Journal of Chromatography, 339 (1985) 414—418
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2522

Note

Determination of cyclobenzaprine in plasma and urine using capillary gas chromatography with nitrogen-selective detection

M.L. CONSTANZER*, W.C. VINCEK and W.F. BAYNE

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

(First received September 13th, 1984; revised manuscript received December 5th, 1984)

Cyclobenzaprine, a tricyclic muscle relaxant, has been analyzed by thin-layer chromatography [1], and packed-column gas chromatography (GC) with either flame ionization [2] or nitrogen-selective detection [3]. With the nitrogen-specific detector, the intra-day coefficient of variation (C.V.) was 11% when 5 ng cyclobenzaprine in 2 ml plasma was quantified. In addition, numerous extractions were required as part of the sample preparation procedure to quantify the drug in the therapeutic range of drug concentrations. In support of pharmacokinetic studies, it was desirable to increase the sample throughput by reducing the sample preparation time and to lower the limit of reliable quantification for a fuller mapping of the plasma concentration—time courses. A capillary GC method with nitrogen-selective detection was developed which employs a single extraction of the biological specimen. The intra-day coefficient of variation at 1 ng/ml was less than 10%.

EXPERIMENTAL

Materials and reagents

Normal hexane (nanograde, Mallinckrodt, Paris, KY, U.S.A.), triethylamine (TEA, sequanal grade, Pierce, Rockford, IL, U.S.A.), methanol, and ethyl acetate (HPLC grade, Burdick & Jackson Labs., Muskegon, MI, U.S.A.), anhydrous sodium carbonate and sodium bicarbonate (reagent grade, Merck, Rahway, NJ, U.S.A.), sodium hydroxide (reagent grade), hydrochloric acid (concentrated, reagent grade), monobasic sodium phosphate, dibasic sodium phosphate (Fisher Scientific, Fair Lawn, NJ, U.S.A.), β-glucuronidase (bovine liver, P.L. Biochemicals, Milwaukee, WI, U.S.A.) and LR101 concentration tubes (Laboratory Research, Los Angeles, CA, U.S.A.) were purchased from

0378-4347/85/$03.30

their respective suppliers. The internal standard, 5-(2-dimethylamino-ethylidene)dibenzo[*a,e*]cyclohepatriene was obtained from Merck.

Assay procedures

Plasma (1.0 ml), internal standard (10 ng in 0.1 ml), carbonate buffer (1 ml, 0.2 *M*, pH 9.8), and hexane (5 ml) were transferred to a glass centrifuge tube (13 ml). The tube was stoppered and the contents shaken and then centrifuged (5 min at 2 *g*). The organic layer was transferred to a LR101 concentration tube and the hexane evaporated to dryness (50°C) under a stream of nitrogen. The residue was reconsituted in TEA—hexane (0.01% TEA, 20 μl) and an aliquot (5 μl) of the TEA—hexane mixture injected onto the GC column.

Urine (1.0 ml), internal standard (500 ng in 0.1 ml), and β-glucuronidase (1 ml = 5000 Fishman units prepared in 0.2 *M* phosphate buffer, pH 6.5) were transferred to a glass centrifuge tube (13 ml). The tube was stoppered and placed in a Teacam Driblock at 37°C for 18 h. At the end of this time period, sodium hydroxide (0.5 ml, 0.5 *M*) and hexane (5 ml) were added. The tube was stoppered and the contents shaken and then centrifuged (5 min at 2 *g*). The hexane was transferred to another tube and drug and internal standard were back-extracted into acid (0.2 ml, 0.1 *M* hydrochloric acid). After removal of the hexane, chromatographic contaminants remaining in the aqueous phase were extracted with additional hexane (3 ml). The hexane layer was discarded and the aqueous layer was made basic by the addition of sodium hydroxide (0.1 ml, 0.5 *M*). Cyclobenzaprine and internal standard were extracted from the basic milieu with ethyl acetate (0.5 ml) and an aliquot (2 μl) of the ethyl acetate was injected onto the chromatographic column.

