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DEVELOPMENT AND VALIDATION OF RATIO SPECTRA DERIVATIVE METHOD FOR THE SIMULTANEOUS ESTIMATION OF BETAHISTINE DIHYDROCHLORIDE AND PROCHLORPERAZINE MALEATE IN TABLET DOSAGE FORM.

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Abstract:

The Ratio spectra derivative spectrophotometric method was developed for the simultaneous determination of Betahistine Dihydrochloride (BET) and Prochlorperazine Maleate (PRO) in tablet dosage form. In this method ratio spectra of one drug is taken then they divided by one of the standard concentration of another drug and then converted into first derivative method. The first derivative amplitudes at 216 and 272.40 nm were selected for the determination of PRO and BET respectively. The wavelength interval (Dl) was selected as 8 nm. 0.1 N HCL was used as the solvent. BET shows linearity in range of 4-24µg/ml concentration while PRO shows linearity range 3-18µg/ml concentration. The method was validated statistically and recovery studies were carried out. It was found to be accurate, precise and reproducible. The method was applied to the assay of the drugs in tablet dosage form, which were found in the range of 98.98% and 98.73% of the labeled value for both Betahistine Dihydrochloride and Prochlorperazine maleate. Hence, the method here in described can be suitable for analysis of BET and PRO in quality control of. combined pharmaceutical dosage forms

Keywords: Betahistine Dihydrochloride , Prochlorperazine Maleate , Ratio spectra derivative, 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 spectrophotometric method based on simultaneous equations for simultaneous estimation of both drugs in their tablet dosage form. The objective of this work was to develop simple, precise and rapid ratio spectra derivative spectrophotometric method for combination drug products containing BET and PRO.

EXPERIMENTAL

Apparatus

A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was 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, 0.1 N HCL as Solvent and Whatman filter paper no. 41 (Millipore, USA) were used in the study

Preparation of Standard Stock Solutions

An accurately weighed quantity of standard BET (10 mg) and PRO (10 mg) powder were weighed and transferred to 100 ml separate volumetric flasks and dissolved in 0.1 N HCL. The flasks were shaken and volumes were made up to mark with 0.1 N HCL to give a solution containing 100µg/ml each of BET and PRO.

Preparation of Working Standard Solution

From the stock solution of BET 1 ml was pipette out in to 10 ml volumetric flask and volume was adjusted to the mark with 0.1 N HCL to get 10µg/ml of BET. Same From the stock solution of PRO 1 ml was pipette out in to 10 ml volumetric flask and volume was adjusted to the mark with 0.1 N HCL to get 10µg/ml of PRO.

Ratio Spectra Derivative Method

This method works on two mechanisms viz. (1) Ratio and (2) Derivatization. In this method, the mixture spectra are divided with the divisor and first derivative spectra of these ratio spectra are generated. The main advantage of the ratio-spectra derivative spectrophotometry is the chance of doing easy measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals (a maximum or a minimum). Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths give an opportunity for the determination of active compounds in the presence of other compounds and excipients which possibly interferes the assay. For the determination of BET, the spectra of BET at increasing concentrations in 0.1 N HCL were divided by previously stored absorption spectrum standard solution of PRO (6µg/ml) to obtain the corresponding ratio spectra. Then the first derivatives of the obtained ratio spectra were traced with interval of 8 nm. In the binary mixtures, content of BET was determined by measuring the first derivative amplitude at 272.40 nm, where there is no contribution or interference from PRO. On the other hand, for the determination of PRO, an analogous procedure was followed. The spectra of PRO at increasing concentrations were divided by previously stored spectrum of 16µg/mL solution of BET and the first derivative of the developed ratio spectra were traced with interval of 8 nm. In the binary mixtures, content PRO was determined by measuring the first derivative amplitude at 216 nm, where there is no contribution or interference from BET.First-derivative technique (D1) traced with $\Delta\lambda$=8 nm was used to resolve the spectral overlapping. The calibration curves were checked for linearity and linear behavior was observed in the concentration range of 4-24µg/ml for BET and 3-18µg/ml for PRO.

