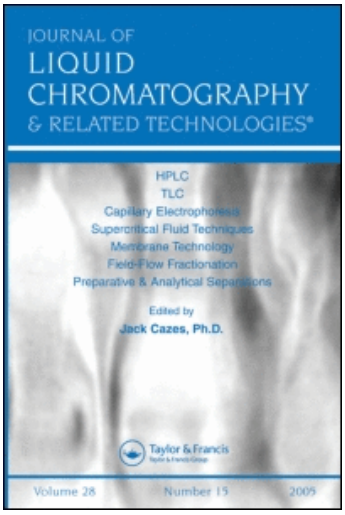

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DETERMINATION OF PROPANTHELINE BROMIDE IN TABLET FORMULATIONS BY REVERSE-PHASE HPLC

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ABSTRACT

The reported reverse-phase HPLC method for the determination of propantheline bromide, xanthanoic acid, xanthone, and 9-hydroxy-propantheline bromide in tablets is fast, sensitive, specific, accurate and reporoducible. Methyl xanthanoate is used as internal standard. The total elution time is 6 min. The method is stability-indicating since it can determine the degradation products. The column utilized was suplecosil LC-8 (5 micron), 250 mm x 4.6 mm i.d. The mobile phase was 0.03 M solution of ammonium acetate in acetonitrile: water: THF (60:38:2); the pH was adjusted to 4.5 with acetic acid, the detection was at 254 nm. A wavelength of 248 nm was used to quantitate xanthanoic acid and the flow rate was 1.5 mL/min. The proposed HPLC method was verified for linearity, accuracy, precision, and applicability.

INTRODUCTION

Propantheline bromide (I) is a quaternary ammonium anticholinergic agent with peripheral effects. It is used in the treatment of gastric duodenal ulcer, and to relieve spasm of the lower gastro-intestinal tract (1).

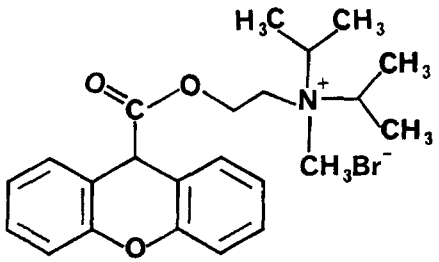
In recent times, little attention has been given to the development of assays to measure propantheline in dosage forms. The compendial USP XXI method (2) and BP method (3) for the analysis of the drug are time-consuming requiring several extractions followed by non-aqueous titration. The measurement of propantheline by direct UV assay is not selective for the drug with the probability of interferences 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 (4,5) and in pharmaceuticals (6). 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 (6).

Propantheline has also been measured in plasma and urine by gas chromatography-mass spectroscopy (7); however, this requires highly expensive and specialized instrumentation and is unsuitable for routine assay of the drug in dosage forms.

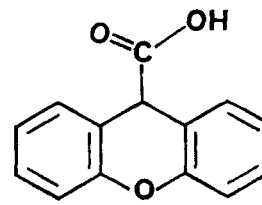
An HPLC assay (8) has been reported for the analysis of propantheline and its degradation product xanthanoic acid (II).

The present paper describes a reverse- phase HPLC method for the simultaneous determination of I,II, xanthone (III) and a major impurity that has been identified in sample tablets by Ford et al

(9) as 9-hydroxypropantheline (IV).The assay has been applied to a commercial product and proved to be free of interferences from excipients normally used in tablet formulations. The elution time was less than six minutes. The assay is fast since it requires only little sample preparation.



I



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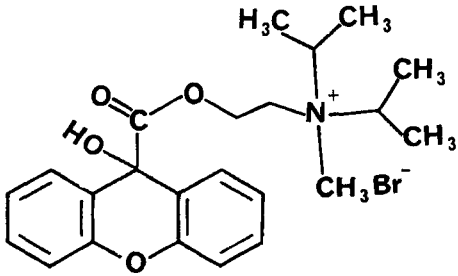
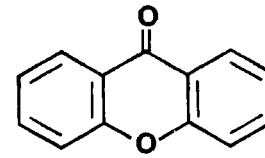


IV



III

EXPERIMENTAL

Materials

Glacial acetic acid, methanol HPLC-grade, and acetonitrile HPLC-grade (Koch-Light) were 99.0%,99.8%, and 99.8%, respectively. Tetrahydrofuran (THF) (Ferak) and ammonium acetate (BDH) were 99.0% and 96.0%respectively. The water used was always distilled and deionized.

