	Positive
sulfametho xypyridazin e (SMP)	No	281.31	Unit/Enh (6490)	91.9	Unit/Enh (6490)	380	36	4	5.6	0.8	Positive
sulfamono methoxine (SMN)	No	281.07	Unit/Enh (6490)	156.1	Unit/Enh (6490)	380	18	4	6	0.8	Positive
sulfamono methoxine (SMN)	No	281.07	Unit/Enh (6490)	107.9	Unit/Enh (6490)	380	30	4	6	0.8	Positive
sulfamono methoxine (SMN)	No	281.07	Unit/Enh (6490)	92	Unit/Enh (6490)	380	34	4	6	0.8	Positive
sulfapyridin e (SPD)	No	250.3	Unit/Enh (6490)	156.9	Unit/Enh (6490)	380	14	4	4.9	0.8	Positive
sulfapyridin e (SPD)	No	250.3	Unit/Enh (6490)	108	Unit/Enh (6490)	380	30	4	4.9	0.8	Positive
sulfapyridin e (SPD)	No	250.3	Unit/Enh (6490)	92	Unit/Enh (6490)	380	26	4	4.9	0.8	Positive
sulfaquinox aline (SQX)	No	301.09	Unit/Enh (6490)	155.9	Unit/Enh (6490)	380	14	4	7.2	1	Positive
sulfaquinox aline (SQX)	No	301.09	Unit/Enh (6490)	107.9	Unit/Enh (6490)	380	26	4	7.2	1	Positive
sulfaquinox aline (SQX)	No	301.09	Unit/Enh (6490)	91.9	Unit/Enh (6490)	380	30	4	7.2	1	Positive
sulfathiazol e (STZ)	No	256.03	Unit/Enh (6490)	155.9	Unit/Enh (6490)	380	14	4	4.8	1	Positive
sulfathiazol e (STZ)	No	256.03	Unit/Enh (6490)	107.9	Unit/Enh (6490)	380	30	4	4.8	1	Positive
sulfathiazol e (STZ)	No	256.03	Unit/Enh (6490)	91.9	Unit/Enh (6490)	380	34	4	4.8	1	Positive
Tetracyclin e (TC)	No	445.4	Unit/Enh (6490)	427.2	Unit/Enh (6490)	380	10	4	5	1.1	Positive
Tetracyclin e (TC)	No	445.4	Unit/Enh (6490)	410.2	Unit/Enh (6490)	380	18	4	5	1.1	Positive
Tetracyclin e (TC)	No	445.4	Unit/Enh (6490)	154	Unit/Enh (6490)	380	34	4	5	1.1	Positive
thiampheni col (TAP)	No	354	Unit/Enh (6490)	289.9	Unit/Enh (6490)	380	16	4	5.35	1.1	Negative
thiampheni col (TAP)	No	354	Unit/Enh (6490)	185	Unit/Enh (6490)	380	22	4	5.35	1.1	Negative
thiampheni col (TAP)	No	354	Unit/Enh (6490)	79	Unit/Enh (6490)	380	32	4	5.35	1.1	Negative
trimethopri m (TMP)	No	291.33	Unit/Enh (6490)	260.8	Unit/Enh (6490)	380	26	4	4.75	1	Positive

Acquisition Method Report

Agilent Technologies

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Cell Acc (V)	Ret Time (min)	Ret Window	Polarity
trimethoprim (TMP)	No	291.33	Unit/Enh (6490)	229.8	Unit/Enh (6490)	380	26	4	4.75	1	Positive
trimethoprim (TMP)	No	291.33	Unit/Enh (6490)	122.8	Unit/Enh (6490)	380	34	4	4.75	1	Positive

Scan Parameters

Data Stg	Threshold
Centroid	0

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	220	220
Gas Flow (l/min)	19	19
Nebulizer (psi)	20	20
SheathGasHeater	300	300
SheathGasFlow	12	12
Capillary (V)	3000	3000
VCharging	500	1500

Ion Funnel Parameters

Pos High Pressure RF	150	Neg High Pressure RF	100
Pos Low Pressure RF	80	Neg Low Pressure RF	60

