ELSEVIER

Journal of Chromatography B, 654 (1994) 159–163

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short Communication

Determination of fenoverine in tissue samples by high-performance liquid chromatography

Robert Ratiney, Alain Astier, Patrick Chariot *

Department of Toxicology, Hôpital Henri Mondor, 94000 Créteil, France

(First received August 3rd, 1993; revised manuscript received December 24th, 1993)

Abstract

Fenoverine is a spasmolytic, non-anticholinergic phenothiazine derivative that inhibits calcium channel currents. We describe a high-performance liquid chromatographic method for the determination of fenoverine in striated muscle, smooth muscle, myocardium, and liver tissue. Reversed-phase liquid chromatography was performed on a 5 μm Nucleosil C_{18} column with acetonitrile–0.015 *M* phosphate buffer (28:72, v/v) as a mobile phase and detection with ultraviolet at 214 nm. The limit of quantitation of fenoverine in tissue samples was 25 ng injected. This method is well suited for the determination of fenoverine in various organs in animal experiments.

1. Introduction

Fenoverine, a spasmolytic, non-anticholinergic phenothiazine derivative that inhibits calcium channel currents [1], has been used in various countries [2,3] in the treatment of some gastrointestinal and gynecological spasmodic disorders [2,4]. Fenoverine has been repeatedly implicated to play a role in the occurrence of rhabdomyolysis; however the mechanism still remains to be elucidated [5–10]. Chromatographic determination of fenoverine in plasma has been presented in one report [11] but a method allowing fenoverine analysis in tissue samples is lacking. In this study, we describe a simple HPLC method for determination of fenoverine in tissue samples. This method has been applied in toxicity studies in rat and human samples.

2. Experimental

2.1. Chemicals

Acetonitrile for HPLC and potassium dihydrogenphosphate obtained from Merck (Darmstadt, Germany) were used for the HPLC mobile phase. Methanol (Carlo Erba, Milano, Italy) and *n*-hexane (Labosi, Paris, France) were used for sample preparation. Fenoverine and MD 230141 (used as internal standard) (Fig. 1) were a gift from Delalande (Paris, France).

* Corresponding author.

0378-4347/94/$07.00
SSDI 0378-4347(94)00016-X

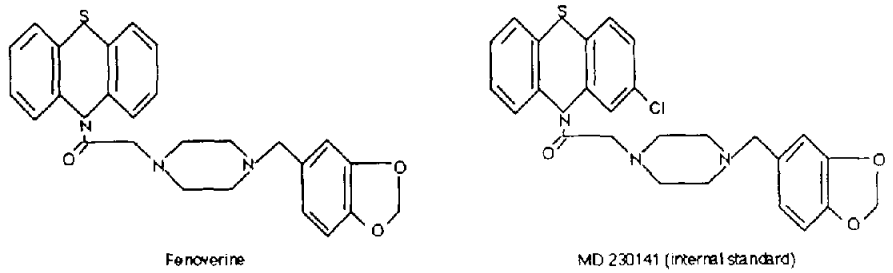

Fig. 1. Structures of fenoverine and the internal standard (MD 230141).

2.2. Apparatus

The HPLC system consisted of a LC-6A pump (Shimadzu, Kyoto, Japan), SPD-6A UV detector (Shimadzu) and CR-6A integrator (Shimadzu). Analyses were performed on a reversed-phase Nucleosil C_{18} column (5 μm particle size, 300 × 3.9 mm I.D.) obtained from the Société Française de Chromato-Colonne (Paris, France). A guard column (2 cm × 2 mm I.D.) filled with 30–40 μm pellicular RP-18 Perisorb material (Touzard et Matignon, Alfortville, France) was used when biological samples were injected.

2.3. Standard solutions

Standard solutions were prepared by dissolving 1 mg of fenoverine and 5.7 mg of internal standard in 100 ml of water and ethanol (50:50, v/v) and stored at 4°C. For tissue samples, 25, 125, 200, 250, and 500 ng of fenoverine with 1.14 μg of internal standard were added to 100 mg of tissue.

2.4. Sample preparation

Fenoverine determinations were performed in striated muscle, smooth muscle, myocardium, and liver obtained from male Wistar rats. A piece of tissue of 100 mg was added to 700 μl of methanol and 20 μl of the internal standard solution, crushed with an homogenizer for microsamples (Bioblock, Illkirch, France) for 10 s, and centrifuged (11 000 *g*, 15 min). The liquid phase was transferred to a capped conical tube, mixed with 200 μl of *n*-hexane, and centrifuged at 3000 *g* for 5 min. The upper phase was discarded. The lower phase was transferred to a borosilicate glass tube and evaporated to dryness under nitrogen at 50°C. The resultant residue was reconstituted with 250 μl of mobile phase, vortex-mixed, and centrifuged at 1000 *g* for 10 min. A 100-μl aliquot was injected onto the HPLC system.

