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A stability-indicating proton nuclear magnetic resonance spectroscopic method for the analysis of propantheline bromide in pharmaceutical samples

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A rapid, specific and accurate proton nuclear magnetic resonance (^{1}H NMR) spectroscopic method was developed for the simultaneous quantitative analysis of propantheline bromide and its degradation product, xanthanoic acid, in bulk materials and tablets. 1,3,5-Trinitrobenzene served as an internal standard and deuterochloroform was used as the solvent for the analytical samples. The quantities of propantheline bromide and xanthanoic acid were calculated on the basis of the integrals for signals of the methine proton of propantheline at 5.09 ppm, the methine proton of xanthanoic acid at 4.99 ppm, and the aromatic protons of the internal standard at 9.39 ppm. The accuracy of the method was established through the analysis of synthetic mixtures containing the parent compound, its degradation product and the internal standard. An excellent agreement was verified between the assay results and the quantities of the various compounds in the mixtures. The mean ± SD recovery values for propantheline bromide and xanthanoic acid from a set of 10 synthetic mixtures were 99.6 ± 0.8% and 98.9 ± 1.8%, respectively. The assay of 10 lots of commercial propantheline bromide tablets by ^{1}H NMR spectroscopy indicated drug and degradate contents in the ranges 97.1–99.8% and 0.1–0.9%, respectively. In addition, the proposed analytical method was found suitable for detecting the formation of xanthanoic acid from propantheline bromide in aqueous media in concentrations below 0.1% of that of the parent compound.

1. Introduction

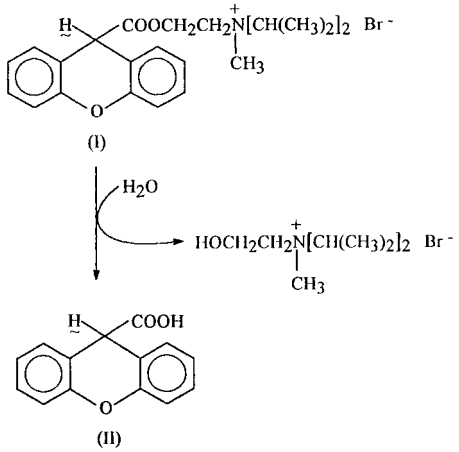
Propantheline bromide, (2-hydroxyethyl)diisopropylmethylammonium bromide xanthene-9-carboxylate, is a synthetic quaternary ammonium compound which, by virtue of its anticholinergic properties, has been used to control gastrointestinal hypermotility in cases of irritable bowel syndrome, to diminish gastric acid secretion in patients afflicted with peptic ulcers, and to suppress ureteral and bladder spasms during urinary incontinence [1–3].

After its oral administration, propantheline bromide undergoes extensive hydrolysis within the gastrointestinal tract and the liver to inactive metabolites such as xanthanoic acid (xanthene-9-carboxylic acid) and (2-hydroxyethyl)diisopropylmethylammonium bromide.

In the presence of traces of moisture, propantheline bromide (I) may hydrolyze in solution and in the solid state to xanthanoic acid (II), an alcoholamine and xanthene [4] (Scheme). The rate of this hydrolysis has been related by empirically derived mathematical equations to the prevalent temperature and humidity and to the concentration of degradate present [4, 5]. When in solution, the breakdown of propantheline bromide is also found to depend on the existing pH and on the concentration of neutral salt present [6–8]. Because of the intrinsic instability of their active component, commercial tablets of propantheline bromide need to be stored in tightly sealed containers, below 30 °C, protected from moisture, and for not more than two years from the date when they were manufactured [3].

