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Short communication

Sensitive method for the determination of the antiarrhythmic drug detajmium in serum by solid-phase extraction and high-performance liquid chromatography with fluorimetric detection

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Abstract

The objective of this study was to develop a very sensitive and selective method for the determination of detajmium (4-3-diethylamino-2-hydroxypropyl-ajmaline), a sodium-channel-blocking drug with antiarrhythmic properties, in serum. A high-performance liquid chromatography (HPLC) method with solid-phase extraction and fluorimetric detection has been applied. Serum samples were diluted with phosphate buffer (pH 3.5) and the extraction of detajmium and ajmaline, which was used as an internal standard, was carried out with Oasis cartridges (Waters). The chromatographic separation was performed on a RP18 column. The limit of quantification for serum samples of detajmium was 1 ng/ml with good reproducibility (R.S.D.<15%) and a linear response from 1 to 200 ng/ml. The described method is highly sensitive and specific for the determination of detajmium in serum of patients and volunteers.

Keywords: Detajmium; Ajmaline

1. Introduction

Ajmaline and its derivative detajmium are antiarrhythmic drugs of class IA/IC (Fig. 1). Detajmium (4-3′-diethylamino-2′-hydroxypropyl-ajmaline; Tachmalcor, Arzneimittelwerk Dresden, Germany) is a sodium-channel-blocking drug, with an extremely long recovery from use-dependent sodium-channel block [1–3]. Very little data are available on detajmium's pharmacokinetics and analytics [4,5]. Commercially available detajmium is a mixture of four stereoisomers that differ in their configuration at C-21 and at the carbon atom of the hydroxyl function of the *N*-diethylamino side chain (Fig. 1). A gas chromatography–mass spectrometric method for the identification of detajmium and its metabolites was described previously [4]. In the same study, a liquid chromatography method was used for the quantification of detajmium. A limit of quantification (LOQ) of 50 ng/ml using fluorimetric detection was found. This study suffered from very complicated or not effective sample preparation methods. In this work, an automated, simple and sensitive method for the measurement of the detajmium concentration in serum samples from patients was developed. The new solid-phase extraction (SPE) material, Oasis (Waters), was found to be suitable for sample preparation. Waters Oasis extraction cartridges contain a special sorbent, a copolymer designed to have a hydrophilic–lipophilic balance (HLB), which gives

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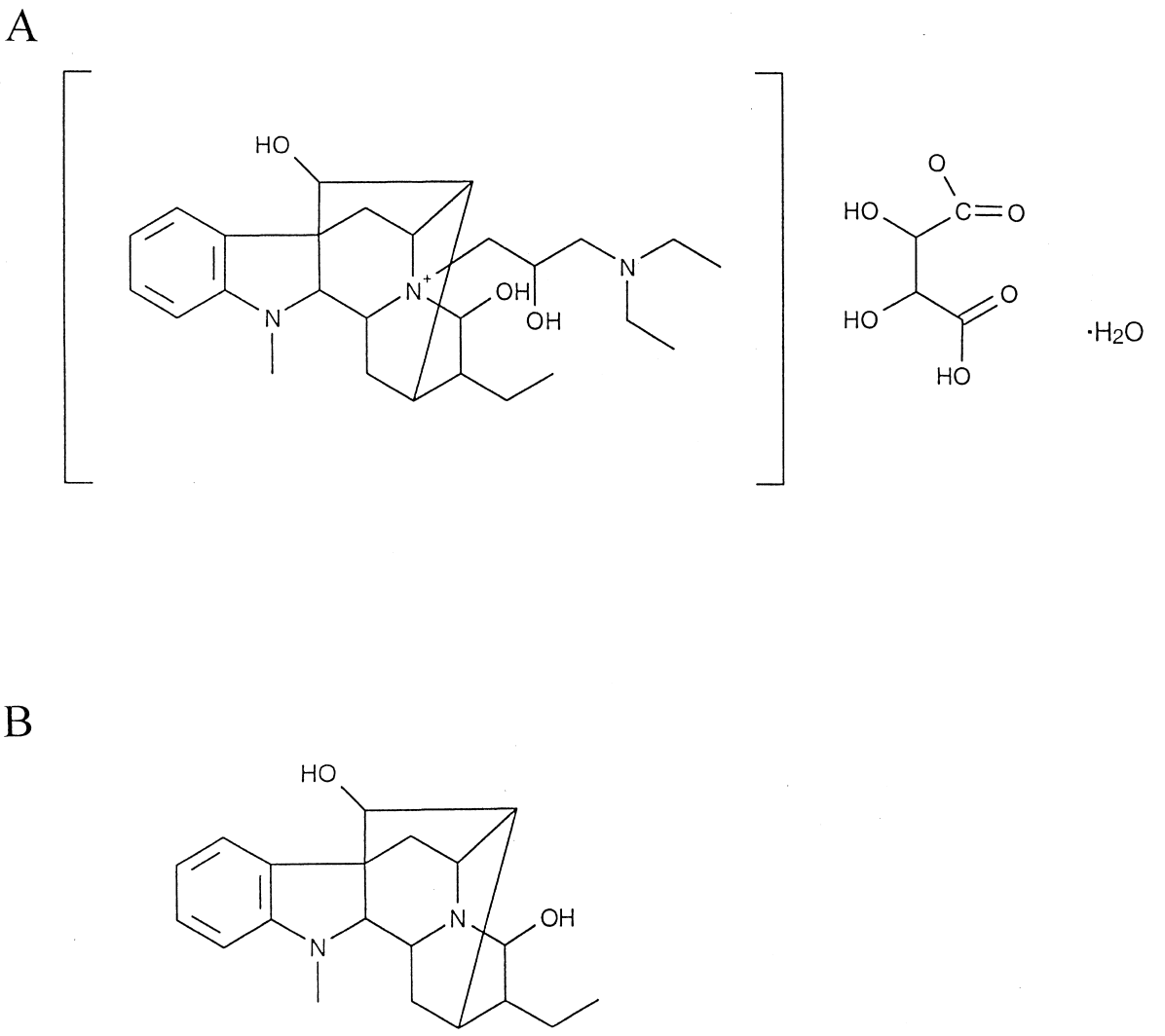

