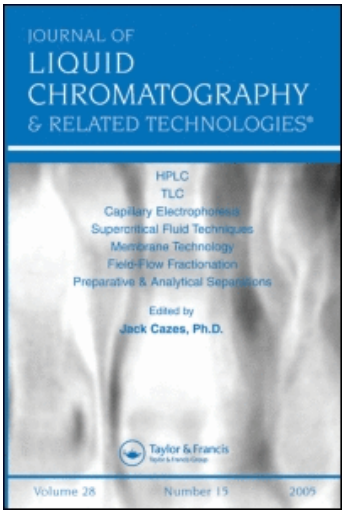

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Analysis of Indenolol In Biological Fluids By High Performance Liquid Chromatography

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To cite this Article Babhair, S. A. , Aboul-enein, H. Y. and El-houfy, Sami(1983) 'Analysis of Indenolol In Biological Fluids By High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 6: 14, 2785 — 2795
To link to this Article: DOI: 10.1080/01483918308064947
URL: http://dx.doi.org/10.1080/01483918308064947

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(14), 2785-2795 (1983)

Analysis of Indenolol In Biological Fluids By High Performance Liquid Chromatography.

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ABSTRACT

The analysis of indenolol in plasma and urine is described. The method involves extraction of the drug from plasma or urine using chloroform at basic pH. The separation was performed on CN column using methanol and 0.01M potassium dihydrogen phosphate solution 50:50. The efficiency of extraction was 97%. Minimum detectable amount by fluorescence was 20 ng/ml.

INTRODUCTION

Indenolol (1) a 1-(7-indenyloxy)-3-isopropylamino-2-propanol a relatively new beta-blocking agent is prescribed for the treatment of angina, cardiac arrhythmias and hypertension. Previous methods of analysis involved gas-liquid-chromatography, non-aqueous potentiometry, U.V. spectrophotometry and HPLC in dosage forms (1,2). The present study reports a simple method for the analysis of the drug in biological fluids using HPLC.

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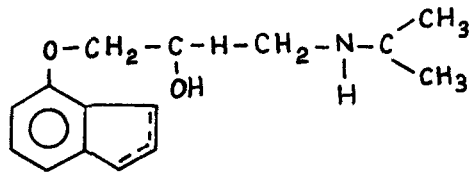

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EXPERIMENTAL

Apparatus

The chromatographic equipment consisted of Model 6000A Pump with Flourescence detector model 420-C and 420-E from Water Associates (Bedford, Massachusetts, USA). The signal output was displayed on a Philipps PM 8251 single-pen recorder.

Chromatographic System

A 3.9ID X 30 cm commercially available stainless steel CN column made by chemically bonding, a cynogroup to PORASIL at 9% w/w was used (Waters Associates). Mobile phase consisted of methanol and 0.01M KH_2PO_4 (50:50). The mixture was degassed for 5 minutes by filteration: Flow rate was 1.5 ml/min. and detector gain was 64.

Reagents

Standard solutions were made by dissolving indenolol in the mobile phase. Methanol and chloroform (spectral grade) were obtained from Merck (61 Darmstadt Germany) and KH_2PO_4 (analytical grade) was obtained from BDH (Poole, England). Authentic sample (labelled purity 99.2% w/w) of indenolol hydrochloride was obtained from Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan.

Standard Curve

20 mg of indenolol hydrochloride was dissolved in 200 ml of distilled water. From this stock solution a series of dilutions were

made ranging from 2 µg/ml to 20 µg/ml. 25 µl of these solutions were injected onto the column in triplicate, the peak height was measured and plotted versus the concentration injected. The results are shown in Figure 1.

Extraction from the urine

Urine samples were collected from an apparently healthy adult male. In each run, various amount of the stock solution was added to 2 ml of urine giving final concentration ranging from 166 to 833 µg/L. Extraction was performed by adding 0.5 ml of 25% ammonia solution and the sample was then extracted with 5 ml of chloroform. The sample was then centrifugated for 10 minutes at 2500 r.p.m. The Chloroform layer was transferred and evaporated at 60° using water bath. The residual was dissolved in 2 ml of the mobile phase and 25 µl of this solution was injected in duplicate.

Extraction from plasma

Plasma was obtained from a whole citrated human blood which was then centrifugated for 10 minutes at 2000 r.p.m. and then pippted to another tube. In each run, various amounts of the stock solution was added to one ml of plasma giving final concentrations ranging also from 166 to 833 µg/L. The same procedure for urine was followed except that 0.1 ml of 25% ammonia solution was added instead of 0.5 ml.

