Confirmation of fluoroquinolone residues in salmon and shrimp tissue by LC/MS: Evaluation of single quadrupole and ion trap instruments

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Abstract

A multiresidue procedure previously developed to confirm fluoroquinolone (FQ) residues in catfish tissue has been used to positively identify the same residues in salmon and shrimp. Using a single quadrupole instrument with in-source collision induced dissociation, ciprofloxacin, enrofloxacin, sarafloxacin, and difloxacin residues were positively identified in salmon muscle and shrimp tissue fortified at 20-80 ppb. These residues were also confirmed in extracts from incurred tissue with final drug concentrations ranging from 10-1000 ppb in salmon and 5-10 ppb in shrimp.

In addition, this method was adapted for use with an ion trap LC/MS^n instrument by collecting data dependent MS^2 and MS^3 scans to yield structurally significant ions. Salmon control, fortified and incurred tissue were reanalyzed for confirmation of FQs using the same extraction and chromatographic conditions developed for the initial LC/MS method. A comparison of the data obtained with a single quadrupole and the ion trap instrument is included.

Introduction

Quinolones are pyridone carboxylic acid derivatives that are effective against gram negative bacteria. Fluoroquinolones (FQs) are a fluorine-containing subclass of the quinolones that have been found to be more effective and also exhibit activity towards some gram-positive bacteria. Sarafloxacin and enrofloxacin were approved for use in poultry in the United States. Residues of fluoroquinolones in food products are of concern due to the development of antibacterial resistance to these drugs in humans. For example, there are reports that show a strong link between the use of FQ antibiotics in chickens and an increase in *Campylobacter* resistant infections in humans (1, 2). Because of these concerns the FDA has recommended withdrawal of prior approval (3) and has banned the extra-label use of these drugs in food producing animals (4). One possible extra-label use would be the use of these drugs for aquacultured species as it has been reported that these drugs are effective against bacterial infections in farm-raised fish (5). Because of the high level of concern with these drugs, residues in food need to be monitored carefully. As a result, several analytical methods have been developed to determine and confirm FQ residues in various food matrices including aquatic species (6-14).

In order to unambiguously identify animal drug residues in a matrix, some structural information must be obtained from mass spectral analysis. The criteria that constitute absolute confirmation have been discussed and general guidelines have been suggested (10,15-16). The mass spectral characteristics of fluoroquinolone drugs needed for drug confirmation have been investigated using several types of mass spectrometers including single (6,11) and triple quadrupole instruments (9-12). There have also been recent reports on the use of an ion trap mass spectrometer for the analysis of these drugs, specifically to screen for FQs in eggs (13) and to characterize FQ residues in chicken (14) tissue.

The focus of the current work is to demonstrate that the method developed for confirmation of FQs in catfish can be used successfully for salmon and shrimp tissue. In addition data obtained using a single quadrupole instrument is compared to that using an ion trap mass spectrometer. Our aim was to develop a rugged regulatory method that can be adapted for use with different type mass spectrometers as long as the resulting data meets defined confirmation criteria.

Method

Sample and Standard Preparation

The procedure used to extract the fluoroquinolones from fish and shrimp tissue was the extraction developed for the determination of these residues by LC/fluorescence (7-8). The tissue was homogenized with ethanol/acetic acid and isolated on a propyl sulfonic acid solid phase extraction cartridge. The residues were eluted from the cartridge with basic methanol which was evaporated and then the extracts were reconstituted in the mobile phase. A more detailed description of all reagents and apparatus used is available in cited references (6-8). A

couple of modifications were found to be acceptable, but are not necessary. For example the tissue extracts could be placed in the freezer (- 20 °C, 30-60 min) and centrifuged again before application to the extraction columns to expedite the flow through the columns. In addition, extracts could be dissolved in either the LC/fluorescence mobile phase or the LC/MS mobile phase described here. In contrast to the previous LC/MS method for catfish (6), the final volume of sample extract was 1 mL as in the determinative method.

For validation of the method, residues were confirmed by comparing extracts of control (positive and negative) and incurred salmon tissue to external standards. A positive control, i.e. fortified salmon tissue, was analyzed along with incurred extracts. Preparation of fortified tissue has been described previously (6-8). The incurred salmon tissues that were used to validate this confirmation method were from fish dosed orally as follows: fish #1 ENR 5 mg/kg; fish #2 ENR 0.5 mg/kg; fish #3 ENR 0.05 mg/kg; fish #4 SAR 5 mg/kg; fish #5 SAR 0.5 mg/kg; fish #6 DIF 5 mg/kg; fish #7 DIF 0.5 mg/kg; fish #8 DIF 0.05 mg/kg and all were then sacrificed after 18 hr. The incurred shrimp tissues were from animals dosed orally as follows: shrimp #1 dosed with ENR at 10 mg/kg, sacrificed after 9.5 hr; shrimp #2 SAR 10 mg/kg, sacrificed after 2 hr; and shrimp#3 DIF 10 mg/kg, sacrificed at 11 hr. The residue levels found in these incurred tissues were determined by the LC/fluorescence method (8) and are also listed here (Tables 4 and 5).

