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Research Papers

Simultaneous determination of otilonium bromide and diazepam by high performance liquid chromatography

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Summary

A reversed-phase high performance liquid chromatographic method for the simultaneous determination of otilonium bromide and diazepam in tablets has been developed. Owing to the residual free silanol groups on the modified silica surface, otilonium bromide eluted from the reversed-phase column without retardation effects, using a methanol-water eluent containing tetramethylammonium bromide and acetic acid. The effects of the tetramethylammonium bromide concentration on the capacity and symmetry factors of otilonium bromide and diazepam were investigated.

Introduction

Otilonium bromide is a potent spasmolytic agent for smooth muscle, but it lacks the side effects of other antimuscarinic drugs. The pharmaceutical combination of otilonium bromide and diazepam (an anxiolytic) is commercially available and widely used in the treatment of spastic pains related to neurovegetative disorders of the gastrointestinal system.

Little analytical work has been done for the quantitation of otilonium bromide: only a gas chromatography/mass spectrometry method (Signorini et al., 1984) for its determination in serum and a reflectance NIRS spectroscopy method (Corti et al., 1990) to quantify simultaneously otilonium bromide and diazepam in pharmaceutical preparations, have been reported.

Reversed-phase high-performance liquid chromatography (HPLC) has become the method of choice for a wide range of analytical applications. In spite of the advanced technology in this area, it is, however, only possible to achieve some 50% coverage of the hydroxyl groups on the silica surface (Roumeliotis and Unger, 1978; Holik and Matejkova, 1981). Ionic interactions between the residual acidic sites and oppositely charged protonated compounds give rise to poor chromatographic efficiency, long retention times and badly tailing peaks (Papp and Vigh 1983, a,b). Several techniques have been assayed to circumventing the problem of unreacted silanol groups (Bidlingmeyer et al., 1979; Bidlingmeyer, 1980). Mo-

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bile phase pH control and modifying agent having the same charge as the sample has been used to improve the analysis of quaternary ammonium ions (Van Der Maeden et al., 1977; Sokolowski and Wahlund, 1980).

This report presents a rapid and selective reversed-phase HPLC method for the simultaneous determination of otilonium bromide and diazepam in two pharmaceutical preparations. Tetramethylammonium bromide was used as mobile phase modifier to enhance the chromatographic performance of otilonium bromide.

TABLE 1

Tablet composition

Ingredients	Amount (mg)	
	Tablet A	Tablet B
Otilonium bromide	20	40
Diazepam	2	2
Avicel	60	98
Maize starch	65	45
Mg stearate	2	2
Silica	2	1
Gelatin	0.35	
Mg oxide	19.8	
Beeswax	0.003	
Saccharose	78.04	

Materials and Methods

Reagents and chemicals

Diazepam, quinine hydrochloride (internal standard) (Sigma) and otilonium bromide (Menarini, Florence, Italy) were used as received. Methanol and water (degassed before use) were HPLC analytical-reagent grade (Carlo Erba). Tetramethylammonium bromide (TMABr) (Janssen Chimica) and acetic acid (Carlo Erba) were of analytical reagent grade.

The composition of the two brands of examined commercial tablets (Menarini) is given in Table 1.

Apparatus and chromatographic conditions

Analyses were performed using a Perkin-Elmer liquid chromatograph (Series 3B) equipped with a Reodyne Injector 7125 (loop 20 μl), a multiple wavelength detector (LC 75 with Autocontrol) and LC 100 integrator. A 10 cm $\times$ 4.6 mm i.d. reverse-phase column (HPLC Technology, Technosphere RP C-8, 5 μm particle size) was used. The elution was isocratic and the mobile phase consisted of methanol (80%) and an aqueous solution (20%, pH 3) containing glacial acetic acid (1.4%) and 20 mM TMABr. A flow rate of 1.4 ml min^{-1} was used; all determinations were performed at room temperature. The column effluent was monitored at 258 nm; an attenuation of 0.08 a.u.f.s. was used and the chart speed was 5 mm min^{-1}. A 20 μl volume was injected.

Standard solutions

All operations were performed with drugs and solutions protected from the light. Stock solutions of otilonium bromide (1 mg ml^{-1}), diazepam (0.1 mg ml^{-1}) and quinine hydrochloride (0.5 mg ml^{-1}) were prepared in methanol. The concentrations of working standard solutions, prepared by appropriate dilution with the mobile phase, ranged from 0.005 to 0.02 mg ml^{-1} for diazepam and from 0.1 to 0.4 mg ml^{-1} for otilonium bromide. The concentration of quinine hydrochloride (internal standard) was fixed at 0.05 mg ml^{-1}.

