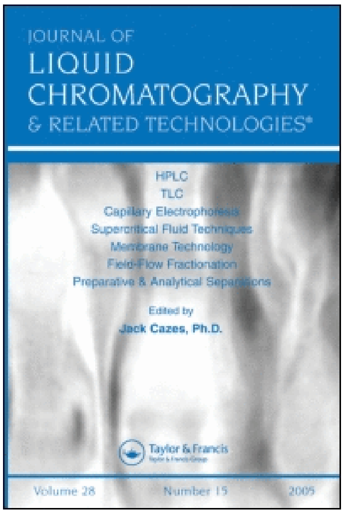

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The Use of Bamifylline as Internal Standard in the Reversed Phase HPLC Analysis of Mefenamic Acid in Pharmaceuticals and Small Volumes of Biological Fluids

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THE USE OF BAMIFYLLINE AS INTERNAL STANDARD IN THE REVERSED PHASE HPLC ANALYSIS OF MEFENAMIC ACID IN PHARMACEUTICALS AND SMALL VOLUMES OF BIOLOGICAL FLUIDS

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ABSTRACT

The reversed-phase liquid chromatographic determination of mefenamic acid in pharmaceutical preparations and small volumes of biological fluids, blood serum (40 μl) and urine (100 μl) is developed. The biological fluids containing internal standard passed through C_{18} cartridges, Bond Elut, for sample clean-up and subsequent separation of bamifylline and mefenamic acid from endogenous interfering compounds. HPLC analyses are performed with a Lichrosorb-RP 18, 10 μm, 250x4 mm I.D. column, employing gradient elution for the development of the chromatogram. The gradient system is formed with the following solutions: A = 0.05 M ammonium acetate and B = methanol. The chromatogram is started with solvent A containing 78% solvent B, followed by a sharp decrease from 78 to 72% in 5 min. The flow rate is 0.81 ml/min and the detection is achieved at 285 nm. The retention time is 3.90 min for mefenamic acid and 4.93 min for the internal standard, bamifylline. Thus the problems existing with caffeine in blood and urine samples from patients are overcomed. The absolute detection limit is 1 pg while the quantitation limit is 1.36 ng and linearity is observed up to 120 ng.

The method outlined in this paper is simple, rapid, sensitive, accurate and can be applied to the determination of mefenamic acid in pharmaceutical preparations (tablets, suppositories and suspensions) and biological fluids (blood serum and urine).

Quantitation limits in blood serum and urine samles, were found to be 0.45 ng and 0.26 ng respectively, due to preconcentration on solid-phase cartridges. The proposed method can be readily utilised for clinical pharmacokinetic studies.

INTRODUCTION

Mefenamic acid, [N-2,3 xylyl]anthranilic acid, is a widely prescribed non-steroidal anti-inflammatory analgesic, acting as a potent prostaglandin synthetase inhibitor with an inhibitory action on prostaglandin receptors too. It's clinical efficacy, like other fenamates, has been documentated in the treatment of rheumatic diseases, dysmenorrhea and migrane[1-6].

It is rapidly absorbed from the gastrointestinal tract with peak concentrations in blood after 2-4 h. The mean plasma half-life of mefenamic acid is 1.8h, found more than 99% protein-bound. The elimination of mefenamic acid occurs principally by extrarenal mechanisms, i.e. by the hepatic metabolic clearance to hydroxylated derivatives and their glucuronide conjugates. The excretion of mefenamic acid is performed through kidneys and faeces.

The therapeutic concentration in blood, ranges between 1-10 ng/μl. Overdosage produces excitement, incoordination, depression and convulsions in mice, while it has been stated that the possible effects of massive overdosage in man include flaccid depression, coma, convulsions, diarrhoea and haematological abnormalities. Especially convulsions have been the most serious manifestation of toxicity[7].

Several analytical methods have been published for the analysis of mefenamic acid, including TLC, fluorimetry, colorimetry, electron capture gas-liquid chromatography and high-pressure liquid chromatography[8-14]. Among these methods others require high sample volumes and others need long separation or sample pretreatment times.

This paper presents a simple, sensitive, accurate and fast method for the analysis of mefanamic acid by gradient reversed phase HPLC, in the presence of bamifylline as internal standard, in tablets, suspension and suppositories, as well as in samples of biological origin, like blood serum and urine.

This work was prompted by the need to develop a simple, rapid, accurate and sensitive procedure for the routine analysis of mefenamic acid, especially due to its short biological half-life, 1.8h. The proposed method requires only a small volume of plasma, (40 µl) and urine, (100 µl) and a rapid sample pretreatment, thus being very suitable for therapeutic monitoring of mefenamic acid in patients undergoing conventional mefenamic acid therapy and for pharmacokinetic studies of the drug as well.