Standards were analyzed with each set of samples (at the beginning and end of a set of samples, and in the middle of the sequence if many samples are being analyzed). Solvent blanks (mobile phase) were analyzed to ensure that there was no carryover from the previous sample or standard.

LC/MS Conditions

Regardless of the instrument used, certain performance criteria were incorporated into the operating parameters. These include mass calibration, tuning, and verification of appropriate fragmentation patterns. Mass axis calibrations were performed according to the instrument manufacturers' specifications. Signal optimization (tuning) parameters were adjusted to maximize the abundance of ions in the mass range of the protonated molecular ions of the FQs (m/z 300-400). While the exact relative abundance ratios of ions will vary depending on the MS technique used, the parameters were optimized so that the fragment ions were of significant abundance and met signal/noise criteria. The conditions for the single quadrupole instrument were the same as those used to confirm FQ residues in catfish. Parameters for the ion trap were optimized for selectivity and sensitivity. Tables 1 and 2 describe the specific operating procedures for the two instruments used to validate this method in salmon and shrimp tissue.

Calculations

The calculations used depended on the type of data collected. The important point was to evaluate the data obtained to determine if they met recognized confirmation criteria (15-16). For data obtained by SIM analysis (single quadrupole), ion chromatograms were generated for each of the four ions monitored for each compound. These ion chromatograms were smoothed using

a Gaussian smoothing function (0.05 min full width half maximum). The resulting peaks in the ion chromatograms were then integrated using the data system. The relative peak areas from the ion chromatograms were calculated and compared to those calculated from standards run the same day.

For data collected on the ion trap, extracted ion chromatograms for the full MS (m/z trace corresponding to MH^+) and MS^2 (m/z trace corresponding to MH-CO_2^+) were generated along with the total ion chromatograms of MS^3 scans. These chromatograms were drawn using a stick to represent each scan or by using the more conventional point to point lines to connect data points. The MS^2 and MS^3 spectra were obtained by averaging across these chromatographic peaks. As scan (as opposed to SIM) data were obtained, the relative abundances were calculated from the tabulated spectra, rather than the integration of ion chromatograms.

Results and Discussion

The purpose of this work was two-fold. First, to determine if the method developed for confirmation of FQ residues in catfish tissue was applicable to salmon and shrimp tissue. Secondly, to expand the scope of this method so that it could be performed using an ion trap LC/MS instrument.

Using the method developed for confirmation of these drug residues in catfish tissue, it was possible to confirm all four FQs in both fortified and incurred salmon and shrimp tissue. The same (single quadrupole) MS program that was used for the confirmation in catfish tissue was used to confirm the FQ residues in these tissues. The in-source collisional induced dissociation (CID) mass spectra for all four FQ compounds contain ions representing MH^+, $[MH - H_2O]^+$, $[MH - CO_2]^+$. There was also a fourth ion available for monitoring in each mass spectrum. These ions corresponded to: CIP $[MH - H_2O - C_3H_4 - NC_2H_5]^+$, m/z 231; ENR $[MH - CO_2 - NC_4H_9]^+$, m/z 245; SAR $[MH - CO_2 - NC_2H_5]^+$, m/z 299; DIF $[MH - CO_2 - NC_3H_7]^+$, m/z 299. In order to adequately confirm these residues at low levels using in-source CID, the internal source voltage (CapEx for this particular instrument) had to be optimized for each ion of each compound using the instrument's ability to dynamically ramp this parameter as a function of m/z. It was not possible to optimize all four ions for all four residues simultaneously using this technique; using the single quadrupole instrument CIP and ENR were done together while DIF and SAR were optimized by another acquisition program.

When using in-source CID with selected ion monitoring (SIM) at least 3 ions must meet the following criteria, which are consistent with published guidelines (14,15) for positive confirmation. The signal height for any ion at the appropriate retention time for that residue must be 3 times the signal for that ion observed in the analysis of control tissue for the same time window. The retention times for all peaks in all ion chromatograms should not vary by more than ± 5% from what is observed for a standard compound on that day. The retention times may drift some during the course of 6-8 runs, but samples should have retention time within ± 5% of standards run either at the beginning and end of a sample set; the peaks must also continue to elute during the appropriate selected ion time window. Finally, the relative abundance of ions

(relative abundance to base peak as calculated by integrating ion chromatograms) must be ±10% of what was calculated for a standard compound run that same day. (For example, if the ion in the standard were 30% relative abundance, the same ion in the samples would be between 20-40% relative abundance.)

Examples of data obtained from the analysis of salmon tissue using a single quadrupole instrument are shown in Figure 1 and in Table 3. Figure 1 shows combined ion chromatograms for CIP and ENR in control, fortified tissue, and in tissue from fish that was incurred with 0.05 mg/kg ENR. For all samples, the relative abundance of each ion (calculated by integrating each ion chromatogram) was compared to those from a standard compound analyzed on the same day under the same conditions. Table 3 is a representative sample of one day's analysis of salmon tissue, showing individual data for relative abundances and retention time for the samples analyzed. All relative abundances were within ±10% of the values calculated for at least one standard analyzed on that day.

