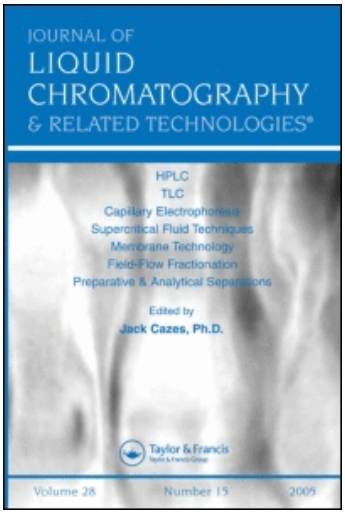


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Prifinium Bromide in Six Pharmaceutical Formulations by Reverse-Phase Hplc

S. I. Sa'sa[a]; I. M. Jalal[b]; H. S. Khalil[a]
[a] Chemistry Department, Yarmouk University, Irbid, Jordan [b] Al-Hikma Pharmaceuticals, Amman, Jordan

To cite this Article Sa'sa, S. I. , Jalal, I. M. and Khalil, H. S.(1988) 'Determination of Prifinium Bromide in Six Pharmaceutical Formulations by Reverse-Phase Hplc', Journal of Liquid Chromatography & Related Technologies, 11: 2, 447 — 462
To link to this Article: DOI: 10.1080/01483918809349951
URL: http://dx.doi.org/10.1080/01483918809349951

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(2), 447-462 (1988)

DETERMINATION OF PRIFINIUM BROMIDE IN SIX PHARMACEUTICAL FORMULATIONS BY REVERSE-PHASE HPLC

S. I. Sa'sa[1], I. M. Jalal[2], and H. S. Khalil[1]

[1]*Yarmouk University*
Chemistry Department
Irbid, Jordan
[2]*Al-Hikma Pharmaceuticals*
P. O. Box 182400
Amman, Jordan

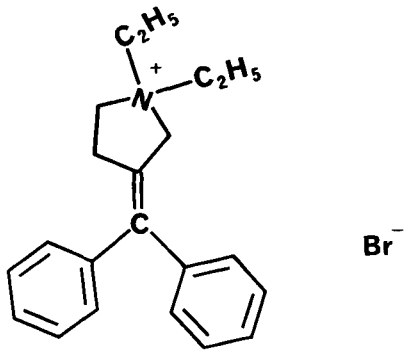
ABSTRACT

Prifinium bromide is an anti-cholinergic drug, available commercially in various pharmaceutical formulations such as tablets, suppositories, syrups, and ampoules. The present available analytical methods are time-consuming and range from non-aqueous titration to UV-spectrophotometry to ion-pair visible spectrophotometry. The method reported here is a fast, reliable, and stability-indicating reversed phase HPLC for prifinium bromide in its various pharmaceutical formulations. The mobile phase was 0.03 M ammonium acetate in acetonitrile : water (65:35); the pH was adjusted to 4.0 with glacial acetic acid. The column utilized was (250 mm x 4.6 mm i.d.) Supelcosil LC-8-DB (5μ) and detection was carried at 254 nm. Benzophenone was used as internal standard. The assay was applied to commercial products and the results expressed in (% label claim ± RSD) are (99.58 ± 0.36), (100.50 ± 0.40), (99.95 ± 0.70) , (99.94 ± 0.29), (100.28 ± 0.52), and (99.99 ± 0.57) for six commercial formulations. The method was tested for linearity, recovery, and specificity and was found fast, stability-indicating, and free from interferences. The method can be extended to separate

potential combinations as prifinium bromide-lorazepam and prifinium bromide-chlordiazepoxide in the presence of 2-amino-5-chlorobenzophenone.

INTRODUCTION

Prifinium bromide (Riabal) is a quaternary ammonium compound possessing anticholinergic properties (1) which has been used for some years in the treatment of gastro-enteritis, gastro-duodenal ulcer, irritable colon syndrome, and other disorders. Riabal is available commercially in various pharmaceutical formulations such as tablets, suppositories, syrups, and ampoules. Each formulation requires certain method for its analysis. Being a quanternary ammonium compound, and UV-absorber , prifinium bromide can be analyzed by various methods which range from non-aqueous titration (2) to UV-spectrophotometry to ion pair visible spectrophotometry (3). These methods lack accuracy , specificity, and are time-consuming. The HPLC method reported here is a fast, reliable, and applicable to all of the various Riabal pharmaceutical combinations. The assay was applied successfully to all commercial products and proved to be free of interferences from excipients normally used. The elution time was found to be less than five minutes and the detection limit was less than 1.0 ng. The assay is fast since it requires only little sample manipulation. It can be extended to be applicable to some potential combinations as Riabal-Lorazepam, and Riabal-chlordiazepoxide in the presence of 2-amino-5-chlorobenzophenone.



