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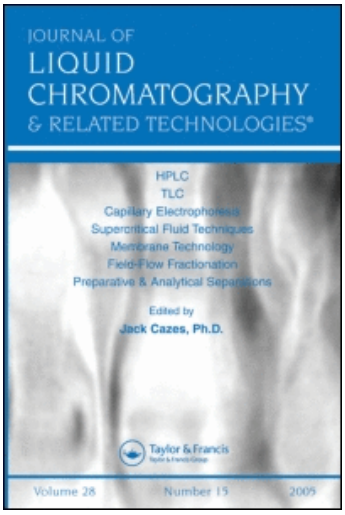

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ASSAY OF N-PROPYLAJMALINE IN BLOOD PLASMA BY ION-PAIR LIQUID-LIQUID CHROMATOGRAPHY

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ABSTRACT

A sensitive method for assay of N-propylajmaline (prajmaline) in human plasma is described. The quaternary ammonium compound exists as a pair of stereoisomers, which are isolated and separated by ion-pair liquid-liquid chromatography on microporous silica particles. An aqueous solution containing perchloric acid and sodium perchlorate is used as stationary phase and a mixture of butanol, dichloroethane and hexane as mobile phase. The procedure involves ion-pair extraction from plasma and evaporation prior to the chromatographic separation. Selective detection is achieved by using a fluorescence detector. The method allows assay of concentrations down to 10 pmol of the two forms of prajmaline in 1 ml of plasma with a relative standard deviation below 5 %.

INTRODUCTION

N-propylajmaline (prajmaline, Fig. 1) is a semisynthetic compound derived from the rauwolfia alkaloid, ajmaline, by quaternization. The drug is used in the treatment of arrhythmia and has pharmacological effect even at low concentrations. So far no method has been published for assay of therapeutic concentrations in plasma. A gas chromatographic method has been employed for analysis of N-propylajmaline in blood and tissue after intoxication (1).

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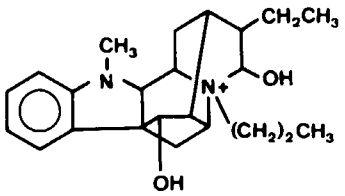

FIGURE 1 Chemical structure of N-propylajmaline.

Our initial liquid chromatographic studies of prajmaline revealed that the reference substance gave two chromatographic peaks. Radioactive prajmaline has previously been reported to give a double spot when analysed in serum using Radio-TLC (2). These observations and the chemical structure indicate that the compound exists as a stereoisomeric pair.

This paper describes an ion-pair liquid-liquid chromatographic method with fluorescence detection for the assay of prajmaline in blood plasma after ion-pair extraction with perchlorate as counter-ion. The assay was used to determine plasma concentrations after oral administration and results were obtained showing a great interindividual variation in the ratio between the two stereoisomers of prajmaline.

EXPERIMENTAL

Chromatographic Equipment

The liquid chromatograph consisted of an LDC Model 711-47 pump, a Rheodyne microsampling valve with a 100-µl loop, a precolumn and separation column (stainless steel, 150 mm in length, 4.5 mm i.d.), an LDC Spectromonitor III photometric detector or a Schoeffel FS 970 fluorescence detector, equipped with a deuterium lamp and a 320-nm cutoff filter. The excitation

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monochromator was set at 242 nm. The precolumn was placed between the pump and the injector. The columns and the mobile phase reservoir were water thermostatted at 25°C.

Chromatographic System

The separation column was packed with LiChrosorb SI 100, 5 µm (E. Merck, Darmstadt, G.F.R.), while 10 µm particles were used in the precolumn.

The stationary phase was an aqueous solution composed of perchloric acid, 0.2 mol/l, and sodium perchlorate, 0.8 mol/l, which was coated on to the silica particles in the separation column by an in-situ method (3).

The particles in the precolumn were spontaneously coated with stationary phase by passing more than 1 litre of mobile phase through the column before the separation column was connected. The precolumn was used to improve the equilibration of the mobile phase.

The mobile phase was a mixture of 1-butanol, 1,2-dichloroethane and hexane (15:40:45, by volume), which was equilibrated with the stationary phase by magnetic stirring for at least 1 h and filtered through glass wool before use.

A flow-rate of 1 ml/min of the mobile phase gave a retention time of 4.5 and 6.0 minutes for the two stereoisomers.

