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Automated multi-residue isolation of fluoroquinolone antimicrobials from fortified and incurred chicken liver using on-line microdialysis and high-performance liquid chromatography with programmable fluorescence detection[1]

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Abstract

Isolation of the quinolones, sarafloxacin (SAR), oxolinic acid (OXA), and flumequine (FMQ), from fortified chicken liver tissues, and SAR incurred chicken liver tissues was achieved by combined liquid–liquid extraction and aqueous on-line microdialysis using the automated trace enrichment of dialysates (ASTED) system. Analysis of tissue isolates after ASTED clean-up was performed using reversed-phase HPLC and programmable fluorescence detection. Overall recoveries of SAR, OXA and FMQ from samples fortified over a concentrations range of 1–100 ppb were 94, 97 and 87% with overall inter-assay variability of 4.2, 4.1 and 3.6%, respectively. Chicken liver samples incurred with SAR at three concentration levels also were tested by the ASTED method. The method exhibited high peak resolution (3.4–4.2 on average), a high signal-to-noise ratio, and demonstrated good precision. The ASTED–HPLC method overall had a lower limit of detection (LOD) of 0.2 ppb, and a limit of quantitation (LOQ) of 1 ppb.

Keywords: Fluoroquinolones; Sarafloxacin; Oxolinic acid; Flumequine

1. Introduction

Fluoroquinolones (FQs) are antibacterial compounds with activity against Gram-positive and Gram-negative microorganisms. These compounds are considered the most important group of synthetic antibacterial agents developed since the discovery of sulfonamides and are widely used in human medical applications [1]. The recent approval of several FQs for use as therapeutic agents in food producing animals in several nations raises serious concerns regarding possible loss of efficacy of these drugs in humans. Increased bacterial resistance to FQs may occur as a result of their presence as residues in the nation's food supply [2,3]. Thus the need exists for rapid, sensitive multi-residues methods to monitor

[1]Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

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and detect FQs in foods such as meat and meat products.

The ''fluoroquinolone'' drug class generally includes both the non-fluoro 4-quinolones and the more recently developed related fluorine containing analogs. Two FQs, flumequine (FMQ) and oxolinic acid (OXA), have been used extensively for treatment of diseases in aquaculture. More recently two additional FQs, sarafloxacin (SAR) in the US specifically for poultry and enrofloxacin (ENR) in the EC for food production animals in general, were approved for treatment of bacterial infections [4,5]. Respective structures for all four compounds mentioned above are presented in Fig. 1. Several solvent based methods have been reported for the isolation of FQ residues in fish tissue [6,7] and chicken serum [8]. However, few methods have been reported for the detection of FQs in meat and meat products [9,10]. Isolation and detection methods reported for the FQs are in most cases limited to single analytes rather than having multi-analyte capability, exhibit low sensitivity, are restricted to plasma or muscle tissue, and are highly labor and solvent intensive [11–13]. Important work performed by Volmer et al. [14] reports the results of an investigation of 15 FQs by electrospray LC–MS–MS in several biological matrices. Even though this work is a major step toward a confirmatory multi-residue method for FQs, its application for the quantitation of these drugs in a regulatory setting is questionable.

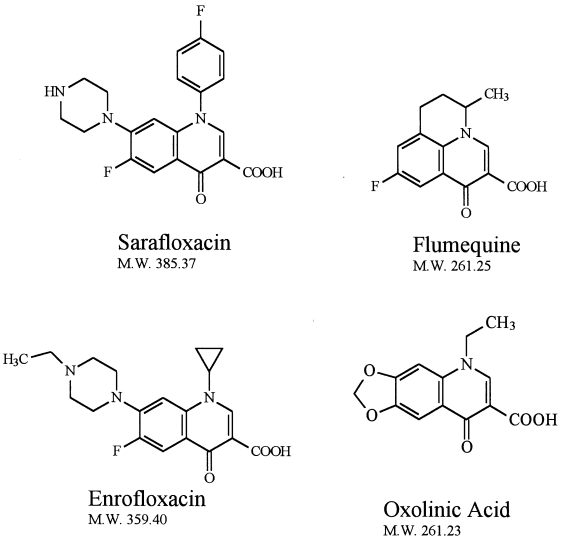

Fig. 1. Molecular structures of selected fluoroquinolones.

