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Simultaneous estimation of Ethyl Alfa Bromisovalerianat and Phenobarbitone in pharmaceutical formulation using HPLC

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ABSTRACT

A simple, sensitive and isocratic reverse phase high performance liquid chromatographic (RPHPLC) method has been developed for the simultaneous determination of Phenobarbitone and Ethyl Alfa Bromisovalerianat in a formulation. HPLC analysis was carried out using reverse phase isocratic elution with a C18 column and a mobile phase of Acetonitrile: Water in the ratio of 65:35, v/v. Detection of the analytes were achieved using UV detector at 203 nm. The retention times of Phenobarbitone and Ethyl Alfa Bromisovalerianat were 3.90 and 9.8 minutes respectively Linearity of the method was found to be in the concentration range of 24-144 μgml^{-1} for Phenobarbitone and 20-120 μgml-1 for Ethyl Alfa Bromisovalerianat. The correlation coefficient value was greater than 0.9999 for both the analytes. The method is suitable for the estimation of both the components simultaneously in pharmaceutical formulations.

KEYWORDS

HPLC;
Acetonitrile;
Phenobarbitone;
Ethyl Alfa Bromisovalerianat.

INTRODUCTION

The product is a barbiturate based heart medication and mild tranquilizer, popular in Eastern Europe and the former Soviet Union. It is a transparent liquid with characteristic strong Aroma.

Ethyl Alfa Bromisovalerianat is a clear liquid; insoluble in water having boiling point in range of 190-192.0°C.It has a specific gravity of 1.2260 g/cm3 and molecular formula $C_7H_{13}BrO_2$ with a molecular weight of 209.8. Some of its synonyms are DL-Ethyl 2-bromovalerate, ethyl ester α Bromovaleric acid ethyl ester, Ethyl Alfa Bromisovalerianat.

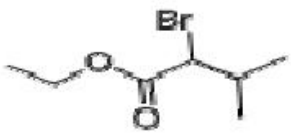

Figure 1 : Chemical structure of Ethyl Alfa Bromisovalerianat

Phenobarbitone is a barbiturate white or colorless crystalline powder having molecular formula as $C_{12}H_{12}N_2O_3$. Its also known as 5-ethyl-5-phenylbarbituric acid. It is very slightly soluble in water; freely soluble in ethanol (96%); sparingly soluble in chloroform; soluble in ether. The melt-

ing point Vrange is in between 174° and 178° ,but the range between beginning and end of melting does not exceed 2°.

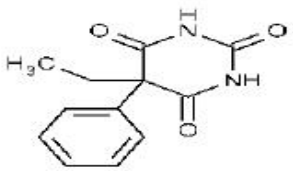

Figure 2 : Chemical structure of Phenobarbitone.

We describe in this paper a simple, sensitive and validated HPLC method for the simultaneous determination of Phenobarbitone and Ethyl Alfa Bromisovalerianat in pharmaceutical formulations.The developed method can be applied successfully to quality control and for other analytical purposes.

EXPERIMENTAL

Chemicals and reagents

Phenobarbitone and Ethyl alfa bromisovalerianat working standards were arranged by Unijules Life Sciences (Pvt) Ltd, Nagpur Maharashtra. Combination product contains 18.0 mg Phenobarbitone and 18.0 mg Ethyl Alfa Bromisovalerianat per ml. Acetonitrile and water (HPLC grade) were purchased from Merck'. Double distilled water, prepared in our laboratory was used throughout the experiment. Mobile phase was filtered using 0.45 µ nylon 6, 6 membrane Ultipore filters made by PALL Corporation. The other filter membrane had a pore size 0.2 µm used for the filteration of sample solutions.

Apparatus and chromatographic conditions

HPLC system consisting of Jasco LC-Net II/ ADC (B211161095) equipped with a pump model PU-2089 plus, an UV- 2075 variable wavelength detector, and Borwin software as system controller injection valve of a 20 µl loop was used for development and evaluation of this method. A Princetonesphre 100 C18 column (250*4.6 mm, 5 µm particle sizes) was selected. The mobile phase was composed of acetonitrile and water in the ratio of 65:35, v/v and flow rate of the mobile phase as 1 ml min^{-1}.The determinations were performed with UV-Vis detector set at 203nm. Peak identity was confirmed by retention time comparison and the HPLC system was operated at room temperature (25±2°C).

