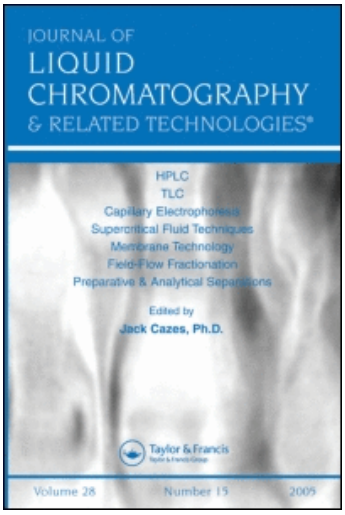

Journal of Liquid Chromatography & Related Technologies

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

Simple and Rapid Analysis of Pefloxacin, Fenbufen and Felbinac in Human Plasma Using High Performance Liquid Chromatography

Giuseppe Carlucci[a]; Giancarlo Palumbo[b]; Pietro Mazzeo[a]

[a] Department of Chemistry, Chemical Engineering and Materials University of L'Aquila, L'Aquila, Italy [b] Department of Internal Medicine Chair of Pharmacology, University of L'Aquila, L'Aquila, Italy

To cite this Article Carlucci, Giuseppe , Palumbo, Giancarlo and Mazzeo, Pietro(1996) 'Simple and Rapid Analysis of Pefloxacin, Fenbufen and Felbinac in Human Plasma Using High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 19: 7, 1107 — 1115

To link to this Article: DOI: 10.1080/10826079608006305

URL: http://dx.doi.org/10.1080/10826079608006305

J. LIQ. CHROM. & REL. TECHNOL., 19(7), 1107–1115 (1996)

SIMPLE AND RAPID ANALYSIS OF PEFLOXACIN, FENBUFEN AND FELBINAC IN HUMAN PLASMA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Giuseppe Carlucci,[1]* Giancarlo Palumbo[2]
Pietro Mazzeo[1]

[1]Department of Chemistry
Chemical Engineering and Materials
University of L'Aquila
Via Vetoio
I-67010 Coppito (L'Aquila), Italy

[2]Department of Internal Medicine
Chair of Pharmacology
University of L'Aquila
L'Aquila, Italy

ABSTRACT

A simple and reproducible method for the simultaneous determination of the antimicrobial agent pefloxacin and the anti-inflammatory drug fenbufen with its metabolite felbinac in human plasma is described. It involves a two steps liquid-liquid extraction and separation using an Adsorbosphere SAX column with ultraviolet detection at 280 nm. 2-[4-(2'-

furoyl)phenyl]propionic acid is used as internal standard. The coefficients of variation are less than 5%. The lower limits of detection are 0.1, 0.3 and 0.1 μg/mL for pefloxacin, fenbufen and felbinac, respectively. This method was found to be applicable to pharmacokinetic and pharmacodynamic studies of each drug after the concomitant administration of pefloxacin and fenbufen.

INTRODUCTION

Fenbufen, or γ-oxo-[1,1'-biphenyl]-4-butanoic acid (Figure 1A), is a potent anti-inflammatory drug with analgesic properties, active by inhibition of prostaglandin synthesis.[1-5] Pefloxacin is a potent quinolone antibacterial agent; it is highly active in *vitro* against a broad spectrum of Gram-positive and Gram-negative organisms, including those resistant to β-lactam antibiotics.[6-9] Its chemical name is 1-ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxoquinolinecarboxylic acid; its formula is shown in Figure 1B. Quinolone carboxylic acid derivatives, used in the treatment of several infections, are known to elicit epileptogenic neurotoxic effects. Several quinolones inhibit the specific binding of [^{3}H]GABA to synaptic plasma membranes from rat brain in a concentration-dependent manner. Since GABA is a major inhibitory neurotransmitter in the mammalian central nervous system, these results indicate that quinolones may induce seizures through the inhibition of postsynaptic GABA function.

Recently, it was reported that several patients had severe chronic convulsions during therapy with quinolones and fenbufen and it is reasonable to assume that, simultaneously administered, fenbufen enhanced the neurotoxic potency of quinolones via some pharmacodynamic interaction in the brain.[10] Many high performance liquid chromatographic methods have been developed for the determination of either pefloxacin[11,12] or fenbufen[13,14] in plasma, but none has described the simultaneous determination of both agents.

