



Development and Validation of UV-Spectrophotometric Methods for Simultaneous Estimation of Cetirizine hydrochloride and Phenylephrine hydrochloride in Tablets

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ABSTRACT

Without resolving mixtures of Cetirizine hydrochloride and Phenylephrine hydrochloride, simultaneous estimation has been successfully achieved by spectrophotometry. First method, simultaneous equation method employs formation and solving of mathematical simultaneous equation using 237.5 nm and 232.0 nm as the λ_{max} of Phenylephrine hydrochloride and Cetirizine hydrochloride respectively in distilled water. Second method is first order derivative spectroscopy, wavelengths selected for quantitation were 232.0 nm for Phenylephrine hydrochloride (zero cross for Cetirizine hydrochloride) and 242.5 nm for Cetirizine hydrochloride (zero cross for Phenylephrine hydrochloride). These methods were validated as per ICH norms. Calibration curves were linear over the concentration ranges of 12-60 $\mu\text{g/ml}$ for both drugs and for both methods. The validation study is statistically significant as all the statistical parameters are within the acceptance range (% RSD < 2.0 and S.D. < 2.0) for both accuracy and precision. The methods are successfully applied to pharmaceutical formulation, with no interference from excipients as indicated by the recovery study. The proposed methods are simple, rapid, economic and accurate for routine simultaneous estimation of Phenylephrine hydrochloride and Cetirizine hydrochloride. Simultaneous equation method was successfully applied to carry out dissolution study of commercial tablet formulation by using USP II dissolution test apparatus.

Keywords: Phenylephrine hydrochloride, Cetirizine hydrochloride, simultaneous estimation, spectrophotometry.

INTRODUCTION

Phenylephrine hydrochloride (PHE), chemically (R)-1-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride (Fig. 1) ^[1], is a direct sympathomimetic agent, a selective α_1 agonist, causing vasoconstriction. It is also a frequent constituent of orally administered nasal decongestant preparations.

Cetirizine hydrochloride (CTZ), chemically [2-[4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl] ethoxy] acetic acid (Fig. 2) ^[2], belongs to the group of second generations antagonists of H_1 -receptors, inhibits the allergic reaction mediated by histamine. It is a non-sedative antihistamine, used in the treatment of seasonal rhinitis, hay fever, running nose, control sneezing of allergic origin.

Phenylephrine hydrochloride and Cetirizine hydrochloride are official in IP and BP, both describes a potentiometric

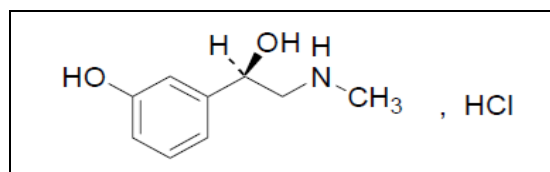


Fig. 1: Structure of Phenylephrine hydrochloride

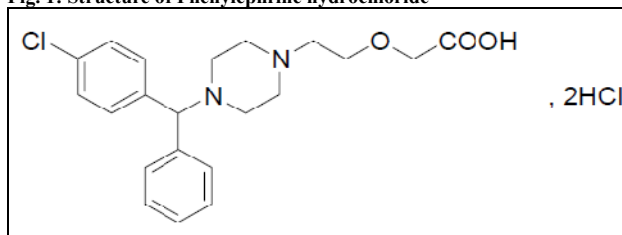


Fig. 2: Structure of Cetirizine hydrochloride.

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method for the assay. Literature reveals that many analytical methods are specified for the determination of PHE and CTZ as individual and combined dosage form with other combination of drugs and also in biofluid viz., UV-visible spectrophotometry ^[3-5], HPLC ^[6-15], fluorometry ^[16], HPTLC ^[17-20] and ion-pair chromatographic method. ^[21]

Since no spectrophotometric method is reported for simultaneous estimation of PHE and CTZ in combined dosage form therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously by the simultaneous equation and first order derivative method.

