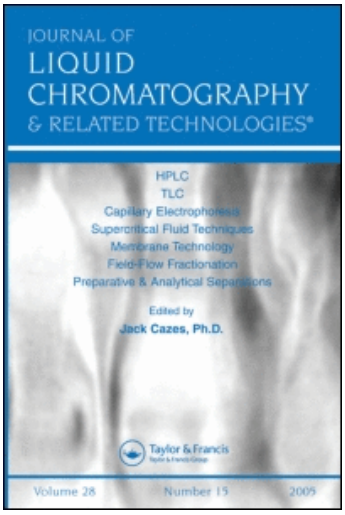

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Rapid Assay for Monitoring Residues of Enrofloxacin in Milk and Meat Tissues by HPLC

Víctor Hormazábal[a]; Magne Yndestad[a]
[a] Division of Food Hygiene, Norwegian College of Veterinary Medicine, Oslo 1, Norway

To cite this Article Hormazábal, Víctor and Yndestad, Magne(1994) 'Rapid Assay for Monitoring Residues of Enrofloxacin in Milk and Meat Tissues by HPLC', Journal of Liquid Chromatography & Related Technologies, 17: 17, 3775 — 3782
To link to this Article: DOI: 10.1080/10826079408013992
URL: http://dx.doi.org/10.1080/10826079408013992

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(17), 3775-3782 (1994)

RAPID ASSAY FOR MONITORING RESIDUES OF ENROFLOXACIN IN MILK AND MEAT TISSUES BY HPLC

VÍCTOR HORMAZÁBAL AND MAGNE YNDESTAD
Division of Food Hygiene
Norwegian College of Veterinary Medicine
P.O. Box 8146-Dep.
N-0033 Oslo 1, Norway

ABSTRACT

A high-performance liquid chromatography method for the determination of enrofloxacin in milk and meat is presented. After homogenization of the milk/tissue, the fat was separated by extraction with organic solvents and the aqueous phase analysed by HPLC. The method is simple and robust, having a limit of quantification of 3 ng/ml and 5 ng/g enrofloxacin in milk and meat respectively. The recovery rate was 86-87% from milk and 89-93% from meat.

INTRODUCTION

Enrofloxacin (EF) belongs to the quinolone carboxylic acid group of antibiotics with a broad antibacterial spectrum. EF has been found to have a satisfactory effect in the treatment of severe intestinal and respiratory infections in turkeys (1), and is useful in the treatment of calves and pigs (2), dogs and cats (3), and poultry (4).

This wide application represents a potential hazard to consumers due to the persistence of residues in milk and meat. Few methods for the determination of EF in biological samples have been published. Waggoner at al. (5) published a spectrofluorometric method for the determination of EF residues in poultry tissues, Tyczkowska et al. (6) an HPLC-method for the simultaneous determination of EF and its metabolite ciprofloxacin in canine serum and prostatic tissue, Rogstad et al. (7) an HPLC method for the determination of EF in fish serum and tissues, Hormazábal et al. (8) a rapid assay for monitoring residues of EF and sarafloxacin (SF) in fish tissues by HPLC, and Steffenak et al. (9) a rapid assay for the determination of SF in fish serum by HPLC.
This study is a continuation of our recent work on EF with the aim of developing sensitive methods for the determination of EF in milk and meat.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and solvents were of analytical or HPLC grade. EF was supplied by Bayer Norge A.S (Oslo, Norway), whereas sarafloxacin (SF), (internal standard) was donated by Abbott Laboratories (Chicago, IL, USA). Stock solutions (1mg/ml) of EF and SF were prepared in 0.03 M sodium hydroxide, and working standards were prepared by dilution with 0.002M phosphoric acic/acetonitrile/methanol (65:27:8). The solutions of 1-heptanesulfonic acid were obtained from Supelco Inc.Supelco Park, Bellefonte, USA.
Spin-X centrifuge filter units from Costar (Cambridge, MA, USA) were used.

Samples of cow`s milk and cow-beef

Whole milk (pasteurized, 3.8% fat) and cow-beef were purchased from local grocery stores, for use as control material and for spiking with EF to conduct recovery experiments.

