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Extractive Spectrophotometric Determination of Cetirizine Dihydrochloride in Pure and Pharmaceutical Preparations

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ABSTRACT

A simple and sensitive extractive spectrophotometric method has been described for the assay of cetirizine dihydrochloride in pure and different pharmaceutical preparations. The method was based on the formation of the ion-pair complex from the reaction between cetirizine dihydrochloride and methyl orange in pH 4.0, which gave a yellow color after chloroform extraction and exhibited a maximum absorbance at 424.5 nm. Beer's law was obeyed in the concentration range of 2.5 - 20 μg/mL. The method was tested and validated for various parameters according to ICH guidelines. The detection and quantification limits were 1.0 and 3.0 μg/mL, respectively. The proposed method was successfully applied for the determination of cetirizine dihydrochloride in pharmaceutical preparations. The results demonstrated that the procedure was accurate, precise and reproducible while statistical analysis of the obtained results showed no significant difference between the proposed method and reference method. No interference was observed for common pharmaceutical preparations.

Key words: spectrophotometry, pharmaceuticals, methyl orange, ion pair

INTRODUCTION

Cetirizine dihydrochloride (CE) is an orally active and selective H_1-receptor antagonist. Its chemical name is (±)- [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid, dihydrochloride (Figure 1). It is a non-sedating type histamine H_1-receptor antagonist used in symptomatic treatment of seasonal rhinitis, conjunctivitis, perennial allergic rhinitis, and pruritus and urticaria of allergic origin[1].

Various analytical techniques have been employed for the determination of CE in pharmaceutical preparations such as spectrophotometry[2,3], high performance thin layer chromatography (HPTLC)[4], high performance liquid chromatography (HPLC)[5,6] and liquid chromatography-mass spectrometry (LC/MS)[7]. CE has been determined in biological fluids by HPLC[8-11] and liquid chromatography - tandem mass spectrometry[12].

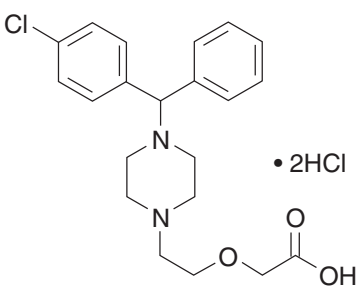

Figure 1. Chemical structure of cetirizine dihydrochloride.

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The present study has shown the development of an accurate, reproducible, fast and adequately sensitive extractive spectrophotometric method based on an ion-pair complex formed between CE and an anionic dye, methyl orange. An important advantage of the extractive spectrophotometric method is that it can be applied to the determination of individual compounds in a multi-component mixture. Unlike gas chromatographic (GC) and HPLC procedures, the instrument is relatively simple and affordable. The sensitivity (in terms of molar absorptivity) and precision (in terms of relative standard deviation, RSD) of the methods are suitable for the determination of the drug in pure and mixed dosage forms. The reagents utilized in the proposed method is relatively cheap and readily available, and the procedure does not involve any critical reaction condition or tedious sample preparation. These advantages, coupled with reasonable accuracy and precision, render the methods suitable for routine quality control. The proposed method was

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applied to the determination of CE in pharmaceutical formulations. No interference was observed in the assay of CE from common excipients in levels found in pharmaceutical formulations. The proposed method was validated by statistical data.

MATERIALS AND METHODS

I. *Materials*

Pure powder of CE was obtained from Sigma (St. Louis, MO, USA). Allerset® and Zyrtec® tablets were obtained from a pharmacy. Methyl orange (MO) was purchased from Merck (Darmstadt, Germany). Solvents and other chemicals were of analytical grade (Merck, Darmstadt, Germany). Water was prepared by using aquaMAX™ ultra (Young instrument, Korea) water purification system.

II. *Preparation of Stock and Standard Solutions*

A stock solution of CE (1.0 mg/mL) was prepared in methanol. A working standard solution was prepared by diluting the stock standard solution with methanol (100 μg/mL). Methyl orange (MO) solutions (0.2% w/v) were prepared in water. Phthalate buffer was prepared by dissolving 1.280 g of potassium hydrogen phthalate in 50 mL of water. The pH was adjusted to 4.0 with 0.2 M HCl solution and the volume was made up to 250 mL with water.