MATERIALS AND METHOD

Phenylephrine hydrochloride and Cetirizine hydrochloride were obtained as a gift sample from Amrut Drug Research Lab Pvt. Ltd., Tarapur and Praveen Laboratories Pvt. Ltd., Surat respectively. Combined dose tablet formulation containing Phenylephrine hydrochloride IP (10 mg) and Cetirizine hydrochloride IP (10 mg), Allercet-DC manufactured by Micro Labs Ltd, was purchased from local market. Distilled water was used to prepare all solutions. Spectroscopic analysis was carried out using a double-beam Shimadzu UV-Visible spectrophotometer, 1601 Pharmaspec, with spectral band width of 2 nm, wavelength accuracy ± 0.5 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of solution.

Preparation of standard stock solution

Accurately weighed about 50 mg of PHE and CTZ was transferred to 100.0 ml volumetric flask and 50.0 ml of distilled water was added to dissolve the drug and diluted up to the mark with distilled water, to get 500 μ g/ml of PHE and 500 μ g/ml of CTZ in separate volumetric flask.

Method I: Simultaneous equation method

For the selection of analytical wavelength, standard solution of PHE (50 μ g/ml) and CTZ (50 μ g/ml) were prepared separately by appropriate dilution of standard stock solution with distilled water and scanned in the entire UV range against distilled water as blank. Absorption spectra of both drugs were recorded and the overlay spectrum is depicted in Fig. 3. The λ_{max} of PHE and CTZ were found to be 273.5 nm and 232.0 nm, respectively. A series of standard solutions were prepared having concentration range of 12-60 μ g/ml for both drugs. The absorbance of resulting solutions was measured at 273.5 nm and 232.0 nm and calibration curves were plotted. Both the drugs obeyed linearity in the concentration range under study. A (1%, 1cm) values were then determined for both the drugs at selected wavelengths. Two simultaneous equations (in two variables C_1 and C_2) were formed using A (1%, 1cm) values obtained and are as follows:

$$A_1 = 76.77C_1 + 5.92C_2 \quad (1)$$

$$A_2 = 36.65C_1 + 277.27C_2 \quad (2)$$

Where, A_1 and A_2 are the absorbance of sample solution at 273.5 nm and 232.0 nm respectively. C_1 and C_2 are the concentrations of PHE and CTZ (in g/100ml), in sample solutions. A (1%, 1cm) values 76.77 & 36.65 are of PHE at 273.5 nm and 232.0 nm, respectively. Similarly, 5.92 & 277.27 are A (1%, 1cm) values of CTZ at 273.5 nm and 232.0 nm, respectively. By applying the Cramer's rule to equation (1) and (2), the concentration C_{PHE} and C_{CTZ} , can be obtained as follows,

$$C_{PHE} = \frac{A_1(277.27) - A_2(5.92)}{21069.05} \quad (3)$$

$$C_{CTZ} = \frac{A_1 - (76.77)C_{PHE}}{5.92} \quad (4)$$

Method II: First order derivative method:

In this method, solutions of PHE and CTZ (50 μ g/ml, each), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm against distilled water as blank. The absorption spectra thus obtained were derivatized for first order (Fig. 4 and 5). From the derivative spectra obtained, the wavelengths were selected in a manner such that PHE had zero crossing point at 242.5 nm and CTZ showed a measurable $dA/d\lambda$ where as the zero crossing point of CTZ at 232.0 nm and PHE showed appreciable $dA/d\lambda$. Hence wavelengths 232.0 nm and 242.5 nm were selected as analytical wavelength for quantitation of PHE and CTZ respectively. The calibration curves for PHE (12-60 μ g/ml) and CTZ (12-60 μ g/ml) were plotted as $dA/d\lambda$ verses concentration at wavelength 232.0 nm and 242.5 nm, respectively.

Analysis of tablet formulation

A quantity of tablet powder equivalent to about 10 mg of PHE was transferred to 100.0 ml volumetric flask; 60.0 ml of distilled water was added and ultrasonicated for 20 min, volume was then made up to the mark with distilled water. The resulting solution was mixed and filtered through Whatmann filter paper no. 42. From the filtrate, 5.0 ml of solution was diluted to 25.0 ml with distilled water so as to obtain final concentration of about 20 μ g/ml of each drug. In method I, the concentration of both PHE and CTZ were determined by measuring absorbance of sample solution at 273.5 nm & 232.0 nm and using equations (3) and (4). In method II, absorbance of sample solution was measured in first order derivative mode at 232.0 nm & 242.5 nm. Concentration of PHE and CTZ in the diluted solution was obtained from calibration curves. Amount of PHE and CTZ in mg/tab was then calculated. Results of tablet analysis are shown in Table 2.

