International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.5, pp 2410-2419, July-Sept 2013

Development and Validation of Simultaneous Estimation of Chlorpheniramine Maleate and Phenylephrine Hydrochloride in Bulk and Capsule Dosage Form by Ultra-Violet Spectrophotometry.

S. J. Wadher*, T. M. Kalyankar, P. P. Panchal

School of Pharmacy, Swami Ramanand Teerth Marathwada University Nanded, Maharashtra, India

***Corres.author: sjwadher@rediffmail.com**
Mobile No: +91 9420683967

Abstract: Simple, accurate, precise, rapid and economical spectrophotometric method has been developed for estimation of chlorpheniramine maleate (CPM) and phenylephrine hydrochloride (PE) in bulk and combined capsule dosage form. Distilled water was used as an economical solvent and all spectrophotometric parameters were optimized. CPM and PE exhibit absorption maxima at 261 nm and 272 nm respectively. The developed simultaneous equation method obeyed Beer-Lambert's law in the concentration range of 2 - 12 µg/ml for CPM and 5 - 30 µg/ml for PE. The method has been validated statically and by recovery studies. The results of analysis have been validated statically.
Keywords: Chlorpheniramine maleate, phenylephrine hydrochloride, UV spectrophotometric, simultaneous equation method, validation.

Introduction

Chemically Chlorpheniramine Maleate (CPM) is (*RS*)-3-(4-chlorophenyl)-3-(pyrid-2-yl) propyldimethylamine hydrogen maleate (Figure 1) [1]. Chlorpheniramine maleate, is an alkylamine derivative with the actions and uses of the antihistamines. It is one of the most potent antihistamines and causes a moderate degree of sedation [2]. For estimation of chlorpheniramine maleate, HPLC chemometric-assisted spectrophotometric methods have been reported [3]. It is used as an antihistaminic in allergic reactions, prevents muscular response of histamine and thereby reducing cough receptors lining the respiratory mucous membrane for symptomatic treatment of common cold.[4]

Chemically Phenylephrine hydrochloride (PE) is (R)-1-(3-hydroxyphenyl)-2 methylaminoethanol hydrochloride (Figure 2) [5], is a direct sympathomimetic agent, a selective α1 agonist, causing vasoconstriction. It is also a frequent constituent of orally administered nasal decongestant preparations [6]. Phenylephrine hydrochloride is widely used as a decongestant drugs and available as an oral medicine or as a nasal spray. Phenylephrine is rarely used as a vasopressor to increase the blood pressure in unstable patients with hypotension.[7]

Literature survey revealed that spectrophotometry [8-10], chromatography [11-13], micellar liquid chromatography [14], methods have been reported for the estimation of phenylephrine hydrochloride in pharmaceutical formulations alone or in combination with other drugs.[4]

Figure 1: Chlorpheniramine Maleate

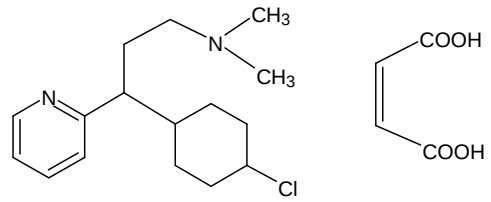
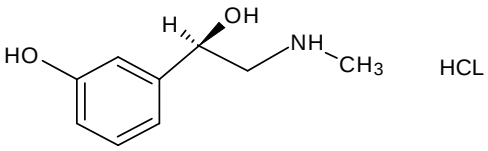


Figure 2: Phenylephrine Hydrochloride



Some procedures have been described for the assay of either chlorpheniramine maleate or phenylephrine hydrochloride in pharmaceutical preparations, such as spectrophotometry [15-25] and HPLC [26–39]. Numerous UV, HPLC and HPTLC based methods have been reported for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms. But no method had yet been reported for simultaneous estimation of these two drugs using UV in bulk drug and capsule dosage forms using water as solvent. This paper describes simple, accurate, precise, rapid and economical method for simultaneous determination of chlorpheniramine maleate and phenylephrine hydrochloride from capsule formulation.