Preparation of standard solution

An amount of 36 mg of Phenobarbitone and 36 mg of Ethyl Alfa Bromisovalerianat were weighed and transferred into a 100 ml volumetric flask with 60 ml of mobile phase. The solution was sonicated for 5 minutes to completely dissolve both the components. It was then cooled and the volume was completed up to the mark with mobile phase. 5 ml of the above solution was diluted to 25 ml with mobile phase to obtain a final concentration of 72 μgml^{-1} for Phenobarbitone and 72 μgml^{-1} for Ethyl Alfa Bromisovalerianat. The solution was filtered through 0.2 µ nylon 6, 6 membrane filters before analysis.

Preparation of sample solution

Take solution equivalent to 36.0 mg of Phenobarbitone and Ethyl AlfaBromisovalerianat and

tranfered into a 100 ml volumetric flask with 60 ml of mobile phase. The solution was sonicated for 5 minutes to completely dissolve both the components and make up the volume to 100 ml with mobile phase. 5 ml of the above solution and dilute it to 25 ml with mobile phase to obtain a final concentration of 72 μgml^{-1} for Phenobarbitone and 72 μgml^{-1} for Ethyl Alfa Bromisovalernate. The solution was filtered through 0.2 µ nylon 6, 6 membrane filters before analysis.

Linearity

Linear calibration plots for the proposed method were obtained over the calibration range of 24-144 μgml^{-1} (24,48,72,96,120,144) for Phenobarbitone and 20-120μgml^{-1} (20,40,60,80,100,120) for Ethyl Alfa Bromisovalerianate. Each level was made in triplicate.

Accuracy

To demonstrate the accuracy of the proposed method recovery studies were employed by the standard addition method. Three levels of solutions were made which corresponds to 80, 100 and 120 % of the nominal analytical concentration. Each level was made in triplicate.

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Selectivity

To check the selectivity of the proposed method a synthetic mixture of phenobarbitone and Ethyl Alfa Bromisovalerianat was prepared with commonly occurring ingredients that are present in most such formulations. The comparison of its chromatograms with the chromatograms of the standard solution was done along with the percentage recovery of both the analytes. Synthetic mixture containing 25 mg phenobarbitone and 20 mg Ethyl Alfa Bromisovalerianat, 0.005 ml and 0.001ml each of mint oil, ethanol, and propyleneglycol, which are present as inactives in the pharmaceutical formulation, were accurately weighed and transferred into 50 ml volumetric flask. The mixture was sonicated for 15 minutes with 30 ml mobile phase and then the volume was completed with mobile phase and filtered. 2 ml of the filtrate was then further diluted to 25 ml to obtain a final solution containing concentration of 40 μgml^{-1} for Phenobarbitone and 32 μgml^{-1} for Ethyl Alfa Bromisovalerianat respectively.

Robustness

Robustness of the method was performed by intentionally varying individually, the chromatographic conditions such as composition of the mobile phase and flow rate of the solution.

The chromatographic parameters of each analyte such as retention time, tailing factor and number of theoretical plates were measured at each changed conditions.

RESULTS AND DISCUSSION

In this work a simple, sensitive and validated HPLC method has been developed for the simultaneous estimation of Phenobarbitone and Ethyl Alfa Bromisovalerianat using liquid chromatography with ultraviolet detection. A number of mobile phases were initially attempted to elute both components simultaneously The main focus was to achieve sharp and gaussian shaped peaks with tailing less than 1.5. In order to achieve this goal, acetonitrile and buffer in different proportions were used but no sharp peaks were observed. water was then added to increase the polarity of the mobile phase. The mobile phase composition of acetonitrile : water in the ratio of 65:35, v/v was strong enough to elute both the components with resolution greater than 5, theoretical plates greater than 7000 and tailing less than 1.5 for both the components. The best mobile phase composition was then found to be acetonitrile: water in the ratio of 65:35 (v/v).

The most suitable mobile phase composition was thus found to be acetonitrile: water in the ratio of 65:35 (v/v).