MATERIALS AND METHODS

Apparatus

Mefenamic acid analyses were carried out on a high performance liquid chromatograph consisted of a ternary gradient pump Spectra Physics, model SP 8800 (California, USA). A Spectra Physics, Spectra Chrom 100, variable wavelength UV-Vis detector operated at 285 nm and a sensitivity setting of 0.002 absorbance units full scale (AUFS). A Rheodyne 7125 (California, USA) injection valve was fitted with a 10 µl loop. The pressure was 1410 psi at a flow rate of 0.81 ml/min. The analytical column was a Lichrosorb RP-18, 10 µm ODS, 250x4 mm I.D., stainless steel from MZ Analysentechnik. Bond Elut C_{18} cartridges were obtained from Analytichem International, a division of Varian (Harbor City, USA)

Computations were performed using a Vip 312 computer.

Materials

Mefenamic acid anhydrous powder (Ph. Eur.) was supplied from ELPEN (Athens, Greece), while bamifylline was supplied from Alfa Wassermann SpA (Bolognia, Italy). These reagents were used as provided without further purification. All standard solutions of these compounds were prepared by dissolving the appropriate amounts in methanol. Ammonium acetate, pro analysi reagent was from Merck (Darmstadt, Germany). Methanol and acetonitrile HPLC-grade reagents were also from Merck. All other reagents used were of analytical grade. Glass distilled water was used throughout.

Chromatographic Conditions

The gradient system was formed with the following solutions: A=0.05M ammonium acetate and B=Methanol. The chromatogram was started with solvent

TABLE 1

Isocratic (Is) and Gradient (Gr) HPLC Conditions Examined in the Present Study.

Eluent System (A:B) Isocratic (Is), Gradient (Gr)	Flow Rate (ml/min)	Retention Time (min)	
		Mefenamic Acid	Bamifylline
(Is) (30:70)	1.00	5.34	5.46
(Is) (23:77)	1.00	3.64	4.14
(Gr) 0 (min) (28:72)			
5 (min) (23:77)	1.00	4.45	4.95
(Gr) 0 (min) (20:80)			
5 (min) (25:75)	1.00	3.28	3.50
	0.90	3.46	4.20
	0.85	3.51	4.42
	0.83	3.69	4.60
	0.80	3.97	4.85
	0.75	4.17	5.13
(Gr) 0 (min) (18:82)			
6 (min) (23:77)	1.00	2.87	3.61
(Gr) 0 (min) (22:78)			
6 (min) (28:72)	1.00	3.45	3.87
	0.80	4.19	5.08
(Gr) 0 (min) (25:75)			
6 (min) (30:70)	1.00	3.72	4.42
	0.80	4.19	5.08
(Gr) 0 (min) (23:77)			
5 (min) (30:70)	1.00	3.65	4.29
(Gr) 0 (min) (28:72)			
5 (min) (33:67)	1.00	4.21	4.97
(Gr) 0 (min) (22:78)	0.85	3.81	4.71
5 (min) (28:72)	0.83	4.09	4.96
	0.82	3.83	4.84
	0.81	3.90	4.94
	0.80	4.07	5.13

A = 0.05 M CH_3COONH_4 B = MeOH

Sensitivity (AUFS) = 0.002

TABLE 2
Chemical Compounds Tested as Internal Standards During the Assay of Mefenamic Acid Determination.

Chemical Compound	Wavelength Used (nm)	Notes
Tolfenamic Acid	285	1
Caffeine	285	2
Theophylline	285	2
Theobromine	285	2
Anthracene	285	3
Anthraquinone	285	1
1-Hydroxyanthraquinone	285	3
Paracetamol (N-acetyl-p-aminophenol)	270	4-5
o-Aminobenzoic Acid	350	5
3,5-Diaminobenzoic Acid	270	5
Bamifylline	285	6

1 = No Sufficient Separation from Mefenamic Acid.
2 = Retention Time lower than 2 min.
3 = Retention Time higher than 6 min.
4 = No Sufficient Separation from Methanol.
5 = No Absorbance at 285 nm.
6 = Suitable for Internal Standard.

B containing 22% solvent A, decreasing from 78 to 72%, by an increase from 22 to 28% of solvent A. This linear gradient was achieved from 0 to 5 min. This gradient eluting system was selected through a number of other mobile phases and systems on the basis of their relative polarities and low absorption at the wavelength used. The flow rate was low, 0.81 ml/min, as well as the sensitivity, 0.002 AUFS.