Materials And Methods

Instrumentation And Glasswares

UV-visible spectrophotometer – Shimadzu 1800 with photomultiplier tube detector with 10 mm matched quartz cell.

Digital ultrasonic cleaner (Sonicator): - Hmq. India.

Chemicals And Reagents

All the chemicals and solvents used during work were of analytical grade. All the solutions were filtered using Whatman filter paper no. 41. The pure CPM and PE were received as a gift samples from Shreya Life Sciences Pvt. Ltd., Waluj, Aurangabad (Maharashtra, India). The solvent used is distilled water.

Simultaneous Equation Method

Selection of Analytical Wavelength

Pure drug sample of CPM and PE were dissolved separately in distilled water so as to get several different dilutions of standard in the concentration range 2 - 12 µg/ml for CPM and 5 - 30 µg/ml for PE. All the dilutions were scanned in the range of 200 - 400 nm. Figure 3 and 4 represents the absorbance spectra of CPM and PE respectively. Figure 5 represents the overlain spectra of both the drugs. Two wavelengths selected for formation of simultaneous equations are 261 nm and 272 nm for CPM and PE respectively.

Determination of E (1%, 1cm) of Drugs at Selected Wavelengths

Aliquot portion of CPM and PE solutions were diluted separately with distilled water to obtain a concentration of 10 µg/ml for CPM and PE. The absorbance of each resulting solution were measured at 261 nm and 272 nm. The E (1%, 1cm) values (ax1, ax2, ay1& ay2) were determined from five different concentrations of 10 µg/ml of CPM and PE using following equation 1.

$$E\ (1\%, 1cm) = \frac{\text{Absorbance}}{\text{Conc. (g/100ml)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Absorptivity values for CPM at 261 nm and 272 nm are shown in Table 1.

Absorptivity values for PE at 261 nm and 272 nm are shown in Table 2.