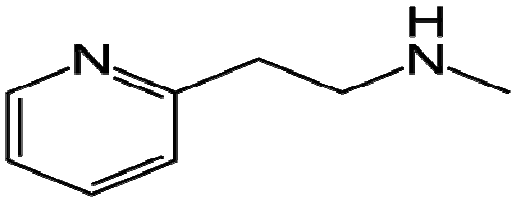

Figure 1- Chemical structure of Betahistine

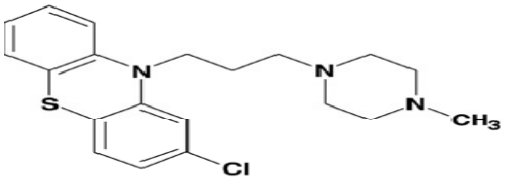

Figure 2- chemical structure ofProchlorperazine

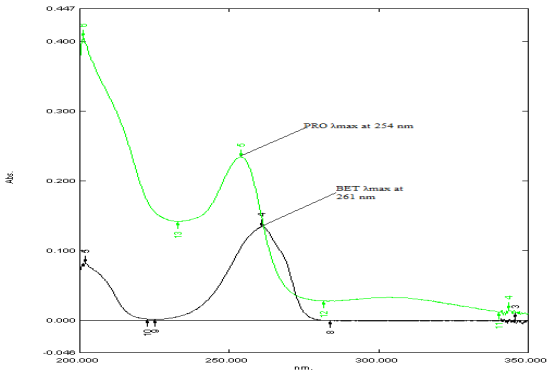

Figure 3: Overlain spectrum of 4µg/ml of BET and 3µg/ml PRO

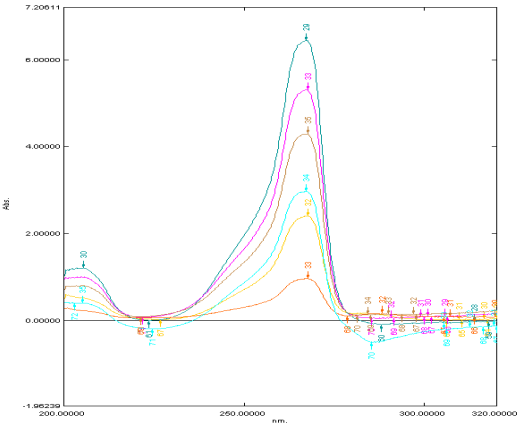

Figure 4a: Ratio spectra of BET when 6µg/ml solution of PRO is used as a divisor

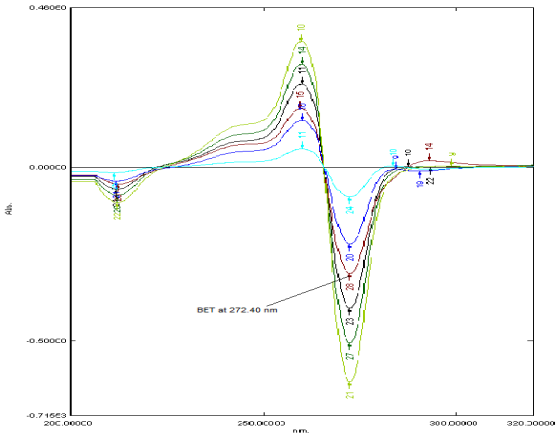

Figure 4b: First derivative ratio spectra for BET at 272.40 nm

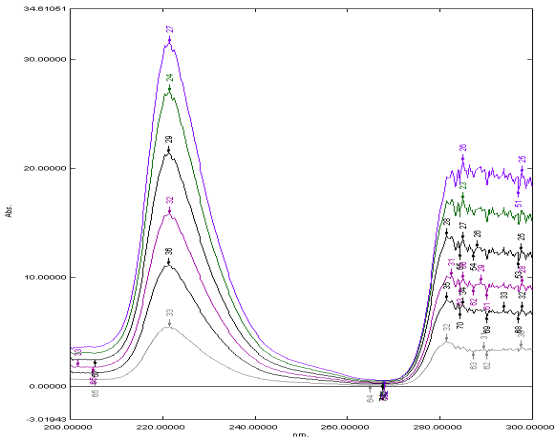

Figure 5a: Ratio spectra of PRO when 16µg/ml solution of BET is used as a divisor

