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Short communication

High-performance liquid chromatography of flufenamic acid in rat plasma

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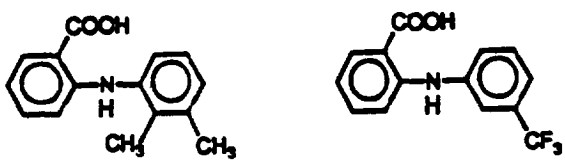
Abstract

A simple high-performance liquid chromatographic (HPLC) method for the determination of flufenamic acid in rat plasma is described. After liquid–liquid extraction, the drug is separated by HPLC on a 5-μm octadecylsilica column (Nucleosil C_{18}) with ultraviolet detection at 280 nm. Linear calibration graphs for flufenamic acid were constructed from 0.5 to 15 μg/ml. The method has been applied to a pharmacokinetic study in animals.

Keywords: Flufenamic acid

1. Introduction

Flufenamic acid, N-(α,α,α-trifluoro-*m*-tolyl)anthranilic acid (Fig. 1), is an analgesic, anti-inflammatory and antipyretic drug. It is used in the treatment of rheumatic disorders and soft tissue injuries [1]. Flufenamic acid inhibits the cyclooxygenase enzyme and thus exerts its anti-inflammatory activity by inhibition of prostaglandin synthesis [2]. This effect seems to be correlated to the appearance of acute protocolitis associated with non-steroidal anti-inflammatory drug therapy [3]. In general, its potency is about half that of phenylbutazone [4]. Flufenamic acid was determined in plasma and tissue of patients with chronic polyarthritis, after a single intramuscular application of etofenamate [5], using a HPTLC method [6]. Jagota and Stewart [7] have reported the separation of non-steroidal anti-inflammatory agents (NSAIDs) using capillary SFC in pharmaceuticals; Salem and Kheir [8] have determined the concentration of flufenamic acid in pharmaceutical preparations using atomic absorption spectrometry. In this paper, a reversed-phase HPLC method, based on liquid–liquid extraction and ultraviolet detection, is proposed for the quantitative determination of flufenamic acid in rat plasma. This assay has been used for pharmacokinetic studies in animals and in man.



mefenamic acid **flufenamic acid**

Fig. 1. Structures of flufenamic acid and mefenamic acid (internal standard).

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2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical grade. Flufenamic acid and mefenamic acid (internal standard = I.S.) were kindly supplied by Sigma (St. Louis, MO, USA). HPLC-grade methanol, dichloromethane and hydrochloric acid were obtained from J.T. Baker (Phillipsburg, NJ, USA). Water was double glass-distilled and Milli-Q filtered.

2.2. Equipment

The HPLC system consisted of a Model 302 LC pump (Gilson, Villiers le Bel, France) equipped with a Rheodyne syringe-loading Model 7125 sample injector with a 20-μl loop. A variable-wavelength ultraviolet detector (Holochrome, Gilson) was used. The detector was set at 280 nm and 0.05 AUFS. The chromatographic response was recorded by a Gilson NI recorder (Gilson).

2.3. Chromatographic conditions

A nucleosil C_{18} analytical column (Macherey-Nagel, Duren, Germany) packed with reversed-phase C_{18} material (5 μm) was used. The mobile phase was prepared from methanol and water (77:23, v/v). The solution was filtered and degassed for 20 min using an ultrasonic water-bath and pumped at a flow-rate of 0.8 ml/min. Chromatographic separations were performed at room temperature.

2.4. Standard solutions

The standard stock solution of flufenamic acid (1 mg/ml) was prepared in methanol and stored at 4°C. Working solutions (100 μg/ml) were made by dilution of the stock solution in methanol. The I.S. stock solution of mefenamic acid (1 mg/ml) was also prepared in methanol and stored at 4°C. A mefenamic acid solution (100 μg/ml) was prepared by dilution of the stock solution with methanol.

The flufenamic acid working solution and the I.S. solution (100 μg/ml) were freshly prepared every working day.