Figure 3: Absorbance spectra of CPM

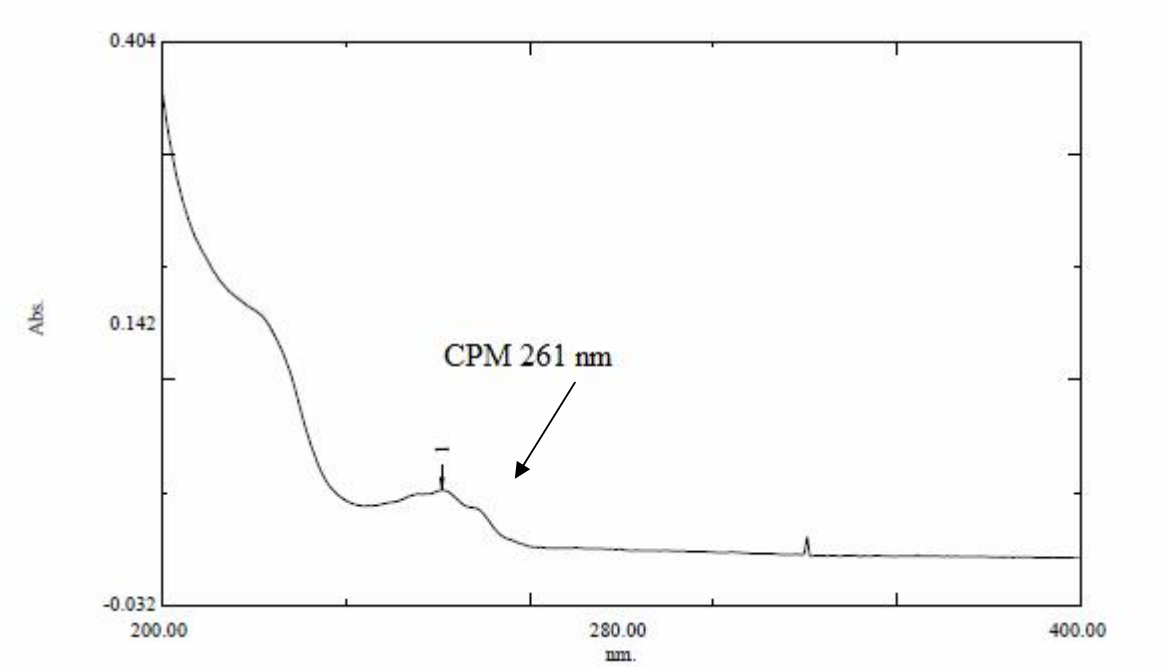


Figure 4: Absorbance spectra of PE

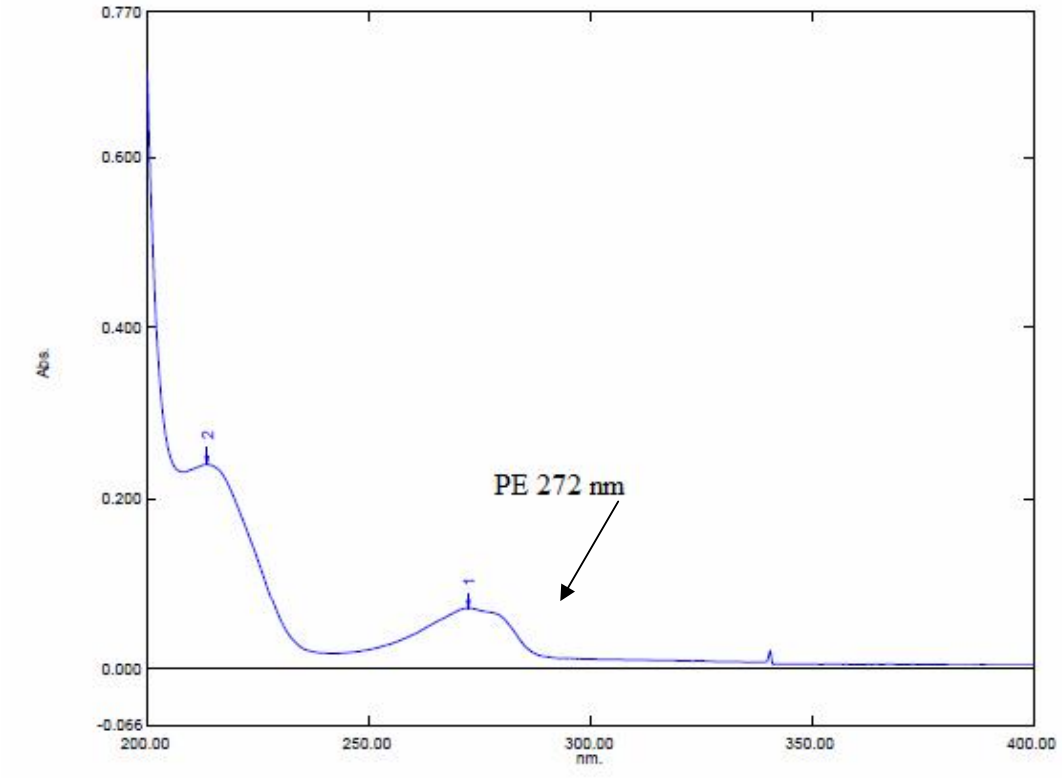


Figure 5: Overlain spectrum of CPM (λ_{max} 261nm) and PE (λ_{max} 272nm) in Distilled water