The ASTED (automated sequential trace enrichment of dialysates) is a technology that may meet regulatory needs for selected polar analytes. The ASTED operation is a combination sample clean-up and concentration device that is based on on-line microdialysis coupled with in-line trace enrichment. This system performs aqueous based, automated sample clean-up prior to high-performance liquid chromatography (HPLC). Sample extracts are injected on the upper side of a microdialysis membrane sandwiched between two sections of a dialysis block. Only low-molecular-mass compounds migrate through the membrane while proteins and other particles (e.g., cellular components) are removed. The analytes of interest, diluted in the dialysis step, then are concentrated on a trace enrichment column, which is connected to an HPLC system through a switching valve. The ASTED has been used for a wide range of water-soluble compounds including: drugs, food additives, toxins, bioamines, vitamins and pesticides [12,15–17]. In addition, the ASTED has been used for the isolation and detection of two FQs, FMQ and OXA, from fish tissues [18,19]. The reported limits of detection (LODs) for these two methods are in the range of 2–4 and 3–7 ppb for oxolinic acid and flumequine, respectively. However, neither method has been applied to the isolation of these drugs from more complex matrices such as meat and meat products.

In an earlier study we reported the use of on-line microdialysis for the isolation of two FQs, FMQ and OXA from fortified chicken liver [20]. In that study the recoveries of FMQ and OXA ranged from 94–96% and 98–99%, respectively, with an LOD of 5 ng/g for FMQ and 2.5 ng/g for OXA and a limit of quantitation (LOQ) of 5 ng/g for both FQs. These FQs have similar fluorescence emission and excitation spectra, which were measured using single wavelength fluorescence detection (FLD). Since other members of the FQ drug class, such as SAR, have fluorescence spectra widely separated from that of FMQ and OXA, their detection required two HPLC runs using the single wavelength FLD. We separately reported the substitution of a programmable for single wavelength FLD in the ASTED–HPLC system. The programmable FLD is effective

for detecting and quantifying FQs, having widely dissimilar fluorescence wavelength characteristics, in a single analysis [21]. In addition, the programmable FLD achieved detection and quantification levels not attainable with earlier single wavelength detectors.

In the present study we reported the development of a multi-residue method for SAR, OXA and FMQ at ppb and sub-ppb levels, in both fortified and incurred chicken liver using the newly configured ASTED–HPLC with programmable FLD.

2. Experimental

2.1. Chemicals and reagents

Sarafloxacin hydrochloride (SAR) (purity= 88.5%) was obtained from Abbott Labs. (North Chicago, IL, USA). Oxolinic acid (OXO) (purity= 100%), flumequine (FMQ) (purity=100%), anhydrous diethyl ether, ammonium hydroxide, sodium hydroxide, sodium dibasic phosphate, sodium monobasic phosphate and Triton X-100 were obtained from Sigma (St. Louis, MO, USA). Triethylamine and phosphoric acid 85% were obtained from Fisher Scientific (Fairlawn, NJ, USA). A Barnstead NANOpure filter & deionizer unit (Dubuque, IA, USA) provided nanopure water. Acetonitrile, hexane, and methanol were from Burdick & Jackson (Muskegon, MI, USA). All solvents were HPLC-grade, and all buffered and non-buffered solutions prepared for this study were filtered through a 0.2-μm pore filter prior to use.

