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Short Communication

Analysis of fentiazac in pharmaceutical dosage forms by reversed-phase high-performance liquid chromatography†

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Introduction

Fentiazac, 2-phenyl-4-*p*-chlorophenyl-thiazol-5-ylacetic acid, is a non-steroidal anti-inflammatory agent [1–5], indicated especially in cases where an analgesic and antipyretic action is required in addition to the anti-inflammatory effect. The drug is relatively new and few reports on its analysis have been published. Methods employed for its determination in biological fluids for pharmacokinetic studies include gas–liquid chromatography [6, 7] and densitometry [8]; analytical procedures applied to pharmaceutical formulations have not been reported. In this paper a rapid, stability-indicating method is presented for the estimation of fentiazac in commercial dosage forms, based on reversed-phase high-performance liquid chromatography (HPLC). The results have been compared with those from a direct ultraviolet spectrophotometric method.

Experimental

Materials

Fentiazac, calcium fentiazac, decarboxylated fentiazac (5-methyl-2-phenyl-4-*p*-chlorophenylthiazole), and dosage forms were kindly supplied by LPB, Milan, Italy, and were used as received. Fentiazac methyl ester was prepared by heating under reflux for 0.5 h an acidic methanolic solution of the parent acid. Its physical properties were: melting point: 74–75°C (MeOH-H_2O); infra-red spectrum (nujol mull): 1730 cm^{-1}, 1195 cm^{-1}, 1000 cm^{-1}, 835 cm^{-1} (doublet) and 760 cm^{-1}. Phenacetin (the internal standard) was

* To whom correspondence should be addressed.
† Dedicated to Professor Michele Amorosa on the occasion of his 70th birthday.

USP grade. Methanol used for chromatography was HPLC grade from Carlo Erba Strumentazione (Milan, Italy); water was doubly-distilled. All other reagents were of analytical grade and used without further purification.

Equipment

HPLC analyses were performed on a Model 5020 liquid-chromatograph (Varian AG, Zug, Switzerland) equipped with a Valco high-pressure injection valve fitted with a 10 μl sample loop. All measurements were made at ambient temperature using a variable wavelength detector UV-50 (Varian) and a chart recorder with integrator (Bryans Southern Instruments). The detector wavelength was adjusted to 254 nm with a sensitivity of 0.05 a.u.f.s.

Spectrophotometric analyses were performed using a digital single-beam spectrophotometer (Jasco, Model UVIDEC-4) with 1 cm cells. The absorbances were determined at the wavelength of maximum absorbance near 247 nm.

Chromatographic conditions

Chromatography was carried out isocratically using a 250 × 4 mm i.d. column of reversed-phase material LiChrosorb RP-8 (7 μm). The mobile phase comprised methanol–0.005M phosphate buffer (pH 3) (80:20 v/v) filtered through a 0.2 μm minicapsule filter (Gelman Sciences) and degassed before use. The flow-rate was kept constant at 1.0 ml/min.

Calibration curve

Fentiazac stock solution (40 μg/ml) and internal standard (phenacetin) stock solution (20 μg/ml) were prepared in methanol. Working standard solutions containing 8–18 μg/ml of fentiazac with 6 μg/ml of internal standard were prepared. The calibration curve was constructed by plotting the ratios of fentiazac peak height to that of internal standard versus their respective concentration ratios. The ratio of the peak areas could be used with equally satisfactory results.

Sample preparation

Coated tablets of fentiazac (strength 200 mg/tablet) and fentiazac calcium suppositories (strength 400 mg/suppository) were treated as follows:

Coated tablets. A quantity of finely powdered tablets, equivalent to about 20 mg of fentiazac and accurately weighed, was extracted four times with 20 ml aliquots of methanol in a 50 ml screw-capped centrifuge tube by vigorous agitation for 10 min. The methanolic extracts were then filtered and combined in a 100 ml volumetric flask and brought to volume with methanol. This solution, further diluted (1 + 24) with methanol, was used for immediate spectrophotometric determination. For HPLC analysis a (1 + 4) dilution was made; then a 3.00 ml aliquot of the resulting solution was transferred to a 10 ml volumetric flask containing 3.00 ml of the internal standard solution and diluted to volume with methanol.

Suppositories. Method A. Five fentiazac suppositories were placed in a tared dish containing a glass rod, heated gently on a steam bath until melted, then cooled while stirring and weighed. An accurately-weighed portion of the mass, equivalent to about 20 mg of fentiazac, was extracted three times with 30 ml portions of the methanol–water

(70:30 v/v) mixture under reflux and with stirring. The combined extracts were cooled in ice–water, filtered into a 100 ml volumetric flask and diluted to volume with methanol (Solution A). For HPLC analysis this solution was further diluted (1 + 4) with the mobile phase. A 3.00 ml aliquot of the resulting solution was transferred to a 10 ml volumetric flask containing 3.00 ml of the internal standard solution and brought to volume with methanol. For spectrophotometric determinations 1 ml of Solution A was transferred to a 25 ml volumetric flask, containing 1 ml of aqueous 0.1 M H_3PO_4, and diluted to volume with methanol.