III. *Apparatus*

All spectrophotometric measurements were performed on a Shimadzu UV-160A UV-Visible spectrophotometer with 1-cm glass cells. UV-visible spectra were automatically obtained by Shimadzu UV-160A system software.

IV. *General Procedure*

Aliquots of 0.25 - 2.0 mL of the standard solution (100 μg/mL) were transferred to 10-mL calibrated flasks. One milliliter of potassium hydrogen phthalate buffer (pH 4.0) was added, followed by 0.5 mL of reagent solution (0.2% w/v). After 2 min vortexing the tubes were allowed to separate the two layers. The complex was extracted three times with 3 mL of chloroform. After the phases had been separated by centrifugation, the combined extracts were adjusted to 10 mL with the same solvent. The absorbance of the yellow-colored complex was measured at 424.5 nm, against a corresponding reagent blank similarly prepared. All measurements were made at room temperature (25 ± 2°C).

V. *Procedure for the Assay of the Tablets*

The contents of twenty tablets (Allerset® and Zyrtec® tablets) were crushed into powder, and the average weight of the tablets was determined. An accurate weight equivalent to 100 mg of CE was dissolved in 50 mL of methanol with shaking for 30 min and filtered. The filtrate was diluted to 100 mL with methanol in a 100-mL measuring flask to give 1.0 mg/mL of stock solution. An aliquot of the diluted drug solution was treated as described under Section IV.

VI. *Method Validation*

Typical analytical performance characteristics for the validation of procedures according to ICH Q2 guidelines were described in this study, including specificity, linearity, limit of detection, limit of quantification, precision, accuracy, recovery and robustness[13].

(I) *Specificity*

To assess the specificity of the method, the effect of diluents, excipients and additives which often accompany CE in its dosage forms (lactose, titanium dioxide, cellulose and microcrystalline) was studied.

(II) *Linearity*

The calibration graph is described by the calibration equation of $A = aC + b$, where A is the absorbance, a *is* the slope, b is the intercept and C *is* the concentration of the ion-pair complex product.

(III) *Limits of Detection (LOD) and Quantification (LOQ)*

The LOD and LOQ of CE by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3r/S and 10r/S, respectively, where r is the standard deviation of y-intercept of the regression equation and S is the slope of the calibration curve.

(IV) *Accuracy*

To determine the accuracy of the proposed method, different levels of drug concentrations (low concentration, medium concentration, and high concentration) in both media were prepared from independent stock solutions and analyzed (n = 6). Accuracy was assessed as the relative mean error.

(V) *Precision*

Precision or repeatability was determined by using different drug concentrations prepared from independent stock solutions and analyzed (n = 6). Inter-day and intra-day variations were taken to determine the intermediate precision of the proposed method. Different levels of drug concentrations in triplicates were prepared thrice in a day and studied for intra-day variation. The same

method was followed for three different days to study inter-day variation (n = 6).

(VI) *Recovery*

The recovery of the proposed method was determined using the standard addition technique, by adding a known amount of standard at three different levels to the pre-analysed sample.

(VII) *Robustness*

The robustness of the proposed method was determined by (a) changing the pH of the media by ± 0.1 unit and (b) changing the concentration of the reagent by ± 0.1 unit.

RESULTS AND DISCUSSION

I. *Absorption Spectra*

The nitrogenous drug was present in positively-charged protonated form, while anionic dyes of sulphonphthalein group were present mainly in anionic form at pH ≥ 3. Thus, when treated with an acid dye at pH 4.0 of potassium hydrogen phthalate buffer, a yellow ion-pair complex (extracted with chloroform) was formed. The absorption spectra of the ion-pair complex, which was formed between CE and MO, were measured in the range of 300-600 nm against the blank solution (Figure 2). The ion-pair complex showed maximum absorbance at 424.5 nm. The optimum reaction conditions for the determination of the ion-pair complex were established.

