SOP No: CLG-FLQ2.00		Page 1 of 24
Title: Confirmation of Fluoroquinolone Antibiotics by HPLC Ion Trap Mass Spectrometry		
Revision: NA	Replaces: NA	Effective: 01/13/2006

Contents

A. INTRODUCTION

1. Theory

Fluoroquinolone antibiotics (FLQs) are extracted from homogenized tissue using a liquid/liquid technique. Upon concentration of the extracts, eight fluoroquinolones are analyzed by HPLC ion trap mass spectrometry. Two fluoroquinolones (desethylene ciprofloxacin and desmethyl danofloxacin) are analyzed using HPLC/MS^2, whereas six other fluoroquinolones (difloxacin, enrofloxacin, norfloxacin, danofloxacin, ciprofloxacin, sarafloxacin) are analyzed by HPLC/MS^3.

2. Applicability

This method confirms desethylene ciprofloxacin, difloxacin, enrofloxacin, norfloxacin, danofloxacin, desmethyl danofloxacin, ciprofloxacin and sarafloxacin at ≥ 25 ppb in bovine liver and muscle.

B. EQUIPMENT

Note: Equivalent equipment may be substituted for that listed below.

1. Apparatus

a. Waring Blender - Model BLH 120, Waring Inc.

b. Robot Coupe® - Model RSI 3Y-1, Robot Coupe Inc.

c. Centrifuge - Model KR22i, Jouan.

d. Homogenizer - Model IKA, Ultra Turrax.

e. Vortex mixer - Fisher-Genie 2.

f. Pasteur pipettes - borosilicate glass, 5.75 inches.

g. Nylon syringe filters - 13 mm, 0.22µm disposable, Cat. No. 9445622, Xpertek.

h. Balance - accurate to 0.0001 g, Cat. No. MT5, Mettler.

i. Nitrogen evaporator - Turbovap LV, Zymark.

j. Plasticware - 50 mL polypropylene centrifuge tubes, 30 x115 mm, Cat. No. 352070, Falcon.

k. Autosampler vials - 750 µL, plastic, Cat. No. 951501, Xpertek.

l. Micropipetters - covering the range from 10 µL – 5000 µL.

m. Vibrax mixer - Model VX8, Janke & Kunkel.

n. Balance - accurate to 0.01 g, Cat. No. PB302, Mettler.

2. Instrumentation

a. Ion trap mass spectrometer - Finnigan LCQ-deca equipped with an APCI LC

interface and Windows NT ver.4.0- LCQ Xcalibur data system, or equivalent.

b. LC system - Quaternary pump equipped with degassing capability and autosampler.

c. LC column - Phenyl 3 x 150 mm containing 3.5 µm particles, Cat. No. XDB, Zorbax.

d. Guard column - 2.1 mm x 12.5 mm containing 5 µm particles, Eclipse XDB-C8.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents and solutions may be substituted for the following unless otherwise indicated:

1. Reagents

a. Ethyl ether - ACS Grade, 99%, Aldrich.

b. Water, LC grade - House distilled water passed through Waters MilliQ deionization system.

c. Acetonitrile - UV Grade, Cat. No. 015-4, Burdick & Jackson.

d. Sodium chloride - ACS Grade, Cat. No. 3624-01, Baker.

e. Ammonium hydroxide 28% - Cat. No. 38,053-9, Aldrich.

f. Hexane - Omnisolv. Cat. No. HX0296-1, EM.

g. Sodium phosphate dibasic, heptahydrate - ACS Grade, Cat. No. S-9390, Sigma.

h. Formic acid - ACS Grade, Cat. No. F-4636, Sigma.

i. Sodium phosphate monobasic, monohydrate - ACS Grade, Cat. No. S-9638, Sigma.

j. Sodium hydroxide - ACS Grade.

k. Methanol - HPLC grade, Mallinckrodt.

2. Solutions

a. 1M sodium chloride solution:

Transfer 58.45 g NaCl to a 1000 mL volumetric flask. Dissolve and dilute to volume with water.

b. 0.2M monobasic sodium phosphate monohydrate:

Transfer 27.6 g NaH_2PO_4 to a 1000 mL volumetric flask. Dissolve and dilute to volume with water. Stable for 6 months at 2-8 ºC.

c. 0.2M dibasic sodium phosphate heptahydrate:

Transfer 53.65 g Na_2HPO_4 to a 1000 mL volumetric flask. Dissolve and dilute to volume with water. Stable for 6 months at 2-8 ºC.

d. 30% sodium hydroxide:

Transfer 60 g NaOH to a 200 mL volumetric flask and dilute to volume with water. Stable for 6 months at 2-8 ºC.

e. 0.1M phosphate buffer, pH 9.0 (Buffer A):

Transfer 27 mL of 0.2M monobasic sodium phosphate monohydrate (a) and 473 mL of 0.2M dibasic sodium phosphate heptahydrate (b) to a 1000 mL beaker. Add water to approx. 900 mL. Adjust to pH 9.0 with 30% NaOH (c). Transfer to a 1000 mL volumetric flask and dilute to volume with water. Stable for 6 months at 2-8 ºC.

f. 0.03M sodium hydroxide:

Transfer 1.2 g NaOH to a 1000 mL volumetric flask and dilute to volume with water.

g. Mobile phase A (15/85 acetonitrile/water containing 1% formic acid):

To a 1000 mL graduated cylinder add 150 mL acetonitrile and 840 mL water. Mix and add 10 mL formic acid. Mix well.

h. Mobile phase B (20/80 acetonitrile/water containing 1% formic acid):

To a 1000 mL graduated cylinder add 200 mL acetonitrile and 790 mL water. Mix and add 10 mL formic acid. Mix well.

i. Mobile phase C (80/20 acetonitrile/water containing 1% formic acid):

To a 1000 mL graduated cylinder add 800 mL acetonitrile and 190 mL water. Mix and add 10 mL formic acid. Mix well.

j. Mobile phase D (60/40 acetonitrile/water):

To a 1000 mL graduated cylinder add 600 mL acetonitrile and 400 mL water. Mix well.

D. STANDARDS

Note: Equivalent standards and solutions may be substituted for any of the following:

1. Source

a. Desethylene ciprofloxacin: Bayer Corp., West Haven, CT.

b. Difloxacin HCl: Abbott Labs, North Chicago, IL.

c. Enrofloxacin: Bayer Corp., West Haven, CT.

d. Norfloxacin: Sigma-Aldrich, St. Louis, MO.

e. Ciprofloxacin: United States Pharmacopoeia (USP).

f. Desmethyl danofloxacin: Pfizer Pharmaceuticals, Groton, CT.

g. Sarafloxacin HCl: Abbott Labs, North Chicago, IL.

h. Danofloxacin Mesylate: Pfizer Pharmaceuticals, Groton, CT.

Note: Chemical structures of fluoroquinolones available in Appendix K.3.

2. Preparation

a. Individual drug stock standard solutions (100 µg/mL):

Using vendor's stated purity, or water and salt content, calculate the amount of material which contains 5 mg drug. Weigh out approximately this amount, accurately recording weight to nearest 0.1 mg. Transfer to 50 mL glass volumetric flask and dilute to mark with 0.03M NaOH (C.2.f). Calculate exact concentration based on purity and actual weight. Stable for 6 months at 2-8 ºC.

b. Mixed working standard solution (2 µg/mL):

Add 1.0 mL of each of the above drug stock solutions to a 50 mL volumetric flask and dilute to mark with Buffer A (C.2.e.). Stable for 1 month at 2-8 ºC.

c. External standard solution (12.5 ng/mL):

Transfer 12.5 µL of the Mixed working standard solution (b) to a 50 mL plastic centrifuge tube and add 2.0 mL of Buffer A (C.2.e). Prepare daily.

d. System suitability standard (12.5 ng/mL):

Transfer 12.5 µL of the mixed working standard solution (b) to a 50 mL plastic centrifuge tube and add 2.0 mL of methanol. Prepare daily.