A summary of all data obtained for the confirmation of FQs in salmon tissue using the single quadrupole instrument are shown in Table 4. All control samples were negative, and all fortified (20- 40 ppb) tissue met the criteria for positive confirmation. Several sets of extracts from incurred tissue were also analyzed using this method. The results from the confirmation analysis of incurred tissue support the data reported earlier from the LC/fluorescence method (8). ENR was confirmed in tissues from salmon dosed with 5, 0.5, and 0.05 mg/kg of the drug and sampled after 18 hr. The results from the LC/fluorescence method indicated the amount of ENR in these tissues ranged from 10-1772 ppb (See Table 4). The small amount of CIP (approximately 10 ppb) found in the tissue of the salmon fed the largest dose of ENR was also confirmed by this LC/MS procedure. The residues of DIF were confirmed in all tissue extracts from salmon dosed with this drug. SAR was measured in the tissue of salmon that had been fed 5 mg/kg DIF by LC/fluorescence at levels around 8 ppb. While this residue was detected by this MS method, the relative abundances of the ions monitored did not meet the ± 10% criteria, and therefore were not positively confirmed. Only 30 ppb of SAR was measured in the fish dosed with 5 mg/kg of this FQ, and these residues were confirmed.

In addition to salmon, this confirmation method was also able to confirm the presence of FQ residues in shrimp tissue. This method was used to confirm residues in shrimp tissue fortified in the 20-40 ppb range. Individual ion chromatograms of SAR in shrimp incurred with this FQ are illustrated in Figure 2. Again, all relative abundances met criteria (±10%). Results from incursion studies indicated that these drugs are poorly absorbed by the shrimp; tissue extracts from animals dosed at fairly high levels showed only low levels of any residues by both determinative and confirmatory methods. However, any residues that were detected at appreciable level (> 5 ppb) in shrimp tissue were confirmed by LC/MS. A summary of the data from the analyses of those shrimp samples is shown in Table 5.

The second aspect of this study was to expand this method to an ion trap LC/MS instrument. Fragmentation to obtain important structural ions can be achieved more efficiently using tandem mass spectrometry, either with an ion trap or triple quadrupole instrument. These techniques have the advantage of providing more sensitive, selective confirmation because the

protonated molecular ion is first isolated before any collisional dissociation is performed, thus minimizing chemical noise and interference in the product ion spectrum. Specific operating conditions and validation data for the confirmation of FQs in salmon tissue using an ion trap instrument were developed (Table 2). Using the ion trap, data dependent (i.e. only obtaining MS^n data when the selected ions are present above a set threshold) MS^2 was performed on the protonated molecular ions for each of the four FQs. With a collision energy of 40% the primary fragment is MH- CO_2^+, which was also observed with in-source CID using the single quadrupole instrument. The value of 40% collision energy was obtained by optimizing the signal for the $[MH - CO_2]^-$ ion as a function of this parameter. Loss of water, a less specific fragment ion, was also observed in the MS^2 spectra, but the abundance is much lower as compared to the in-source CID data. Data dependent MS^3 on the MH-CO_2^+ fragments yields the smaller mass ions. The ions observed as a result of in-source CID in the single quadrupole experiment (MH - CO_2 - NR^+) are present in the MS^3 spectra, but other ions were observed as well including [MH- CO_2 – HF]$^+$. Spectra for MS^2 and MS^3 of the four FQ compounds are shown in Figure 3.

The criteria for positive confirmation of the FQ residues using an ion trap instrument are similar to those for the single quadrupole and are also consistent with published guidelines (15-16). First the ions selected in the single quadrupole method must also be present in the MS, MS^2 and/or MS^3 spectra. Other structurally important ions, such as [MH- CO_2-HF]$^+$, can be substituted for [MH- H_2O]$^+$, but four unique ions should be identified and monitored. It must be determined that these ions are present at a reasonable (> 3:1) signal-to-noise ratio by looking at chromatograms from the data dependent MS^n scans for the appropriate ion ranges or comparing spectra between a control and sample extract analysis. The retention time for the FQs must still be within ± 5% of the retention time of a standard (or fortified sample) injected on that day. Finally, the spectra of the FQs at the appropriate retention time in the sample extract must approximate the spectra of the FQ standards analyzed on the same day. Spectral list (tabulation) data can be used to evaluate this criteria.

Data dependent acquisition was used for the analysis of FQs partially because it has been shown through numerous analyses that the retention times of these compounds can migrate depending on matrix and exact chromatographic conditions (i.e. batch of mobile phase). Using data dependent acquisition eliminates the need to set static time segments or to scan for each compound continuously.