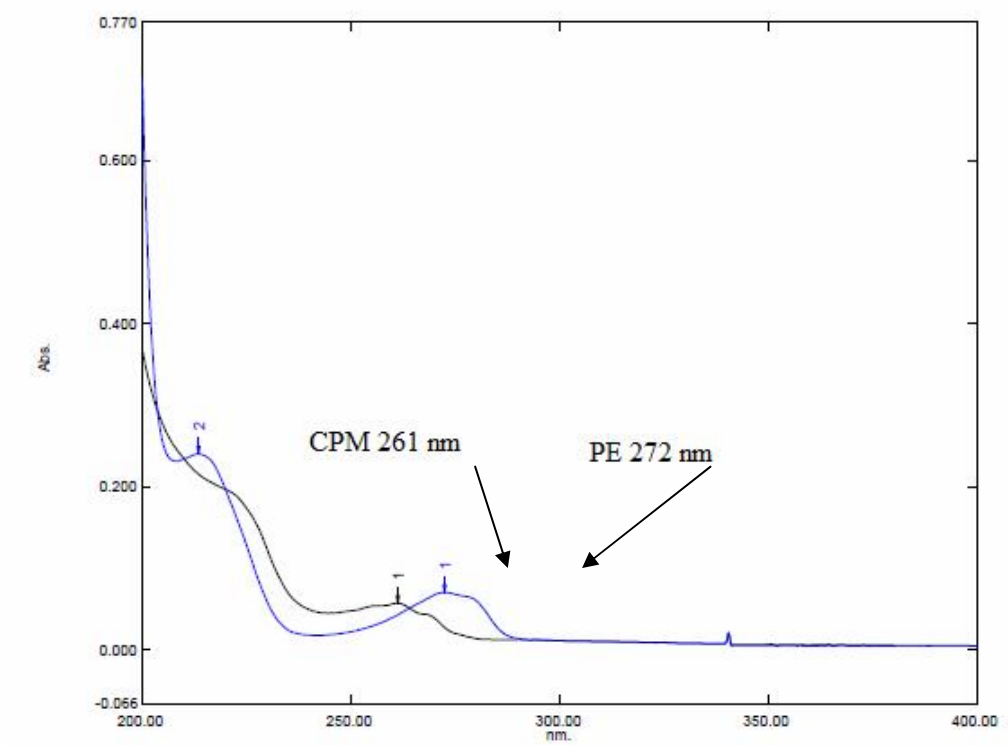


Table 1: Absorptivity values for CPM at 261 nm and 272 nm

Sr. no.	Conc. of CPM (g/100ml)	Absorbance		E (1%, 1cm)	
		261 nm	272 nm	261 nm	272 nm
1	0.001	0.186	0.083	186	83
2	0.001	0.187	0.082	187	82
3	0.001	0.185	0.083	185	83
4	0.001	0.186	0.081	186	81
5	0.001	0.186	0.081	186	81
			Mean	**186**	**82**
			S.D.	**0.707106781**	**1.00**

Table 2: Absorptivity values for PE at 261 nm and 272 nm

Sr. no.	Conc. of PE (g/100ml)	Absorbance		E (1%, 1cm)	
		261 nm	272 nm	261 nm	272 nm
1	0.001	0.047	0.092	47	92
2	0.001	0.048	0.091	48	91
3	0.001	0.049	0.092	49	92
4	0.001	0.050	0.092	50	92
5	0.001	0.051	0.093	51	93
			Mean	**49**	**92**
			S.D.	**1.58113883**	**0.707106781**

Preparation of Sample Solution

Twenty capsules were weighed accurately; shell was removed and average weight of granules was determined and triturated to produce fine powder. A quantity equivalent to 8 mg of CPM and 20 mg of PE was weighed and transferred to 50 ml volumetric flask; dissolved in 25 ml of distilled water. This solution was sonicated for 15 min and filtered. Then filtrate was made 50 ml with distilled water. This solution was appropriately diluted with distilled water to get concentration of 8 µg/ml of CPM and 20 µg/ml of PE. The absorbance A_1 and A_2 of sample solution was measured at 261 nm and 272 nm in 1 cm cell against blank. The contents of CPM and PE in capsule dosage form were calculated using two framed simultaneous equations 2 and 3.[41]

$$Cx = \frac{A_2\, ay_1 - A_1\, ay_2}{ax_2\, ay_1 - ax_1\, ay_2} \quad \text{.....(2)}$$

$$Cy = \frac{A_1\, ax_2 - A_2\, ax_1}{ay_1\, ax_2 - ay_2\, ax_1} \quad \text{.....(3)}$$

Where, A_1 and A_2 are absorbance of mixture at 261 nm and 272 nm respectively, ax_1 and ax_2 are absorptivities of CPM at λ_1 and λ_2 respectively and ay_1 and ay_2 are absorptivities of PE at λ_1 and λ_2 respectively. C_x and C_y are the concentrations of CPM and PE respectively. The results of analysis are given in Table 3.