E. SAMPLE PREPARATION AND CLEANUP

1. Sample Handling and Preparation:

a. Freshly collected samples must be kept cold before and during shipping to laboratory. Once received at laboratory, samples must be frozen (< -10 ºC) prior to processing if they cannot be prepared on the day of receipt.

b. If sample is frozen, allow to thaw, but keep as cold as possible. Dissect away fat and connective tissue from liver and muscle. Homogenize liver in a Waring blender and muscle in a Robot Coupe®.

Note: After each homogenization, rinse the blending jars with tap water and dry.

2. Extraction and Cleanup:

   a. Weigh 1.0 ± 0.10 g of homogenized tissue into a 50 mL disposable polypropylene centrifuge tube.

      Note: Prepare controls (to be included as part of each sample batch) at this time:

      i. Negative controls are tissues from animals known to be free of drugs. If these are not available, tissue from an unknown source may be used provided it is first tested and shown to be free of contaminants.

      ii. Positive controls are negative tissues that have been fortified with fluoroquinolones before extraction. To prepare a 25 ppb fortified sample, add 12.5 µL of a 2 µg/mL mixed drug solution (D.2.b) to 1 g tissue and vortex 30 min on a Vibrax mixer. Store in a dark cabinet (room temp.) for 0.5 hr to allow time for the FLQ's to interact with matrix.

   b. Add approx. 3 mL acetonitrile and 0.25 mL conc. ammonium hydroxide to each tube.

   c. Homogenize tube contents for approx. 20 sec. Rinse homogenizer tip with approx. 0.5 mL of acetonitrile directly into the sample tube. Centrifuge at approximately 5000 rpm (2800 g.) for 10 min.

   d. Decant supernatant into another 50 mL polypropylene centrifuge tube.

   e. Add 0.75 mL of water to the pellet from the centrifugation and repeat steps b-c, adding the supernatant to the tube in step d.

   f. Add approx. 3 mL ethyl ether, 3 mL hexane and 0.25 mL 1M NaCl to the combined supernatants for each sample.

   g. Vortex the tubes for 15 sec.

   h. Using a disposable Pasteur pipette, remove as much of the top layer as possible and discard. There may be 3 layers at this point but discard only the uppermost layer.

   i. Evaporate the combined organic solution to near dryness (approximately 200 µL) in a Turbovap maintained at approximately 50 ºC.

   j. Add 2.0 mL Buffer A (C.2.e) to each tube and vortex for approx 10-20 sec. Filter contents through a 0.2 µm nylon syringe filter into an autosampler vial.

F. ANALYTICAL PROCEDURE

Note: Instrumental parameters yielding equivalent analytical results may be used.

1. Instrument Operating Parameters – LC System:

Note: Typical values listed below. Flows and elution gradient may be optimized, if necessary, for best separation and response.

a. Install and degas mobile phases and install column and guard cartridge per manufacturers' instructions. Flush HPLC column with 20 column volumes (35 mL) of methanol, water, and 60/40 acetonitrile/water (C.2.j) prior to further use of the column. Set initial composition to flow 15/85 acetonitrile/water containing 1% formic acid (C.2.g) at 500 µL/min.

b. Set up the HPLC to run the following gradient:

Time in min.	Flow in mL/min.	Mobile Phase A*	Mobile Phase B*	Mobile Phase C*
0.00	0.50	100%	0%	0%
10.00	0.50	100%	0%	0%
18.00	0.50	0%	100%	0%
20.00	0.50	0%	100%	0%
22.00	0.50	0%	0%	100%
24.00	0.50	0%	0%	100%
27.00	0.50	100%	0%	0%
30.00	0.50	100%	0%	0%

A* = C.2.g, B* = C.2.h, C* = C.2.i.

c. Set injection volume to 20 µL.

d. Use a needle wash step with methanol or water.

2. Instrument Operating Parameters – Mass Spectrometer:

   a. Calibrate the Finnigan LCQ ion trap mass spectrometer with APCI interface according to the manufacturer's specifications.

   b. Set Capillary Temp to 200 °C.

   c. Operate in Pos mode.

   d. Flow inject the system suitability standard through a 5 µL loop and obtain the MS1 precursor ion centroids. The following settings should result in optimal ion intensities:

Capillary temperature	200 °C
APCI vaporizer temperature	470 °C
Sheath gas flow	50
Aux gas flow	5
Capillary voltage	2 V
Tube lens offset	-5 V
Micro scans	2
Ion time	100 msec
Source current	5.00 µA

3. Procedure for Instrumental Analysis of Samples, Controls, and Standards:

   a. Turn on pump and set up mass spectrometer. Equilibrate column in mobile phase at 0.5 mL/min for at least 30 min.

   b. Flow inject the system suitability standard through a 5 µL loop and obtain the MS1 precursor centroids. Using the MS1 mass assignments previously obtained, flow inject a sample under MS/MS conditions for each analyte and obtain the MS2 precursor ion centroids and accompanying collision energies.

   c. Inject the external standard through the HPLC system and determine the retention times of each FLQ. Isolate each peak within a suitable window for acquisition. After setting in the window segments, monitor the following ion transitions:

Fluoroquinolone	Scan Program	Trans.	Amp	Q	Time	IsoW
Desethylene Cip.	306→(80-310)	MS^2	35%	0.250	30	2.0
Norfloxacin	320→276→(75-330)	MS^2	30%	0.350	30	2.0
		MS^3	35%	0.250	30	2.0
Ciprofloxacin	332→288→(75-340)	MS^2	35%	0.350	30	2.0
		MS^3	35%	0.250	30	2.0
Danofloxacin	358→314→(85-370)	MS^2	35%	0.350	30	2.0
		MS^3	35%	0.250	30	2.0
Desmethyl-Dano.	344→(90-350)	MS^2	40%	0.250	30	2.0
Enrofloxacin	360→316→(85-370)	MS^2	40%	0.350	30	2.0
		MS^3	35%	0.250	30	2.0
Sarafloxacin	386→342→(90-400)	MS^2	35%	0.350	30	2.0
		MS^3	38%	0.250	30	2.0
Difloxacin	400→356→(95-410)	MS^2	38%	0.350	30	2.0
		MS^3	35%	0.250	30	2.0

d. Inject the recovered standard and verify retention time, divert valve switching time, and spectral comparison to the external standard.

e. Inject the sample extracts. In order to control carryover, precede each sample analysis with a blank buffer injection as needed.

f. As a test of retention time and instrument response stability, reinject the spiked control extract and one or more chromatographic standards at the end of the sample set. Depending on instrument variability and length of sample set, additional spiked control extract or standard injections may be interspersed throughout the sample set.

g. Column, Pump, and APCI Interface Care: At the end of set of analyses, flush the column for 30 min with mobile phase D (60/40 acetonitrile/water) at 0.50 mL/min.

G. CONFIRMATION

1. Data Processing. Use the QUAL Browser to view total ion current, base ion chromatogram, and/or a total ion chromatogram (TIC) for each drug for each data file. Note retention time of any visible peaks in a drug window. Generate averaged spectra across the retention time window for each drug. This is usually from near the start to near the end of the peak visible in the chromatograms, though a smaller range may be used to avoid a spurious ion spike. Where no peak is visible, use the same settings as in a contemporaneous fortified or positive control extract.