Analytical methods currently available for the assay of propantheline bromide in pharmaceutical samples have relied on the measurement of either the organic cationic component [9–28] or the bromide counterion [29–31]. The first approach has been based on the use of spectrophotometry in the ultraviolet [9–11] or visible [12, 13] spectral region, a combination of gravimetry and polarography [14], amperometry in a flowing stream [15], a selective electrode [16], titrimetry in the potentiometric [17], oscillographic [18] or zero-current oscillopotentiometric [19] mode, and HPLC [4, 7, 20–28]. Quantification of the bromide ion, on the other hand, has been carried out by atomic absorption spectrometry [29], x-ray fluorescence [30], and with a bromide-selective electrode [31]. Furthermore, the detection and measurement of degradation products of propantheline bromide have been largely accomplished by HPLC [4, 7, 20–26].

Scheme



The purpose of the present report is to describe the development and validation of a simple and specific stability-indicating ^{1}H NMR spectroscopic assay method for propantheline bromide as a drug substance and in commercial tablets. In addition to its ability to identify and measure xanthanoic acid, the main degradation product of propantheline bromide, the proposed method offers the added advantage of not requiring the use of pure reference standards as is the case with other analytical techniques for the same purpose.

2. Investigations, results and discussion

Fig. 1 shows the ^{1}H NMR spectrum of propantheline bromide in $CDCl_3$. From this spectrum, the following reso-

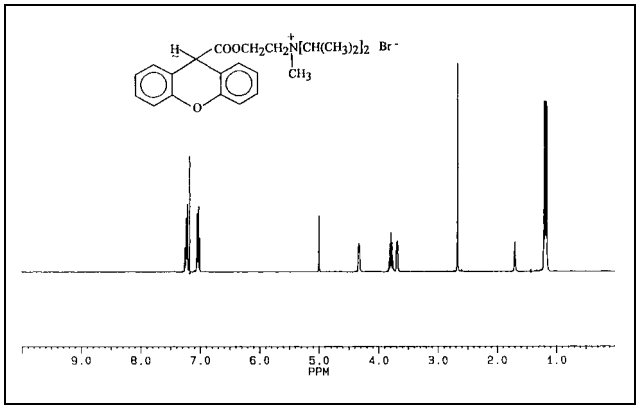


Fig. 1: 400.13 MHz ^{1}H NMR spectrum of propantheline bromide in $CDCl_3$

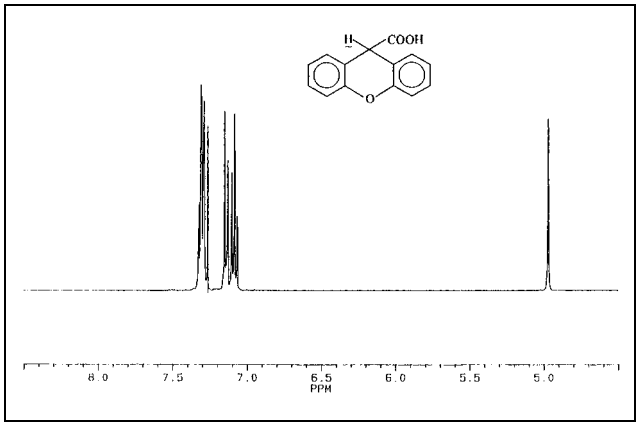


Fig. 2: 400.13 MHz ^{1}H NMR spectrum of xanthanoic acid in $CDCl_3$

nance signals were identified: (a) two unresolved doublets centered at 1.25 ppm and 1.28 ppm representing the twelve methyl protons of the two isopropyl groups; (b) a singlet at 2.74 ppm due to the three $N-CH_3$ protons; (c) two distorted triplets centered at 3.76 ppm and 4.41 ppm due to the two $>N-CH_2$-protons and two $-OCH_2$-protons, respectively; (d) a heptet centered at 3.85 arising from the $-CH$-protons of each of the two isopropyl groups; (e) a singlet at 5.09 ppm from the methine proton at C-9 in the xanthene ring; and (f) two multiplets centered at 7.11 ppm and 7.33 ppm integrating for the eight aromatic protons of the xanthene ring.

