RESEARCH ARTICLE

Determination of Phenobarbital and Pipenzolate-methylbromide in Spastal Oral Drop by High-performance Liquid Chromatography Method

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ABSTRACT

An accurate method has been developed to determine phenobarbital (PHB) and pipenzolate methyl bromide (PMB) in pure form and in pharmaceutical preparations using high-performance liquid chromatography (HPLC) technique. 20 µL of a solution of the drugs with a concentration of 15 µg/mL of PHB and PMB were injected using an L_1 (150 mm × 4.6 mm. 5 µ) separation column at a temperature of 25ºC and acetonitrile: methanol: phosphate buffer (55:10:35) v: v: v as a mobile phase at a flow rate of 1 mL/min and detection at a wavelength of 200 nm. The Linearity of the method ranged from (2.5–50) µg/mL and (2.5–45) µg/mL, the retention time was 4.156 minutes and 6.5 minutes, the detection limit was 0.005 µg/mL and 0.0006 µg/mL, the average Rec% was 99.376% and 99.675%, and the correlation coefficient was 0.9997 for PHB and PMB, respectively. This method was successfully applied for the determination of (PHB) and (PMB) in the pharmaceutical preparation (spastal drop).

Keywords: High-performance liquid chromatography, Phenobarbital, Pipenzolate methyl bromide.

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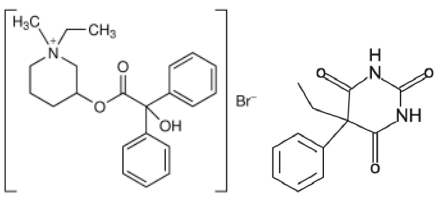
Conflict of interest: None

INTRODUCTION

Phenobarbital is used to treat insomnia, and to help relieve postoperative pain.[1] It is also used in the treatment of all types of disorders, except of coma.[2,3] It is not considered to be less effective than phenytones, but the tolerance and survival of phenobarbital will be less.[4] Chemically known as 5-ethyl-5-phenyl-1,3-diazinane-2,4,6-trione.[5] There are several methods for determination of PHB such as, HPLC,[6,7] TLC,[8] voltammetry,[9] UV-visible spectroscopy.[10]

Pipenzolate methyl bromide, 1-ethyl-3-(2-hydroxy-2,2-diphenylacetoxy)-1-ethylpiperidinium bromide is a quaternary ammonium antimuscarinic (cholinergic) receptors on smooth muscles with peripheral actions like those of atropine and prevents the effect of Acetylcholine. It has been used as an adjunct in the treatment of gastrointestinal disorders characterized by smooth muscle spasm and cramp.[11,12]



Pipenzolate methyl bromide phenobarbital

There are few methods for its determination: HPLC,[13] UV-visible spectroscopy.[14]

EXPERIMENTAL PART

Instruments and Chemicals

The instruments used were Shimadzu DGU-20A5R HPLC, Jenway 3310 pH meter, Ultra Sonic Karl Kolb, Sensitive balance Sartorius 200.

High purity materials were also used, namely methanol (Fisher), phosphoric acid (Scharlau), acetonitrile (J. T. Baker), sodium hydroxide (BDH), Potassium biphosphate (HIMEDIA), Sodium 1-octanesulfonate (TCI), Phenobarbital (China) and pipenzolate methyl bromide (Italy).

Preparation of Solutions

Phenobarbital and Pipenzolate Methyl Bromide Solution (1000 µg/mL)

0.1 gm of each material was dissolved separetly in an amount of solvent (distilled water 50: methanol 50) with good stirring, then completing the volume to the mark in a volumetric flask of 100 mL with the same solvent. Solutions lower concentrations were prepared by appropriate dilution.

Phosphoric Acid Solution (0.1 F)

0.678 mL of concentrated phosphoric acid of 14.74 M was added to distilled water in a volumetric flask of 100 mL.

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Sodium Hydroxide Solution (0.1 F)

0.4 g of solid sodium hydroxide was dissolved by distilled water in a 100 mL volumetric flask.

Buffer Solution

It was prepared by taking 0.6 g of monobasic potassium phosphate, 0.82 g of dibasic sodium phosphate, and 1.25 g of sodium sulfonic acid and transferring to a 1000 mL volumetric flask and completing the volume to the mark by distilled water.

Preparation of the Drugs Solution

The mixture of drug solution was prepared by taking 1.5 mL of the 100 µg/mL working solution of each PHB and PMB solutions in a 10 mL volumetric flask and completing the volume to the mark by the mobile phase.

