RESEARCH ARTICLE

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DEVELOPMENT AND VALIDATION OF REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHYMETHOD FOR SIMULTANEOUS ESTIMATION OF BETAHISTINE DIHYDROCHLORIDE AND PROCHLORPERAZINE MALEATE IN TABLET DOSAGE FORM

PATEL VISHVAS D*, PATEL PARESH U

Department of Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar, Mehsana, Gujarat, India.

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Abstract:

This research manuscript describes simple, sensitive, precise, accurate, economic reverse phase high performanceliquid chromatography method for the simultaneous determination of Betahistine Dihydrochloride (BET) and Prochlorperazine Maleate (PRO) in Tablet dosage form. The sample was analyzed by reverse phase C18 column (X terra C18, 150 mm × 4.6 mm, 5µm) asstationary phase; acetonitrile : methanol : phosphate buffer PH 4 (45 : 45 : 10 , v/v/v) as a mobile phase at a flow rate of 0.8 ml/min. Quantification was achieved with Photo Diode Array detector at 254 nm. The retention time for Betahistine Dihydrochloride was found to be 3.3 min and for Prochlorperazine Maleate was found to be 7.6 min. The linearity was obtained in theconcentration range of 2-30 µg/ml and 2-30 µg/ml for BET and PRO respectively. The method was successfully applied in analysis of tablet dosage form because no chromatographic interferences from formulation excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.

Keywords: Betahistine Dihydrochloride, Prochlorperazine Maleate, RP-HPLC, Tablet dosage form

Introduction

Betahistine (BET) is chemically N-Methyl-2-pyridine-ethanamine (Figure 1) well known Anti Vertigo drug[1]It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), European Pharmacopoeia (EP), and United States Pharmacopoeia (USP). In which USP[2] and IP[3] describe Liquid chromatographic method for estimation. While BP[4] and EP[5]describe potentiometric method for estimation. Literature survey reveals HPLC for estimation Betahistine Dihydrochloride in human serum.[6] it also shows colorimetric method[7], HPLC[8], Voltammetric method[9] for the estimation of Betahistine Dihydrochloride in tablet. Prochlorperazine maleate (PRO) is 2-chloro-10-[3-(4–methyl piperazin-Iyl)propyl]phenothiazine dihydrogen maleate.(figure 2) It is official in IP, BP, USP, EP, and Japanese Pharmacopeia (JP). IP[10], JP[11], USP[12] describe liquid chromatographic method for estimation while BP[13] and EP[14] describe potentiometric method for estimation. Literature survey reveals colorimetric[15] and HPLC[16] methods for determination of PRO in single dosage form. Literature survey also reveals spectrophotometric[17] and HPLC[18] methods for determination of PRO with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia, hence no official method is available for the simultaneous estimation of BET and PRO in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of BET and PRO in synthetic mixture or dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective RP-HPLC method for simultaneous estimation of both drugs in their tablet dosage form. The objective of this work was to develop simple, precise RP-HPLC method for combination drug products containing BET and PRO.

MATERIALS & METHODS

Apparatus

The chromatography was performed on a Shimadzu (Japan) RP-HPLC instrument (LC-2010CHT) equipped with Photo Diode Array(PDA) detector and LC-solution software, X terra (Torrance, CA) C18 column (150 mm × 4.6 mm id, 5µm particle size) was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai, India), Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad) were used in the study.

Reagents and Materials

BET bulk powder was kindly gifted by Astrone Pharmaceuticals Ltd., Ahmedabad, Gujarat, India. PRO bulk powder was kindly gifted by Trios Remedies Ltd., Ahmedabad, Gujarat. Acetonitrile,

Methanol, triple distilled water (S. D. Fine ChemicalsLtd., Mumbai, India) used were of HPLC grade. Potassium dihydrogen ortho-phosphate and Orthophosphoric acid (S.D Fine Chemicals Ltd., Mumbai, India) used were of AR grade. Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India) and Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

Preparation of Phosphate Buffer PH 4

Phosphate buffer (0.02 M KH_2PO_4, pH 4.0) was prepared by dissolving accurately weighed 2.72 g of potassium dihydrogen phosphate in 1000 ml HPLC-grade water and the pH adjusted to 4.0 by diluted ortho-phosphoric acid.

Preparation of standard stock solutions

An accurately weighed quantity of BET(10 mg) and PRO(10mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution each having concentration of 100 µg/ml**.**

Chromatographic Condition

Stationary phase: C_{18} column (150 mm x 4.6 mm id., 5 µm).
Mobile phase: Acetonitrile: Methanol: Phosphate buffer pH 4.0
(45: 45: 10, v/v/v)
Flow rate: 0.8 ml/min
Injection volume: 20 µL
Temperature: 40 °C
Detection: At 254 nm using PDA detector.