2. Confirmation Criteria:

   a. Retention times of extract peaks in one or more of the ion chromatograms must match the peak retention time of a contemporaneous (within same analysis set on same day) fortified control extract chromatogram within ± 4%.

   b. The FLQ peak in the total ion chromatogram (TIC) (see below for ions used for each drug TIC) is present at a S/N ratio of at least 3/1. This is estimated by visual inspection of the TIC.

   c. The spectrum from the extract must visually match spectra from external standards in the same data set. The base ion must be the same. At least two qualifying ions should be present, readily distinguished from background ions, and have relative abundances comparable to those in the standard. There should be a general absence of nonspecific ions.

      Major specific ions for each FLQ are listed below:

Fluoroquinolone	Precursor ion(s)	Spectra Range	Base ion	Qualifying Ions
Desethylene cip.	306	80-310	306	289, 286, 263
Norfloxacin	320, 276	75-330	256	257, 233, 219
Ciprofloxacin	332, 288	75-340	268	245, 231, 205
Danofloxacin	358, 314	85-370	294	283, 245, 219
Desmethyl dano.	344	90-350	344	327, 300, 283
Enrofloxacin	360, 316	85-370	245	296, 288, 268
Sarafloxacin	386, 342	90-400	322	299, 285, 281
Difloxacin	400, 356	95-410	299	336, 311, 285,

   d. The quality assurance positive and negative control samples confirm and fail to confirm, respectively, for the presence of the appropriate drug.

3. Criteria for Repeating an Analysis:

   Note: Sample analyses may be repeated under the following conditions:

   a. The conditions described in G.2.d are not met.

   b. The instrument is suspected to be malfunctioning, as demonstrated by clearly aberrant standard spectra; failure of a calibration check performed shortly after analysis of the sample set; instrumental parameters, especially vacuum readings, outside of normal operating range; or other conditions noted and documented by the analyst.

   c. There is suspected carryover from a previous high concentration sample or standard. In this case, the sample should be reanalyzed after the cause of the carryover has been identified and measures taken to prevent its reoccurrence.

   d. There is strong evidence of FLQ presence, but multiple extraneous ions with relative abundance exceeding that of the FLQ base ion prevent unambiguous confirmation. In this case, it may be appropriate to reanalyze the suspected positive sample together with a chromatographic standard, and negative and positive QA controls.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment — Safety glasses, disposable gloves, lab coats.

2. Hazards:

Procedure Step	Hazard	Recommended Safe Procedures
Acetonitrile, Hexane	Highly flammable and toxic liquid. May cause skin irritation.	Use in a fume hood away from all electric devices and open flames. Avoid breathing vapors.
Formic acid and solutions made from same. Conc. NH_4OH	Corrosive. Danger of chemical burns.	Prepare solutions in a fume hood. Wear PPE and avoid contact with skin.
Ethyl ether	Highly flammable. May detonate due to formation of peroxides.	Order only as much as needed for three months of testing. Test for peroxides before use and monthly thereafter. Discard this solvent upon discontinuation of the project.

3. Disposal Procedures:

Procedure Step	Hazard	Recommended Safe Procedures
Acetonitrile, Hexane	See section 2 above.	Collect waste in a sealed container and store in a cool, well ventilated, flammable liquid storage area/cabinet for disposal in accordance with local, state and federal regulations.
Acids and acidic reagents	See section 2 above.	Collect waste in a sealed container and store in a cool, well ventilated, acid liquid storage area/cabinet for disposal in accordance with local, state and federal regulations.
Bases and basic reagents	See section 2 above.	Collect waste in a sealed container and store in a cool, well ventilated, base liquid storage area/cabinet for disposal in accordance with local, state and federal regulations.
Ethyl ether	See section 2 above.	Collect waste in a separate container. Waste will be disposed of in an alternate manner.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

   Refer to Section G.2 for Confirmation Criteria.

2. Readiness To Perform (FSIS Training Plan):

   a. Familiarization:

      i. Phase I: Standards - Inject external standard solutions (D.2.c.) in duplicate on at least three different days and verify instrument response is adequate for confirmatory purposes.

      ii. Phase II: Fortified samples - Analyze on three separate days, one blank bovine liver, one fortified bovine liver at 25 ppb, one blank bovine muscle, and one fortified bovine muscle at 25 ppb.

      NOTE: Phase I and Phase II may be performed concurrently.

      iii. Phase lll: Check samples for analyst accreditation.

      (a) 6 check samples fortified at levels between 1-2 times Minimum Proficiency Level (MPL) using analytes and concentrations

unknown to the analyst. These six unknowns shall be composed of three bovine liver and three bovine muscle tissues and at least one check sample should be blank . Each set must include a positive control and a negative control.

(b) Notification from QAM is required to analyze official samples.

b. Acceptability criteria:

Refer to I. 1.

3. Intralaboratory Check Samples

a. System, minimum contents:

i. Frequency: One per week per analyst when samples analyzed.

ii. Records are to be maintained for review.

b. Acceptability criteria:

If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst.

ii. Investigate and identify probable cause.

iii. Take corrective action.

4. Sample Acceptability and Stability:

a. Matrices: Bovine liver and muscle.

b. Condition upon receipt: chilled or frozen, minimum weight is 50 grams.

c. Sample storage:

i. Time: 2 weeks for blended/homogenized samples.

ii. Condition: Frozen (less than -10 ºC).

5. Sample Set:

a. Negative liver/muscle control sample (E.2.a.i.). QA samples are to be of the same species and tissues as the samples analyzed.

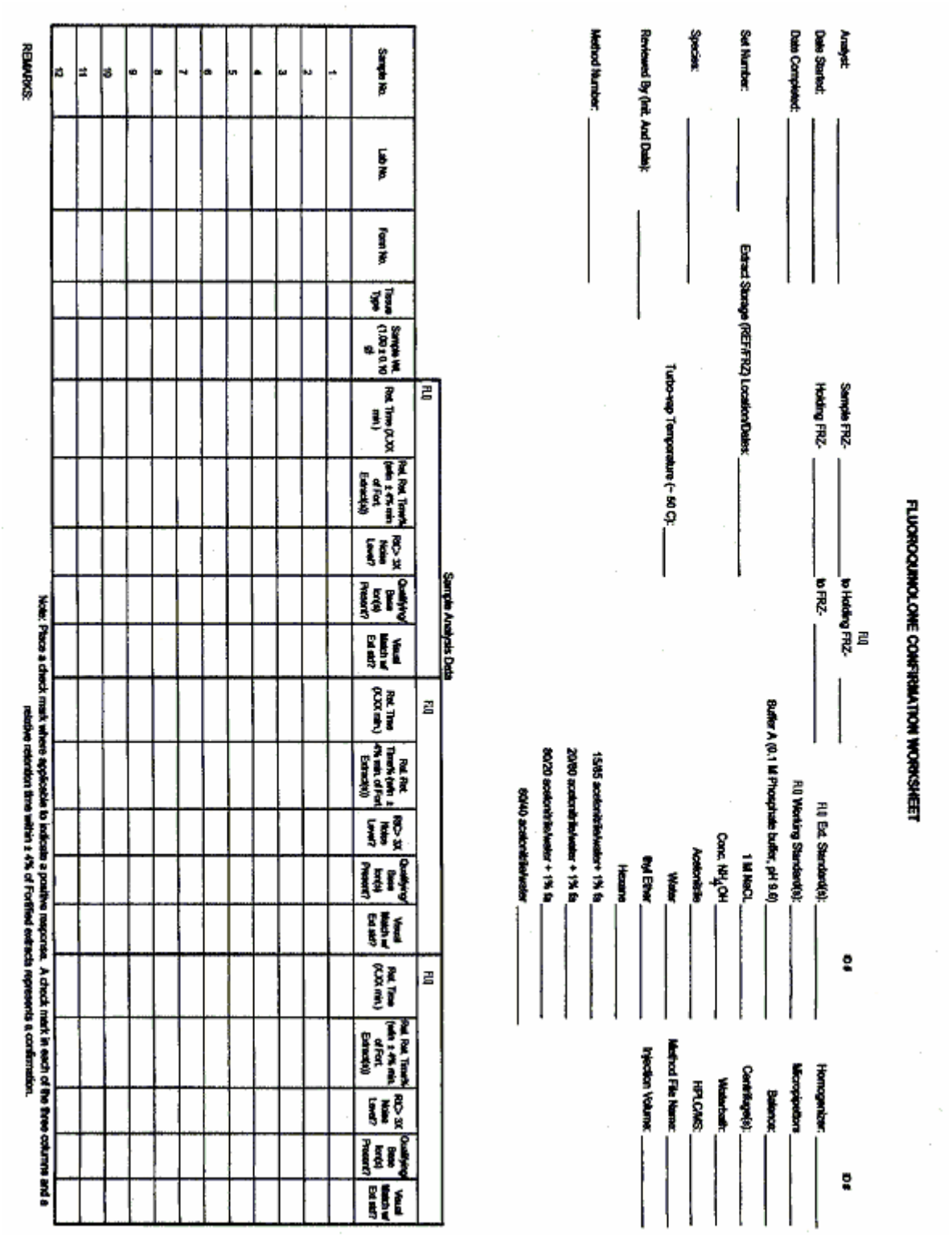
b. Positive liver/muscle control sample (E.2.a.ii.). QA samples are to be of the same species and tissues as the samples analyzed.

