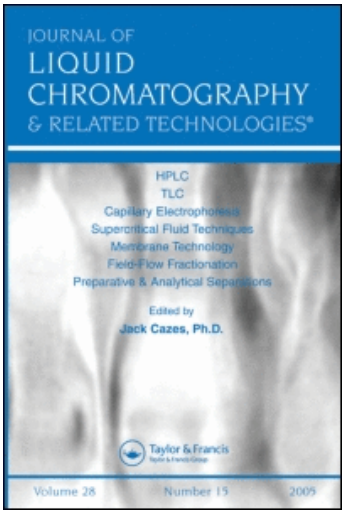

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SIMULTANEOUS DETERMINATION OF BROMVALETONE AND PROPANTHELINE BROMIDE IN TABLETS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple, rapid and accurate high-performance liquid chromatographic (HPLC) procedure was proposed for the simultaneous determination of bromvaletone and propantheline bromide in tablets using carbamazepine as an internal standard. The method employs a reversed-phase column with a mobile phase of acetonitrile/0.05M phosphate buffer pH 2.7 (40 : 60) mixture. The effluent was monitored by a UV detector at 220nm. A simple procedure to extract the active components with acetonitrile was used. The results showed that good resolution and reproducibility can be achieved. A linear relationship was found between peak height ratio and a concentration range of 5 to 30 μg/mL. By varying the active/excipient ratio, we obtained a mean recovery of 99.4% for bromvaletone and 99.2% for propantheline bromide. The chromatographic separation in this study can be completed within ten minutes.

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INTRODUCTION

Bromvaletone, [(a-bromisovaleryl)urea], is a central depressing agent used as a hypnotic or sedative for tension treatment [1]. Propantheline bromide, a widely prescribed anticholinergic drug, is used in the treatment of GI ulceration and urinary incontinence [2]. Combination dosage forms of these two drugs are commercially available in Taiwan.

In the past, pharmaceutical products or biological fluids containing propanthelin bromide were determined by conventional methods such as an extraction procedure followed by gravimetry [3] or non-aqueus titration [4], organic dye-salt partition techniques [5,6], UV spectrophotometry [7], radioactivity [8] and amperometry [9]; and colometry [10], amperometry [11] and gas-liquid chromatography (GLC) [12] were used for determining those containing bromvaletone. These procedures, often tedious and time-consuming and/or subject to interferences from the matrix of the sample, are not suitable for the simultaneous assay.

Although a number of reversed-phase HPLC methods have been developed to determine propantheline bromide in serum [13], intestinal contents [14] and in dosage forms [15-18], and bromvaletone in biological materials [19,20], a search of literatures revealed that no HPLC method has been reported for the simultaneous quantitation of these two active ingredients.

This paper describes a rapid and accurate reversed-phase HPLC method that can be used for the simultaneous determination of bromvaletone and propantheline bromide in pharmaceutical dosage forms.

MATERIALS

APPARATUS

A high-performance liquid chromatograph [21] using a variable wavelength detector [22] and an integrator [23] was used.

COLUMN

A stainless steel, 300 x 3.9mm id, packed with Nucleosil C-18 column, 10 μm particle size was used.

CHROMATOGRAPHIC CONDITIONS

The mobile phase used was a mixture of acetonitrile and 0.05M phosphate buffer, pH 2.7 (40:60 v/v). The mobile phase was filtered and degassed before use. It was pumped through the column at a flow rate of 1.5 mL/min. The detector was set at 220nm. The injection volume was 20 μL and the chromatography was conducted at ambient temperature . All solutions were filtered with a membrane filter before injection.

REAGENTS AND SOLVENTS

All solvents used were of HPLC grade and all reagents used were reagent grade. They were used without further purification. Standard materials of Bromvaletone, Propantheline bromide and Carbamazepine (internal standard) used for the preparation of the calibration graphs and recovery studies were all used as received.

SYSTEM SUITABILITY AND STANDARD CURVES

Prior to each analysis, the suitability of the HPLC system for the separation and determination was evaluated [24, 25]. The following stock solutions were prepared in acidified acetonitrile (0.6% v/v phosphoric acid) using a simple solution method:

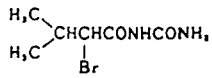
(1) Bromvaletone - 0.276 mg/mL;

(2) Propantheine bromide - 0.268 mg/mL;

(3) Carbamazepine - 0.104 mg/mL.