II. *Optimum Reaction Conditions for Complex Formation*

The reaction conditions of the method was carefully studied to achieve complete reaction, highest sensitivity and maximum absorbance. Reaction conditions for the formation of the ion-pair complex were optimized by studying preliminary experiments involving pH of buffer, type of organic solvent, volume of the dye and shaking time for the extraction of ion-pair complexes.

(I) *Effect of pH*

The effect of pH was studied by extracting the colored complexes in the presence of various buffers such as KCl-HCl (pH 1.5 - 4.5), NaOAc-HCl (pH 2.0 - 5.0), and potassium hydrogen phthalate-HCl (pH 2.0 - 5.0). The maximum color intensity and highest absorbance value were observed in potassium hydrogen phthalate-HCl buffer of pH 4.0 (Figure 3).

(II) *Effect of Time*

The effect of time (Figure 4) and temperature on the above reaction was judged based on the observation of complete colour development between 1 and 2 min and remained stable for one day. It was observed that absorbance reached a maximum at 25°C (room temperature). The optimum reaction time was studied from 1.0 to 15.0 min by following the color 2.0 min and at room temperature. The reaction time was quite short compared with other methods[2-12] .

(III) *Effect of Temperature*

The absorbance-temperature curve of the reaction

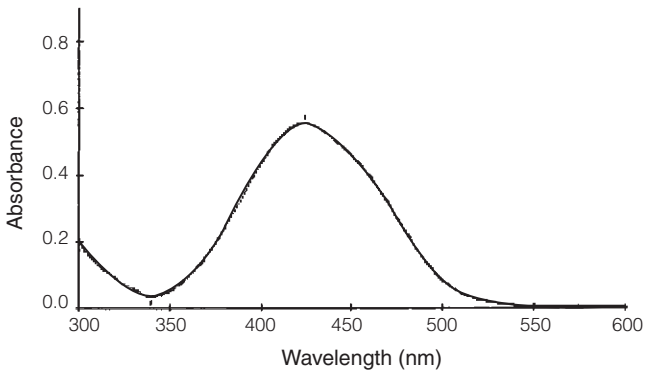

Figure 2. Absorption spectra of CE-MO complex (15 μg/mL).

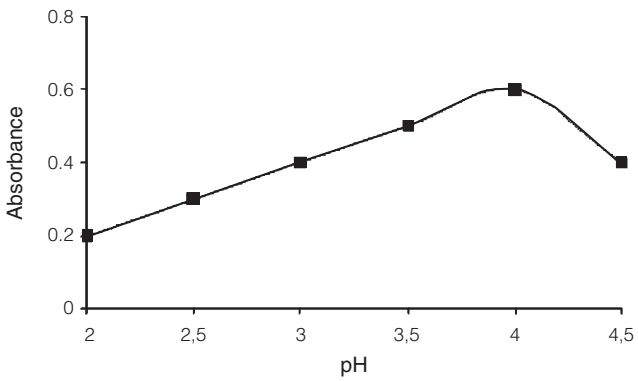

Figure 3. Effect of pH on the reaction of CE with MO.

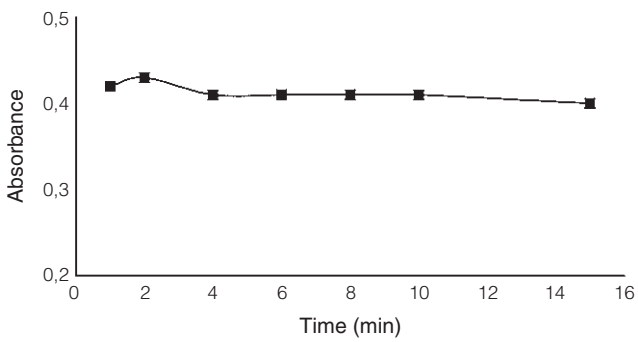

Figure 4. Effect of time on the reaction of CE with MO.

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between CE and MO was constructed at λ_{max} and the results are shown in Figure 5. The absorbance of the extracted ion-pairs was measured at 20 - 60°C and at room temprature. Figure 5 shows that the absorbance generally increased with temperature and peaked at 25°C (room temperature) using MO reagent. The temperature fluctuated slightly about this temperature. The optimum reaction time was studied from 1.0 to 15.0 min. Complete color intensity was attained 2 min at room temperature.