2.5. Chromatography

The mobile phase consisted of acetonitrile–0.015 *M* phosphate buffer (28:72, v/v). Chromatographic analyses were performed at ambient temperature at a flow-rate of 1 ml/min. The detection wavelength was set at 214 nm.

Peak-area ratios of fenoverine to internal standard in unknown samples were used to calculate the fenoverine concentrations from linear regression equations obtained from standards.

2.6. Protein determination

The protein content in liver, striated muscle, colonic smooth muscle, and myocardium was determined as follows: 100 mg of tissue were homogenized in a solution containing 1% sodium dodecyl sulfate and 2 *M* sodium hydroxide, and kept at 60°C for 3 h. Protein concentrations were determined using the method of Lowry, as modified by Hartree [12].

3. Results

Chromatograms of fenoverine extracted from liver and myocardium are shown in Figs. 2 and 3.

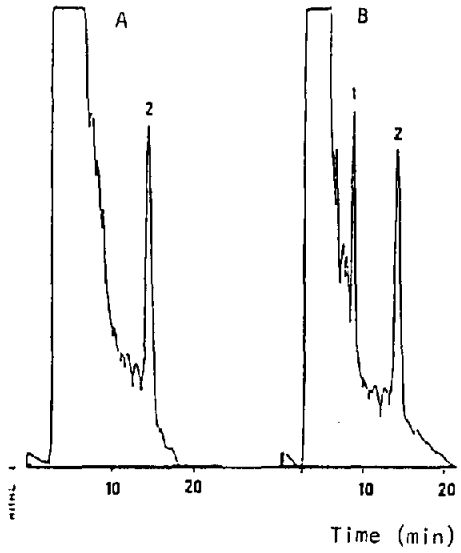

Fig. 2. Chromatograms of (A) drug-free rat liver sample and (B) rat liver containing 250 ng fenoverine per 100 mg. Peaks: 1 = fenoverine; 2 = internal standard.

The retention time of fenoverine was 10.9 min (range: 10.4–11.4) and the retention time of internal standard was 20.3 min (range: 19.9–20.7).

The calibration curves of fenoverine were linear in all tested tissue samples. Calibration curves were (p is peak-area ratio of fenoverine to internal standard, C is added concentration of fenoverine in tissues): C (ng/ml) = 415.8p + 6.3 with a correlation coefficient r = 0.9994 in liver, $C = 791p - 0.91$, $r = 0.9997$ in myocardium, $C = 416p + 0.715$, $r = 0.9997$ in skeletal muscle, and $C = 416p + 3.7$, $r = 0.9996$ in smooth muscle.

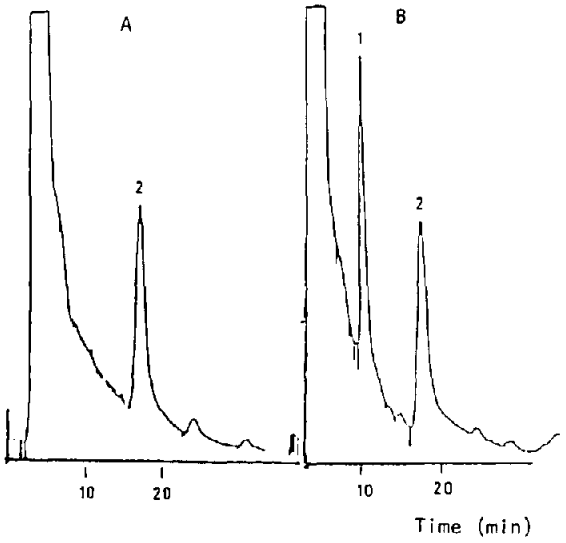

Fig. 3. Chromatograms of (A) drug-free rat myocardium sample and (B) rat myocardium containing 250 ng fenoverine per 100 mg. Peaks: 1 = fenoverine; 2 = internal standard.

The results of inter-day and intra-day reproducibility in liver are reported Table 1. Determinations of fenoverine recovery in striated muscle, colonic smooth muscle, and myocardium are reported Table 2. The limit of quantitation of fenoverine in tissue samples, determined as the smallest measurable value for the concentration of the compound giving a response of at least three times that of the baseline noise, was 25 ng injected.

Protein concentrations in liver, striated muscle, myocardium, and colonic smooth muscle samples were 26.0 mg, 25.6 mg, 32.2 mg, and 35.9 mg/100 mg of tissue, respectively.

4. Discussion

In this study, a simple method was used for the rapid determination of fenoverine levels in tissues. Chlorpromazine had been previously used as internal standard for determination of fenoverine [11]. Under the chromatographic conditions used in this study however, chlorpromazine was not eluted. Intra-day and inter-day reproducibility were satisfactory. Since concentrations of various metabolites that could potentially interfere with fenoverine were higher in liver than in other tissues, reproducibility studies were performed in liver. The variations in the recovery from the different tissues tested showed that a calibration curve must be prepared for each tissue.

