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Note

Analysis of propantheline bromide in serum by high-performance liquid chromatography

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Propantheline is a parasympatholytic drug which has been used for more than twenty years in the treatment of gastrointestinal ulceration and urinary incontinence. Propantheline can be readily measured in vitro [1—3]; however, determination of propantheline in serum has been hampered by the lack of sufficiently sensitive methods, particularly after oral administration of the drug [4]. Until now, the only method available to measure orally administered propantheline involved gas chromatography—mass spectrometry, a highly specialized and expensive technique [5].

The present high-performance liquid chromatographic (HPLC) method involves perchlorate ion-pair extraction of propantheline from serum followed by reversed-phase separation and low-wavelength ultraviolet detection. The assay can be used to measure propantheline in serum following oral or parenteral administration of the drug in normal therapeutic doses.

EXPERIMENTAL

Materials

Propantheline bromide reference material was kindly supplied by G.D. Searle (Chicago, IL, U.S.A.). Potassium dihydrogen orthophosphate was obtained from BDH Chemicals Australia (Port Fairy, Australia). Perchloric acid (70%,

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w/w), hydrochloric acid (32%, w/w) and orthophosphoric acid (85%, w/w) were obtained from Ajax Chemicals (Sydney, Australia). Dichloromethane, acetonitrile and hexane were bought from Waters Assoc. (Sydney, Australia). Diethyl ether (spectral grade) was obtained from E. Merck (Darmstadt, F.R.G.). Unless otherwise stated, all chemicals were analytical grade material.

Standards

For convenience, propantheline standards were routinely prepared in 3% (w/v) bovine serum albumin (BSA) since peak height ratios were similar to those obtained from the assay of propantheline in drug-free serum. Standards containing 100, 50, 20 and 5 ng/ml of propantheline bromide were prepared in bulk, subdivided into 2-ml aliquots and stored at −80°C until assayed by HPLC.

The internal standard (2-dipropylaminoethylxanthene-9-carboxylate methochloride) was synthesized by the following procedure: 2.65 g (16 mmol) of 2-chloroethyldipropylamine [6] and 5.8 g (22 mmol) of potassium xanthene-9-carboxylate were refluxed together in tetrahydrofuran (150 ml) for 13 h. The mixture was filtered and the filtrate evaporated to yield the xanthene ester as a yellow oil (5 g, 88%) which did not distil below 250°C/0.1 mm. (Found: C, 74.5; H, 7.8; N, 4.0. $C_{22}H_{27}NO_3$ requires C, 74.7; H, 7.7; N, 4.0%.) ^{1}H-nmr δ {C^2HCl_3, Me_4Si}, 7.45—6.80, br, 8H; 5.97, s, 1H; 4.66, tr, 2H; 2.52, tr, 2H; 2.30, tr, 4H; 1.26, m, 4H; 0.78, tr, 6H. 2-Dipropylaminoethylxanthene-9-carboxylate (5.1 g, 13 mmol) was refluxed in methyl iodide (25 ml) for 20 h. the pale yellow solid (7.5 g, 87%) was recrystallized from ethanol to give the methiodide, m.p. 130—132°C. (Found: C, 55.4; H, 6.3; I, 24.9; N, 2.6. $C_{23}H_{30}INO_3$ requires C, 55.7; H, 6.1; I, 25.6; N, 2.8%.) ^{1}H-nmr δ {C^2HCl_3, Me_4Si}, 7.5—6.95, br, 8H; 5.08, s, 1H; 4.31, tr, 2H; 3.73, tr, 2H; 3.38—3.02, br, 4H; 2.98, s, 3H; 2.14—1.31, m, 4H; 0.92, tr, 6H. The methiodide (2 g, 5 mmol) was suspended in water (300 ml) and shaken with freshly precipitated and washed silver chloride (from silver nitrate, 8.5 g, 50 mmol) for 1 h. The solid silver chloride—silver iodide was filtered off and the filtrate evaporated in vacuo (0.1 mm, 50°C) to yield a colourless oil (1.4 g, 69%). Trituration of the oil with tetrahydrofuran gave the solid methochloride which was recrystallized as a hygroscopic solid, m.p. 167—169°C, from methanol—ethyl acetate. (Found: C, 68.7; H, 7.3; N, 3.5. $C_{23}H_{30}ClNO_3$ requires C, 68.4; H, 7.5; N, 3.5.) ^{1}H-nmr δ {C^2HCl_3, Me_4Si}, 7.55—7.08, br, 8H; 5.12, s, 1H; 4.52, tr, 2H; 3.92, tr, 2H; 3.28, tr, 4H; 3.00 s, 3H; 2.00—1.30, m, 4H; 0.95, tr, 6H. Melting points were measured in an electrically heated silicone oil bath and are uncorrected. ^{1}H-nmr spectra were recorded on a Varian EM-360 or a Jeol MH-100 spectrometer. Microanalyses were performed by the Department of Chemistry, University of Queensland.