Fig. 1. Structural formulae of detajmium hydrogentartrate monohydrate (A) and ajmaline (B)

high and reproducible recoveries for acidic, basic and neutral compounds. An additional advantage of Oasis is the robustness, especially if the cartridges run dry.

2. Experimental

2.1. Chemicals

Detajmium was kindly provided by Arzneimittelwerk Dresden (Dresden, Germany). Ajmaline was a product of Idenas Milan, Italy. Acetonitrile LiChrosolv (for chromatography), methanol LiChrosolv (for chromatography) and potassium dihydrogen phosphate (for molecular biology) were purchased from Merck (Darmstadt, Germany). Pure water (20 MΩ) was obtained using an ion-exchange system RS 40 E (SG Ionenaustauscher, Barsbüttel, Germany).

2.2. HPLC method

2.2.1. Apparatus and chromatographic conditions

The equipment consisted of a Varian pump 9010, a

Varian 9100 autosampler (Darmstadt, Germany) equipped with a 50-μl loop, a fluorescence detector (Jasco FP 920; Tokyo, Japan) set at an excitation wavelength of 247 nm and an emission wavelength of 353 nm and a Chromeleon (V5.50, Gynkotek, Germeringen, Germany) data system. The chromatographic column [RP18, Nucleosil 50-5C8EC; 125×3 mm, 5 μm (Macherey-Nagel, Düren, Germany) in a LiChroCART HPLC cartridge (Merck)] was maintained in a CTO 10A (Shimadzu) column oven at a temperature of 40°C . The mobile phase consisted of a mixture of acetonitrile–methanol–phosphate buffer (0.1 mol/l adjusted to pH 3.5 with H_3PO_4) (3:22:75, v/v/v) with a flow-rate of 0.7 ml/min.

2.3. Sample preparation

In a tube of the ASPEC XL sample processor, 0.5 ml serum samples, spiked with 10 ng of the I.S. dissolved in 5 μl methanol were diluted with 0.5 ml of 0.05 mol/l phosphate buffer (pH 3.5). The mixture was shaken for 20 s (Heidolph mixer).

The solid-phase extraction of ajmaline and detajmium from serum was carried out with HLB 1 cc Oasis cartridges (Waters, Milford, MA, USA) in an ASPEC XL sample processor (Gilson). All liquids and air were pressed through the cartridges. This is in contrast to most other tools, which draw the liquids through the cartridges.

Extraction procedure

Condition	1 ml methanol
Equilibrate	1 ml phosphate buffer (0.1 mol/l adjusted to pH 3.5 with H_3PO_4)
Load	mixture of 0.5 ml serum and 0.5 ml phosphate buffer
Wash	2 ml phosphate buffer (0.1 mol/l adjusted to pH 3.5 with H_3PO_4) and 6 ml of 5% methanol in water, pushing with 0.5 ml of air
Elute	1 ml methanol.

Eluates were evaporated to dryness at 80°C in an air stream with a Techne DRI Block SC-3 (thermoDUX, Wertheim, Germany) and redissolved in 200 μl of the mobile phase.