Calibration curves were obtained by plotting the peak-height ratios between each compound and the internal standard vs the corresponding analyte concentrations (mg ml^{-1}). The least-square regression equations ($n = 5$) were $y = 164.083x + 0.012$ ($r = 0.9994$) for diazepam and $y = 5.952x + 0.029$ ($r = 0.9995$) for otilonium bromide.

Sample analysis

Twenty tablets were finely powdered and quantities corresponding to the average weight of one tablet were weighed in a 25 ml volumetric flask and brought to volume with methanol. The mixtures were sonicated for 30 min and filtered (0.45 μm); 1 ml of the filtered solution and 1 ml of stock quinine hydrochloride solution were transferred into a 10 ml volumetric flask and diluted to volume with mobile phase.

Results and Discussion

Preliminary experiments carried out in order to select the most suitable composition of the mobile phase for the otilonium bromide and diazepam separation and quantitation, revealed that otilonium bromide elutes from a reversed-phase column using methanol-water mixtures only with a high percentage of methanol and if an electrolyte is added to the mobile phase. It is known (Roumeliotis and Unger, 1978; Holik and Matejkova, 1981; Gomez-Gomar et al., 1989) that RP-modified silica phases always still contain a fraction of unbonded and accessible acidic silanol groups which are ionized at neutral or basic pH. For the strong quaternary ammonium bases, the RP-modified silica would behave like a weak ion-exchange resin. Owing to this effect, otilonium bromide was strongly bound to the column by residual free silanol groups.

The addition of even a small amount of acetic acid to the mobile phase allowed the elution of otilonium bromide, that however, was eluted in very asymmetric band and with a retention time of about 30 min.

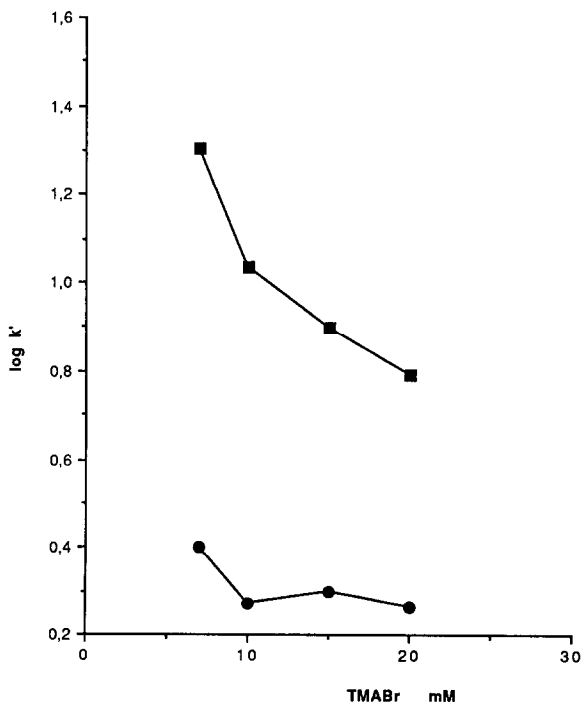

Fig. 1. Influence of TMABr concentration on the logarithm of the capacity factor, k', of diazepam (●) and otilonium bromide (■).

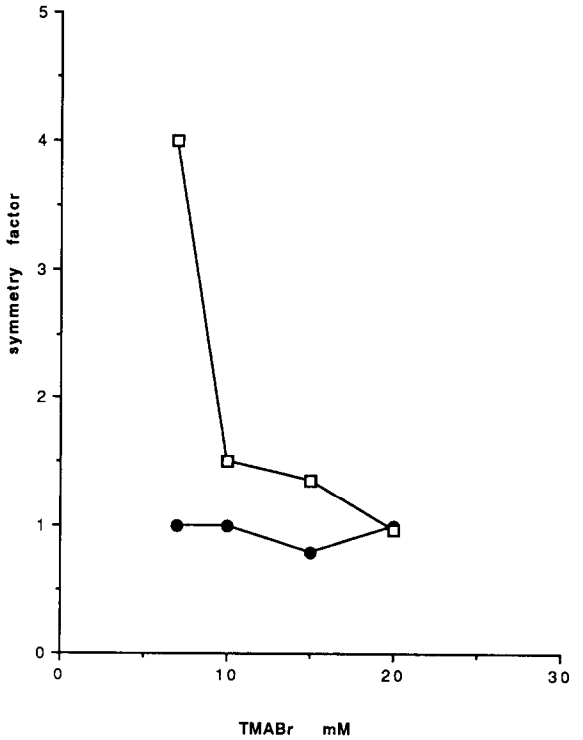

Fig. 2. Influence of TMABr concentration on the symmetry factor of diazepam (●) and otilonium bromide (□).