Chemicals

1-Butanol, 1,2-dichloroethane, dichloromethane (pro analysi, E. Merck) and hexane (HPLC grade, Rathburn Chemicals, Walkerburn, Scotland) were used.

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All other solutions were prepared from analytical grade chemicals.

N-propylajmaline (prajmaline) bitartrate (mol. weight 518.7) was supplied by Giulini Pharma GmbH (Hannover, G.F.R.). In the reference substance used a ratio of 55/45 was found between the two stereoisomeric forms by liquid chromatographic separation and assay by ion-pair extraction with picrate (4).

Determination of Extraction Constants

The extraction constants to dichloromethane were determined at pH 2 with perchlorate as counter-ion. The ion-pair partition experiments were performed with equal volumes of aqueous and organic phases in centrifuge tubes. The tubes were mechanically shaken for 30 min. at room temperature. After centrifugation an aliquot of the organic phase was injected onto the liquid chromatographic column in order to separate the two stereoisomers of prajmaline. The concentration of the counter-ion varied between $0.5 \cdot 10^{-3}$ and $1.0 \cdot 10^{-3}$ mol/l, while the concentration of prajmaline was constant, $0.5 \cdot 10^{-4}$ mol/l. The aqueous phase consisted of a phosphate buffer with an ionic strength of 0.1. The prajmaline content in the original aqueous solution was determined by liquid chromatography after addition of perchlorate to a final concentration of 0.6 mol/l and extraction with dichloromethane.

Analytical Procedure

One millilitre of plasma was mixed with 0.50 ml of sodium perchlorate solution (1.8 mol/l in phosphate buffer pH 7, I = 1.0) in an 8-ml centrifuge tube with a screw cap and extracted with 3.00 ml of dichloromethane. After shaking for

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10 min and centrifugation, the aqueous phase was aspirated and 2.00 ml of the organic phase was transferred to a silanized tapered centrifuge tube. The organic phase was evaporated under a gentle stream of nitrogen. The evaporation residue was dissolved in 100 µl of dichloromethane of which 80 µl was subjected to liquid chromatographic analysis. Quantitation was achieved by analysing reference samples of drug-free plasma to which known amounts of prajmaline had been added.

RESULTS AND DISCUSSION

Extraction Studies

Prajmaline, which is a quaternary ammonium compound, is subjected to ring fission in neutral and alkaline medium, yielding a tertiary amine (5), the transformation being reversible and influenced by pH. The partition of the tertiary amine was studied by extraction of the compound from aqueous phases having different pH into equal volumes of dichloromethane. Fig. 2 shows that the extraction is quantitative above pH 8. However, the corresponding extraction yield for spiked plasma appeared to be only 50 - 75 %. Ion-pair extraction has been used for selective extraction of quaternary ammonium compounds (6-7). The ion-pair partition experiments were performed at pH 2, where the base partition can be disregarded. The extraction constants, $K_{ex(QX)}$, for the stereoisomers to dichloromethane were found to be 340 and 400 using perchlorate as counter-ion. Provided that the concentration of perchlorate is higher than 0.3 mol/l, a theoretical extraction yield of 99 % will be obtained. Plasma samples containing known amounts of prajmaline were extracted at different pH with a perchlorate concentration of 0.6 mol/l and an aliquot of the dichloromethane phase was injected direct onto the separation column. The recoveries at

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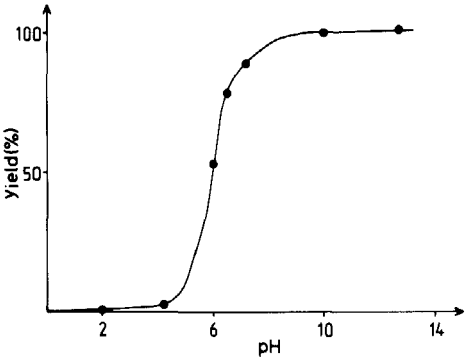

FIGURE 2 Extraction yield of prajmaline to dichloromethane versus pH of the aqueous phase.

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pH 2 and 12 were found to be lower than at pH 7 and 9.5 (> 95 %). Losses owing to adsorption to precipitated plasma have been reported for ion-pairs of a hydrophobic quaternary ammonium compound (7).

