Journal of Chromatography, 430 (1988) 175–179
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4233

Note

High-performance liquid chromatographic determination of enprofylline in human plasma

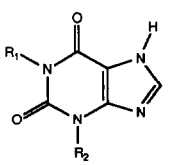
J.Y.-K. HSIEH*, T.J. THORNTON and W.F. BAYNE

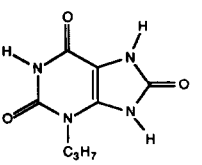
Merck Sharp & Dohme Research Laboratories, West Point, PA 19486 (U.S.A.)

(First received December 31st, 1987; revised manuscript received March 29th, 1988)

Enprofylline (I, Fig. 1) is a potent bronchodilator and has the chemical name 3,7-dihydro-3-propyl-1H-purine-2,6-dione. It may also have other anti-asthmatic activity such as anti-anaphylactic activity in the lung. Enprofylline has been shown to be about five times more potent than theophylline as a bronchodilator in animals [1,2] and asthmatic patients [3]. In animal studies, it lacks the diuretic [1,4] and central nervous system stimulant behavioral effects [4,5] of theophylline. In addition to these differences from theophylline, enprofylline is metabolized to a very low degree, being excreted unchanged through the kidney in rats [4] and in man [3,6]. Clinical studies have shown that enprofylline has quite different pharmacokinetic and pharmacodynamic profiles from those of theophylline [1,3].

A high-performance liquid chromatographic (HPLC) method [6] has been utilized to study enprofylline kinetics in healthy subjects after single oral doses. Assay details, such as sample preparation, chromatographic conditions and val-





I, $R_1 = H$, $R_2 = C_3H_7$

II, $R_1 = C_2H_5$, $R_2 = CH_2CH(OH)CH_3$

III

Fig. 1. Chemical structures of enprofylline (I), the internal standard (II) and 3-propyluric acid (III).

0378-4347/88/$03.50

idation data, e.g., specificity and sensitivity, are not included. The same chemists have recently developed an automated sample work-up method [7] for the analysis of enprofylline in plasma. Although sample throughput and selectivity are greatly increased, the method requires an isolation column, two column-switching valves and two additional pumps.

A rapid and sensitive reversed-phase HPLC method with ultraviolet detection is described for the quantification of enprofylline in plasma. Isolation of enprofylline and the internal standard from plasma matrix is accomplished by a C_{18} bonded-phase extraction cartridge. The extraction efficiencies are greater than 95% for both compounds. This method has been successfully used to determine plasma enprofylline concentrations in humans following incremental oral doses of enprofylline.

EXPERIMENTAL

All reagents were analytical grade. Acetonitrile and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.). A Supelco Visiprep Vacuum Manifold® (Bellefonte, PA, U.S.A.) equipped with Analytichem Bond-Elut® 100-mg octadecylsilane (C_{18}) cartridges (Harbor City, CA, U.S.A.) was used for sample extraction. Heparinized human control plasma was supplied by Ser-Tec Biologicals (North Brunswick, NJ, U.S.A.). Enprofylline, the internal standard II and standard III (Fig. 1) were supplied by Draco (Lund, Sweden).

Apparatus

The chromatographic separation was attained using a Perkin-Elmer Series 4 liquid chromatograph equipped with an ISS-100 autosampler (Norwalk, CT, U.S.A.). Peaks were detected by monitoring absorbance at 278 nm (0.005 a.u.f.s.) with a Kratos Spectroflow 773 detector (Westwood, NJ, U.S.A.). An Applied Science direct-connect 3 cm×2.1 mm guard column (State College, PA, U.S.A.) packed with Whatman Pell ODS (octadecylsilane groups bonded to 30–38 μm glass beads; Clifton, NJ, U.S.A.) was used, along with an ES Industries analytical column [5 μm, M-C_{18} (monolayer octadecyl), 15 cm×4.6 mm, Marlton, NJ, U.S.A.]. A Spectra-Physics Model 4270 integrator (Santa Clara, CA, U.S.A.) was used to obtain peak-height ratios for quantification. Detector noise was filtered by an Anspec Model 1021A filter/amplifier (Ann Arbor, MI, U.S.A.).

