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SIMULTANEOUS DETERMINATION OF ATRACURIUM BESYLATE AND ITS MAJOR DECOMPOSITION PRODUCTS AND RELATED IMPURITIES BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The separation and determination of atracurium besylate and its major decomposition products and related impurities using octadecylsilica columns and acetonitrile–phosphate buffer mobile phases were studied. The influence of the acetonitrile and buffer concentrations and the pH of the mobile phase on the retention was investigated. The results indicated that hydrophobic and silanophilic interactions contribute to the retention of the compounds investigated.

INTRODUCTION

Atracurium besylate {2,2′-(dioxo-4,10-dioxatridecylene)bis[6,7-dimethoxy-1(3,4-dimethoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinolium]dibenzene sulphonate} is a highly selective, competitive (non-depolarizing) neuromuscular blocking agent which undergoes non-enzymic decomposition under physiological conditions, thus having a predictable duration of action[1]. It is highly suitable for endotracheal intubation, especially where subsequent muscle relaxation is required[2]. Atracurium (I) (Fig. 1) has been shown to degrade in solution by two mechanisms[3]. In solutions of pH about 3 or less, ester hydrolysis predominates to yield 2-(2-carboxyethyl)-2-methyl-1,2,3,4-tetrahydropapaverinium (II) and 2-(9-hydroxy-3-oxo-4-oxanoyl)-2-methyl-1,2,3,4-tetrahydropapaverinium (III) as primary decomposition products. Compound III can undergo further degradation to yield II and 1,5-pentanediol. In solutions of higher pH, degradation by Hoffmann elimination predominates to yield laudanosin (IV) and 2-(3,11-dioxo-4,10-dioxatridec-12-enyl)-2-methyl-1,2,3,4-tetrahydropapaverinium (V) as primary decomposition products. Compound V can undergo further degradation by Hoffmann elimination to yield IV and pentamethylene 1,5-diacrylate. Atracurium is obtained by quaternization of 2,2′-(3,11-dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydropapaverinium) (VI)[4]. Therefore, atracurium besylate can contain the potential impurities VI and 2-methyl-2,2′-(3,11-dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydropapaverinium) (VII).

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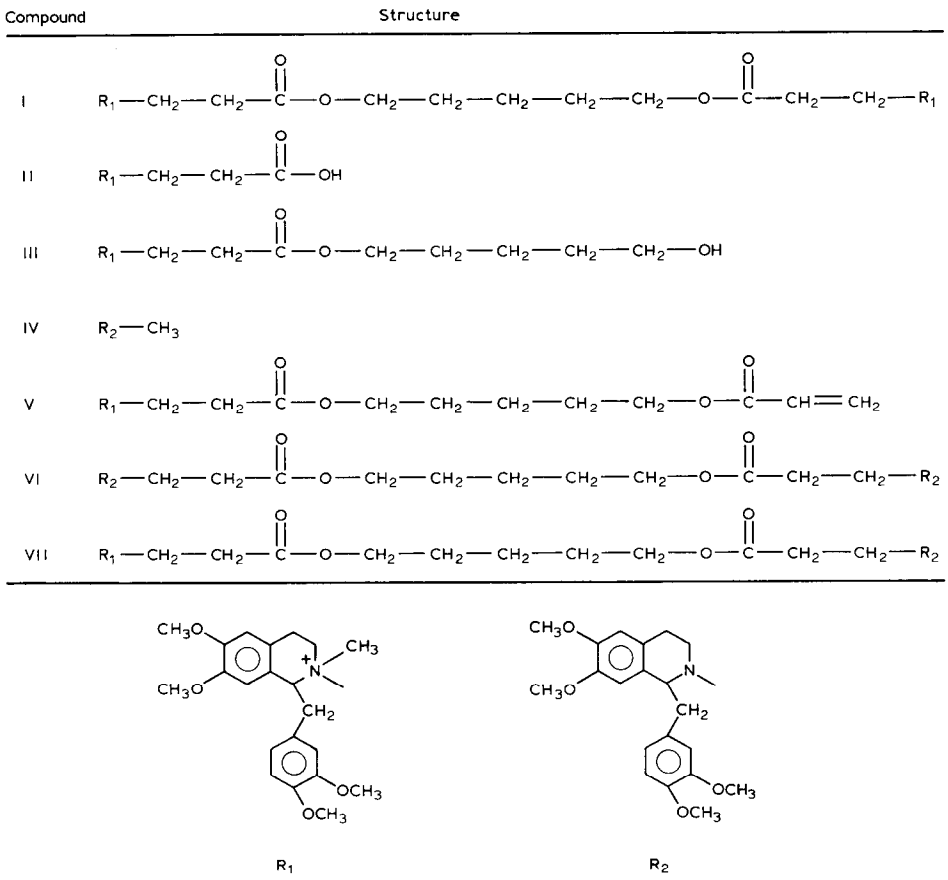

Fig. 1. Structures of atracurium and its major decomposition products and related impurities.

