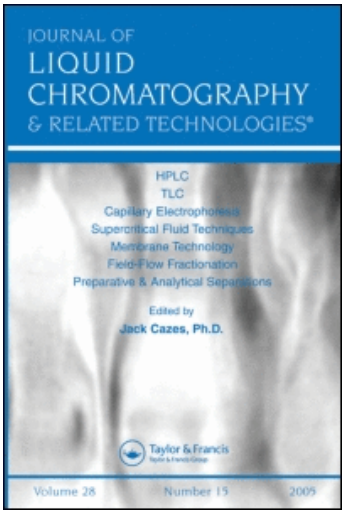

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Determination of Pirenzepine in Dosage Forms and in Biological Fluids

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DETERMINATION OF PIRENZEPINE IN DOSAGE FORMS AND IN BIOLOGICAL FLUIDS

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ABSTRACT

A simple sensitive method for the determination of Pirenzepine in its dosage form and biological fluids by High-Performance Liquid Chromatography with U.V. detector has been developed. A tablet of 25 mg drug was gound, suspended in 10 ml of water, shaken and then filtered. A known volume of the filtrate is adjusted to appropriate concentration. Twenty µl of this solution was injected. Plasma or urine samples were made alkaline with ammonia before extraction with chloroform which was evaporated and the residue was dissolved in the mobile phase, 20 µl of this solution was injected. The determination limit for quantitation was about 1 µg/ml of pirenzepine. Complete separation of the drug was achieved in about 5.4 min. under the present chromatographic conditions.

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INTRODUCTION

Pirenzepine a [5,11 Dihydro-11-[(4 methyl-1-Piperazinyl) acetyl] -6H-Pyrido-[2,3-b][1,4] benzodiazepin-6-one is a selective muscarinic receptor antagonist blocking the acetylcholine receptors of the parietal cells of the stomach and inhibits gastric secretion (1) is a recently developed drug claimed to provide safe and unproblematic treatment for peptic1 ulcer (2).

The only analytical techniques reported for the determination of pirenzepine are radioimmunassay (3) and HPLC (4). The purpose of this study was to develop a simple high performance liquid chromatographic (HPLC) procedure that will provide a method for the determination of pirenzepine in both dosage forms and biological fluids.

EXPERIMENTAL

Apparatus

HPLC was carried out using a Waters Associates System (Milford, Massachusettes, U.S.A.). The system was fitted with a model 6000A solvent delivery system, model 481 LC detector at 285 nm and model 710B WISP automatic injector. Chromatograms were recorded on Waters Data Module Model M730.

Chromatographic System

A 30 x 3.9 cm ID commercially available stainless steel C_{18} column (Waters Associates) was used. Mobile phase consisted of acetonitrile, methanol and 5% acetic acid (70:40:15). The mixture was degassed for 5 minutes by filtration: Flow rate 2 ml/min. and detector range 0.02 (AUFS).

Reagents

Standard solutions were made by dissolving Pirenzepine in the mobile phase. Acetonitrile, methanol and acetic acid (spectral grade)

were obtained from Merck (61 Darmstadt, Germany). Authentic sample (99.2 w/w) of Pirenzepine was obtained from Boehringer, Ingelheim Co., West Germany.

Standard Curve

Ten mg of the drug was dissolved in 100 ml of distilled water. From this stock solution a series of dilutions were made to cover a range of 2.5 to 20 µg/ml. Twenty µl of these solutions were injected onto the column in triplicate, the peak area was recorded and were plotted versus the concentration injected. The results are shown in Figure 1.