2.5. Sample preparation

To 1 ml of plasma, 4 μg of mefenamic acid (I.S.) and 1 ml of 1 *M* HCl were added. The samples were then extracted with 6 ml of dichloromethane on a mechanical shaker for 20 min. After centrifugation (2000 *g*) for 5 min, the upper organic phase was transferred to a 10-ml centrifuge tube and evaporated to dryness under a gentle nitrogen stream, at 40°C. The residue was reconstituted with 50 μl of methanol and 20 μl were injected onto the HPLC column.

2.6. Plasma calibration curve

Standard plasma calibration curves were obtained from spiked plasma samples in the concentration range 0.5–15 μg/ml. Calibration graphs were constructed by plotting the concentration of plasma flufenamic acid (x-axis) (μg/ml) versus the peak-height ratio (flufenamic acid/I.S.) (y-axis) using linear regression.

2.7. Recovery

The absolute recovery of flufenamic acid from rat plasma was determined in triplicate at different plasma concentrations (0.5 and 15 μg/ml) by comparing the peak heights of the drug obtained from extracted plasma samples with those obtained from direct injections of equivalent amounts of the flufenamic acid standards in methanol. The recovery of the I.S. was evaluated by comparing the peak heights of six extracted samples to six standard samples at the same concentration used for the extraction of flufenamic acid.

3. Results and discussion

In Fig. 1 the structures of flufenamic acid and mefenamic acid are reported. Fig. 2 shows chromatograms of extracts from blank rat plasma, blank rat plasma spiked with flufenamic acid and the I.S. and from a rat plasma sample after a single topical administration of flufenamic acid (30 mg), as ointment. Fig. 3 shows the plasma flufenamic acid concentration–time profiles following a topical administration of 30 mg of flufenamic acid (as oint-

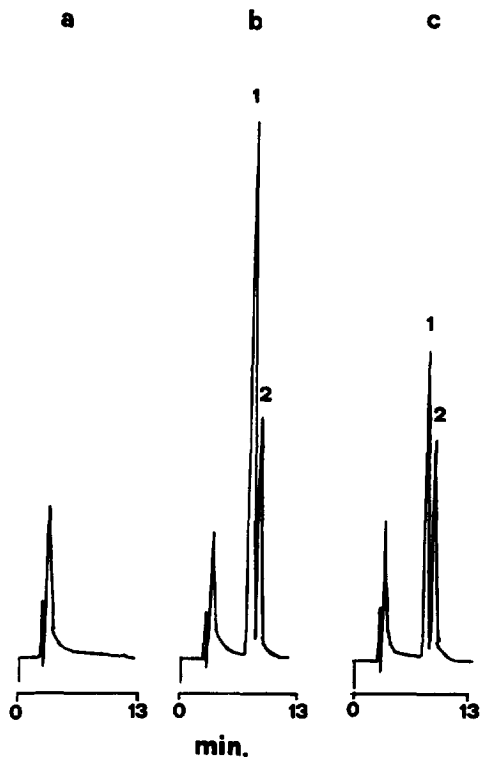

Fig. 2. Typical chromatograms of extracts of rat plasma: (a) blank plasma; (b) plasma spiked with 5 μg/ml flufenamic acid; (c) rat plasma sample after a topical administration of 30 mg of flufenamic acid. Peaks: 1 = Flufenamic acid; 2 = I.S.

ment) on the right leg adductor of the rat. The drug and the I.S. have retention times of 7.2 and 8.6 minutes, respectively. The derived pharmacokinetic parameters are summarized in Table 1.

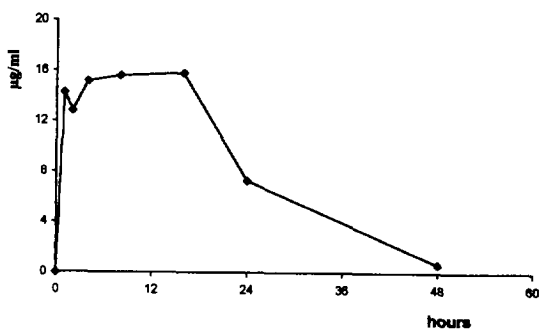

Fig. 3. Concentration–time profile for flufenamic acid in rat plasma following topical administration of 30 mg of flufenamic acid (ointment). Each point is the mean of three determinations from different animals.