The separations were performed at ambient temperature 22°C. The chromatographic conditions concerning solvent systems were selected among several ones as shown in Table 1, since they were found to be the most suitable for the analysis of mefenamic acid in the presence of bamifylline as internal standard.

Bamifylline was selected as internal standard among several chemical compounds tested as shown in Table 2.

As mefenamic acid main metabolites, 3-hydroxymefenamic acid and 3-carboxymethylmefenamic acid, are more polar than the parent compound and thus expected to elute earlier on a reversed phase HPLC system, an internal standard eluted after the drug of interest should be chosen, in order to determine if any metabolites ran in the expected position potentially causing an interference problem[12]. Bamifylline was the only compound among those been tested, that fulfilled this requirement.

System Suitability

The reversed phase analytical column was equilibrated with methanol and the eluting solvent system used, at a flow rate of 0.81 ml/min. After an acceptable stable baseline was achieved, the samples, the internal standard, bamifylline and the standards were analyzed. The resolution factor Rt was calculated and found to be 1.82 for mefenamic acid and bamifylline, which signifies complete separation between the compounds, as shown in Figure 1.

The relative standard deviations of eight replicate analyses of four standards 0.20, 0.50, 1.0 and 2.0 ppm were found to be at the range 5.45 to 8.46% as illustrated in Table 3.

Selectivity

The selectivity of the gradient elution HPLC method was investigated at the retention times of bamifylline and mefenamic acid. No interferences from endogenous compounds were observed in chromatograms of samples extracted from blood serum and urine. Therefore the described method can be used in the analyses of mefenamic acid, using bamifylline as internal standard.

Detection and Quantitation Limits

The detection limit of mefenamic acid was assessed in the presence of the internal standard, bamifylline and was considered to be the quantity producing a

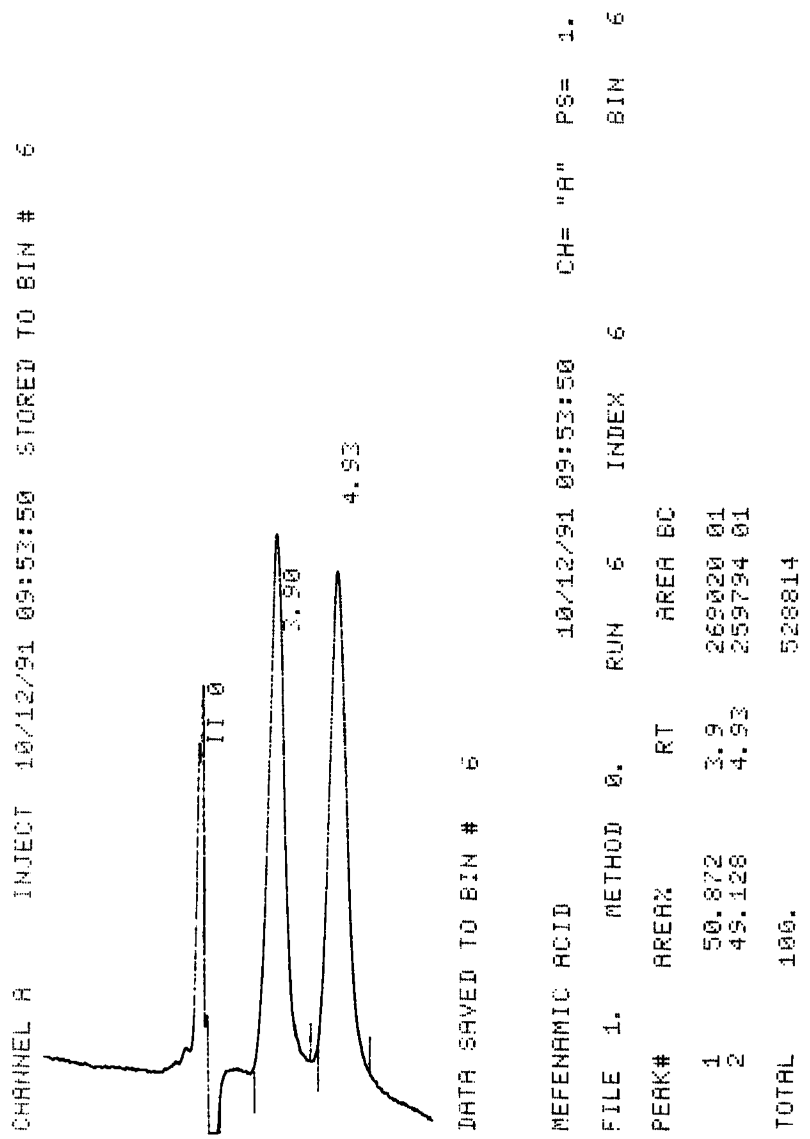

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Figure 1. High-Permormance Liquid Chromatogram of Mefenamic acid (3.90) using Bamifylline (4.93) as Internal Standard. Chromatographic conditions are given in text. [Mefenamic acid] = 1.0 ppm, [Bamifylline] = 1.0 ppm.