The active ingredient and degradation products; I (Siegfried, Switzerland), methantheline bromide, an alternative internal standard, (USP standard). II and III, purum grade (kindly supplied by Fluka) and were recrystallized from 99.0% ethanol (Merck). IV was kindly supplied from NBSL (National Biological Standards Laboratory; Canberra, Australia). The internal standard utilized (methyl xanthanoate) was prepared as described by Ford et al (9). Pro-Banthine tablets (Searle) were purchased locally. The excipients used in the interference study were kindly supplied by Al-Hikma Pharmaceuticals, Amman, Jordan.

Apparatus- The apparatus employed was Varian 5000 LC HPLC system equipped with a 10-µL manual loop injector (Valco instruments Co., Houston, Texas, U.S.A) connected to spectrophotometric detector UV-50 (Varian Associates, Palo Alto, CA, U.S.A.) and Spectra-Physics 4100 digital integrator (Spectra Physics, San Jose', CA., U.S.A.)

Chromatographic Conditions- A reverse phase LC-8 column (5µ) (250 x 4.6 mm i.d.) from Supelco (Switzerland) was utilized at ambient temperature. The mobile phase was prepared by dissolving 2.32 g of ammonium acetate in 600 mL acetonitrile and 20 mL of THF, the volume is then completed to one liter with water. The pH was adjusted to 4.5 with glacial acetic acid. The mobile phase was always filtered through 0.45-µ membrane filters (Supelco). The mobile phase was always degassed by vacuum prior to use. The flow rate was 1.5 mL/min. The wavelength was 254 nm and the sensitivity was set at 0.10 AUFS. For the assay of II, λ =248 nm and the sensitivity was 0.02 AUFS. The chart speed was 0.50 cm/min.

Study Of The Interferences Of Placebo Excipients- A mixture of the following excipients usually incorporated in such a formulation: starch(50 mg), calcium carbonate (50 mg), Iron(III) oxide (2 mg), gelatin (10 mg), acacia (5 mg), lactose (60mg) magnesium carbonate (20 mg),magnesium stearate (1 mg), sodium saccharin (0.5 mg), sugar (50 mg), titanium oxide (5 mg), talc (10 mg),avicel (20 mg), Polyvinylpyrrolidone (3 mg), tartrazine (0.1 mg), sunset yellow FCF (0.1 mg), brilliant blue FCF (0.1 mg), Indigo carmine (0.1 mg), and Erythrosine BS (0.1 mg) were dissolved and treated in the same manner as the sample solution. Ten-µL injections were made under the chromatographic conditions described.

Preparation of Standard Solutions:

Internal Standard Solution- A 6.2 mg of methyl xanthanoate was dissolved in 250 mL methanol.

Standard Solution of I- Fifteen mg of I were accurately weighed and dissolved in 10 mL of the internal standard solution and 0.5 mL of this solution was diluted to 5 mL with the internal standard solution.

Standard Mixture Solution of II, III, and IV- A solution containing 0.025 mg/mL of II, 0.020 mg/mL of III, and 0.025 mg/mL of IV was prepared by dissolving 5.0, 4.0, and 5.0 mg of II, III, and IV, respectively, in 10 mL of the internal standard solution followed by successive dilutions in internal standard solution to obtain the above concentrations. This solution was utilized for stability study only.

Standard Solution of II for HPLC Analysis- A 0.009 mg/mL solution of II was prepared by dissolving 0.9 mg of II in 10 mL of methanol and 0.5 mL of the solution was diluted to 5 mL with methanol.

It is important to mention that xanthanoic acid (II) was determined by the external standard method due to its very low concentration and working at high sensitivity of 0.02 AUFS & 248 nm.

Standard Solutions for Linearity- Standard solutions of I, II, III, and IV were prepared by proper dilution in the internal standard solution to obtain different concentrations in the range of: 0.05-0.2 mg/mL, 4.3×10^{-4} - 1.70×10^{-3} mg/mL, 6.3×10^{-4} - 2.5×10^{-3} mg/mL, and 1.25×10^{-2} - 5.0×10^{-2} mg/mL, for I, II, III, and IV respectively. Linearity study for I,III and IV was carried at 254 nm, and for II, the study was performed at 248 nm.

