A New Method for Assaying Propantheline and Its Degradation Product, Xanthene-9-carboxylic Acid Using High-Performance Liquid Chromatography

B. G. CHARLES ˣ **and P. J. RAVENSCROFT**

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Abstract ☐ A rapid, specific, and precise high-performance liquid chromatographic method is described for the simultaneous analysis of propantheline bromide and its hydrolysis product, xanthene-9-carboxylic acid. Reversed-phase chromatography was conducted using a mobile phase of 40:60, acetonitrile–0.05 *M* phosphate buffer (pH 2.5) delivered at 2 ml/min. Detection was at 254 nm. Methantheline bromide (internal standard), propantheline bromide, and xanthene-9-carboxylic acid gave retention times of 4.1, 5.4, and 8.3 min, respectively. Within-day, between-day, and total precision (*CV*) for assay of 15 mg/10 ml propantheline bromide are 1.2, 1.1, and 1.6%, respectively ($n = 20$). Similar precision was obtained for xanthene-9-carboxylic acid. The limit of detection was 2 ng. The assay is useful for routine quality assurance of propantheline in dosage forms and for stability and kinetic studies.

Keyphrases ☐ Propantheline—new method of high-performance liquid chromatographic assay, degradation product, xanthene-9-carboxylic acid ☐ Xanthene-9-carboxylic acid—degradation product, new method of high-performance liquid chromatographic assay of propantheline ☐ High-performance liquid chromatography—new method for assaying propantheline and its degradation product, xanthene-9-carboxylic acid

Propantheline bromide [(2-hydroxyethyl)diisopropylmethylammonium bromide xanthene-9-carboxylate] is a parasympatholytic agent which has been used to treat conditions such as urinary incontinence and GI ulceration. The development of efficient methods to measure propantheline in dosage formulations has received little attention in recent times. Some of the compendial methods to assay the drug are time consuming, often requiring several extractions followed by nonaqueous titration (1, 2). The measurement of propantheline by direct UV assay is not specific for the drug with the risk of interference from formulation excipients, drug impurities, or decomposition products of propantheline. Several organic dye–salt partition techniques have been used to assay propantheline in biological fluids (3, 4) and in pharmaceuticals (5). Various modifications of these procedures are used in the pharmaceutical industry for the quality assurance of propantheline. Most of these methods are lengthy and complex, requiring multiple extractions with one or more organic solvents before the dye–propantheline complex is measured by visible or fluorescence spectroscopy.

Propantheline has also been measured in plasma and urine by gas chromatography–mass spectroscopy (6); however, this requires highly expensive and specialized instrumentation and is unsuitable for routine assay of the drug in dosage forms.

A method for assaying propantheline by reversed-phase, high-performance liquid chromatography (HPLC) is reported here. The assay requires minimal sample preparation and has the ability to codetermine xanthene-9-carboxylic acid, a major degradation product of propantheline.

