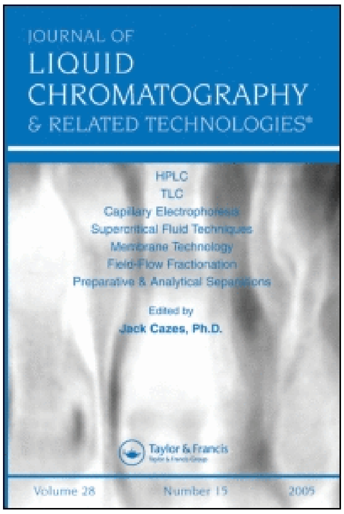

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APPLICATION OF HIGH PRESSURE LIQUID CHROMATOGRAPHY TO THE DETERMINATION OF DIASTEREOISOMER IN METHYLPHENIDATE HYDROCHLORIDE

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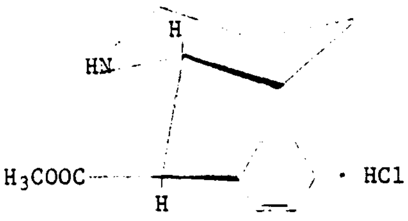
ABSTRACT

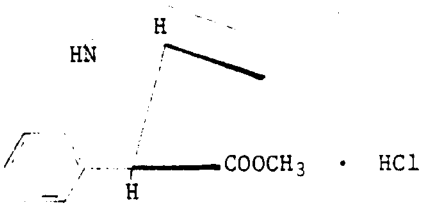
A procedure for the quantitative analysis of the diastereoisomer in methylphenidate hydrochloride is described. The method is based on a high pressure liquid chromatographic separation of the isomers on a Sil-X column with a mobile phase containing ethanol, cyclohexane, chloroform and ammonium hydroxide. The method is simple, precise and accurate and has a limit of detection of 0.1% of the diastereoisomer in the drug substance.

INTRODUCTION

Methylphenidate hydrochloride, USP (Ritalin®) (I) is a mild central nervous system stimulant used extensively for the treatment of narcolepsy and hyperkinesia. The active drug (I), which is a racemic mixture, has two asymmetric centers and hence can have another pair of diastereoisomers (II).



I



II

I Methyl α-phenyl-2-piperidineacetate hydrochloride, (R*, R*)-(±)

II Methyl α-phenyl-2-piperidineacetate hydrochloride, (R*, S*)-(±)

The configurations of the diastereoisomers I and II have been well established (1-3). Isomer II is referred to as erythro isomer in USP XIX (4). Isomer I is the clinically active drug whereas Isomer II is significantly less active (5). Hence, a method was desired to determine the amount of the Isomer II in the active drug.

A quantitative high pressure liquid chromatographic (HPLC) procedure has been developed and is reported in this publication. Similar studies have been reported for the quantitation of enantiomeric purity of naproxen by Johnson et al (6) and for the quantitation of enantiomeric ratio in α-phenethylamine by Helmchen and Stubert (7) by reacting the mixture of optical isomers with specific optically active reagents and then chromatographing the resultant diastereoisomers on silica gel columns. Techniques such as thin-layer and gas-liquid chromatography, phase solubility analysis and nuclear magnetic resonance spectroscopy can also be used for the analysis of the diastereoisomers. However, these methods have certain limitations for the compounds of interest.

Thin-layer chromatography (TLC), which is the technique employed in USP XIX (4) as a limit test, is only semiquantitative and gas-liquid chromatography (GLC) results in partial decomposition of the compounds in the column, tailing and poor resolution. Nuclear magnetic resonance (NMR) spectroscopy is limited by the mutual interference of resonance peaks and hence has a limited application for the quantitation of Isomer II particularly at a concentration level of 1% or below.

EXPERIMENTAL

Apparatus

A DuPont liquid chromatograph, Model 820, with a 254 nm UV detector was used. A stainless steel column, 100 cm x 2.1 mm was dry-packed with Sil-X® (a refined silica gel obtained from Perkin-Elmer Corporation, Norwalk, Connecticut.)

Mobile Phase: Mix 85 mL of Chloroform, 13.5 mL of Cyclohexane, 1.5 mL of Ethanol, and 0.5 mL of Concentrated Ammonium Hydroxide.

Procedure

Extract quantitatively 100 mg of the active drug substance taken in 5 mL of a pH 10 carbonate buffer solution (obtained by mixing appropriate amount of 0.1N sodium hydroxide with 100 mL of 0.1N sodium bicarbonate) with 5 mL of chloroform. Immediately separate the layers and repeat extraction of the aqueous phase with 5 mL of chloroform. Combine the chloroform extracts and evaporate to dryness under dry nitrogen at 35°C. Take the residue in exactly 1 mL of chloroform. Inject 20 µL of the concentrated sample solution into the column.

Standard Preparation

Prepare an aqueous solution of Isomer II reference standard containing 0.1 mg/mL. Add the required volume of the solution to 100 mg of methylphenidate hydrochloride reference standard. Extract this solution as above and inject 20 μL of the concentrated chloroform extract. Compare the peak areas of the Isomer II in the standard and the sample and calculate the amount of the Isomer II in the sample.

RESULTS AND DISCUSSIONS

A chromatogram of the free bases obtained from a methylphenidate hydrochloride sample containing 0.5% Isomer II is shown in Figure 1. The peak areas of samples of methylphenidate to which were added various amounts of Isomer II showed linearity within the concentration range of 0.1 to 5.0%. The limit of detection of Isomer II was found to be 0.1%. While separations are obtainable by TLC and GLC techniques, the HPLC method has the advantage of baseline separation over GLC and better quantitation over both GLC and TLC. The concentration of Isomer II in typical samples of methylphenidate hydrochloride were below 0.1% by the proposed technique.