Validation

The proposed methods were validated as per ICH guidelines.

Accuracy

To ascertain accuracy of the method recovery studies were performed by the standard addition method. Tablet powder equivalent to 10 mg of PHE/CTZ was weighed and transferred to 100.0 ml volumetric flask, added 8 mg, 10 mg and 12 mg of PHE/CTZ pure drug to the tablet powder for 80%, 100% and 120% level of recovery. Extraction and dilutions were performed with distilled water. Solutions were prepared in triplicate and analyzed. The procedure was repeated for three consecutive days. Accuracy was determined and expressed as percent recovery. The result of recovery study is given in Table 3.

Precision

Method repeatability was determined by six times repetitions of assay procedure. The reproducibility of the proposed method was determined by analyzing tablet at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision). Precision of analyst was determined by repeating study by another analyst working in the laboratory. Standard deviation and percent RSD was determined. Results of precision studies are shown in Table 1.

Limit of detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of PHE and CTZ by the proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively,

Table 1: Optical characteristics of the proposed methods and result of precision study

Parameters	Phenylephrine hydrochloride		Cetirizine hydrochloride	
	Method I	Method II	Method I	Method II
Wavelength (nm)	273.5	232.0	232.0	242.5
Beer's law range (µg/ml)	12-60	12-60	12-60	12-60
Regression Slope (m)	0.007	-0.001	0.026	-0.001
Equation Intercept(c)	-0.002	0.000	0.016	-0.001
Correlation coefficient	0.999	0.999	0.999	0.998
LOD (µg/ml)	1.58	2.76	0.64	2.95
LOQ (µg/ml)	4.80	8.36	1.94	8.94
Repeatability*	±0.311, 0.307	±0.584, 0.583	±0.804, 0.809	±0.252, 0.250
Precision (SD±, RSD)	Intra-day*	±0.740, 0.739	±0.433, 0.434	±0.235, 0.234
	Inter-day*	±0.721, 0.717	±0.416, 0.417	±0.610, 0.610
	Analyst**	±0.754, 0.753	±0.725, 0.722	±0.629, 0.625

*mean of six determinations

**mean of three determinations

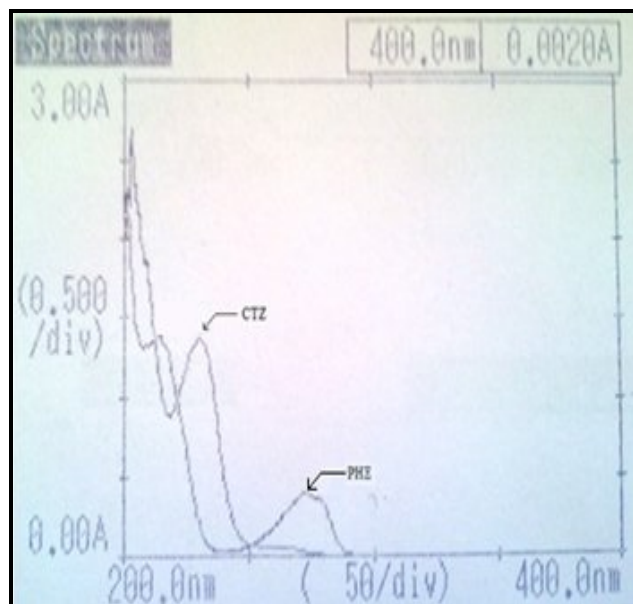
Table 2: Results of analysis of tablet formulation

Method	Drug	Amount of drug estimated (mg/tablet)	% Label Claim*	SD	RSD
Simultaneous Equation Method	PHE	10.09	100.94	±1.042	1.032
	CTZ	9.89	98.99	±0.377	0.380
	PHE	9.99	99.99	±1.359	1.359
First Order Derivative Method	CTZ	9.97	99.76	±1.424	1.427

