Journal of Chromatography, 356 (1986) 468–472
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 453

Note

High-performance liquid chromatographic determination of xipamide and clopamide in pharmaceuticals

R. T. SANE*, G. S. SADANA, G. J. BHOUNSULE, M. V. GAONKAR, A. D. NADKARNI and V. G. NAYAK

Department of Chemistry, Ramnarain Ruia College, Matunga, Bombay 400 019 (India)

(First received May 20th, 1985; revised manuscript received November 2nd, 1985)

Xipamide and clopamide are used in medicine as diuretics and antihypertensive drugs[1]. Sobel and Mutschler[2] have reported a thin-layer chromatographic (TLC) method for the determination of xipamide in urine and plasma, Diembeck *et al.*[3] have reported a high-performance liquid chromatographic (HPLC) method for the estimation of xipamide in urine and Gfeller *et al.*[4] have reported an HPLC method for the estimation of clopamide in pharmaceutical dosage form.

EXPERIMENTAL

Apparatus

A Waters liquid chromatograph equipped with a M-440 absorbance detector, M-45 solvent-delivery system, U6K injector and M-730 data module was employed. The detector wavelength was set at 254 nm with an attenuation of 0.1 a.u.f.s. A Nucleosil C_{18} 10 μm (250 × 4 mm I.D.) column was used.

Preparation of standard solutions

Solutions of xipamide and clopamide of concentration 0.2 mg/ml were prepared in methanol. A mixture of 5 ml of xipamide and 5 ml clopamide made up to 25 ml with the mobile phase constituted the working standard solution for xipamide and a mixture of 5 ml of clopamide and 4 ml of xipamide made up to 25 ml with the mobile phase constituted the working standard solution for clopamide.

Chromatographic conditions

A stable baseline was obtained at a flow-rate of 1.5 ml/min with a mobile phase of methanol–water–acetic acid (69:30:1, v/v). The working standard solutions (25 μl) for xipamide and clopamide were injected separately. The amounts of xipamide and clopamide present in tablet form were computed by comparing the peak area ratios of xipamide and clopamide for the sample and for the standard. A typical chromatogram is shown in Fig. 1.

Tablet formulations analysed

The xipamide tablets contained 20 mg of xipamide only. The clopamide tablets

0021-9673/86/$03.50

contained three components: clopamide (5.0 mg), dihydroergocristine (0.58 mg) and reserpine (0.1 mg).

Linearity of the detector response

Into a series of volumetric flasks, various amounts of drug solutions were placed (0.008–0.040 mg/ml). The corresponding internal standard was added as described earlier, and the volume made up to 25 ml with the mobile phase. A 25-μl volume of the solution from each flask was injected separately for xipamide and clopamide. A plot of the peak area ratio of xipamide and clopamide against the concentration of xipamide was found to be linear in the range of 0.008–0.040 mg/ml of xipamide, and a plot of the peak area ratio of clopamide and xipamide against the concentration of clopamide was found to be linear in the range of 0.008–0.040 mg/ml of clopamide (Fig. 2).

Recovery

The recovery of the added xipamide and clopamide was studied. A fixed amount of the pre-analysed sample was taken and standard drug was added at three different levels. For xipamide, powdered tablet equivalent to 20 mg of xipamide was dissolved in 100 ml methanol. To 2 ml of this solution, 5 ml of the internal standard solution were added and made up to 25 ml with the mobile phase. A 25-μl volume of this solution was injected. Then powder equivalent to 20 mg of xipamide was weighed into three different flasks and 10, 20 and 30 mg standard xipamide respectively were added and dissolved in 100 ml methanol. To 2 ml solution from each flask, 5 ml of the internal standard solution were added, made up to 25 ml with the mobile phase and 25 μl of this solution were injected. Each injection was repeated seven times.

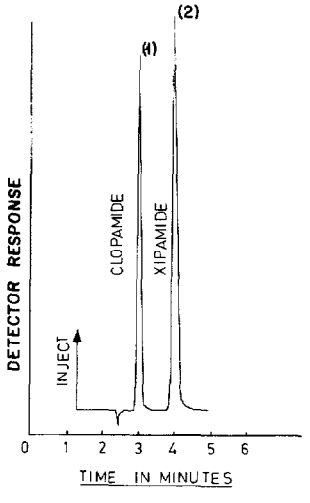


Fig. 1. A typical HPLC chromatogram of clopamide (1) and xipamide (2); each was used as internal standard for the other. Amount injected: 25 μl in each case.