To validate this method using the ion trap, control, fortified and incurred salmon tissue were analyzed. An advantage of the ion trap method is that it is possible to confirm all four analytes in a single chromatographic run. Figure 4 shows the MS^3 chromatograms (isolation of MH^+ followed by transition, isolation and collision of MH - CO_2^+ to obtain MS^3 spectra) for an extract of salmon tissue that was fortified at 10 ppb with all four analytes. The same plot for the control tissue shows no ion abundance at all for this very selective data extraction. Figure 5 shows an example of MS, MS^2, and MS^3 ion chromatograms along with the MS^2 and MS^3 spectra for a tissue extract from fish that had been incurred with DIF. Table 6 shows the relative retention times and relative ion abundances (tabulated from MS^2 and MS^3 spectra) for one day's analysis for SAR. Table 7 is a summary of the confirmation of all the FQ confirmed by using the ion trap LC/MS^n method. The salmon with lower concentrations of residues were reanalyzed

using the ion trap LC/MS. Using this instrument it was possible to confirm FQ residues in all fortified (5-40 ppb) and incurred tissue. All control tissue was negative, with the exception of one day's analysis when the control tissue was contaminated during analysis by salmon tissue with high levels of ENR This control tissue had been found to be negative for ENR every other time (n = 8) that it was analyzed.

The primary advantage of using the ion trap instrument for the confirmation of FQs in fish tissue is that the instrument's ability to perform MS^n allows for more sensitive and selective detection of the residues. In addition, it is possible to confirm all four residues in a single chromatographic analysis using the ion trap, but not when using the quadrupole instrument. Using the single quadrupole instrument, approximately 1 ng of standard could be detected and still meet confirmation criteria. In matrix (salmon tissue) the FQs were confirmed on this instrument at the 10 ppb level using injection volumes of 100 μL (final extract volume of 1.0 mL). The ion trap instrument was able to confirm <50 pg of standards on-column and salmon tissue fortified at 5 ppb was confirmed with a 20 μL injection volume (final extract volume of 1.0 mL). Some of the differences between these two methods are specific to the make and model of each instrument. The single quadrupole used in this study is an older model instrument; newer quadrupole mass spectrometers would be more sensitive and would be able to perform in-source CID experiments more effectively.

The overall goal of our laboratory was to develop a regulatory confirmation method that is of sufficient specificity to be used in other laboratories, but that is general enough to be performed with different types of mass spectrometers. The extraction and chromatographic conditions in this method can be used directly, while the optimized MS parameters are flexible as long as the data obtained meets established confirmation criteria guidelines (15-16). This method was not validated using a triple quadrupole instrument, but others have analyzed FQ residues using that type of mass spectrometer (9-12) and found similar fragment ions in the product ion spectra. A triple quadrupole should be able to be used with this method as long as general confirmation criteria are met. While only salmon tissue was reanalyzed using the ion trap instrumentation, the parameters and criteria should also be applicable to the other aquacultured species, such as catfish and shrimp.

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Figure (1). Single quadrupole instrument combined ion chromatograms for CIP and ENR from extracts of (A) Control salmon. (B) Salmon tissue fortified with FQ at 20 ppb. (C) Salmon dosed with 0.05 mg/kg ENR and sacrificed after 18 hr.

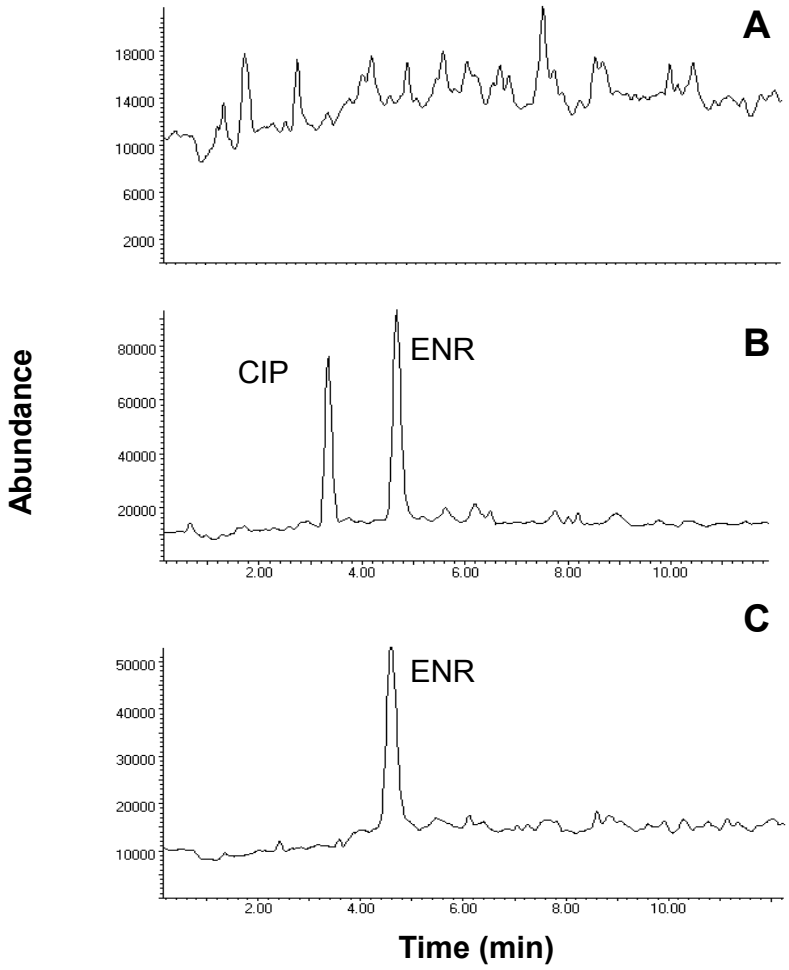

Figure (2). Separate ion chromatograms for SAR in extract of shrimp dosed with 10 mg/kg SAR and sacrificed after 2 hr obtained on single quadrupole instrument.