*mean of six determinations

Table 3: Result of accuracy (recovery studies)

Level of Recovery	Amount of pure added (mg)		Method-I				Method-II			
			Amt of drug recovered (mg)		Percent Recovery		Amt of drug recovered (mg)		Percent Recovery	
	PHE	CTZ	PHE	CTZ	PHE	CTZ	PHE	CTZ	PHE	CTZ
80%	8.1	8.1	8.08	8.13	99.81	100.48	8.10	8.09	100.00	99.87
	8.1	8.1	8.00	8.13	98.84	100.47	8.09	8.09	99.87	99.87
	8.0	8.0	8.04	8.10	100.57	101.35	8.13	7.85	101.62	98.12
100%	10.0	10.1	9.93	10.17	99.31	100.77	10.01	10.09	100.10	99.90
	10.0	10.2	9.87	10.15	98.70	99.54	10.13	10.21	101.30	100.09
	10.0	10.0	9.94	10.13	99.49	101.35	10.16	10.02	101.60	100.20
120%	12.1	12.1	12.18	12.09	100.68	99.92	12.09	12.08	99.91	99.83
	12.2	12.1	12.25	12.11	100.46	100.14	12.30	11.93	100.81	98.59
	12.0	12.0	12.10	101.72	101.88	12.07	11.82	100.58	98.50	
Mean % recovery					99.95	100.54			100.64	99.38
SD					±0.983	±0.615			±0.721	±0.781
RSD					0.983	0.615			0.721	0.781

**Fig. 3: Overlay spectra of Phenylephrine hydrochloride and Cetirizine hydrochloride**

where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation. Results are shown in Table 1.

Dissolution study

The dissolution study was carried out for the above combination. A calibrated dissolution apparatus (USP II) was

used with paddles at 50 rpm and bath temperature maintained at $37 \pm 1^\circ\text{C}$. Nine hundred millilitres freshly prepared and degassed 0.1N HCl solution was used as the dissolution medium. Six tablets were evaluated and dissolution sample were collected at 5, 10, 15, 20, 25 and 30 min interval. At each time point, a 5 ml sample was removed from each vessel with replacement; it was filtered through Whatmann filter paper and analyzed by simultaneous equation method. Percentage release of PHE and CTZ was calculated (Fig. 6).

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and reliable way for quantitative determination of PHE and CTZ in combined dose tablet formulation. Wavelength of maximum absorbance for PHE (273.5 nm) and CTZ (232.0 nm) were selected for analysis by simultaneous equation method (Method I). In first order derivative method (Method II) quantitative determination was carried out at wavelength range 232.0 nm for PHE and 242.5 nm for CTZ. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient are shown in Table 1. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, LOD and LOQ. Linearity for PHE and CTZ was observed in the concentration range of 12-60 µg/ml for both the methods and correlation coefficient was always greater than 0.998 for both the drugs. Percent label claim for PHE and CTZ in tablet