Preparation of calibration curve

Accurately measured standard stock solutions of BET (0.2, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml) were transferred to a series of 10 ml corning volumetric flasks, and the volume was made up to the mark with methanol. Same for the PRO 2-30 µg/ml was prepared.An aliquot (20 µl) of each solution was injected under the operating chromatographic condition asdescribed above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentration, and the regression equations were calculated. Each response was average of three determinations.

Preparation of sample solution

Twenty tablets were weighed individually and powdered. Quantity of the powder equivalent to 8 mg BET & 5 mg PRO was transferred in 100 ml volumetric flask separately and powder was dissolved in 50 ml of methanol with sonication to dissolve drug as completely as possible. The solution was filtered through whatman filter paper No. 41. Than the volume was adjusted up to mark with methanol. Take 1.25 ml of BET & Take 2 ml PRO in 10 ml volumetric Flask From the stock solution. The volume was adjusted up to the mark with methanol to get a final concentration of BET (10 µg/ml) and PRO (10 µg/ml).

Method Validation

The method was validated in compliance with ICH guidelines[19.]

Accuracy (recovery study)

To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (50%, 100%, and 150%). A known amount of drug was added to reanalyzed sample powder and percentage recoveries were calculated.

Method Precision (Repeatability)

The precision of the instrument was checked by repeated injection (n = 6) of standard solutions of BET (15 µg/ml) and PRO (15 µg/ml) under the same chromatographic condition and measurement of peak area, retention time and tailing factor. The low %RSD values (less than 2%) indicates that proposed method is repeatable.

Intermediate Precision (Reproducibility)

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of BET (10, 15, 20 µg/ml) and PRO (10, 15, 20 µg/ml). The result was reported in terms of relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification

LOD and LOQ of drugs can be calculated using the following equations designated by International Conference on Harmonization (ICH) guidelines.[19]

$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

RESULTS AND DISCUSSION

A RP-HPLC method was developed and validated for the determination of BET and PRO in tablet dosage form on a column (X terra C18, 150 mm × 4.6 mm, 5µm) with variable wavelength detection at 254 nm. The retention time of BET 3.322 and PRO 7.615 min, respectively. Linear correlation was obtained between area and concentration of BET and PRO in the concentration range of 2-30 µg/ml for both drugs. The low RSD value of intra-day (0.21-0.33 % for BET and 0.13-0.38 % for PRO) and inter-day (0.40-0.57 % for BET and 0.40-0.60 % for PRO) at 254nm, reveal that proposed method is precise. The limit of detection (LOD) and limit of quantification (LOQ) for BET and PRO were found to be 0.21 and 0.63 µg/ml and 0.24 and 0.72 µg/ml, respectively. These data show that method is sensitive for the determination of BET and PRO. The recovery experiment was performed by the standard addition method. The mean recoveries were 99.50 ± 0.39and 99.74 ± 0.38for BET and PRO, respectively (Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine BET and PRO in tablet dosage form. No interference of the excipients with the retention time of drugs appeared; hence the proposed method is applicable for the routine simultaneous estimation of BET and PRO.

CONCLUSION

In this proposed RP-HPLC method, the linearity is observed in the concentration range of 2-30 µg/ml for both drugs with co-efficient of correlation, (r2) =0.9997 and (r2) =0.9997 for BET and PRO, respectively at 254 nm. The results of the analysis of tablet dosage form by the proposed method are highly reproducible and reliable. The method can be used for the routine analysis of the BET and PRO in tablet without any interference of excipients.

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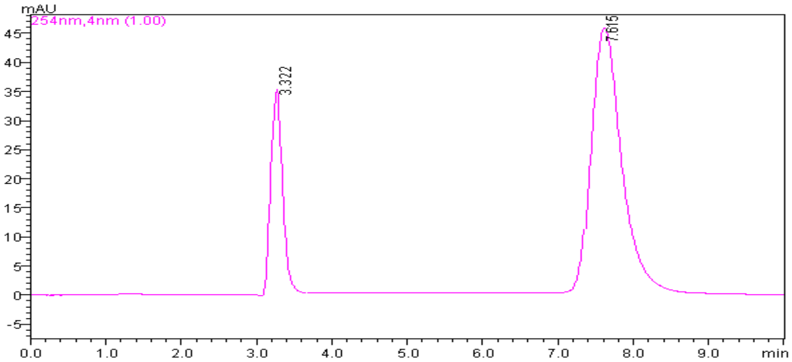