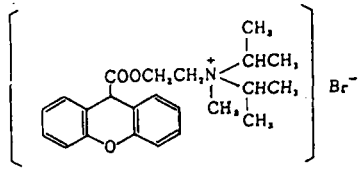
The column was equilibrated with mobile phase at a flow rate of 1.5 mL/min until a baseline was steady. When each of properly prepared solutions for the validation of the proposed method was injected into the chromatograph described above, the resolution factor (R) should be greater than 1.2. The coefficient of variation provided by six replicate injections of the standard

(A) Bromvaletone (Bromvalerylurea)



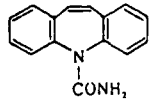
$C_6H_{11}BrN_2O_2$: 223.07

(B) Propantheline Bromide



$C_{23}H_{30}BrNO_3$: 448.40

(C) Carbamazepine



$C_{15}H_{12}N_2O$: 236.27

FIGURE 1. Chemical structure of (A) Bromvaletone: (B) Propantheline Bromide; (C) Carbamazepine.

preparation should be less than 2. The peak height ratios of drug to internal standard were plotted against their respective concentrations to obtain the standard curves.

RECOVERY STUDY

The recovery study was firstly performed by adding reference standards of brovaletone and propantheline bromide in an amount corresponding to 50, 100 and 150% of label claim to accurately weighed portions of powdered placebo mixture. After extraction, suitable amount of stock internal standard solution was added into the extracted solutions. Acetonitrile was used as solvent.

The recovery study was also performed by spiking four equal portions of the commercial tablet powder with different amounts of reference standards of bromvaletone and propantheline bromide.

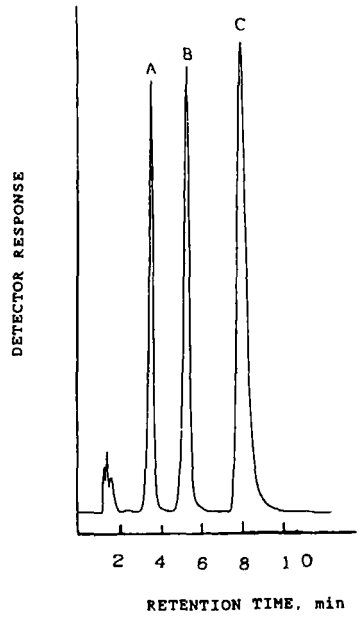


FIGURE 2. Chromatogram of a standard mixture of (A) Bromvaletone: 0.1244 mg/mL, (B) Carbamazepine: 0.0224 mg/mL, (C) Propantheline Bromide: 0.1178 mg/mL at a flow rate of 1.5 mL/min; the injection volume was 20 uL. The detector was set at 220 nm.

RESULTS AND DISCUSSION

Fig. 1 shows the chemical structures of the substances separated on the HPLC system. A typical chromatograpic run of the three compounds is seen in Fig. 2. Table 1 gives some chromatographic parameters of the experiment.

Under the assay conditions described, the retention times for bromvaletone, carbamazepine and propantheline bromide were about 3.57, 5.27 and 7.86 minute, respectively. As expected, it can be seen that propantheline bromide, a quaternary ammonium drug (QAD) of organic nitrogenous bases, was retained longer than the other two drugs on the column. The difference in retention may be correlated with the increased conjugation in this more unsaturated

Table 1. Chromatographic parameters for the experiment :

	Bromvaletone	Cabamazepine	Propantheline
Retention time (min)	3 · 5 6 7	5 · 2 6 7	7 · 8 5 8
Capacity factor * (k′)	1 · 6 4 2	2 · 6 0 1	4 · 8 2 1

	Bromvaletone-Cabamazepine	Propantheline-Cabamazepine
Selectivity factor ** (α)	1 · 7 6 7	1 · 6 6 2
Resolution *** (R)	2 · 0 6 1	2 · 2 5 3

* $k' = (t-t_0) / t_0$

** $\alpha = (t2-t_0) / (t1-t_0)$

*** $R = 2(t2-t1) / (W2+W1)$

molecule [26]. In addition, it is also seen in this study that the extensive interaction of the permanent positive charged quaternary nitrogen of propantheline bromide with residual anionic silanols on the the reversed stationary phase is slightly detrimental to its peak shape.

