Journal of Chromatography, 367 (1986) 434–437
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 870

Note

Determination of clonidine hydrochloride in pharmaceutical preparations by high-performance liquid chromatography

I. WILCZYŃSKA-WOJTULEWICZ* and N. SADLEJ-SOSNOWSKA
Institute for Drug Research and Control, Chełmska 30/34, 00-725 Warsaw (Poland)
(First received May 23rd, 1986; revised manuscript received June 18th, 1986)

For many years gas chromatography (GC) has been the method commonly used for the determination of clonidine hydrochloride (CLH) in biological materials[1–3]. Recently, GC was applied to the measurement of picogram levels of CLH after derivatization[4]; the most suitable derivative was that obtained with 3,5-bis(trifluoromethyl)benzoyl chloride. In the injection and tablet forms the assay of CLH was previously performed by UV spectrophotometry (analytical methods recommended by the manufacturers). Spectrophotometry with bromothymol blue is the official method in the *British Pharmacopoeia*[5] and spectrophotometry with sodium nitroprusside was used by Tawakkol *et al.*[6]. Walters and Stonys[7] determined CLH in tablets by high-performance liquid chromatography (HPLC) using a column with trimethylsilyl-bonded spherical silica and a mobile phase containing 65% of methanol and 35% of pH 7.9 buffer solution. The *United States Pharmacopoeia*[8] requires for the assay of CLH in tablets an HPLC method with a C_8 column deactivated for basic compounds and a mixture of an aqueous solution of triethylamine, acetonitrile and methanol (pH 5.8) as the mobile phase. In this paper an alternative reversed-phase (RP) HPLC method, convenient for routine drug control, is described.

EXPERIMENTAL

Apparatus

A Pye-Unicam liquid chromatograph consisting of an LC-XPD pump, an LC-UV variable-wavelength and a PM 82-51 single-pen recorder was used. It was equipped with a Rheodyne 7125 injector fitted with a 20-μl loop.

Materials

Clonidine hydrochloride and tablets A were produced by VEB Arzneimittelwerk (Dresden, G.D.R.); the eye drops were produced by Boehringer (Ingelheim, F.R.G.). All solvents were of analytical-reagent grade. The eluents were filtered and degassed by sonication.

Chromatographic conditions

Several columns and mobile phases were tried: Nucleosil 10 C_{18} with acetonitrile–water (16:84) and *n*-propanol–water (60:40) as mobile phases and Nu-

0021-9673/86/$03.50

cleosil 10 C_8, Nucleosil 10 C_{18}, Hibar 10 C_{18}, Nucleosil 5 C_{18} and 5 C_8 with methanol–water (80:20) with and without the addition of 0.005% of triethylamine (TEA) as the mobile phase. Finally a Nucleosil 5 C_{18} column (125 mm × 4.6 mm I.D.) with methanol–water (80:20) containing 0.005% of TEA as the mobile phase was selected. The chromatographic conditions were as follows: flow-rate, 1 ml min^{-1}; UV detector, 240 nm; attenuation, 0.02 a.u.f.s. (tablets) and 0.16 a.u.f.s. (eye drops); and recorder chart speed, 0.5 cm min^{-1}.

Standard solutions for calibration graph

The solutions were prepared by weighing about 1 mg of the substance and dissolving it directly in an appropriate volume of methanol–water (80:20). The concentrations ranged from 2.15 to 44.8 μg ml^{-1}.

Determination of CLH in the formulations

Standard solutions. Weighed amounts of 0.7–1.5 mg of CLH were transferred into 50- or 250-ml volumetric flasks. The concentration of the standard for determination of CLH in tablets was 3–4 μg ml^{-1} and in eye drops about 25 μg ml^{-1}.

Sample preparation: tablets — composite assay. A portion of powdered tablets of A or B corresponding to about 100 μg of CLH was weighed, transferred into a 25-ml volumetric flask and extracted with water or with methanol–water (80:20 or

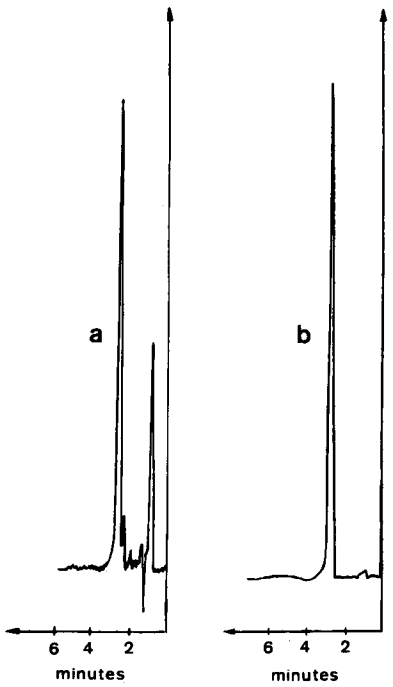

Fig. 1. Chromatograms of methanol–water extract of (a) clonidine hydrochloride tablets and (b) diluted eye drops. Concentration of CLH: (a) *ca.* 4 μg ml^{-1} and (b) *ca.* 25 μg ml^{-1}. Chromatographic conditions: column, Nucleosil 5 C_8 (125 × 4.6 mm I.D.); mobile phase, methanol–water–triethylamine (80:20:0.005); flow-rate, 1 ml min^{-1}; UV detector, 240 nm; recorder chart speed, 0.5 cm min^{-1}; attenuation, (a) 0.02 a.u.f.s. and (b) 0.16 a.u.f.s.