c. Samples.

6. Minimum Proficiency Level: 25 ppb.

J. WORKSHEET

The following worksheet is an example**:**



FLUOROQUINOLONE CONFIRMATION WORKSHEET

Analyst: _____ FLQ ID #: _____

Date Started: _____ Sample FRZ-_____ to Holding FRZ-_____ FLQ Std. Standard(s): _____ Homogenizer: _____ ID #

Date Completed: _____ Holding FRZ-_____ to FRZ-_____ FLQ Working Standard(s): _____ Micropipettors: _____

Set Number: _____ Extract Storage (REF/FRZ) Location/Dates: _____ Buffer A (0.1 M Phosphate buffer, pH 9.6): _____ Balance: _____

1 M NaCl: _____ Centrifuge(s): _____

Species: _____ Conc. NH_4OH: _____ Waterbath: _____

Acetonitrile: _____ HPLC/MS: _____

Reviewed By (Init. And Date): _____ Turbo-vap Temperature (~ 50 C): _____ Water: _____ Method File Name: _____

Ethyl Ether: _____ Injection Volume: _____

Hexane: _____

Method Number: _____ 15/85 acetonitrile/water+ 1% fa: _____

20/80 acetonitrile/water + 1% fa: _____

80/20 acetonitrile/water + 1% fa: _____

60/40 acetonitrile/water: _____

Sample Analysis Data

					FLQ								FLQ								FLQ							
Sample No.	Lab No.	Form No.	Tissue Type	Sample Wt. (1.00 ± 0.10 g)	Ret. Time (XX.XX min.)	Ret. Ret. Time% (with ± 4% min of Fort. Extract(s))	RID> 3X Noise Level?	Qualifying Ions(4) Present?	Visual Match w/ Std?	Ret. Time (XX.XX min.)	Ret. Ret. Time% (with ± 4% min of Fort. Extract(s))	RID> 3X Noise Level?	Qualifying Ions(4) Present?	Visual Match w/ Std?	Ret. Time (XX.XX min.)	Ret. Ret. Time% (with ± 4% min of Fort. Extract(s))	RID> 3X Noise Level?	Qualifying Ions(4) Present?	Visual Match w/ Std?
1																			
2																			
3																			
4																			
5																			
6																			
7																			
8																			
9																			
10																			
11																			
12																			

Note: Place a check mark where applicable to indicate a positive response. A check mark in each of the three columns and a relative retention time within ± 4% of Fortified extracts represents a confirmation.

REMARKS:

K. APPENDIX

1. Reference:

   Schneider, M. J., Donoghue, D. J. (2002), *J. Chromatogr. B* **780***, 83-92.*

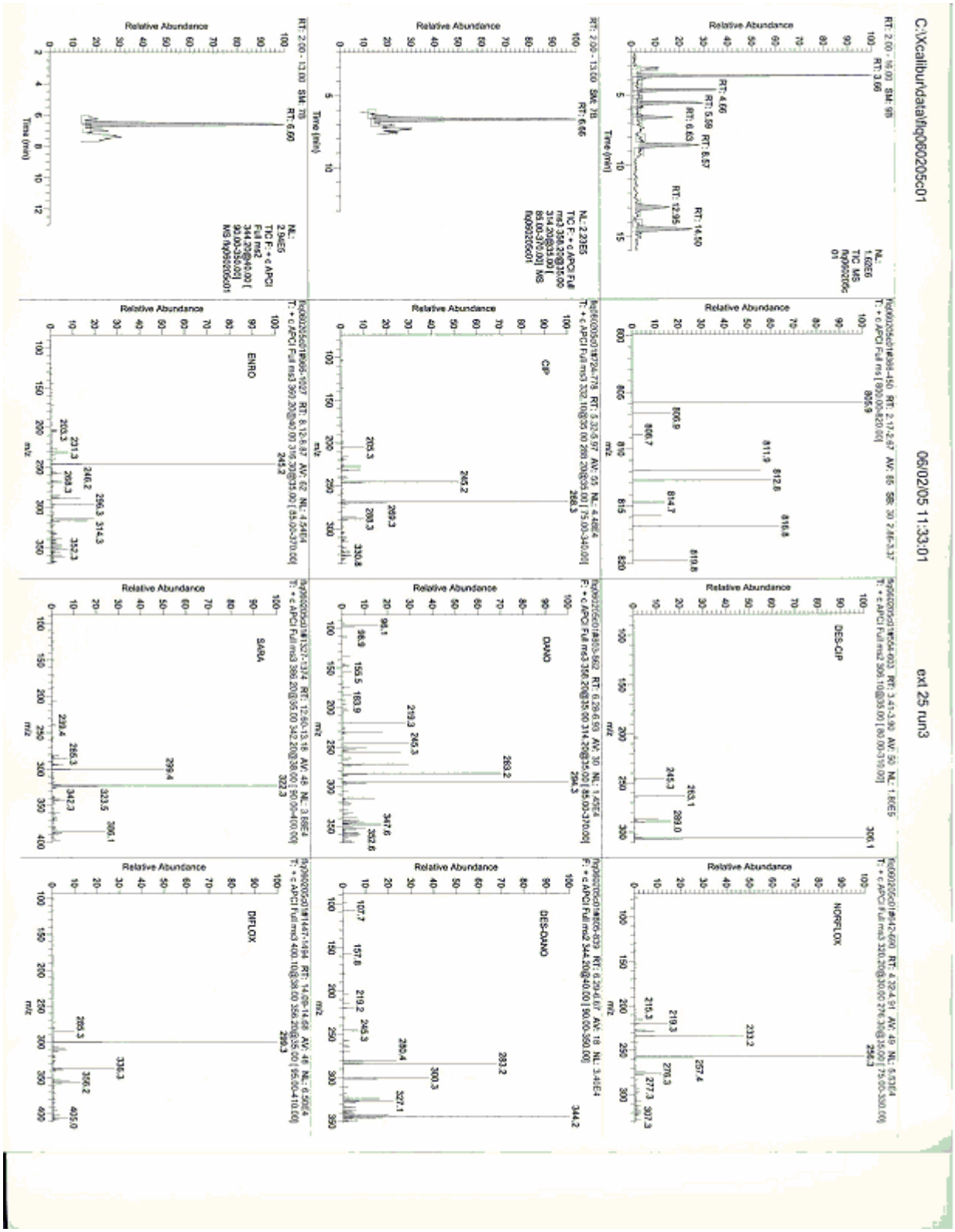
2. Chromatograms and Spectra:

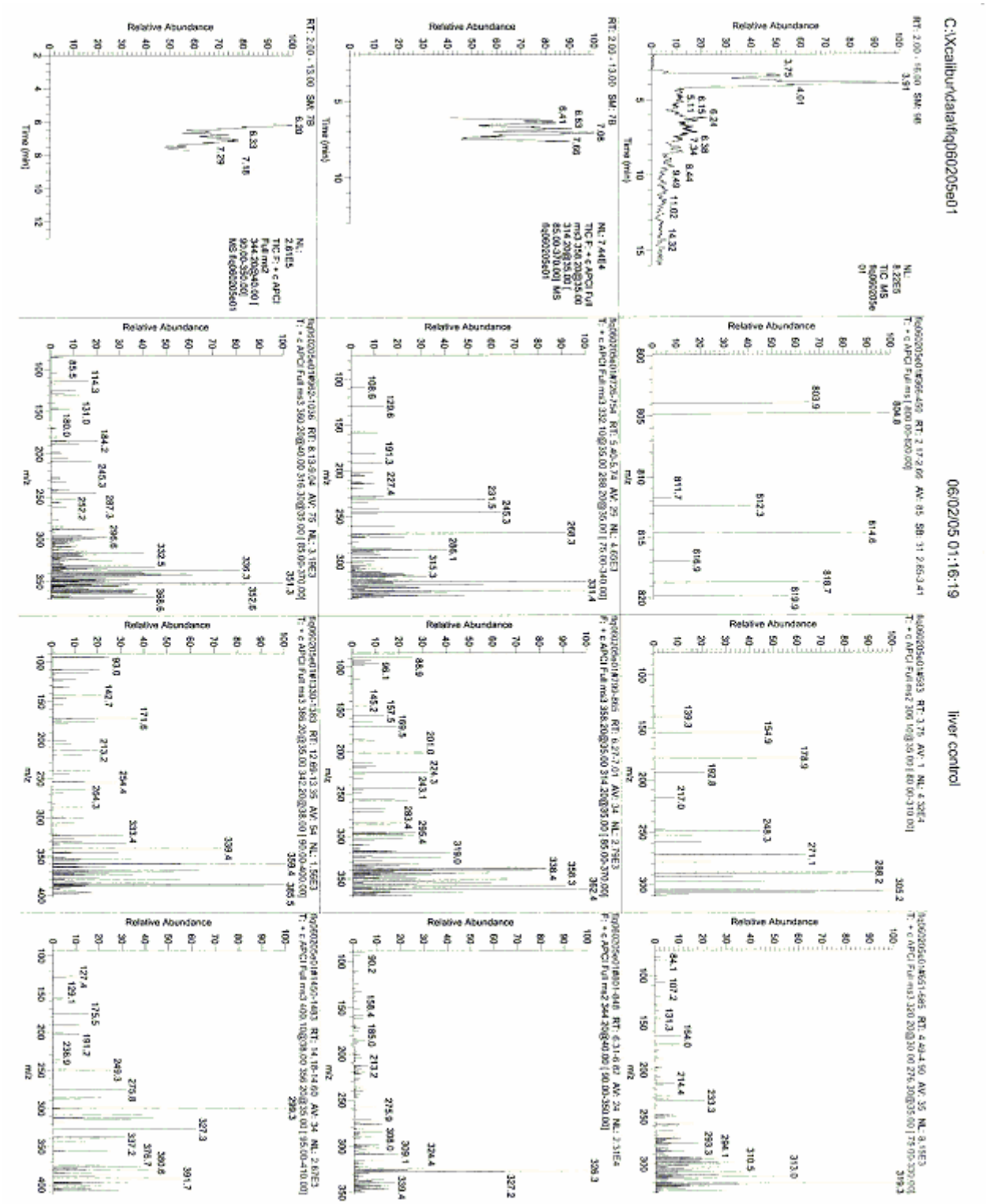
   The following chromatograms and spectra are shown on the next 3 pages:

   a. External standards at 25 ppb.

   b. Blank Beef liver.

   c. Beef Liver Recovery at 25 ppb.