Pharmaceutical Spastal Drop

1-mL of the medicinal preparation Spastal drop produced by the State Company for the Pharmaceutical Industry and Medical Appliances, Samarra, Iraq, which contains 4 mg of pipenzolate methyl bromide and 6 mg of phenobarbital, was placed in a volumetric flask of 100 mL, and the volume was completed to the mark by the solvent distilled water. 50: methanol 50), resulting in a 40 µg/mL concentration and 60 µg/mL for pipenzolate methyl bromide and phenobarbital, respectively.

RESULTS AND DISCUSSION

Maximum Wavelength of PHB and PMB

The scan in the UV-visible Spectrophotometer in the range of (200–400) nm (Figure 1) showed that the λ_{max} is at 202 nm for both drugs, so it was chosen for the determination (Figure 1).

Study of Optimum Conditions

A 20 µL of standard substances at a concentration of 15 µg/mL of each PHB and PMB were injected into a high-performance liquid chromatography apparatus and the optimum conditions were investigated as follows:

Selection of Column

20 µL of the drugs solution were injected into the (HPLC) device and the response (peak area) was recorded at wavelength 202 nm and after a series of practical experiments on the quality of columns (L_1,L_3,L_7 and L_9) and column (C18) L_1 (150 mm x 4.6 mm.5µ) was selected because it gave the best separation with reasonable retention times and sharp peaks and good resolution (Figure 2).

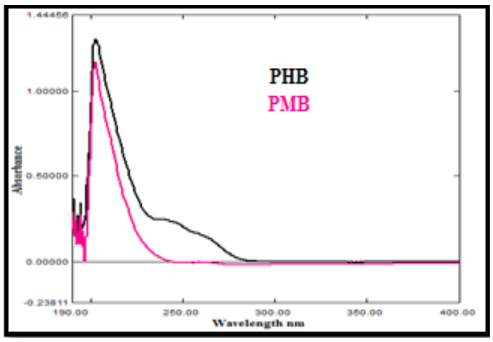

Figure 1: Absorption spectrum with λ_{max} at 202 nm for PHB and PMB

Selection of Wavelength

Using column L1, 20 µL of drugs solution were injected and the response (peak area) was recorded at a different wavelengths (200, 202, 205 and 210) nm. The best separation was obtained at wavelength 200 nm with sharp peaks (Figure 3) and low value of height equivalent to theoretical plate (HETP) and high number of theoretical plates.

Selection of Column Temperature

On column L1, 20 µL of the drugs solution were injected at different temperatures ranging between (25–40)°C and detection at a wavelength of 200 nm. The results showed that the best separation was obtained to a temperature of 25°C, where two sharp peaks appeared with a good retention time of 4.189 minutes and 6.861 minutes for PHB and PMB, respectively, as shown in Figure 4.

4Effect of pH

A group of solutions of different pH values (4, 5, 6, 7) was prepared and the pH was modified using phosphoric acid and sodium hydroxide. The best separation was at pH 6 (Figure 5) where the retention time is less than the separation at other pH's. At pH 7 only one peak for PHB has appeared.

Selection of Volumetric Ratio of Mobile Phase Component.

At optimum conditions (pH 6 and 25°C), 20 µL of the drugs solution was injected and different volume ratios of acetonitrile: methanol: phosphate buffer were used. The best separation (Figure 6) was achieved using the ratio (35 acetonitrile: 10 methanol: 55 phosphate buffer), which gave the lowest HETP and highest N.

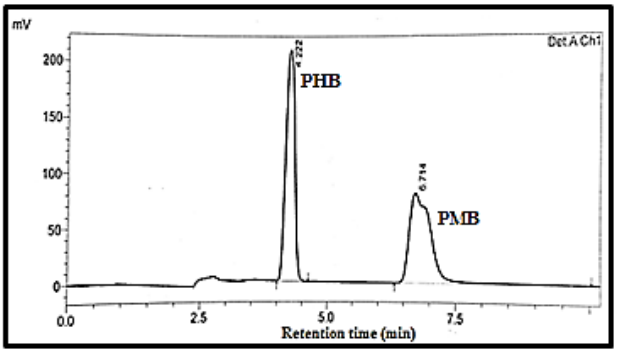

Figure 2: Separation of 20 µL of PHB and PMB on column L1.