Method B. An accurately-weighed portion of the melted mass (Method A), equivalent to about 20 mg of fentiazac, was transferred to a 100 ml volumetric flask containing 30 ml of chloroform, diluted to volume with methanol and shaken. A 1.00 ml aliquot of the resulting solution was transferred to a 25 ml volumetric flask containing 1.00 ml of a (1 + 49) solution of phosphoric acid in methanol, after which methanol was added to volume.

Assay procedure

HPLC. Prior to injection on to the liquid chromatograph, the analytical solutions were filtered through a 0.45 μm Teflon membrane filter (Gelman Sciences). All formulations were first chromatographed without internal standard in order to verify the absence of interfering peaks at the retention position for the internal standard. A 10 μl aliquot of each analytical solution was injected in triplicate. The standard solutions were run concurrently with the unknown samples. The peak-height ratio of drug to internal standard was determined and the amount of fentiazac in each of the samples analysed then calculated by interpolating the calibration curve.

Spectrophotometry. The absorbance of the sample solution and of a standard fentiazac solution having an accurately known concentration of about 8 μg/ml, were determined concomitantly in 1 cm cells at the wavelength of maximum absorbance near 247 nm, using methanol as reference.

Results and Discussion

The separation of fentiazac from its decarboxylated derivative (the major degradation product) and from some related anti-inflammatory agents (aryl- and heteroarylalkanoic acids) was investigated. Using the reversed-phase C_8-column, various mixtures of methanol–buffer solution (pH 3) were tried as mobile phase. Decreases in the methanol/buffer ratios resulted in increased retention with improved separation of fentiazac and related compounds (Table 1). At a lower methanol concentration (70% v/v), only ibuprofen was found to potentially interfere with fentiazac analysis; under these conditions, however, the decarboxylation product was strongly retained. For the stability-indicating assay procedure for fentiazac a mobile phase of 80% v/v methanol was found to permit adequate resolution of fentiazac, internal standard and potential impurity in a reasonable analysis time (Table 1). The resolution factor, R, between the peaks from drug and internal standard was 2.15, as indicated in the representative chromatographic tracing shown in Fig. 1. For the quantitative determination of fentiazac in pharmaceutical formulations, a linear calibration curve was observed over the concentration range studied ($y = 0.411\ x + 0.0264$; $r = 0.998$; $n = 5$). The relative standard deviation of the peak height ratio of the same standard solution was 0.66% ($n =$

Table 1
Effect of the mobile phase methanol content on the retention of fentiazac and some related compounds. Mobile phase: methanol–buffer solution (pH 3.0). Flow rate: 1.0 ml/min

Compound	Retention time (min)*	
	80% MeOH	70% MeOH
Phenacetin	3.6	4.1
Fentiazac	5.2	11.0
Decarboxylated fentiazac	10.9	31.7
Fentiazac methyl ester	7.8	—
Fenbufen	4.2	6.3
Naproxen	4.2	6.3
Ibuprofen	5.6	11.3
Indomethacin	4.8	9.2
Flurbiprofen	4.9	8.8
Tianafac	4.6	7.8
Diclofenac	5.2	10.3
Alclofenac	3.9	5.4

* The column hold-up time, t_0, was 2.8 min.

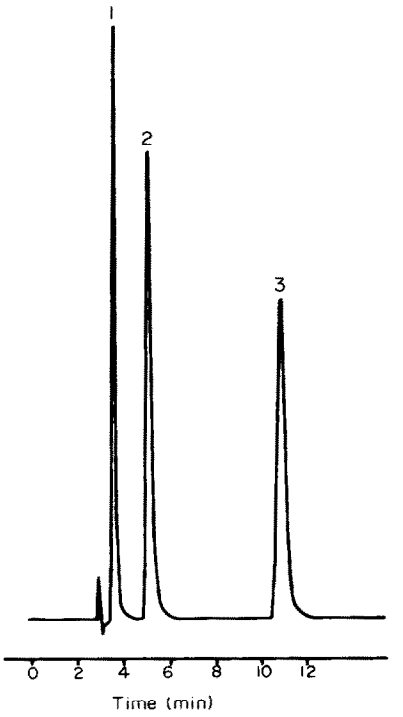

Figure 1
Chromatogram for separation of: 1, phenacetin (internal standard); 2, fentiazac; and 3, decarboxylated fentiazac. Column, Lichrosorb RP 8 (7 μm); mobile phase, methanol–0.005 M phosphate buffer (pH 3) (80:20 v/v) at a flow-rate of 1.0 ml/min.

