Journal of Chromatography, 339 (1985) 457—461
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2521

Note

High-performance liquid chromatographic method for quantification of cyproheptadine in serum or plasma

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(First received August 21st, 1984; revised manuscript received December 20th, 1984)

Cyproheptadine, 1-methyl-4-(5H-dibenzo[*a*,*d*]-cycloheptenylidine) piperidine, is a potent serotonin and histamine antagonist [1] and has a variety of possible therapeutic uses. It has been used as an antipruritic [2], an appetite stimulant [3], an antiabortifacient [4] and is reported to be useful in treating post-gastrectomy dumping syndrome [5].

Cyproheptadine has been analyzed by colorimetric procedures [6, 7], gas chromatography (GC) with flame ionization detection [8, 9], and GC with nitrogen—phosphorus detection (NPD) [10]. This report describes an analytical

0378-4347/85/$03.30

method for low nanogram per milliliter concentrations of cyproheptadine using high-performance liquid chromatography (HPLC) and a variable-wavelength ultraviolet detector. This method is applicable for microliter amounts of plasma or serum.

MATERIALS AND METHODS

Apparatus

The analytical HPLC equipment consisted of a Model 6000A delivery system, a WISP 710B automatic injector, a Model 720 system controller, a Model 730 data module, and a Model 450 variable-wavelength detector (Waters Assoc.). A 10-μm C_{18} reversed-phase column (Waters Assoc., μBondapak) 30 cm × 3.9 mm was used for separations.

Standards and reagents

Cyproheptadine · HCl (Merck Sharpe and Dohme, West Point, PA, U.S.A.) was dissolved in a solution consisting of methanol—0.1 *M* hydrochloric acid (50:50) to produce a concentration of 1 mg/ml cyproheptadine. Four working standards containing 60 ng/ml (A), 100 ng/ml (B), 200 ng/ml (C) and 400 ng/ml (D) were prepared by appropriate dilution of the above stock solution with 0.1 *M* hydrochloric acid. The internal standard was desmethyldoxepin · HCl (Pfizer, Groton, CT, U.S.A.) which was dissolved in 0.1 *M* hydrochloric acid to yield a solution of 1 μg/ml. All standard solutions were stored at 4—8°C. The mobile phase consisted of methanol—acetonitrile—0.1 *M* phosphate buffer and 5 m*M* pentanesulfonic acid at pH 4.7 (41 : 15 : 44).

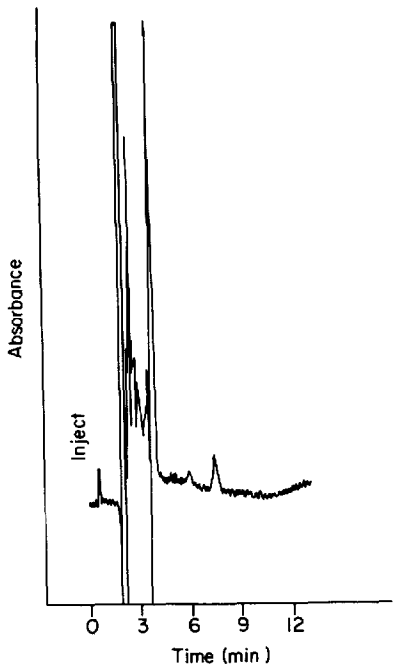
Procedure

A 50-μl aliquot of desmethyldoxepin standard solution and 50 μl of working standards A, B, C or D, were added to four 15-ml conical centrifuge tubes containing 100 μl of plasma. The four tubes contained 3, 5, 10, and 20 ng of cyproheptadine, respectively. To each tube were added 100 μl of 1.5 *M* sodium hydroxide and 3 ml of hexane—isoamyl alcohol (99:1). The extraction was carried out by mixing the solution for 2 min on a vortex mixer, then freezing and thawing to break up the emulsions, and centrifugation for 5 min at 2000 *g* (3000 rpm). The organic layers were transferred to four clean 15-ml conical centrifuge tubes and the aqueous layer was extracted a second time. The organic layers of the second extraction were combined with those of the first and 100 μl of 0.05 *M* sulfuric acid were added to each tube. Cyproheptadine was back-extracted into the acid by vortexing for 2 min. The aqueous solution was separated by centrifugation and the organic layer discarded.

Of the aqueous solution 90 μl were injected onto the HPLC column. The flow-rate of the mobile phase was 1.5 ml/min, the detector was set at 228 nm and the sensitivity was 0.01 a.u.f.s. Using 2 ml of plasma there is sufficient absorbance at 254 nm to run the analysis.

RESULTS AND DISCUSSION

Fig. 1 shows the HPLC curve for the extraction of 200 μl of plasma blank.