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	0	10000000

Instrument Curves

Actual
Pump1Current
Capillary Current
Gas Flow
High Vac

Name: Multisampler **Model: G7167B**

Sampling Speed	
Draw Speed	200.0 µL/min
Eject Speed	200.0 µL/min
Wait Time After Drawing	2.0 s
Injection	
Needle Wash Mode	Standard Wash
Injection Volume	10.00 µL
Standard Needle Wash	
Needle Wash Mode	Flush Port
Duration	30 s
High Throughput	
Injection Valve to Bypass for Delay Volume Reduction	No
Sample Flush-Out Factor	5.0
Overlapped Injection	
Overlap Injection Enabled	No
Needle Height Position	
Draw Position Offset	0.0 mm
Use Vial/Well Bottom Sensing	No
Thermostat Settings	
Thermostat On	Yes
Temperature	5 °C
Stop Time	
Stoptime Mode	No Limit
Post Time	
Posttime Mode	Off

Acquisition Method Report

Agilent Technologies

Name: Binary Pump 1 Model: G7120A

Flow	0.450 mL/min
Use Solvent Types	Yes
Stroke Mode	Synchronized
Low Pressure Limit	0.00 bar
High Pressure Limit	600.00 bar
Max. Flow Ramp Up	100.000 mL/min²
Max. Flow Ramp Down	100.000 mL/min²
Expected Mixer	No check
Stroke A	
Automatic Stroke Calculation A	Yes
Stop Time	
Stoptime Mode	Time set
Stoptime	13.50 min
Post Time	
Posttime Mode	Off

Solvent Composition

	Channel	Ch. 1 Solv.	Name 1	Ch2 Solv.	Name 2	Selected	Used	Percent
1	A	100.0 % H2O (migrated)	0.1% formic	100.0 % H2O (migrated)		Ch. 1	Yes	95.00 %
2	B	100.0 % ACN (migrated)		100.0 % H2O (migrated)		Ch. 1	Yes	5.00 %

Timetable

	Time	A	B	Flow	Pressure
1	0.00 min	95.00 %	5.00 %	0.450 mL/min	600.00 bar
2	1.11 min	95.00 %	5.00 %	0.450 mL/min	600.00 bar
3	6.67 min	50.00 %	50.00 %	0.450 mL/min	600.00 bar
4	7.23 min	50.00 %	50.00 %	0.450 mL/min	600.00 bar
5	8.89 min	0.00 %	100.00 %	0.450 mL/min	600.00 bar
6	10.56 min	0.00 %	100.00 %	0.450 mL/min	600.00 bar
7	10.84 min	95.00 %	5.00 %	0.450 mL/min	600.00 bar
8	12.22 min	95.00 %	5.00 %	0.450 mL/min	600.00 bar

Name: Column Comp. Model: G7116B

Valve Position	Port 1 -> 10
Ready when front door open	Yes
Position Switch After Run	Do not switch
Left Temperature Control	
Temperature Control Mode	Temperature Set
Temperature	30.00 °C
Enable Analysis Left Temperature	
Enable Analysis Left Temperature On	No
Right Temperature Control	
Right temperature Control Mode	Temperature Set
Right temperature	30.00 °C
Enable Analysis Right Temperature	
Enable Analysis Right Temperature On	No
Enforce column for run	
Enforce column for run column type	
Enforce column for run enabled	No
Stop Time	
Stoptime Mode	As pump/injector
Post Time	
Posttime Mode	Off

Appendix C: AB SCIEX QTRAP 5500 LC-MS/MS instrument method

Comment:	A: 0.1% formic in water; B: ACN; column: WatersXSelect HSS T3 s/n:01143507015506 : lot #:0114350701 Inst ID: 1701725
Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Acquisition Duration:	13min12sec
Number Of Scans:	3630
Periods In File:	1
Acquisition Module:	Acquisition Method
Software version	Analyst 1.6.2
MS Method Properties:	
Period 1:	
Scans in Period:	3630
Relative Start Time:	1000.00 msec
Experiments in Period:	1
Period 1 Experiment 1:	
Scan Type:	MRM (MRM)
Scheduled MRM:	Yes
Polarity:	Positive
Scan Mode:	N/A
Ion Source:	Turbo Spray
MRM detection window:	60 sec
Target Scan Time:	0.2000 sec
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	5.0070 msec
MCA:	No
Step Size:	0.00 Da

