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HPLC determination of papaveraldine and papaverinol in papaverine injection

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Abstract: A simple, specific, and sensitive high-performance liquid chromatographic method for the determination of papaveraldine and papaverinol in papaverine hydrochloride injection has been developed. The compounds were chromatographed on a reversed-phase C-18 column using a water–methanol–acetonitrile solvent system containing sodium lauryl sulphate ion-pair reagent.

Keywords: *Papaverine injection; papaverinol; papaveraldine; HPLC.*

Introduction

Papaverine (Fig. 1) (I), a smooth-muscle relaxant of the larger blood vessels, is used chiefly as peripheral vasodilator. Its decomposition products include papaverinol (II) and papaveraldine (III). The USP [1] reports under impurities, a non-specific test for cryptopine or other organic impurities, whilst the European Pharmacopoeia [2] reports a test for extraneous alkaloids, determined by TLC by comparison with codeine standard solution. These methods are non-specific for the determination of papaverinol and papaveraldine in injections. Two methods have been reported for the simultaneous determination of papaverinol, papaveraldine and papaverine, that have employed a TLC

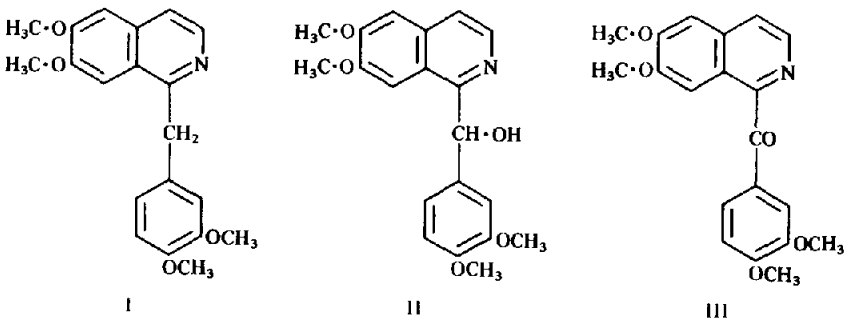

Figure 1
I = papaverine, II = papaverinol, III = papaveraldine.

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separation followed by extraction from the plate and then quantitation by UV absorbance [3] or a combination of UV absorbance and polarography [4]. One HPLC method reported [5] for the separation of papaverinol and papaveraldine from papaverine injection did not provide a quantitative procedure.

This report describes an ion-pair HPLC procedure for the separation of papaverinol and papaveraldine from papaverine in injections, and an assay for the determination of the two impurities at levels of less than 1%.

Experimental

Reagents and chemicals

Acetonitrile, methanol (Rudi Pont, Eurobase S.p.a., S. Giuliano Milanese, Italy) were HPLC grade, glacial acetic acid (Reagents ACS, Riedel de Haën, Seize, FRG), lauryl sulphate (Janssen Chimica, Beerse, Belgium), isopropylantipyrine (Hoechst–Riedel de Haën Ph.Helv. VI, Italy), papaverine hydrochloride (Recordati S.p.a., Milano), were used without further purification.

Papaveraldine hydrochloride and papaverinol hydrochloride were obtained through synthesis [6, 7].

Apparatus

A high-performance liquid chromatograph (Series 4, Perkin–Elmer, Norwalk, CN) equipped with a 6 μl-loop injector (Model 7125 Rheodyne, Calif., USA), was connected to a variable-wavelength UV detector (Model LC-75 variable wavelength detector), and recorder (Hitachi Perkin–Elmer Recorder 165, Tokio Hitachi Ltd., Japan).

Chromatographic conditions

A 250 × 4.6 mm i.d. column packed with 10 μm C18-SIL-10 (Bodenseewerk Perkin–Elmer and Co., G.m.b.H., Uberlingen, FRG) was used.

The mobile phase was water–methanol–acetonitrile (41:40:19, v/v/v) containing 0.002 M sodium lauryl sulphate and 6 ml glacial acetic acid, at 2.0 ml min^{-1} and room temperature. Detector sensitivity was 0.02 and 0.16 AUFS at 238 nm, and chart speed 30 cm/h.

Calibration procedure

Stock solutions of papaveraldine hydrochloride (0.10 mg/ml), papaverinol hydrochloride (0.10 mg/ml), and isopropylantipyrine (0.15 mg/ml, as internal standard) were prepared in ethanol.

Calibration solutions were prepared by pipetting 5 ml of the internal standard solution into each of four 50-ml volumetric flasks and adding, respectively 3.0, 4.0, 5.0, and 6.0 ml of both the papaverinol HCl and papaveraldine HCl stock solutions, and making to volume with ethanol. The solutions were stored at 0°C until they could be analysed. The calibration solution containing 5 ml each of the three stock solutions was designated the reference standard solution.