Method Validation

Validation of an analytical procedure is the process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirements for the intended analytical application. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The proposed method was validated for various parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantisation (LOQ) according to ICH Q2 (R1) guidelines [42].

Table 3: Results of Analysis of Capsule Formulation

Drug	Label Claim(mg)	Amount Found(mg)	Mean % Drug Recovered ± SD*	% RSD
CPM	8	7.89	98.75 ± 0.443	0.45
PE	20	19.80	99.02 ± 0.971	0.98

*Denotes average of 3 determinations

Linearity

Linearity was studied by preparing solutions at different concentration levels. Calibration curve of Absorbance vs. Concentration was plotted using standard solutions of 2 - 12 µg/ml of CPM and 5 - 30 µg/ml of PE and regression line equation and correlation coefficient was determined (Figure 6,7).

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [42]. Precision of the method was studied by intraday and interday variations in the test method of CPM and PE. Method repeatability (intra-day precision) was evaluated by assaying six samples, prepared as described under sample preparation. Inter day precision was performed by assaying six samples in different days as described in the sample preparation. The results are presented in Table 4 and 5.

Figure 6: Linearity Graph of CPM at 261 nm.

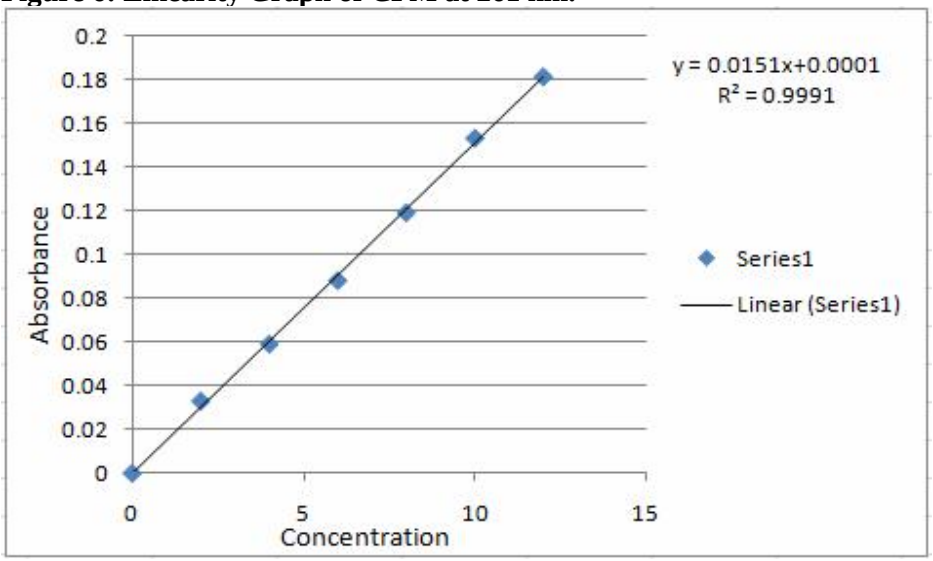


Figure 7: Linearity Graph of PE at 272 nm.