TABLE 3

Peak Area Ratio of Mefenamic Acid to Bamifylline Over the Concentration Range 0.20-2.0 ppm in Methanolic Solutions, Equivalent Quantity Injected 2.0-20.0 ng.

Concentration of Mefenamic Acid (ppm)	Equivalent Quantity Injected (ng)	Peak Area Ratio of Mefenamic Acid to Internal Standard Bamifylline										
		1	2	3	4	5	6	7	8	Mean Value ($\bar{x}$)	SD	RSD (%)
0.20	2.0	0.1564	0.1446	0.1310	0.1215	0.1240	0.1444	0.1368	0.1408	0.1374	0,01	8,46
0.50	5.0	0.3900	0.3710	0.4037	0.3627	0.3789	0.3236	0.3905	0.3811	0.3752	0,02	6,49
1.0	10.0	0.7537	0.7760	0.6710	0.6602	0.7053	0.7625	0.7005	0.7200	0.7186	0,04	5,91
2.0	20.0	1.3680	1.4732	1.3356	1.2762	1.444	1.4346	1.4882	1.3373	1.3946	0,08	5,45

signal of peak height twice the size of background noise. The minimum detectable quantity, expressed in pg injected on the column, was found to be 1.0 pg.

The quantitation limit was assessed in the presence of the internal standard and considered to be the quantity producing a signal five times more the peak height the detection limit produces, was found to be 1.36 ng.

Calibration Curves for the Determination of Mefenamic Acid in Methanol

Calibration curve for the determination of mefenamic acid was constructed in the presence of internal standard bamifylline.

Mefenamic acid and bamifylline were accurately weighed and dissolved in methanol to give stock solutions of 100 ng/μl each. Standard mefenamic acid solutions of 0.20, 0.30, 0.50, 1.0, 2.0, 3.0, 5.0, 10.0 and 12.0 ng/μl were prepared in 50 ml volumetric flasks by serially diluting the stock solution in methanol. 10 ml of a 10 ng/μl methanolic bamifylline solution giving a final concentration of 2.0 ng/μl were always added to the solutions during dilutions.

Aliquots of 10 μl of each solution were injected onto the analytical column. The peak area ratios of mefenamic acid to those of bamifylline were recorded and plotted as function of mefenamic acid concentrations.

All determinations were repeated eight times and the results were treated statistically.

Determination of Mefenamic Acid in Pharmaceutical Preparations

The developed High Pressure Liquid Chromatographic method was applied to the analysis of pharmaceutical formulations such as tablets, suspension and suppositories.

Ten Tablets of 500 mg (labelled amount) in mefenamic acid were weighed and the average tablet weight was found to be 726.2 mg. The tablets were finely powdered and a portion of 14.5 mg was quantitatively transferred into a 100 ml volumetric flask. The content was diluted to volume with methanol after sonication and gave a concentration of 100 ng/μl according to labelled amount of mefenamic acid. From this stock solution, dilute solutions 0.50, 1.0, 1.50 and 2.50 ng/μl were prepared by sequential dilutions with methanol. To the last solutions bamifylline was added as internal standard at the final concentration level of 2.0 ng/μl.

Aliquots of 10 μl were analyzed by HPLC.

Suspension, 10 ml, of labelled concentration 50 mg/5 ml, was transferred into a 100 ml volumetric flask and diluted to volume with methanol. According to labelled amount, the concentration of this solution was 1000 ng/μl. The solution was sonicated in ultrasonic bath for about 10 min, then a portion was centrifuged at 3500 rpm for 10 min. A volume of 10 ml of the supernatant layer was diluted with methanol to 100 ml. By sequential dilutions, final solutions of 0.50, 1.0 and 2.50 ng/μl in mefenamic acid and 2.0 ng/μl in bamifylline, were prepared.

Aliquots of 10 μl were analyzed by HPLC.