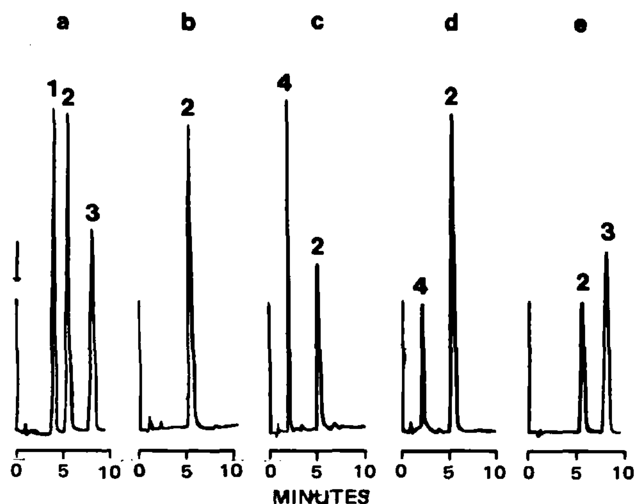

Figure 1—*Representative chromatograms for the HPLC assay of: (a) methantheline bromide, propantheline bromide, and xanthene-9-carboxylic acid reference compounds; (b) propantheline bromide tablet, 15 mg (brand A); (c) propantheline bromide tablet, 15 mg (brand B); (d) propantheline bromide ampule, 30 mg (brand A); (e) propantheline bromide hydrolysis reaction, (pH 10.1, 0.05* M *carbonate buffer, room temperature, 25 min). Key: (1) methantheline bromide; (2) propantheline bromide; (3) xanthene-9-carboxylic acid; (4) unidentified peak (see text). The injection event is represented by the arrow.*

EXPERIMENTAL

Apparatus—High-performance liquid chromatography was performed on a modular system consisting of pump[1], injector[2], bonded-phase microparticulate column[3], fixed wavelength detector[4], and chart recorder[5].

Reagents—Methantheline bromide[6], propantheline bromide[6], and xanthene-9-carboxylic acid[7] were used as received. Potassium dihydrogen orthophosphate, orthophosphoric acid, sodium carbonate, and sodium bicarbonate were analytical grade. Acetonitrile and methanol, HPLC grade[8], and reagent grade water[9] were used throughout the study.

Liquid Chromatography—Phosphate buffer (0.05 *M*) was prepared by dissolving potassium dihydrogen orthophosphate (6.805 g) in 900 ml of water, adjusting to pH 2.5 with orthophosphoric acid, and making up to 1 liter with water. The mobile phase was prepared by slowly adding 400 ml of acetonitrile to 600 ml of rapidly stirred phosphate buffer. Solutions were routinely filtered[10] and vacuum deoxygenated before chromatography. The mobile phase was delivered at 2 ml/min at a pressure of 1500 psi. Injections were made with a special high-performance liquid chromatographic (HPLC) syringe[11], and the column eluent

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Model 7120, Rheodyne Inc., Berkeley, Calif.
[3] μBondapak C_{18}, Waters Associates.
[4] Model 440, Waters Associates.
[5] Omniscribe, Houston Instruments, Austin, Tex.
[6] G.D. Searle & Co., Chicago, Ill.
[7] Aldrich Chemical Co., Ltd., Gillingham, Dorset, U.K.
[8] Waters Associates.
[9] Milli-Q System, Millipore Corp., Bedford, Mass.
[10] 0.5-μm FH filter pads, Millipore Corp.
[11] 10 μl, Hamilton Corp., Reno, Nev.

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Table I—Estimates of the Precision of the Assay of Propantheline Bromide [a] and Xanthene-9-carboxylic Acid [a] by High-Performance Liquid Chromatography

Source of Variation	Propantheline Bromide		Xanthene-9-Carboxylic Acid	
	SD	*CV*, %	*SD*	*CV*, %
Between-day	0.0116	1.1	0.0148	0.8
Within-day	0.0121	1.2	0.0230	1.2
Total	0.0168	1.6	0.0273	1.4

[a] 15 mg/10 ml.

was monitored at 254 nm with the detector set at 0.05 or 0.1 aufs. Chromatograms were obtained with the recorder set for a 10-mV input and a chart speed of 2.5 mm/min. All chromatography was conducted at ambient temperature (~22°).

Analytical Procedure—*Calibration Standards*—Methanolic stock solutions (100 mg in 10 ml) of propantheline bromide, methantheline bromide, and xanthene-9-carboxylic acid were prepared daily. Working standards were prepared by pipetting 3, 2, 1, and 0.5 ml of the propantheline and xanthene-9-carboxylic acid stock solutions into four 10-ml volumetric flasks. One milliliter of methantheline bromide stock solution was added to each flask and the solutions were adjusted to volume with methanol. Thus, the working standards contained 30, 20, 10, and 5 mg of both propantheline bromide and xanthene-9-carboxylic acid in 10 ml, with 10 mg of methantheline bromide as the internal standard. These standards were analyzed by HPLC. Calibration graphs were constructed daily by plotting the ratios of the peak heights of both propantheline and xanthene-9-carboxylic acid to methantheline *versus* the weights of propantheline and xanthene-9-carboxylic acid in the standards. The calibration data were analyzed by standard, linear least-squares regression methods.