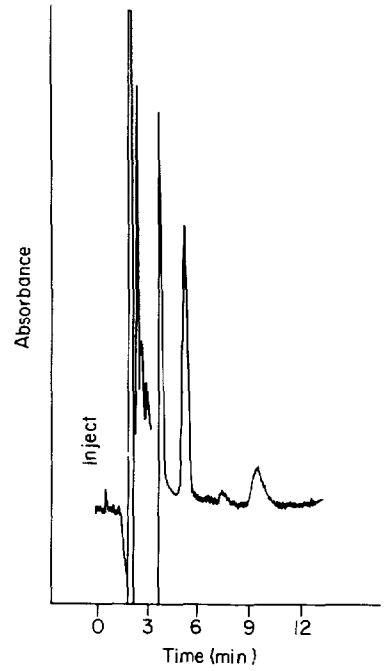


Fig. 1. Chromatogram of plasma blank extracts.

Fig. 2. Chromatogram of plasma extract containing 50 ng of desmethyldoxipin (5.19 min) and 7.84 ng of cyproheptadine (9.40 min).

Peaks at retention times of 5.85 and 7.36 min represent some endogenous compounds in the plasma. Fig. 2 represents the HPLC curve for the extraction of 100 μl of plasma to which were added 50 ng of desmethyldoxipin and 7.84 ng of cyproheptadine. The retention time for desmethyldoxepin is 5.19 min and for cyproheptadine 9.40 min. Although there is some overlap between desmethyldoxepin and the peak at 5.85 min, the error in measurement from this interference is less than 1%. A sensitive GC—NPC method [10] was unable to separate the desmethylcypropheptadine, a possible metabolite from the internal standard, 1-ethyl-4-(5H-dibenzo[*a*,*d*]-cycloheptenylidene)piperidine. In a separate analysis it was determined that under the conditions described here desmethylcyproheptadine had a retention time of 7.84 min. These HPLC conditions are suitable for determinations of cyproheptadine and its metabolite.

TABLE I

RECOVERY OF CYPROHEPTADINE ADDED TO PLASMA (n = 11)

Added (ng)	Recovered (ng)			R.S.D. (%)	Percentage error
	Mean	Range	S.D.		
3	3.02	3.36— 2.68	0.23	7.5	+0.67
5	4.87	5.34— 4.52	0.29	6.0	—2.60
10	10.16	10.70— 9.62	0.36	3.6	+1.60
20	19.95	22.30—17.40	1.52	7.6	—0.25

Quantification of the data was accomplished by measuring peak heights and establishing a ratio of cyproheptadine to desmethyldoxepin. Eleven separate determinations of the cyproheptadine/desmethyldoxepin ratios were obtained over a two-month period. The results were linear from 3 to 20 ng. The equation for the resulting straight line was $y = 0.016x + 0.005$ and the Pearson Product Moment correlation coefficient was 0.999. Calculation of the recoveries is presented in Table I.

The day-to-day variation in the precision and accuracy ranged from 3.6% to 7.6% and from −2.60% to 1.60% error, respectively. The within-day variations were determined on ten 100-μl plasma samples spiked with 7.84 ng of cyproheptadine. The average of recovered cyproheptadine was 7.57 ng ± 3.9%. The analytical recovery was 96.6%.

Serum levels were measured in two groups of Sprague—Dawley rats (weighing 150—250 g) that were given 10 and 20 mg/kg cyproheptadine per day via osmotic pumps (Alza, Palo Alto, CA, U.S.A.) which delivered at a constant rate for four weeks. A control group received saline alone. Steady-

TABLE II

DOSE VERSUS SERUM LEVEL (n = 11)

Dose (mg/kg per 24 h)	Serum concentration (ng/ml)
10	28.3 ± 14.9
20	42.0 ± 23.1

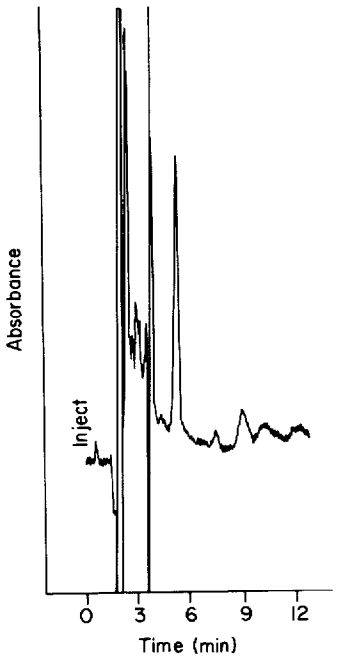


Fig. 3. Chromatogram of serum extract from a rat that received 20 mg/kg cyproheptadine per day. The serum cyproheptadine concentration is 37.9 ng/ml.

state serum concentrations were determined from tail bloods (100—200 μl serum) on the fourth week of treatment (Table II).

A typical chromatogram of serum analysis after dosing the rats with cyproheptadine is presented in Fig. 3.

CONCLUSIONS

In summary, the present method was shown to be sensitive and reliable for low nanogram level determinations of cyproheptadine. The use of desmethyldoxepin as an internal standard serves to minimize error in quantitation resulting from sample preparation. This method is more sensitive than previous colorimetric [6, 7] and flame ionization GC methods [8, 9]. A similar sensitivity for cyproheptadine was reported using GC—NP [10], but the internal standard used in that assay had the same retention time as the desmethylcyproheptadine metabolite. In the present method, there is no interference with this metabolite.

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