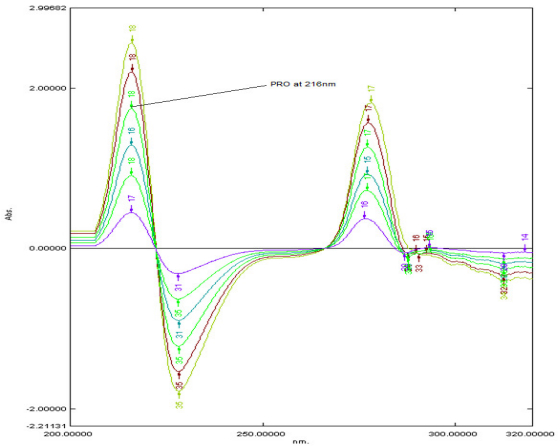

Figure 5b: First derivative ratio spectra for PRO at 216 nm

Method Validation

The method was validated as per ICH guidelines[19] for parameters like Linearity, Accuracy and Precision. The accuracy studies were carried out at different concentrations by spiking a known concentration of standard drug to the pre-analyzed sample and contents were reanalyzed by the developed method. Precision was studied by analyzing six replicates of sample solutions. Intermediate precision was determined in a similar manner on the next day using a different instrument.

RESULTS AND DISCUSSION

Zero-order absorption spectra of 4µg/ml of BET and 3µg/ml of PRO showed overlapping peaks that interfere with the simultaneous determination of this formulation as shown in Figure 3. So it was thought of interest to develop the ratio spectra derivative spectrophotometry method for the simultaneous estimation of BET and PRO in dosage forms. 0.1 N HCL was used as the solvent. Since both the drugs exhibit good solubility in it and no interference due to excipients of the tablet formulation were observed.

Ratio spectra Derivative Spectrophotometry Method

The absorption spectra of BET prepared at increasing concentrations in 0.1 N HCL were recorded in the spectral region of 200.0-400.0 nm and divided by the previously stored spectrum of 6µg/mL PRO in the same solvent and their ratio spectra were obtained as seen in the Figure 4a. Then, the first derivatives of ratio-spectra were recorded as shown in Figure 4b which were plotted with the interval of nm and the values of the derivatives were measured at suitably selected wavelength for the determination of BET. The influence of the obtaining the first derivative was tested and Δλ=8nm was considered as suitable. The concentration of divisor can be modified, and different calibration graphs are then obtained. A concentration of 6µg/ml of PRO was considered as suitable. The calibration graph was established by measuring at the amplitude at 272.40 nm corresponding to a maximum wavelength (Figure 6).

For determining PRO, an analogous procedure was followed. The ratio spectra were obtained by dividing the spectra of PRO with previously stored spectrum of a 16µg/ml BET solution as shown in Figure 5a and their first derivatives were calculated with the interval of 8 nm as shown in Figure 5b. The values of the derivatives were measured at suitably selected wavelength for the determination of PRO. The concentration of divisor can be modified, and different calibration graphs are then obtained A concentration of 16µg/ml of BET was considered as suitable. The calibration graph was established by measuring at the amplitude at 216 nm corresponding to a maximum wavelength (Figure 7).

