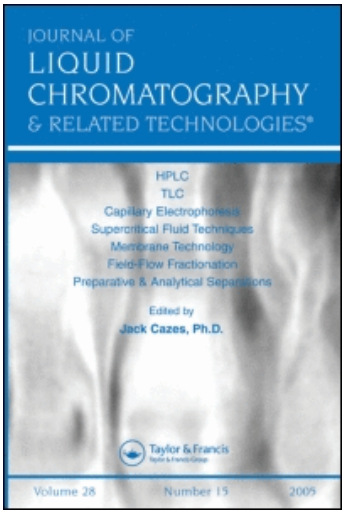

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HPLC DETERMINATION OF PINDOLOL BENZOATE AND PINDOLOL 2-METHOXYPHENYLACETATE

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ABSTRACT

In order to investigate the formation of organic salts of drugs, two new salts of pindolol were precipitated using benzoic acid and 2-methoxyphenylacetic acid. The reversed-phase high-performance liquid chromatographic (HPLC) method was developed for the determination of the mole ratio of the salt components. The method permits the simultaneous quantitation of the cationic and anionic parts of the salts prepared. The method was validated with respect to linearity and precision of the chromatographic run and the assay. Also, the precisions at the lowest and the highest levels of the linearity curves were tested. The system suitability was studied by calculating the chromatographic parameters, such as capacity factor (k'), number of theoretical plates (N), tailing factor (T) and resolution (R) between pindolol and organic acids used.

INTRODUCTION

Pindolol is a widely used 13-adrenergic blocker, commercially marketed in pharmaceutical preparations as pure base. It is classified as non-cardioselective and it has intrinsic sympathomimetic actions, but little membrane-stabilising activity. It is used mainly in the treatment of hypertension, cardiac arrhythmias and glaucoma.

Different methods for the quantitation of pindolol are described in the various pharmacopoeias. The most simple method for assaying pindolol is to titrate with hydrochloric acid and to determine the end point potentiometrically.[1,2] Also, spectrophotometric determination at a wavelength of 264 nm has been used.[3] Different reversed-phase HPLC methods have been reported for the quantitation of pindolol. For example, reversed-phase columns with nitrile groups or dimethylsilane chemically bound to porous silica have been used with the mobile phase of pH 5.0.[4] A mobile phase of pH 6.7 has been reported for screening basic nitrogenous drugs.[5]

The HPLC methods have also been used for the simultaneous quantitation of the pharmaceutical salt components in order to find out the mole ratio of the salts.[6-8] The present method was developed by modifying the method reported for separation of nine β-blocking agents.[9]

In order to investigate the formation of organic salts, two salts of pindolol were precipitated using benzoic acid and 2-methoxyphenylacetic acid. The syntheses, elemental analyses and physical studies of the salts formed have been described elsewhere.[10] The present study was undertaken to develop and validate a rapid, simple and precise reversed-phase HPLC method for the determination of the mole ratio of two new salts precipitated.

EXPERIMENTAL

Chemicals

Pindolol (Sandoz Pharm AG, Switzerland) was kindly supplied by Orion-Farmos Pharmaceuticals (Espoo, Finland) and it was Ph. Eur. grade. Analytical grade benzoic acid (Darmstadt, Germany) and 2-methoxyphenylacetic acid (Munchen, Germany) were obtained from Merck. Sodium 1-heptanesulfonic acid 1-hydrate was from Eastman Kodak (Rochester, N.Y., U.S.A) and it was HPLC grade. The methanol used for the mobile phase and sample preparation was of HPLC quality. Uracil (50 ,ug/ml) was used for

the determination of residence time of the unretained compound for the calculation of capacity factors (k').

Chromatographic Conditions

The HPLC consisted of a Hewlett-Packard 1090 (Avondale, PA, U.S.A.) equipped with a diode array UV-VIS detector and a Hewlett-Packard 79994A workstation. Separation of the salt components was carried out using a 5-μm Hypersil RP-18 column (200 x 4.6 mm I.D. stainless steel).

The mobile phase consisted of methanol (pump A) and 2 % acetic acid solution containing sodium 1-heptanesulfonate (1.1 %) as an ion pair (pump B). The elution was carried out with a gradient program by increasing the amount of methanol 6% / min from 20 to 80 % (v/v).

The oven temperature was +40°C and the flow rate 1.5 mL/min. The detection was carried out for pindolol, benzoic acid and 2-methoxyphenylacetic acid at 254 nm, 273 nm and 270 nm, respectively. The volume iniected was 20 μL.

Calibration Graphs

Stock solutions of pindolol (0.9 mg/ml), benzoic acid (0.4 mg/ml) and 2-methoxyphenylacetic acid (0.5 mg/ml) were prepared in methanol. The calibration graphs were examined by performing measurements on five different concentrations diluted from the stock solutions in methanol-water (1:1); concentration ranges were 35-175 μg/mL for pindolol, 15-80 μg/mL for benzoic acid and 20-100 μg/mL for 2-methoxyphenylacetic acid. Each solution was injected twice.