Preparation Of The Sample Solutions:

Propantheline Bromide Assay- Twenty tablets (one tablet if content uniformity was to be determined) were weighed and powdered. Accurately weighed portions of the powder (each equivalent to one tablet containing 15 mg of I) were dissolved in 10 mL of the internal standard solution. The solution was sonicated for 5.0 minutes, filtered through 0.45-μ membrane filter, then 0.5 mL was diluted to 5 mL with the internal standard solution and 10-μL injections were made.

Xanthanoic Acid Assay- Accurately weighed portions of the powder (each equivalent to one tablet containing 15 mg of I) were

dissolved in 10 mL of methanol. The solution was filtered through 0.45-μ membrane filter and 10-μL injections were made.

Percent Recovery Study- This study was performed by adding 3.8 mg of the standard of I to accurately weighed portions of the tablet powder equivalent to 0.25, 0.75, 1.00, and 1.25 tablets. The resulting mixtures were assayed and the results obtained were compared with the expected ones. The active ingredient concentration levels covered were in the range of 50-150% of the expected assay values. In order to obtain 50% of the expected assay value (i.e. 7.55 mg), one fourth of the powdered tablet was mixed with 3.80 mg of standard I , and to obtain 150% of the assay value (i.e. 22.55 mg), a portion of the powdered tablets equivalent to 18.75 mg of I (1.25 Tablet) was mixed with 3.80 mg of standard of I.

Assay Method- Equal volumes (10-μL) and approximately equal concentrations of freshly prepared standard and sample solutions were injected into the HPLC and chromatographed under the conditions described above. The standard and sample solutions contained the same concentrations of the internal standard. The quantity of each component injected was always within the linearity range.

Calculations- The results of I were calculated using response ratios (RR) relative to internal standard based on peak areas.

$$\text{Percent of the label claim found} = \frac{RR_{sa}}{RR_{st}} \times 100$$

Where RR_{sa} = sample response ratio; RR_{st} = standard response ratio .

It is useful to mention that the concentration of the standard solution of I is exactly equal to the theoretical concentration of the sample solution based on label claim.

The results of II were calculated based on peak height ratio of the sample to external standard solution:

$$\text{mg of II/tablet} = \frac{\text{Peak height of II (sample)}}{\text{Peak height of II (standard)}} \times \text{Concentration of standard} \times \text{dilution factor.}$$

The results of III & IV were calculated using the internal standard method as follows:

$$\text{mg of III or IV/tablet} = \frac{RR_{sa}}{RR_{st}} \times \text{concentration of standard} \times \text{dilution factor.}$$

RESULTS AND DISCUSSION

A preliminary study for optimization of mobile phase parameters was conducted. The effects of acetonitrile composition, pH, and ionic strength on the capacity factor (k') were studied (Figures 1-3). The capacity factor (k') values for I, II, III, and the internal standard were affected by the variation of acetonitrile composition in the mobile phase (Figure 1). At high acetonitrile composition, all four compounds gave very sharp peaks and very close to each other with low k' values; this means that the resolution was inadequate. Lowering acetonitrile composition below 55%, the peaks became broader with larger retention times. The 60% acetonitrile composition was selected as optimum composition since

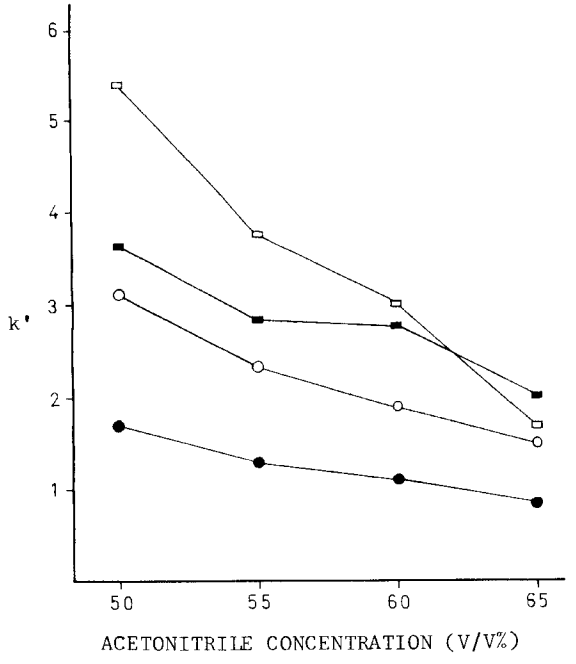


Figure 1. Plots of the capacity factor versus the acetonitrile Composition in the mobile phase. Key: ●, II; o, III; ■ I; □, internal standard.