Table 1
Flufenamic acid pharmacokinetic parameters after a single 30-mg topical administration (as ointment) on the rat leg

Parameter	Value
C_{max} (μg/ml)	15.6
t_{max} (h)	8.0
$t_{1/2}$ (h)[a]	7.7
$AUC_{0-\infty}$ (μg h/ml)	400.0

[a] $t_{1/2}$ calculated starting at the 16[th] h.

3.1. Linearity and precision

The standard curve of flufenamic acid in plasma was linear over the concentration range (0.5–15 μg/ml) examined. The regression equation for flufenamic acid was $y = 0.30064x - 0.10785$ ($r = 0.992$), were y is the peak-height ratio (drug/I.S.) and x was the plasma concentration of flufenamic acid. The intra-assay precision was determined using quadruplicate spiked samples at four different concentrations (1, 2, 4 and 10 μg/ml) (Table 2).

The intra-assay C.V. for every studied concentration, was less than 7%, illustrating the precision of the method for routine purposes. The inter-assay precision was studied using spiked plasma samples at four different concentrations, that were analysed at least four times within a 20-day period. The results show that the concentration values are reproducible with an inter-assay C.V. at the studied concentrations of less than 9% (Table 1). The stability of flufenamic acid in rat plasma was investigated after storage for 7 h at room temperature (ca. 25°C) and after up to 30 days at −20°C. No loss of drug was seen under these conditions.

3.2. Recovery

The mean (± S.D.) recovery for flufenamic acid from plasma samples was 89.2 ± 1.8% at 15 μg/ml and 77.8 ± 3.6% at 0.5 μg/ml. The recovery for the I.S., mefenamic acid, was 76.2 ± 4.8%.

3.3. Limit of detection

Diluted standard solutions of flufenamic acid were injected directly onto the HPLC system, to estimate the minimum amount of detectable drug. The sensitivity of the assay was 0.1 μg/ml when a signal-to-

Table 2
Inter- and intra-assay accuracy and precison of the method for determining flufenamic acid concentration in rat plasma

Concentration (μg/ml)	Mean (μg/ml)		C.V. (%)	
	Inter-assay[a]	Intra-assay[b]	Inter-assay[a]	Intra-assay[b]
1	1.16	1.11	8.9	6.1
2	2.13	2.09	5.3	3.4
4	4.20	4.15	1.7	5.4
10	10.20	10.15	2.3	2.9

[a] $n = 4$.
[b] $n = 4$.

noise ratio of three was used as a criterion for a significant response.

4. Conclusions

A simple and selective HPLC assay with ultraviolet detection has been developed for the determination of flufenamic acid in plasma samples. Because of its specificity, good reproducibility, accuracy, inexpensiveness, relative rapidity and sufficient sensitiveness, the method was successfully used in pharmacokinetic studies in rats [9] and to quantify therapeutic concentrations of flufenamic acid in human plasma samples for single- or multiple-dose clinical studies (unpublished data).

References

[1] A. Seligra and F. Ingles, Curr. Med. Res. Opin., 12 (1990) 249–253.
[2] H.O.J. Collier and W.J.F. Sweatman, Nature, 219 (1968) 864–865.
[3] S. Ravi, A.C. Keat and E.C.B. Keat, Postgrad. Med. J., 62 (1986) 773–776.
[4] C.V. Winder, Ann. Physic. Med., (Suppl), (1966) 7–16.
[5] G. Köhler, W. Tressel, H.D. Dell, M. Doersing, W. Fisher, R. Kamp and M. Langer, Arzneim. Forsh. Drug Res., 42 (1992) 1487–1491.
[6] B. Beckermann and H.D. Dell, in E. Kaiser (Editor), Institute of Chromatography, Bad Dürkeim, Proc. 3rd Int. Symp. on Instrumental HPTLC, Würzburg, 1985, pp. S 15–24.
[7] N.K. Jagota and J.T. Stewart, J. Chromatogr., 604 (1992) 255–260.
[8] H. Salem and A.A. Kheir, Anal. Lett., 28 (1995) 1833–1848.
[9] D. Cerretani, L. Micheli, A.I. Fiaschi, M.R. Romeo, P. Dal Pra and G. Giorgi, in Swedish Academy of Pharmaceutical Science (Editor), Stockholm, Sweden, Proc. Symposium on Topical Administration of Drugs. Stockholm, January 27–29, 1992, p. 1.