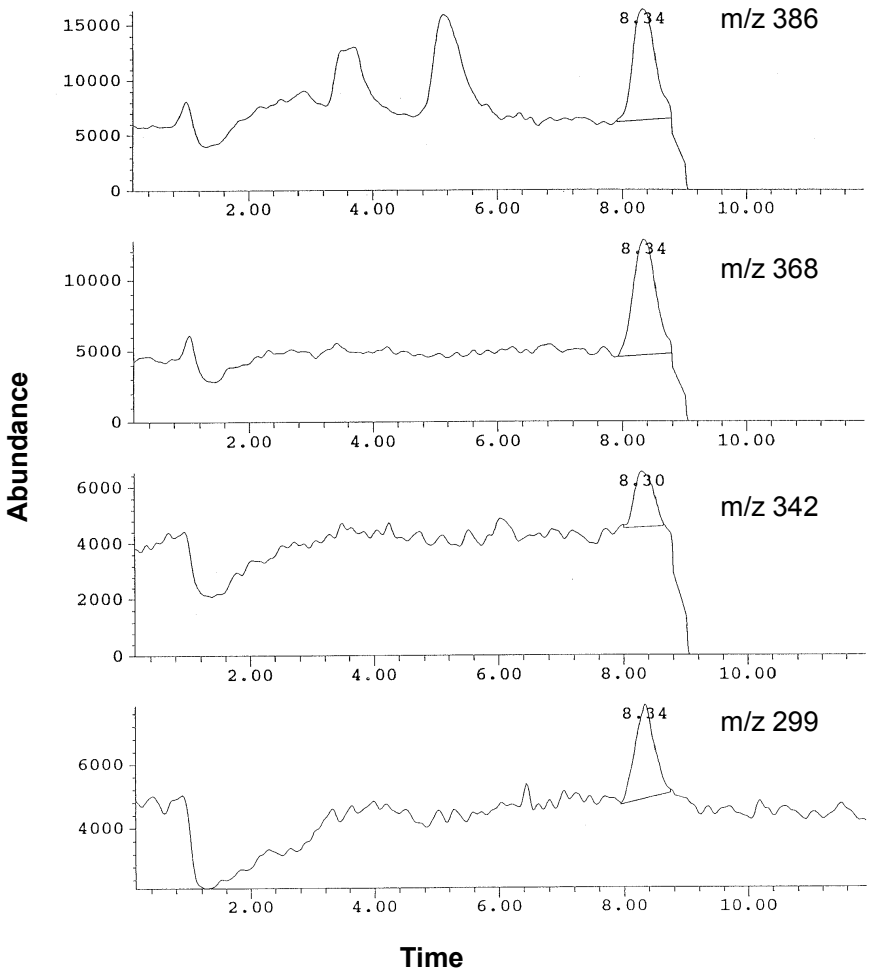

Figure (3). MS^2 and MS^3 spectra of FQs obtained on ion trap LC/MS.