Standard mixture

A standard mixture solution was prepared by dissolving 41.2 mg of papaverine HCl, 22.1 mg of isopropylantipyrine, 14.6 mg of papaverinol HCl and 14.1 mg of papa-

veraldine HCl in 10 ml of ethanol and bringing the solution to 100 ml with ethanol. Mixture solutions were prepared immediately before use.

Sample preparation

The total content of an ampoule (40 mg papaverine HCl in 2 ml water) was transferred into a 20 ml volumetric flask and adjusted to volume with ethanol. A 5 ml aliquot of this solution was pipetted into a 10 ml volumetric flask, 1 ml of the internal standard solution added and made up to volume with ethanol.

Assay method

Aliquots (6 μl) of reference standard solution and of sample solution were injected alternatively into the chromatograph and the peak responses measured as peak heights.

The reference standard solution was used to determine the response using:

$$\delta = \frac{h_2'}{h_1'} \frac{p_1'}{p_2'},$$

where h_1' is the peak height of the examined substance, h_2' is the peak height of the internal standard, p_1' is the weight (mg) of the single impurity and p_2' is the weight (mg) of the internal standard.

The amount of papaveraldine and papaverinol were then calculated using:

$$P_i = \frac{h_1}{h_2} p_s \delta,$$

where P_i is the weight of impurity (mg), h_1 is the peak height of the single examined impurity, h_2 is the peak height of the internal standard and p_s is the weight (mg) of the isopropylantipyrine in 1.0 ml of the stock solution.

The same procedure was followed for the quantitation of papaverine HCl. The reference standard solution was ethanol containing papaverine HCl and isopropylantipyrine (internal standard) in the concentration 0.15 and 0.20 mg/ml, respectively.

The papaverine HCl samples were prepared as follows: an aliquot of 10 ml of the solution obtained by dissolving the contents of one ampoule in 20 ml of ethanol, was transferred into a 100-ml volumetric flask containing 10 ml of isopropylantipyrine aqueous solution (2 mg/ml) and then brought to volume with ethanol.

Results and Discussion

The separation of the standard mixture of papaverine, papaverinol, papaverine and internal standard is shown in Fig. 2. All components are well resolved due to optimization of the mobile phase, which included sodium laurylsulphate as an ion-pair reagent. Good separation of papaverine HCl from its degradation products has also been reported [3] using sodium dodecylsulphate or camphorsulphonic acid. The standard curves were constructed by plotting the ratio of papaveraldine to internal standard and of papaverinol to internal standard peak heights versus the concentration of the impurity expressed in mg/100 ml. The standard curves obtained were linear over the concen-

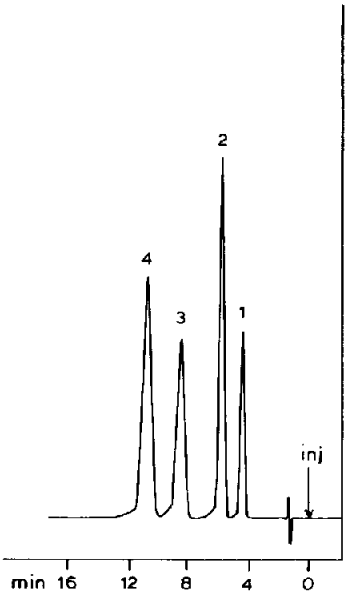

Figure 2
Chromatogram of a standard mixture of isopropylantipyrine (1, internal standard), papaveraldine HCl (2), papaverinol HCl (3) and papaverine HCl (4).

tration range 0.025–26 mg/100 ml with slope, intercept, and correlation coefficient, respectively of 4.3, −0.055, and 0.9973 for papaveraldine and 1.65, −0.035, and 0.9994 for papaverinol.

The accuracy and precision of the proposed procedure were tested by the analysis of six aqueous solutions containing papaverine HCl at the same concentration as the ampoules, whilst the concentrations of papaverinol HCl and papaveraldine HCl were between 150–200 μg/ml and 75–100 μg/ml, respectively. The samples were prepared for analysis as described under the section Sample Preparation, and the results are summarised in Table 1.

Table 1
Analysis of papaverine HCl, papaverinol HCl and papaveraldine HCl in known mixtures

	Papaverine HCl (mg ml^{-1})			Papaverinol HCl (μg ml^{-1})			Papaveraldine HCl (μg ml^{-1})		
Sample	Added	Recovery (%)	SD* (%)	Added	Recovery (%)	SD* (%)	Added	Recovery (%)	SD* (%)
1	20	100.8	1.4	200	96.6	2.5	100	96.9	2.1
2	20	101.5	1.6	190	98.1	2.3	95	98.4	1.8
3	20	99.3	1.5	180	101.0	1.6	90	100.5	1.6
4	20	98.0	1.8	170	99.0	1.8	85	98.0	2.2
5	20	98.7	1.3	160	102.2	2.4	80	100.8	1.4
6	20	102.0	1.6	150	97.2	1.5	75	98.8	1.6
Mean recovery (%)		100.05			99.0			98.9	

*Number of estimation = 4.