Calculations

A standard curve of cyclobenzaprine in plasma or in urine was run daily with the clinical specimens. The calibration curves for plasma and urine were linear from 1 to 30 ng/ml and 2 to 8 μg/ml, respectively. The equation for the resulting line was $y = 0.0890x + 0.05309$ (with $r^2 = 0.99939$) for plasma and $y = 0.73663x - 0.04853$ (with $r^2 = 0.99947$) for urine. The peak ratio of the drug to internal standard from the unknown sample was employed to calculate their concentrations from the standard curve.

Instrumentation

Analyses were performed on a Varian 6000 gas chromatograph equipped with a nitrogen—phosphorus detector (thermal specific detector). For plasma and urine, respectively, a 40- and 20-m length standard bore, 0.25-μm film thickness, DB-5, J & W. Scientific capillary column was used. A fritted glass liner with the top portion packed with 1% OV-17 on Gas-Crhom Q (80—100 mesh), which has been conditioned previously, was installed in the injection port to act as a guard column for plasma analysis. A fritted glass liner without packing was installed in the injection port for urine analysis. The splitter was off and the septum purge was capped.

Instrumental conditions

The injection port temperature and detector temperature were set at 300°C. The oven temperature programme for plasma was (a) 140°C initial temperature, 0 min hold; (b) 50°C/min to 230°C; (c) 4°C/min to 245°C; (d) 50°C/min to 300°C, 3 min hold. The oven temperature programme for urine was (a) 140°C initial temperature, 0 min hold; (b) 50°C/min to 230°C; (c) 4°C/min to 245°C, 2 min hold. Flow-rates of the hydrogen, air, and helium (make-up) gases were 4.5, 175 and 20 ml/min, respectively. The helium (carrier) gas had a column head pressure of 2.068 bars.

RESULTS AND DISCUSSION

The intra-day and inter-day precision of the methods are presented in Table I. Typical chromatograms are presented in Figs. 1 and 2.

With the thermionic bead operating at 80% maximum current, a minimum of 10 pg of drug could be detected easily (signal-to-noise ratio > 10). In the presence of endogenous interferences, 1 ng/ml of plasma could be quantified reliably as indicated by Table I. Since one fourth of the prepared plasma sample was injected, approximately 250 pg of the drug were being quantified in the precence of the endogenous interferences with absolute recovery of 90%.

Following cyclobenzaprine administration in man, unchanged cyclobenzaprine constitutes only a minor fraction in urine, the major fraction is a glucuronide-like conjugate of cyclobenzaprine [4]. After conversion of the glucuronide back to the parental form reliable quantification of 0.2 μg of cyclobenzaprine per ml of urine can be achieved with recoveries of 95%.

A clean-up procedure which was more efficient in reducing the endogenous interferences relative to the drug would, in principle, yield an even more sensitive method. However, as with the structurally similar tricylic antidepressants, the clean-up procedures can lead to losses of the drug due to adsorptive phenomena [5]. To minimize these losses, a single extraction with hexane was employed to maintain high drug recovery. High-efficiency capillary

TABLE I

INTRA- AND INTER-DAY PRECISION OF THE METHOD

Plasma			Urine		
ng/ml	C.V. (%)	*n*	μg/ml	C.V. (%)	*n*
Intra-day precision					
1	8.1	6	0.2	5.1	6
10	3.6	6	0.5	5.0	6
30	3.6	6	1.0	2.6	6
			2.0	2.7	6
			4.0	2.6	6
			8.0	2.6	6
Inter-day precision					
2.0	10.0	16	0.80	6.6	20
20.0	10.9	16			

