Journal of Chromatography, 366 (1986) 321–328
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 751

SEPARATION AND DETERMINATION OF ISOPROPAMIDE IODIDE IN PHARMACEUTICAL FORMULATIONS BY REVERSED-PHASE ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received April 24th, 1986)

SUMMARY

A stability-indicating determination for isopropamide iodide, an anticholinergic quaternary ammonium drug, in several pharmaceutical dosage forms by reversed-phase ion-pair liquid chromatography is reported. The use of eluents containing both an amine as well as an alkylsulphonate proved to be very efficient for separating and determining quaternary ammonium drugs and related other basic drugs: adequate selectivity, excellent peak shape and good reproducibility (coefficient of variation, 1–2%) were obtained in a short analysis time.

INTRODUCTION

Isopropamide iodide, I (2,2-diphenyl-4-diisopropylaminobutyramide methiodide), is the active ingredient of several pharmaceutical formulations. It is a quaternary ammonium anticholinergic agent with peripheral effects similar to those of atropine[1].

Few papers deal with the separation and quantitation of quaternary ammonium drugs by reversed-phase column liquid chromatography[2–5]. A tedious and time-consuming method for determination of compound I involving ion-exchange separation and subsequent spectrophotometry has been reported[6], but no other chromatographic method is available for the estimation of I in pharmaceuticals. This paper reports such a method, involving reversed-phase ion-pair liquid chromatography (RP-IPC) with an eluent containing both positively and negatively charged ion-pairing agents.

EXPERIMENTAL

Chemicals and solvents

Isopropamide iodide, isopropamide, cinnarizine and haloperidol reference compounds were kindly supplied by Janssen Pharmaceutica (Beerse, Belgium). Fenpiverinium bromide was a gift from Hoechst (Brussels, Belgium). Priamide® tablets

0021-9673/86/$03.50

and Priamide oral solution (Janssen Pharmaceutica) stated to contain 5 mg isopropamide iodide per tablet and 5 mg per ml, respectively, were analyzed.

N,N-Dimethyloctylamine (DMOA) was obtained from Aldrich (Milwaukee, WI, U.S.A.) and was used as received; anhydrous sodium 1-octanesulphonate (SOS) from Janssen, 85% phosphoric acid from Merck (F.R.G.) and analytical grade methanol from UCB (Belgium). Water was purified by ion-exchange chromatography and subsequent distillation.

HPLC

Chromatography was performed on a SP 8000 liquid chromatograph (Spectra-Physics, Darmstadt, F.R.G.) equipped with a Model 770 variable-wavelength detector (Spectra Physics) and a BD 8 single channel recorder (Kipp & Zonen, Delft, The Netherlands).

A 5-μm RSil C_{18} column (150 × 4.1 mm) (RSL, Eke, Belgium) was used throughout. The mobile phase was pumped at 1.0 ml/min and the column eluent was monitored at 220 nm with the detector set at 0.1 a.u.f.s. All separations were conducted at 25°C (heated air oven) and the chromatograms were recorded with settings for a 10-mV input at a chart speed of 0.5 cm/min. The injections were made with a Valco six-port injection valve and a 10-μl sample loop.

The mobile phase was prepared by dissolving 4.325 g SOS (20 m*M*) and 1.0 ml DMOA (5 m*M*) in *ca.* 990 ml of methanol–water (55:45). The mixture was adjusted to pH 3.0 with orthophosphoric acid and diluted in methanol–water (55:45) to exactly 1000 ml. Before chromatography, the mobile phase was filtered through a 5-μm filter and degassed with helium.

Calibration standards

Stock solutions containing *ca.* 60 mg I per 100.0 ml and *ca.* 20 mg fenpiverinium bromide (internal standard) per 100.0 ml were prepared in methanol–water (45:55). Working standards were preparated by pipetting 10.0, 9.0, 8.0 and 7.0 ml of the stock solution of I and 10.00 ml of the internal standard stock solution in a 50-ml volumetric flask and diluted to volume in the methanol–water mixture. Thus, these solutions cover a range of 84–120% of the concentration claimed on the label. Calibration graphs were constructed by plotting the peak-area ratios of compound I to fenpiverinium bromide *versus* the weights of I in the standard solutions and analyzed by least-squares regression.

Quality control

Determination of the isopropamide content of tablets. An intact tablet was suspended in 10.0 ml internal standard stock solution and 35 ml of methanol–water (45:55) by ultrasonic dispersion for 10 min in a 50-ml volumetric flask. After complete disintegration of the tablet, the suspension was adjusted to volume with the methanol–water mixture and centrifuged at 3020 *g* for 10 min. Aliquots of the supernatant were injected into the liquid chromatograph.

