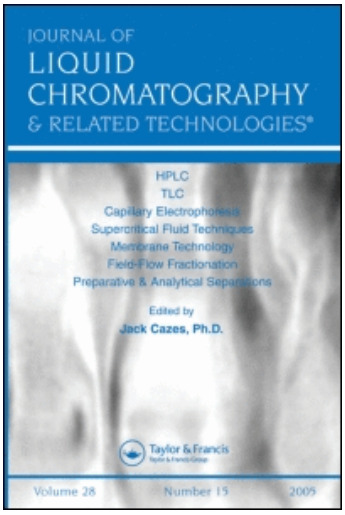

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Rapid Assay for the Determination of Sarafloxacin (A-55620) in Fish Serum by High Performance Liquid Chromatography

Ingrid Steffenak[a]; Victor Hormazabal[a]; Magne Yndestad[a]
[a] Department of Food Hygiene, Norwegian College of Veterinary Medicine, Oslo, Norway

To cite this Article Steffenak, Ingrid , Hormazabal, Victor and Yndestad, Magne(1991) 'A Rapid Assay for the Determination of Sarafloxacin (A-55620) in Fish Serum by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 14: 10, 1983 — 1988
To link to this Article: DOI: 10.1080/01483919108049668
URL: http://dx.doi.org/10.1080/01483919108049668

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(10), 1983-1988 (1991)

A RAPID ASSAY FOR THE DETERMINATION OF SARAFLOXACIN (A-55620) IN FISH SERUM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

INGRID STEFFENAK, VICTOR HORMAZABAL, AND MAGNE YNDESTAD
Department of Food Hygiene
Norwegian College of Veterinary Medicine
P.O. Box 8146 - Dep.
N-0033 Oslo 1, Norway

ABSTRACT

A simple, rapid, and sensitive method for the determination of sarafloxacin (A-55620) in fish serum using enrofloxacin as internal standard is described. The serum sample and internal standard enrofloxacin are loaded onto the extraction column packed with C2 sorbent material. The column is rinsed and then eluted. The detection limit is 5 ng/g, the recovery rate varying from 92 to 100 %.

INTRODUCTION

Sarafloxacin (A-55620) is one of the new quinolones that might prove suitable for use in fish farming. Stamm (1) tested in vitro resistance of fish pathogens to sarafloxacin, and found that the drug has excellent efficacy against such fish pathogens. Resistance is not expected to develop easily (1).

Fernandes et al. (2) determined sarafloxacin in mice serum and urine by HPLC. Sera and urine were injected directly with no pretreatment.

Fernandes et al. (2,3) assayed sarafloxacin in mice blood, serum and kidney homogenates by an agar diffusion bioassay prosedure using *Bacillus subtilis* 6633 as the assay organism. Thadepalli et al. (4) determined sarafloxacin by bioassey with *E. coli* ATCC 25922 as the test organism. Mader et al. (5) determined sarafloxacin in rabbit serum and bone by agar disk diffusion assay using *Bacillus subtilis*. The lower limit of detection was 200 ng/ml sarafloxacin in serum (5).

The purpose of the present study was to find a sensitive method for the determination of sarafloxacin in fish serum.

MATERIALS AND METHODS

This work is based upon the the method by Rogstad et al. (6) for the determination of enrofloxacin in serum.

Materials and Reagents

Sarafloxacin (A-55620) were supplied by Abbot Norge (Asker, Norway). Enrofloxacin (BAY Vp 2674) were supplied by Bayer Norge A.S. (Oslo, Norway).

All solvents except H_3PO_4 were of HPLC grade, H_3PO_4 (85 %) was p.a. grade. Stock solutions of sarafloxacin (1 mg/ml) and enrofloxacin were prepared in 0.03 M sodium hydroxide. Working standards were prepared by dilution with mobile phase. The solutions were stored in a refrigerator in dark stoppered flasks. Solutions of sarafloxacin and enrofloxacin are stable for months.

Solid phase extraction (SPE) columns were of the Bond Elute^R type, size 1CC, with C2 sorbent material.

Spin-X^{TM} centrifuge filter units (low type) with 0.45 µm cellulose acetate binding from Costar (Cambridge, MA, USA) were also employed.

Chromatographic conditions

The analyses were performed on a Perkin-Elmer HPLC system, consisting of a Series 400 solvent delivery system, an ISS 100 sampling system equipped with a Lauda RMT6 cooler (14 °C) from Messgeräte Werk Lauda, (Lauda-Königshafen, Germany), a LS 4 fluorescence detector, and an Omega-2 programme from Perkin-Elmer Corporation in an Olivetti M 300 PC connected to a Star LC24-10 printer. The detector was operated at an excitation wavelength of

278 nm and emission wavelength of 440 nm. The analytical column (stainless steel, 15 cm x 4.6 mm I.D.) and guard column (stainless steel, 1.0 x 3.0 mm I.D.) were both packed with 5 µm particles of PLRP-S polymer adsorbent (Polymer Laboratories, Amherst, MA, USA).

The mobile phase was 0.002 M phosphoric acid-acetonitrile-methanol (72:20:8, v/v/v) at a flow rate of 0.9 ml/min. Aliquots of 20 µl of both standards and samples were injected onto the column.

Extraction and Clean-up.

