Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 5, Issue 2, 2013

Research Article

DEVELOPMENT AND VALIDATION OF A RAPID RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CETIRIZINE AND PSEUDOEPHEDRINE IN PHARMACEUTICAL DOSAGE FORMS

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Received: 12 December 2012, Revised and Accepted: 05 January 2013

ABSTRACT

The objective of the present study was to develop a simple, precise and accurate reversed-phase HPLC method and subsequent validation of the same as per the ICH guidelines. The present study deals with the estimation by RP HPLC of two different drug components pseudoephedrine hydrochloride (PSE) and cetirizine hydrochloride (CET) present in a tablet formulation.

The chromatographic separation of PSE and CET was done using phosphate buffer along with acetonitrile as mobile phase, in the proportion of 60:40. The separation is done on a C_{18} column and it is estimated at a λ max of 220 nm with a flow rate of 1ml/min. The retention times were 2.99 and 8.856 min for PSE and CET, respectively. The specificity for interference of any peak with main peak of interest is checked. The repeatability was checked by system precision with relative standard deviation less than 1% in all instances, along with method reproducibility for PSE and CET. The linearity ranges from a 0.48 to 1.68 mg/ml for PSE and 0.02 to 0.07 mg/ml for CET respectively. Correlation coefficients (*r*) of the regression equations were greater than 0.999 in all cases. The system suitability by precision is also checked to ensure that the analytical method is precise. The precision of the method was demonstrated using intra- and inter-day assay R.S.D. values which were less than 1%. The method was found to be accurate and precise for estimation of the two drugs simultaneously.

According to the validation results, the proposed method was found to be specific, accurate, precise and rapid. Hence the same can be applied to the quantitative analysis of tablets containing PSE–CET binary mixtures.

Keywords: RP-HPLC, Simultaneous, Validation, Pseudoephedrine and Cetirizine.

INTRODUCTION

Pseudoephedrine hydrochloride is chemically 2-methylamino-1-phenyl-1-propanol hydrochloride (Fig. 1) and is official in the United States Pharmacopoeia [1], British Pharmacopoeia [2], and Indian Pharmacopoeia [3]. Pseudoephedrine hydrochloride is a white, crystalline powder and the molecular mass of Pseudoephedrine hydrochloride is 201.69 g/mol [4]. Pseudoephedrine is a decongestant that shrinks blood vessels in the nasal passages. It is used to relieve nasal congestion caused by colds, allergies, and fever. Pseudoephedrine occurs naturally as an alkaloid in certain plant species, the majority of pseudoephedrine produced for commercial use is derived from yeast fermentation of dextrose in the presence of benzaldehyde. The salts pseudoephedrine hydrochloride and pseudoephedrine sulfate are found in many over the counter preparations either as single-ingredient preparations, or more commonly in combination with antihistamines active substances including cetirizine [5] in capsule or coated tablet forms for the treatment of seasonal allergic rhinitis.

Cetirizine hydrochloride is (±) - [2- [4- [(4-chlorophenyl) phenylmethyl] -1- piperazinyl] ethoxy]acetic acid, dihydrochloride (Fig.2) and is official in the United States Pharmacopoeia [6], British Pharmacopoeia [7], and Indian Pharmacopoeia [8]. Cetirizine hydrochloride is a white, crystalline powder and is water soluble. The molecular mass of cetirizine hydrochloride is 461.81 g/mol. Cetirizine hydrochloride an antihistamine, is a major metabolite of hydroxyzine, and a racemic selective H_1 receptor inverse agonist used in the treatment of allergies, fever, angioedema, and urticaria. Like many other antihistamine medications, cetirizine is commonly prescribed in combination with pseudoephedrine hydrochloride, a decongestant.

Analytical methods for simultaneous estimation of Pseudoephedrine hydrochloride and cetirizine hydrochloride in different combinations by reverse phase chromatography have been reported [9-15]. There is a derivative spectrophotometric method reported for the determination of pseudoephedrine in combination with antihistamines including cetirizine, fexofenadine and loratadine [16]. There are also some methods used for estimating individual Pseudoephedrine hydrochloride and cetirizine hydrochloride [17-22]. Some methods are also available for estimation of Cetrizine in different combination [23].

The HPLC methods using the most commonly used mobile phase, shorter run time, easily available columns and simple detectors like the UV detectors are preferred. The present study describes the determination of Pseudoephedrine hydrochloride and cetirizine hydrochloride by using reverse phase chromatography, a C_{18} column, simpler mobile phase and a UV detector.