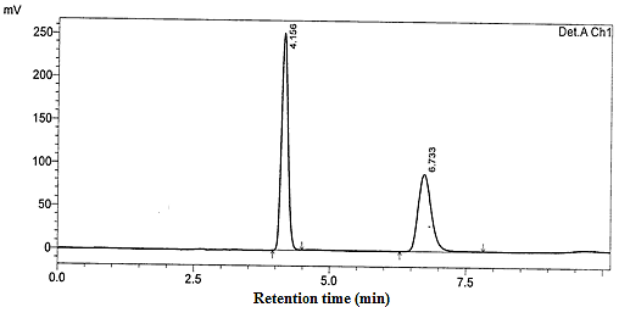

Figure 3: Separation of 20 µL of PHB and PMB with detection at wavelength of 200 nm

Flow Rate of Mobile Phase

At optimum conditions, 20 µL of the drugs solution was injected at different flow rates (0.5–2) mL/min. The best separation was obtained at a flow rate of 1 mL/min. It gave with a sharp peak with a suitable retention time (Figure 7). The values were: N= 4505 and 4225, H= 0.033 and 0.035 of PHB and PMB, respectively.

Procedure and Construction of Calibration Curves

In a series of 10 mL volumetric flasks, concentrations (1–65) µg/mL of PHB and PMB drugs solution were prepared by dilution with the mobile phase. 20 µL for each concentration were injected in the HPLC apparatus using L1 column and applying the previous optimum conditions (L1, temperature 25 ºC, pH 6, mobile phase 35 Acetonitrile: 10 Methanol: 55 Phosphate buffer, flow rate 1-mL/min and detection at 200 nm). The response (peak area) was recorded at wavelength 200 nm, and calibration curves were drawn between the peak area and concentration (Figures 8 and 9). The linearity of the method was 2.5 to 50 µg/mL and 2.5 to 45 µg/mL for PHB and PMB, respectively and the correlation coefficient was 0.9997 for both drugs.

Method Validation

The proposed method was validated by calculating limit of detection (LoD), limit of quantitation (LoQ), accuracy, precision, robustness test, and ruggedness test.

Limit of Detection and Limit of Quantitation

The response (peak area) was recorded for both drugs for the lowest concentration of the calibration curves. The results are shown in Table 1.

Accuracy and Precision

The accuracy and precision of the results for four concentrations within the calibration curve (2.5, 10, 25, 45) µg/mL were studied by taking an average of six readings for each concentration, and the relative standard deviation, percentage recovery relative error were calculated. Table 2 shows that this method has high accuracy (Rec% and RE%) and good precision (RSD%) for both drugs.

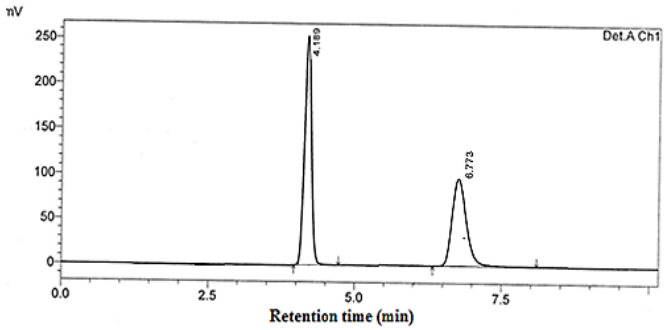

Figure 4: Separation of 20 µL of PHB and PMB by column L1 at 25ºC

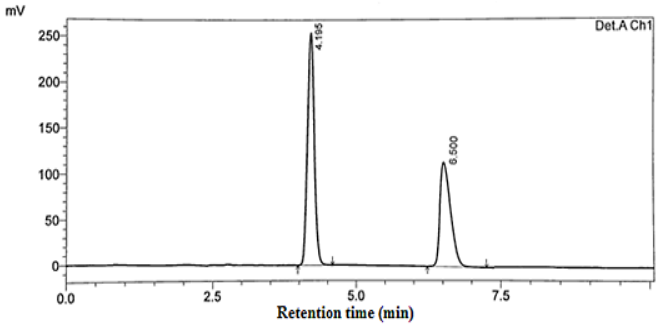

Figure 5: Separation of 20 µL of PHB and PMB at pH 6.0

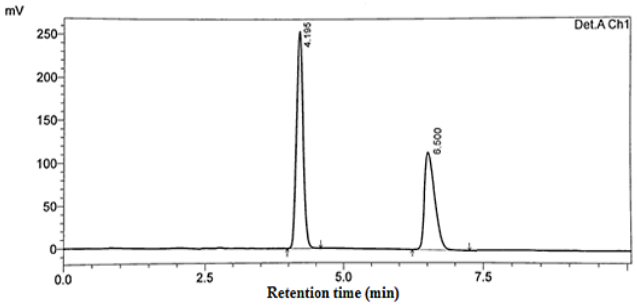

Figure 6: Separation of 20 µl of PHB and PMB using the mobile phase with volumetric ratio (35 acetonitrile: 10 methanol: 55phosphate buffer)