2.4. Standard solutions

Stock solution of detajmium were prepared by dissolving the drug in phosphate buffer (0.1 mol/l, adjusted to pH 3.5 with H_3PO_4) to a final concentration of 1 mg/ml. Working solutions were obtained by further dilution of the stock solutions with phosphate buffer. Stock and working solutions of the internal standard ajmaline were prepared by dissolving and dilution with methanol.

3. Results and discussion

3.1. Sample preparation and chromatography

The aim of the method development was to separate detajmium from compounds of the biological matrix without resolution of the detajmium isomers. Solid-phase extraction with Oasis cartridges showed better results relative to recovery, reproducibility and selectivity compared to other cartridges, like C_8 or C_{18}, or to liquid–liquid extraction. Many endogenous substances were retained on the sorbent together with detajmium and ajmaline under the selected conditions. The goal of sample preparation was the complete elimination of matrix components. Two wash steps with relative large volumes were used to clean the samples. A higher proportion of organic modifier or larger volumes would reduce the recovery of detajmium. The residual part of the matrix had to separate on the analytical column. Independent of the type of column used or the composition of the mobile phase, it was not possible to find chromatographic conditions that gave a small peak for detajmium without interferences from serum. A higher resolution in the chromatogram to separate the biological matrix resulted in a peak with a shoulder for detajmium. The peak for the internal standard ajmaline was symmetrical (Fig. 2). It could be shown that the height of the main peak of detajmium is suitable for measurement of the drug concentration. Typical chromatograms obtained from extracted serum samples are shown in Fig. 3a–c. The retention times of detajmium and the internal stan-

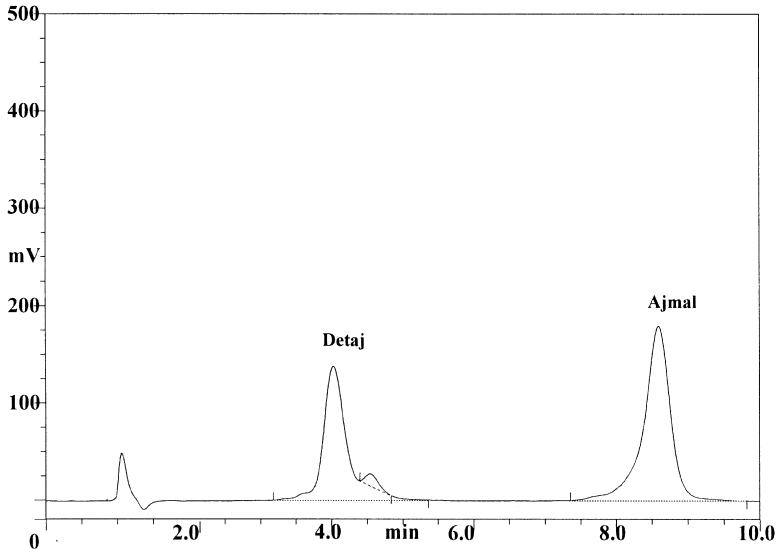

Fig. 2. Chromatograms after direct injection of standard solutions of detajmium (4.0 min) and ajmaline (8.6 min). Chromatographic conditions: RP 18, 125×3 mm, 5 μm; mobile phase, acetonitrile–methanol–phosphate buffer (pH 3.5) (3:22:75, v/v/v); flow-rate, 0.7 ml/min.

dard were 4.0 and 8.6 min, respectively. The overall chromatographic run time was 10.5 min.

3.2. Validation

The linearity and precision were tested using spiked serum samples. The linearity of the method was confirmed in the range of 1–200 ng/ml using a 0.5-ml sample. The precision of the method was assessed by determination of eight concentrations in six independent series of samples, as shown in Table 1. The lower limit of quantification, i.e. a coefficient of variation <20% for six repeated measurements, is 1 ng/ml. The correlation coefficient of regression lines was 0.992 or higher. Fig. 3a shows a representative chromatogram of a processed serum blank and indicates that no endogenous compounds exist at the retention times of detajmium or internal standard. Day-to-day precision data were obtained over a period of ten working days by taking aliquots of serum with 7, 30 and 65 ng/ml detajmium, respectively, and processing them daily. Adequate coefficients of variation were found: 9.7% (lowest concentration), 14.7% (medium concentration) and 11.9% (highest concentration).

Recoveries of 70% for detajmium and 78% for ajmaline from the serum matrix were found, independent of the concentration in the range from 1 to 200 ng/ml. Detajmium has been shown to be stable in serum maintained at −20°C for up to six months and at ambient temperature for 24 h. Stability has also been established through two freeze–thaw cycles for spiked serum samples.