The interest in this group of drugs has prompted us to develop a simple and sensitive assay method for both these substances in human plasma, which could be applied to pharmacokinetic studies. The procedure, based on the use of the high performance liquid chromatography, allows accurate and precise results, and permits also the determination of felbinac or 4-biphenylacetic acid (Figure 1C), the major metabolite of fenbufen.

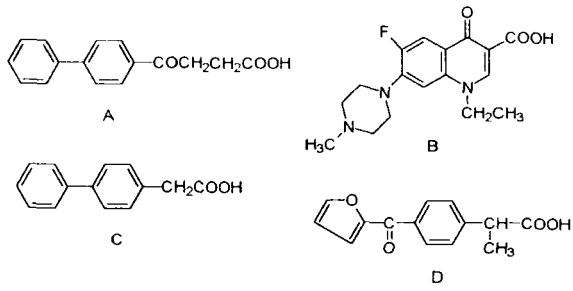

Figure 1. Chemical structures of fenbufen (A), pefloxacin (B), felbinac (C) and internal standard (D).

EXPERIMENTAL

Chemicals and Reagents

Fenbufen was purchased from Sigma (Sigma, St Louis, MO, U.S.A.). Felbinac was purchased from Aldrich (Aldrich Chimica, Milan, Italy). Pefloxacin was extracted from tablets. The internal standard (2-[4-(2'-furoyl)phenyl]propionic acid) (Figure 1D) was a gift of the Chair of Pharmacology of our University. HPLC-grade acetonitrile was obtained from Farmitalia-Carlo Erba (Farmitalia-Carlo Erba, Milan, Italy). Dichloromethane, diethylether and all other analytical-grade reagents (sodium hydroxide, sodium hydrogen phosphate and potassium dihydrogen phosphate) were purchased from Fluka Chemie (Fluka Chemie, Buchs, Switzerland). Water (HPLC-grade) was obtained by distillation in glass and passage through a Milli-Q Water Purification System (Millipore Corporation, Bedford, MA, USA).

Standard Solutions

Stock solutions of pefloxacin, fenbufen and felbinac were prepared by dissolving 10 mg of each compound in 10 mL of 0.01M sodium hydroxide. These solutions could be stored at -20°C for over 1 month with no evidence of decomposition. Stock solution of the internal standard was prepared by dissolving 20 mg of compound in 10 mL of methanol. Standard solutions, each containing the three drugs, were prepared with control human plasma in the concentration range of 0.2-10 μg/mL for pefloxacin, 0.5-30 μg/mL for

fenbufen, and 0.2-10 μg/mL for felbinac. For each solution the concentration of the other two drugs was kept constant at 5 μg/mL , and an aliquot (20 μl) of the internal standard stock solution was added. These standards were treated concurrently in the same manner as the samples to be analysed. The calibration curves were obtained by plotting the peak-area ratios of each drug to internal standard versus its concentration.

Instrumentation and Chromatographic Conditions

The HPLC system consisted of a model 7125 sample injector Rheodyne (Rheodyne, Cotati, CA, U.S.A.) equipped with a 20μl loop, a Waters Associates constant flow reciprocating pump (model 510) with Data Module integrator model 740, and a Tunable absorbance detector model 486 (Milford, MA, USA). The separation system was on an analytical 250 x 4.6 mm I.D. anion-exchange Adsorbosphere SAX (5 μm particle size) column (Alltech Associates, Deerfield, IL, U.S.A.) protected by a 20 x 4.6 mm anion-exchange Vydac AXGU (10 μm particle size) precolumn (Separations Group, Hesperia, CA, U.S.A.). Separations were performed at room temperature and the detector set at 0.1 absorbance unit full scale. The mobile phase consisted of a mixture of acetonitrile, 0.1M phosphate buffer pH 7.0 (10:90, vol/vol). Phosphate buffer was filtered through an HA 0.45 μm filter, while the acetonitrile was filtered through a FA 0.5 μm filter (Millipore, Bedford, MA, USA). The mobile phase was prepared daily and delivered at a flow rate of 1.2 mL/min. Column eluate was monitored at 280 nm.

Extraction Procedure

Pefloxacin, fenbufen, felbinac and the internal standard were extracted from plasma using a simple two-steps extraction procedure. An aliquot (20μl) of the internal standard stock solution was added to 1.0 mL of plasma sample and mixed into a 16x150mm screw-capped tube. The mixture was vortex-mixed for 1 min with 4mL of dichloromethane-diethylether (80:20, vol/vol). The sample was centrifuged at 1000 x **g** for 10 min and the organic layer was separated and transferred into a second tube. Fresh dichloromethane-diethylether (4mL) was added to the first tube and the same extraction procedure was repeated twice. The organic phase was evaporated to dryness under a stream of nitrogen. 100μl of 0.01M sodium hydroxide were then added to the residue. The tube was shaken for 5 min and 20μl aliquots were used for HPLC analysis.