In the bioanalytical method we have preferred to do the extraction at pH 7 with a perchlorate concentration of 0.6 mol/l and a phase volume ratio of 2.

Chromatographic System - Separation and Detection

Different chromatographic systems were examined in order to separate the two stereoisomeric forms of prajmaline. Diastereoisomers have previously been separated on conventional normal phase systems (8), but the two forms of prajmaline could not be sufficiently separated on a silica gel column using a mixture of perchloric acid (or methane-sulfonic acid), methanol and dichloromethane as mobile phase, cf. (9). On a reverse-phase column (LiChrosorb RP 8) prajmaline was considerably retained but the retention time could be decreased

by adding tetrabutylammonium to the mobile phase. The stereoisomers eluted, however, as a double peak. The separation of the stereoisomers was achieved by an ion-pair liquid-liquid chromatographic system. The separation factor, α, was 1.4 using a mixture of 1-butanol, 1,2-dichloroethane, hexane (15:40:45, by volume) as mobile phase.

The chosen separation system, in combination with UV-detection, was not sufficiently selective against coextracted plasma components. However, the interferences were significantly reduced by introducing a fluorescence detector instead of the UV-detector (Fig. 3). The signal to noise ratio for prajmaline was of about the same magnitude with the two detectors when the mobile phase consisted of 1-butanol, 1,2-dichloroethane, hexane.

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Bioanalysis

In order to increase the sensitivity of the analytical method, the organic extract was concentrated by evaporation in a silanized glass tube before injection onto the separation column. No adsorption losses of prajmaline were observed during this procedure, nor were interfering peaks in the chromatogram substantially increased. The analytical method allows assay of plasma concentrations as low as 10 nmol/l of each stereoisomer. At this level the repeatability, determined by performing analyses of spiked plasma samples, was 3.1 % (S.D. n=7), while it was 1.6 % (S.D. n=10) for 1 µmol/l. The linearity was checked and the peak heights and concentrations were found to be linearly related over the range of 0.02 - 10.0 µmol/l plasma.

The procedure was employed to measure the steady-state level of prajmaline in patients. Blood samples were drawn

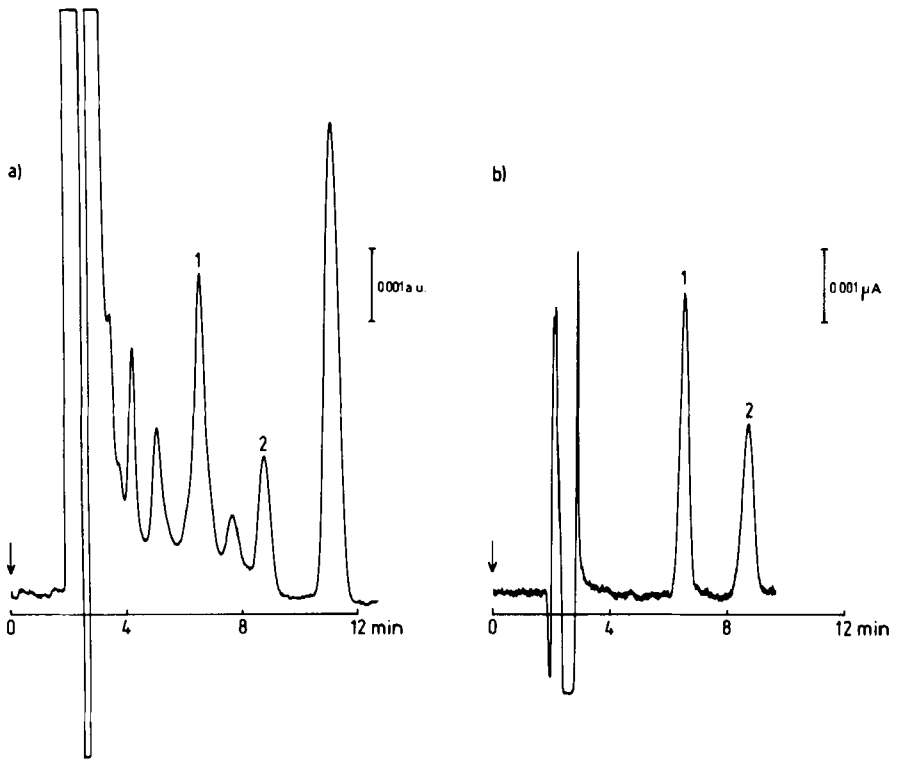

FIGURE 3 Chromatogram of a plasma sample spiked with 0.97 μmol/l of prajmaline. The concentration ratio between the two forms is 1.22. Mobile phase: 1-Butanol, 1,2-dichloroethane, hexane (10:45:45, by volume). a) UV-detection at 246 nm. b) Fluorescence detection.

