Journal of Chromatography, 228 (1982) 372—376
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1111

Note

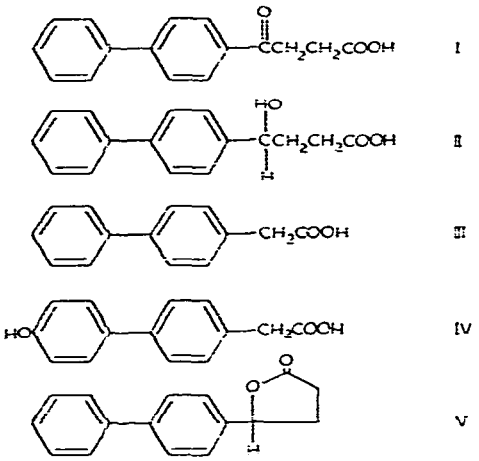
High-performance liquid chromatography assay for fenbufen and two serum metabolites

JEFFERY S. FLEITMAN*, STEPHEN G. SCHULMAN and JOHN H. PERRIN

College of Pharmacy, Department of Pharmaceutics, University of Florida, Gainesville, FL 32610 (U.S.A.)

(First received July 9th, 1981; revised manuscript received September 29th, 1981)

Fenbufen, 3-(4-biphenylcarbonyl)propionic acid (I), a non-steroidal anti-inflammatory drug, has been found to possess the same spectrum of activity as aspirin, phenylbutazone and indomethacin. Its two major serum metabolites, 3-(4-biphenylhydroxymethyl)propionic acid (II) and 4-biphenylacetic acid (III) have a similar degree of activity [1].



Previous gas chromatographic (GC) [2] and high-performance liquid chromatographic [3] methods have been developed for the determination of fenbufen and its metabolites in biological fluids. The GC method is very time consuming, involving double extraction, derivatization and thin-layer chromatographic separation before GC injection. The established HPLC method

0378-4347/82/0000—0000/$02.75

has retention times up to 22 min for the compounds on interest. A column heated to 45°C and a 2-ml plasma sample are also necessary.

In the present method the chromatographic procedure was improved to keep the total retention time for all compounds under 9 min (Table I). A plasma sample of only 1 ml is necessary and the column is operated at ambient temperature.

EXPERIMENTAL

Materials

Fenbufen, its metabolites and an internal standard, 4'-hydroxy-4-biphenylacetic acid (IV), were kindly supplied by Lederle Labs. (Pearl River, NY, U.S.A.). HPLC grade acetonitrile and isopropanol were obtained from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Analytical grade phosphoric acid, hydrochloric acid, sodium hydroxide and methanol were supplied by Mallinckrodt (St. Louis, MO, U.S.A.). All chromatographic solvents including deionized water, were filtered through 0.45-μm filters (Millipore, Bedford, MA, U.S.A.).

Sample analyses were carried out on a Varian Model 5000 liquid chromatograph equipped with a 10-μl manual loop injector and a column heater (Varian, Walnut Creek, CA, U.S.A.). An alkylnitrile column, MicroPak CN-10 (Varian), particle size 10 μm, 30 cm × 4.0 mm I.D., of medium polarity was used. Detection was made on a Vari-Chrom variable-wavelength UV detector.

During the extraction procedure, metabolite II forms a lactone (V) [3], as will be described later. The lactone was prepared as follows: metabolite II was saturated in water at 40°C. An excess of concentrated hydrochloric acid was added and the mixture was allowed to stand for 5 min. The white precipitate was extracted into cyclohexane—diethyl ether (7:3, v/v). The organic layer was evaporated under a nitrogen stream and the lactone crystals were collected. Purity was determined chromatographically to be greater than 99%.

Chromatographic conditions

The mobile phase consisted of water—isopropanol—acetonitrile—phosphoric acid (84.5:10:5:0.5). The flow-rate was 2.5 ml/min and the column was kept at 25°C. Detection was made at 265 nm at 0.02 a.u.f.s. A 10-μl samples was injected onto the column.