The use of HPLC is now a day's very much preferred in routine quality control analysis. It is important that well validated HPLC methods are to be developed for simultaneously estimating Pseudoephedrine hydrochloride and cetirizine hydrochloride. The aim of this study is development of a simple, precise, rapid and accurate reverse phase HPLC method for the simultaneous estimation of Pseudoephedrine hydrochloride and cetirizine hydrochloride in pharmaceutical tablet dosage form. The method was validated in compliance with ICH guidelines [24-25].

MATERIALS AND METHODS

Materials and reagents

The pharmaceutical grade Pseudoephedrine hydrochloride and cetirizine hydrochloride were provided by the Bhavan's college and were certified to contain 100.03% (w/w) and 99.99% (w/w) respectively. HPLC grade Acetonitrile, Water and Orthophosphoric acid of AR grade was procured from Merck, Mumbai, India. Monobasic potassium phosphate AR grade was procured from S. D. Fine Chemical, Mumbai, India.

Instrumentation

A liquid chromatography system consisting of a Shimadzu, VP LC-10AT equipped with a binary solvent delivery pump, column thermostat and UV detector was used for the study. A Rheodyne syringe loading manual injector with a 20 µl sample loop was used for the injection of analyte. The system was controlled, data was collected and processed by Class VP software. The separation was performed at ambient temperature, on reverse phase column used

was Waters Bondapak C_{18}, 300 mm, 3.9mm ID, packed with 10 µ particle size.

Chromatographic conditions

Preparation of Mobile Phase: A mixture of monobasic potassium phosphate buffer and acetonitrile was used as mobile phase. Buffer was prepared by accurately weighing 6.8 g of monobasic potassium phosphate in to a 1000 mL volumetric flask, dissolved by adding 500mL of distilled water to it and sonicated. Adjusted the pH to 3.0 with dilute orthophosphoric acid, and diluted to volume with distilled water. The mobile phase with a mixture of monobasic potassium phosphate buffer, and acetonitrile in the ratio of 60:40 v/v was prepared. This mobile phase was filtered through 0.45 µ Nylon 6, 6 membrane filter and degassed in ultrasonic water bath. The mobile phase solvent was delivered at a flow rate of 1.0mL per min. All the experiments were performed in the isocratic mode. Detection of the analytes PSE and CET was done at a wavelength of 220 nm. Injection volume of the analytes was set at a constant volume of 20 µl by using a fixed sample loop.

Preparation of Standard stock solutions and construction of calibration curves

In a 25ml volumetric flask 12.5 mg of cetirizine hydrochloride was added and mixed with 10 ml of mobile phase. This solution was sonicated to dissolve completely and diluted to 25 ml with mobile phase to form a solution of 0.5mg/mL. Similarly, in a 50ml volumetric flask 60 mg of pseudoephedrine hydrochloride was added and mixed with 15 ml of mobile phase and the contents were sonicated to dissolve. Then using a graduated pipette 5 ml of previously prepared 0.5mg/mL of cetirizine hydrochloride solution was added to it. This solution was mixed and diluted to 50 ml with the same mobile phase. The final concentration obtained for pseudoephedrine hydrochloride and cetirizine hydrochloride was 1200 ppm and 50 ppm respectively.

Calibration curves for pseudoephedrine and cetirizine

Twenty microlitres of the above standard solution of pseudoephedrine hydrochloride and cetirizine hydrochloride was injected each time in to the stream of mobile system at a flow rate of 1 ml/min. The solution was injected seven times into the column and the corresponding chromatograms were obtained. From these chromatograms the area under the peaks and respective retention time of the drug were noted. The retention time of pseudoephedrine hydrochloride and cetirizine hydrochloride observed was 3.011 and 8.701 min respectively. A model chromatogram is shown in the Fig. 3, 4 and 5. Using these values of the two drug substances the precision was checked for the area and retention time of both the drugs. The system suitability was carried out by injecting standard solution of pseudoephedrine hydrochloride (1200 ppm) and cetirizine hydrochloride (50 ppm) into the chromatographic system to check the reproducibility of peak areas. The % RSD observed was 0.64 and 0.40 for pseudoephedrine hydrochloride and cetirizine hydrochloride respectively. The results of system precision are as shown in (Table 1).