Standard Solution for the Assay

Standard solutions for the assay were made from stock solutions to yield 145 μg/mL of pindolol and 60 μg/mL of benzoic acid (= Standard solution A) and 145 μg/mL of pindolol and 80 μg/mL of 2-methoxyphenylacetic acid (= Standard solution B). Dilutions of stock solutions were made in methanol-water (1:1).

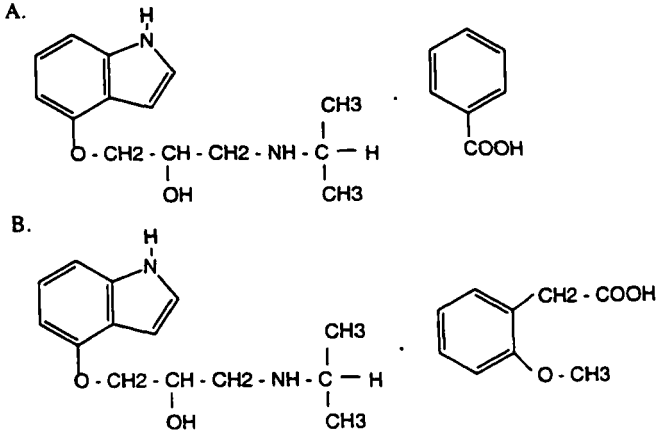

Figure 1. Structure of pindolol benzoate (A) and pindolol 2-methoxyphenylacetate (B).

Sample Preparation

The samples were diluted to a concentration of about 200 μg/mL with a mixture of methanol-water (1:1). The mole ratio of the components of the product was determined by assaying six samples of the salts against the standards.

RESULTS AND DISCUSSION

In order to investigate the formation of organic salts, two salts of pindolol were synthesized. Syntheses and physical studies of the salts have been described elsewhere.[10]

Stuctures of the salts formed are shown in Fig. 1. The compositions of the products were first established by HPLC. For that reason, an appropriate HPLC method was developed.

The HPLC method developed gave a baseline resolution for the cationic and anionic components of the salts (see Fig. 2). The retention times for benzoic acid, 2-methoxyphenyl-acetic acid and pindolol were 5.9, 6.1 and 7.8 min, respectively.

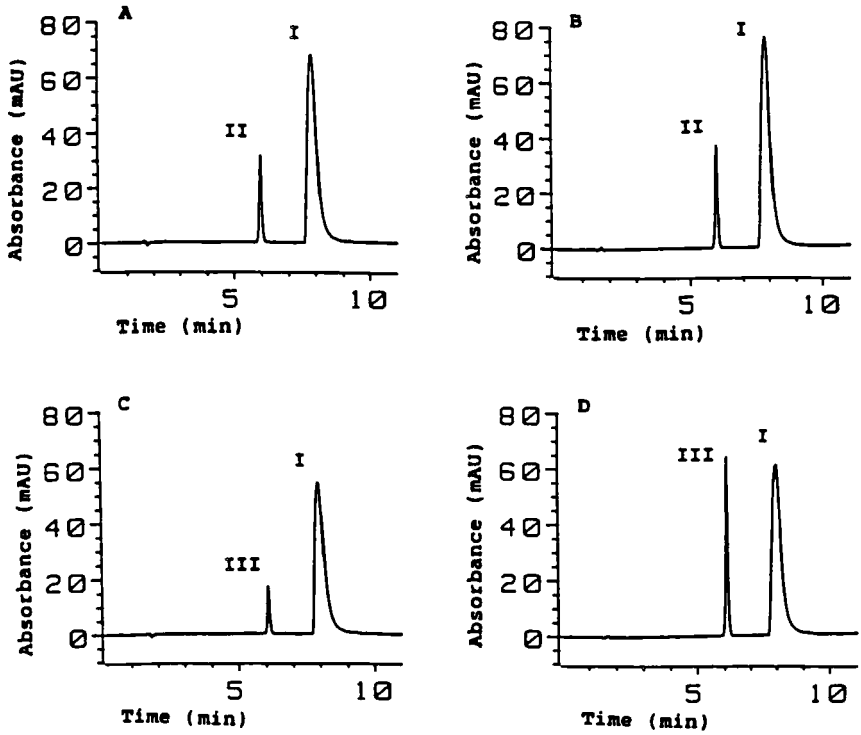

Figure 2. HPLC traces of pindolol benzoate (200μg/mL) at wavelengths 254 nm (A) and 272 nm (B) and pindolol 2-methoxyphenylacetate (200μg/mL) at wavelengths of 254 nm (C) and 270 nm (D).
Peaks: I. Pindolol; II. Benzoic Acid; III. 2-Methoxyphenylacetic acid. For chromatographic conditions, see Experimental section.