Prifinium bromide

EXPERIMENTAL

Materials - Prifinium bromide (Riabal) was obtained from Fujisawa Pharmaceutical Co. (Osaka, Japan) (99.5%), and lorazepam, chlordiazepoxide, and 2-amino-5-chlorobenzophenone were USP reference standards (Rockville, MD, U.S.A.). Methanol and acetonitrile-HPLC grade (99.8 %) were from May and Baker Ltd. (Dagenham, England), ammonium acetate (98.0%) and glacial acetic acid (99.0 %) were from Fluka, Switzerland, and Koch Light, England, respectively; the internal standard benzophenone (99.0%) was from BDH, England. The water used was always distilled and deionized. Commercial tablets, suppositories, ampoules, syrups, and the excipients usually used in manufacturing the pharmaceutical combinations were kindly supplied by Al-Hikma Pharmaceuticals, Amman, Jordan.

Apparatus - The apparatus employed was a Varian 2010 pump (Varian, California, U.S.A.), equipped with a 10-µL manual Rheodyne

loop injector (Rheodyne, California, U.S.A.), a Varian 2050 UV variable wavelength detector and a Varian 4290 integrator.

Chromatographic Conditions - A reversed phase column (250 mm x 4.6 mm i.d.), Supelcosil LC - 8- DB (5 µm) (Supelco, Inc., Pennsylvania, U.S.A.) was utilized at ambient temperature. The mobile phase was 0.03 M ammonium acetate in acetonitrile : water (65 : 35) solution, the pH was adjusted to 4.0 with glacial acetic acid.The mobile phase was always filtered using 0.45 µm-membrane filters (Supelco, Inc.), and 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 0.2 AUFS. The chart speed was 0.25 cm/min.

Study Of The Interferences of Placebo Excipients - A mixture of the following excipients usually incorporated in such formulations : lactose , starch,polyvinyl pyrrolidone, colloidal silicon dioxide , magnesium stearate, shellac, talc, titanium oxide, gelatin, sucrose, wax, paraffin and various coloring agents were dissolved and treated in the same manner as the sample solution. Ten-µL injections were made under the chromatographic conditions described.

Preparation of The Standard Solutions:

Internal Standard Solution - Five mg of benzophenone was dissolved in 500 mL methanol to yield a concentration of 10-µg/mL.

Prifinium Bromide Standard Solution For Tablets And Suppositories - Thirty mg of prifinium bromide was dissolved in 25 mL of the internal standard solution. This was further diluted with the internal standard solution to obtain a final concentration of 60 µg/mL.

Prifinium Bromide Standard Solution For Ampoules - Fifteen mg of prifinium bromide was dissolved in 2 mL methanol and diluted to 50 mL with the internal standard solution. This was further diluted with the internal standard solution to obtain a final concentration of 60 µg/mL.

Prifinium Bromide Standard For Syrup -Since each 5.0 mL of the syrup contains 7.5 mg prifinium bromide, 7.5 mg of prifinium bromide was placed in 25 mL volumetric flask, five mL of methanol was added and then completed to volume with the internal standard solution. This was further diluted with the internal standard solution to obtain a final concentration of 60 µg/mL.

Standard Solutions For Linearity - Forty five mg of prifinium bromide was dissolved in 25 mL of the internal standard solution. The following concentrations of prifinium bromide in the internal standard solution were prepared :90, 75, 60, 45 , 30, and 15 µg/mL.

Preparation Of The Sample Solution :

Riabal Tablets and Suppositories - Twenty units (one unit if content uniformity was to be determined) were weighed and powdered.

Accurately weighed portions of the powder(each equivalent to the weight of one unit) were placed in 25 mL-volumetric flask. Each sample was sonicated for five min. with 15 mL of the internal standard solution (in case of suppositories the solution was slightly warmed) then diluted to volume with the internal standard solution. Samples were further diluted with the internal standard solution to obtain a concentration of 60 µg/mL of prifinium bromide. The solutions were filtered through 0.45 µm-membrane filters.

