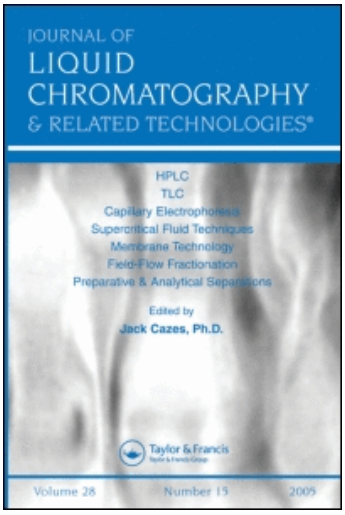

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High-Performance Liquid Chromatographic Determination of Indenolol Hydrochloride and Its Pharmaceutical Formulation

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF INDENOLOL HYDROCHLORIDE AND ITS PHARMACEUTICAL FORMULATION

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ABSTRACT

A high-performance liquid chromatographic assay was developed for the analysis of the beta-adrenergic blocking agent indenolol as a bulk material and in tablet dosage form (Pulsan(R)). Chromatography was done on a reversed-phase system using a Micropak CN-10 column. The mobile phase consisted of 66.7% v/v methanol and 33.3% v/v aqueous acetate buffer of pH 7.0. Detection was performed at the peak maximum of 250 nm utilizing a variable U.V. wavelength detector. Authentic indenolol hydrochloride was employed to establish the calibration curve; and linearity between peak area and concentration proved to be in the concentration range of 2.0 µg/ml to 20 µg/ml. The purity of authentic indenolol hydrochloride powder was checked by non-aqueous potentiometric titration using standard acetous perchloric acid in presence of mercuric acetate.

INTRODUCTION

Indenolol exists as a mixture of two isomers chemically known as 1-(7-indenyloxy)-3-isopropyl amino-2-propanol and 1-(4-indenyloxy) -3-isopropyl amino-2-propanol. The drug is a relatively new beta-

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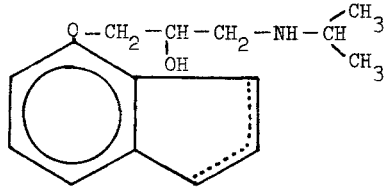
adrenergic blocking agent prescribed for the treatment of angina pectoris, arterial hypertension and cardiac arrhythmias. The manufacturer's methods for determination of indenolol involves gas-liquid-chromatography (1), non-aqueous potentiometry (1) and U.V.-spectrophotometry (1).

The investigation reported in this communication is a result of application of high-performance liquid chromatographic study for the quantitative determination of authentic indenolol hydrochloride and indenolol tablets (Pulsan$^{(R)}$). This procedure can be adopted as a basis for the analysis of indenolol in biological fluids.



Indenolol

EXPERIMENTAL

Apparatus:

The HPLC basic unit is Waters Associates model 6000A. Accessory units attached to the basic unit are Waters Associates absorbance detector model 440, and a Waters data module.

The optimum values of the HPLC parameters established are assembled in Table (1).

The non-aqueous potentiometric titrations were conducted using a combined glass electrode assembly and an automatic potentiograph model E576, Metrohom, Herisau, Switzerland.

TABLE (1)

The Optimum Values of the HPLC Parameters.

Volume injected manually	20 µl
Column	Commercially available stainless steel (3.9 mm I.D. X 30 cm) made by chemically bonding a cyanogroup to PORASIL at 9% w/w.
Mobile phase	Methanol 66.7% v/v, aqueous acetate buffer (pH = 7) 33.3% v/v.
Flow rate	2 ml/min.
Detector	Variable, 250 nm
Sensitivity	0.02 AUFS
Chart speed	0.5 cm/min.

MATERIALS

Authentic indenolol hydrochloride (labelled purity of 99.2% w/w) and indenolol hydrochloride tablets (Pulsan(R) 10 mg and 30 mg tablets) were kindly obtained from Yamanouchi Pharmaceutical Co., Ltd. 1-1-8, Azusawa, Itabashi-ku, Tokyo, Japan.