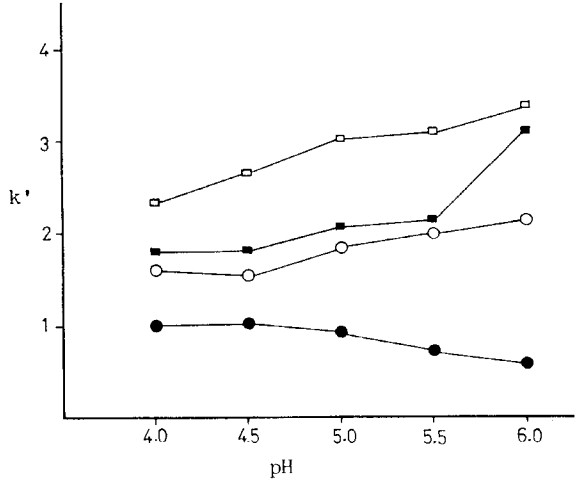


Figure 2. Plots of the capacity factor versus the pH of the mobile phase. Key: ●, II; o, III; ■, I; □ , internal standard.

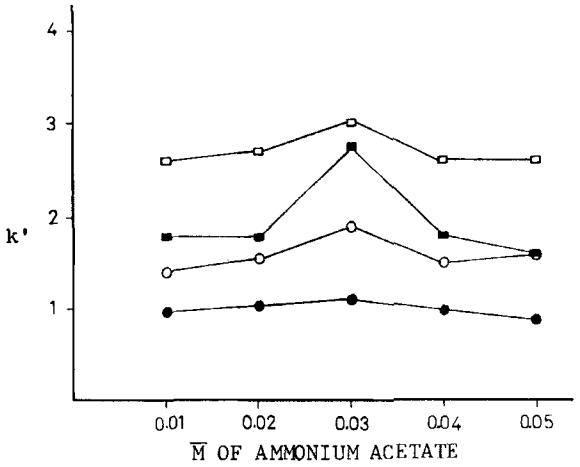


Figure 3. Plots of the capacity factor versus molarity of ammonium acetate in the mobile phase Key: ●, II; o, III; ■ , I; □ internal standard.

it provided baseline separation and sharp peaks in a reasonable retention time.

Variation of pH (Figure 2) yielded maximum k' values for I, III, and the internal standard at pH 6.0. However, k' values for II decreased with increasing pH due to the increased solubility of the xanthanoic acid. At lower pH values, k' for II increased to value >1.0 with some interference between the peaks of internal standard and III at pH 4.0. Therefore, a pH 4.5 was selected as optimum pH at which baseline separation was achieved in a reasonable time.

Variation of the ionic strength yielded optimum k' values with 0.03 M ammonium acetate (Figure 3). Therefore the optimum composition of the mobile phase used was 0.03 M solution of ammonium acetate in

acetonitrile: water: THF (60:38:2), the pH was adjusted to 4.5 with acetic acid. The THF (2%) in the mobile phase was added to reduce peak tailing.

To determine the linearity of the detector response, calibration standard solutions of I-IV were prepared as previously described in the text. A plot of peak area ratio vs. amount injected was linear up to 2.0 µg for I, 17.0 ng for II, 25.0 ng for III, and 0.5 µg for IV with a correlation coefficient of 0.999 or better.

A study of interferences shown in Figure 4 supports the specificity of the HPLC method since the peaks of excipients in the chromatogram are eluted at the solvent front.

To determine the accuracy of the method, recovery study was conducted by standard addition method and subjected to HPLC analysis. In all cases, excellent recoveries and reproducibility of peak heights and areas were obtained. A linear regression of the data shows excellent linearity with a slope of about 1 over the analysis range studied (Table I). No interference due to excipients was detected in the chromatograms produced. The detection limits based on signal-to-noise of 2 were 150, 2.5, 2, and 25 ng for I, II, III, and IV, respectively.