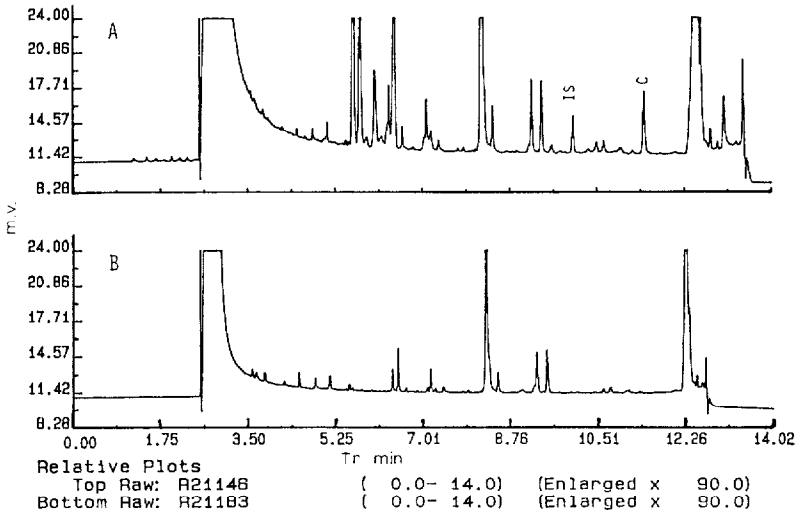

Fig. 1. Representative chromatograms of cyclobenzaprine (C) and internal standard (IS) in plasma: (A) 20.0 ng/ml cyclobenzaprine and internal standard; (B) blank plasma.

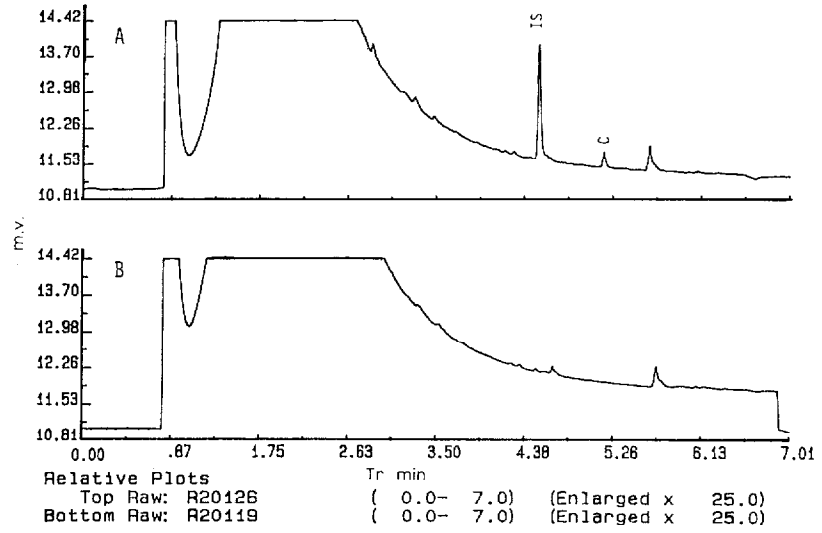

Fig. 2. Representative chromatograms of cyclobenzaprine (C) and internal standard (IS) in urine: (A) 0.2 μg/ml cyclobenzaprine and internal standard; (B) blank urine.

GC was then employed to resolve the drug from the endogenous interferences. In order to insure efficient reconstitution of the prepared samples containing nanogram quantities of the drug, it was necessary to add small amounts of triethylamine to the hexane. The triethylamine minimized adsorptive losses. In fact triethylamine in hexane has previously been used as an extracting solvent in a packed-column method for amitriptyline using a nitrogen-selective detector [6]. For cyclobenzaprine there was no advantage in using triethylamine in hexane compared to using hexane alone for the extracting solvent. However, as mentioned above it was a requisite for the reconstituting solvent. Neither

in the newly developed method for cyclobenzaprine nor in the previously reported method [6] for amitriptyline did the disturbance of the nitrogen detector by triethylamine prevent the quantification of the drug. The triethylamine eluted much earlier than the drug of interest.

This present method has been employed for the routine analysis of 600 plasma and 500 urine samples.

REFERENCES

1 D. Faber, J. Chromatogr. Sci., 12 (1974) 675.
2 H. Hucker and S. Stauffer, J. Pharm. Sci., 65 (1976) 1253.
3 H. Hucker and S. Stauffer, J. Chromatogr., 124 (1976) 164.
4 A. Till, M. Constanzer, J. Demetriades, J. Irvin and R. Lee, Biopharm. Drug Dispos., 3 (1982) 19.
5 P. Edelbroek, E. deHaas and F. Wolff, Clin. Chem., 28 (1982) 2143.
6 A. Jorgensen, Acta Pharmacol. Toxicol., 36 (1975) 79.