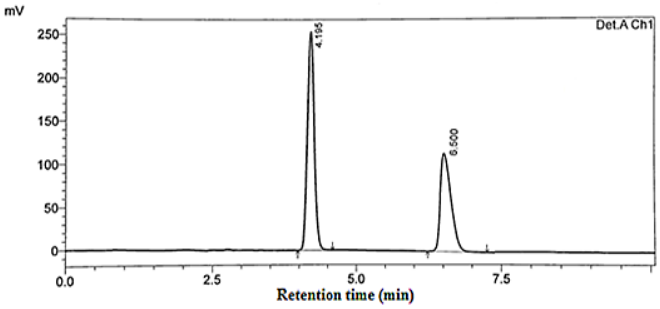

Figure 7: Separation of 20 µL of PHB and PMB at mobile phase flow rate of 1 mL/min

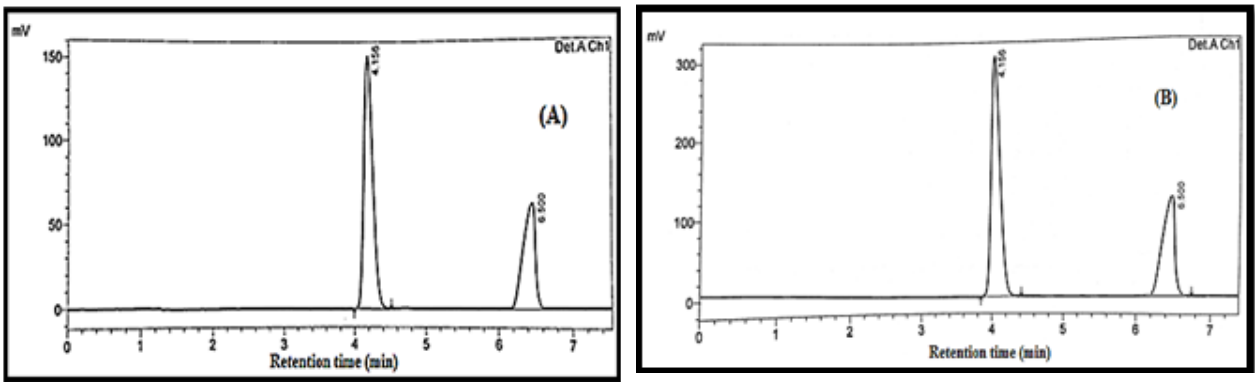

Figure 8: Standard chromatogram of PHB and PMB (A) at a concentration of 10 µg/mL (B) at a concentration of 20 µg/mL.

Study the Robustness (Rob.) and Ruggedness (Rug.) tests. Rob. test verifies the ability of the proposed method is not affected by changing one variable of the method and provides an indication of its reliability.[15] The test was conducted for this method by measuring the peak area of the solutions at the pH 5.0, a series of 10 mL volumetric flasks containing increasing concentrations (1–60) µg/mL of PHB and PMB drug solution were prepared by dilution with the mobile phase. The peak area of each concentration was measured after separation with a column L1 (150 mm× 4.6 mm, 5µm) and a mobile phase consisting of acetonitrile: methanol: phosphate buffer volumetric ratio (35: 10: 55 v/v/v) with a flow rate of 1-mL/min and a temperature of 25°C. The response was recorded (peak area) at wavelength 200 nm. Figure 10 represents the calibration curve for PHB and PMB, where the method linearity was (2.5-50) µg/mL and (2.5–45) µg/mL and the correlation coefficient was 0.9994 and 0.9997 for PHB and PMB, respectively. These results agree and close in value with the previous results obtained for the original calibration curve at pH 6.0.