Quality Assurance—For determination of the propantheline bromide content of tablets, the following procedure was followed: A 15-mg tablet of propantheline bromide was pulverized and a 10-ml solution of methanol containing 10 mg of the internal standard was added. The solution was stirred for at least 15 min and filtered or centrifuged to obtain a particle-free solution. One microliter of the solution was injected onto the liquid chromatograph. Alternatively, intact tablets may be dissolved in 0.1 *M* hydrochloric acid or an aqueous buffer of <pH 5.0 containing the internal standard. For the assay of freeze-dried ampules of propantheline bromide (30 mg), 1 ml of methanol (containing 20 mg of internal standard) was injected into the vial. The solution was dissolved by shaking, and 0.5 ml was withdrawn and diluted to 10 ml with methanol. One microliter was injected.

Assay Precision—Methanolic stock solutions of both propantheline bromide and xanthene-9-carboxylic acid (75 mg in 5 ml) and the internal standard (50 mg in 5 ml) were prepared. Four replicate dilutions of the stocks were made in methanol so that each contained 15 mg of both propantheline bromide and xanthene-9-carboxylic acid and 10 mg of the internal standard in 10 ml. The four replicates were analyzed by HPLC, as described previously. The entire procedure was repeated daily, for 5 days, using fresh mobile phase each day. The data were treated statistically using one-way ANOVA.

Propantheline Hydrolysis—One hundred microliters of a methanolic stock solution of propantheline bromide (15 mg/10 ml) was dried under a stream of air and reconstituted in 1.0 ml of carbonate buffer (0.05 *M*, pH 10.1). A sample (1.0 μl) was withdrawn after 25 min and injected into the liquid chromatograph.

RESULTS AND DISCUSSION

Figure 1 shows chromatograms obtained following the HPLC analysis of propantheline bromide, methantheline bromide, and xanthene-9-carboxylic acid reference compounds, commercially available propantheline bromide tablets and ampules, and a propantheline hydrolysis reaction. Retention times of 4.1, 5.4, and 8.3 min were obtained for methantheline, propantheline, and xanthene-9-carboxylic acid, respectively, as seen in Fig. 1(a). For each compound, sharp, symmetrical peaks were obtained with baseline resolution and minimal tailing. Methantheline bromide served as an ideal internal standard, since it has similar spectral and chromatographic properties to propantheline. Interestingly, methantheline bromide and propantheline bromide belong to the quaternary nitrogen class of compounds, the members of which often require the presence of heptane- or pentanesulfonic acid counterions in the mobile phase for reversed-phase HPLC analysis. With the present method, paired-ion chromatography is unnecessary for the assay of propantheline bromide. The minimum detectable amount of propantheline bromide and xanthene-9-carboxylic acid was ~100 ng, although this could be decreased to ~2 ng using a 50-μl injection with the detector set to 0.01 aufs.

The chromatograms in Fig. 1(b, c) were obtained during the analysis of two commercially available brands of propantheline bromide tablets (15 mg). The peak height obtained with brand A (Fig. 1b) was similar to that obtained when a standard solution of propantheline bromide (15 mg/10 ml) was assayed. However, the results in Fig. 1(c) indicate that only half the nominal amount of the drug was present in the brand B tablets. Repeated assays of tablets from different batches of this preparation confirmed that the propantheline content was consistently <15 mg. Furthermore, an extra peak with a retention time of 2 min appeared in these chromatograms, indicating the presence of a breakdown product or impurity. As seen in Fig. 1(d), a peak with similar retention time appeared when propantheline bromide ampules (brand A) were assayed, although the height in this case was considerably less than found in the brand B tablets. While the identity of the unknown peak is yet to be established, it is interesting to note that all products tested were from new stock.