Sample preparations

Analysis of marketed formulation

A commercial brand, Cetiriz-D tablet was procured for testing suitability of the proposed analytical method to estimate pseudoephedrine and cetirizine in tablet formulation. The label claim was 120 mg and 5 mg for pseudoephedrine and cetirizine respectively. Twenty tablets were weighed and average weight was determined. These tablets were crushed to a fine powder, and weighed quantity of powder equal to the average weight was transferred in the 100 ml volumetric flasks. Then 50ml of mobile phase is added in this volumetric flask. The contents of the flask were allowed to dissolve with intermittent sonication to ensure complete solubility of the drug. The mixture was diluted to 100ml with mobile phase, thoroughly mixed and then filtered through 0.45 µ nylon filter. The final concentration of the solution was 1200 ppm for pseudoephedrine hydrochloride and 50 ppm for cetirizine hydrochloride. 20 µl of each of this solution was injected into the HPLC system. The drug content in the test preparation was quantified by comparing with the known amount of standard injected. The results obtained are as shown in (Table 2). The amounts of CET–PSE in binary mixtures or dosage forms were individually calculated using the related linear regression equations.

Recovery studies

This was performed to demonstrate that the proposed analytical method is accurate and to evaluate whether there is interference from excipients used in the dosage forms. This recovery studies were employed by the standard addition method. Known amount of CET & PSE was added to the powdered commercial tablets contents. Placebo solutions (mixture of excipients), diluent used for preparation of standard solution and sample solution were injected in the chromatographic system and checked for interference at retention time corresponding to the retention time of pseudoephedrine hydrochloride and cetirizine hydrochloride. HPLC samples were then prepared and the resulting mixtures were analysed as described for pharmaceutical dosage forms.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

During the optimization of this method for better separation three different columns were tried (Hypersil SAS, 25mm x 4.6 mm, 5µ; Kromasil C8, 100mm x 4.6mm, 5µ; Waters Bondapak C_{18}, 300 mm, 3.9mm ID, packed with 10 µ), different organic solvents viz. acetonitrile and methanol were tried with water at different pH values from 3 to 5, with and with-out ion pairing agent (hexane sulphonate). A desirable response was not achieved for PSE even after moderate variations in chromatographic conditions as in case of CET due to its very polar nature. Of the stationary phases experienced, Waters Bondapak C_{18}, 300 mm, 3.9mm ID, packed with 10 µ gave the best results in terms of peak shape, resolution and analysis time. To overcome the early elution of PSE, formation of its ion pair with hexane sulphonate was tried; but this resulted in very late elution or no peaks for CET.

After trying various mobile phases containing acetonitrile and methanol with different buffers, the one consisting of buffer and acetonitrile proved to be useful for better resolution and peak symmetry. Different concentrations of potassium hydrogen phosphate salt were tried. Later different pH of buffer was also tried and adjusted with orthophosphoric acid.

To optimize this mobile phase, proportions of acetonitrile were systematically changed from 10 to 40%, also concentration of salt in buffer was varied (0.01M to 0.1M, with pH 3 to 5). Higher acetonitrile ratio resulted in shorter retention times of all analytes. For further optimization, methanol + acetonitrile (20 + 30%) were mixed with buffer (0.05M) of different pH values varied in the range of 3.0–5.0. As a result of pH screening, the optimum mobile phase was chosen as buffer (0.05M) pH 3.0), acetonitrile (60:40, v/v). The flow rate used was set to 1.0 ml min for all experiments. Using this mobile phase, best results were obtained in terms of resolution, peak symmetry, selectivity and analysis time for both analytes.

Detection wavelengths were chosen considering the ratios of active ingredients in pharmaceutical dosage forms. Thus, it was aimed at maintaining the peak heights close to each other as much as possible. The detector was set to 218 nm for determination of the tablet sample which contained 120 mg of PSE and 5 mg of CET. This wavelength was used but the peak of CET was very small in comparison to PSE peak, as the tablet contained 120 mg of PSE and only 5 mg of CET. Therefore, detection was performed at 220 nm where CET had a higher absorbance in spite of its low amount in the formulation.

The aim of this experiment was to develop a rapid and sensitive liquid chromatographic method for the quality control analysis of cetirizine hydrochloride in binary mixtures with pseudoephedrine hydrochloride in pharmaceutical dosage forms. From this method it was possible to separate these two compounds with a good resolution and a short run time.