Determination of the isopropamide content of the oral solution. A 1.0-ml volume of the oral solution and 10.0 ml of the internal standard stock solution were pipetted into a 50.0-ml volumetric flask and diluted to volume with the methanol–water mixture. Aliquots of this solution were injected into the liquid chromatograph.

Precision

The precision of the assay was tested by subjecting portions of the appropriate sample preparation to the entire procedure and calculating the coefficient of variation (C.V.) of the results.

Accuracy

The accuracy of the assay was tested using synthetic samples. A placebo mixture was prepared by combining all ingredients except compound I. Portions of the placebo were taken and an accurate weighed amount of compound I, approximately equivalent to the label claim, was added. The samples were analysed and the recoveries of compound I were calculated.

System suitability

The suitability of the method, determined with ten consecutive standard responses and expressed as the coefficient of variation of the results obtained, was excellent (C.V. = 0.63%).

Peak asymmetry

The peak asymmetry (A_s) was measured by drawing a perpendicular from the peak maximum to the baseline and by dividing the rear portion of the peak by the front portion at 10% of the peak height.

RESULTS AND DISCUSSION

Chromatography

Quaternary ammonium compounds are among the most difficult compounds to separate by RP-HPLC due to the presence of the quaternary nitrogen atom. The latter results in strong interactions with active sites, the so-called residual silanol groups, present at the surface of most silica-based reversed-phase stationary phase materials[7–9]. Thus, although being quite polar compounds, they were not eluted from an octadecyl column with acidic aqueous methanolic eluents. The addition of DMOA (5 m*M*)[10–12] to the eluent strongly reduces the capacity ratios and greatly improves the performance of the separations through an increased efficiency and improved peak shape. However, there was insufficient separation between compound I, the internal standard and other related quaternary ammonium drugs. Changing the water content, the pH and/or the DMOA concentration in the eluent did not yield the desired separations.

A chromatographic system with much higher separation power was needed for a detailed study of the column liquid chromatographic separation of structurally closely related quaternary ammonium drugs. The idea was to elute the solutes with moderate water concentrations (40–60%) and relative high DMOA concentrations (5–20 m*M*) at low pH to obtain sharp, symmetrical peaks. Sodium octanesulphonate (10–20 m*M*) was added to the eluent to obtain reasonable capacity ratios (k'); consequently, a gain in resolution was also expected.

This approach enables the separation of complex mixtures of quaternary ammonium drugs, pharmaceutically important 2-imidazolines, butyrophenones, local anaesthetics, etc. The mobile phase used for the determination of compound I (see

Experimental) was selected to meet following criteria: primarily, high resolution, *i.e.*, a baseline separation of I and the related internal standard; secondly, reasonable k' values. The latter criterion implies a short analysis time, the former improves the reproducibility of the assay. Other important requirements are satisfactory selectivity towards accompanying constituents in the pharmaceutical preparations (methylparaben, phenylpropanolamine hydrochloride, chlorpheniramine maleate, etc.) or degradation products (stability-indicating) and sufficient specificity towards related quaternary ammonium drugs (identification). Finally, good peak symmetry and an adequate number of theoretical plates (N) are needed as these parameters again influence the reproducibility.

Typical chromatograms for a Priamide tablet (Fig. 1a) and a Priamide oral solution (Fig. 1b) illustrate the convenience of the proposed method. Symmetrical peaks ($A_s = 1.6$) and sufficient efficiency ($N = 2500$) are obtained in spite of the extensive use of the column, and the analysis is completed within 8 min. No excipient peaks interfered with the determination, and the preservative methylparaben, present in the oral solution, can be determined simultaneously if desired.

Stability

Although compound I proved to be a stable molecule, some stability tests were performed. Solutions of compound I in 0.2 M sodium hydroxide stored for 10 days at room temperature show no degradation at all; heating these solutions for 5 min at 75°C again yields no degradation. Finally, solutions of I in 1 M sodium hydroxide were evaporated to dryness and reconstituted in the eluent. A complete degradation was now observed (Fig. 2a). Chromatography of a degradation solution spiked with I showed a complete separation of the latter from its potential decomposition products (Fig. 2b). The identity of these products has not yet been determined. The selectivity of the method is further illustrated in Fig. 3. A baseline separation between compound I and its non-quaternary analogue, isopropamide, an intermediate in the synthesis, is easily achieved.

Specificity

Since compound I is frequently used in combination with other pharmaceuticals (Table I), it is necessary to establish whether these drugs interfere in this analytical system. Table I also shows the k' values for isopropamide iodide, phenylpropanolamine hydrochloride (norephedrine hydrochloride), chlorpheniramine maleate, haloperidol and cinnarizine. None of these compounds interfered with the determination. The present method might even be adapted for the quantitation of chlorpheniramine maleate as this compound often shows considerable tailing in other chromatographic systems[13,14]. Fig. 4 depicts a separation of compound I, norephedrine hydrochloride and chlorpheniramine maleate, obtained after extraction of an Ornade® spansule® by ultrasonification for 15 min in methanol–water mixture (45:55). Minimum peak tailing ($A_s = 1.6$) and acceptable efficiency ($N = 2400$) for chlorpheniramine maleate were obtained.