@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
215	156	5	DP	81	Sulfacetamide (SAA) 1
			CE	13	
			CXP	18	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
215	92.1	5	DP	81	Sulfacetamide (SAA) 2
			CE	33	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
215	108	5	DP	81	Sulfacetamide (SAA) 3
			CE	27	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
251	156	5.7	DP	41	Sulfadiazine (SDZ) 1
			CE	21	
			CXP	18	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
251	92.1	5.7	DP	41	Sulfadiazine (SDZ) 2
			CE	31	
			CXP	14	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
251	108.1	5.7	DP	41	Sulfadiazine (SDZ) 3
			CE	35	
			CXP	14	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
256	156	6.1	DP	56	Sulfathiazole (STZ) 1
			CE	19	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
256	92	6.1	DP	56	Sulfathiazole (STZ) 2
			CE	33	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID

256	108.1	6.1	DP	56	Sulfathiazole (STZ) 3
			CE	31	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
250	92	6.1	DP	61	Sulfapyridine (SPD) 1
			CE	35	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
250	156	6.1	DP	61	Sulfapyridine (SPD) 2
			CE	27	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
250	108.1	6.1	DP	61	Sulfapyridine (SPD) 3
			CE	30	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
291	230.2	6.3	DP	66	Trimethoprim (TRI)1
			CE	31	
			CXP	16	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
291	261	6.3	DP	66	Trimethoprim (TRI) 2
			CE	33	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
291	123.1	6.3	DP	66	Trimethoprim (TRI) 3
			CE	31	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
265	156	6.4	DP	36	Sulfamerazine (SMR) 1
			CE	23	
			CXP	14	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
265	108	6.4	DP	36	Sulfamerazine (SMR) 2
			CE	31	

			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
265	92	6.4	DP	36	Sulfamerazine (SMR) 3
			CE	19	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
320.1	276.1	6.45	DP	81	Norfloxacin (NOR) 1
			CE	23	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
320.1	302.1	6.45	DP	81	Norfloxacin (NOR) 2
			CE	29	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
320.1	233	6.45	DP	81	Norfloxacin (NOR) 3
			CE	53	
			CXP	16	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
332.1	245.1	6.6	DP	106	Ciprofloxacin (CIP) 1
			CE	33	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
332.1	314.2	6.6	DP	106	Ciprofloxacin (CIP) 2
			CE	20	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
332.1	231.1	6.6	DP	106	Ciprofloxacin (CIP) 3
			CE	47	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
360.1	316.1	6.8	DP	36	Enrofloxacin (ENR) 1
			CE	29	
			CXP	12	

@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
360.2	342.1	6.8	DP	36	Enrofloxacin (ENR) 2
			CE	31	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
360.2	245.1	6.8	DP	36	Enrofloxacin (ENR) 3
			CE	41	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
279	186	7	DP	61	Sulfamethazine (SMZ) 1
			CE	25	
			CXP	14	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
279	124	7	DP	61	Sulfamethazine (SMZ) 2
			CE	33	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
279	92.1	7	DP	61	Sulfamethazine (SMZ) 3
			CE	35	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
280.9	156.1	7.1	DP	66	Sulfamethoxypyridazine (SMP) 1
			CE	23	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
280.9	92	7.1	DP	66	Sulfamethoxypyridazine (SMP) 2
			CE	37	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
280.9	108.1	7.1	DP	66	Sulfamethoxypyridazine (SMP) 3
			CE	33	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
374.2	358.3	7.55	DP	65	Leucogentian violet (LGV)1

			CE	39	
			CXP	18	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
374.2	239.1	7.55	DP	65	Leucogentian violet (LGV) 2
			CE	51	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
374.2	253	7.55	DP	65	Leucogentian violet (LGV)3
			CE	43	
			CXP	34	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
284.9	156	7.95	DP	56	Sulfachloropyridazine (SCP) 1
			CE	25	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
284.9	92.1	7.95	DP	56	Sulfachloropyridazine (SCP) 2
			CE	35	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
284.9	108.1	7.95	DP	56	Sulfachloropyridazine (SCP) 3
			CE	33	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
310.9	156	8.1	DP	26	Sulfadoxine (SDX) 1
			CE	25	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
310.9	108.1	8.1	DP	26	Sulfadoxine (SDX) 2
			CE	33	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
310.9	92	8.1	DP	26	Sulfadoxine (SDX) 3
			CE	39	
			CXP	10	