The chromatograms shown in Figure 5 and 6 indicate the possibility of separation of I, II, III, and IV using methantheline bromide as internal standard (Figure 5) and using methyl xanthanoate

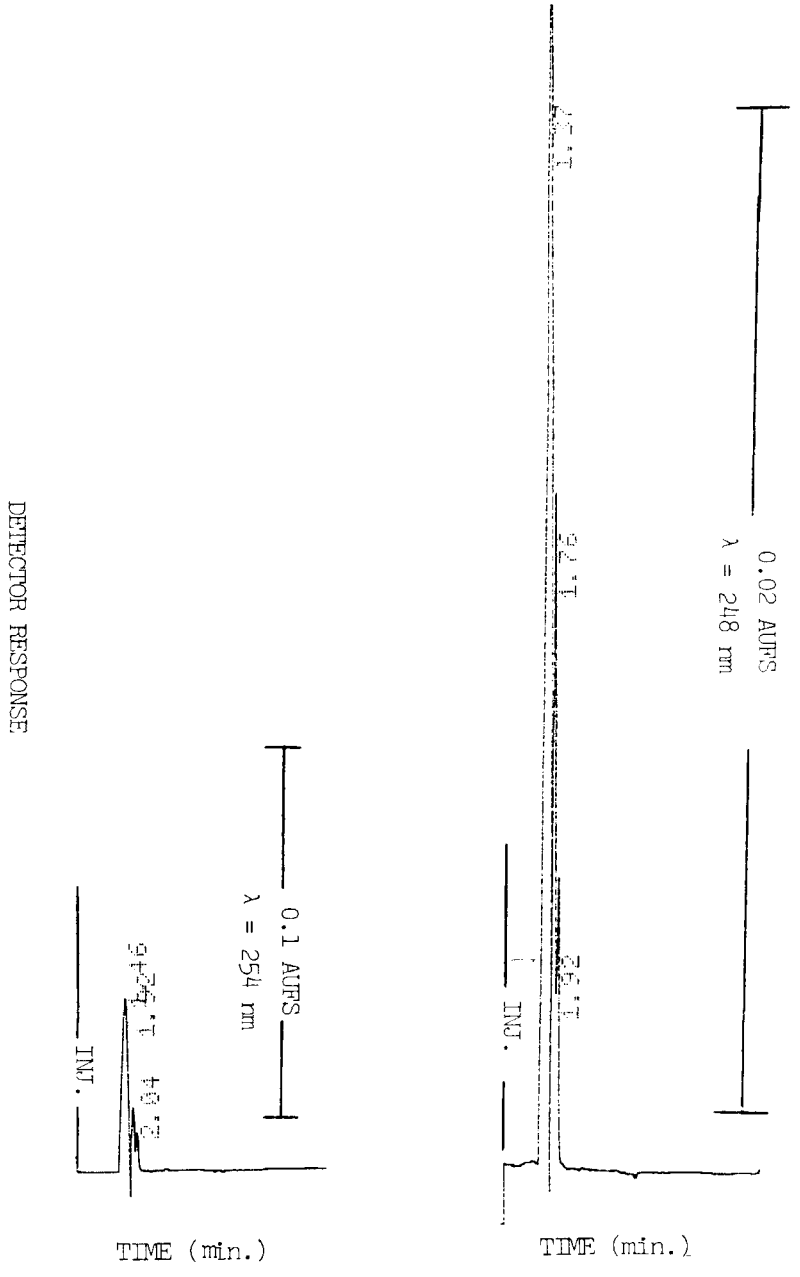


Figure 4. Typical chromatograms for a placebo mixture.