Riabal Ampoules -The contents of ten ampoules were mixed. Accurately measured volume of the solution (equivalent to 1.0 mL) was diluted to 25-mL with the internal standard solution, and further dilution was made with the internal standard solution to obtain a concentration of 60 µg/mL of prifinium bromide, and filtered through 0.45 µm-membrane filters.

Riabal Syrup- Five mL of the syrup (containing 7.5 mg prifinium bromide) was placed in a 25 mL volumetric flask and diluted to volume with the internal standard solution. Further dilution with the internal standard solution was made to obtain a concentration of 60 µg/mL of prifinium bromide, and filtered through 0.45 µm-membrane filters.

Percent Recovery Study - The study was performed by preparing synthetic mixtures identical to the pharmaceutical formulations and were spiked with known amounts of prifinium bromide (45.0, 37.0, 30.0, 22.5, and 15.0 mg) spanning the range of 50-150% of the expected assay

values. The resulting mixtures were assayed and the results obtained were compared with the expected results.

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

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

$$\text{Percent of the label claim found} = \frac{RR_x}{RR_s} \times 100$$

Where RR_x = sample response ratio ; RR_s = standard response ratio.

RESULTS AND DISCUSSION

Figure 1 shows the possibility of separating prifinium bromide (t_R = 3.34 min.) from the internal standard benzophenone (t_R = 4.94 min.) and the excipients. These excipients eluted with the solvent peak. The assay was found applicable to six Riabal formulations without any change (Figure 1.).

The linearity of the detector response was determined by injecting standard solution of prifinium bromide in internal standard solution as described previously in the text. The peak area ratios to the internal standard were measured and plotted vs