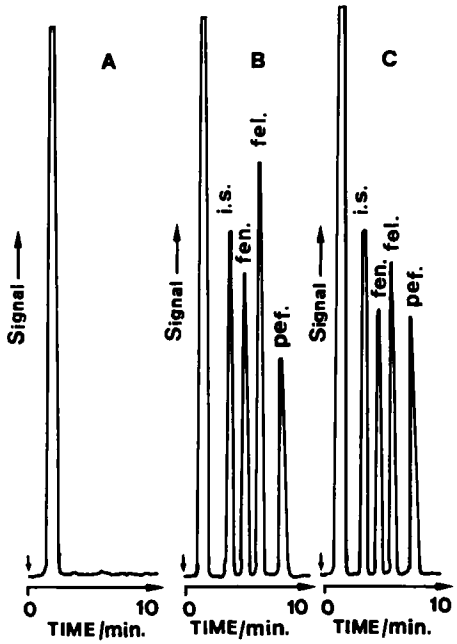

Figure 2. HPLC profiles of human plasma extracts. (A) drug-free human plasma; (B) drug-free human plasma spiked with 2.0 μg/mL of pefloxacin (pef.), 5.0 μg/mL of fenbufen (fen.), 3.0 μg/mL of felbinac (fel.) and 5.0μg/mL of internal standard (i.s.); (C) plasma sample from a volunteer (two hours after an oral dose of 400 mg of pefloxacin and 300 mg of fenbufen) containing 3.1 μg/mL of pefloxacin, 4.3μg/mL of fenbufen and 2.2 μg/mL of felbinac. Vertical axis: UV detector response (280 nm). Horizontal axis: retention time (min).

RESULTS AND DISCUSSION

Figure 2 shows a typical chromatogram of an extracted drug-free blank plasma (A), an extracted plasma sample (B), and a two hours post dose sample from a volunteer (C). The elution order is internal standard, fenbufen, felbinac and pefloxacin, respectively. The retention times for internal standard, fenbufen, felbinac and pefloxacin were 3.9, 5.3, 6.8 and 9.2 min, respectively. There were no interfering peaks detected in either the blank plasma sample or the plasma standard. The validity of the liquid chromatographic assay was established through a confirmatory study of calibration curves specificity, sensitivity, accuracy and precision. The calibration graphs were linear from 0.2

Table 1

Precision and Accuracy of Perfloxacin Calibration Standards in Human Plasma

Conc. (μg/mL)	N	Conc. Found (μg/mL)	SD	CV (%)	RE (%)
0.2	7	0.19	0.007	3.6	5.2
0.3	7	0.29	0.01	3.4	3.4
0.5	7	0.49	0.02	4.0	2.0
1.0	7	0.97	0.05	5.1	3.1
2.0	7	1.95	0.08	4.1	2.5
5.0	7	4.81	0.2	4.1	3.9
10	7	9.84	0.4	4.0	1.6

SD: standard deviation; CV: coefficient of variation; RE: relative error.

to 10 μg/mL for pefloxacin, from 0.5 to 30 μg/mL for fenbufen and from 0.2 to 10 μg/mL for felbinac, respectively. All correlation coefficients were more than 0.998. The mean of seven different calibration graphs yielded the following equations: $y = 0.041x + 0.264$, $y = 0.052x + 0.573$ and $y = 0.039x + 0.632$, where y is the peak area ratio of either pefloxacin, fenbufen and felbinac to the internal standard and x is the concentration of each drug (μ g/mL).

The precision and accuracy of the method was determined by preparing pools of plasma containing pefloxacin, fenbufen and felbinac at seven different concentrations. The values for pefloxacin, fenbufen and felbinac for each standard concentration were determined by nine repeated analyses, using spiked human plasma. Results are given in Table 1-3. The method was found to be reproducible and accurate. The coefficients of variation were less than 4.8% for pefloxacin (Table 1), 4.6% for fenbufen (Table 2) and 4.7% for felbinac (Table 3). The mean extraction efficiencies calculated by comparison of the peak-area ratios of the extracted samples with those of aqueous standards of same concentration, were 95, 96 and 95% for pefloxacin, fenbufen and felbinac. The extraction efficiency of the internal standard was 97%.