2.2. Preparation of standards, buffers and fortification solutions

Stock solutions of FMQ, OXA and SAR (all 100 μg/ml) were prepared in 100 ml of a 0.03 *M* sodium hydroxide solution. These solutions were stored at 4°C in amber glass bottles and were stable for at least three months [22]. Working standards (1 μg/ml) were prepared daily by dilution with 0.03 *M* sodium hydroxide. Buffer solutions used were: buffer A – 0.1 *M* sodium phosphate (pH 9.0), and buffer B – 0.02 *M* sodium phosphate (pH 5.0). Fortification solutions were prepared by diluting the working standards with buffer A to final concentrations of 100, 50, 25, 10, 5 and 1 ng/100 μl using the ASTED dilution program.

2.3. Tissue fortification and incurred tissue samples

Tissue fortification was performed using control chicken livers free of FQs. These livers were obtained from FDA/CVM (Laurel, MD, USA) and stored at −70°C until needed. Prior to fortification, the livers were thawed for 5 h at 4°C, then blended with dry ice and homogenized using a Janke & Kunkel Ultra-Turrax T25 homogenizer (IKA Labortechnic, Staufen, Germany). The homogenized tissues were refrozen as bulk samples and stored at −20°C. One-gram portions of the frozen homogenized tissue were weighed, as needed, into 50-ml plastic centrifuge tubes. Fortification was performed by delivering 100 μl aliquots of the fortification solutions (Section 2.2) containing a mixture of SAR, OXA and FMQ at each concentration level onto the tissue portions in the centrifuge tubes. Six sample replicates were prepared for each concentration level. The centrifuge tubes then were shaken vigorously for 30 min at 25°C using a Janke & Kunkel IKA Vibrax VXR shaker and incubated at 4°C overnight to allow drug permeation into the homogenized tissue. Two additional samples were added to each set of six replicates of fortified samples at a specific concentration: a non-tissue control sample containing 1 ml of 0.1 *M* sodium phosphate (pH 9.0) fortified with a 100-μl aliquot of the same fortification solution, and a negative control containing 1 g of control liver to which 100 μl of a 0.1 *M* sodium phosphate (pH 9.0) buffer was added.

Chicken livers incurred with SAR were obtained from FDA/CVM. The SAR was administered to the chickens via an intramuscular injection at two dosing levels: 50 mg dose (Group I), and 5 mg dose (Group II) repeated on three consecutive days. After the chickens were sacrificed, their livers were stored at −70°C, until needed.

Prior to use, the incurred livers were slightly defrosted at 4°C, separately homogenized in a blender with powdered dry ice, and refrozen. Separate portions of livers from Group II were mixed and homogenized with equal portions of homogenized control livers in order to prepare a third set of

samples (Group III) at a SAR concentration approximately one half that of the original Group II set. The three groups of samples were individually divided into one-gram portions each, placed in 50-ml centrifuge tubes and stored at −20°C until needed (between 1–12 days).

2.4. Extraction – pre-ASTED debinding and defatting

The method used for debinding and defatting of FQs from tissue prior to ASTED clean-up was adapted from a solvent-based method by Hormazabal et al. [13]. The fortified tissues amples, stored in centrifuge tubes, were first deproteinated in the following manner: 0.5 ml ammonium hydroxide and 3 ml acetonitrile were added to the tubes which then were shaken on a Vortex Genie-2 (Scientific Industries, Bohemia, NY, USA) for 1 min. Additional mixing was carried out by blending the samples for 1 min on an Ultraturax homogenizer followed by centrifugation at 3000 *g* for 2 min. The supernatants were transferred into new centrifuge tubes. The solid residue pellets in the first tubes were re-extracted using the same steps as above and the resulting supernatants were combined, while solid residues were discarded. Defatting and transfer of the analytes from an organic to an aqueous phase were achieved in the following manner: 1 ml of a 1 *M* sodium chloride solution, 3 ml diethyl ether, and 2 ml hexane, were added to the samples, followed by vigorous mixing and centrifugation at 3000 *g* for 1 min. The upper organic layers were aspirated and discarded. The lower aqueous layers were centrifuged at 2000 *g* for 5 min, and the clear supernatants were transferred to autosampler vials prior to ASTED clean-up. Thirty-five samples were processed by this procedure in 1.5 h.