Validation of the method

Method Validation

The aim of method validation was to confirm that the present method was suitable for its intended purpose as described in ICH guidelines Q2A and Q2B [24-25]. The method was validated as per the recommendation of ICH and USP for the parameters like precision, specificity, accuracy, linearity etc. Intraday and interday precision study of pseudoephedrine hydrochloride and cetirizine hydrochloride was carried out by estimating the corresponding responses on the same day and on different days for the concentration of 1200 ppm pseudoephedrine hydrochloride and 50 ppm cetirizine hydrochloride. The precision (% relative standard deviation) was expressed with respect to the intraday and interday variation in the expected drug concentrations. The recovery studies were carried out by adding known amounts of pseudoephedrine hydrochloride and cetirizine hydrochloride and then analyzing them by the proposed HPLC method. Subsequent dilutions of the solutions ranging from 480 ppm to 1680 ppm for pseudoephedrine hydrochloride and 20 ppm to 70 ppm for cetirizine hydrochloride were made and linearity was checked. System suitability tests are an integral part of chromatographic method which is used to verify the reproducibility of the chromatographic system. The calibration curves for CET and PSE binary mixtures were constructed by plotting the peak area of the drug against the drug concentration. After validation, the developed method has been applied to pharmaceutical dosage forms containing CET and PSE, respectively.

System suitability

The system suitability tests are an integral part of analytical method validation for liquid chromatography and they are basically used to verify that the proposed method is able to produce good resolution between the peaks of interest with high reproducibility [26]. The system suitability was determined by injecting seven replicate injections from freshly prepared standard solutions and analyzing each solute for their peak area, theoretical plates (*N*), resolution (*R*) and tailing factors (*T*). System suitability requirements for CET and PSE were a R.S.D. of peak areas and retention times less than 1%, peak resolution (*R*) greater than 2.0 between two adjacent peaks for two analytes, theoretical plate numbers (*N*) at least 2000 for each peaks and USP tailing factors (*T*) less than 1.5.

Stability/specificity

Specificity can be described as the capability of the method to accurately measure the response of the analysed compound with no interferences originating from sample matrix. High percentage recovery observed with assay samples of pharmaceutical dosage forms, including standard addition experiments, indicates that the proposed method was not affected by interferences from excipients used in formulations. The peaks obtained from recovery experiments of dosage forms, were checked for uniformity using UV spectra taken from different points of the peak of interest. These spectra were super-imposable when overlaid; showing that there was no other co-eluting peaks, at the peak of analytes, PSE and CET.

Linearity

The calibration curves for PSE and CET in binary mixtures were constructed by plotting the peak area of PSE or CET against the respective concentration. The Linearity curve was made using standard solutions containing PSE and CET at six different concentrations ranging from 40 to 140% of nominal concentrations such as 480 to 1680ppm for PSE and 20 to 70ppm for CET. Twenty microlitres were injected each time in the stream of mobile phase, at a flow rate of 1 ml/min. Each of these dilutions of different concentration was injected in duplicate into the column and the corresponding chromatograms were obtained. From these chromatograms the area under the peak of the drug were noted. Using these values, the mean area of the drug was calculated. The regression of the drug concentration was completed. The linear concentrations range of 480 to 1680 ppm of pseudoephedrine and 20 to 70 ppm of cetirizine was selected for routine analysis purpose. Six levels were prepared and each level was injected in duplicate in to the chromatographic system. Mean peak area of each level was calculated. Graph of mean peak area vs. concentration was plotted and the best-fit line was determined by regression. % Intercept and Correlation coefficient was obtained. The % co-relation co-efficient was 0.9992 pseudoephedrine and 0.9999 for cetirizine. The results of Linearity are shown in (Table 3 (a) & (b). The plot of peak area vs. concentration of each analyte was found to be linear within the concentration ranges stated above.

Limit of detection (LOD) and limit of quantitation (LOQ)

ICH guideline Q2B [25] describes several approaches for determination of detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. The LOD and LOQ values obtained for these compounds of the developed method are presented in (Tables 4).

Precision

The precision of the proposed method were assessed as repeatability and intermediate precision performing seven replicate injections of standard and three different sample solutions, which were freshly prepared and analyzed (Tables 1, 2 and 5). These experiments were repeated over a period of 24 hrs to evaluate day-to-day variability (intermediate precision). As can be seen in (Tables 2 and 5), the % R.S.D. values of the measurements ranged between 0.35 and 0.61%. The % R.S.D. of assay results obtained in intermediate precision study were not greater than 2%, confirming good precision of the proposed method between days.

Accuracy

Accuracy of the proposed method was established by recovery experiments using standard addition method. This study was employed by addition of known amounts of PSE and CET onto known concentration of commercial tablets sample. The pure standards at these three levels were added to the sample. The resulting mixtures were analyzed as described in Section 3.3. The experiment was carried out at three different levels i.e. 110 %, 120 %, and 130 % of the working concentration of pseudoephedrine hydrochloride (1200 ppm) and cetirizine hydrochloride (50 ppm). From the amount estimated, the percentage recovery was calculated. The recovery experiments, using Cetrizet-D tablets containing PSE & CET, showed recovery from 99.24 to 100.43% with mean recovery of 99.86 and from 98.31 to 101.60% with mean recovery of 100.02% with R.S.D. values of 0.39 and 1.02% for PSE and CET, respectively. The percent recovery is in between 98 % to 102 % which indicates specificity and accuracy of the method. Results obtained from recovery studies are as shown in (Table 6).