Table 2

Precision and Accuracy of Fenbufen Calibration Standards in Human Plasma

Conc. (μg/mL)	N	Conc. Found (μg/mL)	SD	CV (%)	RE (%)
0.5	7	0.48	0.01	2.1	4.1
1.0	7	0.95	0.03	3.1	5.2
2.0	7	1.92	0.05	2.6	4.1
5.0	7	4.9	0.15	3.0	2.0
10.0	7	9.57	0.3	3.1	4.5
20.0	7	19.4	0.85	4.3	3.1
30.0	7	29.0	1.4	4.8	3.4

SD: standard deviation; CV: coefficient of variation; RE: relative error.

Table 3

Precision and Accuracy of Felbinac Calibration Standards in Human Plasma

Conc. (μg/mL)	N	Conc. Found (μg/mL)	SD	CV (%)	RE (%)
0.2	7	0.19	0.01	4.7	5.2
0.5	7	0.48	0.02	5.1	2.0
1.0	7	0.96	0.05	5.2	4.1
2.0	7	1.95	0.08	4.1	4.1
3.0	7	2.92	0.14	4.7	2.7
5.0	7	4.78	0.18	3.7	4.6
10	7	9.88	0.35	3.5	1.2

SD: standard deviation; CV: coefficient of variation; RE: relative error.

The lower limits of detection, using a signal-to-noise ratio of three, were 0.1 μg/mL for pefloxacin, 0.3 μg/mL for fenbufen and 0.1 μg/mL for felbinac, respectively. The comparison of the peak-area ratios for pefloxacin, fenbufen and felbinac between fresh plasma samples and samples frozen at -20°C for a

period of four weeks showed no differences. The HPLC method described in this paper allows the simultaneous determination of pefloxacin, fenbufen and felbinac in human plasma. Since the inhibition of GABA receptor binding to synaptic membranes is dose dependent, it is useful to know the simultaneous concentrations of pefloxacin, fenbufen and felbinac. This method permits its application to pharmacodynamic studies of these compounds and will facilitate detailed investigations on the interactions between new quinolones and fenbufen.

Acknowledgement

This research was supported by a grant from the Ministero della Università e della Ricerca Scientifica e Tecnologica (40%) and the Consiglio Nazionale delle Ricerche.

REFERENCES

1. F. S. Chiccarelli, H. J. Eisner, G. E. Van Lear, Arzneim. - Forsch., **30**, 728-735 (1980).

2. A. E. Sloboda, E. L. Tolman, A. C. Osterberg, J. Panagides, Arzneim. - Forsch., **30**, 716-721 (1980).

3. H. F. Bolte, A. V. Koralec, C. E. Traitor, Arzneim. - Forsch., **30**, 721-725 (1980).

4. B. A. Jackson, G. Tonelli, F. Chiesa, L. Alvarez, Arzneim. - Forsch., **30**, 725-728 (1980).

5. R. G. Child, A. C. Osterberg, A. E. Sloboda, A. S. Tomcufcik, Arzneim. - Forsch., **30**, 695-702 (1980).

6. S. Nakamura, A. Minami, H. Katae, S. Inoue, J. Yamagishi, Y. Tacase, M. Shimizu, Antimicrob. Agents Chemother., **23**, 641-648 (1983).

7. Y. Goueffon, G. Montay, F. Roquet, M. Pesson, C. R. Acad. Sci. Sér., III, **292**, 37-40 (1981).

8. M. Thibault, B. Coumare, C. J. Soussy, J. Duval, Ann. Microbiol. (Paris), **132A**, (1981).

9. G. Montay, Y. Goueffon, F. Roquet, Antimicrob. Agents Chemother., **25**, 463-472 (1984).

10. Ministry of Health and Welfare. Information on Drug Adverse Reactions Tokyo, **81**, 1 (1986).

11. G. Montay, J.P. Tassel, J. Chromatogr., **339**, 214-218 (1985).

12. G. Montay, Y. Blain, F. Rouquet, A. Le Hir, J. Chromatogr., **272**, 359-365 (1983).

13. G. Van Lear, F. Chiccarelli, P. Bonenfant, A. Barr, J. Pharm. Sci., **67**, 1662-1664 (1978).

14. J. S. Fleitman, S. G. Schulman, J. H. Perrin, J. Chromatogr., **228**, 372-376 (1982).

Received September 15, 1995
Accepted October 6, 1995
Manuscript 4000