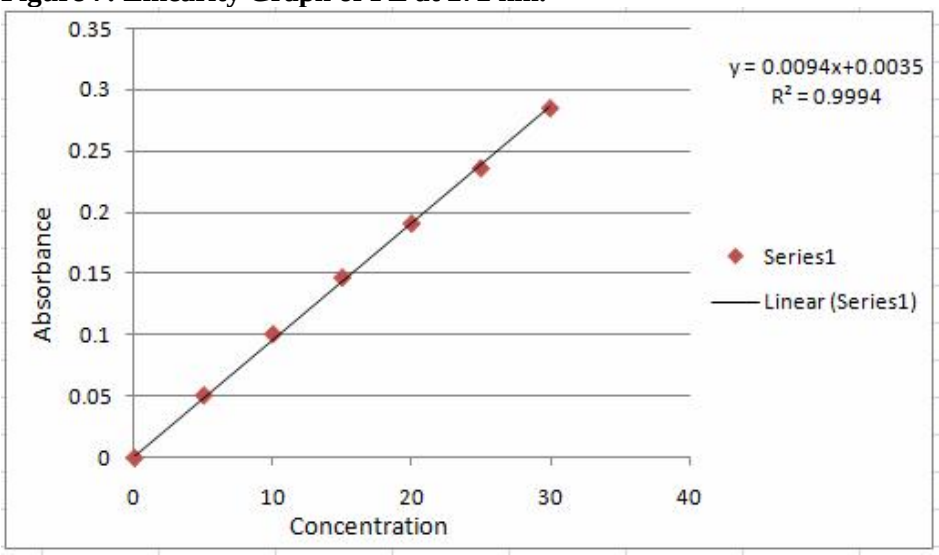


Table 4: Intraday Precision

Drug	Concentration(µg/ml)	Mean % Assay ± SD*	% RSD
CPM	8	99.15 ± 0.644	0.65
PE	20	99.60 ± 1.036	1.04

*Denotes average of 6 determinations

Table 5: Interday Precision

Drug	Concentration(µg/ml)	Mean % Assay ± SD*	% RSD
CPM	8	98.95 ± 0.719	0.73
PE	20	100.76 ± 1.263	1.25

*Denotes average of 3 determinations

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness [42]. Accuracy of the method was determined by calculating recovery of CPM and PE at 80 %, 100 % and 120 % level of sample solutions of CPM and PE. The results are presented in Table 6.

Limit Of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value [42]. The detection limit was determined by using six sets of calibration curves and estimating the standard deviation of the response and slope of the calibration curve. The results were calculated using following equation and the results are presented in Table 7.

$$LOD = 3.3 \frac{\sigma}{S}$$

Where,
σ = Standard deviation of y-intercept of the calibration curves.
S = Slope of calibration curve.

Limit Of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy [42]. The quantitaion limit was calculated using six sets of calibration curves and estimating the standard deviation of the response and slope of the calibration curve. The results were calculated using following equation and results are presented in Table 7.

$$LOQ = 10 \frac{\sigma}{S} \quad \text{---------- (6)}$$

Where,
σ = Standard deviation of y-intercept of the calibration curves.
S = Slope of calibration curve.

Table 6: Recovery Study

Drug	Amount Added(µg/ml)	Amount Recovered(µg/ml)	% Recovery ± SD*	% RSD
CPM	80%(6.4 µg/ml)	6.30	98.61% ± 0.276	0.28
	100%(8 µg/ml)	7.92	99.09% ± 0.670	0.68
	120%(9.6 µg/ml)	9.55	99.51% ± 0.568	0.57
PE	80%(16 µg/ml)	16.03	100.25% ± 0.975	0.97
	100%(20 µg/ml)	19.77	98.90% ± 0.406	0.41
	120%(24 µg/ml)	23.79	99.18% ± 0.435	0.44

*Denotes average of 3 determinations

Table 7: Analytical Method Validation Results

Sr. No	Parameter	CPM	PE
1	λ max, nm	261nm	272 nm
2	Beer's law limit(µg/ml)	2 – 12	5 – 30
3	Regression Equation	Y= 0.0151x + 0.0001	Y= 0.0094x + 0.0035
4	Slope	0.0151	0.0094
5	y-intercept	0.0001	0.0035
6	Correlation Coefficient	R^2 = 0.9991	R^2 = 0.9994
7	% R.S.D of Intraday Precision	0.65	1.04
8	% R.S.D of Interday Precision	0.73	1.25
9	% Recovery	98.61-99.51	98.90-100.25
10	LOD(µg/ml)	0.115	0.200
11	LOQ(µg/ml)	0.348	0.608

Results And Discussion

The validation parameters for the proposed analytical spectrophotometric method are given in Table 7. Linearity was found to be in the concentration range of 2 - 12 µg/ml for chlorpheniramine maleate and 5 - 30 µg/ml for phenylephrine hydrochloride. Regression analysis was made for the slope (m), intercept (c) and correlation coefficient (R^2) as shown in Table 7. Higher values of correlation coefficient (R^2) indicate good linearity of the calibration curve for both the drugs as shown in Fig.4 and 5.The proposed method was found to be precise as % R.S.D values for intraday as well as interday precision were less than 2 % (Table 4 and 5) . The accuracy of the method was proved by performing recovery studies on the commercial formulation at 80, 100 and 120% level. Recovery ranges from 98.61-100.25% (Table 6). The results of recovery study indicate that these drugs could be quantified simultaneously and that there is no interference of the excipients present in the formulation. Sensitivity of the method was determined by calculating limit of detection (LOD) and limit of quantification (LOQ). Drug content in the capsule was directly calculated from the given equations (Eq.2 and 3) and the results ranges from 98.75 - 99.02% in as shown Table 3.