A previous study (7) has shown that propantheline is hydrolyzed to xanthene-9-carboxylic acid in aqueous solutions of pH >5.0. In this regard, the present assay may be useful when the stability of propantheline is under investigation, particularly since both the substrate and hydrolysis product can be measured in a single chromatographic run. To support this, the results in Fig. 1(e) show the decrease in propantheline concentration and appearance of xanthene-9-carboxylic acid following exposure of propantheline bromide (15 mg/10 ml) to pH 10.1 carbonate buffer for 25 min at room temperature.

The analysis of standard solutions of propantheline bromide and xanthene-9-carboxylic acid gave calibration graphs which were linear from 5 mg/10 ml to at least 30 mg/10 ml. The average of five replicate sets of standards (one replicate per day) gave calibration graphs of $y = 0.066x + 0.026$ ($r > 0.999$) for propantheline and $y = 0.122x + 0.052$ ($r > 0.999$) for xanthene-9-carboxylic acid, where y is the peak height ratio and x is the analyte concentration. Methanolic stock solutions of methantheline bromide, propantheline bromide, and xanthene-9-carboxylic acid were stable for at least 3 weeks when stored at room temperature.

The precision of the assay was assessed by analyzing solutions of propantheline bromide and xanthene-9-carboxylic acid, four times daily, on each of 5 days using new stock solutions and mobile phase each day. We chose a solution of 15 mg in 10 ml, since this is the nominal concentration obtained when a 15-mg propantheline bromide tablet is assayed by the recommended procedure. Estimates of the day-to-day, within-day, and total precision of the assay were calculated from ANOVAs of the data as described previously (8). These results appear in Table I. Coefficients of variation for the assay were determined from the mean peak height ratio for all assays of propantheline (1.021) and xanthene-9-carboxylic acid (1.903) and the appropriate standard deviation. It is preferable to use an internal standard in the assay, since the injection volume (1 μl) is less than the maximum volumes accommodated by commercially available sample loops. If samples >1 μl are to be injected, it is recommended that the samples be diluted appropriately, otherwise nonlinearity in the calibration graphs may result. Moreover, if the internal standard is not used, it is advisable that at least two duplicate injections be made per sample.

In conclusion, the present assay provides a rapid, specific, and precise method for the simultaneous determination of propantheline bromide and xanthene-9-carboxylic acid by HPLC.

This novel assay would be useful for the routine, quality assurance of propantheline and for assessing the stability and reaction kinetics of the drug.

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COMMUNICATIONS

Assessment of 75/75 Rule: FDA Viewpoint

Keyphrases □ Bioavailability—studies involving subjects with intersubject coefficient of variation, assessment of 75/75 Rule, FDA viewpoint □ Bioequivalence—bioavailability studies involving subjects with intersubject coefficient of variation, assessment of 75/75 Rule, FDA viewpoint.

To the Editor:

Recently, the new 75/75 specification proposed by the FDA for Bioequivalency Studies came under criticism (1) as being scientifically invalid and unpredictable in bioavailability studies involving subjects with intersubject coefficient of variation (*CV*) of 60% and intrasubject *CV* of 20–30%. Similar criticism also has been launched at the FDA for application of the 75/75 Rule in establishing an *in vitro–in vivo* correlation. Although the FDA does not disagree with the calculated results on hypothetical problems in the published article and the application of the Pittman–Morgan test when appropriate, the authors of the proposed FDA rule do disagree with the underlying assumptions of the author, *i.e.*, that such large variations are the norm in bioequivalency studies.