@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
295	156	8.2	DP	51	Sulfaethoxypyridazine (SEP) 1
			CE	23	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
295	92	8.2	DP	51	Sulfaethoxypyridazine (SEP) 2
			CE	41	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
295	108	8.2	DP	51	Sulfaethoxypyridazine (SEP) 3
			CE	39	
			CXP	14	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
254	156	8.3	DP	66	Sulfamethoxazole (SMX) 1
			CE	21	
			CXP	16	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
254	108	8.3	DP	66	Sulfamethoxazole (SMX) 2
			CE	35	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
254	92	8.3	DP	66	Sulfamethoxazole (SMX) 3
			CE	21	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
262.1	244	8.6	DP	16	Oxolinic Acid (OXO) 1
			CE	23	
			CXP	14	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
262.1	216	8.6	DP	16	Oxolinic Acid (OXO) 2
			CE	41	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID

262.1	160	8.6	DP	16	Oxolinic Acid (OXO) 3
			CE	49	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
300.9	156	8.9	DP	51	Sulfaquinoxaline (SQX) 1
			CE	23	
			CXP	14	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
300.9	92.1	8.9	DP	51	Sulfaquinoxaline (SQX) 2
			CE	41	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
300.9	108	8.9	DP	51	Sulfaquinoxaline (SQX) 3
			CE	23	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
310.8	156.1	8.95	DP	71	Sulfadimethoxine (SDM) 1
			CE	27	
			CXP	22	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
310.8	92	8.95	DP	71	Sulfadimethoxine (SDM) 2
			CE	41	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
310.8	108.1	8.95	DP	71	Sulfadimethoxine (SDM) 3
			CE	37	
			CXP	14	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
233.1	215	9.6	DP	26	Nalidixic Acid (NAL) 1
			CE	25	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
233.1	187.1	9.6	DP	26	Nalidixic Acid (NAL) 2
			CE	33	

			CXP	26	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
233.1	104.1	9.6	DP	26	Nalidixic Acid (NAL) 3
			CE	55	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
262.1	244.1	9.8	DP	46	Flumequine (FLU) 1
			CE	27	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
262.1	202	9.8	DP	46	Flumequine (FLU) 2
			CE	40	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
262.1	126	9.8	DP	46	Flumequine (FLU) 3
			CE	63	
			CXP	14	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
329	313.1	10	DP	65	Malachite Green (MG) 1
			CE	53	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
329	208.1	10	DP	65	Malachite Green (MG) 2
			CE	55	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
329	241.1	10	DP	65	Malachite Green (MG) 3
			CE	73	
			CXP	14	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
372.2	356.2	10.3	DP	65	Gentian violet (GV)1
			CE	55	
			CXP	18	

@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
372.2	340.3	10.3	DP	65	Gentian violet (GV) 2
			CE	73	
			CXP	18	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
372.2	251.1	10.3	DP	65	Gentian violet (GV) 3
			CE	40	
			CXP	6	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
331.1	239.1	10.55	DP	65	Leucomalachite Green (LMG) 1
			CE	45	
			CXP	16	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
331.1	316.1	10.55	DP	65	Leucomalachite Green (LMG) 2
			CE	30	
			CXP	4	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
331.1	223.1	10.55	DP	65	Leucomalachite Green (LMG) 3
			CE	69	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
385.4	341.2	10.45	DP	65	Brilliant Green (BG) 1
			CE	51	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
385.4	297.1	10.45	DP	65	Brilliant Green (BG) 2
			CE	71	
			CXP	16	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
385.4	241	10.45	DP	65	Brilliant Green (BG) 3
			CE	79	
			CXP	20	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
303.2	97.1	10.65	DP	16	Methyltestosterone (MT) 1