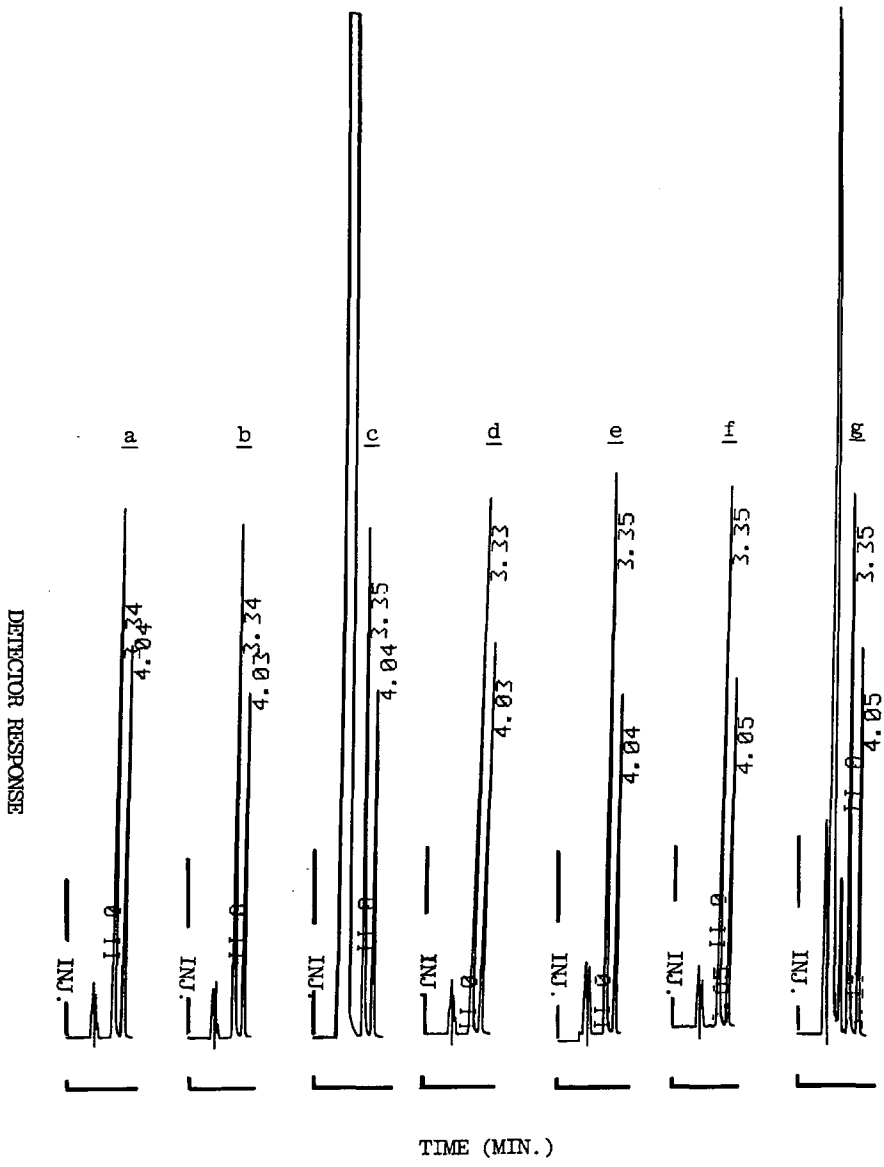


Figure 1. Typical chromatograms of 10-µL injections of prifinium bromide (t_R = 3.34 min.) and the internal standard benzophenone (t_R = 4.04 min.).
a. Standard solution ; b. Sample of Riabal 30 tablets
c. Sample of Riabal compound tablets; d. Sample of Riabal 30 suppositories; e. Sample of Riabal 60 suppositories; f. Sample of Riabal ampoules; and g. Sample of Riabal syrup.

the amount injected. The peak area ratios were found linear for the range of 0.15-0.90 μg of prifinium bromide with a correlation coefficient of 0.9995.

The accuracy of the method was determined by treating the sample by spiked placebo method, then subjecting it to HPLC analysis. In all cases, satisfactory recoveries and reproducibility of peak areas were obtained. A linear regression of the data shows excellent linearity over the analysis range studied (Table I).The selectivity of the method is validated by the fact that no interference due to excipients was detected in the chromatograms produced.

The detection limit based on signal-to-noise of 2 was less than 1.0 ng for prifinium bromide as determined by diluting a standard solution with methanol and injecting 10-μL into the column.

Table I. Recovery Of Prifinium Bromide From Spiked Placebo Samples

mg Added	mg Found[a]	% Recovery[b]
45.00	45.47 ± 0.15	101.04 ± 0.33
37.50	36.68 ± 0.10	97.81 ± 0.27
30.00	30.23 ± 0.26	100.78 ± 0.86
22.50	22.47 ± 0.08	99.85 ± 0.36
15.00	14.82 ± 0.02	98.81 ± 0.13

R = 0.9992 Intercept =-0.2700 Slope = 1.0068

[a]Mean ± SD for 6 determinations

[b]Mean ± RSD. for 6 determinations

TABLE (II)

HPLC Assay Results Of Prifinium Bromide In Commercial Products[a] (% Label Claim ± RSD)

Sample	Riabal Tablets		Riabal Suppositories		Riabal Ampoule[f]	Riabal Syrup[g]
	Riabal 30[b]	Riabal Compound[c]	Riabal 30[d]	Riabal 60[e]		
1	99.92 ± 0.09	100.26 ± 0.28	100.68 ± 0.58	99.61 ± 0.74	100.81 ± 0.39	100.18 ± 0.63
2	99.21 ± 0.55	100.28 ± 0.21	99.29 ± 0.96	100.17 ± 0.68	99.77 ± 0.49	99.35 ± 0.47
3	99.61 ± 0.65	100.96 ± 0.74	99.89 ± 0.52	100.03 ± 0.81	100.27 ± 0.97	100.45 ± 0.34
Mean	99.58	100.50	99.95	99.94	100.28	99.99
RSD	0.36	0.40	0.70	0.29	0.52	0.34

(n = 3x6 determinations)

[a] Al-Hikma Pharmaceuticals - Amman - Jordan
[b] (Lot 0386 C)
[c] (Lot 0386 A)
[d] (Lot 4352)
[e] (Lot 4348)
[f] (Lot 1400)
[g] (Lot 200)

TABLE (III)

Assay Results Of Prifinium Bromide In Commercial Products Using Various Methods (% Label Claim ± RSD)

Product[a]	HPLC[b]	Other Methods[c]
Riabal 30 Tablets	99.58 ± 0.36	97.58 ± 0.70[d]
Riabal Compound Tablets	100.50 ± 0.40	98.05 ± 0.87[d]
Riabal 30 Suppositories	99.95 ± 0.70	98.75 ± 0.78[d]
Riabal 60 Suppositories	99.94 ± 0.29	98.91 ± 0.67[d]
Riabal ampoules	100.28 ± 0.52	101.30 ± 1.68[e]
Riabal Syrup	99.99 ± 0.34	97.98 ± 2.23[e]

[a]The same Lots in Table (II).
[b]Mean ± RSD for 3x6 determinations (three samples each injected six times)
[c]Mean ± RSD for 6 determinations
[d]Non-aqueous titration.(2)
[e]Ion-pairing visible spectrophotometry (3)

The results of analysis of six commercial products (Table II) indicate the versatility of the proposed assay for the quantitation of prifinium bromide in commercial formulations. Comparison of the results of the HPLC with other methods (Table III) shows that the proposed method is more accurate and precise.