Figure 1 Chromatogram of BET (8µg/ml) and PRO (5µg/ml) at 254 nm

PARAMETERS	BET	PRO
Concentration range (µg/ml)	2-30	2-30
Regression equation (y = mx + c) Slope (m) Intercept (c)	y = 19140x + 12274 19140 12274	y = 63769x - 7969.4 63769 7969.4
Correlation Coefficient (r^2)	0.9997	0.9997
Accuracy (Recovery ± S.D.) (n = 3)	99.50 ±0.39	99.74 ±0.38
Method precision (Repeatability) (% RSD, n= 6)	0.326	0.269

Intraday (n = 3) (% RSD)	0.21-0.33	0.13-0.38
Interday(n = 3) (% RSD)	0.40-0.57	0.40-0.60
LOD(µg/ml)	0.21	0.24
LOQ (µg/ml)	0.63	0.72
Assay ± S. D. (n = 3)	99.58±0.219	99.6±0.227

Table 1: Data Showing Linearity and Precision of the Developed Method

Drug	**Level**	**Amount taken (µg/ml)**	**Amount added (%)**	**% Mean recovery ± S.D. (n = 3)**
BET	I	6	50	99.64 ± 0.29
	II	6	100	99.34 ± 0.21
	III	6	150	99.52 ± 0.67
PRO	I	6	50	99.81 ± 0.34
	II	6	100	99.73 ± 0.26
	III	6	150	99.68 ± 0.56

Table 2: Recovery Data

SR NO.	**Label claim (mg)**		**Amount found (mg)**		**% Label claim (mg) (n = 6)**	
	BET	**PRO**	**BET**	**PRO**	**BET**	**PRO**
1	8	5	7.97	4.99	99.62	99.8
2	8	5	7.95	4.97	99.37	99.4
3	8	5	7.93	4.95	99.12	99
4	8	5	7.99	5.02	99.87	100.2
5	8	5	8.02	5	100.25	100
6	8	5	7.94	4.96	99.25	99.2
MEAN					99.58	99.6
SD					0.219	0.227

Table 3: Results of Analysis of Tablet Dosage Forms Containing BET and PRO

Parameters	**BET ± CV % (n=6)**	**PRO ± CV % (n=6)**
Retentiontime (min)	3.322 ± 0.1378	7.615 ± 0.1012
Tailing factor	1.018 ±1.211	1.387 ± 1.578
Theoretical plates	2379 ± 1.892	3943 ± 1.943
Resolution	5.312 ± 1.273	

Table 4 System suitability test parameters for the proposed RP-HPLC method

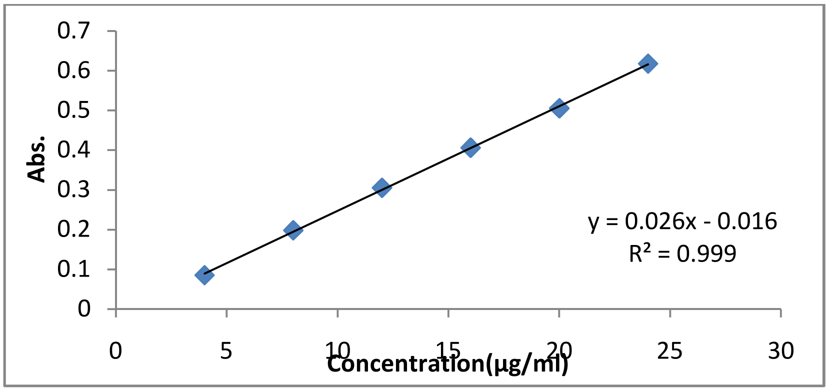

Figure 2 Linearity curve of BET (2-30µg/ml)

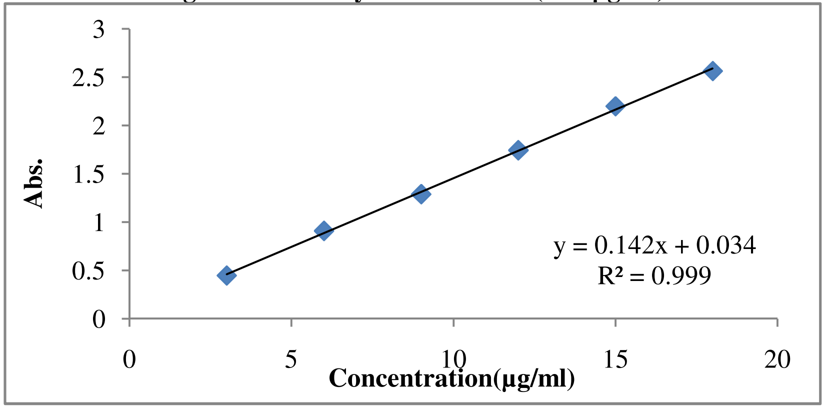

Figure 3 Linearity curve of PRO (2-30µg/ml)

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