Determination of Pirenzepine in Tablets

Twenty tablets of 25 mg content were weighed accurately and the average weight of each tablet was calculated. The tablets were then ground and an accurate weight equivalent to about 20 mg of the active material was taken. The powder was then transformed quantitatively into 100 ml volumetric flask with aid of distilled water. The suspension was shaken for 10 minutes and the volume was then adjusted to the mark with distilled water. The suspension was then filtered through 0.22 µ millipore filter. From the filtrate a series of dilutions were made to cover concentration ranging from 2.5 - 20 µg/ml. From each solution, 20 µl was injected onto the column in triplicate. The average area under the peak was calculated for each sample. The drug concentration was then calculated from Figure 1.

Extraction from Urine and Plasma

Urine and plasma are collected from healthy adult male. In each run various amount of the stock solution of the drug were added to 2 ml or urine or 1 ml of plasma giving final concentration ranging from 2.5 to 20 µg/ml. Extraction was performed by adding 3 drops of concentrated

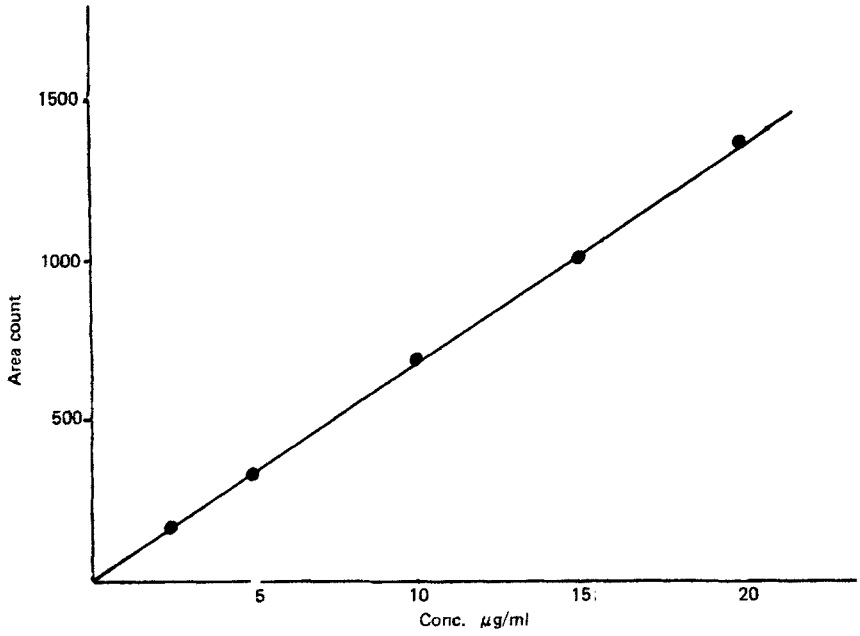

Figure 1. Calibration curve for Pirenzepine in solution under the present chromatographic conditions.

ammonia solution and the drug was then extracted with two portions of chloroform 10 ml each. The sample was centrifuged for 10 minutes at 2000 r.p.m. The combined chloroform layer was then evaporated at 60° using rotavaporator. The residue was dissolved in 1 ml of the mobile phase and 20 µl of the resulted solution was injected in triplicate.

RESULT AND DISCUSSION

An HPLC method for the determination of Pirenzepine in dosage form and in biological fluids was developed. A typical graphs of the results when the peak area was plotted versus concentration from urine and plasma are shown in Figures 2 and 3. Typical chromatograms using this