Rug. test can be verified by measuring the absorbance of solutions under a variety of conditions such as different laboratories, analyzers and different instruments and devices.[15] In the present study, Rug. test was carried out by measuring the peak area by Shimadzo Lc-20A. At pH 6, the solutions were prepared in the same manner as in Rob. test study (Figure 11). represents the calibration curve for PHB and PMB, where the method linearity was 2.5 to 50 µg/mL and 2.5 to 45 µg/mL and the correlation coefficient was 0.9994 and 0. 9995 for PHB and PMB, respectively. The linearity, slope and correlation coefficient values are close to that when using Shimadzu DGU-20A5R.

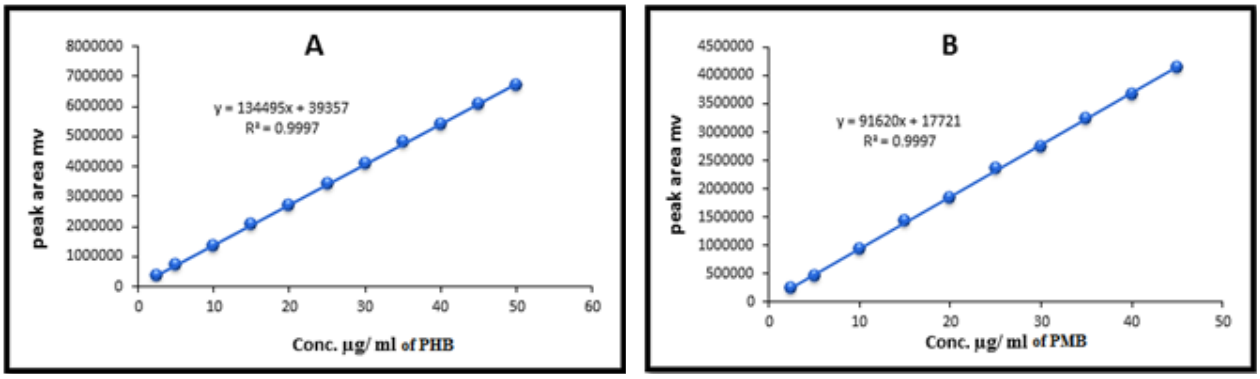

Figure 9: Calibration curve (A) for determination of PHB drug; (B) for determination of PMB drug.

Table 1: Detection limit and quantitation limit

Drug	*Concentration µg/mL*	$\bar{X}$ *	*S*	*LOD µg/mL*	*LOQ µg/mL*
PMB	2.5	240906.8	19.370	0.0006	0.002
PHB	2.5	368721.8	272.167	0.005	0.018

*Average of six determinations

Table 2: Accuracy and Precision for PHB and PMB drugs estimation

		PHB			
Conc. taken µg/mL	*Conc. found µg/mL*	**RE (%)*	**RSD%*	**REC%*	**Ave. REC%*
2.5	2.449	2.040-	0.073	97.960	99.376
10	9.853	1.470-	0.029	98.530	
25	25.246	0.984	0.006	100.984	
45	45.014	0.031	0.007	100.031	
		PMB			
Conc. taken µg/mL	Conc. found µg/mL	*RE (%)	*RSD%	*REC%	*Ave. REC%
2.5	2.436	2.560-	0.008	97.440	99.675
10	9.916	0.840-	0.008	99.160	
25	25.535	2.140	0.024	102.140	
45	44.982	0.040-	0.005	99.960	