Hydrolysis of the ester linkage of propantheline bromide can take place slowly in the solid-state under appropriate conditions of humidity and temperature, and more readily in an alkaline aqueous medium [4–6]. In either case, the products are xanthanoic acid and an alcoholamine (Scheme). The appearance of xathanoic acid was ascertained from the singlet at 4.99 ppm (due to the methine proton at C-9 of the xanthene ring) and the two mutliplets centered at 7.10 ppm and 7.30 ppm (from the aromatic protons). The ^{1}H NMR spectrum of an authentic sample of xanthanoic acid is shown in Fig. 2.

As a test for accuracy, the proposed method was used to analyze a set of ten synthetic formulations made from known quantities of propantheline bromide, xanthanoic acid and the internal standard. From the results summarized in Table 1, it was concluded that the accuracy of the method was maintained at the various levels of the analytes present and the fixed amount of internal standard added. The amount of xanthanoic acid was calculated taking into account the intensity of the resonance for its lone methine proton at C-9 and that of the parent compound. In this regard, although the lowest level of xanthanoic acid tested was 0.1%, visual assessment of the signal intensity for this compound indicated the possibility of detecting much lower concentrations. Overall, the mean $\pm$ SD% recovery of propantheline bromide was $99.6 \pm 0.8\%$ (range 98.5–100.7%) and that of xanthanoic acid $98.9 \pm 1.7\%$ (range 97.9–102.8%).

No apparent changes in composition were noted after allowing solutions of propantheline bromide, xanthanoic acid and internal standard in $CDCl_3$ to stand at ambient temperature for up to 14 days. In contrast, propantheline bromide decomposed in aqueous solutions at a rate and to an extent that were related to both the pH and temperature of the solution. Thus, at about 100 °C hydrolysis was complete in 10 min at a pH of 11.0, and it required about 30 min at a pH of 7.0. In contrast, a very limited breakdown (~3%) occurred at pH 2.0 after 3 h of heating. Fig. 3 depicts the time course of the hydrolysis of an aqueous solution of propantheline bromide of pH 7.0 that had been heated for up to 1 h on a boiling water bath. Fig. 4 represents the ^{1}H NMR spectrum of an aqueous solution of propantheline bromide that had become hydrolyzed to xanthanoic acid after standing at 80 °C for 1 h. In all instances, the xanthanoic acid that had formed was extracted quantitatively into $CHCl_3$.

Ten lots of commercial tablets of propantheline bromide were assayed by ^{1}H NMR spectroscopy for their contents

Table 1: Results of the assay of propantheline bromide and xanthanoic acid in synthetic mixtures by ^{1}H NMR spectroscopy[a]

Sample No.	Internal standard added[b] (mg)	Propantheline bromide			Xanthanoic acid[c]		
		Added (mg)	Found (%)	Recovery (%)	Added (mg)	Found (%)	Recovery (%)
1	0.79	4.05	3.99	98.5	0.5000	0.4895	97.9
2	0.79	4.56	4.51	98.9	0.4375	0.4305	98.4
3	0.79	5.54	5.55	100.2	0.3750	0.3675	98.0
4	0.79	6.07	6.02	99.2	0.3125	0.3066	98.1
5	0.79	6.47	6.46	99.2	0.2500	0.2453	98.1
6	0.79	7.04	7.06	100.3	0.1875	0.1839	98.1
7	0.79	7.51	7.56	100.6	0.1250	0.1285	102.8
8	0.79	8.03	8.09	100.7	0.0625	0.0613	98.0
9	0.79	8.51	8.42	98.9	0.0500	0.0506	101.2
10	0.79	9.02	8.94	99.1	0.0375	0.0368	98.1
Mean				99.6			98.9
SD				0.8			1.7

[a] Recoveries were calculated from (100 × amount found)/amount added
[b] Added from a 37.1 μM solution of TNB in $CDCl_3$ (100 μl)
[c] Added from an 11.1 μM solution of xanthanoic acid in $CDCl_3$