Preparation of standard solutions and reagents

The mobile phase consisted of 0.01 *M* sodium acetate (pH 4.0)–acetonitrile (90:10, v/v) and was delivered at a flow-rate of 1.5 ml/min at ambient temperature. A stock standard solution of enprofylline (1 mg/ml) was prepared in deionized water. This solution was further diluted with deionized water to give a series of enprofylline working standards. The concentrations were 100, 50, 20, 10, 5 and 2 μg/ml. This produced equivalent plasma concentrations of 10, 5, 2.5, 1, 0.5, 0.25 and 0.1 μg/ml. The stock internal standard (1 mg/ml) was also prepared in deionized water. A working internal standard of 30 μg/ml was used for plasma analysis.

Preparation of plasma samples

Healthy volunteers were given enprofylline every 12 h, beginning at 150 mg and progressing at 150-mg increments to 600 mg. Pre- and post-dose blood samples were drawn as specified in the protocol. After separation, plasma samples were stored at −15°C until the time of analysis.

Plasma specimens were prepared for extraction by placing 0.5 ml of plasma, 0.5 ml of 0.001 *M* phosphoric acid and 0.75 μg of working internal standard in a 12 mm × 75 mm glass disposable tube. After vortexing, the plasma sample was applied to a Bond-Elut C_{18} cartridge pre-washed with 1 ml of methanol followed by 1 ml of 0.001 *M* phosphoric acid. The cartridge was further washed twice with 1 ml of 0.001 *M* phosphoric acid and all washings were discarded. Each cartridge was then eluted with 0.5 ml of a mixture of acetonitrile–0.01 *M* sodium acetate (30:70, v/v) and the eluates were collected. An aliquot (70 μl) of this solution was injected for HPLC analysis. Samples for standard curves were prepared by adding different amounts (0.1–10 μg) of enprofylline and 0.75 μg of internal standard to 0.5 ml of human control plasma. Sample extraction and HPLC analysis were carried out as described above. Unknown concentrations of enprofylline in plasma were calculated from the linear regression equation of the daily standard curves constructed by plotting the peak-height ratios of enprofylline to internal standard versus their actual concentration.

RESULTS AND DISCUSSION

A reversed-phase HPLC method using UV detection at 278 nm has been developed for the determination of enprofylline in human plasma. Under the chromatographic conditions utilized, enprofylline was baseline-resolved from the internal standard (Fig. 2B). Enprofylline eluted at 4.9 min and the internal standard at 6.2 min. Typical chromatograms of pre-dose subject plasma and plasma collected after the administration of enprofylline are shown in Fig. 2. As can be seen from the pre-dose plasma sample, there is minimal endogenous interference (Fig. 2A). The limit of detection for enprofylline was 50 ng/ml (based on a signal-to-noise ratio of > 5:1).

A C_{18} extraction cartridge was activated with methanol and then washed with 0.001 *M* phosphoric acid. Under these acid conditions, enprofylline and the internal standard were all retained. A further washing with 0.001 *M* phosphoric acid would not elute these compounds, but will remove polar and many basic compounds that would otherwise interfere with the chromatographic analysis. A mixture of acetonitrile–0.001 *M* sodium acetate (30:70, v/v) was found to be the best eluting solvent for these compounds from the extraction cartridges. It gave excellent recoveries (> 95%) of both drug and internal standard and minimal elution of interfering endogenous compounds. Direct injection of enprofylline-dosed plasma sample (100 μl) onto a Pinkerton internal-surface reversed-phase HPLC column (Regis, Morton Grove, IL, U.S.A.) resulted in poor peak shapes for both compounds and a long-tailing solvent peak using a mobile phase of 0.1 *M* monobasic potassium phosphate (pH 6.5) and a flow-rate of 1 ml/min.