			CE	31	
			CXP	12	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
303.2	109.1	10.65	DP	16	Methyltestosterone (MT) 2
			CE	37	
			CXP	10	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
303.2	79	10.65	DP	16	Methyltestosterone (MT) 3
			CE	63	
			CXP	4	
@Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	ID
285.1	186.1	7	DP	106	13 C6 SMZ
			CE	23	
			CXP	17	

Parameter Table(Period 1 Experiment 1):

CUR:	30	
CAD:	Medium	
TEM:	600	
GS1:	50	
GS2:	60	
IS:	5000	
EP	10	
Valco Valve	Diverter	
	Total Time (min)	Position
1	0	to Waste
2	4.4	to MS
3	11.2	to Waste
	Agilent LC Pump Method Properties	
Pump Model:	Agilent 1260 Binary Pump	
Minimum Pressure (psi):	0	
Maximum Pressure (psi):	8702	
Dead Volume (µl):	40	
Maximum Flow Ramp (ml/min²):	100	
Maximum Pressure Ramp (psi/sec):	290	
Max Flow Ramp Up (ml/min²):	100	
Max Flow Ramp Dn (ml/min²):	100	

Step Table:

@Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
0	0	450	95	5
1	1.11	450	95	5
2	6.67	450	50	50
3	7.23	450	50	50
4	8.89	450	0	100
5	10.8	450	0	100
6	11	450	95	5
7	13.2	450	95	5

Left Compressibility:	50
Right Compressibility:	115
Left Dead Volume (µl):	40
Right Dead Volume (µl):	40
Left Stroke Volume (µl):	-1
Right Stroke Volume (µl):	-1
Left Solvent:	A2
Right Solvent:	B2

Agilent LC Pump Method Properties

Pump Model:	Agilent 1260 Binary Pump (Upper Pump)
Minimum Pressure (psi):	0
Maximum Pressure (psi):	8702
Dead Volume (µl):	40
Maximum Flow Ramp (ml/min²):	100
Maximum Pressure Ramp (psi/sec):	290
Max Flow Ramp Up (ml/min²):	100
Max Flow Ramp Dn (ml/min²):	100

Step Table: (Lower Pump)

@Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
0	0	0	50	50
1	13.2	0	50	50

Left Compressibility:	50
Right Compressibility:	115
Left Dead Volume (µl):	40
Right Dead Volume (µl):	40
Left Stroke Volume (µl):	-1
Right Stroke Volume (µl):	-1
Left Solvent:	A1
Right Solvent:	B2

Agilent Column Oven Properties

Left Temperature (°C):	30
Right Temperature (°C):	30
Temperature Tolerance +/- (°C):	1
Start Acquisition Tolerance +/- (°C):	1
Time Table	(Not Used)
Column Switching Valve	Installed 10Port2Pos
Position for first sample in the batch:	Left
Use same position for all samples in the batch	

CTC PAL Autosampler Method Properties

Loop Volume1 (µl):	20
Loop Volume2 (µl):	20
Injection Volume (µl):	10
Barcode Reading:	Disabled
Method Description:	
Syringe: 100ulDLW	
Cycle date: 9/9/2010 2:26:06 PM	
Cycle name: Analyst LC-Inj DLW Fast_Rev05	

Airgap Volume (µl)	3
Front Volume (µl)	5
Rear Volume (µl)	5
Filling Speed (µl/s)	5
Pullup Delay (ms)	3
Inject to	LC Vlv1
Injection Speed (µl/s)	5
Pre Inject Delay (ms)	500
Post Inject Delay (ms)	500
Needle Gap Valve Clean (mm)	3
Valve Clean Time Solvent 2 (s)	3
Valve Clean Time Solvent 1 (s)	4
Post Clean Time Solvent 1 (s)	3

Appendix D: SPEXertificate for SPEX CertiPrep mixed dyes standard (for ordering reference)

ACCREDITED
Reference Materials Producer
Cert #2495.01

SPEXertificate®
Certificate of Reference Material

ilac-MRA ACCREDITED
Chemical Testing
Cert #2495.02

Catalog Number: GO-FDACO-21 **Lot No.** BW171206013

Description: Custom Organic Standard

Matrix: LCMS Acetonitrile

Ship Date: 12-8-2017
Expiration Date: 12-8-2018

This SPEXOrganics® Certified Reference Material, CRM, is intended primarily for use as a calibration standard or quality contr standard for organic chromatography instrumentation such as GC, GC-MS, LC, and LC-MS. It can be employed in USEPA, ASTI and other methods relevant to the certified properties listed below.