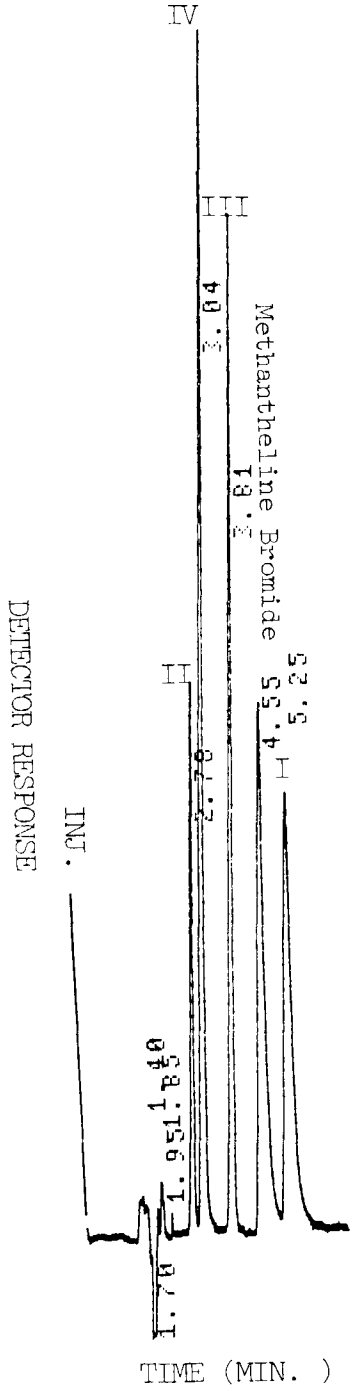


Figure 5. A typical chromatogram of a synthetic mixture of I, II, III, IV, and methantheline bromide as internal standard.

as an alternative internal standard (Figure 6). The chromatograms are free of interferences from excipients normally used in tablet formulations as it is clear from the content uniformity chromatograms (Figure 7). Figure 8 shows chromatograms of the assay of II. The total elution time is less than 6 min.

The results of analysis of the commercial product (Pro-Banthine) for I and II (Table II) indicate that the proposed assay can be used for the quantitation of I and II. The accuracy of the HPLC method is further supported by the closeness of the results in Table II to the label claim. The higher results obtained for II by HPLC can be attributed to the fact that the assay does not require extraction, thus no possible loss is involved. Furthermore, this indicates that the HPLC method is more sensitive in detecting degradation products than the USP XXI.

The precision of the HPLC method is confirmed by the very small relative standard deviation (RSD) shown in table I and II.

The specificity of the method is further confirmed by comparing the results of content uniformity of I which were performed on a commercial product using the HPLC and the spectrophotometric USP XXI methods (Table III). The lot passes the specifications of the content uniformity by both methods. However, the USP XXI method is not specific which would lead to higher deviation if the product contained excipients or degradation products that interfere spectrophotometrically.

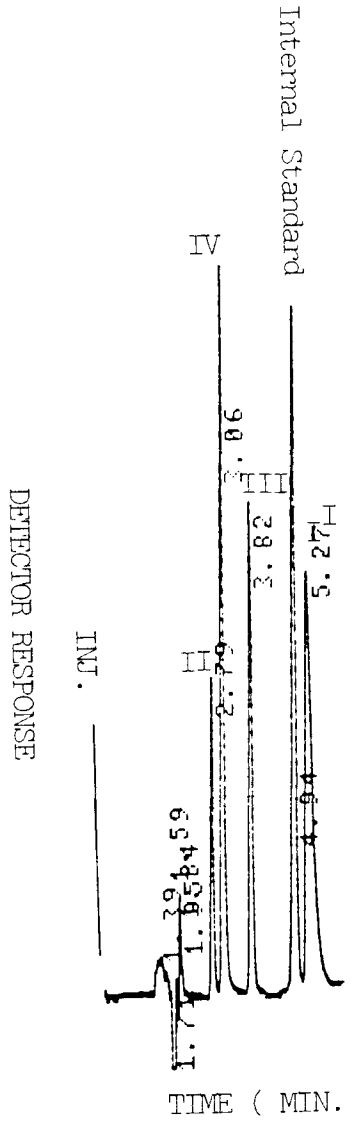


Figure 6. A typical chromatogram of a synthetic mixture of I, II, III, IV, and methyl xanthanoate as internal standard.