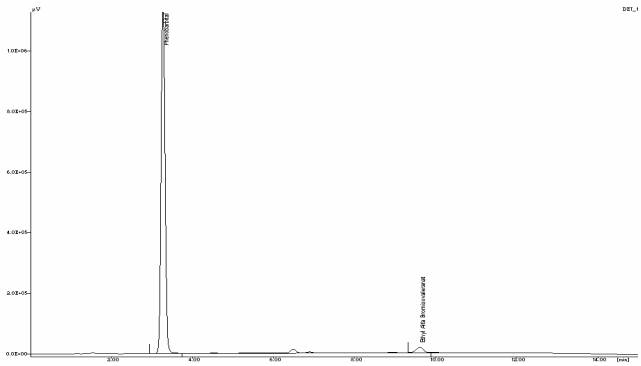

Figure 3 : Under the mentioned chromatographic conditions sharp peaks belonging to Phenobarbitone and Ethyl Alfa Bromisovalerianat were obtained at retention times of 3.90 and 9.8 minutes respectively.

The proposed chromatographic method was validated using ICH guidelines3 by performing linearity, limits of detection and quantitation, selectivity, robustness accuracy and repeatability Linear calibration plots for the proposed method were obtained by analyzing five solutions in the concentration range of 24 - 144μgml^{-1} for Phenobarbitone and 20 - 120μgml^{-1} for Ethyl Alfa Bromisovalerianat. The peak area of drugs was plotted against the corresponding concentration to construct a linear regression equation and to calculate the value of correlation coefficient. The linear regression equation for Phenobarbitone was found to be Y= 91752X - 7381 with correlation coefficient equal to 0.9998. The linear regression equation for Ethyl Alfa Bromisovalerianat was found to be Y= 2732.3 X + 1836.4 with value of correlation coefficient equal to 0.9993.

The limit of detection (LOD) and quantification (LOQ) were measured by calculating the minimum level at which the analyte can be readily detected and quantified with accuracy, respectively. In this study, the LOD was found to be 0.08 and 0.06 μgml^{-1} for Phenobarbitone and

Ethyl Alfa Bromisovalerianat respectively (signal to noise ratio of 3:1). The LOQ was found to be 0.13 and 0.10 $\mu g ml_{-1}$for Phenobarbitone and Ethyl Alfa Bromisovalerianat respectively (signal to noise ratio of 10:1).

The accuracy of the method was performed by the standard addition technique. Three levels of solutions were made which correspond to 80, 100 and 120 % of the nominal analytical concentration. Each level was made in triplicate. The recovery and percent recovery for each of the analytes are given in TABLE 1. From the recovery studies

TABLE 1 : Results of accuracy experiment.

Amount of Sample		Amount of drug added		Amount recovered		% Recovery	
EA*	PB **	EA*	PB**	EA*	PB**	EA*	PB*
µg/ml	*µg/m*	*µg/ml*	*µg/ml*	*µg/ml*	*µg/ml*	*µg/ml*	*µg/ml*
180	360	80	80	258.80	438.99	99.53	99.77
180	360	80	80	259.76	438.96	99.90	99.76
180	360	80	80	257.99	439.56	99.22	99.9
180	360	100	100	279.55	459.56	99.83	99.90
180	360	100	100	278.99	457.99	99.63	99.56
180	360	100	100	279.65	458.99	99.87	99.78
180	360	120	120	299.85	479.65	99.95	99.92
180	360	120	120	298.76	479.56	99.58	99.90
180	360	120	120	298.69	480.10	99.56	100.02
		Average				99.67	99.83

EA* -Ethyl Alfa Bromisovalerianat
PB -Phenobarbitone**

it is evident that the method is highly accurate and can give excellent results.

Precision of method was studies by analysis of multiple sampling of homogeneous sample. The precision of analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogenous authenticated sample. Precision Expressed as % RSD is given in table which should be less than 2%.Within-day precision was determined by injecting five standard solutions of three different concentrations on the same day (n=6) and between-day precision was determined by injecting the same solutions for consecutive five days. Relative standard deviation (RSD %) of the peak area was calculated to represent precision. The

TABLE 2 : Precision for Ethyl Alfa Bromisovalerinate EA and phenobarbitone PB.