Analytical procedure

The extraction procedure is similar to the one previously described [3]. Stock solutions containing 200 μg/ml of fenbufen, metabolites and internal standard were prepared in methanol. Accurate volumes are pipetted into test-tubes which will give final concentrations of 0.5, 1.0, 3.0, 5.0, 10.0 and 25.0 μg/ml of plasma. The internal standard IV (2 μg) is then added. The methanol is evaporated in a stream of dry nitrogen. Samples are reconstituted in 125 μl of 0.1 *N* sodium hydroxide and 1 ml of plasma is added. Limited solubilities of fenbufen and its analogue necessitate this procedure rather than direct spiking of plasma samples from an aqueous stock solution.

After the contents of the tube are thoroughly mixed, 1 ml of concentrated hydrochloric acid is added and the samples are vortexed for 15 sec. The samples are allowed to stand for 2 min and then 8 ml of cyclohexane–diethyl ether (7:3, v/v) are added and the samples are vortexed for 30 sec. The samples are centrifuged at 2000 *g* for 12 min and the organic layer is transferred to another tube and evaporated under a stream of dry nitrogen. Samples were reconstituted in 100—200 μl of methanol and 10 μl were injected into the column.

For absolute recovery experiments, spiked plasma samples were compared to unextracted stock solutions. Peak height ratios were calculated and amounts found were compared.

Precision determinations were performed by comparing the peak height ratios of five extractions at each concentration.

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of plasma containing fenbufen (I), two metabolites (II and III) and internal standard (IV). The retention times are listed in Table I.

Table II lists recovery data for compounds I, II and III. In the 0.5—25 μg/ml range, the average recovery for I was 88.9 ± 9.2%; for II, 82.4 ± 9.0%; and for III, 100.4 ± 2.6%.

Precision data listed in Table III give the range, average ± S.D. and coefficient of variation of peak height ratios.

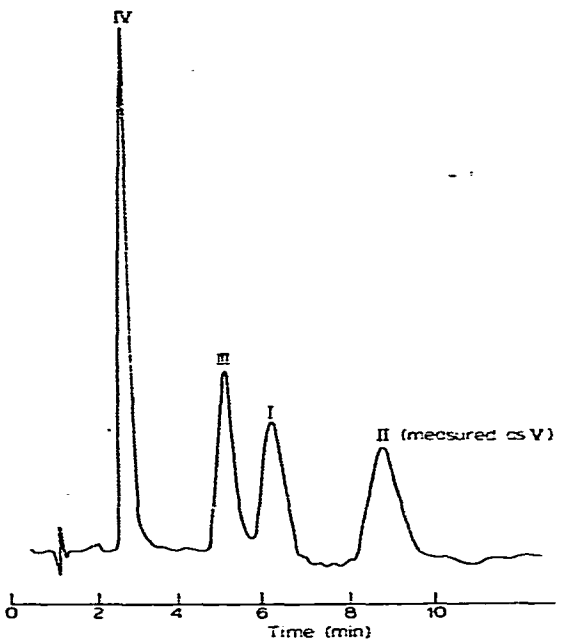


Fig. 1. Chromatogram of plasma extract containing 10 μg/ml fenbufen (I), two serum metabolites (II, III) and internal standard (IV).

TABLE I

RETENTION TIMES OF FENBUFEN, METABOLITES AND INTERNAL STANDARD

Compound	Retention time (min)
I	6.1
II	8.8
III	5.1
IV	2.9
V	4.2

TABLE II

ABSOLUTE RECOVERY OF FENBUFEN (I) AND TWO SERUM METABOLITES (II, III) FROM 1-ml PLASMA SAMPLE (n = 3)

Amount added (μg)	Recovery (%), mean ± S.D.)		
	I	II as V	III
0.5	98.3 ± 3.5	64.8 ± 5.6	101.7 ± 0.5
1.0	104.0 ± 2.0	82.6 ± 1.9	103.7 ± 1.9
3.0	86.7 ± 3.1	80.2 ± 2.2	101.3 ± 0.9
5.0	77.8 ± 3.3	94.4 ± 6.4	95.2 ± 8.2
10.0	82.1 ± 1.1	86.3 ± 5.4	100.0 ± 1.6
25.0	84.6 ± 1.3	86.3 ± 4.3	100.6 ± 6.1
Average	88.9 ± 9.2	82.4 ± 9.0	100.4 ± 2.6