RESULTS AND DISCUSSION

A simple method for the analysis of indenolol in biological fluids was developed using HPLC and fluorescence detector. A typical graph of the results when peak height was plotted versus concentration injected from both urine and plasma are shown in Figures 2 and 3 respectively. Typical chromatograms using this method are shown in Figures 4 and 5. The retention time for the drug was 4.2 min. The method is

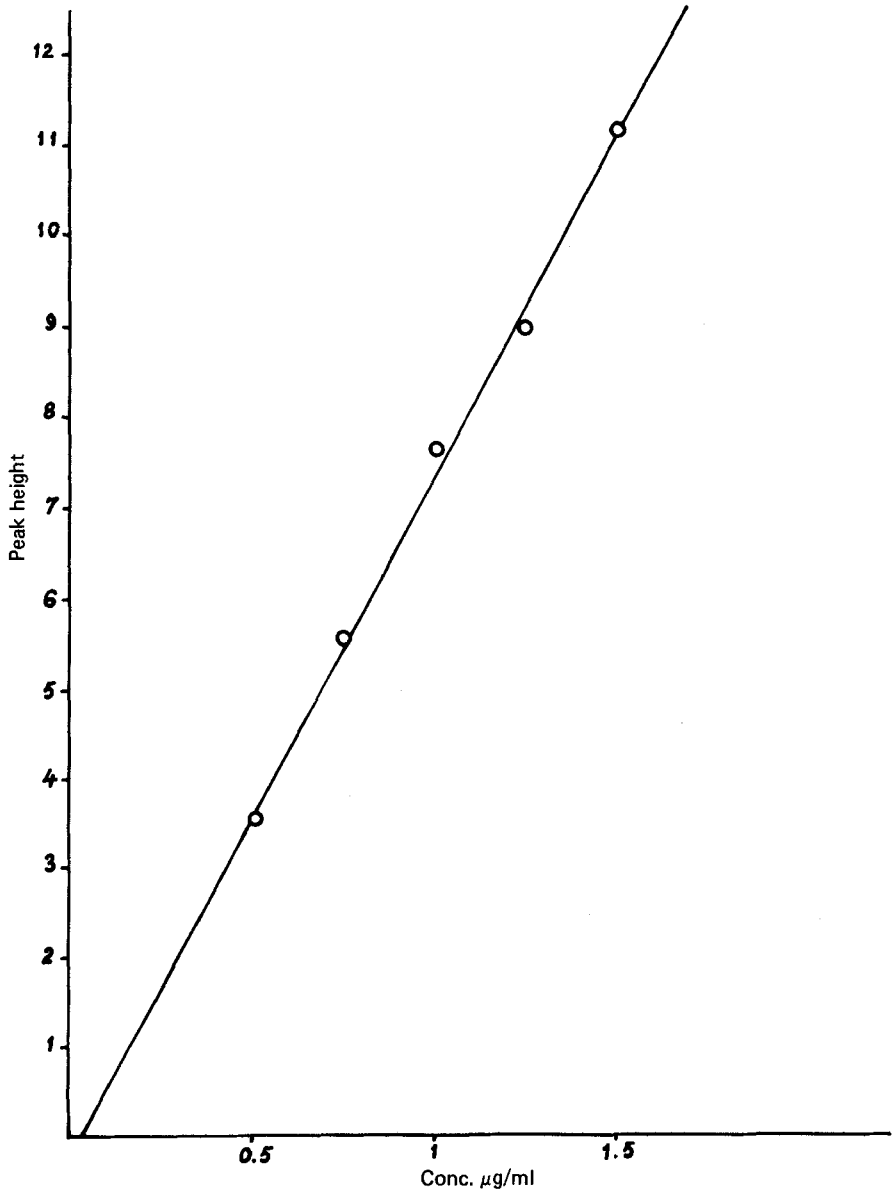

Fig. 1: Standard curve of indenolol when the peak height was plotted versus the Conc. injected.