C:\Xcalibur\data\flq060205c01
06/02/05 11:33:01
ext 25 run3
Relative Abundance
Time (min)
RT: 3.66
RT: 4.66
RT: 5.59
RT: 6.63
RT: 8.57
RT: 12.95
RT: 14.50
RT: 6.66
RT: 6.60
DES-CIP
NORFLOX
CIP
ENRO
DANO
DES-DANO
SARA
DIFLOX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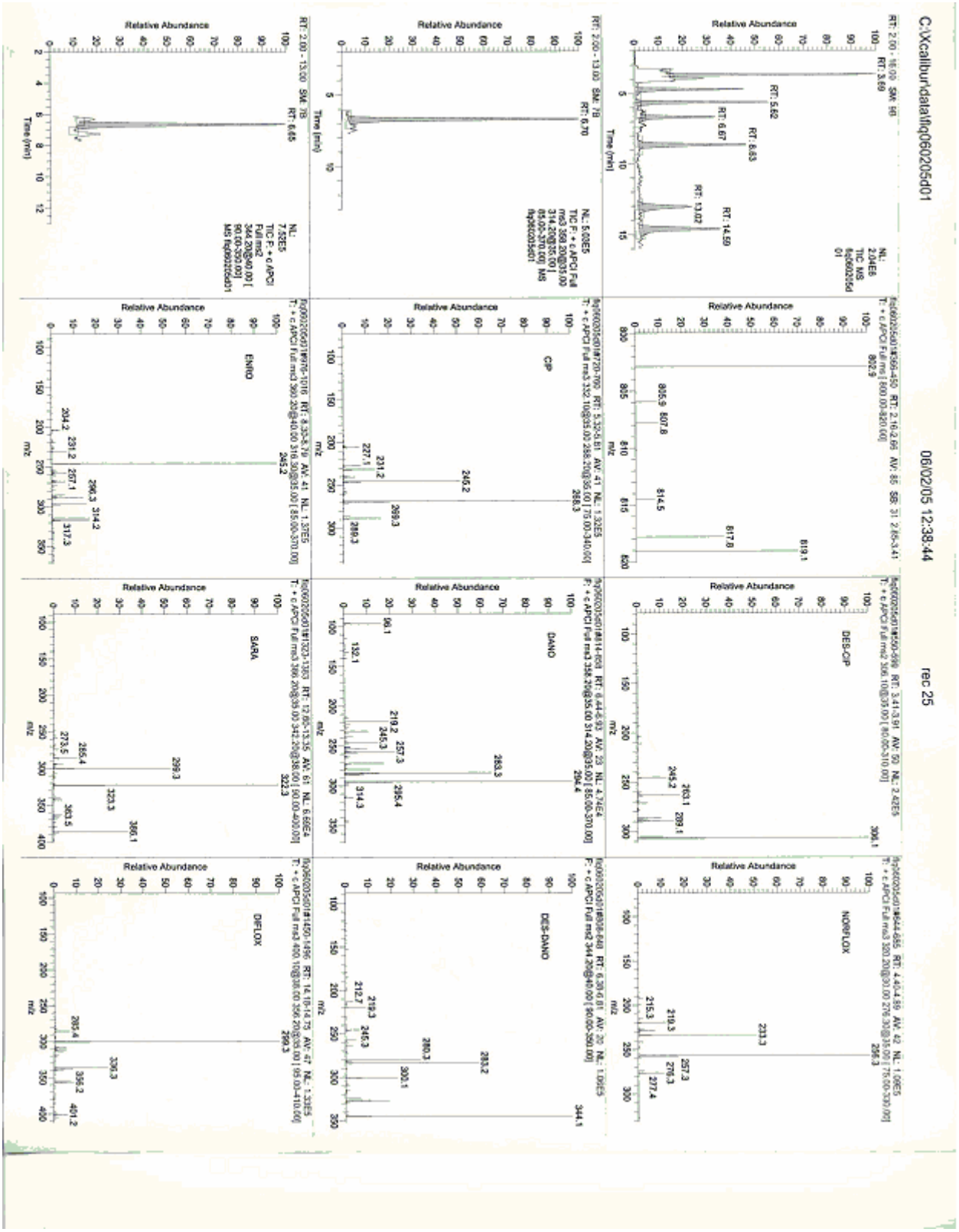
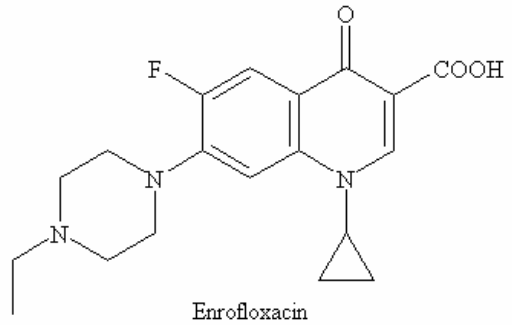
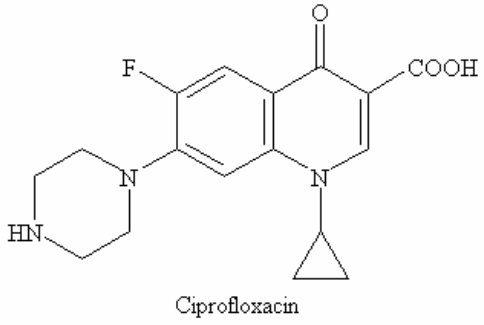
liver control


C:\Xcalibur\data\flq060205d01
06/02/05 12:38:44
rec 25
Relative Abundance
Time (min)
DES-CIP
CIP
ENRO
NORFLOX
DES-DANO
DANO
SARA
DIFLOX

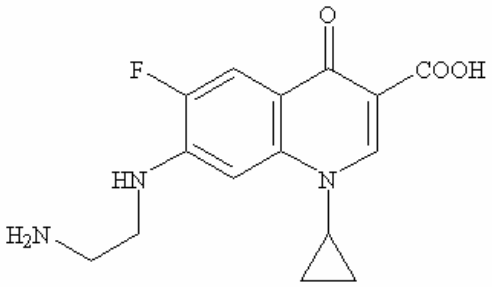
3. Structures of Fluoroquinolones:



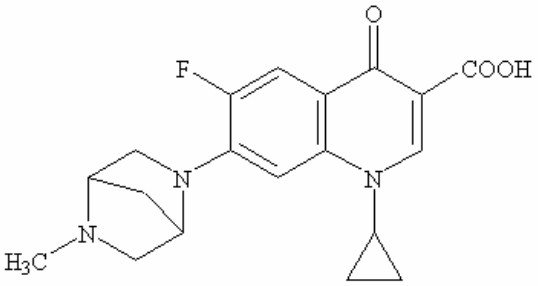
Enrofloxacin



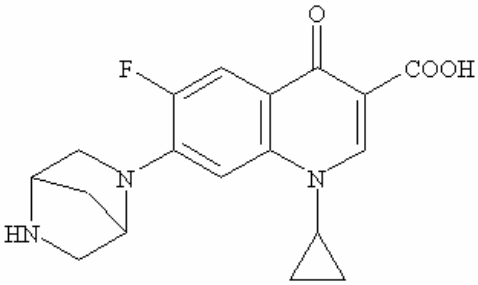
Ciprofloxacin



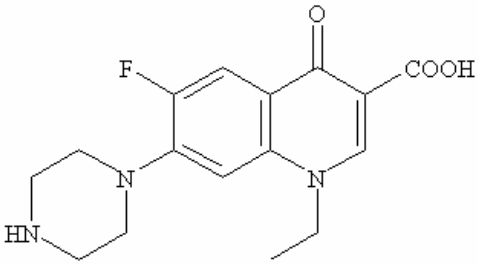
Desethylene ciprofloxacin



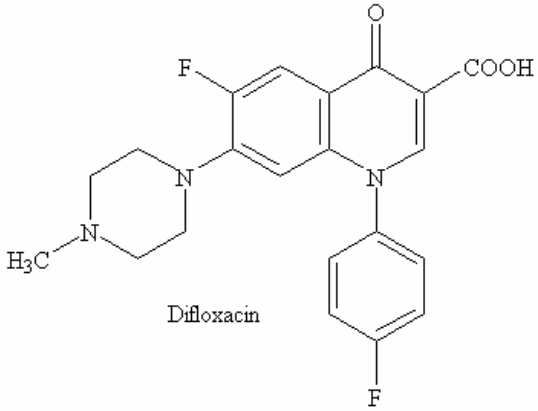
Danofloxacin



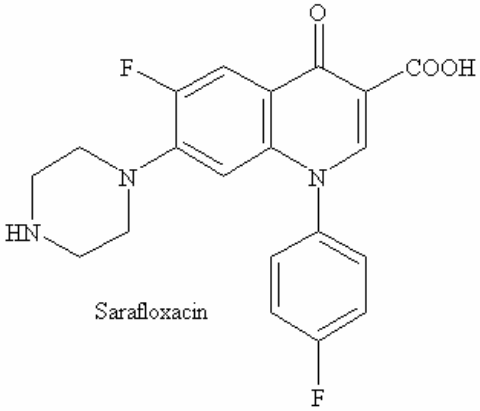
Desmethyl danofloxacin



Norfloxacin



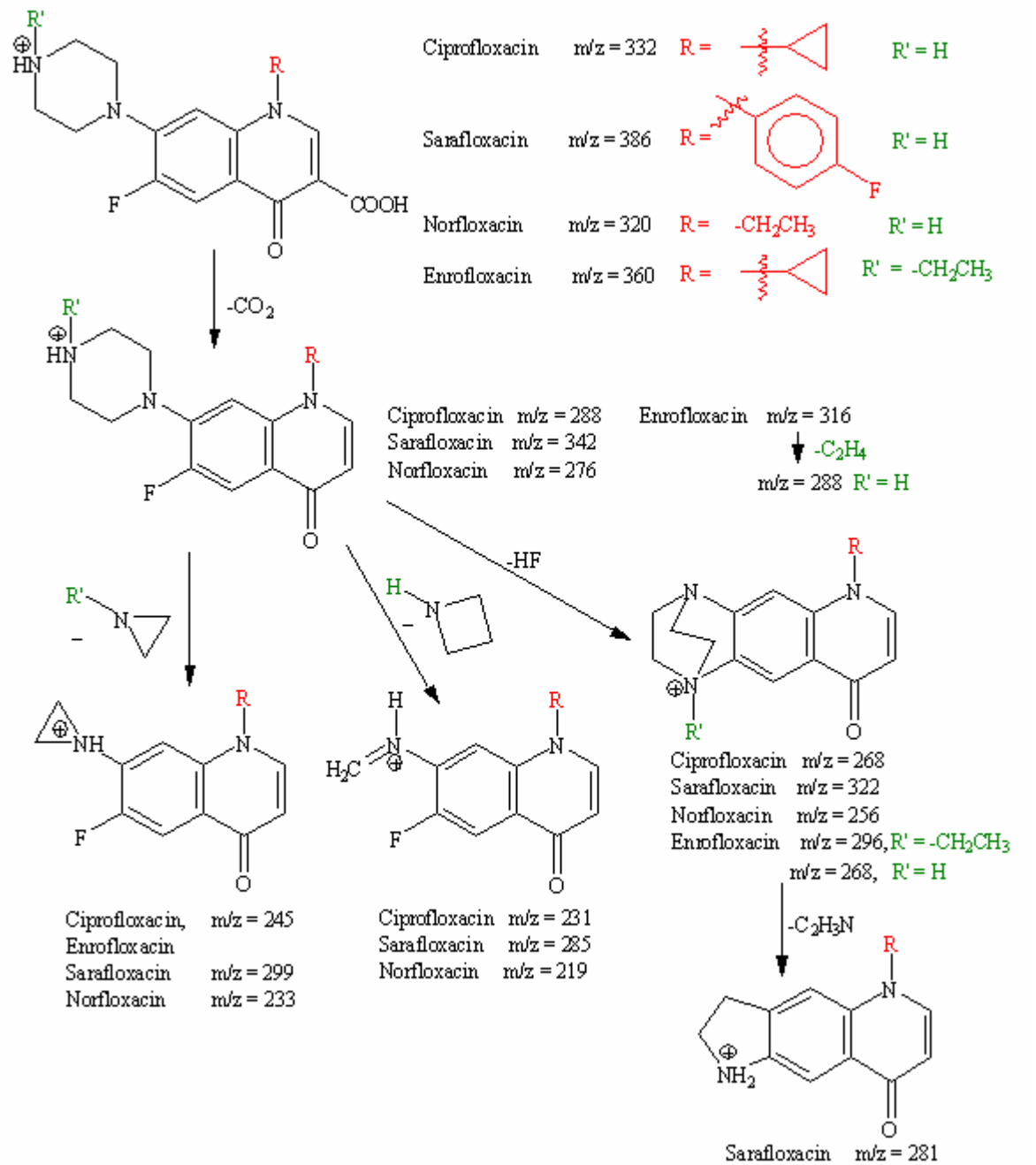
Difloxacin



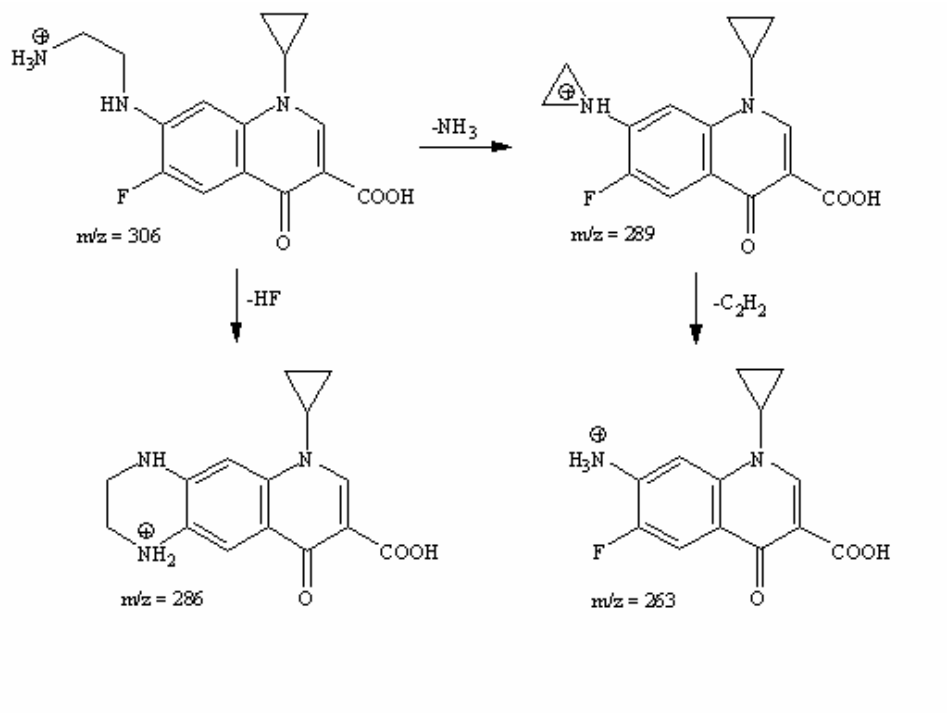
Sarafloxacin

4. Proposed Fragmentation Patterns of Fluoroquinolones:

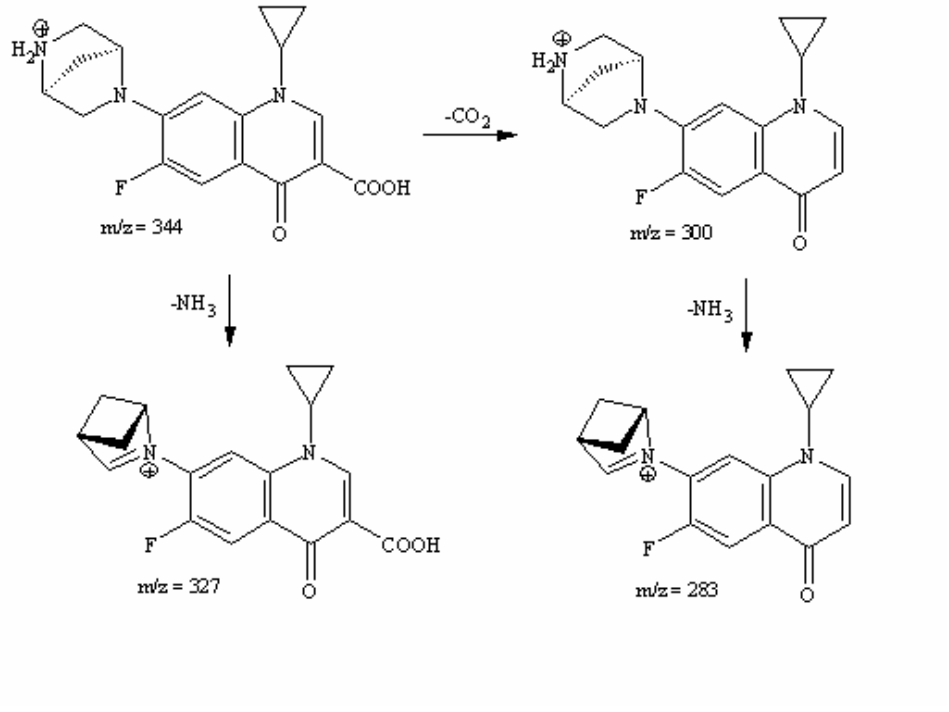
   a. Ciprofloxacin, Sarafloxacin, Norfloxacin, and Enrofloxacin