Eight **Suppositories** of 500 mg labelled amount in mefenamic acid were weighed and the average weight was found to be 2.0746 g. The suppositories were put into a glass beaker and the beaker was incubated at 38°C for 5 min. A quantity of 2.0721 g was dissolved by 50 ml methanol and sonicated in ultrasonic bath for 5 min. Then the entire quantity was transferred into a 250 ml volumetric flask diluted to volume with methanol to give a mefenamic acid solution of 2000 ng/μl according to labelled amount. From this solution and the bamifylline standard solution, final solutions of 0.50, 1.0, 1.50 and 2.50 ng/μl in mefenamic acid and 2.0 mg/μl in bamifylline were prepared.

Aliquots of 10 μl were analyzed by HPLC.

Determination of Mefenamic Acid in Biological Fluids, Blood Serum and Urine

Stock mefenamic acid methanolic solutions (100 μl) of 0.20, 0.30, 0.50, 1.0 and 1.50 ng/μl with bamifylline as internal standard at a concentration of 2.0 ng/μl and acetonitrile (80 μl) for protein precipitation were added to the plasma sample (40 μl). After vortex mixing for 2 min and centrifugation at 3500 rpm for 5 min, the supernatant was evaporated to remove the solvents under a stream of nitrogen on a water bath at 45°C. The aqueous phases were subsequently treated by solid-phase liquid extraction using C_{18} cartridges (Bond Elut). These phases were slowly forced through the cartridges which were previously conditioned, by passing 3 ml of methanol, and washed with 3 ml of water. The cartridges were fitted in a Vacuum system (Vac Elut) and washed twice with 2 ml of water before the final elution. Mefenamic acid and bamifylline were eluted with 2.5 ml of methanol. These methanolic solutions were evaporated to dryness on a water bath under a nitrogen stream at 45°C. The residues were redissolved in 50 µl of methanol and aliquots of 10 µl were injected into the chromatograph.

For urine assay a 100 µl volume of mefenamic acid, stock methanolic solutions 0.50, 1.0, 1.50, 2.0, 3.0 ng/µl with bamifylline as internal standard at a concentration of 2.0 ng/µl and 200 µl acetonitrile were added to 100 µl of urine sample. After centrifugation for 20 min at 3500 rpm, methanol and acetonitrile were removed from the supernatant by evaporation, under a stream of nitrogen on a water bath at 40°C. The mixtures were purified on C_{18} cartridges Bond Elut. After washing five times with 2 ml of water, elutions were performed with 2.5 ml of methanol. The methanolic solutions were evaporated to dryness as mentioned above. The residues were reconstituted with 100 µl of methanol and, after mixing, 10 µl were injected into the analytical column.

Quantitation limits for blood serum and urine samples were found to be 0.45 ng and 0.26 ng respectively.

RESULTS AND DISCUSSION

The majority of the methods for the determination of mefenamic acid reported in literature are either time consuming or require a complicate sample pretreatment, while the determination of mefenamic acid in blood serum requires large sample volumes, except for a recent method reporting a sample volume of 50 µl[13].

As only small plasma samples can be taken, we propose a new reversed-phase gradient HPLC method of mefenamic acid analysis in blood serum, urine and anti-inflammatory drugs. A very small volume (40 µl) of plasma, from which proteins were isolated by acetonitrile precipitation, is required. The proteins were also removed from urine samples by employing the same procedure, using only 100 µl of sample.

To improve the accuracy and precision of the method, at low concentration levels of mefenamic acid, the samples of biological fluids, after the precipitation of proteins and centrifugation, were subsequently treated by solid - phase liquid extraction and analyzed.

The total chromatographic time for analysis of the mefenamic acid was less than 5 min, with reproducible retention times, under the experimental conditions used.

The linearity of the method was studied and the areas of the chromatographic peaks for mefenamic acid were related to concentration. The linear regression equations and correlation coefficients were found to be:

STATISTICAL EVALUATION

Samples of Mefenamic Acid	Regression Equation	Correlation Coefficient
Methanolic Solutions in the Presence of Bamifylline	$Y = (-0.01807 \pm 0.0482) + (0.0718 \pm 8.594 \cdot 10^{-4})X$ QL = 1.36 ng	0.99991
Blood	$Y = (0.0329 \pm 0.03513) + (0.0934 \pm 0.0033)X$ QL = 0.45 ng	0.99999
Urine	$Y = (-1.4476 \cdot 10^{-3} \pm 0.01729) + (0.07279 \pm 7.6688 \cdot 10^{-4})X$ QL = 0.26 ng	0.00007

Where Y = Peak Area ratio of Mefenamic acid to Bamifylline for methanolic solutions, blood and urine samples.

X = Concentration of Mefenamic Acid in ng/µl.

QL = Quantitation Limit.