4. Conclusions

The new extraction cartridges (Oasis from Waters) are suitable for the determination of detajmium in serum. The method is simple and more sensitive than liquid–liquid extraction. The high recovery for acidic, basic and neutral compounds using Oasis cartridges requires a careful washing procedure to remove the serum matrix, especially for very low drug concentrations and a good separation of residual matrix components on the analytical column. The chromatograms presented show a main peak with a shoulder for the detajmium isomers. The height of the main peak is suitable for measurement of the drug concentration.

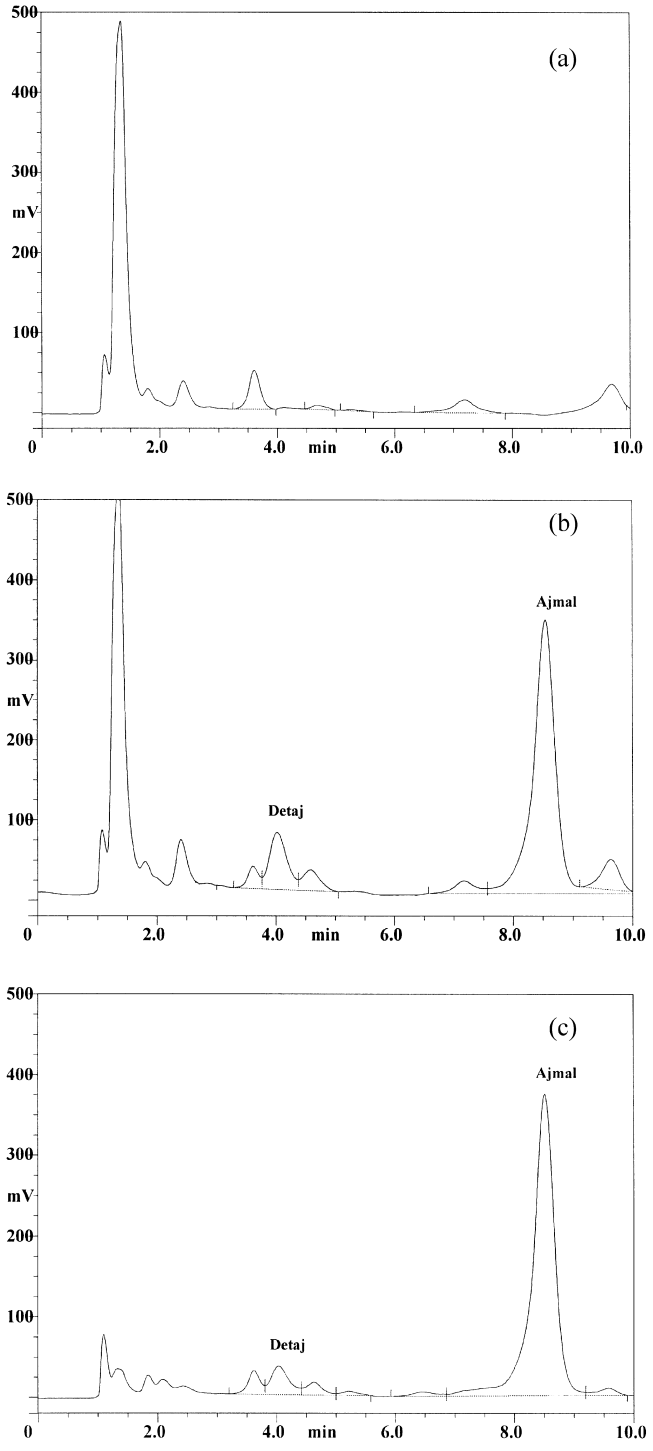

Fig. 3. Chromatograms of (a) blank serum; (b) blank serum spiked with detajmium (50 ng/ml) and ajmaline (20 ng/ml); (c) serum sample from a patient, which contained 25 ng/ml detajmium and 20 ng/ml ajmaline. Chromatographic conditions: RP18, 125×3 mm, 5 μm; mobile phase, acetonitrile–methanol–phosphate buffer (pH 3.5) (3:22:75, v/v/v); flow-rate, 0.7 ml/min.

Table 1
Precision of the analytical method to determine detajmium

Concentration added (ng/ml)	Concentration found mean±S.D. (ng/ml)	R.S.D. (%)
1	1.00±0.11	10.7
2	2.06±0.22	10.6
5	5.32±0.19	3.49
10	9.29±0.53	5.65
20	18.9±1.55	8.22
50	52.0±3.89	7.48
100	99.2±12.74	12.8
200	200.2±13.5	6.76

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