The method can be simplified by injecting a methanolic solution of methylphenidate hydrochloride instead of a solution of the free base directly into the column without going through the extraction step (Figure 2). However, in this procedure the peaks appearing before the Isomer II make the peak area determination difficult below a 1% concentration level. Hence, the direct injection of the hydrochloride salt solution procedure is recommended only for a 1% level limit test.

As one would expect, the resolution of Isomers I and II is subject to column conditions and, hence, one could expect the separation to be adversely affected due to the deactivation of the column, particularly when the mobile phase contains both water and a base, namely ammonium hydroxide (8). In such instances, we found that resolution could be improved by eliminating the concentrated ammonium hydroxide completely

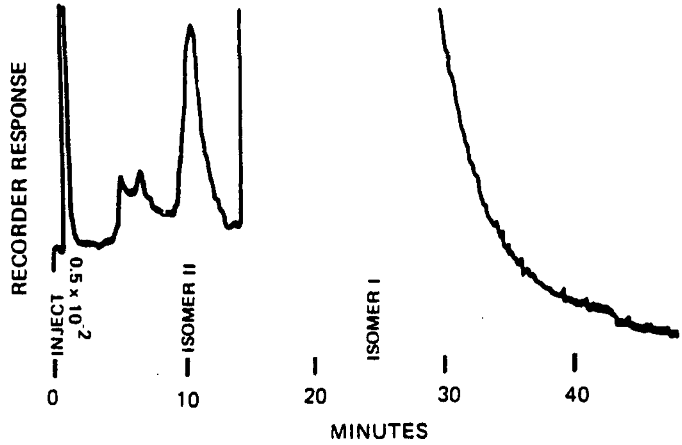


FIGURE 1

Chromatogram showing the separation of a mixture containing 0.5% Isomer II in methylphenidate HCl by the extraction procedure. Sample, 20 µL of a solution containing 100 mg of Isomer I and 0.5 mg of Isomer II in 1 ml of chloroform extract; column, Sil-X® at ambient temperature; mobile phase, ethanol:cyclohexane: chloroform:concentrated ammonium hydroxide (1.5:13.5:85:0.5); flow rate, 0.4 mL/min.

from the mobile phase and by adjusting the ratio of chloroform and cyclohexane to obtain the optimum resolution. A mobile phase containing chloroform, cyclohexane and ethanol (80:20:1.5) was one such system successfully employed with a prepacked 50 cm x 2.1 mm (i.d.) MicroPak-SI-10 column obtained from Varian Associates, Palo Alto, California. However, this modified mobile phase is not suitable for the direct injection of the salt solution.

Since α-phenyl-2-piperidineacetic acid, which is the major degradation product of methylphenidate hydrochloride, is retained by the aqueous phase in the chloroform pH 10 carbonate buffer extraction step, the method reported in this communication can be readily adapted to the development

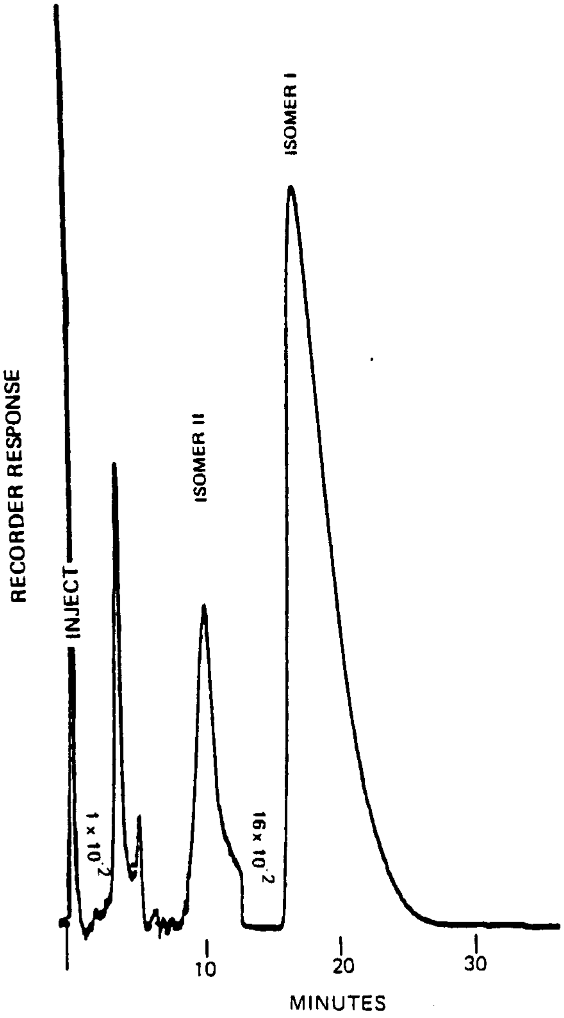


FIGURE 2

Chromatogram showing the separation of a mixture containing 1% Isomer II in methylphenidate HCl by direct injection. Sample, 20 μL of a solution containing 100 mg Isomer I and 1 mg of Isomer II in 1 mL of methanol; column Sil-X® at ambient temperature; mobile phase, ethanol:cyclohexane: chloroform:concentrated ammonium hydroxide (1.5:13.5:85:0.5); flow rate, 0.4 mL/min.

of a stability-indicating assay procedure for the methylphenidate HCl formulations (9). This investigation is being carried out in our laboratories.

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