Validation of the HPLC Method for the Salt Components

The method was validated with respect to linearity and precision of the chromatographic run and the assay. Also, the precisions at the lowest and the highest levels of the linearity curves were tested.

The system suitability was studied by calculating the chromatographic parameters, such as capacity factor (k'), number of theoretical plates (N), tailing factor (T) and resolution (R) between pindolol and organic acids used.

Table 1

Results of the System Suitability Test in the Method Developed

Std Sol'n	Compound	Precision of the Chrom. Run (RSD %)	Chromatographic Parameter k'	N	T	R
A	Pindolol	0.3	3.8	2600	1.4	
	Benzoic Acid	0.7	2.6	17500	1.0	5.1*
B	Pindolol	0.3	3.8	2600	1.4	
	2-Methoxy-phenylacetic acid	0.1	2.7	18600	1.0	4.7*

Symbols: k' = capacity factor; N = theoretical plates; T = tailing factor; R = resolution. Residence time of an unretained compound (uracil) was 1.6 min (= t_o; used for k' calculations).
* = Resolution of pindolol and benzoic acid
** = Resolution of pindolol and 2-methoxyphenylacetic acid

System Suitability

The system suitability was studied by calculating chromatographic parameters from the chromatograms obtained with the standard solutions A and B containing uracil (about 50 μg/mL). The uracil was used for determining the holdup time (t_o). The data demonstrates that the chromatographic parameters were acceptable (Table 1).

Linearity

The results of the linear regression analyses are described by the equations

$y = 12.05\ x - 4.72$ ($r = 1.0000$) for pindolol,
$y = 3.81\ x - 2.78$ ($r = 0.9999$) for benzoic acid and
$y = 5.44\ x + 0.96$ ($r = 1.0000$) for 2-methoxyphenylacetic acid.

The precisions at the lowest and the highest levels were 0.3 and 0.6 % for pindolol, 0.6 and 0.1 % for 2-methoxyphenylacetic acid and 1.3 and 0.2 % for

Table 2

Determination of Pindolol Benzoate (I) and Pindolol 2-Methoxyphenylacetate (II)

Sample No.	Found (%)							
	I				II			
	Pindolol		Benzoic Acid		Pindolol		2-Methyxyphenyl-Acetic Acid	
	A	B	A	B	A	B	A	B
1	66.5	66.1	32.9	32.8	58.4	59.0	40.1	40.3
2	66.5	65.8	32.9	32.7	58.3	58.4	40.0	40.1
3	66.5	66.2	33.1	32.6	58.6	59.1	40.2	40.4
4	66.4	66.2	32.7	32.7	59.3	59.5	40.1	40.3
5	66.4	66.2	32.4	32.5	59.2	58.6	40.0	39.6
6	66.4	66.4	32.5	32.9	59.1	59.8	40.0	40.5
Mean	66.45	66.15	32.75	32.70	58.82	59.07	40.07	40.20
S.D.	0.05	0.20	0.27	0.14	0.44	0.53	0.08	0.32
R.S.D. (%)	0.1	0.3	0.8	0.4	0.7	0.9	0.2	0.8

A = Precipitation of the salt was made in ethanol.
B = Precipitation of the salt was made in acetone-water (98+2).

benzoic acid, respectively. The data demonstrates that the responses were linear for all salt components and verifies that single point calibration is suitable.

Precision of the Chromatographic Run and the Assay

Precision of the chromatographic run was examined by making six injections from standard solutions A and B. Relative standard deviations for pindolol, benzoic acid and 2-methoxyphenylacetic acid were 0.3, 0.7 and 0.1 %, respectively (Table 1). Precision of the assay was studied by analysing six samples of the salts. Samples were prepared from homogeneous sample material and assayed. Relative standard deviations for all compounds were

under 1.0 % (Table 2). Both the precision of the chromatographic run and the assay were good for all compounds studied.

Determination of Pindolol Benzoate and Pindolol 2-Methoxyphenylacetate

The mole ratio was determined by assaying six samples of the salts versus standards. The mean portions for the salts are shown in Table 2. Those values were close to the theoretical values 67.0 and 33.0 % for pindolol and benzoic acid in pindolol benzoate and 59.9 and 40.1 % for pindolol and 2-methoxyphenylacetic acid in pindolol 2-methoxyphenylacetate, respectively. There was no great difference whether the salt was made in ethanol or in a mixture of acetone and water. This indicates an approximate stoichiometry of 1:1 for both organic salts of pindolol formed. Timolol maleate and metoprolol tartrate also had a 1:1 stoichiometry as determined by elemental analysis.[11,12] Those salts were not, however, quantitated by HPLC.

CONCLUSION

The HPLC method developed for the determination of the mole ratio of the prepared salts permits the simultaneous quantitation of both components of pindolol benzoate and pindolol 2-methoxyphenylacetate. The validation data was acceptable and the results obtained agree with the results from analytical and physical studies[10] of the salts, confirming that two new organic salts of pindolol were formed in the syntheses.

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