High recovery results obtained from the proposed method for the analysis of CET–PSE in tablets indicate that this assay procedure can be used for quantitation and routine quality control analysis of these binary mixtures in commercial samples.

Robustness

For testing the robustness of method a few parameters like flow rate, percent of composition of acetonitrile in the mobile phase were deliberately changed. One parameter was changed at one time to evaluate the effect in results. Each parameter was changed at two levels –ie. -5% & +5% with respect to optimized parameter. The results obtained with changed parameter were compared with those of method precision. The % RSD of results of samples obtained for robustness with respect to change in flow & change in composition were within 2% of method precision & thus ensures that the method is Robust.

Application of the validated method to pharmaceutical products

Thus based on the above results, the proposed method was applied to the determination of CET and PSE in tablet dosage forms which comprised the binary mixture (120 mg PSE and 5 mg CET). The representative chromatograms obtained from the analysis of PSE and CET in tablet sample is shown in (Fig. 5). The differences between the amount claimed and those assayed were very low and the R.S.D. values were within the acceptable range

mentioned by pharmacopoeias. The mean values of 119.8 and 5.0 mg with R.S.D. % of 0.37 and 0.72 were obtained for PSE and CET, respectively.

Recovery of the procedure was determined by standard addition method. The previously analyzed samples of tablets were spiked with the known amounts of standard PSE and CET. The mean percentage recoveries obtained after nine repeated experiments found were 99.86 and 100.02 (Table 6) for PST & CET respectively, indicating that the results are accurate and precise and there is no interference from the common excipients used in the pharmaceutical dosage forms.

Table 1: Method validation - System precision

PSE - 1200 ppm		**CET - 50 ppm**	
Injection No.	**Area count**	**Injection No.**	**Area count**
1	35547	1	13673
2	35146	2	13700
3	35886	3	13700
4	35731	4	13680
5	35540	5	13626
6	35527	6	13703
7	35612	7	13600
Average	35570		13669
Std. Dev.	228		0.30
% RSD	0.64		0.40

PSE is Pseudoephedrine, CET is Cetirizine, ppm is the parts per million, Std. Dev. is the standard deviation of the mean, %RSD is relative standard deviation for n = 7 observations.

Table 2: Assay of commercial sample: Method precision.

PSE			**CET**		
Avg std area : 35570			**Avg std area : 13669**		
Std conc. (ppm): 1274			**Std conc. (ppm): 52**		
Std % purity: 100.03			**Std % Purity: 99.99**		
Area	mg/tab	% Assay	Area	mg/tab	% Assay
Sample1.	120.23	100.19	Sample1.	5.01	100.22
Sample2.	119.35	99.46	Sample2.	4.96	99.27
Sample3.	119.94	99.95	Sample3.	5.03	100.68
Avg.	119.8	99.87	Avg.	5.0	100.06
Std. Dev.	0.4487	0.3739	Std. Dev.	0.0357	0.7149
% RSD	0.37	0.37	% RSD	0.72	0.72

Table 3 (a): Method validation - Linearity of PSE

Conc. in ppm	**Area count Inj-1**	**Area count Inj-2**	**Average area**
480	27224	27216	27220
720	33749	33729	33739
960	38811	38966	38889
1200	43936	43998	43967
1440	48987	49006	48997
1680	54705	54725	54715
Correlation			0.9991
Intercept			17039
Regression (R^2)			0.998

Table 3 (b): Method validation - Linearity of CET

Conc. in ppm	**Area count Inj-1**	**Area count Inj-2**	**Average area**
20	5827	5843	5835
30	8712	8758	8735
40	11764	11573	11669
50	14591	14530	14561
60	17359	17381	17370
70	20326	20397	20362
Correlation			0.9995
Intercept			-46.64
Regression (R^2)			0.999

Concentration for linearity evaluation levels which was 480 ppm to 1680 ppm for pseudoephedrine and 20 ppm to 70 ppm for cetirizine, with a correlation co-efficient of 0.9991 and 0.9999 respectively.