2.5. ASTED and other apparatus

The ASTED XL system (Gilson Medical Electronics, Villiers-leBel, France) used in these studies was comprised of the following components: Gilson keypad with software version 722 V2.00 controlled the operating parameters; a sample injector (Model 231) and two dilutors (Model 401C) equipped with 5-ml and 1-ml syringes; a flat-bed dialysis block with a 370-μl donor channel and a 650-μl recipient channel volume, which was fitted with a cellulose dialysis membrane (Cuprophan) having a molecular mass cut-off of 15 000; a custom made trace enrichment column (TEC) (5.8 mm×4.6 mm I.D.) fitted with 5 μm frits and packed with 70 mg of a 10 μm, Hypersil ODS sorbent (Keystone Scientific, Bellefonte, PA, USA) which was connected to the recipient channel of the dialysis cell or to the HPLC system through an auto-switching Model 7010 six-port valve (Rheodyne, Berkeley, CA, USA). Prior to initial sample introduction, the dialysis block donor channel was washed with a solution of 0.01% (w/v) Triton X-100 in buffer B and then primed with buffer A. The recipient channel was flushed with buffer B and the TEC was washed with 500 μl of a 50% acetonitrile–water mixture and finally conditioned with 500 μl of buffer B.

The supernatants in the autosampler vials (Section 2.4) were loaded into the ASTED sampler tray. The automated syringe delivered 370 μl of each into the donor channel – upper portion of the dialysis block – at a rate of 0.3 ml/min (Fig. 2 – pathway 1). The sample was programmed to remain in a stationary state in the donor channel for 5 min. At the end of that period, three pulses of 650 μl each of buffer B, for a total of 1950 μl, were pumped through the recipient channel and subsequently flowed onto the TEC at a rate of 0.6 ml/min (Fig. 2 – pathway 2). A second aliquot of the sample (370 μl) was delivered into the donor channel, dialyzed, and similarly flowed onto the TEC. The TEC then was washed with 500 μl of buffer B after which a valve switching transferred HPLC mobile phase onto the TEC. The mobile phase carried the analytes in a narrow band onto the analytical column for chromatographic separation and detection (Fig. 2 – pathway 3).

As the initial sample was being processed by HPLC, the dialysis donor channel was washed with 2 ml of a solution of 0.01% Triton X-100 in buffer B, and the recipient channel washed with 3 ml of plain buffer B to prepare the system for the next sample. The dialysis membrane then was regenerated by flowing 2 ml of buffer A through the donor channel, and 3 ml of buffer B through the recipient channel followed by loading of the donor channel with the second sample to be dialyzed. These steps were