The assay for determining enprofylline in plasma was evaluated for intra-day

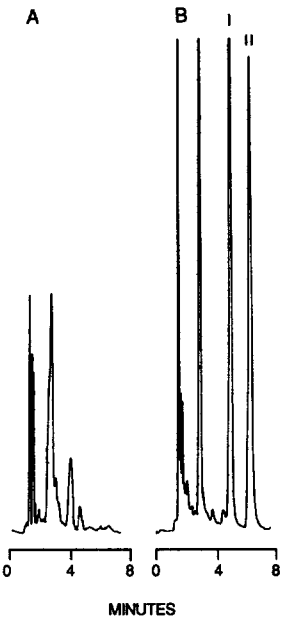


Fig. 2. Typical chromatograms for enprofylline (I) and the internal standard (II) in plasma. (A) Blank human plasma; (B) subject plasma containing 1.4 μg/ml enprofylline after the administration of enprofylline.

TABLE I

INTRA-DAY AND INTER-DAY PRECISION FOR THE ASSAY OF ENPROFYLLINE IN PLASMA

Concentration (μg/ml)	Relative standard deviation (%)	
	Intra-day ($n=6$)	Inter-day ($n=3$)
0.10	5.3	5.8
0.25	3.3	6.0
0.50	7.5	3.9
1.00	2.6	3.2
2.50	2.3	3.0
5.00	3.3	1.0
10.00	3.0	2.8

and inter-day variation. Standard concentrations ranged from 0.1 to 10 μg/ml. The precision of the assay was checked by comparing the intra- and inter-day variability of each standard point. The data are summarized in Table I. The accuracy of the assay was established by preparing quality control samples at low (0.25 μg/ml) and high (5.0 μg/ml) points on the standard lines. When 1-ml plasma samples containing 0.25 and 5.0 μg of enprofylline were assayed with healthy volunteers' samples, the calculated amounts were 0.28 μg (R.S.D.=9%, $n=6$) and 4.6 μg (R.S.D.=3%, $n=6$) of enprofylline, respectively.

TABLE II

RETENTION TIMES FOR SOME XANTHINES

Xanthine	Retention time (min)
Enprofylline	4.9
Theophylline	4.1
Theobromine	3.1
Caffeine	8.4
1,7-Dimethylxanthine	4.0
3-Methylxanthine	2.1
1,3-Dimethyluric acid	2.4
7β-Hydroxytheophylline	7.2
8-Chlorotheophylline	7.5

The linearity of each standard curve was confirmed by plotting the drug concentration versus the peak-height ratio of drug to internal standard. Correlation coefficients of 1.000 were consistently obtained. The specificity of the assay was checked by assaying control human plasma and volunteer's pre-dose plasma. No endogenous interference was encountered.

Several xanthine compounds have been checked for potential interferences with the analysis of enprofylline in plasma. Pure xanthine standards (concentrations 10 μg/ml) were chromatographed individually under the conditions described for the analysis of enprofylline in plasma. Table II lists various xanthines and their HPLC retention times. None of the xanthines checked were found to interfere with the analysis of enprofylline. 3-Propyluric acid (III, Fig. 1), an identified urinary metabolite in humans, eluted at 2.6 min and also did not interfere with the assay under the conditions described.

This method has been used to assay clinical samples from human volunteers administered therapeutic doses of enprofylline. The assay has proven to be reliable and rugged. The short analysis time has allowed a rapid turn-around of large numbers of clinical samples.

REFERENCES

1 C.G.A. Persson, I. Erjefält and J.-A. Karlsson, Acta Pharmacol. Toxicol., 49 (1981) 317.
2 C.G.A. Persson and G. Kjellin, Acta Pharmcol. Toxicol., 49 (1981) 313.
3 E. Lunell, N. Svedmyr, K.-E. Andersson and C.G.A. Persson, Eur. J. Clin. Pharmacol., 22 (1982) 395.
4 C.G.A. Persson, I. Erjefält, L.-E. Edholm, J.-A. Karlsson and C.-J. Lamm, Life Sci., 31 (1982) 2673.
5 C.G.A. Persson and I. Erjefält, Arch. Int. Pharmacodyn. Ther., 258 (1982) 267.
6 O. Borgå, K.-E. Andersson, L.-E. Edholm, P.-O. Fagerström, E. Lunell and C.G.A. Persson, Clin. Pharmacol. Ther., 334 (1983) 799.
7 L. Ögren, E. Albertsson and L.-E. Edholm, 10th International Symposium on Column Liquid Chromatography, San Francisco, CA, May 18–23, 1986, p. 2708.