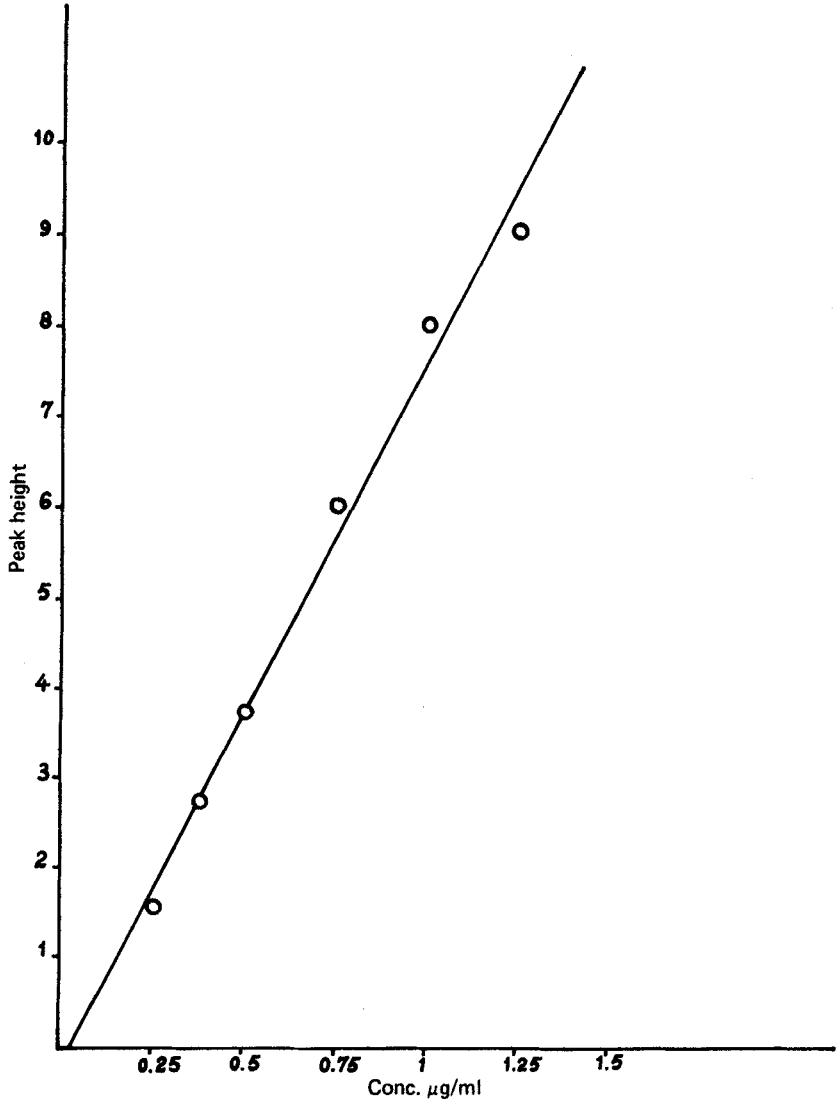

Fig. 2: Standard curve of indenolol extracted from plasma.