Acknowledgement

The authors would like to convey our regards to Shreya Life Sciences Pvt. Ltd., Waluj, Aurangabad (Maharashtra, India) for providing gift sample of CPM and PE for research. The authors would also like to thank the Director, School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded for providing all the facilities to carry out the work.

References

1. Indian pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Vol- II, published by controller of publication, Delhi, 2007, 301-303.
2. Nevin Erk. Quantitative analysis of chlorpheniramine maleate and phenylephrine hydrochloride in nasal drops by differential-derivative spectrophotometric, zero-crossing first derivative UV spectrophotometric and absorbance ratio methods. Journal of Pharmaceutical and Biomedical Analysis 2000:23; 1023–1031.
3. Sawant R., Joshi RP, and Bhangale L.. Simultaneous estimation & validation of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate in tablets by spectrophotometric. JPRHC ;Volume 3 Issue 2 : Page 23-28.

4. Sawant R, Joshi R, Sawant M, Lanke P and Bhangale L. Mathematical and Multiwavelength Spectrophotometric Methods for Simultaneous Estimation of Paracetamol, Phenylephrine Hydrochloride, Chlorpheniramine Maleate and Caffeine. International Journal of Pharmaceutical Frontier Research, July-Sep 2011; 1(2): 31-38
5. Indian pharmacopoeia, Govornment of India, Ministry of health and family welfare, Vol- III, published by controller of publication, Delhi, 2007, 936-937..
6. Wankhede SB. Lad KA, Chitlange SS. Development and Validation of UV-Spectrophotometric Methods for Simultaneous Estimation of Cetirizine hydrochloride and Phenylephrine hydrochloride in Tablets. International Journal of Pharmaceutical Sciences and Drug Research 2012; 4(3): 222-226
7. Othman NS. Fatah NT. Indirect Spectrophotometric Determination of Phenylephrine Hydrochloride in Pharmaceutical Preparations. Tikrit Journal of Pure Science :2011 ;16 (2) ; 1813 – 1662
8. Giglio KJ. Flow-injection determination of phenylephrine hydrochloride in pharmaceutical dosage forms with on-line solid-phase extraction and spectrophotometric detection phenylephrine hydrochloride and orphenadrine citrate in pure and in dosage forms. Talanta. 2004; 64: 1226.
9. Erk N. Quantitative analysis of chlorpheniramine maleate and phenylephrine hydrochloride in nasal drops by differential-derivative spectrophotometric, zero-crossing first derivative UV spectrophotometric and absorbance ratio methods. J.Pharm. Biomed. Anal. 2000; 23: 1023.
10. Ahmed IS, Amin AS. Spectrophotometric microdetermination of phenylephrine hydrochloride in pure pharmaceutical formulations using haematoxylin. J. Molecul.Liq. 2007; 130: 84.
11. JO DB, Vandenbroucke CV, Massart DL. Experimental design for the rapid selection of separation conditiones for methyl and propyl parahydroxybeyoate, phenylephrine hydrochloride and chlorphenamine maleate by ion-pair liquid chromatography. J. Pharm. Biomed. Anal. 1994; 12: 1379.