HPLC equipment

Chromatography was performed on a modular system consisting of a Model M45 pump (Waters Assoc., Milford, MA, U.S.A.) connected to a Model 7125 sample injector (Rheodyne, Berkeley, CA, U.S.A.) and a Model 480 Lambda-Max variable-wavelength detector (Waters Assoc.). A 10-μm particle size, 300 × 3.9 mm μBondapak C_{18} column (Waters Assoc.) was used for all

separations. Chromatograms were recorded on a Model R-02 chart recorder (Rikadenki Kogyo, Tokyo, Japan).

Chromatography

The HPLC mobile phase was prepared by slowly adding 400 ml of acetonitrile to 600 ml of rapidly stirred potassium dihydrogen orthophosphate solution (0.1 *M*, previously adjusted to pH 3 with orthophosphoric acid). All HPLC solvents were filtered (0.5 μm, Millipore, Bedford, MA, U.S.A.) and degassed before use. Reagent grade water (Milli-Q, Millipore) was used throughout. The mobile phase was delivered at 1.5 ml/min and the detector set at 210 nm with a 2-sec time constant. Chromatograms were recorded at a chart speed of 10 cm/h using a 10-mV input from the detector.

Extraction procedure

Two millilitres of serum were pipetted into 100 mm × 16 mm Kimax tubes and vortexed for 10 sec with 0.1 ml of aqueous perchloric acid (1 *M*). Dichloromethane (10 ml) containing 300 ng of internal standard was accurately dispensed into the tubes which were capped (PTFE seals) and rotated at 24 rpm on a blood mixer for 10 min. The tubes were then centrifuged (850 *g*, 15 min); the upper (aqueous) phase was aspirated and the lower (organic) phase filtered through 7-cm diameter Whatman 1PS phase separators into 15-ml glass centrifuge tubes.

The organic phase was evaporated to dryness at 35°C under reduced pressure and agitation in a Vortex-Evaporator (Buchler Instruments, Fort Lee, NJ, U.S.A.) and reconstituted in 0.1 ml of hydrochloric acid (0.1 *M*). The acidic solution was washed first with 5 ml of diethyl ether (pre-saturated with 0.1 *M* hydrochloric acid) and then with 5 ml of hexane. A 50-μl aliquot of the lower (aqueous) phase was injected onto the HPLC column.

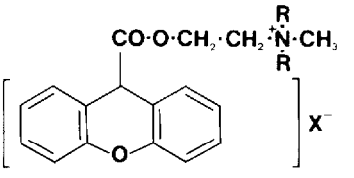
To determine optimum conditions for ion-pair extraction, the above procedure was repeated using 100 ng/ml propantheline bromide in serum with 0.0, 0.25, 0.5, 1.0, 1.5, 2.0 and 4.0 *M* perchloric acid solutions (0.1 ml).

RESULTS AND DISCUSSION

Propantheline and the internal standard (Fig. 1) gave retention times of 6.75 and 9.0 min, respectively. Both compounds displayed symmetrical peaks with baseline resolution and minimal tailing. The chromatograms in Fig. 2 are from the assay of (a) serum from a patient administered 60 mg of propantheline bromide tablets, (b) a propantheline bromide standard (100 ng/ml) in 3% BSA, and (c) drug-free serum. No endogenous serum components interfered with the analysis.

2-Dipropylaminoethylxanthene-9-carboxylate methochloride (Fig. 1) served as a suitable internal standard since it is not a drug and has similar extractive, chromatographic and spectral properties to propantheline. Methantheline bromide (2-diethylaminoethylxanthene-9-carboxylate methobromide, G.D. Searle) can also be used as an internal standard [1]; however, we noted interference with this peak in samples from patients on propantheline who were also taking tricyclic antidepressants.

There was a linear relationship between propantheline bromide concentration and peak height ratio, from 5 ng/ml to at least 100 ng/ml. Calibration plots were constructed daily; the mean data from ten such plots gave the regres-



	R	X
Propantheline Bromide	$CH{\cdot}(CH_3)_2$	Br
Internal Standard	$CH_2{\cdot}CH_2{\cdot}CH_3$	Cl

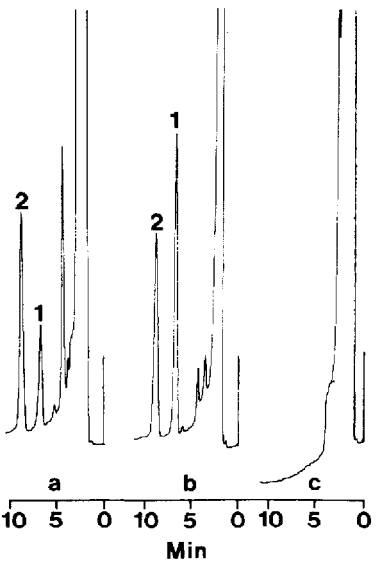


Fig. 1. Structure of propantheline bromide and of the internal standard (2-dipropylamino-ethylxanthene-9-carboxylate methochloride).