(IV) *Effect of MO Concentration*

The effect of the concentration of the dye on the intensity of the color developed at the selected wavelength and constant drug concentration was tested using different volumes of MO (0.5 - 4.0 mL). It was observed that 0.5 mL of 0.2% (w/v) MO was necessary for maximum color development of the ion-pair complex. Above this volume, the absorbance remained constant.

(V) *Effect of Solvent*

Solvents like *n*-butanol and benzene could not be used for the extraction of the ion-pairs formed, while acetone, dichloromethane, methylene chloride and chloroform extracted those ion-pairs quantitatively. The molar absorptivity value for the ion-pair in chloroform using MO was $\varepsilon = 1.5 \times 10^4$ L/mol/Xcm at λ_{max} = 424.5 nm. This value made chloroform a suitable media for extraction.

(VI) *Stoichiometric Ratio*

Job's Method of Continuous Variation[14] of equimolar solutions was employed. A 2.0×10^{-4} M standard solution of drug and 2.0×10^{-4} solution of CE were used. A series of solutions was prepared in which the total volume of drug and reagent was kept at 10 mL. The absorbance was measured at the optimum wavelength. The molar ratio of the reagent (drug : dye) in the ion-pair complex was determined by the Method of Continuous Variation (Job's Method) and the results indicated that 1 : 2 (drug : dye) was the optimum ratio for the reaction (Figure 6).

III. *Method Validation*

(I) *Specificity*

A study of some potential interfering substances in the spectrophotometric determination of CE was performed by selecting some excipients commonly used in tablet formulations. Samples containing a fixed amount of CE (20 μg/mL) and variable concentrations of excipients were analyzed. There was no interference from most of the common ingredients such as lactose, titanium dioxide, cellulose and microcrystalline. It was shown that these compounds did not interfere with the proposed method.

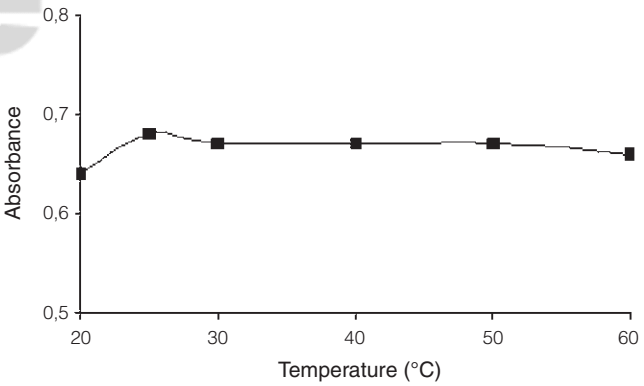

Figure 5. Effect of temperature on the reaction between CE and MO.

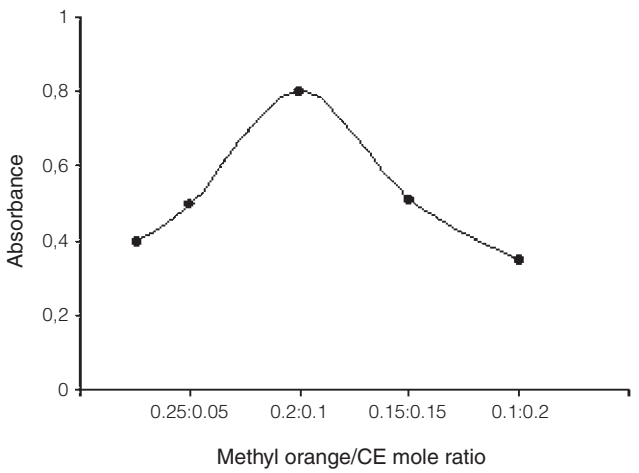

Figure 6. Effect of molar ratio of MO to CE on the reaction.

(II) *Linearity*

A calibration graph was constructed by measuring the absorbance at five concentration levels. It showed a linear response of absorbance in relation to the concentration of CE over the range of 2.5 - 20 μg/mL. As shown by the data, the method was much more sensitive than most of the reported methods[2-3,6]. The representative linear equations relating A (absorbance) to C (concentration μg/mL) was A = 0.034C + 0.114 (r = 0.9998) (Table 1).