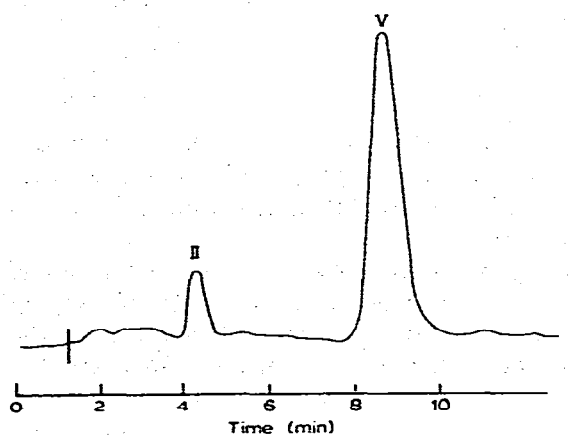


Fig. 2. Chromatogram of plasma extract containing metabolite II incompletely converted to lactone V.

During the acidic extraction procedure, metabolite II forms the lactone V. It is actually the lactone which is the chromatographed species. If compound II is injected directly onto the column from a methanolic stock solution, it has a retention time of 4.2 min. After acidic extraction from plasma, the lactone formed has a retention time of 8.8 min. If the acidification of the

TABLE III

METHOD PRECISION AND REPRODUCIBILITY FOR FENBUFEN (I) AND TWO SERUM METABOLITES (II, III) (n = 5)

Amount added (μg)	Ratio of peak height of compound to peak height of I.S.		Coefficient of variation (%)
	Mean ± S.D.	Range	
Compound I			
0.5	0.101 ± 0.003	0.097—0.106	2.97
1.0	0.208 ± 0.009	0.194—0.220	4.33
3.0	0.578 ± 0.043	0.515—0.647	7.44
5.0	0.903 ± 0.042	0.857—0.975	4.65
10.0	1.820 ± 0.040	1.760—1.880	2.36
25.0	5.980 ± 0.250	4.760—5.350	4.92
	m = 0.240, b = —0.179, r = 0.9950		
Compound II (measured as compound V)			
0.5	0.050 ± 0.007	0.042—0.063	14.0
1.0	0.078 ± 0.008	0.067—0.089	10.2
3.0	0.251 ± 0.028	0.216—0.289	11.1
5.0	0.443 ± 0.053	0.375—0.538	11.9
10.0	1.030 ± 0.140	0.914—1.290	13.4
25.0	2.000 ± 0.120	1.880—2.190	5.8
	m = 0.081, b = 0.042, r = 0.9929		
Compound III			
0.5	0.081 ± 0.003	0.078—0.085	3.70
1.0	0.163 ± 0.007	0.155—0.173	4.29
3.0	0.467 ± 0.039	0.433—0.543	8.35
5.0	0.742 ± 0.034	0.686—0.778	4.58
10.0	1.560 ± 0.131	1.410—1.800	8.40
25.0	4.190 ± 0.267	3.860—4.500	6.37
	m = 0.168, b = —0.045, r = 0.9995		

sample is not complete, both the free acid and the lactone will be seen in the chromatogram (Fig. 2). The lactone was therefore prepared as a reference for the absolute recovery experiments. This compound is not stable in methanol and new solution must be prepared daily.

This assay has been shown to be an improvement over the existing method [3] in that the overall assay time for the fenbufen and its metabolites has been decreased from 22 min to 9 min. The lowest detectable level of the compounds was 0.5 μg/ml and the range studied was within the expected therapeutic range [4, 5].

REFERENCES

1 R. Child, A. Osterberg, S. Sloboda and A. Tomcufcik, J. Pharm. Sci., 66 (1977) 466.
2 G. Cuisinaud, J. Legheand, C. Belkahia and J. Sassard, J. Chromatogr., 148 (1978) 509.
3 G. Van Lear, F. Chiccarelli, P. Bonenfant and A. Barr, J. Pharm. Sci., 67 (1978) 1662.
4 G. Cuisinaud, J. Legheand, G. Llorca, C. Belkahia, E. Lejeune and J. Sassard, Eur. J. Clin. Pharmacol., 16 (1979) 59.
5 H. Yoshizawa, Y. Tada, T. Naruke and M. Mizumura, Basic Pharmacol. Ther., 2 (1974) 31.