6). Commercial dosage forms of fentiazac were analysed and the assay results (Table 2) were in good agreement with the label claim. The inactive excipients did not interfere with the analysis. A typical chromatogram of the extract from commercial tablets is shown in Fig. 2, which is exactly comparable with the chromatogram for a suppository extract.

Table 2
Analysis of fentiazac in commercial dosage forms

Drug	Dosage form	HPLC*		Spectrophotometry*	
		Recovery†	RSD (%)‡	Recovery†	RSD (%)‡
Fentiazac	Coated tablets	100.1	1.80	99.7	2.12
Fentiazac calcium	Suppositories	100.2§	0.77	99.8§ 101.1‖	0.89 0.75

* Average of five determinations.
† Recovery is expressed as a percentage of label claim.
‡ RSD = relative standard deviation (%).
§ Sample preparation by method A.
‖ Sample preparation by method B.

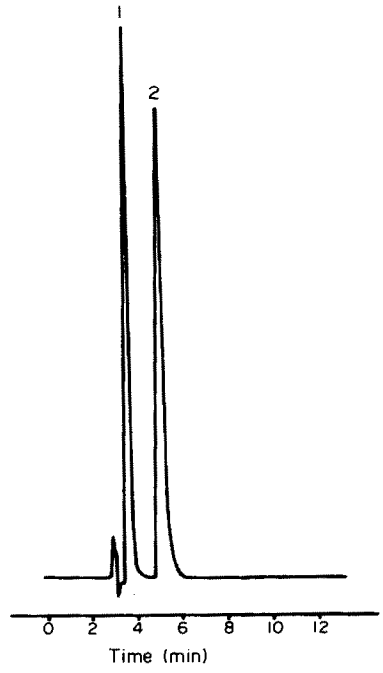

Figure 2
Typical chromatogram obtained for a sample of fentiazac tablets: 1, phenacetin (internal standard); and 2, fentiazac. Chromatographic conditions as in Fig. 1.

For comparison purposes, fentiazac was also assayed by a direct spectrophotometric procedure. A linear relationship between absorbance and concentration was found over the concentration range of 5–16 μg/ml ($y = 0.0622\,x - 0.0077$; $r = 0.9999$; $n = 5$). No significant differences were found between results by HPLC and by spectrophotometry for the same batch at the 90% confidence limit level (Student's *t*-test). The difference between the means of the results obtained by the spectrophotometric method A and B for the analysis of suppositories was found to be significant ($t_{obs} = 2.418$, $t_{tab} = 2.306$) at the 95% confidence level, but not for higher confidence levels.

Recovery studies over the range of 80–120% of the label claim were performed by adding known amounts of the drug to amounts of the excipients equivalent to those used in the dosage forms: these included lactose, saccharose, starch, microcrystalline cellulose, magnesium stearate and talc for tablets; and semisynthetic triglycerides for

suppositories. Quantitative recovery of fentiazac was obtained by both the HPLC and the spectrophotometric method (Table 3).

Since methanol was used as the working solvent, the possibility of fentiazac methyl ester forming in the analytical solutions was checked. Under the chromatographic conditions employed, adequate separation of fentiazac and its methyl ester was observed (Table 1). In analytical solutions maintained at room temperature for up to three days, no traces of the methyl ester were detected.

Table 3
Recovery of fentiazac added to placebo preparations

Method	Tablets		Suppositories†	
	Recovery*	RSD (%)	Recovery*	RSD (%)
HPLC	99.6	1.07	100.3	0.86
Spectrophotometry	99.2	1.10	99.3	0.98

* Recovery is expressed as a percentage of drug added to placebo; each is an average of four determinations.
† Extraction by method A.

Two different procedures were used for sample preparation from suppositories. Although method A based on extraction with the methanol–water solvent system is time-consuming, it does permit the removal of most of the excipients (semisynthetic glycerides), to give clean samples for injection. Furthermore, this procedure proved to be precise and accurate. Complete dissolution of the sample in chloroform–methanol (30:70 v/v) mixture (method B) can be used for a rapid spectrophotometric assay; it is not, however, to be recommended for HPLC, because of the frequent column cleaning required.

In summary, both the described HPLC and spectrophotometric methods are suitable for the determination of fentiazac in pharmaceutical formulations. The HPLC procedure, however, offers a more specific, stability-indicating assay method and could serve also as a rapid identity test for fentiazac in commercial dosage forms.

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