from patients, given prajmaline (Neo-Gilurytmal®, 20 mg) three times a day for six days and the plasma concentration was assayed with the described analytical procedure. Fig. 4 shows a chromatogram from one of these plasma samples. Examples of concentrations found are listed in Table 1. It will be seen that the concentration ratio between the stereoisomers varies and differs from that of the reference compound. This indicates that the two stereoisomers do not have identical pharmacokinetics and an identical rate of metabolism. The method presented should make it possible to carry out thorough investigations on this question.

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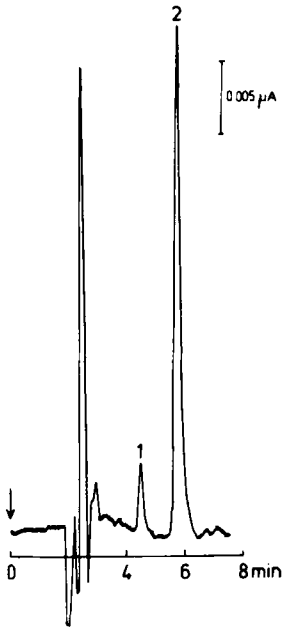

FIGURE 4 Chromatogram of an authentic sample. 2.00 ml of plasma was used for the analysis. The sample contained 0.041 µmol/l and 0.383 µmol/l of the respective stereoisomer.

TABLE 1

Concentration of Prajmaline in Plasma and the Ratio between the Two Forms Found in Patients Treated with Neo-Gilurytmal® (20 mg t.i.d.).

Peak 1 (µmol/l)	Peak 2 (µmol/l)	Ratio between 1 and 2
0.327	0.610	0.536
0.044	0.459	0.096
0.720	1.190	0.605
0.489	0.800	0.611
0.049	0.247	0.198
0.138	0.590	0.234
0.042	0.330	0.127
0.154	0.485	0.318
0.063	0.236	0.267
0.030	0.405	0.074

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REFERENCES

1. Gelbke, H.P., Schlicht, H.J., Suicide by an Overdosage of N-Propylajmalinium Bitartrate, Arch. Toxicol., 37, 135, 1977.

2. Schaumlöffel, E., Pharmakokinetische Studien mit Radioaktiv Markiertem N-(n-propyl)-Ajmaliniumhydrogentartrat, Med. Welt, 25, 2008, 1974.

3. Persson, B.-A., Karger, B.L., High Performance Ion Pair Partition Chromatography: The Separation of Biogenic Amines and their Metabolites, J. Chromatogr. Sci., 12, 521, 1974.

4. Gustavii, K., Schill, G., Determination of Amines and Quaternary Ammonium Ions as Complexes with Picrate, Acta Pharm. Suecica, 3, 241, 1966.

5. Keck, J., Synthese von N(b)-Alkyl-Ajmalinium-Verbindungen mit Starker Antiarrythmischer Wirkung, Z. Naturforsch., 18b, 177, 1963.

6. Eksborg, S., Persson, B.-A., Vessman, J., Enell, B., Determination of a Quaternary Ammonium Compound, Emepronium Bromide, in Human Urine by an Ion-Pair Extraction Method, J. Pharm. Sci., 60, 475, 1971.

7. Westerlund, D., Karset, K.H., Fluorimetric Determination of Propantheline in Human Blood Plasma by an Ion-Pair Extraction Method, Anal. Chim. Acta, 67, 99, 1973.

8. Selby, D.W., Munden, R.P., Separation of the Diastereoisomers of Labetalol Hydrochloride by High-Performance Liquid Chromatography, Proc. Analyt. Div. Chem. Soc., 14, 296, 1977.

9. Lagerström, P.-O., Persson, B.-A., Liquid Chromatography in the Monitoring of Plasma Levels of Antiarrythmic Drugs, J. Chromatogr., 149, 331, 1978.

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