Methods published to date for the determination of atracurium besylate by high-performance liquid chromatography (HPLC) include cation-exchange chromatography for pharmacokinetics[5–7], adsorption chromatography for stability studies of atracurium and the separation of its three diastereoisomers[3] and reversed-phase (RP) HPLC for pharmacokinetics[8]. Carthy and Hill[3] discussed some analytical aspects of the determination of atracurium and some of its decomposition products, but no quantitation details were given and no chromatogram or retention data were presented. The chromatographic conditions gived[3] did not separate II and IV from each other, the potential impurity VII was not clearly resolved from the atracurium peak and the other potential impurity VI was not taken into consideration.

This paper describes an RP-HPLC method for the simultaneous determination of atracurium and its major decomposition products and related impurities.

EXPERIMENTAL

Chemicals

Atracurium besylate and compounds II–VII (Fig. 1) were synthesized in our Institute.

Acetonitrile (HPLC grade) and dibasic potassium phosphate trihydrate were obtained from Merck (Darmstadt, F.R.G.) and orthophosphoric acid from Fluka (Buchs, Switzerland).

Equipment

The HPLC equipment consisted of a Perkin-Elmer Series 3B chromatograph linked to a Perkin-Elmer LC 75 spectrophotometric detector with autocontrol and a Waters 740 Data Module. The UV detector was set at 280 nm. A Rheodyne 7120 injection valve (10-μl sample loop) was used. Pre-packed LiChrosorb RP-18 columns (25 cm × 4 mm I.D.), particle size 5 and 10 μm, were obtained from Merck and a Nova-Pak C_{18} Radial-Pak cartridge (10 cm × 8 mm I.D.), particle size 5 μm, in a Radial Compression Module RCM-100 was purchased from Waters Assoc. (Milford, MA, U.S.A.). A Radelkis Model OP-211/1 pH meter equipped with a glass electrode and a calomel reference electrode was used.

Mobile phases

The mobile phases were prepared from acetonitrile and aqueous buffers containing potassium hydrogenphosphate. Acetonitrile and the buffer solutions were filtered, mixed in the desired volume ratios and degassed ultrasonically. The pH of the mobile phases was adjusted with orthophosphoric acid under pH-metric control. The mobile phases were pumped through the column at ambient temperature.

The retention time of an unretained compound, t_0, was determined using sodium nitrate.

Sample preparation

About 5 mg atracurium besylate were dissolved in 10 ml of mobile phase. Sample solutions of atracurium besylate of 10 mg/ml were prepared by diluting 1 ml of the above solution to 20 ml with mobile phase. The reaction mixtures were also diluted with mobile phase to obtain an atracurium concentration of about 0.5 mg/ml. The stability of atracurium in the mobile phases was examined. In all instances atracurium was stable for 1 h or more under the mobile phase conditions.

RESULTS AND DISCUSSION

The influence of mobile phase conditions (concentrations of the organic component and buffer and pH) and concentration on retention was studied. Different octadecylsilica columns were also compared.

Effect of acetonitrile content in the mobile phase

Fig. 2 illustrates the effect of the acetonitrile concentration in the mobile phase on the capacity factors (k') of the components investigated. A constant mobile phase pH (pH 5.0) and a constant buffer concentration in the aqueous component of the mobile phase (0.1 mol/l potassium phosphate) were maintained.