Table 4: Method validation -LOD and LOQ

	PSE		**CET**	
	ppm	**area**	**ppm**	**area**
Linearity	480	27220	20	5835
LOD	12	688	2	578
LOQ	31	1707	5	1430

LOD is Limit of detection & LOQ is Limit of quantitation

Table 5: Method validation -Intermediate precision

Method precision	Area	mg/tab	% Assay	Area	mg/tab	% Assay
Sample1.	35291	120.23	100.19	13595	5.01	100.22
Sample2.	35033	119.35	99.46	13571	4.96	99.27
Sample3.	34929	119.94	99.95	13551	5.03	100.68
Intermediate precision						
Sample1.	36576	120.37	100.31	13540	5.02	100.40
Sample2.	36363	119.67	99.73	13706	5.04	100.84
Sample3.	36009	119.44	99.53	13701	5.04	101.00
	Avg.	119.83	99.86	Avg.	5.02	100.40
	Std. Dev.	0.42	0.35	Std. Dev.	0.03	0.62
	% RSD	0.35	0.35	% RSD	0.61	0.62

Table 6: Method validation - Recovery data

% Recovery					
PSE			**CET**		
Level-%	**Result**	**%Recovery**	**Level-%**	**Result**	**%Recovery**
110	110.12	100.14	110	109.58	101.60
110	109.63	99.69	110	109.13	99.70
110	110.43	100.42	110	109.73	100.47
120	119.05	99.24	120	120.05	100.09
120	119.37	99.50	120	120.67	99.67
120	119.85	99.90	120	120.77	100.11
130	130.34	100.29	130	131.06	100.49
130	129.87	99.93	130	129.81	99.72
130	129.47	99.62	130	130.21	100.52
	Average	99.86		Average	100.02
	Std. dev	0.38		Std. dev	0.34
	% RSD	0.39		% RSD	1.02

The % recovery observed for PSE and CET is 99.24 to 100.42 & 99.67 to 100.52 respectively.