Certified Compounds:

Compound	CAS #	Labeled	Purity	Certified†	Uncertainty
Malachite Green (from Malachite Green oxalate salt)	569-64-2	10 µg/mL	71%	9.94 µg/mL	± 0.085 µg/mL
C.I. Basic Violet 3	548-62-9	10 µg/mL	86%	9.89 µg/mL	± 0.085 µg/mL
Brilliant Green	633-03-4	10 µg/mL	86%	10.1 µg/mL	± 0.087 µg/mL
Leucomalachite green	129-73-7	10 µg/mL	95%	10.1 µg/mL	± 0.087 µg/mL
Leucocrystal violet	603-48-5	10 µg/mL	95%	9.88 µg/mL	± 0.085 µg/mL

Final Solution Verification:

Gravimetrically certified.

† Certified concentration based on gravimetric weights and corrected for the purity of the compound(s) used to prepare the standard. Analytical balance calibration is verified daily with C1 weight set #23-190006 which is registered with Atlantic Scale, traceable to NIST and NJ Division of Weights and Measures.

This CRM is guaranteed stable and accurate to within the uncertainty listed for the certified value. This includes uncertainty components due to preparation, homogeneity, short term and long term stability. During the stated period of validity, the purchaser will be notified if this product is recalled due to any significant changes in the stability of the solution. For further information, contact the Sales Support Department at crmsales@spexcsp.com.

Date of Certification: 12-8-2017 Certifying Officer: Shannon Moss

Page 1 of 2

Appendix E: SPEXertificate for SPEX CertiPrep mixed Sulfonamides/trimethoprim/methyl testosterone standard (for ordering reference)

ACCREDITED
Reference Materials Producer
Cert #2495.01

SPEXertificate®

Certificate of Reference Material

ilac-MRA ACCREDITED
Chemical Testing
Cert #2495.02

Catalog Number: GO-FDACO-16 **Lot No.** BW171204010

Description: Custom Organic Standard

Matrix: LC/MS Methanol

Ship Date: 12-7-2017
Expiration Date: 12-7-2018

This **SPEXOrganics®** Certified Reference Material, CRM, is intended primarily for use as a calibration standard or quality contrc standard for organic chromatography instrumentation such as GC, GC-MS, LC, and LC-MS. It can be employed in USEPA, ASTI and other methods relevant to the certified properties listed below.

Certified Compounds:

Compound	CAS #	Labeled	Purity	Certified†	Uncertainty
17a-Methyltestosterone	58-18-4	8 µg/mL	100%	8.16 µg/mL	± 0.070 µg/mL
Sulfacetamide	144-80-9	100 µg/mL	99%	99.0 µg/mL	± 0.85 µg/mL
Sulfachloropyridazine	80-32-0	100 µg/mL	99%	100 µg/mL	± 0.86 µg/mL
Sulfadiazine	68-35-9	100 µg/mL	99%	100 µg/mL	± 0.86 µg/mL
Sulfadimethoxine	122-11-2	100 µg/mL	99%	100 µg/mL	± 0.86 µg/mL
Sulfadimidine-Sulfamethazine	57-68-1	100 µg/mL	99%	101 µg/mL	± 0.87 µg/mL
Sulfadoxin	2447-57-6	100 µg/mL	98%	101 µg/mL	± 0.87 µg/mL
Sulfaethoxypyridazine	963-14-4	100 µg/mL	99.4%	99.4 µg/mL	± 0.85 µg/mL
Sulfamerazine	127-79-7	100 µg/mL	99%	99.0 µg/mL	± 0.85 µg/mL
Sulfamethoxazole	723-46-6	100 µg/mL	99%	99.0 µg/mL	± 0.85 µg/mL
Sulfamethoxypyridazine	80-35-3	100 µg/mL	99%	102 µg/mL	± 0.88 µg/mL
Sulfapyridine	144-83-2	100 µg/mL	99%	100 µg/mL	± 0.86 µg/mL
Sulfaquinoxaline	59-40-5	100 µg/mL	98.8%	98.8 µg/mL	± 0.85 µg/mL
Sulfathiazole	72-14-0	100 µg/mL	99%	99.0 µg/mL	± 0.85 µg/mL
Trimethoprim	738-70-5	100 µg/mL	98%	101 µg/mL	± 0.87 µg/mL
Sulfamonomethoxine	1220-83-3	100 µg/mL	98%	101 µg/mL	± 0.87 µg/mL

Final Solution Verification:

Gravimetrically certified.