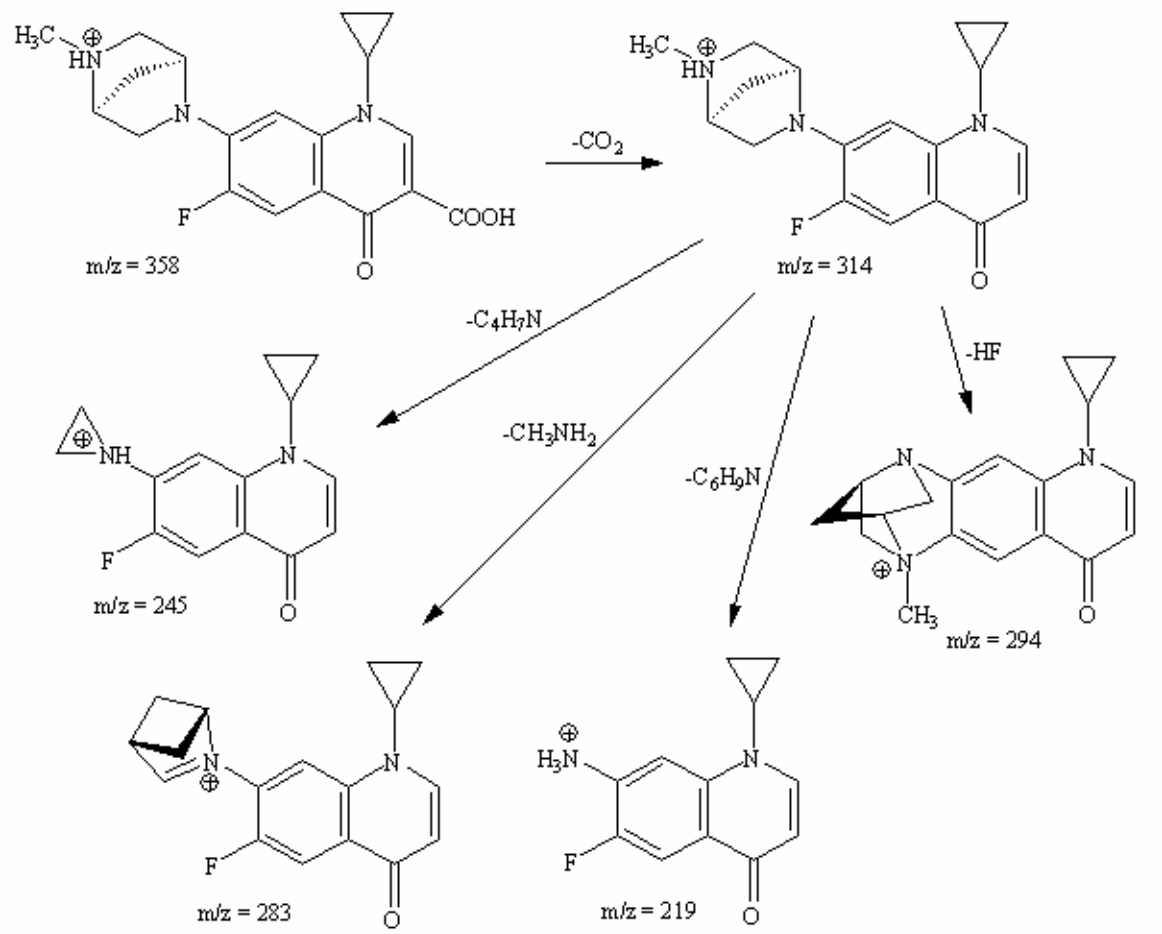
b. Desethylene ciprofloxacin



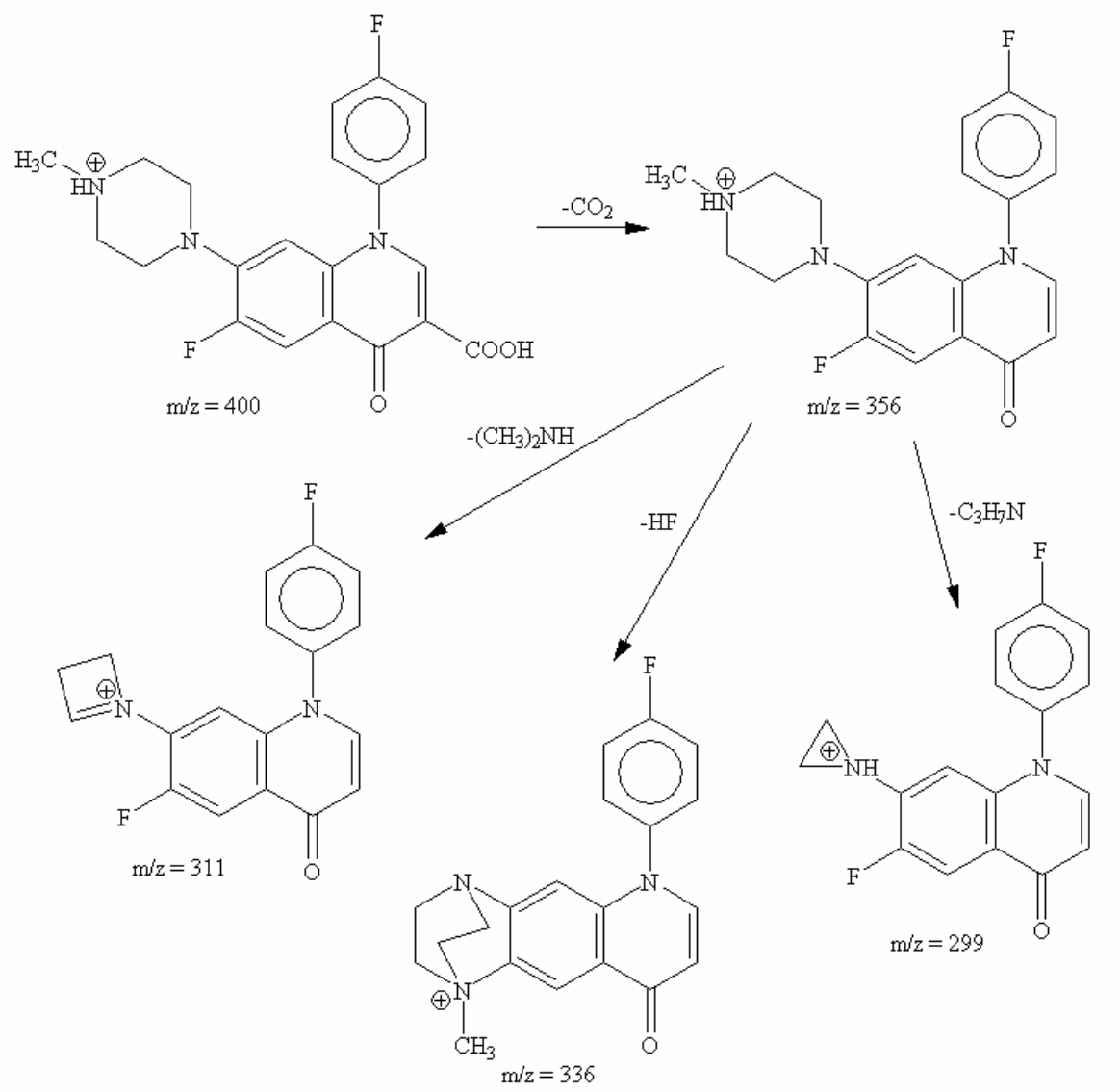
c. Desmethyl danofloxacin



d. Danofloxacin



e. Difloxacin



L. APPROVALS AND AUTHORITIES

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