Secondary mobile phase modifiers used to enhance the chromatographic analysis of pharmaceuticals have recently been reviewed (Gilpin et al., 1988). Tetraalkylammonium salts have been used as ion-pairing additives as well as to reduce residual silanophilic interactions on reversed-phase surfaces (Wolff et al., 1985; Gilpin et al., 1988).

Upon addition to the mobile phase of a 20 mM TMABr concentration, the otilonium peak performance was substantially improved with reduction of the peak tailing and retention time. The chromatographic performance of positively charged samples is influenced in two ways by the addition of quaternary modifying agents to the mobile phase (Kiel et al., 1985): the retention time is decreased by the formation of a positively charged layer of the ion interaction reagent adsorbed on the alkyl chains of the stationary phase, while the

peak symmetry is mainly controlled by ion exchange interactions.

The effects of the TMABr concentration on the capacity factor and on the symmetry factor (calculated according to Lonardi and Mosconi, 1980) are shown in Figs 1 and 2, respectively.

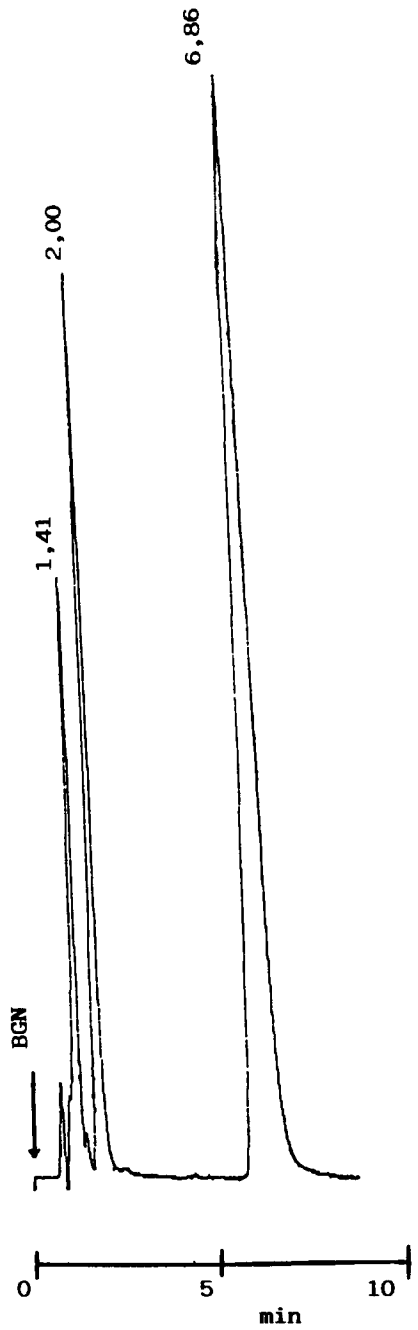

Fig. 3. Typical chromatogram of diazepam (Rt 1.41 min), quinine hydrochloride (i.s., Rt 1.97 min) and otilonium bromide (Rt 6.86 min).

TABLE 2

Analyses of standard mixtures and commercial tablet batches of diazepam and otilonium bromide

Batch	Diazepam		Otilonium bromide	
	Taken quantity (mg)	Percent average recovery (RSD, $n = 5$)	Taken quantity (mg)	Percent average recovery (RSD, $n = 5$)
1	2	98.1 (1.8)	40	102.5 (2.1)
2	2	102.3 (1.3)	40	99.5 (1.1)
3	2	98.7 (2.1)	20	99.1 (1.9)
4	2	103.3 (1.5)	20	102.4 (1.8)
5 [a]	2.2	100.2 (1.3)	41.2	99.1 (0.9)
6 [a]	2.3	99.3 (1.6)	19.8	99.7 (0.7)

[a] Authentic sample.

As might be expected, the retention time and the symmetry of the diazepam peak were not significantly influenced by the TMABr concentration. Therefore, under the described chromatographic conditions, diazepam and otilonium bromide could be separated and quantitated (Fig. 3).

Because of the good molar absorptivity of diazepam and otilonium bromide, it is possible to detect both substances at 258 nm, without changing the a.u.f.s.

The analytical results from the assay of four commercial tablet batches indicate that the proposed method can be used for the simultaneous quantitation of diazepam and otilonium bromide. Two mixtures containing known quantities of diazepam and otilonium bromide in ratios equivalent to those labelled in commercial dosage forms, were studied. The good accuracy and precision of the proposed method are demonstrated in Table 2, which summarizes the results of the analyses of two authentic samples and four commercial batches.

The proposed method can be used for the control of pharmaceutical preparations containing otilonium bromide and diazepam with good accuracy and reproducibility.

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