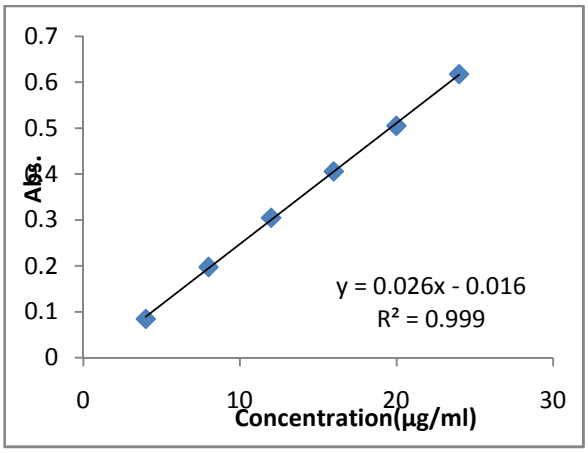

Figure 6: calibration curve of BET at 272.40nm

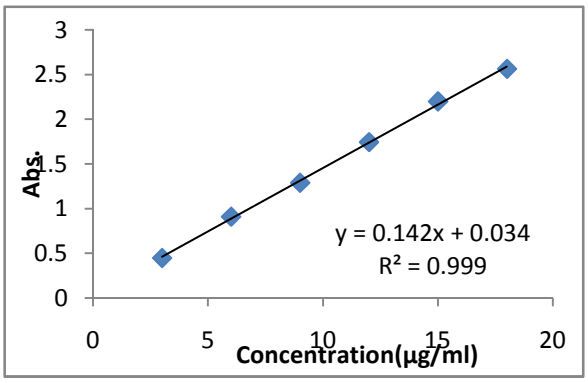

Figure 7: calibration curve of PRO at 216nm

Method Validation

The developed method was validated for parameters like linearity, precision and accuracy. The method was found to be linear in the range of 4-24µg/ml for BET and 3-18µg/ml for PRO with correlation coefficient of 0.9995 and 0.999 for BET and PRO respectively. The data for linearity and precision are presented in the Table 1. The data for recovery study are shown in the Table2. The low value of %R.S.D. indicates that the method is precise and accurate.

CONCLUSION

Ratio derivative method was found to be simple, sensitive, accurate and precise for determination of BET and PRO in tablet dosage form. The method also economic for estimation of BET and PRO in tablet dosage form. Excipients and additives present in the tablet are not interfere in the analysis of BET and PRO in 0.1 N HCL, hence the method is useful for the determination of BET and PRO in tablet dosage form.

PARAMETERS	BET	PRO
Wavelength (nm)	272.40	216
Beer's law limit (µg/ml)	4-24	3-18
Regression equation (y = mx + c) Slope (m) Intercept (c)	Y=0.0263X-0.016 0.0263 0.016	Y=0.142X+0.034 0.142 0.034
Correlation Coefficient (r^2)	0.9995	0.999
Accuracy (Recovery ± S.D.) (n = 3)	99.05±0.51	99.13±0.54
Method precision (Repeatability) (% RSD, n= 6)	0.571	0.474
Interday (n = 3) (% RSD[a])	1.25-1.35	1.02-1.47
Intraday(n = 3) (% RSD)	0.37-1.04	0.49-0.99
LOD(µg/ml)	0.39	0.37
LOQ (µg/ml)	1.19	1.14
Assay ± S. D. (n = 3)	98.98±0.368	98.73±0.512

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	99.14 ± 0.49
	II	6	100	99.09 ± 0.34
	III	6	150	98.92 ± 0.69
PRO	I	6	50	99.26 ± 0.37
	II	6	100	99.19 ± 0.71
	III	6	150	98.96 ± 0.59

Table 2: Recovery Data for the Ratio Spectra Derivative Method (n=3)