Reproducibility tests were performed by chromatographing five standard solutions of each drug ranging in concentration from 5 to 30 μ g/mL in the presence of the internal standard carbamazepine. Standard curves were obtained by plotting peak height ratios versus concentrations (Figs. 3 and 4). Good precisions can be obtained (Table 2 and Table 3) and the linear correlation coefficients were 0.9998 and 1.0000 for brovaletone and propantheline bromide, respectively.

The effectiveness of extraction step and the accuracy of the proposed method were evaluated by adding reference standard to

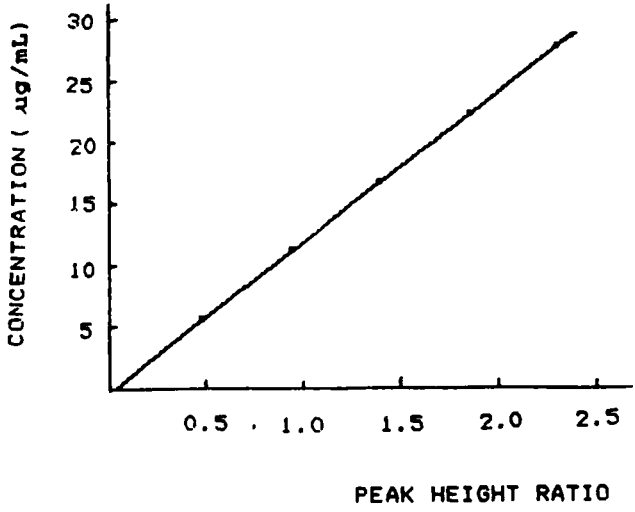


FIGURE 3. Standard Curve for Propantheline Bromide (Y = 10.45 X - 0.37; r = 0.9998).

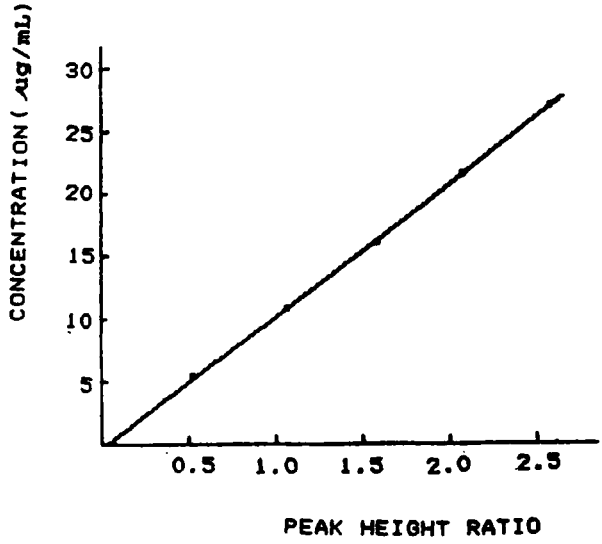


FIGURE 4. Standard Curve for Bromvaletone (Y = 11.99 X - 0.41; r = 1.0000).

Table 2. Peak Height Ratios(P.H.R.) of bromvaletone to carbamazepine in standard solutions

	concentration of bromvaletone (ug/ml)				
	5.52	11.04	16.56	22.08	27.6
P.H.R.					
1	0.4923	0.9599	1.4269	1.8828	2.3405
2	0.4862	0.9609	1.4193	1.8931	2.3204
3	0.4891	0.9438	1.3865	1.9016	2.3293
4	0.5022	0.9707	1.4201	1.8944	2.3624
5	0.5031	0.9501	1.3975	1.8620	2.3480
6	0.4879	0.9557	1.4205	1.8676	2.2951
Mean	0.4935	0.9568	1.4118	1.8836	2.3326
S.D.	0.0068	0.01	0.0141	0.0141	0.0224
C.V.(%)	1.37	1.05	1.03	0.75	0.96