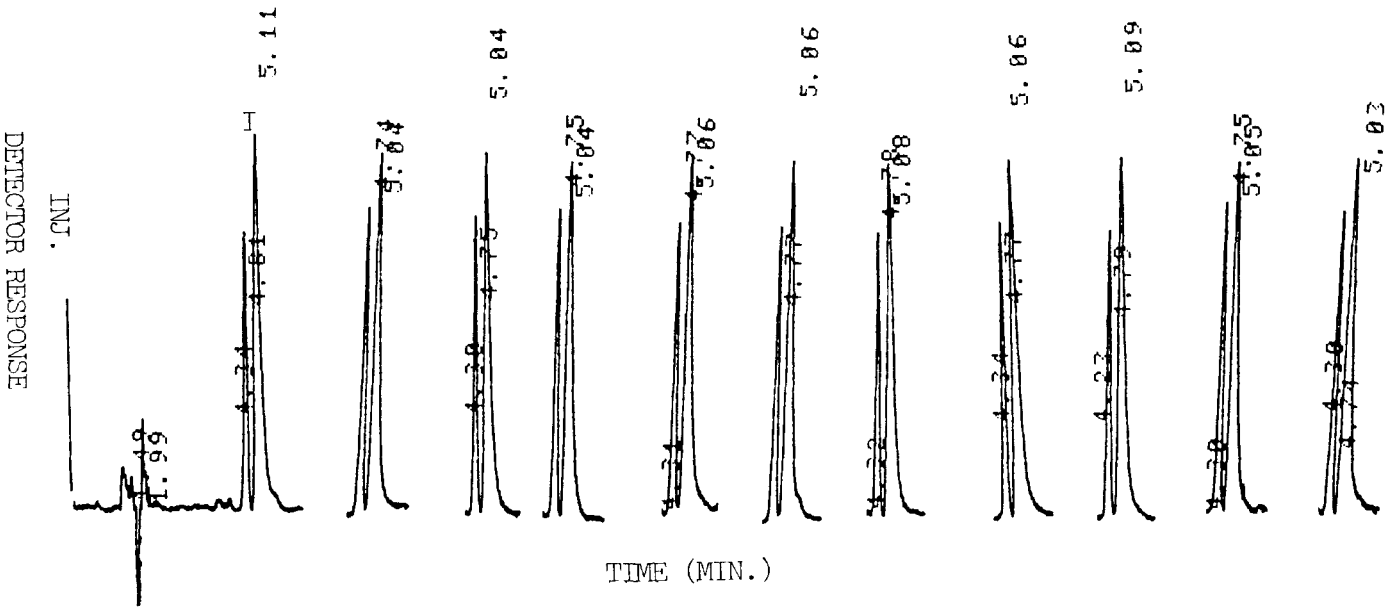


Figure 7. Content uniformity chromatograms for 10 tablets of Pro-Banthine.

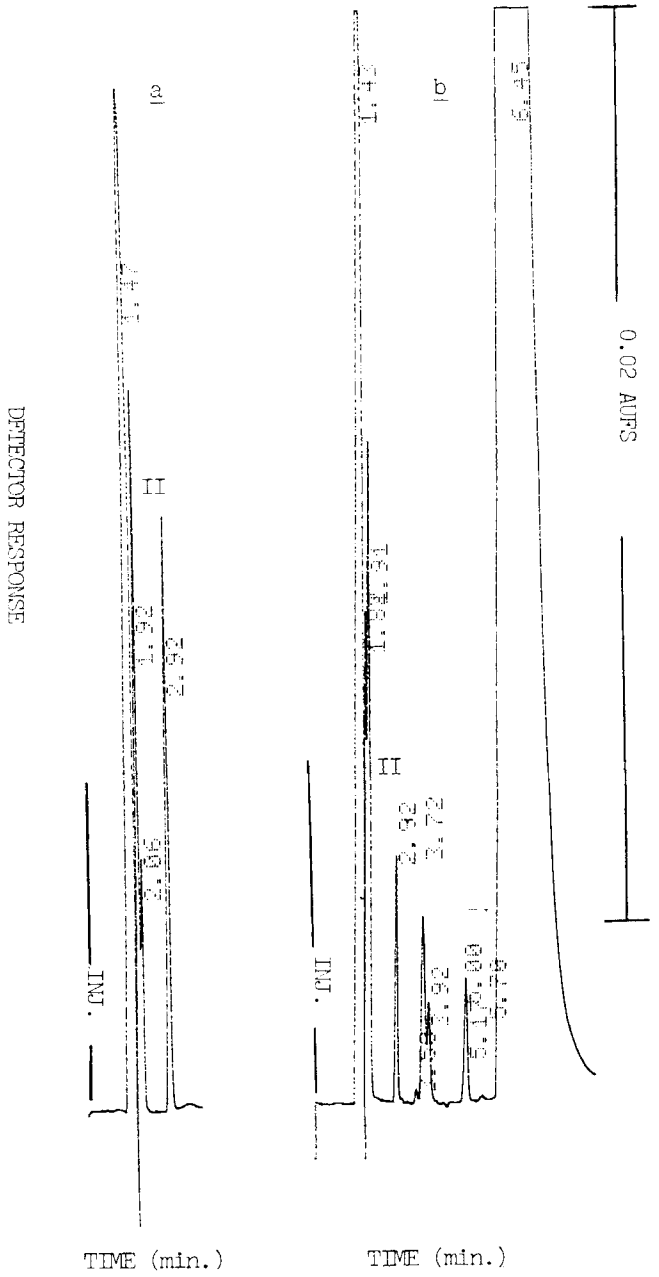


Figure 8. a. A typical chromatogram of a standard 10-µL injection containing 0.09 µg xanthanoic acid (t_R= 2.92 min.)

b. A chromatogram for one tablet of Pro-Banthine sample/ 10 mL methanol under the same conditions.