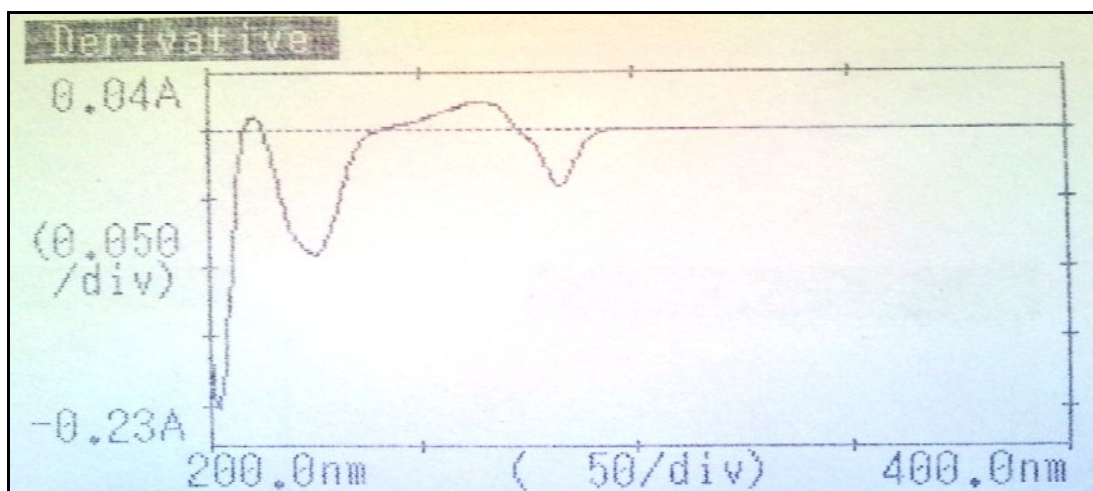


Fig. 4: First order derivative spectra of Phenylephrine hydrochloride

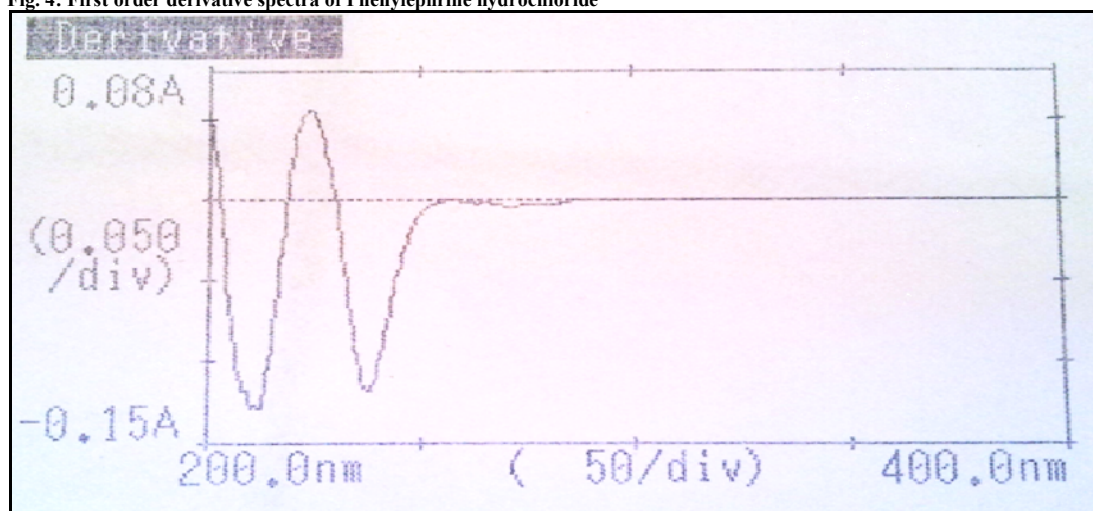


Fig. 5: First order derivative spectra of Cetirizine hydrochloride

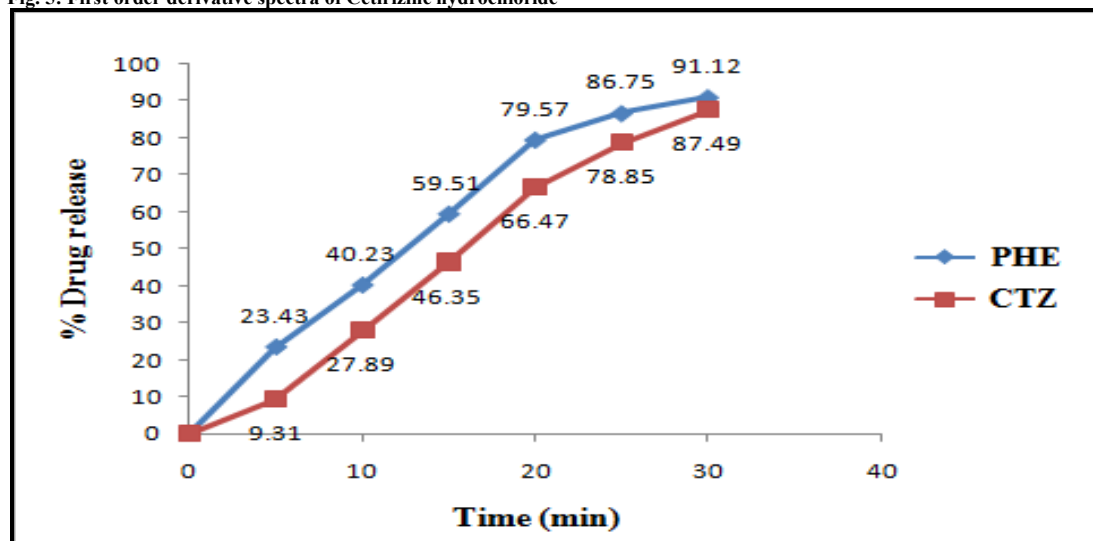


Fig. 6: Dissolution profile of Phenylephrine hydrochloride and Cetirizine hydrochloride tablet formulation by simultaneous equation method

analysis, by both the methods, was found in the range of 98.99% - 100.94%. Percent recovery for PHE and CTZ, by both methods, was found in the range of 99.38 % to 100.64 % with standard deviation well below 2 indicating accuracy of the methods. Intra-day and Inter-day precision studies were carried out by analyzing tablet formulation, by both

methods. Percent RSD for intra-day and inter-day precision studies for both drugs were well within the acceptable range (< 2 %) indicating that both the methods have excellent repeatability and reproducibility. LOD and LOQ were studied and found to be 1.58 µg/ml and 4.80 µg/ml for PHE respectively and 0.64 µg/ml and 1.94 µg/ml for CTZ

respectively by simultaneous equation method and by first order derivative method, LOD and LOQ were found to be 2.76 μ g/ml and 8.36 μ g/ml for PHE respectively and 2.95 μ g/ml and 8.94 μ g/ml for CTZ respectively. Simultaneous equation method was applied for dissolution study and percentage release during dissolution study was always greater than 85% within 30 minutes for both drugs in the tablet formulation under study (Fig. 6).

Based on the results obtained, it can be concluded that the proposed UV-Spectrophotometric methods (simultaneous equation method and first order derivative method) for simultaneous determination of Cetirizine hydrochloride and Phenylephrine hydrochloride is rapid, economical, accurate, precise and reproducible. The utility of the developed methods have been demonstrated by analysis of combined dose tablet formulation. Hence, the proposed method can be employed for quantitative determination of Cetirizine hydrochloride and Phenylephrine hydrochloride in combined dose tablet formulation. Simultaneous equation method can be used to carry out dissolution study in combination tablet formulation.