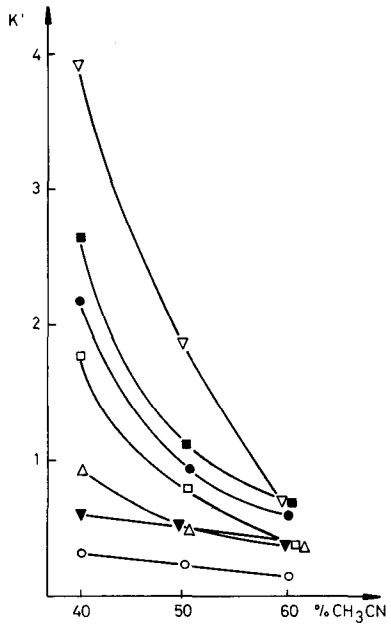

Fig. 2. Effect of acetonitrile content in the mobile phase on k'. Column, LiChrosorb RP-18 (25 cm × 4 mm I.D.), particle size 5 μm; mobile phase, mixtures of acetonitrile and 0.1 M potassium phosphate buffer (pH 5.0); flow-rate, 1 ml/min. □ = I; ○ = II; △ = III; ▼ = IV; ▽ = V; ■ = VI; ● = VII.

It is clear from the data that a reduction in the percentage of acetonitrile in the mobile phase improves the resolution. A comparison of the retention behaviours of I, VI and VII indicates that the capacity factors of compounds containing ternary amino groups in the molecule (VI and VII) depend more strongly on the acetonitrile content of the mobile phase than a component containing only quaternary amino groups (I). This result can be explained by the higher hydrophobicity of compounds containing ternary amino groups under the mobile phase conditions.

Because the buffer concentration in the aqueous component of the mobile phase was constant, a reduction in the percentage of acetonitrile leads to an increase in the ionic strength of the mobile phase. Therefore, the effect of the acetonitrile content of the mobile phase on the retention behaviour of the compounds studied can be affected by the combined effects of the variation of the acetonitrile content and the ionic strength of the mobile phase.

Effect of pH of the mobile phase

Fig. 3 shows the dependence of k' of the compounds investigated when using 40% acetonitrile in the mobile phase and 0.1 M potassium phosphate buffer solution. The effect of pH on k' differ for the different compounds. It was found that the effect of the mobile phase pH on the retention of I, VI and VII depends on the number of quaternary amino groups in the molecule. The strongest dependence of k' on the pH of the mobile phase was found for VI, which has no quaternary amino groups but two ternary amino groups in its molecule. VII has one quaternary and one ternary

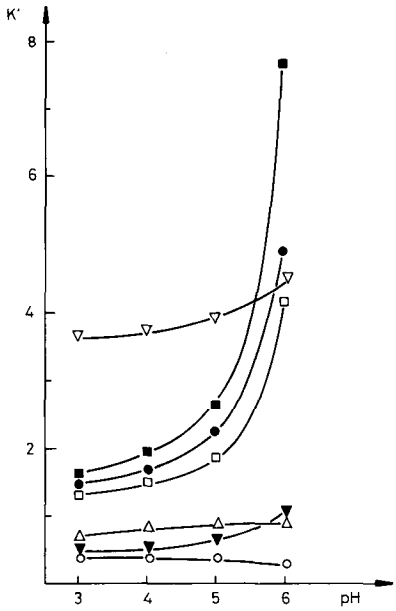

Fig. 3. pH dependence of k'. Mobile phase, acetonitrile–0.1 *M* potassium phosphate buffer (4:6). Other conditions and symbols as in Fig. 2.

amino group in the molecule and shows a dependence of k' on the pH of the mobile phase between those of VI (without quaternary amino groups) and atracurium (with two quaternary amino groups). A comparison of the retention behaviour of VII and V, also containing one quaternary amino group in the molecule, indicated that the ternary amino group in the molecule of VI and VII plays the main role in the retention behaviour under the mobile phase conditions. The k' value of compounds containing ternary amino groups depends on the proportion of the protonated species. At lower pH, the proportion of protonated species increases, and the retention of the solute will decrease.

The pH dependence of k' was used for the separation of atracurium, V, VI and VII. It can be seen that a changed elution order of V, VI and VII was obtained when the mobile phase pH was increased from 3 to 6.

Effect of buffer concentration in the mobile phase

Capacity factors were determined for atracurium and its major decomposition products and related compounds (II–VII) using mobile phases consisting of 40% acetonitrile and 60% aqueous solutions of potassium phosphate in concentrations of 0.15, 0.1, 0.075, 0.062 and 0.032 mol/l. The mobile phases were maintained at pH 5.0.

