ELSEVIER

Journal of Chromatography B, 664 (1995) 468–471

JOURNAL OF
CHROMATOGRAPHY B:
BIOMEDICAL APPLICATIONS

Short communication

Reversed-phase high performance liquid chromatographic determination of cyproheptadine from urine by solid-phase extraction

John E. Kountourellis*, Kenneth O. Ebete

School of Pharmacy, Laboratory of Pharmaceutical Analysis, Aristotelian University, 54006 Thessaloniki, Greece

First received 1 July 1994; revised manuscript received 11 October 1994

Abstract

A simple, sensitive and reliable high-performance liquid chromatographic method for the determination of cyproheptadine in urine by solid-phase extraction (SPE) has been developed. The sample matrix was passed through a pre-conditioned C_{18} cartridge, washed with methanol–water solution (4:1) and eluted with methanol. The methanolic solution was evaporated to dryness, reconstituted with methanol and chromatographed using a C_{18} reversed-phase column. The mobile phase consisted of acetate buffer (constant ionic strength of 0.005 *I*)–methanol (56:44, v/v). Detection was performed at 254 nm with the sensitivity set at 0.002 AUFS. Concentrations as low as 50 ng/ml could be quantitatively determined by an external standard method and the overall recovery was found to be 76.16%, whereas the limit of detection was estimated as 15 ng/ml.

1. Introduction

Cyproheptadine, 1-methyl-4-(5*H*-dibenzo[a,d]-cycloheptenylidine) piperidine, is a potent antihistaminic and antiserotonergic agent [1]. It is also an inhibitor of platelet aggregation [2] and has been used as an antipruritic agent [3].

Several analytical methods have been reported for the separation of cyproheptadine using gas chromatography [4,5], gas–liquid chromatography [6] and high-performance liquid chromatography [7,8]. However, the previously described HPLC methods are time-consuming and require a tedious liquid–liquid extraction procedure. This report describes a rapid and simple method for the determination of cyproheptadine in urine using solid-phase extraction technique coupled with a reproducible HPLC method which is suitable for determination in the low nanogram range.

* Corresponding author.

2. Experimental

2.1. Apparatus and chemicals

A solvent delivery system with two Model LC-6A high-pressure pumps coupled with a Model SPD-6AV UV spectrophotometric detector operated at 254 nm was used. The reversed-phase octyl-ODP column (150 × 6 mm I.D.), placed in a Model CTO-6A oven at 40°C, and equipped with an injector with a 20-μl loop, was operated isocratically (all products from

0378-4347/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0378-4347(94)00476-5

Shimadzu Instruments, Kyoto, Japan). Chromatograms were recorded on chart paper with a Chromatopac Model C-R 6A thermal printer–plotter (Shimadzu) at a speed of 2 mm/min. Supelclean C_{18} cartridges and vacuum glass manifold were obtained from Supelco (Rohm and Haas, Bellefonte, PA, USA).

Cyproheptadine hydrochloride was supplied by Sigma Company (St. Louis, MO, USA) and used without further purification. All standard solutions of cyproheptadine were prepared by dissolving the appropriate amount in methanol. Ammonium acetate and glacial acetic acid analytical reagent grade were obtained from Merck (Darmstadt, Germany). HPLC grade methanol and water were used throughout.

2.2. Chromatography

The mobile phase was acetate buffer (ionic strength 0.005 I)–methanol (56:44, v/v). The pH was adjusted to 3.6 using acetic acid. The column was equilibrated with the eluting solvent by pumping the mobile phase at a flow-rate of 0.3 ml/min for 24 h and degassed by slowly bubbling with helium gas until a stable baseline was achieved. The flow-rate was set at 1.4 ml/min during analysis and detection was performed at 254 nm. Although one of the reported HPLC methods [7] has a similar approach with respect to the chromatographic system, both methods described in Refs. [7,8] were less sensitive. The proposed method is reproducible with a limit of detection of 15 ng/ml at a sensitivity of 0.002 AUFS.