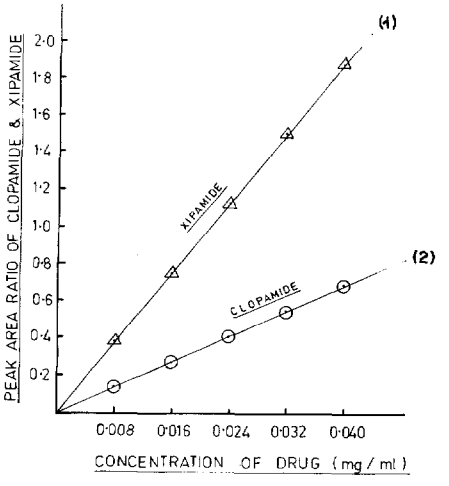


Fig. 2. Linearity of detector response with concentration of xipamide (1) and clopamide (2).

For clopamide, powder equivalent to 5 mg clopamide was dissolved in 25 ml methanol. To 2 ml of this solution, 4 ml of internal standard solution were added and made up to 25 ml with the mobile phase. A 25-μl volume of this solution was injected. Then sample powder equivalent to 5 mg clopamide was weighed into three different flasks and 2.5, 5.0 and 7.5 mg standard clopamide respectively were added and dissolved in 25 ml methanol. To 2 ml solution from each flask, 4 ml of the internal standard solution were added, made up to 25 ml with the mobile phase and 25 μl of this solution were injected. Each injection was repeated seven times.

Plots of the amount of drug found by the proposed method (y axis) against the amount of the standard drug added (x axis) for clopamide and xipamide are shown in Figs. 3 and 4. The intercepts on the y axis indicate the amount of drug present per tablet. From the amount of the drug found, the percentage recovery was calculated using the formula

$$\% \text{ recovery} = \text{slope} \cdot 100$$

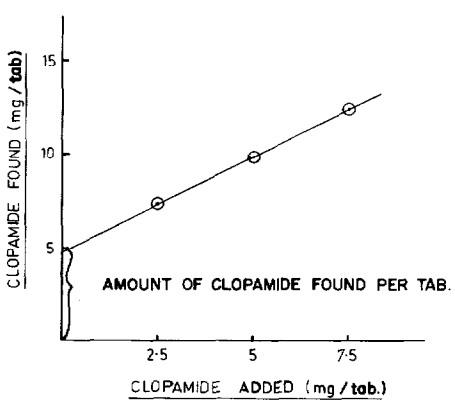
$$= \frac{N(\Sigma xy) - (\Sigma x)(\Sigma y)}{N(\Sigma x^2) - (\Sigma x)^2} \cdot 100$$

where x = amount of the standard drug added, y = amount of the drug found by the proposed method and N = number of observations. The results are shown in Tables I and II.

Various parameters such as the coefficient of variation and the relative mean deviation were studied to determine the reliability of the assay for xipamide and clopamide. The calculations were based on the data given in Tables I and II. The results are shown in Table III.

RESULTS AND DISCUSSION

The proposed method was devised with the purpose of developing a fast and accurate technique, devoid of any interferences from common excipients, thereby eliminating any elaborate sample treatment.



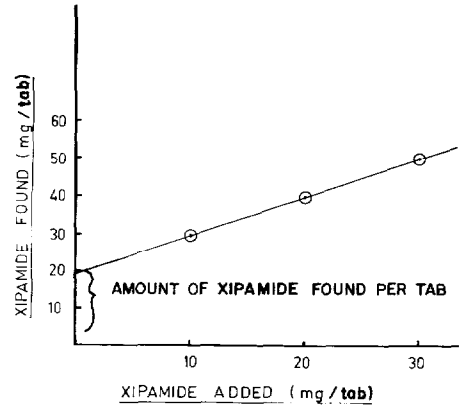


Fig. 3. Recovery experiment for clopamide. Each point on the y axis is an average of seven determinations.

Fig. 4. Recovery experiment for xipamide. Details as in Fig. 3.

TABLE I

RESULTS OF REPLICATE ANALYSES OF DRUGS AT THREE DIFFERENT LEVELS OF ADDITION

For xipamide, weight taken at zero level is 20.0 mg, for clopamide it is 5.0 mg.