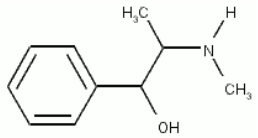

Fig. 1: structure of pseudoephedrine

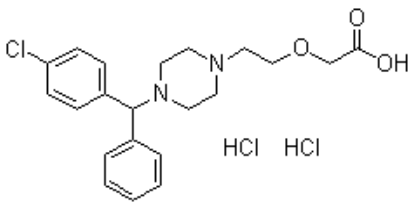

Fig. 2: structure of cetirizine hydrochloride

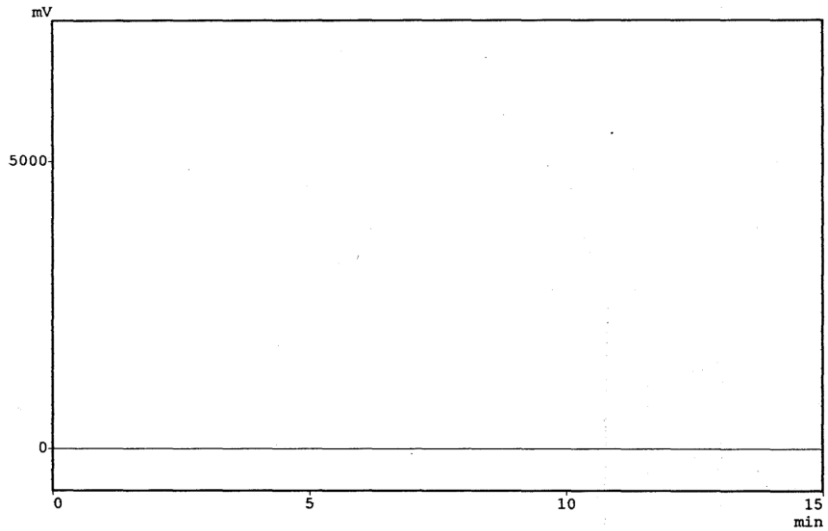

Fig. 3: Chromatogram of blank sample

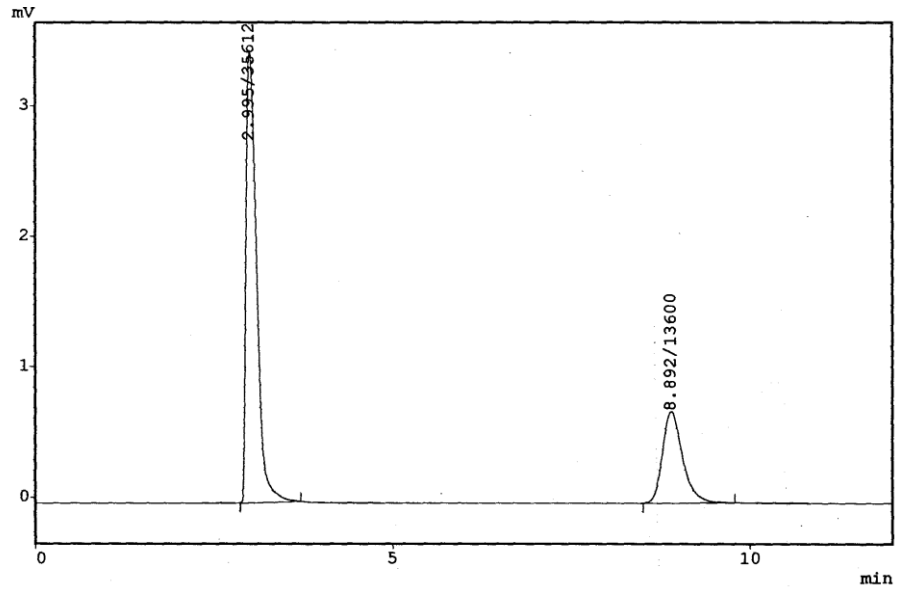

Fig. 4: Chromatogram of mixture of pseudoephedrine and cetirizine standard I

The two components were eluted at a retention time of 2.995 min and 8.892 min with an area of 35612 and 13600 for PSE and CET respectively

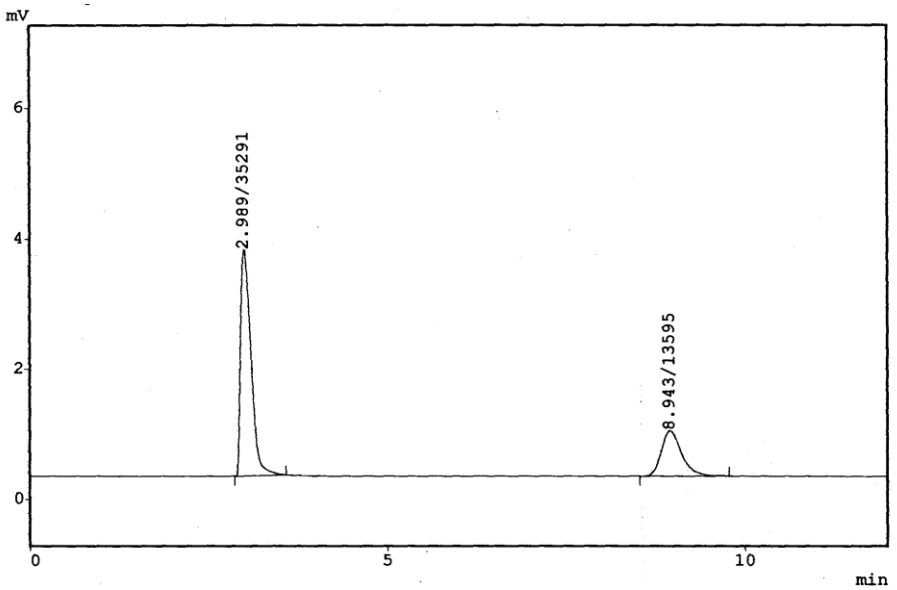

Fig. 5: Chromatogram of mixture of pseudoephedrine and cetirizine sample II

CONCLUSION

This validated HPLC method has been proved to be simple, precise, rapid and reliable. To achieve sharp peaks with good resolution under isocratic conditions, mixture of monobasic potassium phosphate salt solution and acetonitrile in different proportion were tested as mobile phase on a C_{18} stationary phase. The mixture of monobasic potassium phosphate salt solution and acetonitrile in the proportion (60: 40 v/v) proportions was proved to be the most suitable for estimation.

The UV overlay spectra showed that both the drugs absorbed appreciably at 220nm & hence this wavelength was selected for detection of both the compounds. Since the chromatographic peaks were better defined, resolved with this system, under the above mentioned chromatographic conditions, the retention time obtained for pseudoephedrine hydrochloride and cetirizine hydrochloride were 3.011 and 8.701 min respectively. The calibration curve for pseudoephedrine and cetirizine was found to be linear over the range of 480 to 1680 ppm of pseudoephedrine and 20 to 70 ppm of cetirizine, respectively. The data of regression analysis of the calibration curves is shown in (Table 3 (a) & (b)). The proposed method provides a good resolution between PSE and CET. Using this single procedure, it is possible to perform quantitative analysis of two different analytes from a tablet dosage forms within a short analysis time. The developed method reported herein was validated by evaluation of the validation parameters as described in ICH-Q2B guideline. System suitability, specificity, linear- ity, LOD, LOQ values, precision and accuracy of the proposed technique were obtained during the validation studies. The results of the validation and the precision test parameters are summarised in (Table 1). The developed method was found to be simple, sensitive and selective for analysis of pseudoephedrine and cetirizine in combination without any interference from excipients. Thus the method was successfully used for determination of pseudoephedrine and cetirizine in pharmaceutical formulation. The results of assay for combined dosage form using proposed method are summarised in (Table 2).