2.3. Extraction procedure

A 1-ml volume of stock cyproheptadine methanolic solution (53, 106, 212, 318, 428 and 530 ng/ml) was evaporated to dryness in a 15-ml glass centrifuge tube under nitrogen. Then a 0.10–0.12 ml volume of methanol together with 1 ml of urine was added; the solution was thoroughly mixed and subsequently processed by solid-phase extraction using 3-ml C_{18} supelco cartridges. It was found that, when the cyproheptadine methanolic solution was evaporated to dryness before addition of the urine sample, the amount of cyproheptadine recovered was reduced. Addition of 0.10–0.12 ml of methanol prior to extraction helps to increase the recovery. The mixture was slowly forced through the cartridge that had been conditioned with 2 ml of methanol and washed with 2 ml of water. The cartridges were fitted in a vacuum glass manifold system and washed twice with 2 ml of methanol–water (4:1, v/v), before the final elution of cyproheptadine with 6 ml of methanol. It was observed that washing the cartridges with only water results in overlap of the cyproheptadine peak with endogenous material from urine while washing with methanol alone considerably reduces the amount of cyproheptadine recovered. However, an appropriate combination of methanol–water was very useful as the water helps to retain cyproheptadine on the column material while methanol removes the endogenous compounds before the final elution of cyproheptadine. The methanolic solution was evaporated to dryness on an evaporating unit (Pierce Reacti-Therm, Model 18780) under a stream of nitrogen at 40°C. The residue was redissolved in 1 ml of methanol and aliquots of 20 μl were injected onto the analytical column.

3. Results and discussion

The retention time of cyproheptadine was found to be 8.30 min with a k' value of 3.09 and was reproducible under the chromatographic conditions used. The optimum conditions were selected by also using a number of other mobile phases on the bases of their relative polarities. The proportion of the constituents of the mobile phase, the pH and ionic strength I were varied until a good peak symmetry and acceptable separation were achieved.

Fig. 1A illustrates a typical chromatogram of cyproheptadine standard in methanolic solutions while Fig. 1B, represents a cyproheptadine extract from a spiked urine sample processed by solid-phase extraction.

The plot of peak height against concentration of cyproheptadine was linear over the range 53–

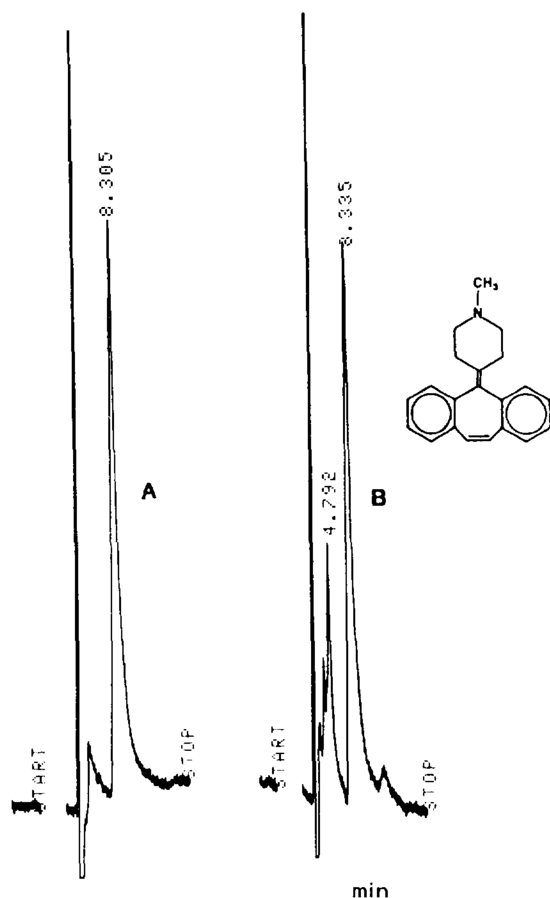

Fig. 1. (A) Typical chromatogram of cyproheptadine standard in methanolic solution. (B) Cyproheptadine extract from spiked urine sample processed by solid-phase extraction procedure and detection at 254 nm, t_R 8.30 min, t_0 2.03 min.

530 ng/ml. The linear regression and correlation analysis were found to be $y = 0.2414x - 0.253$, $r = 0.9998$ and $y = 0.1842x + 0.60$, $r = 0.9987$ for cyproheptadine in methanolic solution and after extraction of spiked samples, respectively. Recovery data are presented in Table 1.