The specificity of the analysis for related quaternary ammmonium drugs is illustrated in Fig. 5. The sufficiently high separation factor obtained allows the specific identification of compound I in such mixtures. The excellent peak shape observed for the eluted drugs is to be noted. These related quaternary ammonium drugs are

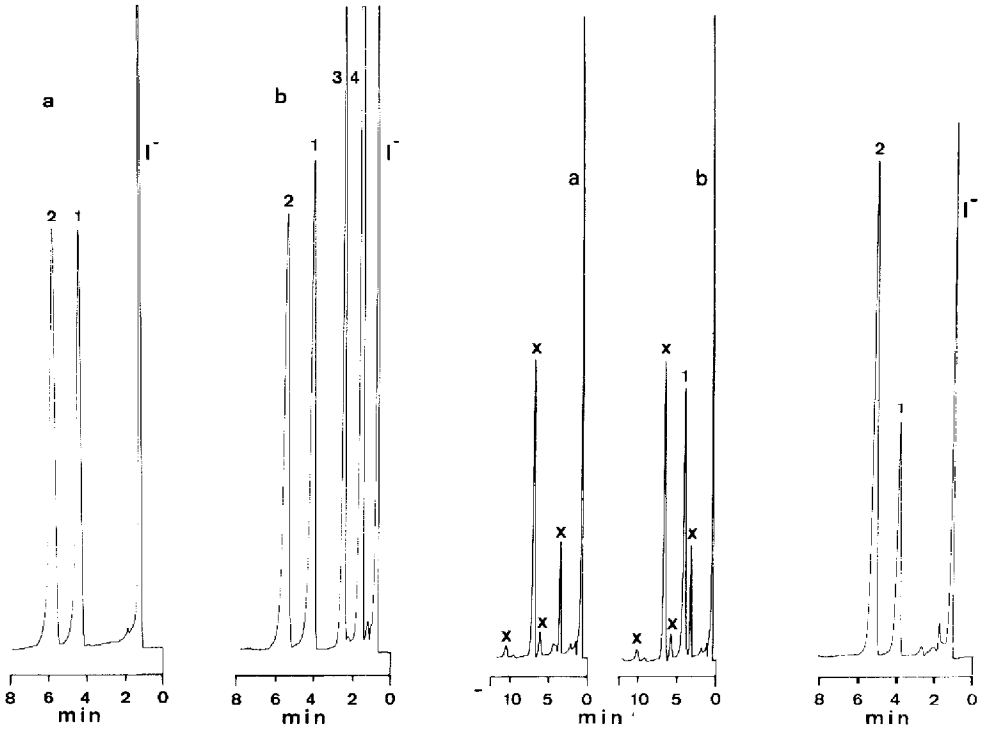


Fig. 1. Representative chromatograms for the HPLC analysis, after appropriate sample preparation, of an isopropamide iodide tablet (a) and of an isopropamide oral solution (b). Mobile phase, stationary phase and chromatographic conditions as in Experimental. Peaks: 1 = isopropamide iodide; 2 = fenpiverinium bromide; 3 = methylparaben; 4 = cocoa flavour.

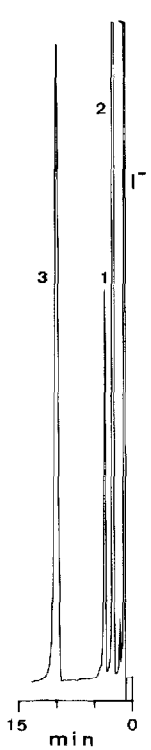
Fig. 2. Separation of isopropamide iodide and its possible degradation products: (a) after complete (accelerated) degradation; (b) as (a) but spiked with isopropamide iodide reference compound. Mobile phase, stationary phase and chromatographic conditions as in Experimental (chart speed: 0.2 cm/min). Peaks: 1 = isopropamide iodide; × = unidentified degradation products.

Fig. 3. Separation of isopropamide iodide and its non-quaternary analogue, isopropamide. Mobile phase, stationary phase and chromatographic conditions as in Experimental. Peaks: 1 = isopropamide iodide; 2 = isopropamide.