The developed method can be used for the simultaneous estimation of pseudoephedrine hydrochloride and cetirizine hydrochloride. It can serve as an easy, cost effective and efficient HPLC method for routine quality control analysis of tablet dosage forms containing pseudoephedrine and cetirizine.

ACKNOWLEDGEMENTS

The authors are grateful to Bhavan's college, Andheri, for providing the necessary facilities for the research work.

REFERENCES

1. The United States Pharmacopoeia 2012, volume III, The United States Pharmacopeial Convention, Rockville, 4477, 4476.
2. British Pharmacopoeia 2012, volume II, British Pharmacopoeia Commission Office: MHRA, London, 1854, 1853.
3. Indian Pharmacopoeia 2010, volume III, The Indian Pharmacopoeia Commission, Ghaziabad, 1999 to 2001, 2000.
4. Budavari S., Eds., In, The Merck Index, 12th Edn., Merck and Co., Inc., Whitehouse Station, NJ, 1996, 3647.
5. K. Wellington, B. Jarvis. Drugs, 61, (2001), 2231-2240.
6. The United States Pharmacopoeia 2012, volume II, The United States Pharmacopeial Convention, Rockville, 2598, 2597.
7. British Pharmacopoeia 2012, volume I, British Pharmacopoeia Commission Office: MHRA, London, 458, 457.
8. Indian Pharmacopoeia 2010, volume II, The Indian Pharmacopoeia Commission, Ghaziabad, 1038, 1037.
9. Kumudhavalli, M. V., Saravana, C., Kumar, M., Jayakar, B, Journal of Global Pharma Technology, 2010: 01(2): 97-101.
10. Lakshmi Sivasubramanian, K. S. Lakshmi, Der Pharma Chemica; 2009, 1 (1):37-46.
11. S.S. Merukar, P.S. Mhaskar, S.R. Bavaskar, K.B. Burade, P.N. Dhabale, Journal of pharmaceutical sciences and research, J. Pharm. Sci. & Res. 2009, 1(2), 38-42.
12. Mayte Gil-Agusti, Llorenc Monferrer-Pons, Maria Celia Garcia–Alvarez-Coque, Josep Esteve-Romero. Talanta 54 (2001) 621–630.
13. Singhvi I, Bhatia N. Indian J Pharm Sci. 2006; 68:72-5.
14. Sandeep Rajurkar. International Journal of Life science & pharma research. 2011; 1 (1).
15. The United States Pharmacopoeia 2012, volume II, The United States Pharmacopeial Convention, Rockville, 2600, 2601.
16. H. Mahgoub, A.A.Gazy, F.A.El-Yazbi, M.A.El-Sayed, R.M.Youssef, J.Pharm.Biomed.Anal. 2003 (31) 801-809.
17. Mohammad Reza Ganjali, Aidin Alipour, Siavash Riahi, Bagher Larijani, Parviz Norouzi. Int. J. Electrochem. Sci., 2009 (4) 1262-1276.
18. Muhammad Imran Khan, Ghulam Murtaza, Sher Awan, Muhammad Iqbal, Muhammad Khurram Wagas, Akhtar Rasool, Urooj Fatima, Muhammad Hassham Hassan Bin Asad, Anwar Kahlid, Faisal Usman, Qazi Najam-us-Saqib, Shujaat Ali Khan, kalsoom Farzana, Seema Mahmood, and Izhar Hussain. Africal Journal of Pharmacy and Pharmacology vol. 2011 5 (2), pp. 143-149.
19. Arayne MS, Sultana N, Siddiqui FA., Pak. J. Pharm. Sci., 2005, 18 (3): 7-11.
20. The United States Pharmacopoeia 2012, volume II, The United States Pharmacopeial Convention, Rockville, 2599, 2598.
21. The United States Pharmacopoeia 2012, volume III, The United States Pharmacopeial Convention, Rockville, 4478.
22. Moncrieff J, J.Chromatogr., 1992 :583(1): 128-30.
23. Maithani M et al. / Pharmacie Globale (IJCP) 2010, vol. 01, 2 (03).
24. [24 ICH-Q2A, Guidelines for industry: Text on validation of analytical methods, March-1995.
25. ICH, Q2B, Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Nov-1996.
26. I.S. Krull and M.E. Swartz. LC/GC N. Am. Magazine, 1999, 17 (3) 244-46.