Distilled water was doubly distilled and stored in glass. Methanol was spectral grade Fluka AG., and all other reagents were analytical grade.

For the non-aqueous potentiometric determination of authentic indenolol hydrochloride, glacial acetic acid and potassium hydrogen phthalate were BDH products and were analytical grade. Perchloric acid and mercuric acetate were products of Riedel -De Haen AG., Seelze, Hannover.

METHODS

(1) Assay of authentic indenolol hydrochloride by non-aqueous potentiometry:-

Weigh accurately three pre-dried samples each about 100 mg indenolol hydrochloride. Dissolve each sample in about 25 ml glacial acetic acid. Add 15 ml of 5% w/v acetous mercuric acetate. Record the potentiometric titration curve using the potentiograph. Determine the end point of titration for each sample using the method of parallel tangents (4). Calculate the percentage of indenolol hydrochloride from the following expression:

$$\text{Percentage of indenolol hydrochloride} = \frac{V \times F \times 7.09}{\text{Weight of indenolol hydrochloride sample}} \times 100$$

where V is the volume (in millilitres) of the standard 0.025 N - acetous perchloric acid, F is the factor of the standard acetous perchloric acid and 7.09 is the number of milligrams of indenolol hydrochloride chemically equivalent to 1 ml of 0.025N - perchloric acid.

The mean value of the percentages for the three samples is calculated and used for correction of indenolol hydrochloride concentrations employed to establish the calibration curve.

(2) Establishment of the calibration curve:

Dissolve about 20 mg of indenolol hydrochloride accurately weighed in about 25 ml of distilled water; transfer quantitatively into 200 ml volumetric flask and complete up to the mark. From

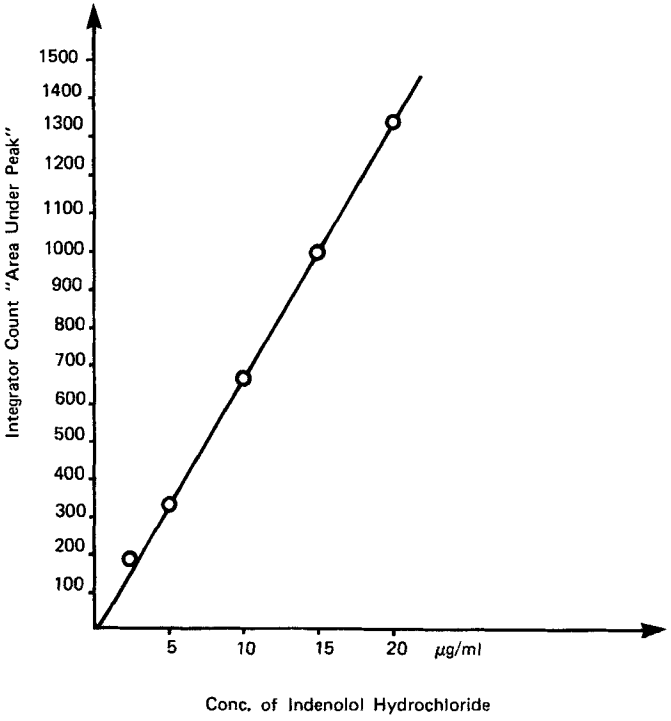


FIG. 1 Calibration Curve of Indenolol Hydrochloride

this stock solution prepare by serial dilution a series of standard solutions ranging between 2 µg/ml to 20 µg/ml. Triplicate injections of 20 µl each were made on to the column. The average integrator count of area under the peak is then plotted versus the corrected concentration of indenolol hydrochloride. The results are presented in Fig. 1.

(3) For indenolol hydrochloride tablets (Pulsan(R) 10 mg and 30 mg tablets:

Weigh accurately twenty tablets for the 10 mg tablets and for the 30 mg tablets and calculate the average weight of tablet.