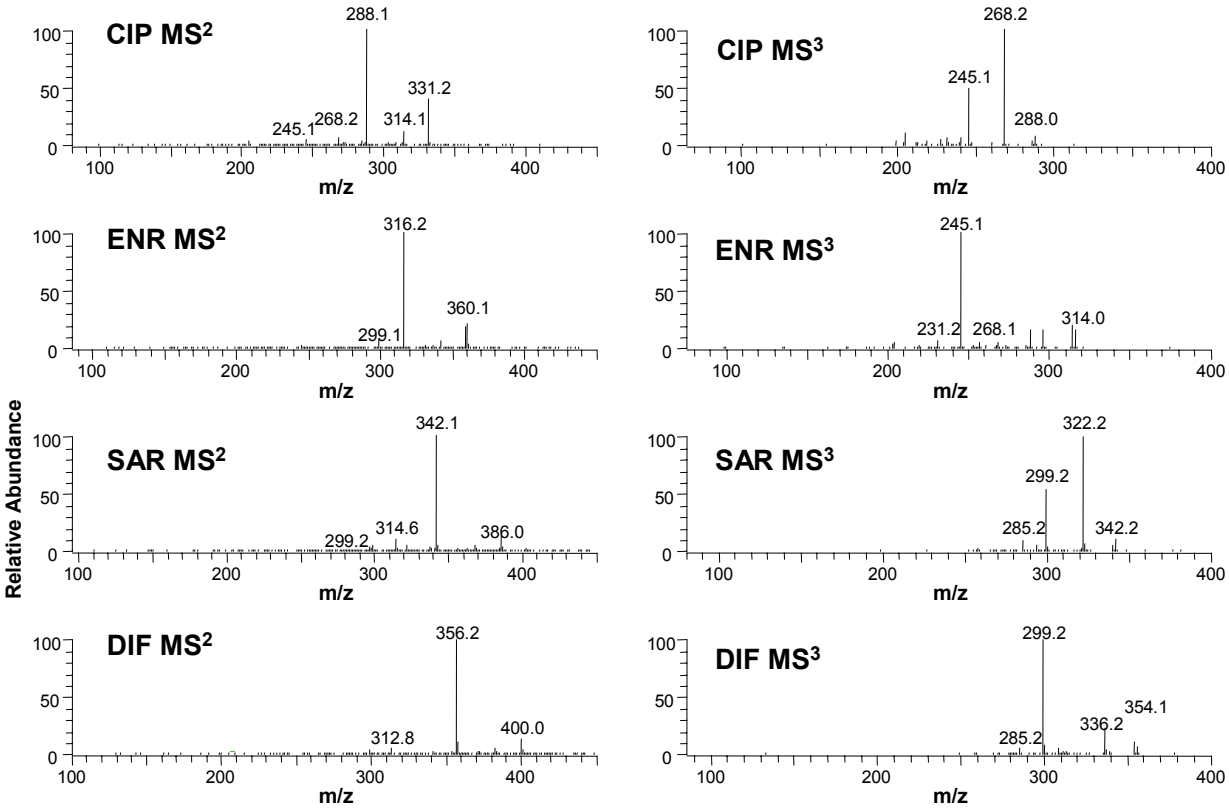

Figure (4) Salmon extracts fortified at 10 ppb. Ion trap data showing MS^3 ion transitions for (A) DIF (m/z 400 -> m/z 356 ->). (B) SAR (m/z 386 -> m/z 342 ->). (C) ENR (m/z 360 -> m/z 316 ->). (D) CIP (m/z 332 -> m/z 288 ->).

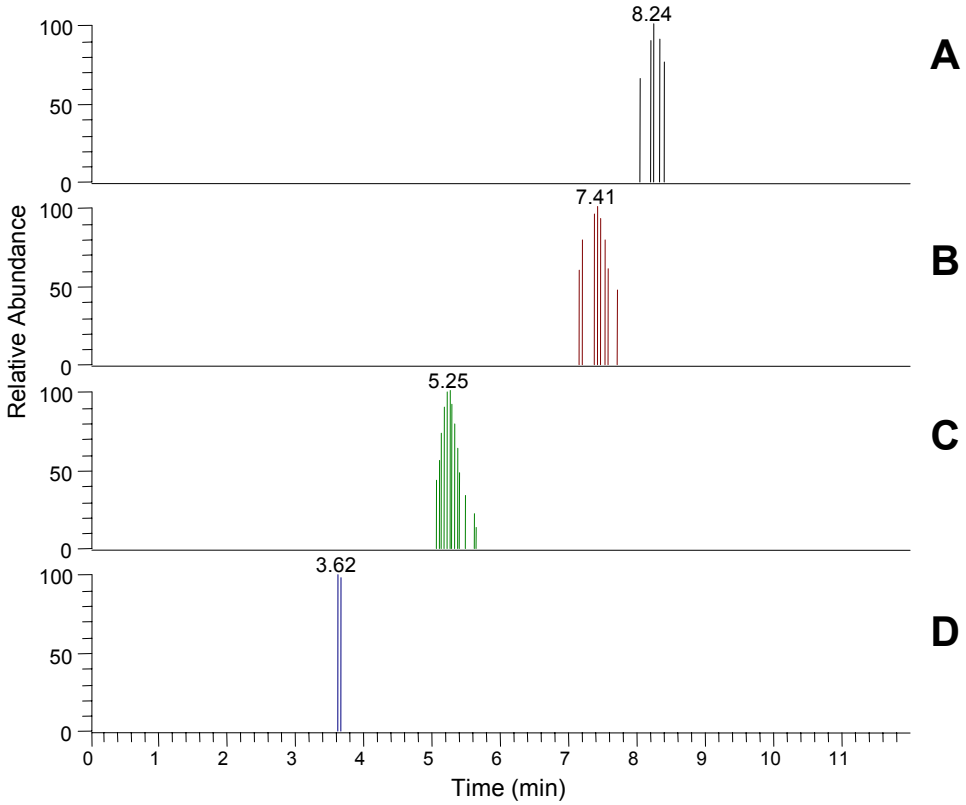

Figure (5) Extract from salmon dosed orally with 0.05 mg/kg DIF and sacrificed after 18 hr. Ion trap data showing (A) Extracted ion chromatograms for (1) full MS (m/z 400) and (2) MS^2 (m/z 356) and (3) total ion chromatogram for MS^3. (B) MS^2 spectrum for peak at 8.1 min. (C) MS^3 spectrum for peak at 8.1 min.