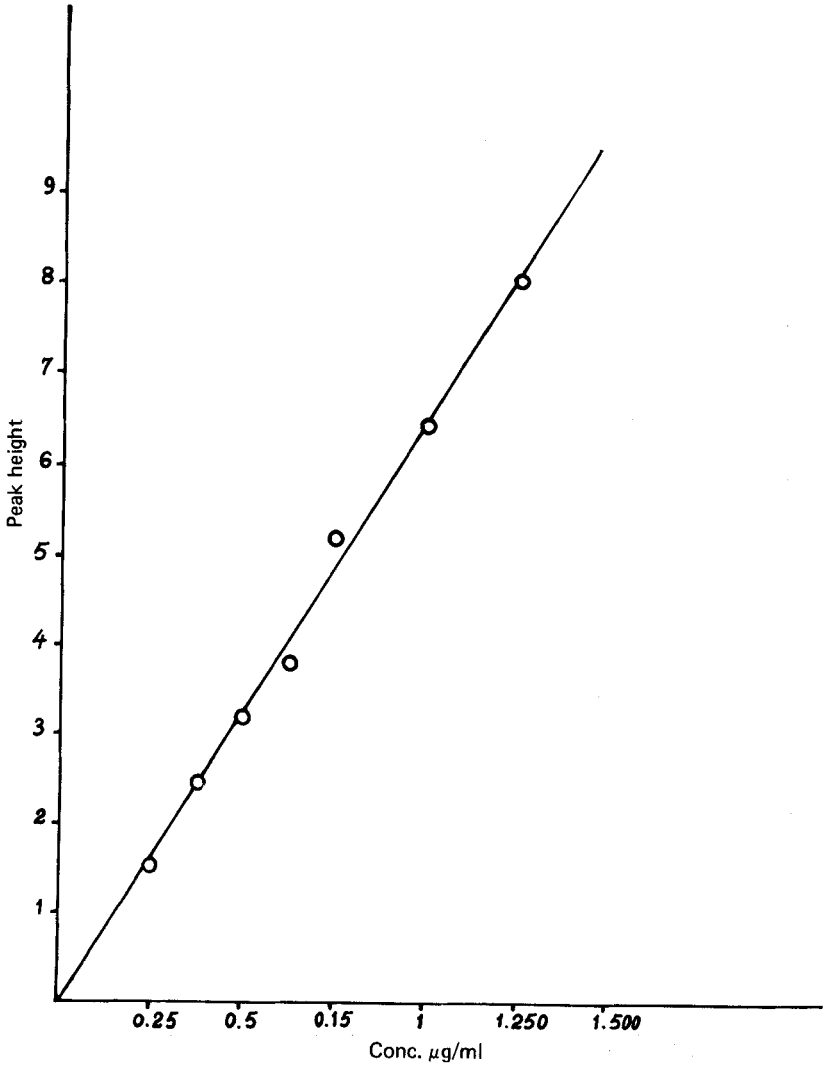

Fig. 3: Standard curve of indenolol extracted from urine.

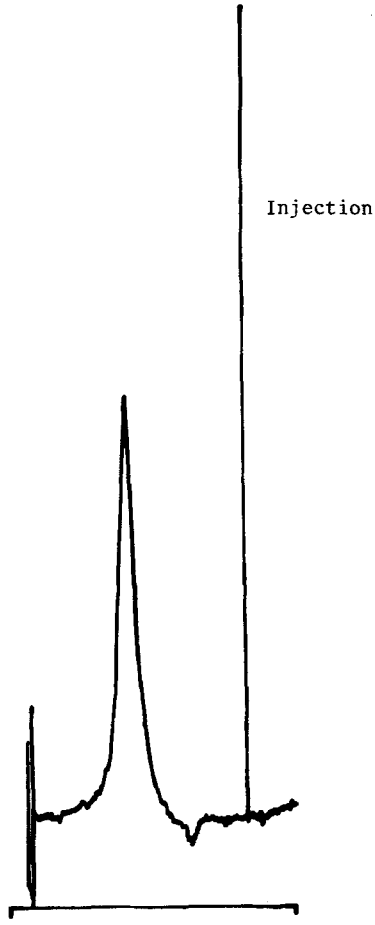

Fig. 4: A typical chromatogram of indenolol when 25 µl of the drug was injected.

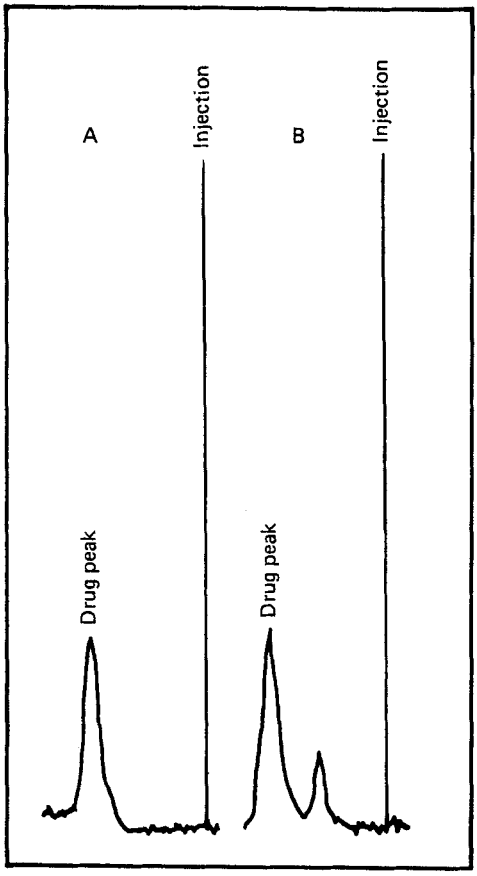

Fig. 5: Typical chromatograms of Indenolol extracted from Plasma (A) and Urine (B)