Chromatographic Conditions

The analyses were performed on a Perkin-Elmer HPLC system, consisting of a Series 410 Bio solvent delivery

system, an ISS 100 sampling system equipped with a Lauda RMT6 cooler (14°C) from Messgeräte Werk Lauda, (Lauda Königshafen, Germany), and a LS 240 fluorescence detector (Perkin-Elmer, Norwalk, Conn., USA). The detector was operated at an excitation wavelength of 278 nm and emission wavelength of 440 nm, and with a response of 5 and an attenuation factor of 256. The integration was carried out using the software programme Omega-2 (Perkin-Elmer), wich was operated on an Olivetti M300 personal computer connected to a Bj-330 printer (Canon).
The analytical column (stainless steel, 150 x 4.6 mm I.D.) and guard column (stainless steel, 5.0 x 3 mm I.D.), were packed with 5 µm particles of PLRP-S polymer adsorbent (Polymer Laboratories, Amherst, MA, USA).
The mobile phase was a mixture of three solutions, A, B and C (65:27:8). Solution A was 0.02 M heptane sulphonate and 0.002 M phosphoric acid, made by dissolving 4.45 g/l 1-heptane sulphonic acid sodium salt (Supelco) in ca. 750 ml of 0.002 M phosphoric acid when preparing 1 litre of solution, the solution being made up to volume with 0.002 M phosphoric acid. Solution B was acetonitrile, and solution C was methanol. The flow-rate was 0.7 ml/min for 6 min followed by 1.0 ml/min for 3 min. The samples were injected at intervals of 10 min. Aliquots of 15 and 10 µl for milk and meat, respectively, were injected onto the column for the determination of EF.

Sample pretreatment

Milk. The pretreatment of milk samples is shown in Fig. 1. To 1 ml milk was added 100 µl of internal standard solution (SF, 2.5 µg/ml), 100 µl water (or standard), 0.5 ml 1M NaOH, and 3 ml acetonitrile. The sample was mixed for approx. 5 sec. and 5 ml diethyl ether - hexane (3:2) then added. The sample was again mixed for 5 sec. and was then centrifuged for 4 min. (4000 rpm.). The upper layer of organic solvents was discarded. 850 µl of the aqueous solution (corresponding to 0.5 ml milk) was pipetted into a glass-stoppered centrifuge tube, and 250 µl phosphoric acid (1M) and 6.9 ml methanol were added. The sample was again mixed for 5 sec. and then centrifuged for 3 min. (4000 rpm.).

Meat. The sample pretreatment of tissues is shown in Fig.2, and is based upon the method by Hormazábal et al. (8) for the determination of EF in fish tissue. The tissue sample, 3g of ground muscle, was weighed

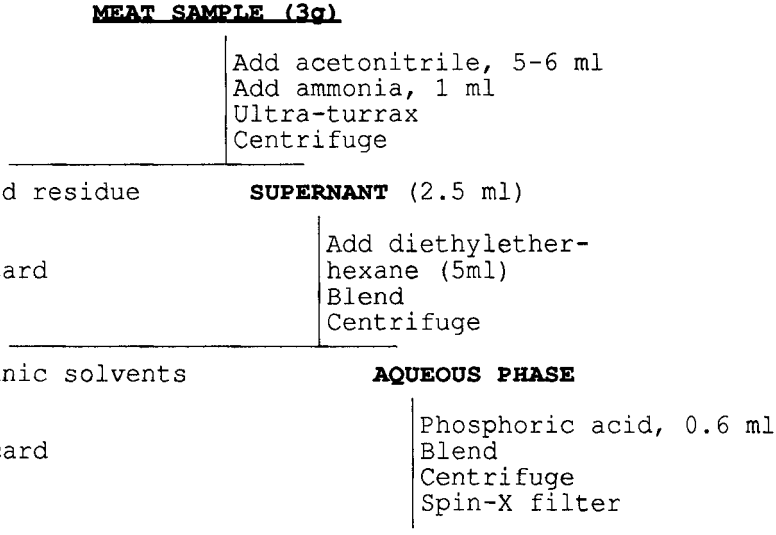

FIGURE 1
Extraction and Clean-up Procedure for Enrofloxacin from Meat Tissue.