The specificity of the method is further confirmed by comparing the results of content uniformity test of prifinium bromide which were performed on four different formulations (Table IV and Figure 2). The results of content uniformity show compliance to specifications of all dosage forms and support the

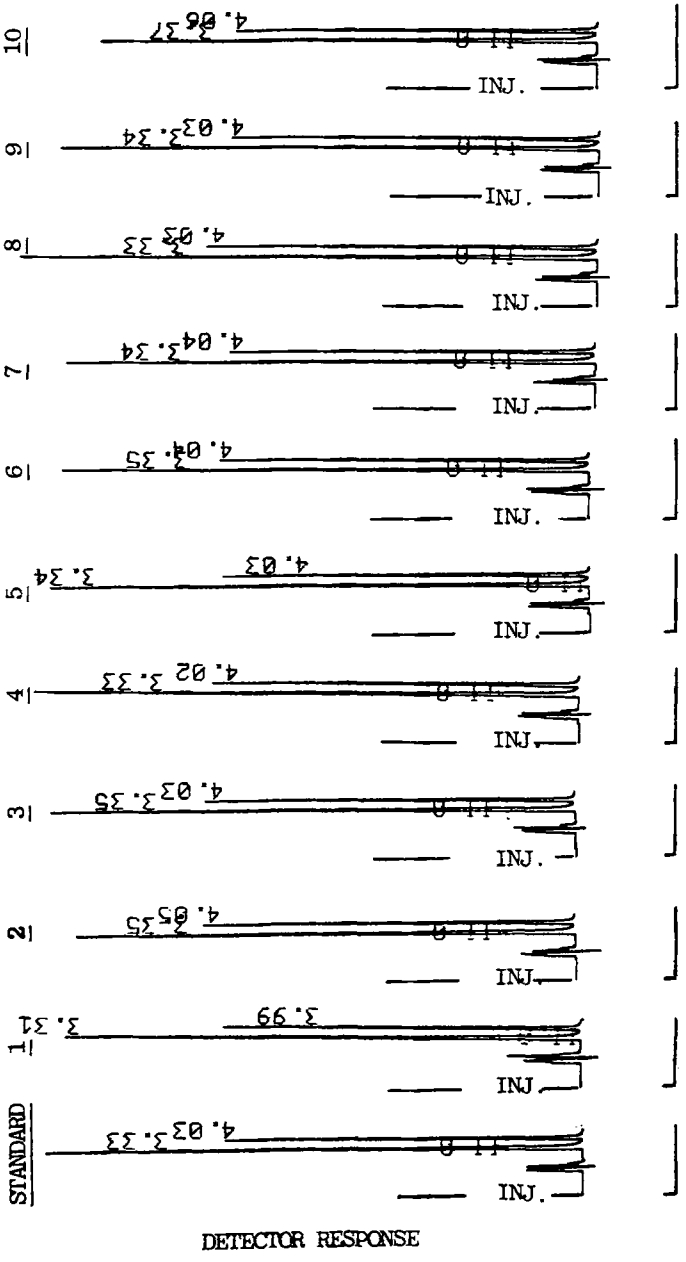


Figure 2. Content Uniformity chromatograms for 10 tablets of Riabal 30 containing 0.6 µg prifinium bromide (t_R = 3.34 min.) and 0.1 µg internal standard benzophenone (t_R = 4.04 min.).

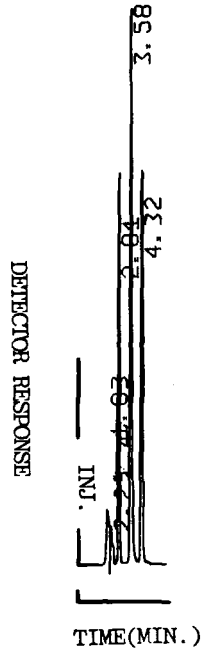


Figure 3. A typical chromatogram of a 10-μL injection of a synthetic mixture containing lorazepam (t_R = 2.01 min.), prifinium bromide (t_R = 3.58 min.), and benzophenone as internal standard (t_R = 4.32 min.).

specificity of the HPLC results. However, the HPLC method is superior to the ion-pairing and spectroscopic methods due to its being faster, simpler, and more versatile.