TABLE 4
Experimental Results for the Determination of Mefenamic Acid in Methanolic Solutions Using Bamifylline as Internal Standard, by HPLC.

Compound	RT (min)	Added (ng)	Found[a] (ng)
Bamifylline	4.94	-	-
Mefenamic Acid	3.90	2.00	1.90±0.26
		5.00	4.10±0.55
		10.0	7.40±1.20
		20.0	16.4±0.08

a = Average Value of Ten Determinations ± Standard Deviation.

TABLE 5
Experimental Results for the Determination of Mefenamic Acid in Blood Serum, of Patients Receiving Mefenamic Acid Medication, by Reversed-Phase HPLC, in the Presence of Bamifylline as Internal Standard.

Sample Case No	Found[a] (ng)	RSD (%)
1	1.72±0.19	11.0
2	0.62±0.08	12.9
3	2.88±0.38	13.0
4	0.99±0.12	12.1
5	0.36±0.03	8.3
6	$0.11\pm 9.08\cdot 10^{-3}$	8.3
7	0.31±0.04	12.9

a = Mean Value of Eight Determinations ± Standard Deviation.

TABLE 6
Mean Recovery Data for Mefenamic Acid Assay in Urine in the Presence of Bamifylline as Internal Standard.

Urine Mefenamic Acid Quantity (ng)	Sample 1		Sample 2		Sample 3	
	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)
5.0	102.68±3.57	3.5	100.82±4.42	4.4	83.25±1.13	1.3
10.0	86.03±3.16	3.7	98.98±5.11	5.2	78.87±0.48	0.6
15.0	109.60±4.12	3.8	89.05±4.80	5.4	107.14±6.66	6.2
20.0	108.48±3.78	3.5	100.25±8.64	8.6	99.11±10.01	10.1
30.0	96.31±2.41	2.5	116.17±6.74	5.8	108.0±11.57	10.7

Mean Recovery = $\bar{x} \pm (t \cdot SD/\sqrt{n})$/amount added X 100
Where $\bar{x}$ = mean value for n=6 determinations at 95% confidence level.

Linearity was observed up to 120 ng of mefenamic acid injected.

Experimental results for the determination of mefenamic acid in the presence of bamifylline as internal standard are given in Table 4.

Seven samples of blood serum from patients receiving mefenamic acid medication were analyzed and the results obtained are shown in Table 5.

The mean recovery data of mefenamic acid were determined by comparing the peak area ratios of mefenamic acid to bamifylline obtained from the injection of known quantities of the pure compound with those obtained from the direct injection of extracted urine samples spiked with five different concentrations of mefenamic acid in the presence of bamifylline as internal standard. The mean percentage recoveries of mefenamic acid at the various concentrations averaged 98.98% for urine samples analyses.

These results are laid out in Table 6.

TABLE 7
Experimental Results for the Determination of Mefenamic Acid in Pharmaceutical Preparations by Reversed-Phase HPLC in the Presence of Bamifylline as Internal Standard.

Sample	Labelled Amount (mg)	Found[a] (mg)	RSD (%)	Analyzed Quantity ng	Found[b] (ng)	RSD (%)
Tablets	500[c]	626.86±46.35	7.4	5.0	6.85±0.08	1.2
				10.0	11.94±0.96	8.0
				15.0	18.53±0.32	1.7
				25.0	29.41±1.40	4.7
Suspension	50[d]	59.96±4.67	7.8	5.0	6.4±0.50	7.8
				10.0	11.8±1.05	8.9
				15.0	17.31±1.03	5.9
				25.0	29.61±1.56	5.3
Suppositories	500[e]	620.61±62.95	10.1	5.0	6.63±0.84	12.6
				10.0	11.98±1.34	11.2
				15.0	19.32±1.45	7.5
				25.0	29.95±1.08	3.6

a = Mean Value of 24 Determinations ± Standard Deviation.

b = Mean Value of 6 Determinations ± Standard Deviation.

c = mg/Tablet, d = mg/5ml of Suspension, e = mg/Suppository

TABLE 8
Day-to-day Precision and Accuracy of Mefenamic Acid Assay in the Presence of Internal Standard (n = 12)

Quantity Injected (ng)	Mean[a] ± SD	RSD (%)
2.0	2.32±0.18	7.7
5.0	5.17±0.49	9.5
10.0	10.29±0.53	5.1
20.0	19.92±0.40	2.0

a = Mean value of Eight Determinations.