Fig. 2. Chromatograms from the analysis of: (a) serum from a patient administered 60 mg of propantheline bromide 45 min previously, (b) propantheline bromide (100 ng/ml) in 3% BSA, and (c) drug-free serum. Peaks: 1 = propantheline bromide; 2 = internal standard.

sion equation $Y = 0.0138X - 0.009$ ($r^2 > 0.999$), where Y is the peak height ratio of propantheline to internal standard and X is the concentration of propantheline bromide.

The minimum serum propantheline concentration that could be measured was about 2 ng/ml. Further increases in sensitivity might be obtained using larger injection volumes or by using HPLC columns with higher resolution and smaller particle sizes than used here. In our experience, columns which lack silanol "end-capped" packings are unsatisfactory for the assay, due to excessive retention and marked tailing of the propantheline peak. The sensitivity of the method is due in part to the excellent signal-to-noise ratio of the Lambda-Max detector. Further, propantheline has an absorption maximum at 246 nm; however, a marked increase in response is obtained at 210 nm. Since many endogenous components in serum also absorb in this region, it was necessary to develop suitable clean-up procedures. Acid back-extraction followed by ether and hexane washes successfully removed interfering peaks.

As reported by others [4], we found that dichloromethane extraction of propantheline from serum was improved considerably using perchlorate ion-pairing. In our studies, 0.1 ml of 1 *M* perchloric acid added to 2 ml of sample gave optimum extraction of the drug (Fig. 3). Perchloric acid concentrations above 1 *M* gave decreased propantheline extraction, presumably due to occlusion of the drug in protein precipitates [4].

Concentrations of propantheline bromide from 5 ng/ml to 100 ng/ml were stable in 3% BSA for at least three months at −80° C in the presence of 1 *M* perchloric acid (0.1 ml per 2 ml of sample). Assay precision was assessed by repeatedly assaying propantheline bromide standards on the same day. These results appear in Table I.

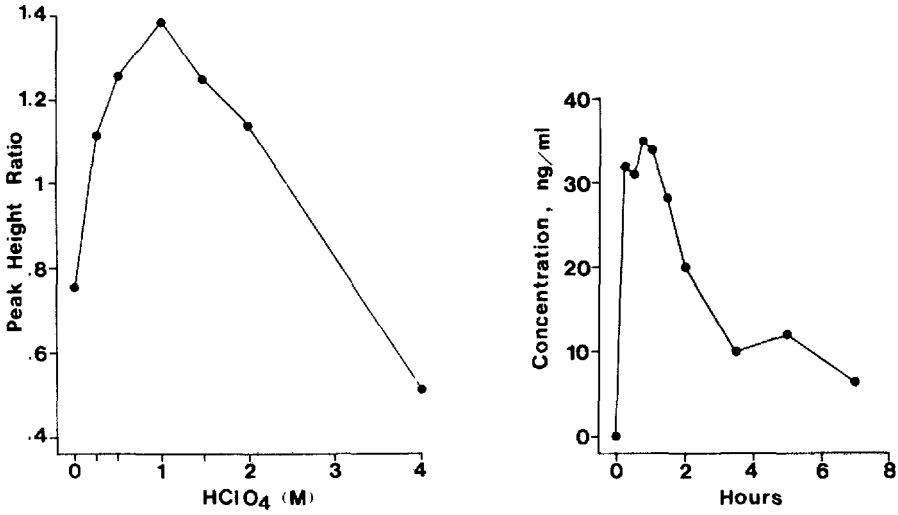


Fig. 3. Effect of perchloric acid concentration on the dichloromethane extraction of propantheline bromide from serum.

Fig. 4. Serum concentrations of propantheline bromide following administration of propantheline bromide tablets (4 × 15 mg) to a fasting, healthy subject.

TABLE I

WITHIN-DAY PRECISION OF THE HPLC ASSAY OF PROPANTHELINE BROMIDE IN SERUM

Propantheline bromide (ng/ml)	*n*	Mean peak height ratio	Standard deviation	C.V. (%)
100	10	1.370	0.1059	7.7
50	10	0.695	0.0502	7.2
20	9	0.253	0.0171	6.8
5	7	0.066	0.0037	5.6

The data in Fig. 4 show propantheline concentrations in serum measured by HPLC following administration of propantheline bromide (60 mg) tablets to a fasting, healthy volunteer. Propantheline could be measured for at least 7 h after drug administration. The assay has been extensively employed in bioavailability studies as well as in propantheline urodynamic investigations in hospital patients.

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