In a previous study [5] the authors reported the condition required to separate papaveraldine and papaverinol from papaverine and the quantitation of papaverine injections. However the simultaneous quantitation of papaveraldine and papaverinol was not possible due to the lack of sensitivity of the procedure (at 0.2 AUFS) and the chromatograms did not show the peaks corresponding to the two decomposition products.

In this study the sensitivity was increased to 0.05 AUFS for the impurities whilst a sensitivity of 0.16 AUFS was used for the papaverine quantitation. The results reported in Table 2 indicate that very small quantities of papaveraldine and papaverinol can be assayed in papaverine injections with this method.

The chromatogram presented in Fig. 3 was obtained from a 10 year old sample of papaverine injection. It clearly shows the presence of the two major degradation products (2, 3), and also an unidentified compound (x). About 3.3% of the papaverine concentration has been degraded to papaverinol and about 0.54% to papaveraldine. The method was also able to determine the levels of these two degradation products in ampoules subjected to accelerated stability testing conditions (Table 2) (Fig. 4).

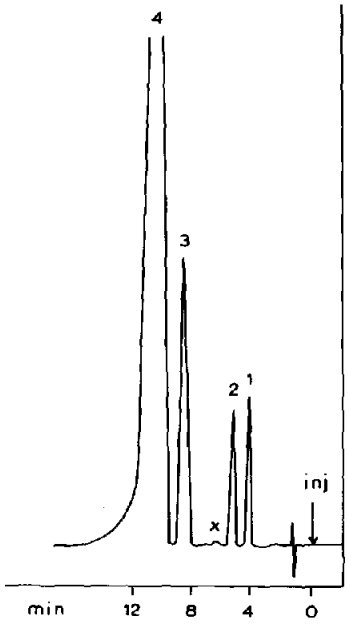

Figure 3
Chromatogram of a ten year old sample of papaverine HCl injection. Peak identification as Fig. 2, plus an unidentified component (x).

Table 2

Sample	Papaverine HCl			Papaveraldine HCl		Papaverinol HCl	
	Label claim per dosage form (mg)	Found (mg)	% Label claim	Found (mg)	% Based on papaverine found	Found (mg)	% Based on papaverine found
Ampoule 10 years old	40	38.64	94.6	0.21	0.54% (1.4)	1.28	3.31% (1.2)
Ampoule 5 years old	40	39.44	98.6	0.024	0.06 (2.1)	0.30	0.76 (1.4)
Ampoule (5 years old) kept at 100°C (oven) for 48 h	40	38.13	95.3	0.33	0.86 (1.5)	0.936	2.45 (1.6)
Ampoule, fresh	40	41.68	104.2	0.01	0.02 (2.4)	0.144	0.34 (2.1)
Ampoule (5 years old) kept at 55°C (oven) for 169 days	40	40.2	100.5	0.128	0.3 (1.8)	0.896	2.2 (1.6)

Values reported in parentheses are the relative standard deviations based on five injections.

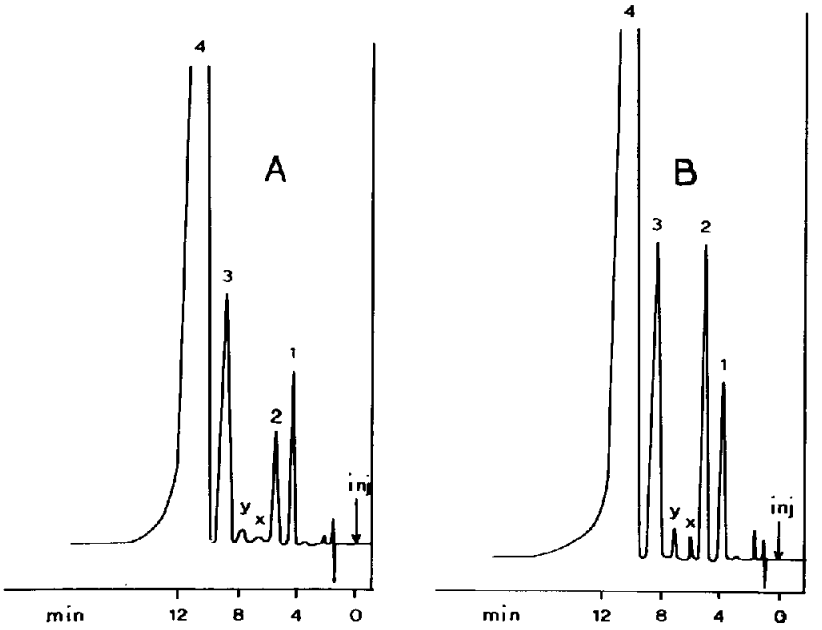

Figure 4

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