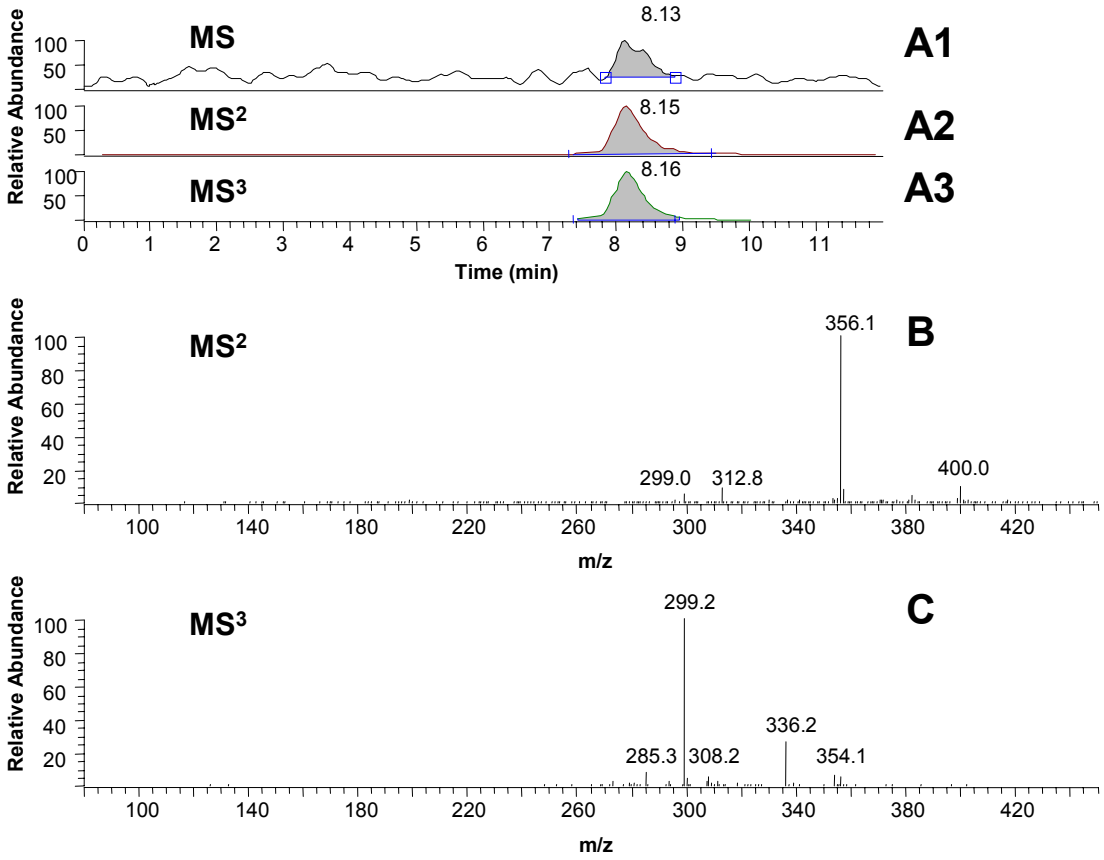

Table 1. Operating Conditions for Single Quadrupole Instrument

Instruments:	Hewlett Packard (HP) 5989 Mass Spectrometer with HP Atmospheric Pressure Ionization Interface (HP, Palo Alto, CA)	
	HP 1090 Liquid Chromatograph	
Data System:	Chemstation G1034C version C.03.00 for data acquisition and Chemstation G1701BA version B.01 for data processing.	
LC Conditions:	Column	Inertsil Phenyl (150 x 2.0 mm, 5 μm) Metachem Technologies (Torrance, CA)
	Mobile Phase	86:14 2% Formic Acid (88%, Baker): acetonitrile
	Flow rate	0.35 mL/min
	Injection	100 μL, manual
	Column Temp	40 °C
MS Conditions:	Polarity	Positive ion
	Source Temp	260 °C
	Nebulizer Gas	N_2 80 psi
	Drying Gas	N_2 40 psi
	Dwell Time	200 ms
	Resolution	Low
Acquisition parameters:		
Program 1 (CIP, ENR)	Time	Ions (CapEx value at each ion)
	0-4.5 min	332 (128), 314 (178), 288 (174), 231 (202)
	4.5 -12 min	360 (128), 342 (184), 316 (178), 245 (202)
Program 1 (DIF, SAR)	Time	Ions (CapEx value at each ion)
	0-7.5 min	386 (150), 368 (190), 342 (190), 299 (220)
	7.5 -12 min	400 (150), 382 (190), 356 (190), 299 (220)

Table 2. Operating Conditions for Ion Trap LC-MS: FQ Confirmation

LC Conditions:	Column	Inertsil Phenyl (150 x 2.0 mm, 5 µ) Metachem Technologies (Torrance, CA)
	Mobile Phase	86:14 2% Formic Acid (88%, Baker): acetonitrile
	Flow rate	0.35 mL/min
	Injection	20 µL, automatic injector
	Column Temp	Ambient
Gradient:	none	
MS Conditions:	Polarity	Positive ion
	Source Temp	350 °C
	Gas	Sheath:N_2 78.6, Auxillary: N_2 18.8 (arbitrary units)
	Spray Voltage	5 kV
	#Prescan/Max Inj Time (ms)	MS: 3/50 MS^2: 2/400 MS^3: 2/400
Data dependent acquisition parameters:		
Scan Event 1 (MS)	MS	Scan [m/z 150-500]
Scan Event 2 (MS^2)	MS^2 of most intense ion of following ions (m/z 400, 386, 360,332) from scan event 1, above threshold of 100000. Collision Energy 40%, Isolation width: 2 amu, Q=0.25, scan [m/z 80-450]	
Scan Event 3 (MS^3)	MS^3 of most intense ion from scan event 2, above threshold of 100000. Collision Energy 40%, Isolation width: 2 amu, Q=0.25, scan [m/z 80-400]	