*Average of Six determinations

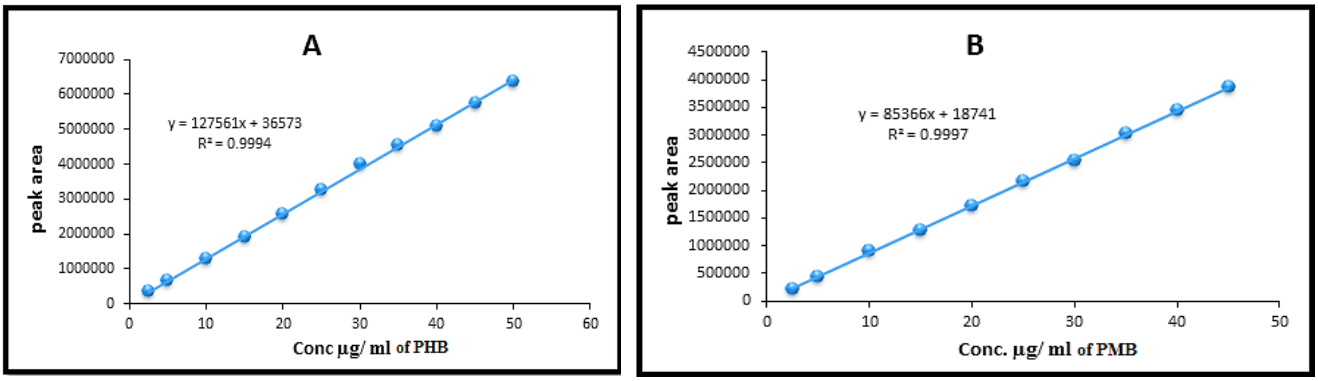

Figure 10: Calibration curve of Rob test (A) for determination of PHB; (B) for determination of PMB

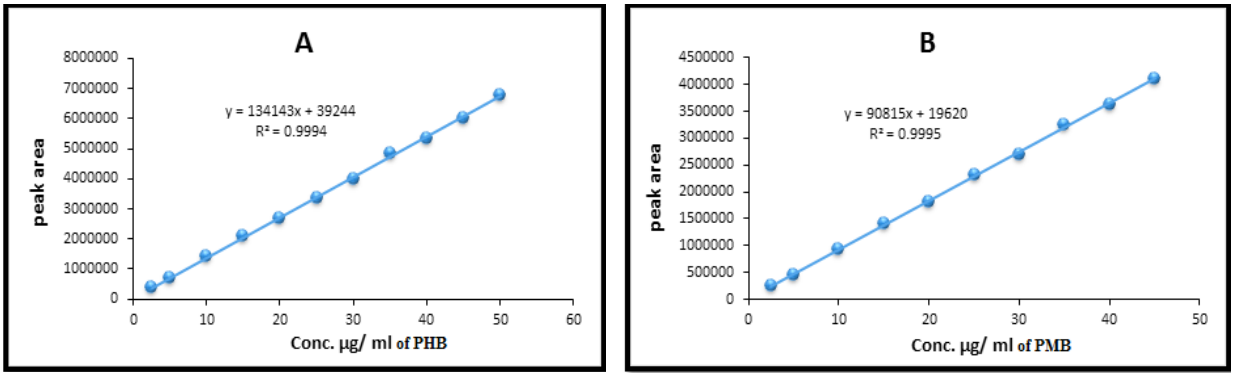

Figure 11: Calibration curve of Rug test (A) for determination of PHB; (B) for determination of PMB

Table 3: Application of the method for determination of PHB and PMB in Spastal drop

			PHB			
Conc. taken µg/mL		*Conc. found µg/mL*	**RE (%)*	**RSD%*	**REC%*	**Ave. REC%*
6.0		5.960	0.667-	0.051	99.333	98.874
	24		23.667	1.388-	0.594	98.612
	45		44.405	1.323-	0.007	98.677
PMB						
Conc. taken µg/mL		Conc. found µg/mL	*RE (%)	*RSD%	*REC%	*Ave. REC%
4.0		4.072	1.800	0.122	101.800	101.359
	16		16.292	1.825	0.038	101.825
	30		30.136	0.453	0.017	100.453

*Average of Six determinations

Application of Method

The proposed method was applied to detemine PHB and PMB in pharmaceutical preparation (Spastal drop), produced by the State Company for Pharmaceutical Industry and Medical Appliances/Samarra-Iraq (SDI). In three volumetric flasks of 10 mL volume, (0.5, 2.0, 3.75) mL of the previously prepared (40 PMB + 60 PHB) µg/mL solution were taken. The mobile phase completed the volume, resulting in different concentrations of (4, 16, 30) µg/mL of PMB and (6, 24, 45) µg/mL of PHB. Under optimum conditions, the peak area war recorded and the results are shown in Table 3.

The value of RE is not more than 1.825 and the value of REC is not less than 98.612 proving the success of this method for the determination of PHB and PMB in its pharmaceutical preparation (spastal drop).[16]

CONCLUSIONS

In this study, HPLC technique was applied to separate and quantify the active substances: phenobarbital and pipenzolate methyl bromide in their pure forms and pharmaceutical preparations using (column L_1, detection at wavelength 200 nm, mobile phase consisting of acetonitrile: methanol: phosphate

buffer with a volumetric ratio (55:10:35), pH 6.0, flow rate 1-mL/min and temperature 25ºC). The results obtained for the relative standard deviation, percentage recovery, detection limit, and quantitation limit showed that the method has good accuracy, precision, and high sensitivity.

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