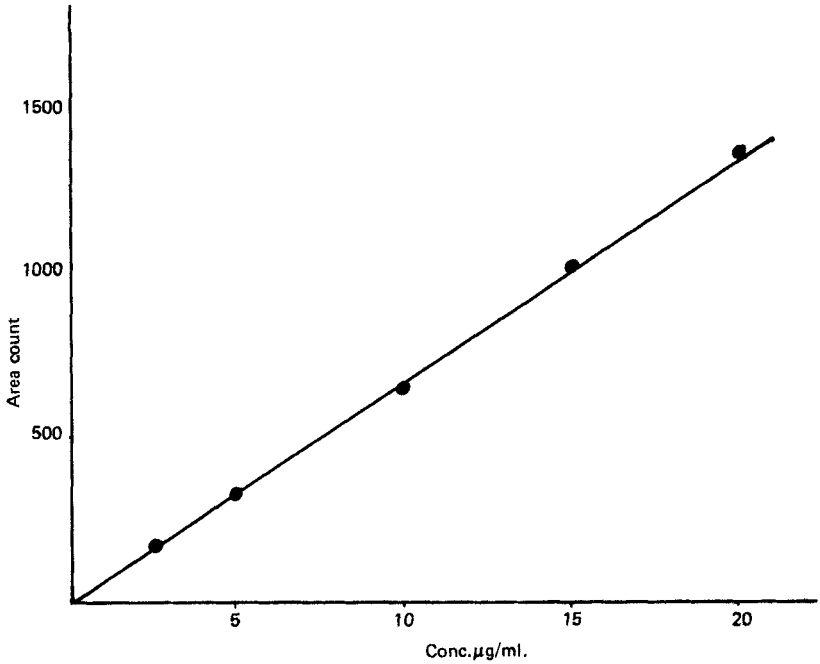

Figure 2. Calibration curve for Pirenzepine from urine under the present chromatographic conditions.

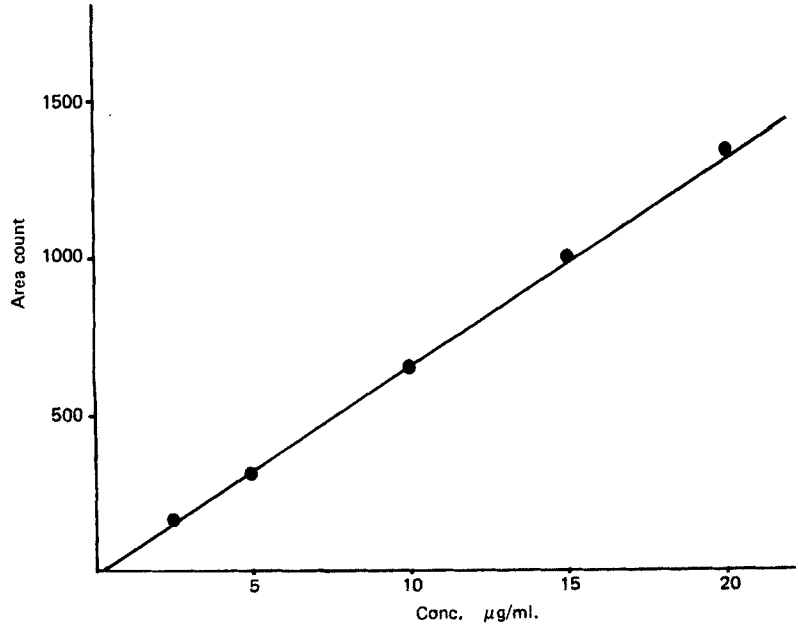

Figure 3. Calibration curve for Pirenzepine from plasma under the present chromatographic conditions.