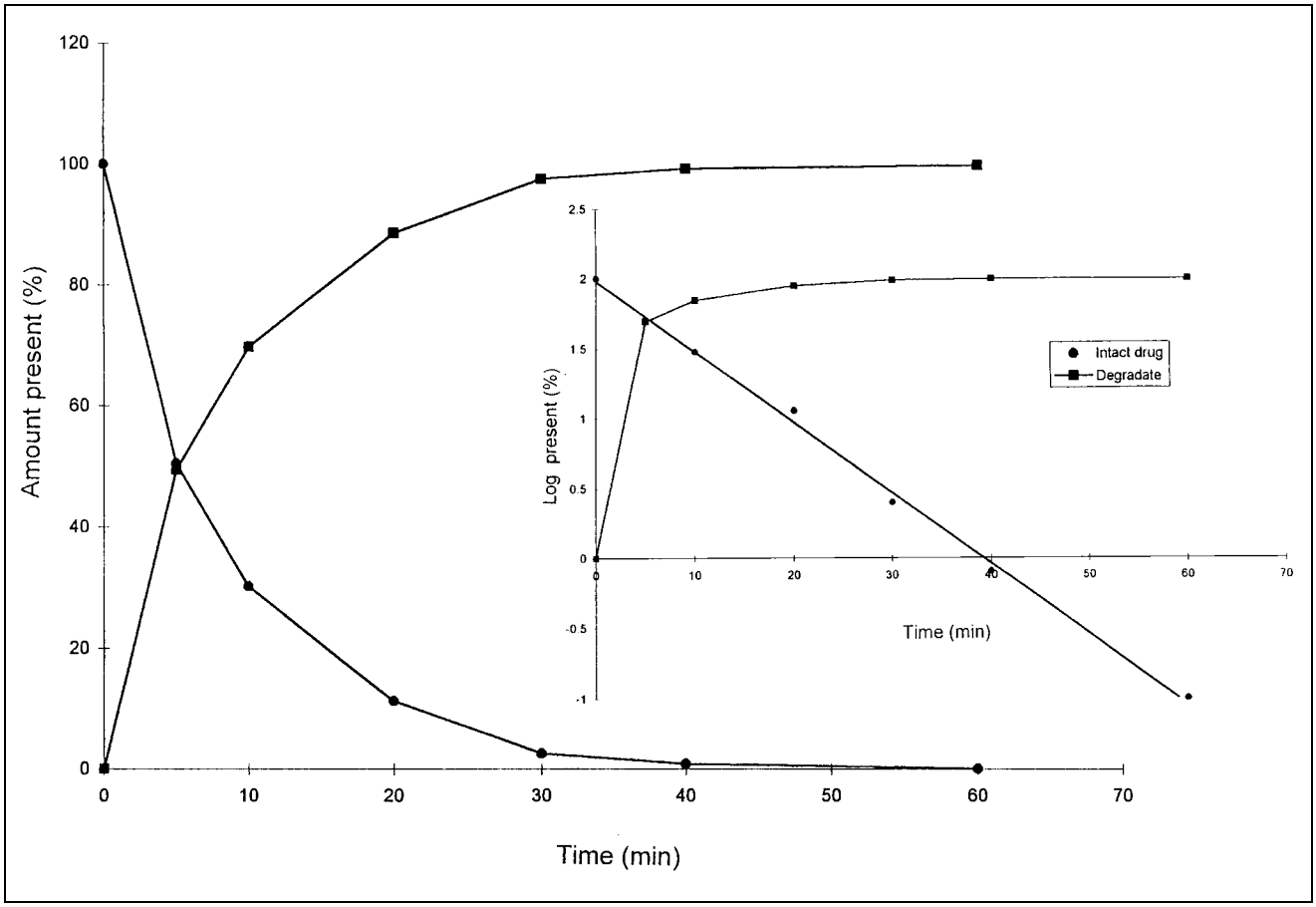


Fig. 3: Linear and logarithmic (inset) plots for the time course hydrolysis of propantheline bromide to xanthanoic acid in water at 100 °C. The equation for the logarithmic line representing decomposition was $y = -0.505x + 1.9808$ ($r^2 = 0.9974$)