TABLE I

COMMERCIAL PHARMACEUTICAL PREPARATIONS CONTAINING ISOPROPAMIDE IODIDE AND OTHER ACTIVE INGREDIENTS

Name	*Manufacturer*	*Labelled amount of active ingredients (mg/tablet)*		*k'*
Ornade®	Smith Kline RIT (Belgium)	Chlorpheniramine maleate	8	5.7
		Isopropamide iodide	3.395	1.8
		Phenylpropanolamine hydrochloride	50	1.0
Rinomar®	Janssen Pharmaceutica (Belgium)	Isopropamide iodide	2.5	
		Cinnarizine	10	35.8
		Norephedrine hydrochloride	50	
Vesalium®	Janssen Pharmaceutica (Belgium)	Haloperidol	0.3	12.5
		Isopropamide iodide	2	



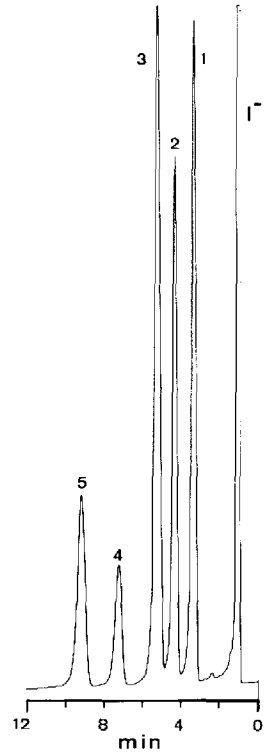


Fig. 4. Representative chromatogram for the HPLC analysis of an Ornade spansule, after appropriate sample preparation. Mobile phase: methanol–water (55:45) containing 20 m*M* SOS and 10 m*M* DMOA, adjusted to pH 3.0 with phosphoric acid. Stationary phase and other chromatographic conditions as in Experimental (chart speed: 0.2 cm/min). Peaks: 1 = isopropamide iodide; 2 = phenylpropanolamine hydrochloride; 3 = chloropheniramine maleate.

Fig. 5. Chromatographic separation of isopropamide iodide and related quaternary ammonium drugs. For mobile phase see Fig. 4. Stationary phase and chromatographic conditions as in Experimental. Peaks: 1 = isopropamide iodide; 2 = tiemonium iodide; 3 = mepenzolate bromide; 4 = pentienate bromide; 5 = glycopyrolate.

potentially useful internal standards; that used in this paper (fenipiverinium bromide) overlaps with some degradation products (Fig. 2).

Quantitative analysis

A plot of the peak area ratios of compound I *versus* standard weights was linear with a correlation coefficient of 0.9998 over the concentration range studied (84–120% of the label claim).

Standard addition–recovery experiments performed on placebo mixtures showed a mean recovery of 99.9% (n = 4, C.V. = 1.81%) for the oral solution and of 98.8% (n = 3, C.V. = 0.97%) for the tablets. More detailed results are given in Table II.

The results of the quantitation, calculated for four independently prepared samples of both the tablets and the oral solution, are summarized in Table III. Suf-

TABLE II

RECOVERIES OF ISOPROPAMIDE IODIDE FROM THE ORAL SOLUTION AND THE TABLETS

	Sample	*Added (% of label claim)*	*Recovered (%)*
Oral solution	1	108.4	101.3
	2	94.2	101.3
	3	84.8	97.5
	4	75.4	99.5
Average			99.9
C.V.			1.81
Tablets	1	114.7	99.4
	2	121.9	99.3
	3	67.3	97.7
Average			98.8
C.V.			0.97

TABLE III

RESULTS OF THE DETERMINATION OF ISOPROPAMIDE IODIDE IN COMMERCIALLY AVAILABLE TABLET AND ORAL SOLUTION FORMULATIONS

	Sample	*% found of the label claim*
Oral solution	1	102.7
	2	103.0
	3	103.0
	4	102.5
Average		102.8
C.V.		0.24
Tablets	1	100.7
	2	99.5
	3	102.3
	4	99.1
Average		100.4
C.V.		1.43

ficient precision (C.V. = 1–2%) is obtained and in both cases the analysis results were in good agreement with the amount claimed on the label.

CONCLUSION

The reversed-phase ion-pair liquid chromatographic method described resolves isopropamide iodide from degradation compounds, preservatives, intermediates of the synthesis, related quaternary ammonium drugs and from accompanying (active) ingredients of isopropamide iodide-containing dosage forms.

Successful application to different commercially available dosage forms was performed (C.V. = 1–2%). The method can be adapted to quantitate chlorpheniramine maleate as well as related quaternary ammonium drugs (tiemonium iodide, pipenzolate bromide, pentienate bromide and glycopyrolate).

The great separation power, adequate efficiency and excellent peak shape are achieved by the combined effects of a positively charged (DMOA) and a negatively charged (SOS) ion-pairing agent, both present in the acidic aqueous methanolic mobile phase.

ACKNOWLEDGEMENT

The authors are grateful to Mr. P. Demoen, Janssen Pharmaceutica, Beerse, Belgium for providing valuable information concerning the inactive ingredients of the dosage forms investigated.

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