(III) *Accuracy and Precision*

The intra-day and inter-day relative standard deviation (RSD) values obtained by the proposed method were found to be within 0.36 - 2.38% for CE. Accuracy of the method expressed as relative mean error (RME) was between 2.0 - 7.0%. The statistical parameters are given in Table 2.

(IV) *Limits of Detection and Quantification*

LOD and LOQ were found to be 1.0 and 3.0 μg/mL,

Table 1. Statistical data of the regression equations for determination of CE

Parameters	Spectrophotometric method
Linearity range (μg/mL)	2.5 - 20
Molar absorptivity (L/mol/Xcm)	1.5×10^4
Sandell's sensitivity (μg/cm^2)	2.6×10^{-2}
Regression equation	A = 0.034C + 0.114
Intercept (*b*)	0.114
Slope (*a*)	0.034
Correlation coefficient (*r*)	0.9998

Table 3. Determination of CE by standard addition method

Proposed method	Concentration (μg/mL)		Recovery ± RSD%
	Theoretical	Spiked	
	5.0	5.0	98.40 ± 2.28
	5.0	10.0	98.70 ± 0.67
	5.0	15.0	99.30 ± 0.45

Table 2. Intra- and inter-day precision and accuracy of the assay for CE

Nominal concentration (μg/mL)	No. of replicates (*n*)	Found concentration (mean ± SD, μg/mL)	Precision (RSD %)	Accuracy (RME %)
Intra-day				
2.5	6	2.45 ± 0.041	1.67	-2.00
10	6	9.64 ± 0.056	0.58	-3.60
20	6	19.1 ± 0.069	0.36	-4.50
Inter-day				
2.5	6	2.40 ± 0.057	2.38	-4.0
10	6	9.57 ± 0.068	0.71	-4.3
20	6	18.6 ± 0.071	0.38	-7.0

respectively. These values were much lower than those obtained by many other methods.

(V) *Recovery*

The recoveries are given in Table 3. The observed CE values were in good agreement with the theoretical values in the three model mixtures. The recovery data ranged from 98.40 to 99.30% in the proposed method.

(VI) *Robustness*

The robustness of the method was checked by examining the effect of slight condition changes on the quantification result. No significant difference could be observed. The results are shown in Table 4.

VI. *Determination of CE in Pharmaceutical Formulations*

The applicability of the developed method was

Table 4. Robustness of the proposed method

Conditions	Mean ± S.D. CE-MO ion pair (10.0 μg/mL)
Optimum	9.88 ± 0.231
Dye concentration	
0.3% w/v	9.83 ± 0.341
0.1% w/v	9.86 ± 0.323
Dye pH	
4.1	9.86 ± 0.421
3.9	9.81 ± 0.312

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checked by analyzing two commercially available pharmaceutical formulations. The formulations selected were Allerset® and Zyrtec® tablets. Comparing the results obtained by the proposed method with the reference method[6] using the *t*-test for accuracy and *F*-test for precision assessment, the calculated values did not exceed the corresponding theoretical values indicating insignificant differences between the results and showing robustness of the proposed procedure (Table 5).

Table 5. Assay results of CE in pharmaceutical preparations by the proposed method

Item	Spectrophotometric method		Reference method[6]
Allerset® tablet (10 mg CE per tablet)			
Recovery (%)	99.25		99.18
S.D.	0.31		0.43
t		0.51	
F		1.92	
Zyrtec® tablet (10 mg CE per tablet)			
Recovery (%)	99.23		99.18
S.D.	0.37		0.43
t		0.34	
F		1.35	

n = 6, Theoretical values for *t* and *F* at P = 0.05 are 2.23 and 5.05, respectively.

CONCLUSIONS

The proposed method made use of a simple reagent, which most ordinary analytical laboratories can afford. The method is sufficiently sensitive to permit determinations as low as 1.0 μg/mL. Unlike GC and HPLC procedures, the spectrophotometer is relatively simple to handle and affordable. The proposed method is simple, precise, accurate and convenient. Hence, the proposed methods should be useful for routine quality control purposes.

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