Level of addition	*Standard drug added per tablet (mg)*	*Xipamide found by the proposed method (mg/tablet)*							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*Av.*
Zero	0	20.00	19.89	20.02	19.96	19.86	19.74	20.25	19.96
1	10	29.59	29.59	29.59	29.59	30.11	29.85	30.38	29.81
2	20	40.15	39.63	40.15	40.15	39.63	40.15	40.15	40.00
3	30	49.93	49.14	50.72	50.19	50.19	50.72	49.66	50.07
		Clopamide found by the proposed method (mg/tablet)							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*Av.*
Zero	0	4.94	4.93	4.97	4.98	5.04	4.97	5.02	4.97
1	2.5	7.45	7.39	7.45	7.43	7.45	7.39	7.41	7.42
2	5.0	9.89	9.92	9.83	9.89	9.83	9.89	9.89	9.87
3	7.5	12.43	12.52	12.32	12.32	12.32	12.32	12.43	12.38

The chromatographic results showed that graphs of the peak area ratios of xipamide to clopamide and of clopamide to xipamide *versus* the amounts of xipamide and clopamide injected are linear up to 0.040 mg/ml. These linear relationships can be expressed by the equations

$$y = 46.83\,x + 0.002 \qquad r = 0.998 \text{ (xipamide)}$$
$$y = 16.99\,x + 0.002 \qquad r = 0.998 \text{ (clopamide)}$$

where x represents the amount injected (mg/ml) and y the peak area ratio.

The amount of drug per tablet was calculated using the formula

$$\text{Amount} = A \cdot \frac{R_{\text{samp}}}{R_{\text{std}}} \cdot \frac{D}{W} \cdot \text{Av. wt.}$$

where A = concentration of standard drug in mg/ml, R_{samp} = peak area ratio of drug to internal standard from sample, R_{std} = peak area ratio of drug to internal standard from standard, D = dilution factor and W = weight of sample taken for estimation. The amount of xipamide found was 20.06 mg per tablet as against the labelled claim of 20.0 mg. The amount of clopamide found was 4.93 mg per tablet as against the labelled claim of 5.0 mg. The percentage recovery of the added standard was 100.3% for xipamide and 98.7% for clopamide, indicating that there was no

TABLE II

CALCULATION OF PERCENTAGE RECOVERY

See Figs. 3 and 4.

Level of addition	x	y	x^2	xy
For xipamide				
0	7 × 0	7 × 19.96	7 × 0	7 × 0
1	7 × 10.0	7 × 29.81	7 × 100.0	7 × 298.1
2	7 × 20.0	7 × 40.0	7 × 400.0	7 × 800.0
3	7 × 30.0	7 × 50.07	7 × 900.0	7 × 1502.1
	$\Sigma x = 420.0$	$\Sigma y = 978.8$	$\Sigma x^2 = 9800$	$\Sigma xy = 18201.4$
For clopamide				
0	7 × 0	7 × 4.97	7 × 0	7 × 0
1	7 × 2.5	7 × 7.42	7 × 6.25	7 × 18.55
2	7 × 5.0	7 × 9.87	7 × 25.0	7 × 49.35
3	7 × 7.5	7 × 12.38	7 × 56.25	7 × 92.85
	$\Sigma x = 105.0$	$\Sigma y = 242.48$	$\Sigma x^2 = 612.5$	$\Sigma xy = 1125.25$

TABLE III

PRECISION OF THE PROPOSED METHOD AT THREE DIFFERENT LEVELS OF ADDITION FOR XIPAMIDE AND CLOPAMIDE

Level of addition	*Coefficient of variation (%)*		*Relative mean deviation (%)*	
	Xipamide	*Clopamide*	*Xipamide*	*Clopamide*
0	0.75	0.30	0.55	0.56
1	1.039	0.36	0.83	0.32
2	0.625	0.34	0.52	0.30
3	1.12	0.64	0.85	0.48

interference from other excipients present in the tablet. The coefficient of variation and relative mean deviation are low, indicating that the proposed HPLC method is precise and reproducible.

REFERENCES

1 *Merck Index, An Encyclopedia of Chemicals and Drugs*, Merck, Rahway, NJ, 9th ed., 1976, 9741, 2356.
2 M. Sobel and E. Mutschler, *J. Chromatogr.*, 183 (1980) 124–130.
3 W. Diembeck, R. Dies and N. Heinz, *Arzneim.-Forsch.*, 32 (1982) 1482–1485.
4 J. C. Gfeller, R. Haas, J. M. Troendle and F. Erni, *J. Chromatogr.*, 294 (1984) 247–259.