Table 3. Peak Height Ratios(P.H.R.) of propantheline bromide to carbamazepine in standard solutions

	concentration of propantheline bromide (ug/ml)				
	5.36	10.72	16.08	21.44	26.80
P.H.R.					
1	0.5335	1.0710	1.5937	2.1168	2.6745
2	0.5407	1.0868	1.5677	2.1528	2.5419
3	0.5358	1.0779	1.5684	2.0935	2.6186
4	0.5284	1.0837	1.6451	2.0683	2.6201
5	0.5216	1.0673	1.5822	2.0491	2.5321
6	0.5205	1.0401	1.5974	2.0469	2.5185
Mean	0.5301	1.0711	1.5924	2.0879	2.5843
S.D.	0.01	0.0141	0.0265	0.0374	0.0566
C.V.(%)	1.89	1.32	1.66	1.79	2.19

Table 4 Recovery of Bromvaletone and Propantheline Bromide in Spiked Placebo Mixtures

Active ingredient	Amount added(mg)	Amount found(mg)*	Slope**	Recovery (%)
Bromvaletone	7.9	7.77	0.994	99.4
	15.9	16.06		
	23.9	23.68		
Propantheline Bromide	3.6	3.55	0.992	99.2
	7.3	7.18		
	10.9	10.79		

* Calculated from three replicate determinations.

** "Slope" means the slope of linear regression line.

placebo mixture powder in this study. Results were listed in Table 4. Because recovery values were 99.2% and 99.4% for the two drugs, obviously, no interference due to the excipients could be detected. The correlation coefficients of two recovery curves (Fig. 5 and Fig. 6) for propantheline bromide and bromvaletone were 0.9999 and 0.9997 respectively . Therefore, this method is linear and accurate between 50 and 150% for the tablet formulations tested.

Another recovery study was implemented by spiking sample powders with additional reference standards (Table 5). Figs. 7 and 8 obtained by plotting the amount of drugs found by the proposed method against the amount of the standard drugs added were for brovaletone and propantheline bromide respectively. The percentage recoveries were calculated using the following formula:

$$\% \text{ recovery} = \text{slope}.100$$

in which the slope was calculated by regressional analysis. The intercepts on the y axis can indicate the amount of drugs found.

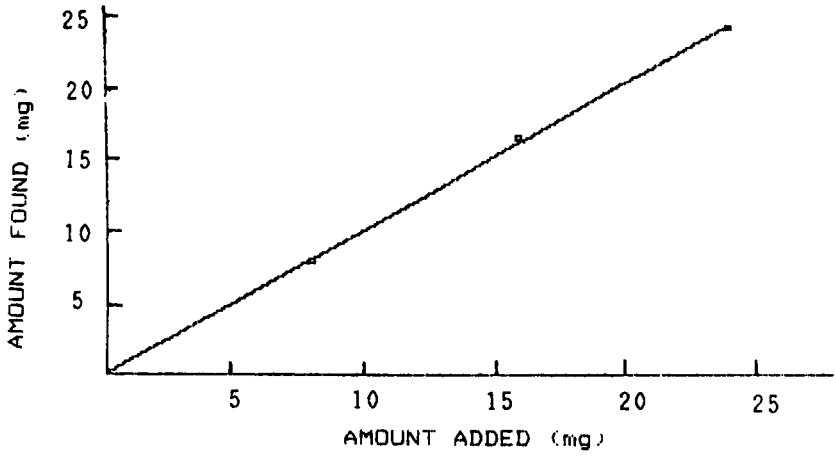


FIGURE 5. Recovery Curve for Bromvaletone from Spiked Placebo Mixtures (Y = 0.9944 x + 0.026; r = 0.9997).