Table I: Recovery of Propantheline Bromide by the Standard Addition Method.

Total mg Present	mg Found[a]	% Recovery[a]
7.55	7.62 ± 1.25	101.1 ± 1.2
11.30	11.44 ± 0.75	101.2 ± 0.7
15.05	15.16 ± 0.73	100.7 ± 0.7
18.80	18.34 ± 1.01	97.6 ± 1.0
22.55	22.19 ± 0.82	98.3 ± 0.8
Slope = 0.9611	Intercept = 0.4859	R^b = 0.9996

a. Mean ± RSD for 6 determinations.

b. R is the correlation coefficient.

Table II: Assay Results for Propantheline Bromide and Xanthanoic Acid By HPLC and USP XXI

Percent Label Claim Found			mg/tablet	
	Propantheline Bromide[a]		Xanthanoic Acid[a]	
Sample	HPLC	USP XXI	HPLCx10^3	USF XXIx10^3
one	100.0±1.8	98.9	25.3±1.3	3.2
two	99.8±0.5	99.6	29.4±3.4	2.9
three	100.1±1.3	99.2	22.3±1.8	4.1
four	98.7±1.3	98.9	22.3±3.3	3.7
five	99.3±1.2	99.2	25.6±3.1	2.1
	99.8±0.8[b]	99.2±0.3[c]	25.2±2.7[e]	3.2±0.8[d]

a. Pro-Banthine lot (349).

b. Mean ± RSD for 5x6 determinations (five samples, each injected 6 times)

c. Mean ± RSD for 5 determinations

d. Mean ± SD for 5 determinations

e. Mean ± SD for 5x6 determinations

Table III: Content Uniformity For Propantheline Bromide In Commercial Tablets (Lot 349)

Tablet	Percent Label Claim Found	
	HPLC	USP
1.	107.0	102.7
2.	105.9	106.7
3.	99.6	102.0
4.	95.7	97.3
5.	96.1	104.7
6.	102.8	102.4
7.	101.1	97.3
8.	94.7	92.7
9.	104.9	104.7
10	99.0	107.3
Mean	100.7	101.8
RSD	4.4	4.6
High	107.0	107.3
Low	94.7	92.7

Table IV: Stability of Propantheline Bromide Tablets.[a]

Period Elapsed, Days	mg I	mg II	mg III	mg IV
0	15.0	———	———	———
3	12.2±0.2	0.110±0.003	0.026±0.001	0.430±0.012
6	11.6±0.2	0.150±0.005	0.051±0.004	0.670±0.019
10	11.1±0.1	0.069+0.004	0.130±0.006	1.800±0.072
15	9.0±0.1	0.066±0.001	0.180±0.004	3.400±0.080
20	7.5±0.1	———	0.241±0.003	4.400±0.034

[a] Mean ± SD for 2x3 determinations.
(two samples, each injected three times).

The stability study was conducted to determine I, II, III, and IV by placing samples in tubes in a humid atmosphere at 70°C. Three samples were withdrawn at the intervals indicated and were assayed. The data shown in Table IV indicate the capability of the assay to determine all four compounds. It is important to note that the total number of milligrams of I,II,III, and IV is less than the initial milligrams of I due to the loss of the quaternary ammonium part of I (which is not detected) on hydrolysis. It is also worth to note the considerable increase of IV with time indicating that IV is one of the degradation products of I. The same compound was reported by Vose et al (10) as a phenolic metabolite of I and by Ford et al (9) as a major impurity in some tablets.

In conclusion,the HPLC assay described here has been shown to be applicable to commercially available products. The method is accurate, precise, rapid, and easy to perform. It is of stability-indicating capability, easily applied for the determination of degradation products and commonly found impurities.

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