in active component and in xanthanoic acid. The results of this study are summarized in Table 2. Whereas the amounts of propantheline bromide found ranged from 97.1 to 99.8% of declared, those of xanthanoic acid ranged from 0.1 to 0.9%. These values met the requirements set by USP 24 [20] for the active ingredient (i. e., not less than 90.0% and not more than 110.0% of the labeled amount of $C_{23}H_{30}BrNO_3$) and for xanthanoic acid (i. e., not more than 1.0% of the sample weight).

As a conclusion, it can be stated that the ^{1}H NMR spectroscopic method described in this report will permit the identification and assay of propantheline bromide in the presence of xanthanoic acid and vice versa in a simple, accurate and specific manner. In addition to obviating the need for separate analytical approaches or for different experimental conditions for each of the analytes, this method does not rely on the use of pure reference standards.

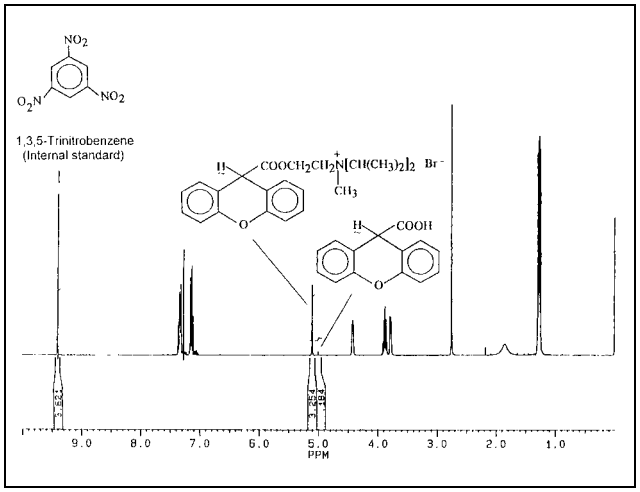


Fig. 4: 400.13 MHz ^{1}H NMR spectrum in $CDCl_3$ of a sample of propantheline bromide that had been stored for 1 h inside an oven maintained at 80 °C. A very small amount of xanthanoic became detectable under these conditions

Table 2: Results of the assay of propantheline bromide and xanthanoic acid in propantheline bromide (15 mg) tablets by ^{1}H NMR spectroscopy

Sample No.	Propantheline bromide	Xanthanoic acid
	Amount found (% of declared)	Amount found (% of sample weight)
1	99.5	0.7
2	99.8	0.5
3	97.1	0.9
4	98.2	0.5
5	97.3	0.3
6	98.7	0.3
7	99.7	0.1
8	98.9	0.2
9	99.8	0.5
10	98.6	0.4
Mean	98.8	0.44
Range	97.1–99.8	0.1–0.9
SD	0.99	0.24

3. Experimental

3.1. Apparatus

All ^{1}H NMR spectra were obtained on a model AM-400 spectrometer operating at 400.13 MHz (Bruker Instruments, Inc., Billerica, MA) and 28 °C. For each spectrum, 320 individual scans were collected at a relaxation delay of 10 s, a data point resolution of 0.215 Hz/point, and omitting the without function. The chemical shifts were referenced to $CHCl_3$, taken as 7.26 ppm.

3.2. Materials

Deuterochloroform ($CDCl_3$, 99.8 atom% D, stabilized with Ag foil), tetramethylsilane (TMS) and 1,3,5-trinitrobenzene (TNB, >99.9%) were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Propantheline bromide USP reference standard was from U.S. Pharmacopeial Convention, Inc. (Rockville, MD, USA). Xanthanoic acid was from Sigma Chemical Co. (St. Louis, MO, USA).

3.3. Samples

Propantheline bromide bulk material and tablets (15 mg/tablet) were obtained from local commercial sources.