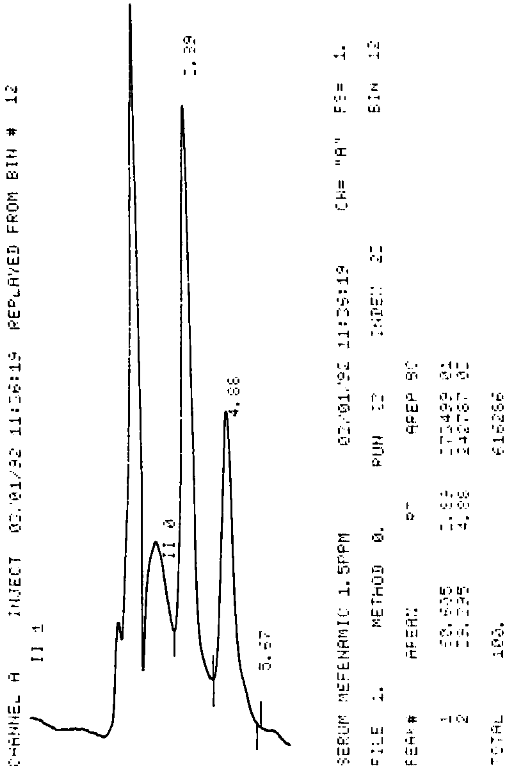

Figure 2. High-Performance Liquid Chromatogram of Mefenamic acid Extracted by Solid Phase Cartridge from Blood Serum samples. Peaks: 3.89 = [Mefenamic Acid] = 1.50 ppm, 4.88 = [Bamifylline] = 2.0 ppm. Chromatographic conditions are described in text.

The developed method was applied to the analyses of mefenamic acid in pharmaceutical preparations using bamifylline as internal standard. Therefore mefenamic acid was determined in tablets, suspension and suppositories. The results taken are given in Table 7.

The day-to-day precision and accuracy of the proposed method were assessed by the repeated analyses of methanolic solutions in the presence of the internal standard over 12 days. The concentrations of mefenamic acid ranged from 0.2 to 2.0 ng/μl. Eight replicate samples at each of the four concentrations were used in the