Although no precise data are available concerning fenoverine binding to proteins *in vivo*, we may consider that fenoverine, like other phenothiazine derivatives [13], is probably highly bound to proteins. No correlation was found, however, between fenoverine recovery from liver, striated muscle, myocardium, and colonic

Table 1
Inter-day and intra-day reproducibility of fenoverine analysis in liver samples[a]

Fenoverine added (ng)	Intra-day ($n = 6$)		Inter-day ($n = 6$)	
	Mean recovery (%)	C.V. (%)	Mean recovery (%)	C.V. (%)
25	60	1.5	57	2.6
125	58	1.6	70	7.2
250	ND	ND	58	6.3
500	56	2.4	61	6.7

[a]Fenoverine was added to liver samples of 100 mg; ND = not done; C.V. = coefficient of variation.

smooth muscle tissue and the protein content of these tissues.

The tissues evaluated in this study are the main target organs for the drug: smooth muscle is the main therapeutic target of fenoverine [1], the manifestations of fenoverine toxicity in humans include striated muscle [5–10] and myocardium involvement (Chariot, unpublished data); the liver, the organ responsible for fenoverine metabolism, may be a site of accumulation of the drug. The same chromatographic profiles were obtained for all four organs studied. The method described here might therefore be applied to other organs. Biomedical applications of this method include determinations of fenoverine concentrations in tissues in experimental animal studies. In one patient with fenoverine-induced rhabdomyolysis, the fenoverine level in skeletal muscle was found to be 120 ng/100 mg of tissue. In rats fed with 100 mg/kg of fenoverine, mean fenoverine levels in tissue varied from 90 ng/100 mg in skeletal muscle to 2000 ng/100 mg in liver (Chariot, unpublished data). Further knowledge of distribution of fenoverine in tissues could be helpful in understanding the pathogenesis of muscular toxicity of the drug in humans.

Table 2
Recovery of fenoverine from muscle samples[a]

Amount added (ng)	Recovery[b] (%)		
	Striated muscle	Smooth muscle	Myocardium
25	64 (4.2)	63 (2.4)	57 (1.0)
125	80 (1.6)	86 (3.4)	117 (3.2)
200	79 (2.0)	79 (1.1)	100 (2.5)
250	70 (3.3)	70 (1.6)	113 (1.5)
500	61 (0.8)	75 (1.2)	102 (3.1)

[a]Fenoverine was added to tissue samples of 100 mg.
[b]Values in parentheses are coefficients of variation.

5. Acknowledgement

This study was supported in part by a grant to Dr. Chariot from the Association Française contre les Myopathies.

6. References

[1] J. Mironneau, S. Arnaudeau and C. Mironneau, *Br. J. Pharmacol.*, 104 (1991) 65.
[2] E. Camarri, *Curr. Med. Res. Opin.*, 10 (1986) 52.
[3] J.E.F. Reynolds (Editor), *Martindale: the Extra Pharmacopeia*, The Pharmaceutical Press, London, 29th ed., 1989, p. 1571.
[4] F. Bader, *Pharmatherapeutica*, 4 (1986) 422.
[5] R.M. Chichmanian, J.G. Fuzibet, G. Mignot and P. Dujardin, *Ann. Med. Interne (Paris)*, 141 (1990) 490.
[6] X. Hebuterne, R.M. Chichmanian, H.L. Cohen and P. Rampal, *Gastoenterol. Clin. Biol.*, 15 (1991) 861.
[7] J.M. Hardin, J.C. Guillebaud, P.Y. Lallement, B. Matta and M. Andrejak, *Thérapie*, 47 (1992) 165.
[8] R. Benamouzig, S. Chaussade, H. Roche, A. Aubert, J.N. Fiessinger, J. Carlet, F.E. Dazza and D. Couturier, *Gastoenterol. Clin. Biol.*, 16 (1992) 719.
[9] R.M. Chichmanian, A. Spreux, M. Bouillet, A. Chiffoleau, S. Baudot and C. Larousse, *Rev. Med. Interne*, 13 (1992) S114.

[10] S. Sultan, B. Lesgourgues, Y. El Attar, F. Fauvelle and N. Delas, *Thérapie*, 47 (1992) 433.
[11] O. Yoa-Pu Hu, P.-H. Chen, Y.-J. Fang, H.-S. Tang, L.-H. Pao, K.-M. Kwork and M.-L. King, *J. Pharm. Sci.*, 81 (1992) 91.
[12] E.F. Hartree, *Anal. Biochem.*, 48 (1972) 422.
[13] R.J. Baldessarini, in A.G. Gilman, T.W. Rall, A.S. Nies and P. Taylor (Editors), *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, Pergamon Press, New York, 8th ed., 1990, Ch. 18, p. 383.