3.4. Standard solutions

Stock solutions of xanthanoic acid (2.5 mg/ml) and TNB (7.9 mg/ml) were prepared in $CDCl_3$, transferred to glass vials, and immediately crimper-sealed with Teflon-coated rubber septa and aluminum seals. When needed, these solutions were withdrawn through the septa by means of a liquid-tight microliter syringe.

3.5. Assay procedure

Synthetic formulations, containing propantheline bromide, xanthanoic acid, and TNB, were prepared by adding an accurately weighed quantity of propantheline bromide to a 5 mm NMR tube, followed by appropriate aliquots of the xanthanoic acid and TNB stock solutions. The final volume of the mixture was adjusted with $CDCl_3$ to 0.75 ml, after which the NMR tube was capped with a Teflon cap, and inverted several times to effect solution. The tube was placed in the NMR spectrometer, to obtain the spectrum. The sample for the analysis of propantheline bromide bulk material was prepared as described for the synthetic formulations, after taking an accurately weighed amount of powder equivalent to 5 mg of the active compound. To analyze commercial propantheline bromide tablets, a group of 20 tablets was accurately weighed, placed in a glass mortar, and ground to a fine powder. An accurately weighed portion of the powder, equivalent to 5 mg of active ingredient, was transferred to a 2 ml polypropylene microtube with a snap cap, and mixed with 0.65 ml of $CDCl_3$ and 0.1 ml of TNB solution. After capping the microtube, its contents were sonicated for about 1 min, and next centrifuged at 3000 rpm for 5 min. The clear supernatant was transferred to a 5 mm NMR tube, which was then placed in the spectrometer.

The quantity of propantheline bromide and its impurity were calculated by measuring the intensity of the signals for the C-9 methine proton of the drug (5.09 ppm, singlet), C-9 methine proton of xanthanoic acid (4.99 ppm, singlet), and aromatic protons of the internal standard (9.39 ppm, singlet), and using the following equations:

$$\text{Propantheline bromide (mg)} = [A_p/A_t] \times [EW_t/EW_p] \times M_t$$

$$\text{Xanthanoic acid (\%)} = 100 \times [A_x/A_p] \times [EW_p/EW_x]$$

where A_p is the integral value of propantheline bromide, A_t is the integral value of the internal standard, A_x is the integral value of xanthanoic acid, EW_p is the formula weight of propantheline bromide divided by the number of absorbing protons (i. e., 448.40/1 = 448.40), EW_t is the formula weight of the internal standard divided by the number of absorbing protons (i. e., 213,11/3 = 71.04), EW_x is the formula weight of xanthanoic acid divided by the number of absorbing protons (i. e., 226.08/1 = 226.08), and M_t is the amount of internal standard added, mg.

3.6. Stability studies

Solutions of propantheline bromide, xanthanoic acid and TNB in $CDCl_3$ (~ 1 mg/ml) were placed in individual screw-capped amber glass vials, and allowed to stand at room temperature (22 ± 1 °C) for 14 days. From each sample, a ^{1}H NMR spectrum was obtained on days 1 and 14, and the spectra compared with each other for any possible changes. In another study, a stock solution of propantheline bromide was prepared in distilled water to contain ~ 1 mg/ml. Aliquots of this stock solution (~ 5 ml) were placed in individual screw-capped amber glass vials, and their pH adjusted to either 2.0, 7.0 or 11.0 with HCl or sodium carbonate. After capping, the vials were placed in a boiling water bath, and periodically checked spectroscopically for any evidence of xanthanoic acid formation. For this purpose, the contents of one vial was cooled under running water, and then extracted into $CHCl_3$. After evaporation of the $CHCl_3$ using a stream of dry nitrogen, the residue was dissolved in $CDCl_3$. This solution was transferred to an NMR tube, mixed with internal standard solution, and then analyzed as described.

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Received February 21, 2001
Accepted March 15, 2001

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