TABLE (2)

High-Performance Liquid Chromatographic Determination of Indenolol Hydrochloride and its Tablets.

Indenolol Hydrochloride tablets	Stated Amount of Indenolol Hydrochloride µg/ml	Added amount of authentic Indenolol Hydrochloride µg/ml	Percentage recovery	Mean and standard deviation
(Pulsan(R)) (10 mg tablets)	2.5	-	102.5	
	5	-	98.5	
	8	-	97.3	
	10	-	99.5	
	15	-	99.1	Mean = 99.7
	20	-	101.5	S.D. = ±1.9
(Pulsan(R)) (30 mg tablets)	2.5	-	97.8	
	5	-	98.6	
	8	-	101.3	
	10	-	100.5	
	15	-	102.1	Mean = 100.0
	20	-	99.8	S.D. = ± 1.6
(Pulsan(R)) 10 mg tablets	10	5	101.0	-
(Pulsan(R)) 30 mg tablets	5	10	98.6	-

Pulverize each type of tablets and weigh accurately aliquot portions of powder each containing about 20 mg of active ingredient. Transfer quantitatively into 200 ml volumetric flasks. Add 100 ml distilled water. Shake well for about 10 minutes. Adjust to volume using distilled water and filter through millipore filter. From the filterate prepare by serial dilution six samples for each type of tablet such that the concentration of indenolol hydrochloride ranges between 2 µg/ml and 20 µg/ml. Triplicate injections of 20 µl each were made onto the column; and the average integrator count of area under the peak is then calculated for each sample. The concentration of indenolol hydrochloride is then found graphically from Fig. 1.

Similarly, spiked samples have been prepared and the total amount of indenolol hydrochloride is found graphically from Fig. 1. The results are assembled in Table (2).

RESULTS AND DISCUSSION

A tracing of a typical chromatogram obtained by HPLC under the experimental conditions previously described in shown in Fig. 2. The retention time for indenolol hydrochloride was 4.75 min. The relationship between the integrator count and concentration of indenolol hydrochloride is linear for concentration ranging between 2 µg/ml to 20 µg/ml as shown in Fig. 1.

The sensitivity of the method can be improved considerably if a fluorescence detector is used. Unpublished data by the authors reveals that indenolol is a fluorescent compound. Indeed the fluorescence activity is predictable from the aromatic nucleus of indenolol struc-

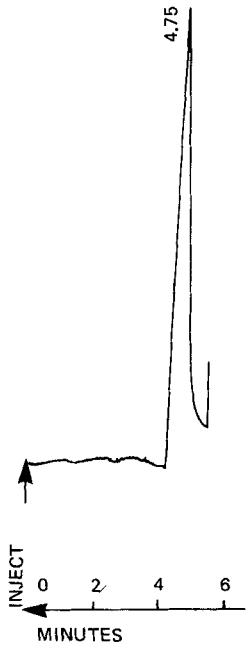


FIG. 2 High-Performance Liquid Chromatogram of Indenolol Hydrochloride

tural formula. With a fluorescence detector the method is recommendable for the evaluation of indenolol in biological fluids.

The results of applying the HPLC method and U.V. detector to the determination of indenolol hydrochloride and its dosage form of tablets (Pulsan$^{(R)}$) are assembled in Table (2). The results of tablets indicate percentages of 99.7 ± 1.9 and 100 ± 1.6 , whereas the results of spiking shown also in Table (2) indicate percentages of recovery 101.0 and 98.6 for the 10 mg and the 30 mg tablets respectively.

The stated purity of authentic hydrochloride powder was 99.2% w/w. This agreed with the results of 99.1% w/w found by non-aqueous potentiometric titration.

Although indenolol is a mixture of two isomers, only one peak emerges under the HPLC conditions adopted. This has simplified the procedure of calculation since a single peak represents the total quantity of the two isomers in the sample.

Acknowledgement:

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