The results are shown in Fig. 4. Over the region examined, k' decreased with increasing buffer concentration in the mobile phase. The presence of an inorganic salt in the eluent can decrease the silanophilic effect of the stationary phase supported by the influence of the salt concentration in the mobile phase on the retention of the components.

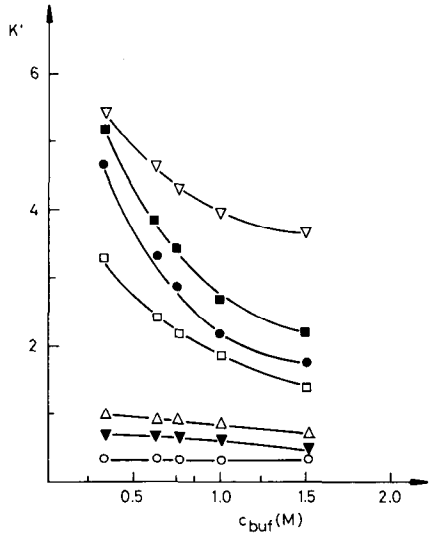

Fig. 4. Effect of potassium phosphate buffer concentration ($c_{buf.}$) on k'. Mobile phase, acetonitrile–buffer (4:6), pH 5.0; Other conditions and symbols as in Fig. 2.

Effects of different octadecylsilica columns

Table I shows the relative retention times of I–VII for different octadecylsilica columns. The results suggest that the differences in the retention behaviour of the octadecylsilicas used are silanophilic interactions which, together with hydrophobic interactions, contribute to the retention of the sample components. Variation of the particle size of the octadecylsilica had no significant effect on the retention behaviour of the compounds studied.

Fig. 5 shows typical chromatograms for the determination of atracurium in the presence of its major decomposition products and related impurities using different columns. The chromatographic conditions shown in Fig. 5 were used for the analysis of substances of atracurium besylate and for stability studies of its solu-

TABLE I

RELATIVE RETENTION TIMES OF COMPOUNDS INVESTIGATED USING DIFFERENT OCTADECYLSILICA COLUMNS

Mobile phase, acetonitrile–0.1 *M* potassium phosphate buffer (4:6); pH, 5.0.

*Column**	*Relative retention times*						
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*
A	1.00	0.38	0.56	0.49	1.62**	1.33	1.18
B	1.00	0.41	0.59	0.50	1.59**	1.31	1.13**
C	1.00	0.53	0.74	0.65	2.21**	1.44	1.24**

* A, LiChrosorb RP-18 (10 μm); B, LiChrosorb RP-18 (5 μm); C, Nova-Pak C_{18} Radial-Pak (5 μm).

** Major isomer.

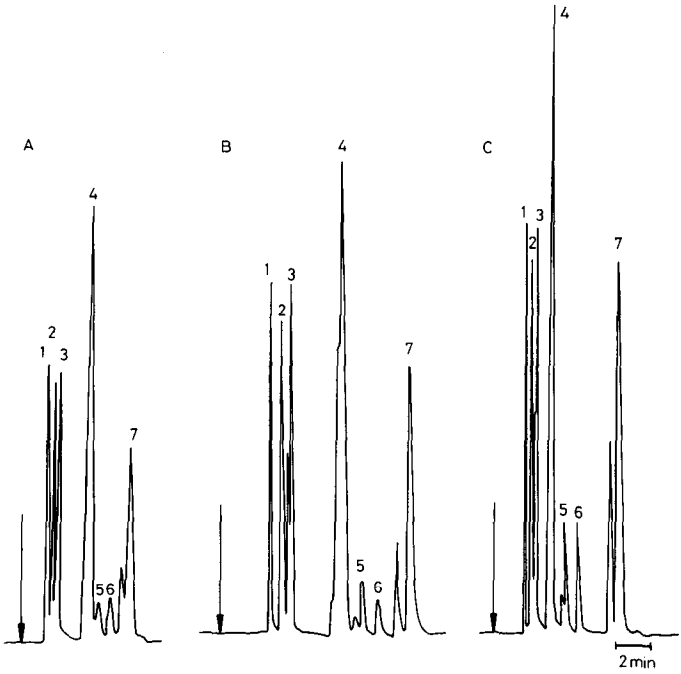

Fig. 5. RP-HPLC results showing the separation of atracurium and its major decomposition products and related impurities. Columns: (A) LiChrosorb RP-18 (10 μm); (B) LiChrosorb RP-18 (5 μm); (C) Nova-Pak Radial-Pak (5 μm). Mobile phase, acetonitrile–0.1 *M* potassium phosphate buffer (4:6), pH 5.0; flow-rate, (A) 2 ml/min, (B) 1 ml/min and (C) 1.5 ml/min; detection, 280 nm (0.16 a.u.f.s.). Peaks: 1 = II; 2 = IV; 3 = III; 4 = I; 5 = VII; 6 = VI; 7 = V.