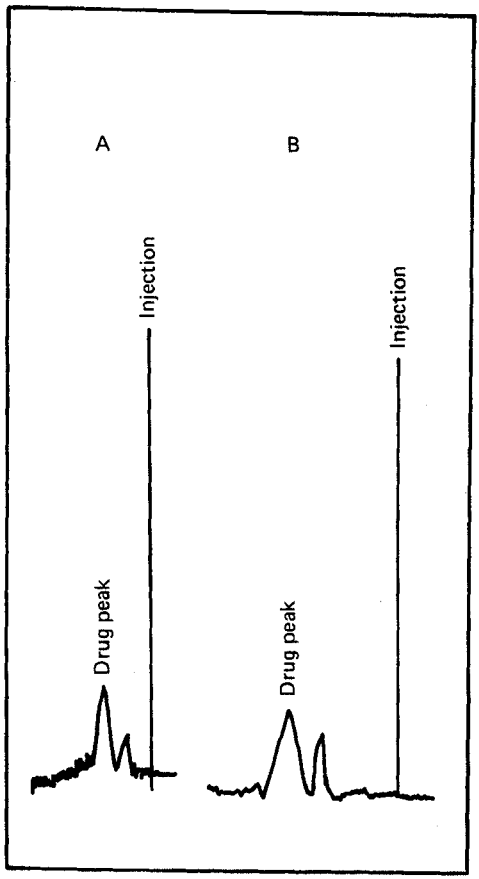

Fig.6: Typical chromatograms when 20 µg/L of Indenolol extracted from Plasma (A) and Urine (B)

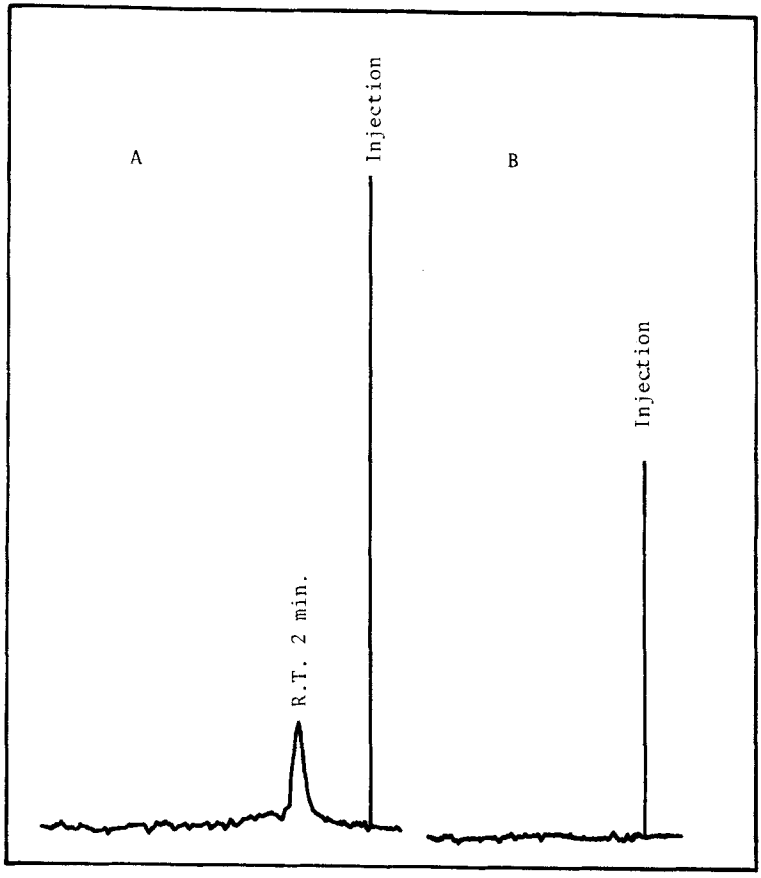

Fig. 7: Typical chromatograms when blank Urine (A) and blank Plasma (B) were extracted, and then injected onto the column.

very sensitive and concentrations as low as 20 µg/L (S/N > 2) can be detected (Figure 6). Peak height was linearily correlated to the drug concentration for the standard curves, urine and plasma with correlation coefficients of 0.995, 0.994 and 0.994. Intercepts of -0.35, -0.4, -0.1 and slopes of 0.007, 0.0066 and 0.0076 respectively. The interference from other plasma constituents was minimal and the recovery of the drug from the plasma or urine using this method of extraction was 97%. The sensitivity of the method can be considerably improved by dissolving the residual after evaporating the chloroform in 500 µl or even 100 µl of the mobile phase and injecting 50 or 75 µl of the reconstituted solution onto the column (Figure 7).

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