12. Galmier MJ, Frasey AM, Meski S, Beyssac E, Petit J, Aiache JM, Lartigue C. High performance liquid chromatographic determination of phenylephrine and tropicamide in human aqueous humor. Biomed. Chromatogr 2000; 14: 202.
13. Erk N, Kartal M. Simultaneous high performance liquid chromatographic and derivative ratio spectra spectrophotometry determination of chloropheniramine maleate and phenylephrine hydrochloride. Il Farmaco. 1998; 53: 617.
14. Gil-Agusti M, Monferrer-Pons L, Celia Garcia-Alvarez-Coque M, Esteve-Romero. Determination of active ingredients in cough-cold preparations by micellar liquid chromatography. J. Talanta. 2001; 54:621.
15. Lucy CA, Cantwell FF., Anal. Chem. 58 (1986) 2727–2731.
16. Murtha JL, Julian TN, Radebaugh GW, J. Pharm. Sci. 77 (1988) 715–718.
17. Hoover JM, Soltero RA, Bansal PC, J. Pharm. Sci. 76 (1987) 242–244.
18. El-Bolkiny MN, Hana S, El-Henawee MM, Ayad MM, Egypt J. Pharm. Sci. 33 (1992) 31–42.
19. Abdelmageed OH, Bull. Fac. Sci. Assiut Univ. B 22 (1993) 179–195.
20. Tuszynska E, Podolska M, Kwyatkowska-Puchniarz B, Kaniewska T, Acta Pol. Pharm. 51 (1994) 317–323.
21. Yang G, Qing H, Li P, Liu Y, Shenyang Yaoxueyuan Xuebao 9 (1992) 168–171.
22. Barary MH, Wahbi AM, Drug Dev. Ind. Pharm. 17 (1991) 457–461.
23. Lin LM, Zhang NJ, Wang HY, Yaoxue Xuebao 26 (1991) 451–457.
24. Shoukrallah I, Anal. Lett. 24 (1991) 2043–2045.
25. Leung CP, Law CK, Analyst 114 (1989) 241–242.
26. El Sadek M, El Shanawany A, Aboul Khier A, Ruecker G, Analyst 115 (1990) 1181 1184.
27. Kountourellis JE, Markopoulou CK, Georgakopoulos PP, Anal. Lett. 23 (1990) 883 891.
28. Fermandez Otero GC, Lucangioli SE, Carducci CN, Chromatogr J. 654 (1993) 87– 91.
29. Cui X, Li H, Longshui J, Yanbian Yixueyuan Xuebao 17 (1994) 129–130.
30. Thomas BR, Fang XG, Shen P, Ghodbane S, J. Pharm. Biomed. Anal. 12 (1994) 85- 90.
31. Guo D, Yang S, Lu W, Yaowu Fenxi Zazhi 14 (1994) 32–35.
32. Akiyama S, Nakashima K, Yamada K, Shirakawa N, Bull. Chem. Soc. Jpn. 64 (1991) 3171–3172.
33. Lau OW, Mok CS, J. Chromatogr. 693 (1995) 45–54.
34. Raju NR, Srilakshmi P, Krishna UM, Indian Drugs 29 (1992) 408–411.
35. Liang G, Qian H, Zhu L, Wang Y, Sun L, Zhongguo Yaoxue Zazhi 29 (1994) 46–48 (CA 121, 308487g, 1994).
36. Indrayanto G, Sunarto A, Adriani Y, J. Pharm. Biomed. Anal. 13 (1995) 1555–1559.
37. De Beer JO, Vandenbroucke CV, Massart DL, J. Pharm. Biomed. Anal. 12 (1994) 1379–1396.
38. Erk N, Kartal M, Il Farmaco 53 (1998) 617–622.

39. Das-Gupta V, Jagdish JT, Drug Dev. Ind. Pharm. 13 (1987) 473–486.
40. Maithani M, Raturi R, Gautam V, Kumar D, Chaudhary AK, Gaurav A and Singh R. Development and validation of a RP-HPLC method for the determination of chlorpheniramine maleate and phenylephrine in pharmaceutical dosage form. International Journal of Comprehensive Pharmacy 2010, Vol. 01, Issue 05.
41. Beckett AH, Stanlake JB. Practical Pharmaceutical Chemistry.4th ed. New Delhi: CBS Publishers and Distributors; 1997.p. 284.
42. ICH, Q2 (R1) Validation of Analytical Procedures: Text and Methodology, November; 2005.

*****