Table 3. Example of Single Quadrupole Confirmation Data: Analysis of SAR in Incurred Salmon Tissue

Sample	Ret. Time	% 368	% 342	% 299	Conf?
40 FQ Std	8.51	75	19	31	
Control Salmon	ND				
Fort. 20 ppb	8.63	65	25	25	Y
Incurred #4 -1[1]	8.64	67	29	34	Y
Incurred #4 -2	8.67	69	26	31	Y
Incurred #4 -3	8.59	72	18	26	Y
Incurred #4 -4	8.68	81	19	30	Y
40 FQ Std	8.54	75	18	26	

[1] Incurred fish was dosed with 5 mg/kg SAR and sacrificed after 18 hr.

Table 4. Summary of Confirmation of FQ in Salmon using Single Quadrupole

Sample (amount residue found by LC/Fluor.)	Number Confirmed			
	CIP	**ENR**	**SAR**	**DIF**
Control				
Fortified 20 ppb	4 of 4	6 of 6	7 of 7	7 of 7
Fortified C 40 ppb	5 of 5	4 of 4	4 of 4	4 of 4
Incurred #1 (1772 ppb ENR,10 ppb CIP)	4 of 4	4 of 4	NA	NA
Incurred #2 (95 ppb ENR)	ND	4 of 4	NA	NA
Incurred #3 (10 ppb ENR)	ND	4 of 4	NA	NA
Incurred #4 (30ppb SAR)	NA	NA	4 of 4	ND
Incurred #5(5 ppb SAR)	NA	NA	2 of 4	ND
Incurred #6 (1535 ppb DIF, 8 ppb SAR)	NA	NA	0 of 4	4 of 4
Incurred #7 (126 ppb DIF)	NA	NA	ND	4 of 4
Incurred #8 (28 ppb DIF)	NA	NA	ND	4 of 4

Table 5. Summary of Confirmation of FQ in Shrimp using Single Quadrupole

Sample(amount residue found by LC/Fluor.)	Number Confirmed			
	CIP	**ENR**	**SAR**	**DIF**
Control				
Fortified 20 ppb	3 of 3	4 of 4	5 of 5	4 of 4
Fortified 40 ppb	2 of 2	2 of 2	2 of 2	2 of 2
Incurred #1 (7 ppb ENR)	ND	2 of 2	NA	NA
Incurred #2 (6 ppb SAR)	NA	NA	2 of 2	ND
Incurred #3 (8 ppb DIF, 2 ppb SAR)	NA	NA	0 of 2	4 of 4

Table 6. Example of Ion Trap LC-MS Confirmation Data: Analysis of SAR in Incurred Salmon Tissue

Sample	**Ret. Time (min)**	**% Relative Abundance in MS [2] Spectrum**		**% Relative Abundance in MS [3] Spectrum**		
		342	**368**	**322**	**299**	**285**
FQ standard [1]	5.91	100	4.7	100	50.7	8.0
Control	ND					
Fortified 20 μg/kg	6.71	100	10.3	100	63.4	17.9
Incurred [2] -1	6.73	100	6.6	100	56.2	9.9
Incurred -2	6.93	100	7.6	100	54.8	9.4
Incurred -3	6.92	100	6.0	100	56.9	9.6
Incurred -4	7.16	100	6.5	100	60.0	11.0
Incurred -5	7.16	100	8.8	100	51.9	10.3
Incurred -6	7.18	100	8.7	100	56.8	10.1
FQ standard	7.06	100	11.9	100	50.2	8.8

[1] FQ standard consists of mixture of CIP, ENR, SAR, DIF at 0.02 ng/μL each

[2] Incurred fish was dosed orally with SAR 5 mg/kg and then sacrificed after 18 hr and was found to contain 30 μg/kg SAR by LC/fluorescence

Table 7. Summary of Confirmation of FQs in Salmon using Ion Trap LC-MS

Sample	Number Confirmed			
	CIP	**ENR[1]**	**SAR**	**DIF**
Control Tissue	0/9	1/9	0/9	0/9
Fortified 5 μg/kg	4 / 4	4 / 4	4 / 4	4 / 4
Fortified 10 μg/kg	5 / 5	5 / 5	5 / 5	5 / 5
Fortified 20 μg/kg	6 / 6	6 / 6	6 / 6	6 / 6
Fortified 40 μg/kg	2/ 2	2/ 2	2/ 2	2/ 2
Incurred #1 (1772 μg/kg ENR ;10 μg/kg CIP)	3/3	3/3	ND	ND
Incurred #2 (10 μg/kg ENR)	ND	3/3	ND	ND
Incurred #3 (30 μg/kg SAR)	ND	ND	6/6	ND
Incurred #4 (28 μg/kg DIF	ND	ND	ND	6 / 6

[1] One control was contaminated during sample preparation with ENR