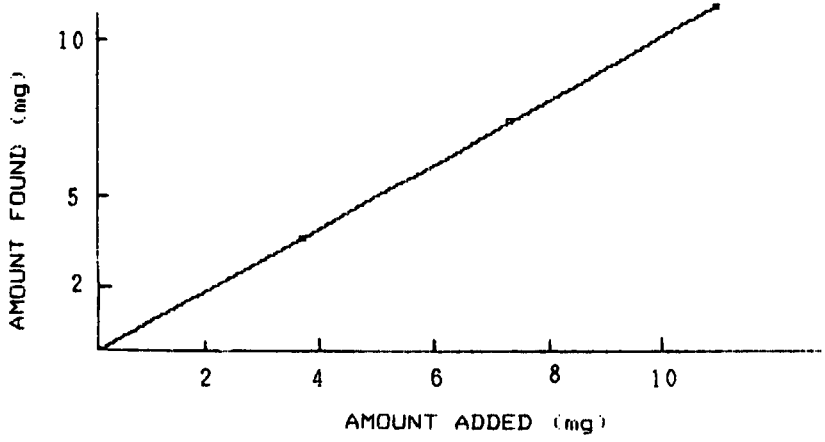


FIGURE 6. Recovery Curve for Propantheline Bromide from Spiked Placebo Mixtures (Y = 0.9917 X + 0.033; r = 0.9999).

Table 5. Recovery of Bromvaletone and Propantheline Bromide in Spiked Commercial Products

Active ingredient	Amount added(mg)	Amount found(mg)*	Slope**	Recovery (%)
Bromvaletone	9.2	10.5	1.023	102.3
	13.7	15.2		
	15.6	16.8		
	17.3	18.9		
Propantheline Bromide	9.0	17.8	1.017	101.7
	10.6	18.7		
	13.2	21.9		

* Calculated from two or three replicate determinations.

** "Slope" means the slope of linear regression line.

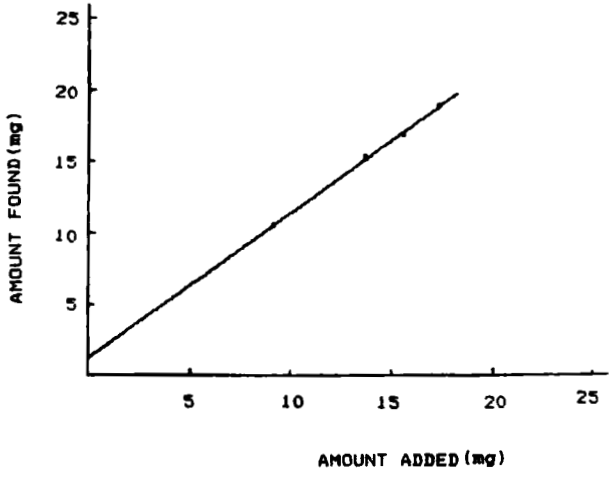


FIGURE 7. Recovery Curve for Bromvaletone from Spiked Commercial Samples (Y = 1.023 X + 1.101; r = 0.9987).

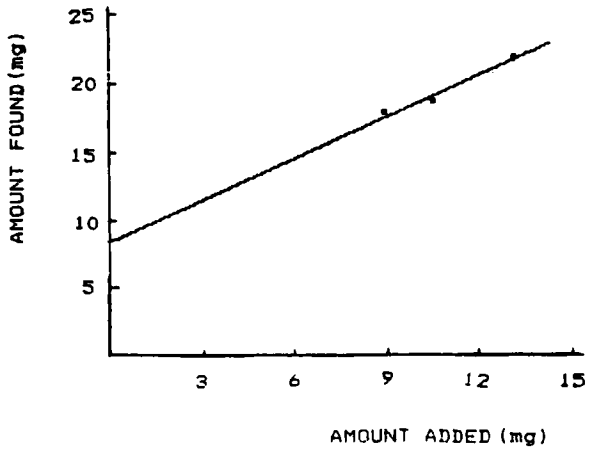


FIGURE 8. Recovery Curve for Propantheline Bromide from Spiked Commercial Samples (Y = 1.017 X + 8.340; r = 0.9829).

Therefore, the percentage of drug found in the tablet against the label claim per tablet was calculated by the following equation:

$$\% \text{ of drugs found to the label amount} = \frac{Wc}{slope} \times \frac{Wav}{Wt} \times \frac{100}{LA}$$

where Wc = amount of the drug found by the method;

Wt = amount of powder used;

Wav = average weight per tablet;

and LA = label amount of active ingredient per commercial tablet.

CONCLUSIONS

The HPLC method described in this paper has been proven to be fast, simple and reliable for simultaneous quatitation of brovaletone and propantheline bromide. And its precision is sufficient for routine analysis and now in regular use in our laboratory.

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