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REFERENCES

1. Indian Pharmacopoeia, Volume III, Government of India, Ministry of Health and Family Welfare, Published by The Indian Pharmacopoeia Commission, Ghaziabad 2007, pp. 1550-1551.
2. Indian Pharmacopoeia, Volume II, Government of India, Ministry of Health and Family Welfare, Published by The Indian Pharmacopoeia Commission, Ghaziabad 2007, pp. 893-894.
3. Beyene NW, Van Staden JF. Sequential injection spectrophotometric determination of Phenylephrine hydrochloride in pharmaceutical preparations. *Talanta* 2004; 63: 599-604.
4. Drozd J, Hopkala H, Misztal G, Paw B. Applications of derivative UV- spectrophotometry for the determination of Cetirizine dihydrochloride in preparations. *Acta Poloniae Pharmaceutica-Drug Research* 2002; 59(1): 3-7.
5. El Walily AFM, Korany MA, El Gindy A, Bedair MF. Spectrophotometric and high performance liquid chromatographic determination of Cetirizine dihydrochloride in pharmaceutical tablets. *Journal of Pharmaceutical and Biomedical Analysis* 1998; 17: 435-442.
6. Maithani M, Raturi R, Gautam V, Kumar D, Chaudhary AK, Gaurav A, Singh R. Development and validation of a RP-HPLC method for the determination of Chlorpheniramine maleate and Phenylephrine in pharmaceutical dosage form. *Pharmacie Globale International Journal of Comprehensive Pharmacy* 2010; 5: 5.
7. Wagh RS, Hajare RA, Tated AG, Gadbail PA, Khan FA, Kayal SD. Method development and validation for simultaneous determination of Ebastine and Phenylephrine hydrochloride in tablet formulation by RP-HPLC. *International Journal of Pharmaceutical Research and Development* 2011; 3(7): 214-220.
8. Demiralay E, Mehmet G, Canbay H. Validation of method for simultaneous determination of Paracetamol and Phenylephrine in pharmaceutical formulation by reversed phase liquid chromatography. *Pharmacie Globale International Journal of Comprehensive Pharmacy* 2011; 09: 6.
9. Pirol O, Sukuroglu M, Ozden T. Simultaneous determination of Paracetamol, Phenylephrine hydrochloride, Oxolamine citrate and Chlorpheniramine maleate by HPLC in pharmaceutical dosage forms. *E-Journal of Chemistry* 2011; 8(3): 1275-1279.
10. Maithani M, Raturi R, Gautam V, Kumar D, Gaurav A, Singh R. Simultaneous estimation of Ambroxol hydrochloride and Cetirizine hydrochloride in tablet dosage form by RP-HPLC method. *Pharmacie Globale International Journal of Comprehensive Pharmacy* 2010; 03: 2.
11. Sultana N, Arayne MS, Siddiqui FA. Determination and quantification of Cetirizine hydrochloride in dosage formulations by RP-HPLC. *Pakistan Journal of Pharmaceutical Sciences* 2005; 18(3): 7-11.
12. Küçükgül I, Karakus S, Küçükgül SG. Development and validation of a rapid RP-HPLC method for the determination of Cetirizine or Fexofenadine with Pseudoephedrine in binary pharmaceutical dosage forms. *Journal of Pharmaceutical and Biomedical Analysis* 2008; 46: 295-302.
13. Jaber AMY, Al Sherife HA, Al Omari MM, Badwan AA. Determination of Cetirizine dihydrochloride, related impurities and preservatives in oral solution and tablet dosage forms using HPLC. *Journal of Pharmaceutical and Biomedical Analysis* 2004; 36: 341-350.
14. El Walily AFM, Korany MA, El Gindy A, Bedair MF. Spectrophotometric and high performance liquid chromatographic determination of Cetirizine dihydrochloride in pharmaceutical tablets. *Journal of Pharmaceutical and Biomedical Analysis* 1998; 17: 435-442.
15. Bajerski L, Sangoi MS, Barth T, Diefenbach IF. Determination of Cetirizine in tablets and compounded capsules: comparative study between CE and HPLC. *Quim. Nova* 2010; 33(1): 114-118.
16. Chien D, Schoenwald RD. Fluorometric determination of Phenylephrine hydrochloride by liquid chromatography in human plasma. *Journal of Pharmaceutical Sciences* 1985; 74(5): 562-564.
17. Devarajan PV, Adani MH, Gandhi AS. Simultaneous determination of Lignocaine hydrochloride and Phenylephrine hydrochloride by HPTLC. *Journal of Pharmaceutical and Biomedical Analysis* 2000; 22: 685-690.
18. Makhija NS, Vavia RP. Stability indicating HPTLC method for the simultaneous determination of Pseudoephedrine and Cetirizine in pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis* 2001; 25: 663-667.
19. Dhongle SP, Sahare JS, Dhongle SS, Mundhey SA, Wate PS. Development and validation of HPTLC method for simultaneous estimation of Cetirizine hydrochloride and Phenylpropanolamine hydrochloride in tablet dosage form. *Der Pharmacia Lettre*. 2011; 3 (4): 111-119.
20. Patel BA, Patel GS, Patel PD, Patel HB, Patel MM. Stability indicating HPTLC method development and validation for estimation of Ambroxol hydrochloride and Cetirizine dihydrochloride in combine tablet dosage form. *International Research Journal of Pharmacy* 2011; 2(3): 95-99.
21. Schieffer WG, Hughes ED. Simultaneous stability-indicating determination of Phenylephrine hydrochloride, Phenylpropanolamine hydrochloride, and Guaifenesin in dosage forms by reversed-phase paired-ion high-performance liquid chromatography. *Journal of Pharmaceutical Sciences* 1983; 72(1): 55-59.