Sample*	EA (%)	PB (%)
Sample 1	100.3	98.6
Sample 2	101.1	98.9
Sample 3	101.5	100
Sample 4	100.9	98.8
Sample 5	102.0	98.7
Sample 6	101.7	98.6
Average	**101.2**	**98.93**
%RSD	0.255%	0.541%

results of within-day and between-day precision are presented in TABLE 2.

To be considered selective, a method should demonstrate its ability to separate the analyte peaks from the excipients peaks and should not result in co-elution of peaks. The selectivity was tested by making the synthetic mixture of both the analytes with commonly used pharmaceutical excipients.

Then its chromatograms were compared with that of standard solution along with the recovery of analytes. No excipient interfered with the main peaks of analytes. The results of recovery study are given in TABLE 3, which shows that excipients have no interference with the analyte peaks and the method is found to be highly selective for its intended use.

Robustness of the method was performed by slightly varying the chromatographic conditions. The results showed that the slight variations on the chromatographic conditions have negligible effect on the chromatographic parameters showing the method is highly robust for its intended use. The results are given in TABLE 4 and 5

The application of the method was checked by analyzing the Ethyl alfa brom Isovaleriate (EA) and Phenobarbitone (PB) in commercial pharmaceutical formulation. The results are given in TABLE 6 which shows high percentage recoveries and low RSD (%) values.

CONCLUSION

A simple and accurate reverse phase HPLC method has been described for the simultaneous

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TABLE 3 : Shows Selectivity study of the proposed HP.

S.No	Ethyl Alfa Bromisovalerianat (EA)			Phenobarbitone (PB)		
	Amount of drug added (μgml^{-1})	Amount recovered (μgml^{-1})	% Recovery	Amount of drug Added (μgml^{-1})	Amount recovered (μgml^{-1})	% Recovery
1	72	72.35	100.48	72	72.20	100.27
2	72	72.58	100.80	72	71.98	99.97
3	72	72.46	100.63	72	72.56	100.77
4	72	72.51	100.70	72	72.65	100.90
5	72	72.60	100.83	72	71.80	99.72
6	72	72.29	100.40	72	72.11	100.15
	Mean % Recovery		100.64	Mean % Recovery		100.29
	R.S.D		0.172%	R.S.D		0.171%

TABLE 4 : Shows robustness study of Ethyl Alfa Bromisovalerianat (EA).

Conditions	Retention time	Tailing	Theoretical Plates
Mobile Phase			
Acetonitrile: Water (62:38)	9.791	1.02	16981.01
Acetonitrile: Water (65:35)	9.600	1.00	16866.09
Acetonitrile: Water(67:33)	9.552	1.03	16766.09
Flow rate			
0.9 *ml/min*	9.698	1.04	17050.98
1.0 *ml/min*	9.600	1.00	16866.09
1.1 *ml/min*	9.596	1.01	16820.51

TABLE 5 : Shows robustness study of Phenobarbitone (PB)

Conditions	Retention time	Tailing	Theoretical Plates
Mobile Phase			
Acetonitrile: Water (62:38)	3.365	1.02	7416.26
Acetonitrile: Water (65:35)	3.258	1.00	7155.27
Acetonitrile: Water (67:33)	3.125	1.06	7081.85
Flow rate			
0.9 *ml/min*	3.526	1.00	7401.16
1.0 *ml/min*	3.258	1.00	7155.27
1.1 *ml/min*	3.098	1.01	7078.00

determination Ethyl Alfa Bromisovalerianat (EA) and Phenobarbitone (PB). The method was validated by testing its linearity, accuracy, precision, limits of detection and quantitation and selectivity the run time of less than five minutes allows its application for the routine determination of Ethyl Alfa Bromisovaleranate (EA) and Phenobarbitone (PB). In pharmaceutical formulations.

TABLE 6 : Shows result of analysis of Ethyl Alfa Bromisovaleranate (EA) and Phenobarbitone (PB) in ml.

Drug	Amount of the drug claimed *mg per ml*	Amount of the Drug found *mg per ml*	Mean recovery %	% RSD
Ethyl AlfaBromisovalerianat	18.0	18.65	103.6	0.255%
Phenobarbitone	18.0	18.26	101.4	0.541%

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