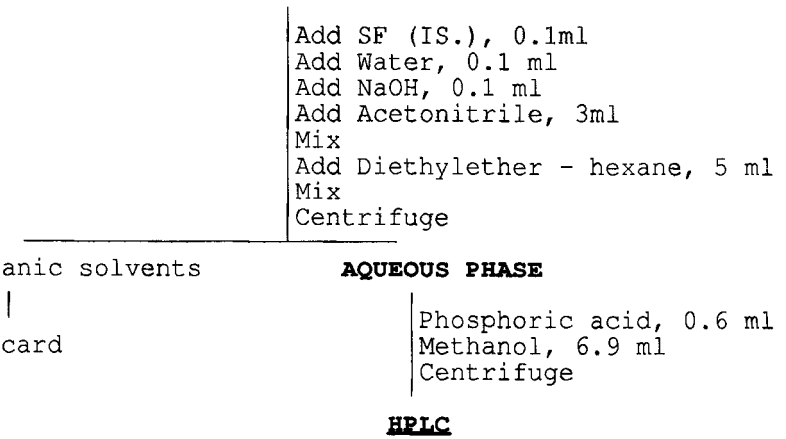

FIGURE 2
Extraction and Clean-up Procedure for Enrofloxacin from Milk.

into a 50 ml centrifuge tube with a screw cap (NUNC, Roskilde, Denmark). Volumes of 300 µl of internal standard solution (SF, 2.5 µg/ml), 1 ml ammonia and 5-6 ml acetonitrile were added. The total volume of added solvents should amount to 7 ml. The mixture was homogenized for approx. 6 sec. in an Ultra-Turrax TP 18/2 (Janke & Kunkel KG, Ika Werk, Staufen, F.R.G.). After centrifugation (3 min. at 4000 rpm.), 2.5 ml of the supernatant was pipetted into a glass-stoppered centrifuge tube and 1 ml NaCl-solution (1M), and 5 ml diethyl ether - hexane (3:2) were added. The mixture in the tube was blended well and centrifuged (2 min. at 3000 rpm.). The upper layer of organic solvents was discarded, and the aqueous solution was acidified using 0.6 ml phosphoric acid (5M). The sample was mixed and centrifuged for 3 min at 3000 rpm. Approximatly 500 µl were then filtered through a Costar Spin-X centrifuge filter unit with 0.2 µm nylon membrane, and centrifuged for 4 min. at 10000 rpm. (5600g).

Validation of the pretreatment procedure

The precision, recovery and linearity of the pretreatment procedure were determined by analyses of spiked milk and meat in the concentration range 3-200 ng/ml and 5-200ng/g, respectively.
The spiked samples were extracted using the above procedures. Duplicated samples were used. The recovery rates were determined by comparing results of analysis of the spiked milk and muscle sample with those of standard solution. The linearity of the standard curves for EF in milk and meat was tested using peak-height measurements and internal standard.

RESULTS AND DISCUSSION

The chromatographic system that had appeared to be efficient for analysis of EF in fish tisssue is not applicable to milk and meat. Minor modifications of the aqueous phase/organic phase ratio of the mobile phase did not improve the separation of the peaks of the drugs from those of the endogenous compounds. However, the problem was overcome by adding an anion-pairing agent to the mobile phase.
Chromatograms of extracts of blank samples, and spiked samples from milk and meat are shown in Fig. 3.
The limit of quantification was 3 ng/ml and 5 ng/g for EF in milk and meat, respectively. However, the