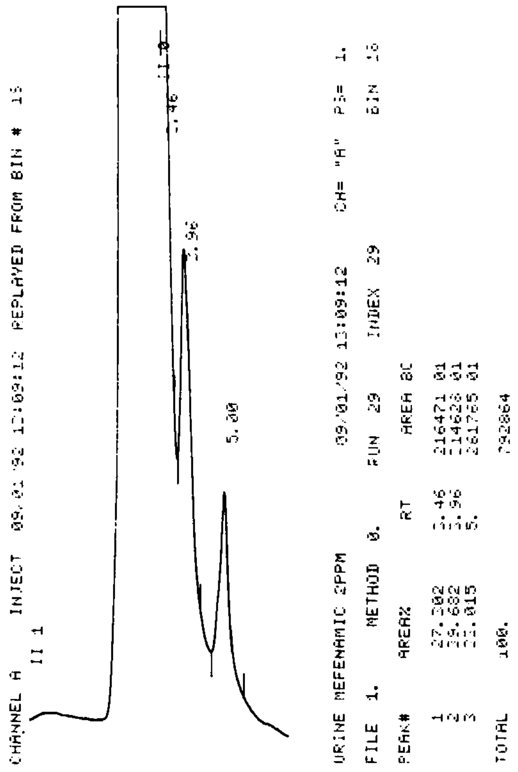

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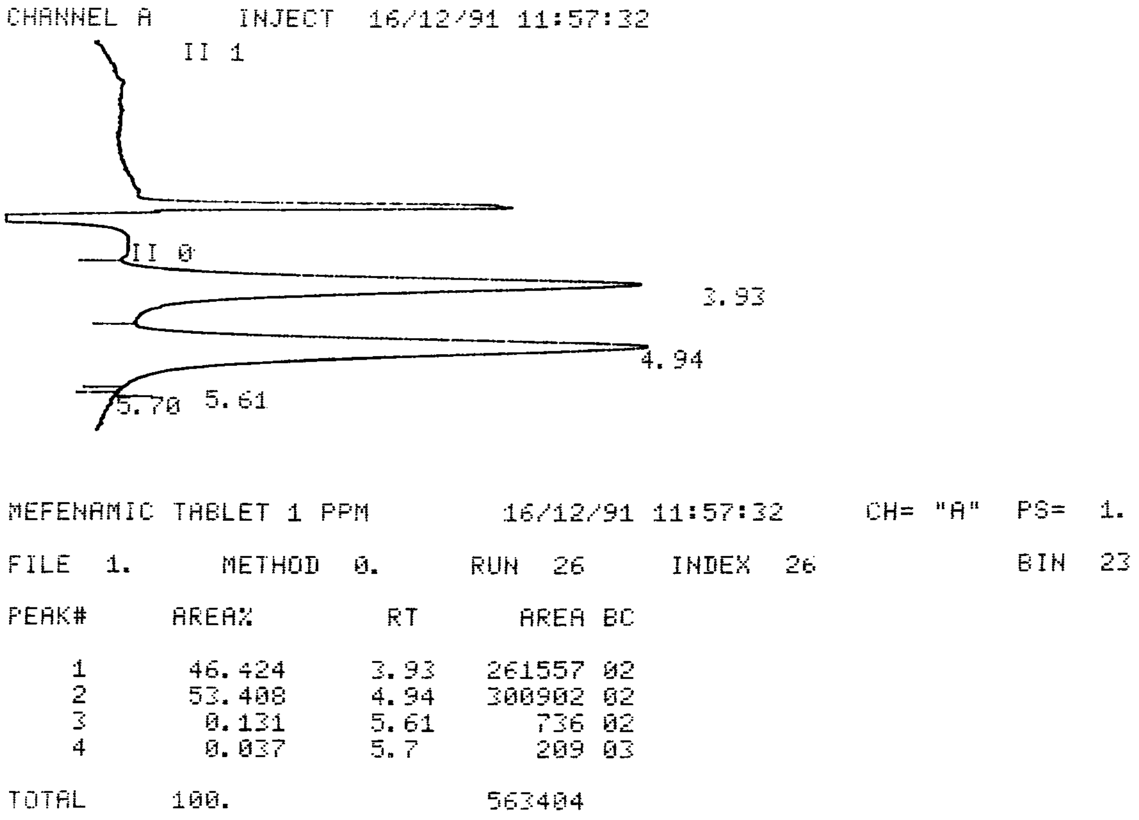
Figure 3. High-Performance Liquid Chromatogram of Mefenamic acid Extracted by Solid Phase Cartridge from Urine samples.
Peaks: 3.96 = [Mefenamic Acid] = 2.0 ppm, 5.00 = [Bamifylline] = 2.0 ppm. Chromatographic conditions are given in text.

assessment of the between - day variability of the method. The results taken of this procedure are laid out in Table 8.

Ten drug-free plasma and urine samples were analyzed for possible interferences from endogenous constituents. From these analyses no background interferences were observed.

Using the standard addition technique it is possible to analyze mefenamic acid in 40 μl of blood serum and 100 μl of urine samples in under five minutes time. Mefenamic acid was successfully determined employing the procedures described



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MEFENAMIC TABLET 1 PPM        16/12/91 11:57:32     CH= "A"  PS=  1.
FILE  1.     METHOD  0.     RUN  26     INDEX  26              BIN  23
PEAK#     AREA%        RT       AREA BC

    1      46.424      3.93   261557 02
    2      53.408      4.94   300902 02
    3       0.131      5.61      736 02
    4       0.037      5.7       209 03

TOTAL     100.                563404
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Figure 4. High-Performance Liquid Chromatogram of Mefenamic acid in Pharmaceutical Formulations, Tablets.
Peaks 3.93 = [Mefenamic Acid] = 1.0 ppm, 4.94 = [Bamifylline] = 2.0 ppm. Chromatographic conditions are described in text.

using bamifylline as internal standard for blood serum and urine samples. The chromatograms taken are given in Figures 2 and 3.

Bamifylline used as internal standard, was found to be very convenient, due to its later elution than mefenamic acid and thus not being overlapped with urine endogenous substances, as opposed to caffeine previously used[15,16].

The proposed procedure was then applied to the analyses of mefenamic acid in pharmaceutical preparations such as tablets, suspension and suppositories. Chromatograms are given in Figure 4.

The results taken were in good agreement with the nominal concentrations according to the labelled content of the pharmaceutical preparations and no interferences from the excipients were observed in the chromatograms.

CONCLUSION

In conclusion, the proposed HPLC method of mefenamic acid analysis provides high accuracy, precision and reproducibility, good selectivity and high sensitivity. It requires only a small sample volume, 40 μl for blood serum and 100 μl for urine and a separation time under 5 minutes, thus being very suitable for therapeutic drug monitoring of mefenamic acid in patients undergoing conventional mefenamic acid therapy and for pharmacokinetic studies of the drug as well.

It is also very useful for the routine analysis of mefenamic acid in pharmaceutical formulations.

Bamifylline was found to be very suitable as internal standard, eluting after mefenamic acid, avoiding any overlapping with potential mefenamic acid metabolites or urine sample endogenous compounds, as opposed to caffeine, previously used as internal standard.

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