SR NO.	Label claim (mg)		Amount found (mg)		% Label claim (mg) (n = 6)	
	BET	PRO	BET	PRO	BET	PRO
1	8	5	7.93	4.93	99.19	98.6
2	8	5	7.91	4.9	98.87	98.0
3	8	5	7.87	4.88	98.37	97.6
4	8	5	7.94	4.96	99.37	99.2
5	8	5	7.92	4.98	99.00	99.6
6	8	5	7.93	4.97	99.12	99.4
MEAN					98.98	98.73
SD					0.368	0.512

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

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REFERENCES

1. Maryadele J O' Neil ed, Merck Index, 14th Edition, Merck & Co. Inc, White House Station NJ: 2005.1177.
2. U. S. Pharmacopoeia/ NF Vol. II. The United states pharmacopeial convention, Twinbrook pathway Rockvillae, 2013.2635.
3. Indian Pharmacopoeia. Vol. II. The Controller of Publication. 6th edition. Govt. of India. New Delhi, 2010.897-898.
4. British Pharmacopoeia. Vol. I. Stationary office. London Medicines and Healthcare product regulatory agency, 2010. 254-255.
5. European Pharmacopoeia 6.0. vol. 2. 6th edition. Starboary:Council of Europe,2008.1292.
6. EnsafiA., Doozandeh F., AllafchianA.,Potentiometric Sensor for Betahistine Determination in Pharmaceuticals, Urine and Blood Serum, J. Braz. Chem. Soc., 2010;21(12):2446-2453.
7. KumarA., ChomwalR.,Nanda S., Spectrophotometric estimation of Betahistine hydrochloride in tablet formulations, J Pharm Bio all Sci 2010;2:121-123.
8. Khedr A., Mahmoud S., Stress degradation studies on Betahistine and development of a validated stability-indicating assay method, Journal of Chromatography B, 2008; 869: 111–117.
9. Jain R., Yadav R., Voltammetric assay of anti-vertigo drug Betahistine hydrochloride in sodium lauryl sulphate, Colloids and Surfaces A: Physicochem. Eng. Aspects 2010; 366; 63–67.
10. Indian Pharmacopoeia. Vol. II. The Controller of Publication. 6th edition. Govt. of India. New Delhi, 2010.1972.
11. Japanese Pharmacopoeia. 15th edition, Shibuya Tokyo Japan; Society of Japanese Pharmacopeia; 2006.1033.
12. U. S. Pharmacopoeia/ NF Vol. II. The United states pharmacopeial convention, Twinbrook pathway Rockvillae, 2013.4912.
13. British Pharmacopoeia. Vol. II. Stationary office. London Medicines and Healthcare product regulatory agency, 2010.1769.
14. European Pharmacopoeia 6.0. Vol. II. 6th edition. Starboary: Council of Europe, 2008.2756.
15. Aman T., Majeed Z., Kazi A., KhanI., Spectrophometric determination of Prochlorperazine Maleate in Pure and pharmaceutical preparation, MikrochimicaActa, 2002;138: 13-17.
16. Venkatesh D., Sundaram S., Meyyanathan S.,Muralidharan S., Shanmugam R., Kannan E., Bhojraj S., and Santhi S., Bioavailability Studies on Developed Prochlorperazine Maleate Sustained Release Tablets by HPLC, Journal of Bioanalysis& Biomedicine, 2009;1: 54-59.
17. Bhagwat G. B., Wate S. P. and Mundhey A. S., Development and Validation of UV Spectrophotometric Method for Estimation of Prochlorperazine Maleate and Pyridoxine Hydrochloride in Tablet Dosage Form by UV Using Multi-Component Mode of Analysis,2013; 3:20-25.
18. Bhosale D., Nikalje A., Development and validation of RP-chromatography for simultaneous determination of paracetamol, caffeine, ergotamine and Prochlorperazine in tablets, Asian Journal of Pharmaceutical Sciences,2013; 7 (5): 364-370.
19. ICH, Q2(R1) Validation of Analytical Procedure:Text and Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland.[Cited 2013 Feb 5], Available from:www.ich.org/ fileadmin/ Public.../ ICH.../ Q2_R1__Guideline.pdf