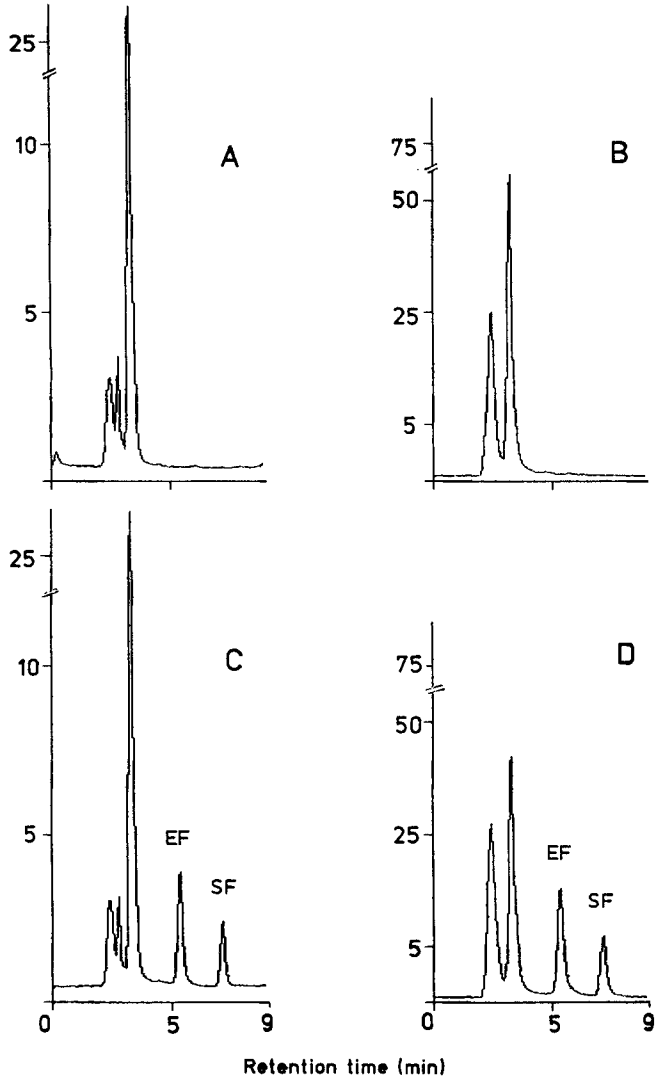

FIGURE 3

Chromatograms of extracts from milk and meat. **A**: drug-free milk, **B**: drug-free meat, **C**: milk spiked with enrofloxacin (100 ng/ml), **D**: meat spiked with enrofloxacin (100 ng/g).

TABLE 1.

Recovery and repeatability for enrofloxacion from spiked samples of milk and meat.

Sample	No of Samples	Amount of drug added (ng/ml or g)	Recovery % EF Mean	SD*
Milk (1ml)	8	10	86	2.8
	8	100	87	0.9
Meat (3g)	8	10	89	0.5
	8	50	93	2.0

*SD-standard deviation

sensitivity may be enhanced by using a larger amount of sample.

The extraction procedures were validated, and the results are shown in Table 1, showing good recovery of EF in milk and meat. The average recoveries for EF over the concentration range of the standard curve were 86.5% for milk and 91% for meat.

The precision and recovery of the internal standard were calculated, the average recovery of SF being found to be 85.5% from milk (standard deviation (SD)= 1.9%) and 90% from meat (SD= 1.65%).

The linearity of the standard curve was 0.9996 for EF both in milk and meat when using the internal standard method. The external standard method of calculation gave a linearity coefficient of 0.9990 and 0.9993 for EF in milk and meat, respectively.

The results also showed that the precision and accuracy of the quantification of EF are good.

The simplified extraction and clean-up procedure makes it possible to monitor drug concentrations in 30 or 40 samples of meat or milk, respectively per day.

CONCLUSIONS

This study has shown that EF levels in milk and meat can be determined after the samples have been subjected

to certain very simple clean-up steps. Good recovery, precision and sensitivity were obtained.

ACKNOWLEDGEMENT

We are grateful to the Norwegian Research Council for financial support.

REFERENCES

1) Braunius, W. W. Tijdschrift voor Diergeneeskunde , deel 112, afl.9, 1987.

2) Bauditz, R. (1987), Vet. Med. Rev. No. 2, 122 - 129.

3) Bauditz, R. (1987), Vet. Med. Rev. No. 2, 137 - 140.

4) Bauditz, R. (1987), Vet. Med. Reev. No. 2, 130 - 136.

5) Waggoner, T. B. and Bowman, M. C., J. Assoc. Off. Anal. Chem. 70, 813, 1987.

6) Tyczkowska, K., Hedeen, K. M., Aucoin, D. P. and Aronson, A. L., J. Chromatogr. 493, 337-346, 1989.

7) Rogstad, A., Hormazábal, V. and Yndestad, M., J. of L. Chromatogr., 14 (3), 521-531, 1991.

8) Hormazábal, V., Rogstad, A., Steffenak, I. and Yndestad, M., J. of L. Chromatogr., 14 (8), 1605-1614, 1991.

9) Steffenak, I., Hormazábal, V. and Yndestad, M., J. of L. Chromatogr., 14 (10), 1983-1988, 1991.

Received: March 17, 1994
Accepted: May 6, 1994