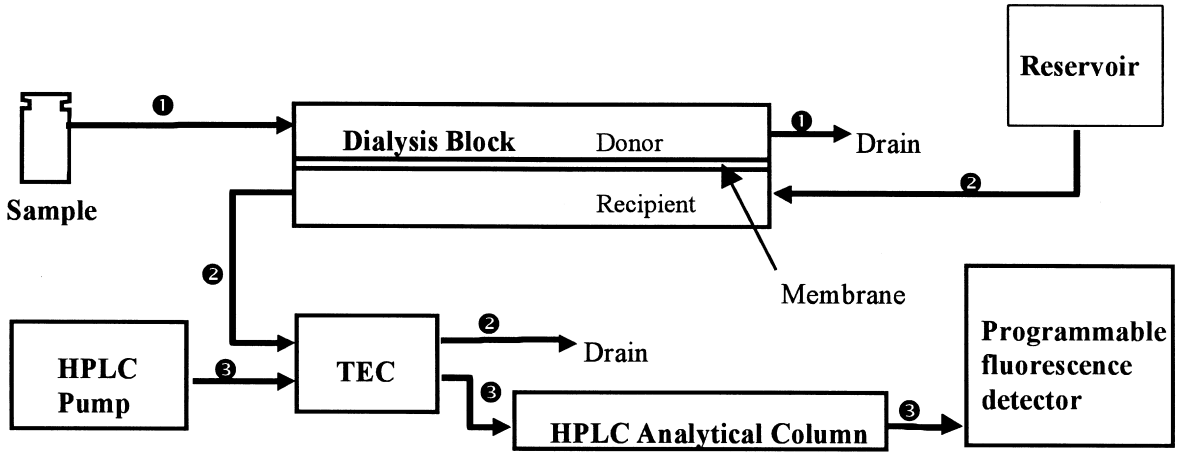

Fig. 2. Schematic diagram of the ASTED XL dialysis, TEC and HPLC flow pathways.

repeated sequentially until all samples were processed. A complete microdialysis cycle required 37.9 min which included 27.2 min for sample processing and 10.7 min for membrane and TEC wash operations.

The analytes concentrated on the TEC cartridge were injected into a Rainin HPLC system (Rainin Instrument, Woburn, MA, USA) consisting of two Model HPXL pumps controlled by a Macintosh computer (Apple Computers, Cupertino, CA, USA) using Rainin Dynamax system software V.1.3 which also processed data from the programmable FLD. Analytical separations were achieved on a Supelcosil-ABZ+ Plus column, 5 μm, 250 mm×4.6 mm I.D. (Supelco, Bellafonte, PA, USA) at a flow-rate of 1 ml/min. A gradient mobile phase was formed from (a) 0.025 *M* phosphoric acid buffer solution adjusted with triethylamine (TEA) to pH 2.7 and (b) acetonitrile. The two solvents were continuously degassed using an in-line degasser (Degasys DG-1310, Rainin Instrument). The initial mobile phase composition was a mixture of buffer–acetonitrile (65:35, v/v) which was programmed over 9 min to reach a composition of 42:58 (v/v). This composition was held for 1 min and then returned to its initial composition over a 5 min interval, and held for 3 min until the next sample injection.

The FQs were detected and quantified on a Jasco FP-920 programmable FLD (Jasco International, Easton, MD, USA) interfaced to the Macintosh computer used to operate the Rainin HPLC system. The excitation/emission maxima wavelengths were programmed in ascending elution order for each FQ (SAR – ex. 278 nm, em. 440 nm, standard response, gain 1, and attenuation 256; OXA and FMQ – ex. 318 nm, em. 368 nm, standard response, gain 1, and attenuation 256).

2.6. Automation

Complete automation of the ASTED and HPLC systems was accomplished by means of a programmable microprocessor controller (ChronTrol Corporation, San Diego, CA, USA). The controller coordinated and integrated operations between the ASTED, the HPLC software run on the Macintosh, and Jasco F-920 detector including automatic system shut down after the final analysis was completed.

3. Results and discussion

3.1. Sample pretreatment

For samples such as plasma and milk, debinding and defatting operations may be performed directly on the ASTED sample tray platform prior to dialysis and TEC [23,24]. However, because of the complex nature of tissue samples, these operations must be carried out off-line prior to loading onto the ASTED sample tray. In the present study a non-ASTED liquid–liquid extraction method of Hormazabal et al.

[13] for the isolation of SAR and ENR from fish tissues, was used to prepare fortified and incurred chicken liver samples for subsequent clean-up on the ASTED system. Their method was reported to have an LOQ of 10 ng/g and 5 ng/g for SAR and ENR, respectively. In addition, the authors claimed their method was applicable for monitoring residues of FMQ and OXA, in Atlantic salmon, although no data for these analytes was reported [13]. Several steps in the original Hormazabal method were eliminated or modified since we included the ASTED as part of the overall sample clean-up procedure. These modifications included eliminating an acidification step, reducing the tissue sample size from 3 g to 1 g, and reducing the total amount of organic solvents used in their procedure.

3.2. Microdialysis

One advantage of the ASTED system over conventional dialysis techniques is its ability to maintain a steep concentration gradient across the dialysis membrane when operating in the static donor channel pulsed recipient channel mode. This technique has been reported to give high dialysis efficiencies in short time periods [25,26] and was used in the present study to dialyze the aqueous chicken liver extracts containing the FQs.