tions. It can be seen that the diastereoisomers of some decomposition products (III and V) and the related compound VII were separated. Using a highly efficient column (Fig. 5B), the peak shape of atracurium was affected by the partial resolution of its diastereoisomers. With 30% acetonitrile in the mobile phase the three diastereoisomers of atracurium can be separated. These results will be published later.

For determining atracurium in reaction mixtures (quaternization of VI to atracurium) a gradient technique was applied. A representative chromatogram obtained by gradient elution of such a reaction mixture is shown in Fig. 6. To increase the detection limits of V–VII gradient elution was used in the analysis of atracurium besylate substances and its solutions. The detection limits of V–VII can also be increased by increasing the acetonitrile content in the mobile phase. Of course, in this way the resolution of II, III and IV will be decreased.

Quantitation, detection limits and linearity

Calculations of the concentrations of atracurium and its major decomposition products and related impurities were carried out by internal normalization using the equation

$$C_i = A_i/\Sigma A_i \cdot 100\ (\%) \qquad (1)$$

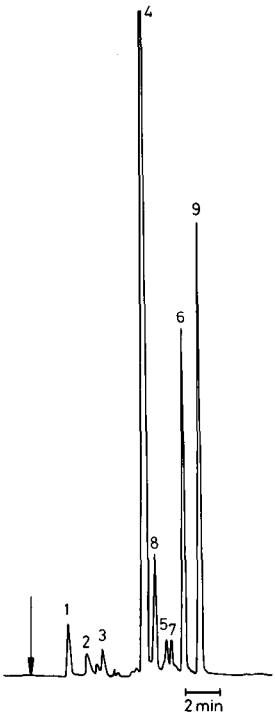

Fig. 6. Chromatogram obtained by gradient elution of a quaternization reaction mixture. Column, LiChrosorb RP-18 (5 μm); eluents, (A) acetonitrile, (B) 0.1 *M* potassium phosphate (pH 5.0); solvent programme, 6 min linear gradient from 35 to 60% A, 5 min isocratic at 60% A, 1 min linear gradient from 60 to 35% A, 5 min isochratic at 35% A for re-equilibration; flow-rate, 1.5 ml/min; detection, 280 nm (0.16 a.u.f.s.). Peaks: 8 = methyl benzenesulphonate (quaternization agent); 9 = benzenesulphonylchloride (byproduct of methyl benzenesulphonate); other peaks as in Fig. 5.

TABLE II

REPRODUCIBILITY OF PEAK AREAS OF A MIXTURE CONTAINING COMPONENTS I–VII

Column, LiChrosorb RP-18 (5 μm); mobile phase, acetonitrile–0.1 *M* potassium phosphate buffer (4:6); pH, 5.0; flow-rate, 1 ml/min.

Component	*Concentration (μg/ml)*	*Coefficient of variation (%) (n = 5)*
I	104	0.42
II	49	0.51
III	34	0.34
IV	42	0.82
V	46	1.58*
VI	35	0.40
VII	38	0.72

* Major isomer.

where C_i is the percentage content and A_i the peak area of sample component i. For reaction mixtures the reaction kinetics were studied and no quantitation was carried out.

The results for the reproducibility of the peak areas of the compounds investigated are given in Table II. The linearity of the detector response was examined in the range 0.05–10 μg for all components investigated. The regression coefficients of the linearity tests were 0.999 or better. The detection limits (signal-to-noise ratio = 2) of the compounds studied were 6–25 ng for atracurium about 10 ng for II, III and IV, 8–35 ng for V, 6–25 ng for VI and 9–25 ng for VII.

CONCLUSION

An RP-HPLC method has been developed for the separation and simultaneous determination of atracurium and its major decomposition products and related impurities. The method can be applied to the analysis of atracurium besylate substances, its solutions in stability tests and its reaction mixtures obtained from the quaternization reaction. The results indicate that hydrophobic and silanophilic interactions contribute to the retention of the compounds investigated.

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