A stability study was performed on Riabal syrup by placing samples in water-glycerin bath at 70°C. Three samples (each contains 5.0 mL syrup) were taken and assayed. The data presented in Table (V) reveal no significant loss in activity through the study.

Table (IV)

Content Uniformity (% of Prifinium Bromide) in Commercial Riabal Products[a].

Tablet No	Riabal 30 Tablets		Riabal Compound Tablets		Suppositories			
					Riabal 30		Riabal 60	
	HPLC	Ion-Pairing	HPLC	Ion-Pairing	HPLC	Spect.	HPLC	Spect.
1	100.38	96.84	100.42	100.00	101.97	98.33	101.66	103.93
2	92.99	98.02	99.83	103.95	100.69	95.65	99.51	100.17
3	97.13	104.74	101.60	101.98	101.69	96.32	96.25	104.12
4	103.49	104.74	101.75	96.84	102.11	104.01	99.10	98.97
5	102.21	100.79	96.10	98.81	94.94	97.99	98.03	101.03
6	99.58	96.05	98.91	101.19	96.04	98.66	98.31	95.70
7	100.68	94.07	98.28	104.74	90.58	98.66	100.22	94.67
8	102.22	92.89	95.22	100.79	94.64	105.02	101.16	98.80
9	102.24	100.00	101.09	106.72	105.58	101.67	102.97	97.25
10	95.24	90.91	101.10	94.07	96.83	96.32	99.78	99.83
Mean	99.71	97.91	99.43	100.91	98.51	99.26	99.70	99.45
RSD	3.40	4.81	2.30	3.73	4.66	3.27	1.95	3.13
High	103.49	104.74	101.75	104.74	105.58	105.02	102.97	104.12
Low	92.99	90.91	95.22	94.07	90.58	95.65	96.25	94.67

[a] The same lots indicated in table (I).

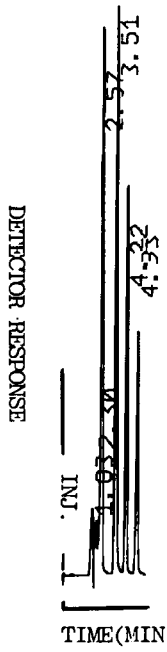


Figure 4. A typical chromatogram of a 10-µL injection of a synthetic mixture containing chlordiazepoxide (t_R = 2.57 min.), prifinium bromide (t_R = 3.51 min.), benzophenone as internal standard (t_R = 4.22 min.), and 2-amino-5-chlorobenzophenoone (t_R = 4.93 min.).

TABLE (V)

Stability Study For Prifinium Bromide In Commercial Syrup Formulation[a].

Period Elapsed, Weeks	% Prifinium Bromide[b]
1	100.36 ± 0.36
2	99.47 ± 0.78
3	99.53 ± 0.85
4	99.44 ± 0.78

[a] Al-Hikma Pharmaceuticals - Amman - Jordan

[b] Mean ± RSD for 3x6 determinations . (three samples each injected six times).

The method can be further expanded to include the analysis of some potential combinations such as (prifinium bromide with lorazepam, and prifinium bromide with chlordiazepoxide in the presence of 2- amino-5-chlorobenzophenone). Such combinations could be anticipated and separations shown in Figures (3 and 4) could be a basis for their analysis.

The assay presented here has been shown to be applicable to commercially available products. The method is quite simple, accurate , precise, rapid , versatile, and easy to perform. It is experimentally much simpler than other methods. The same chromatographic conditions can be applied to some additional separations.

ACKNOWLEDGEMENTS

The financial support provided by Ministry of Planning-Jordan, is gratefully acknowledged. The authors would also like to thank Al-Hikma Pharmaceuticals, Amman, Jordan, for providing the raw materials and standards.

REFERENCES

1. Kumada S. and Hitomi M., Jap. J. Pharmacol., 17, 90(1967).

2. British Pharmacopoeia, London Her Majesty's Stationary,. Appendix VIIIC A 89 Method I, 1980.

3. Connors K.A., A textbook of Pharmaceutical Analysis, John Wiley and Sons, New York, 1982, P. 407 .