Spiked fish serum was prepared by adding working standard of sarafloxacin to fish serum. The extraction column packed with C2 sorbent material was conditioned with 2 X 1 ml methanol and 3 x 1 ml of 0.002 M orthophosphoric acid. The column was loaded, in this sequence, with 1) 0.5 M orthophosphoric acid (300 µl), 2) serum (250 µl), 3) internal standard enrofloxacin (50 µl, 0.5 µg/ml), and 4) distilled water (250 µl), all being sucked through at 3 psi. The column was rinsed with distilled water (100 µl) and 0.1 M orthophosphoric acid (100 µl). The sample was eluted with 5 x 100 µl 0.5 M orthophosphoric acid/methanol, 3:7 v/v. The collected elute was diluted with 0.5 ml distilled water.

Validation of the Extraction Method.

The precision, recovery and linearity of the extraction method were determined by spiking serum samples with standard solutions of sarafloxacin and internal standard enrofloxacin to yield 5, 10, 20, 50, 100, 150, 200 and 400 ng sarafloxacin pr ml in serum samples. Each concentration level was assayed in duplicate. The recovery rates were determined by comparing analysis of spiked serum with those of standard solutions.

RESULTS AND DISCUSSION

Chromatograms of a clean serum sample, and a sample of serum from fish treated with sarafloxacin, are shown in Figure 1. There were no interferences in the area of the sarafloxacin and internal standard enrofloxacin peaks.

Chromatograms of samples of serum from fish treated with sarafloxacin (Fig. 1 B) were similar to chromatograms of samples with standard addition of

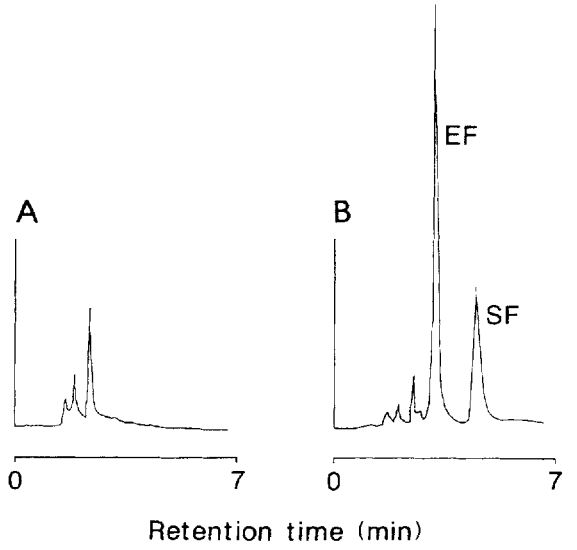

FIGURE 1
Chromatograms of extracts from fish serum for the determination of sarafloxacin (A-55620), 20 µl injected onto the HPLC.
A - Unspiked serum sample.
B - Serum sample from fish treated with sarafloxacin (SF). The sample contain 170 ng sarafloxacin (SF) and 100 ng enrofloxacin (EF) pr ml sample.

TABLE 1.

Recovery of Sarafloxacin (A-55620) from Spiked Samples of Fish Serum.

No. of samples	Amount in spiked samples (ng/ml)	Recovery %	
		Mean	SD
8	200	99.5	3.0
8	10	92.2	4.8

sarafloxacin. Chromatograms of real samples gave no additional peaks or interferences.

The linearity of the standard curve for sarafloxacin in serum was tested using peak height measurements and the internal standard. The standard curve was linear in the investigated area, 5-400 ng/ml. The correlation coefficient for sarafloxacin in serum was r=0.9991.

Table 1 shows the recovery and repeatability of sarafloxacin from serum. The recovery of samples containing 200 ng/ml was 99.5% with SD 3.0, and for samples containing 10 ng/ml the recovery was 92.1% with SD 7.0. The detection limit for sarafloxacin was 5 ng/g in serum.

The method is well suited for the determination of sarafloxacin in fish serum, and is sensitive, simple, rapid and robust. A technician could easily analyse 40 samples a day.

ACKNOWLEDGEMENTS

We are grateful to The Agricultural Research Council of Norway for financial support, grant no. 102.963.

Drug free serum and authentic samples (serum containing sarafloxacin) were provided by EWOS AQUA AS (Norway).

REFERENCES

1. Stamm, J.M. In vitro resistance by fish pathogens to aquacultural antibacterials, including the quinolones difloxacin (A-56619) and Sarafloxacin (A-56620). Journal of Aquatic Animal Health, 1, 135, 1989.

2. Fernandes, P.B., Chu, D.T.W., Bower, R.R., Jarvis, K.P., Ramer, N.R. and Shipkowitz, N. In vivo evaluation of A-56619 (Difloxacin) and A-56620: new aryl-fluoroquinolones. Antimicrob. Agents Chemother., 29, 201, 1986.

3. Fernandes, P.B., Hanson, C.W., Stamm, J.M., Vojtko, C., Shipkowitz, N.L. and Martin, E.S. The frequency of in-vitro resistance development to fluoroquinolones and the use of a murine pyelonephritis model to demonstrate selection of resistance *in vivo*. J. Antimicrob. Chemother., 19, 449, 1987.

4. Thadepalli, H., Gollapudi, S.V.S. and Chuah, S.K. Therapeutic evaluation of difloxacin (A-55619) and A-55620 for experimentally induced *Bacteroides fragilis*-associated intra-abdominal abcess. Antimicrob. Agents Chemother., 30, 574, 1986.

5. Mader, J.T., Morrison, L.T. and Adams, K.R. Comparative evaluation of A-55619, A-55620 and Nafcillin in the treatment of experimental *Staphylococcus aureus* osteomyelitis. Antimicrob. Agents Chemother., 31, 259, 1987.

6. Rogstad,A., Hormazabal, V. and Yndestad, M. Extraction and high performance liquid chromatographic determination of enrofloxacin in fish serum and tissues. J.Liq.Chromatog. Accepted.