It has been observed by the FDA that for the large majority of drugs for which bioavailability bioequivalence data are submitted as part of a New Drug Application, the coefficient of variation is generally <40%, assuming that a properly validated analytical assay is employed. To substantiate the latter claims, a review of FDA reports over a 5-year period by a primary FDA contractor (2) is summarized in Table I. The FDA review reveals that the coefficients of variation among 106 total drug products involving 12 drug entities were generally well within the 40% range. Only in the case of diphenhydramine (Table I) was

Table I—Summary of Bioavailability Studies [a]

Drug	Number of Products Tested	*CV*, % (C_{max})[b]	*CV*, % (*AUC*)[b]	Number of Products with *CV* >40%
Phenytoin	a. 6	14–33	16–45	1
	b. 6	19–24	19–26	0
Meprobamate	a. 6	6–21	12–26	0
	b. 6	15–24	22–37	0
Chlorothiazide	6	—	30–46[c]	1
Acetazolamide	4	19–36	17–30	0
Propylthiouracil	6	16–23	16–29	0
Warfarin	5	10–20	9–13	0
Griseofulvin	a. 6	21–40	14–26	0
	b. 6	27–46	24–30	1
Diphenhydramine	a. 6	14–54	35–67	4
	b. 6	36–48	40–62	5
Tolbutamide	7	12–20	18–24	0
Phenobarbital	6	16–23	21–34	0
Sulfisoxazole	a. 6	5–13	7–16	0
	b. 6	15–25	20–32	0
Trichlormethiazide	a. 5	30–35	26–35	0
	b. 5	17–25	11–26	0

[a] Studies performed under FDA Contract 223-77-3011. The total number of drug products tested was 106. The total number of drug products exceeding 40% *CV* was 12. [b] Range of *CV* values for peak plasma level (C_{max}) and area under the curve (*AUC*). [c] Range of *CV* for total cumulative urinary excretion.

Table II—Proportion of 1000 Simulated Studies with 12 Hypothetical Drugs Meeting 75/75 Rule [a]

Drug	N[b]	Intersubject *CV*: *CV* TP[c]	Intersubject *CV*: *CV* RP[d]	Intrasubject *CV*	Proportion of 1000 Studies Meeting 75/75 Criterion: p[e] = 0	$p = 0.3$	$p = 0.4$	$p = 0.5$	$p = 0.6$	$p = 0.7$	$p = 0.8$	$p = 0.9$
Case 1	24	60	60	30	0.15	0.19	0.22	0.26	0.30	0.35	0.43	0.52
Case 2	24	40	40	30	0.72	0.78	0.79	0.81	0.84	0.86	0.88	0.90
Case 3	24	60	40	30	0.29	0.32	0.34	0.36	0.38	0.39	0.41	0.43
Case 4	12	60	60	20	0.24	0.29	0.32	0.35	0.40	0.47	0.54	0.66
Case 5	12	40	40	20	0.55	0.63	0.66	0.69	0.73	0.79	0.82	0.88
Case 6	12	60	40	20	0.38	0.44	0.45	0.47	0.51	0.53	0.56	0.59
Case 7	12	30	30	15	0.74	—	—	0.87	—	0.91	—	0.98
Case 8	12	15	15	13	1.00	1.00	—	1.00	—	1.00	—	1.00
Case 9	12	30	15	13	0.84	—	—	0.86	—	0.88	—	0.89
Case 10	12	30	30	10	0.68	—	—	0.86	—	0.94	—	0.99
Case 11	12	15	15	10	1.00	1.00	—	1.00	—	1.00	—	1.00
Case 12	12	30	15	10	0.80	—	—	0.86	—	0.89	—	0.91

[a] Portion of Table II, *i.e.*, drugs 1–6, published by Haynes (1) drugs 7–12 was generated by Dr. Haynes at the request of Dr. Purich for presentation at the 1981 International Industrial Pharmacy Conference, Austin, Tex. [b] N is the number of subjects. [c] TP is the test product. [d] RP is the reference product. [e] Correlation coefficients between *AUC* values for test and reference products in the same individual.

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