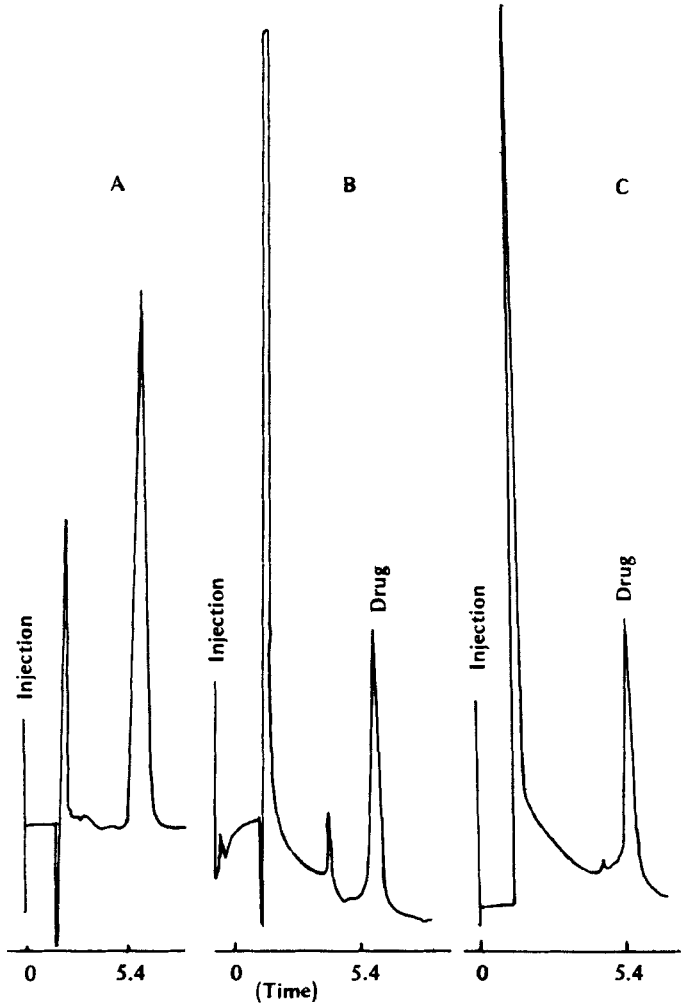

Figure 4. Typical chromatograms obtained using the procedure given. (A) Drug-free plasma, (B) Drug extracted from plasma, (C) Drug extracted from urine.

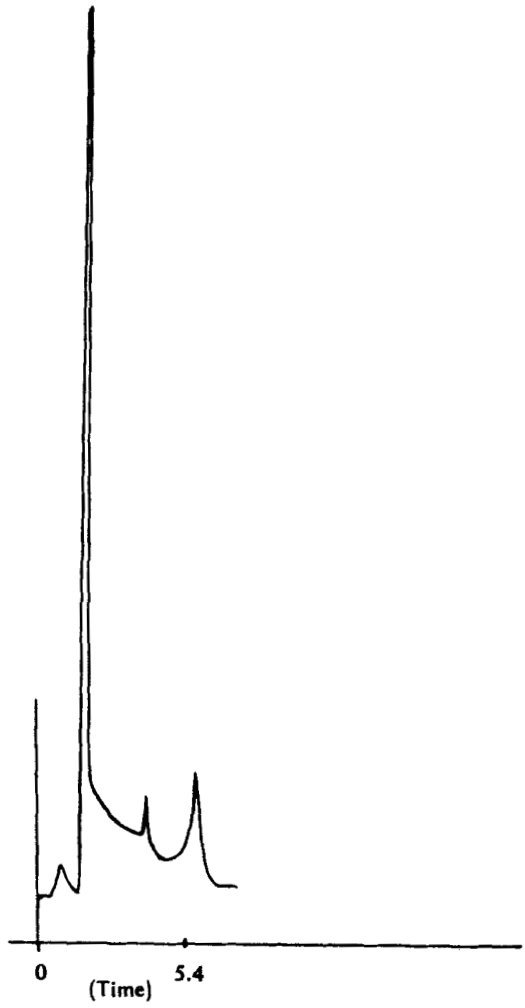

Figure 5. A chromatogram of 1µg/ml drug extracted from plasma.

method are shown in Figure 4 with a retention time of 5.4 minutes. The method is relatively sensitive and concentration as low as 1 µg/ml (which covers the range found in clinical studies) could be detected (S/N > 2) Figure 5.

Peak areas were linear to the drug concentration in the range used with corelation coefficients from water, urine and plasma of 0.996, 0.9936, 0.9944 and intercepts of -1.09, 0.27, - .27 respectively. Interference from other constituents of urine and plasma were minimal with a recovery of 95-98% and a standard error of ± 0.7 calculated from the triplicate injections using this method of extraction.

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