3.3. Trace enrichment

The TEC cartridge in the ASTED system performs the important function of trapping and concentrating the dialyzed analytes on a small sorbent bed prior to their separation by the HPLC analytical column. Initially, we used the TEC cartridge supplied by Gilson to trap and concentrate the dialyzed FQs. This column contained 70 mg of 10 μm Hypersil ODS sorbent, and was fitted with 2 μm frits at both ends of the column. We found no evidence of breakthrough of the three analytes on this column at the volumes, flow-rate, and mobile phase used in our procedure. However, we did notice carryover of analytes from one sample analysis to another. In addition, when we bypassed the dialysis block and injected samples directly onto the TEC, we observed the accumulation of droplets of the sample on the ASTED injection port. We attributed these problems to the development of backpressure when the dialysate was transported from the dialysis block to the TEC, or during a direct injection. This problem was previously observed by Snippe et al. [27] who substituted a conventional LC pump in place of the Gilson dilutor pump in order to overcome backpressure buildup. However, we determined that the cause of the problem could be attributed instead to the pore size of the frits installed in the original Gilson TEC cartridges. The reduced porosity of these 2 μm frits apparently caused the development of the observed backpressure. Obtaining custom-made TEC cartridges fitted with 5 μm frits from another supplier eliminated this problem.

3.4. HPLC analysis and programmable fluorescence detection

In the present study we found that we could detect SAR, OXA and FMQ at their optimal chromatographic parameters using a programmable FLD in conjunction with the ASTED–HPLC system. We were able to devise a chromatographic separation and detection scheme for the three FQs in a single analysis with this newly configured system, which also provided excellent peak separation, a higher signal/noise ratio, and a greater sensitivity than was possible with the single wavelength FLD used in our earlier investigation [21].

3.5. ASTED–HPLC system performance with fortified tissue

System calibration curves for the ASTED were constructed using standard mixtures of SAR, OXO and FMQ and tissues fortified with drug mixtures at concentrations of 0.5–100 ng/g by plotting detector response (peak areas) vs. concentration. Correlation coefficients were 0.9998 or better. Relevant analytical data are summarized in Table 1.

After the initial performance evaluation, the system was tested with tissue samples fortified with the three FQs. Recovery data for the FQs are reported in Table 2. Mean recoveries with RSDs were calculated for each analyte at fortification levels from 1 to 100 ppb. The data show that recoveries were similarly high for all three analytes over the range of fortification levels studied. The LOD for all three FQs was

Table 1
Analytical performance parameters for the ASTED system with standard mixtures and fortified chicken liver tissues

Analyte	SAR	OXO	FMQ
LOD (ng/ml)	0.2	0.2	0.2
Linearity[a]			
Range (ng/ml)	0.5–100	0.5–100	0.5–100
Slope (±SD)	50583 (±970)	14538 (±458)	16731 (±2157)
Intercept (±SD)	4689 (±5519)	3947 (±171)	11690 (±3436)
R^2	0.9999	0.9998	0.9998
Precision (RSD, %)[b]			
Level, 0.5 ng/ml	10.0	8.4	11.6
Level, 1.0 ng/ml	8.4	6.2	5.8
Level, 5.0 ng/ml	7.3	2.1	3.4
Level, 10.0 ng/ml	3.1	4.3	2.7
Level, 25 ng/ml	2.0	3.4	1.2
Level, 50 ng/ml	2.5	2.7	2.3
Level, 100 ng/ml	2.0	3.2	1.2

[a] Seven data points for each curve averaged from triplicate standard mixture determinations.
[b] $n=6$, based on fortified samples.