The overall recovery of cyproheptadine was estimated by plotting a standard calibration of the spiked versus found concentrations of cyproheptadine in a urine sample which gave a linear relationship with $y = 0.7616x + 4.26$, $r = 0.9987$. Therefore the slope 0.7616 of this regression line was used as an estimate of the overall recovery for cyproheptadine: 76.16% in urine samples.

Precision and accuracy were assessed by spiking urine samples with cyproheptadine standards at three concentration levels (53, 318 and 530 ng/ml). The intra-day precision for each concentration level was determined from four determination of samples with the same concentration while the overall accuracy and precision for each concentration level was calculated from twelve determinations of samples with the same concentration at three different days (4×3) within one month. Therefore for the assessment of the overall precision within one month at three concentration levels 36 urine samples were used. The results are presented in Table 2.

Concentrations as low as 50 ng/ml could be quantitatively determined by the proposed method and the limit of detection was estimated as 15 ng/ml, corresponding to 2 times the signal-to-noise ratio.

Table 1
Standard calibration and recovery data of cyproheptadine from spiked urine samples ($n = 4$)

	Added (ng/ml)	Mean peak height Standard (mm)	Mean peak height Recovery (mm)	Mean concentration found (ng/ml)	Recovery (%)
1	53	13.0 ± 0.49	10.0 ± 0.48	42.48	80.15
2	106	25.5 ± 0.97	20.0 ± 0.89	85.57	80.73
3	212	51.0 ± 1.58	41.0 ± 1.40	170.92	80.62
4	318	75.5 ± 1.87	57.0 ± 1.31	237.21	74.59
5	428	102.0 ± 1.32	82.0 ± 1.25	340.79	79.62
6	530	129.0 ± 1.45	97.0 ± 1.22	402.94	76.03

Table 2
Accuracy and precision of the determination of cyproheptadine in spiked urinary samples (n = 4)

Spiked amount ng/ml	Day	Accuracy Mean found (ng/ml)	Precision		Overall (mean ± S.D.) (ng/ml)
			S.D. (ng/ml)	C.V. (%)	
53	1	43.1	1.55	3.60	42.7 ± 1.77
	2	41.9	1.73	4.13	
	3	43.1	2.17	5.03	
318	1	237.2	5.96	2.51	238.6 ± 5.09
	2	238.1	4.79	2.01	
	3	240.5	5.25	2.18	
530	1	405.0	6.20	1.53	404.9 ± 6.32
	2	404.1	4.63	1.14	
	3	405.8	9.21	2.27	

5. Conclusions

The results of the present study show that the proposed HPLC method is an efficient, simple and accurate means of quantitating cyproheptadine in urine by using solid-phase extraction. It is therefore recommended for routine analysis of cyproheptadine in biological fluids.

Acknowledgement

The authors would like to thank Mr. A.E. Tsolakopoulos for his technical assistance.

References

[1] C.A. Stone, H.C. Wenger, C.T. Ludden, J.M. Stavorski and C.A. Ross, *J. Pharmacol. Exp. Ther.*, 131 (1961) 73.

[2] *Physicians Desk Reference, 34th ed.*, Medical Economics Company, Oradell, NJ, 1980, p. 1195.

[3] B. Goldman, L.M. Aledort, E. Puszkin and L. Burrows, *Circulation*, 44 (1971) 11.

[4] J.S. Wold and L.J. Fischer, *J. Pharmacol. Exp. Ther.*, 183 (1972) 188.

[5] R.T. Sane, K.A. Karkhanis and P.G. Anaokar, *Indian J. Pharm. Sci.*, 43 (1981) 111.

[6] H.B. Hucker and J.E. Hutt, *J. Pharm. Sci.*, 72 (1983) 1069.

[7] N.H. Foda, H.W. Jun and J.W. McCall, *J. Chromatogr.*, 9 (1986) 817.

[8] E.A. Novak, S.M. McIntyre and L.M. Hryttorczuk, *J. Chromatogr.*, 339 (1985) 457.