70:30) by mechanical shaking for 30 min. The mixture was then diluted to volume and filtered. For CLH determination according to USP XXI, the samples were prepared according to the indications given there.

Sample preparation: tablets — content uniformity. One tablet was placed in a 25-ml volumetric flask containing 5 ml of water. After complete disintegration of the tablet (5 min), 15 ml of methanol were added. The flask was then sonicated for 5 min and shaken mechanically for a further 15 min, made up to volume with methanol and filtered.

Sample preparation — eye drops. Eye drops were diluted 100 times with methanol–water (80:20).

RESULTS AND DISCUSSION

The chromatographic method described here gives an adequate separation from the few components (unidentified excipients) present in the water–methanol extract of the tablets (Fig. 1) and so makes detection possible in the lower ultraviolet region in which the absorptivity of CLH is higher than that in the region used in the spectrophotometric assay.

Of all the column–mobile phase combinations tried, the one finally selected gave the highest ratio of peak height to concentration of CLH. This is especially important when the content of active substance is low, as in the case of CLH (0.075 μg per tablet).

For five consecutive standard responses, the relative standard deviation (R.S.D.) was not more then 1.5%, but after several dozen working hours the reproducibility of the peak height decreased and the column had to be washed to remove any traces of adsorbed species. Regeneration with the use of the solvent sequence water, dimethyl sulphoxide, water, methanol, dichloromethane and methanol restored the previous efficiency of the column.

Calibration graph

The calibration is linear graph over the chosen concentration range. The mean

TABLE I

RELATIONSHIP BETWEEN PEAK HEIGHT (*h*) AND CONCENTRATION OF CLH SOLUTION (*c*)

c (μg ml^{-1})	*Detector range (a.u.f.s.)*	*h (mm)*	*h at 0.01 a.u.f.s./c*
2.15	0.02	81.0	75.3
3.81	0.02	136.0	71.4
6.14	0.04	112.7	73.4
10.95	0.08	98.0	71.6
19.94	0.08	181.0	72.6
44.80	0.16	204.5	73.0
			$\bar{x}$ = 72.9
			R.S.D. = 2%

TABLE II

RESULTS OF THE DETERMINATION OF CLH IN TABLETS (COMPOSITE ASSAY) AND EYE DROPS

Formulation	*Found (mg)*	*R.S.D. (%)*	*n*
Tablets, 0.075 mg:			
Type A:			
Lot 1	0.0703	4	6
Lot 2	0.0712	3	4
Lot 3	0.0739	3	3
Lot 4	0.0736	4	4
Type B	0.0859	3	4
Eye drops, 0.25 mg ml^{-1}	0.2690	3	4

ratio of the peak height calculated for an atenuation of 0.01 a.u.f.s. to the CLH concentration in the solutions in methanol–water (80:20) was 72.6 mm to 1 $\mu g\ ml^{-1}$ and the R.S.D. was 2% for $n = 6$ (Table I).

Quantitative determination

The results of the determination of CLH in tablets (composite assay) and in the eye drops are shown in Table II. The R.S.D. obtained for the formulations was 3–4%.

The method for the determination of CLH presented here is rapid and simple. Its precision is sufficient for routine drug control and it is now in regular use in our laboratory.

ACKNOWLEDGEMENT

This work was supported by a grant from the Ministry of Health and Social Welfare (Poland), MZ VII, U.115.

REFERENCES

1 A. K. Cho and S. H. Curry, *Biochem. Pharmacol.*, 18 (1969) 511.
2 C. T. Dollery, D. S. Davies, G. H. Draffan, H. J. Dargie, C. R. Dean, J. L. Reid, R. A. Clare and S. Murray, *Clin. Pharmacol. Ther.*, 18 (1976) 11.
3 P. O. Edlund, *J. Chromatogr.*, 187 (1980) 161.
4 S. Murray and D. S. Davies, *Biomed. Mass Spectrom.*, 11 (1984) 435; *Anal. Abstr.*, 47 (1985) 4D84.
5 *British Pharmacopoeia*, HMSO, London, 1980.
6 M. S. Tawakkol, A. I. Jado and H. Y. Aboul-Enein, *Arzneim.-Forsch.*, 31 (1981) 1064.
7 S. M. Walters and D. B. Stonys, *J. Chromatogr. Sci.*, 21 (1983) 43.
8 *United States Pharmacopeia XXI*, Mack, Easton, PA, 1985.