0.2 ng/g, based on a determination of the mean value of the matrix blank readings plus three standard deviations of the mean. We found that the S/N ratio was very high (135/1) even at such extreme low concentration levels. Inter-assay precision was determined at seven fortification levels. The relative standard deviations (RSDs) ranged from ca. 1.2–3.2% at a concentration of 100 ng/g to ca. 8.4–11.6% at a concentration of 0.5 ppb. A representative chromatogram of an extract from chicken liver fortified at 10 ppb with SAR, OXA, and FMQ is shown in Fig. 3. The advantages of ASTED–HPLC combined with programmable FLD are apparent from this chromatogram where the complete absence of any background interference may be observed. The heightened sensitivity with this system is due in part to the analyte concentration step, which occurs on the TEC prior to HPLC analysis. The peak for SAR in this chromatogram is significantly greater in area than that of OXA and FMQ for equivalent concentrations owing to its enhanced fluorescence absorption at its excitation and emission maxima.

An important measure of a system's performance is sample throughput. In general sample throughput depends on the total processing time per sample. In this study the time required for sample pretreatment (debinding and defatting prior to ASTED–HPLC) was 17 min/sample using hand pipetting. Switching

Table 2
ASTED–HPLC multi-residue recovery of three fluoroquinolones from fortified chicken liver

Fortification level[a] (ppb)	Mean recovery (%) (±RSD)[b]		
	SAR	OXA	FMQ
100	83.1 (2.5)	87.7 (1.8)	80.2 (1.3)
50	96.0 (8.1)	100.1 (7.7)	90.7 (7.3)
25	96.6 (5.3)	102.2 (3.1)	91.1 (1.8)
10	85.1 (3.4)	85.4 (5.3)	78.3 (5.3)
5	113.6 (2.0)	114.5 (2.5)	106.2 (2.2)
1	95.0 (3.9)	85.2 (3.9)	80.8 (3.6)

[a] $n=6$ except 10 and 1 ppb level samples which were $n=7$.
[b] Recoveries determined based on standard curve responses (peak areas).

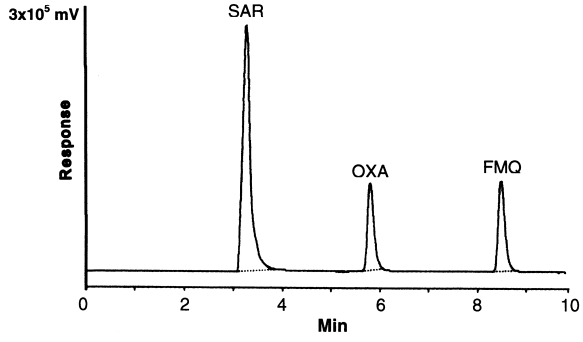

Fig. 3. HPLC programmable fluorescence chromatogram of an extract from chicken liver fortified with sarafloxacin, oxolinic acid and flumequine at 10 ppb each, after ASTED sample clean-up.

to automated reagent dispensing cut this time requirement to less than 3 min/sample. Subsequent automated sample clean-up and detection by the ASTED–HPLC system enabled us to process 35 samples over a 24 h period.

3.6. Performance of the ASTED–HPLC system with incurred tissues

The ASTED–HPLC method developed for fortified tissue samples next was tested with chicken livers containing incurred SAR. No modifications to the ASTED–HPLC method were needed to isolate the FQs from the incurred samples. Group I and II chicken liver samples were obtained by administering SAR to chickens at two dosing levels (Section 2.3). Group III chicken liver samples were prepared by mixing livers from Group II with control livers according to a previously reported dilution method [28]. This dilution was performed in order to obtain a set of incurred samples with a SAR concentration theoretically one half that of the original Group II concentration. The diluted samples were needed to test the performance of the ASTED–HPLC method at a level lower than would be possible using only Group I and II samples. Mean SAR recoveries reported in Table 3, for the three sample groups, are proportional to the original dosing profile. The RSDs for the experimental values are within acceptable error limits at the three concentration levels using plots of concentration vs. coefficients of variation as described by Horwitz et al. [29]. Our original experimental design called for analyzing the incurred livers individually, and averaging the resultant recoveries. Had we pooled the livers in each group prior to analysis, we would have expected lower RSDs, since animal to animal variation would have been eliminated as a source of error.