† Certified concentration based on gravimetric weights and corrected for the purity of the compound(s) used to prepare the standard. Analytical balance calibration is verified daily with C1 weight set #23-190006 which is registered with Atlantic Scale, traceable to NIST and NJ Division of Weights and Measures.

This CRM is guaranteed stable and accurate to within the uncertainty listed for the certified value. This includes uncertainty components due to preparation, homogeneity, short term and long term stability. During the stated period of validity, the purchaser will be notified if this product is recalled due to any significant changes in the stability of the solution. For further information, contact the Sales Support Department at crmsales@spexcsp.com.

Date of Certification: 12-7-2017 Certifying Officer: Shannon Mora

Page 1 of 2

Appendix F: Appendix D: SPEXertificate for SPEX CertiPrep mixed fluoroquinolones/quinolones standard (for ordering referen

ACCREDITED
Reference Materials Producer
Cert #2495.01

SPEXertificate®
Certificate of Reference Material

ilac-MRA ACCREDITED
Chemical Testing
Cert #2495.02

Catalog Number: LC-FDACO-15 **Lot No.** TS170323027

Description: Custom Organic Standard

Matrix: LC/MS Methanol

Ship Date: 3-30-2017
Expiration Date: 3-30-2018

This **SPEXOrganics®** Certified Reference Material, CRM, is intended primarily for use as a calibration standard or quality control standard for organic chromatography instrumentation such as GC, GC-MS, LC, and LC-MS. It can be employed in USEPA, ASTM and other methods relevant to the certified properties listed below.

Certified Compounds:

Compound	CAS #	Labeled	Purity	Certified†	Uncertainty
Norfloxacin	70458-96-7	50 µg/mL	98%	49.7 µg/mL	± 0.48 µg/mL
Ciprofloxacin	85721-33-1	50 µg/mL	98%	49.6 µg/mL	± 0.48 µg/mL
Enrofloxacin	93106-60-6	50 µg/mL	98%	49.8 µg/mL	± 0.48 µg/mL
Danofloxacin	112398-08-0	50 µg/mL	99.8%	50.3 µg/mL	± 0.48 µg/mL
Sarafloxacin	98105-99-8	50 µg/mL	94.4%	50.2 µg/mL	± 0.48 µg/mL
Difloxacin (from Difloxacin Hydrochloride)	91296-86-5	50 µg/mL	98%	50.0 µg/mL	± 0.48 µg/mL
Oxolinic acid	14698-29-4	100 µg/mL	99%	101 µg/mL	± 0.97 µg/mL
Nalidixic acid	389-08-2	100 µg/mL	98%	100 µg/mL	± 0.96 µg/mL
Flumequine	42835-25-6	100 µg/mL	99%	99.1 µg/mL	± 0.95 µg/mL

Final Solution Verification:

Final solution integrity of this CRM has been verified and confirmed by LC/MS. Sarafloxacin has a purity of 94.4%, balance unknown.

† Certified concentration based on gravimetric weights and corrected for the purity of the compound(s) used to prepare the standard. Analytical balance calibration is verified daily with C1 weight set #23-190006 which is registered with Atlantic Scale, and traceable to NIST and NJ Division of Weights and Measures.

This CRM is guaranteed stable and accurate to within the uncertainty listed for the certified value. This includes uncertainty components due to preparation, homogeneity, short term and long term stability. During the stated period of validity, the purchaser will be notified if this product is recalled due to any significant changes in the stability of the solution. For further information, contact the Sales Support Department at crmsales@spexcsp.com.

Date of Certification: 3-30-2017 Certifying Officer: Julian Burton

Page 1 of 2