Table 3
ASTED–HPLC recovery of incurred sarafloxacin from chicken liver

Group	Mean recoveries[a] (ng/g)	RSD (%)
I	178.3	21.6
II	22.2	16.8
III	10.2	32.5

[a] $n=6$.

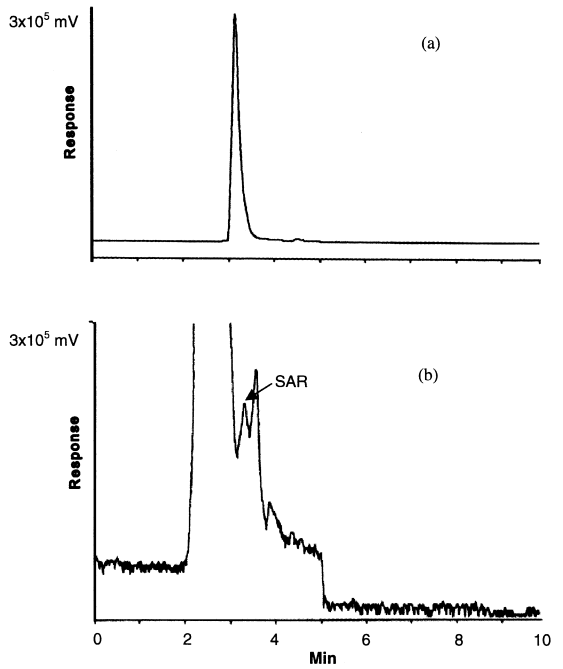

Fig. 4. HPLC programmable fluorescence chromatograms of incurred sarafloxacin chicken livers from Group II (a) by ASTED method – (recovery 22.2 ppb); (b) by Hormazabal method – (recovery not determined).

An HPLC chromatogram, after ASTED clean-up, of a SAR incurred chicken liver extract from Group II is shown in Fig. 4a. Comparison of this chromatogram with that of the fortified sample (Fig. 3) indicates no observable differences in the levels of background interference which may otherwise be attributed to artifact material although the peak for SAR displays a skewed appearance which is a characteristic of this drug's behavior on the analytical column used in this study. We also compared the chromatogram from an incurred sample after ASTED clean-up (Fig. 4a) with one obtained after isolation of the incurred drug from chicken liver using the complete Hormazabal et al. solvent extraction procedure [13]. Sample preparation and analysis by this method employs debinding and defatting steps followed by direct injection of the defatted extract into an HPLC. The defatted extracts were injected into the ASTED–HPLC system connected to the programmable FLD, bypassing the dialysis block and the TEC. An HPLC chromatogram of this extract is shown in Fig. 4b. The arrow in this figure indicates the peak for SAR, which was

so poorly resolved, that it could only be identified from the complex chromatographic background interference with difficulty. We also used the Hormazabal method to extract and analyze Group III level (10 ppb) samples, however we were unable to detect SAR in those chromatograms because background interference completely obstructed its peak at that concentration.

4. Conclusions

This report describes the development of an automated microdialysis procedure for the isolation of three FQ residues in fortified and incurred chicken liver tissues. Isolation of the three analytes was achieved using a published liquid–liquid extraction method, followed by sample clean-up using the ASTED system. Separation, detection and quantification was accomplished using HPLC with programmable FLD. The entire system was integrated on-line and automated to achieve analysis of all three FQs within a single chromatographic run. In addition, our analysis included the isolation and detection of SAR at levels of regulatory concern from incurred chicken liver tissue. The proposed method was shown to be more sensitive than a conventional solvent-based method, due in part to the exceptional clean-up performed by the microdialysis system. The incorporation of the programmable FLD into the ASTED system greatly increases the potential performance capabilities of on-line microdialysis for isolating and detecting a variety of trace level chemical residues in biological samples.

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