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Spectrophotometric and high performance liquid chromatographic determination of cetirizine dihydrochloride in pharmaceutical tablets

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Abstract

Derivative spectrophotometric, colorimetric and high performance liquid chromatographic methods, for the determination of the antihistaminic cetirizine dihydrochloride in tablet form were described. Spectrophotometrically, cetirizine was determined by the measurement of its first (^{1}D) and second (^{2}D) derivative amplitudes at 239 (peak) and 243–233 nm (peak-to-trough), respectively. The aqueous solutions obeyed Beer's law in the concentration ranges of 1.2–10.0 and 0.8–10.0 μg ml^{-1} for ^{1}D and ^{2}D measurements, respectively. The colorimetric procedure was based on measuring the absorbency of the coloured chromogen resulted from the reaction between cetirizine sodium salt in polar solvent (DMF) and chloranil at 556 nm. The relation with concentrations was linear over 120–250 μg ml^{-1}. Optimization of the reaction conditions was studied. At the same time, investigation of the complex formed was made with respect to its composition and the associated constant. A simple liquid chromatographic assay has been developed for the determination of cetirizine dihydrochloride in the presence of one of its synthesis precursor (hydroxyzine hydrochloride). A Bondapak-C_{18} column was used with a mobile phase consisting of acetonitrile/0.01 M ammonium dihydrogen phosphate (32:68, v/v) containing 0.1% w/v tetrabutyl ammonium hydrogen sulphate adjusted to pH 3 with phosphoric acid at a flow rate of 2 ml min^{-1}. With salicylic acid as internal standard, quantitation was achieved with UV detection at 230 nm based on the peak height ratios. Beer's law was obeyed in a concentration range of 3–35 μg ml^{-1} and the regression line equation was derived with a correlation coefficient of 0.9999. The validity of the methods was further confirmed using the standard addition method. The proposed procedures were successfully applied to the determination of cetirizine in bulk and tablet form, with high percentage of recovery, good accuracy and precision.

Keywords: Cetirizine dihydrochloride; First and second derivative; Chloranil; HPLC; Tablets

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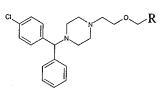
1. Introduction

Cetirizine dihydrochloride (CZ), the dihydrochloride of 2-(4-(4-chlorobenzhydryl)piperazin-1-yl)ethoxyacetic acid, a non-sedating type histamine H_1-receptor antagonist is used, mainly, in symptomatic treatment of seasonal rhinitis and conjunctivitis, perennial allergic rhinitis as well as pruritus and urticaria of allergic origin [1]. Cetirizine is not official in any pharmacopoeia and a literature survey reveals a high performance liquid chromatographic method and a direct UV absorbance measurement after chloroformic clean-up for its determination in pharmaceutical tablets [2] and syrups [3], respectively. Chromatographic procedures for the determination of CZ have been described, but these GLC [4] and HPLC [5,6] methods were all used for the analysis of the drug in biological fluids. No colourimetric or other spectrophotometric methods are available for the analysis of CZ in pharmaceutical tablets.



R_1 COOH Cetirizine

R_2 CH_2OH Hydroxyzine

For single component preparations, the simplest assay method involves the direct measurement of the UV absorption at the maximum. Cetirizine is relatively weak UV absorbing compound, therefore, the direct UV absorbance measurements at low concentration (dissolution testing) will be unreliable. Fortunately, the derivative transformation of spectral data has been proved to be valuable procedure for the identification and quantitation of several drugs [7,8]. In the present paper we develop a rapid, accurate and precise first (^{1}D) and second (^{2}D) derivative spectrophotometric method for CZ determination in tablet dosage form.

For more selectivity, a simple colourimetric procedure, which utilized the reaction between the sodium salt of CZ and 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil) in DMF medium, was described. For more specificity, validation and comparison of the proposed spectrophotometric procedures, a simple isocratic high performance liquid chromatographic method (HPLC) was developed. A study of several combinations of different solvents and buffer systems, different pH and suitable internal standards for proper quantitation, led to the described analytical conditions giving sharp and well resolved peaks. The proposed methods were successfully applied to the determination of CZ in commercial tablets. The results obtained from the photometric methods were compared statistically with those of the HPLC procedure.

2. Experimental

2.1. Instrumentation

A double beam, self recording Shimadzu (Duisburg, Germany) UV-VIS spectrophotometer model UV-160A with 1 cm matched quartz cells was used. The spectral band width was 2 nm and the wavelength scanning speed was 480 nm min^{-1}. The response time was 0.02 s in the spectrum mode.

The HPLC (Waters Associates-Milford, MA) instrument was equipped with a model 501 pump, automated gradient controller model 680, U6K universal injector and model 481 variable wavelength UV detector. Chromatographic peaks were electronically integrated and recorded with a model 740 (Waters Associates) computing integrator.

2.2. Materials

Pharmaceutical grade of CZ and hydroxyzine hydrochloride (HZ)(UCB Pharma, Brussels) were kindly supplied by Pharco Pharmaceutical (Alexandria, Egypt) and were certified to contain 99.50 and 99.35%, respectively. They were used without further purification. Salicylic acid, used as an internal standard (1 mg ml^{-1} solution in methanol) in the HPLC procedure, was an in-house standard and its purity was certified to be 99.00%. Chloranil (Aldrich Chem, Milwaukee, WI) was analytical grade reagent and

used as 1.5×10^{-2} M solution in acetone. Acetonitrile (BDH, Poole) was of HPLC grade and water was doubly-distilled from all glass apparatus. Analytical reagent grade ammonium dihydrogen phosphate, tetrabutyl ammonium hydrogen sulphate, acetone and phosphoric acid were used throughout these experiments.

2.3. Chromatographic conditions

The mobile phase was prepared by mixing acetonitrile and 0.1 M ammonium dihydrogen phosphate solution in the ratio of 32:68. To each liter of mobile phase, 1 g of tetrabutyl ammonium hydrogen sulphate was added and the apparent pH of the solution was adjusted to 3 using phosphoric acid. All determinations were performed at ambient temperature (20°C) using C_{18}, 300×3.9 mm i.d., reverse phase column (Waters Bondapak, 10 μm). The column effluent was monitored at 230 nm, which represents the wavelength of maximum absorbancy of CZ and the sensitivity was set at 0.1 AUFS (Absorbance units full scale). The injection volume was 20 μl with a flow rate of 2 ml min^{-1}. The chart speed of the integrator was set at 0.5 cm min^{-1} with an attenuation of 32.

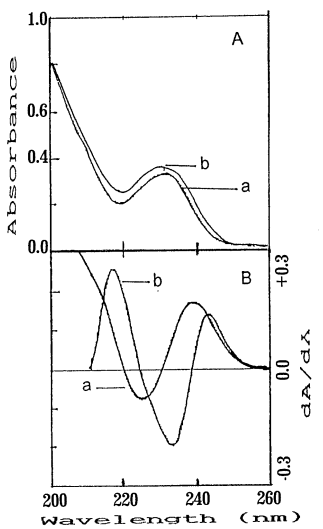


Fig. 1. (A) Spectrophotometric scans corresponding to 10 μg ml^{-1} CZ (in water) as the standard (a) and extracted tablet (b). (B) (^{1}D) first derivative (a) and (^{2}D) second derivative (b) scans corresponding to 10 μg ml^{-1} of standard CZ in water.

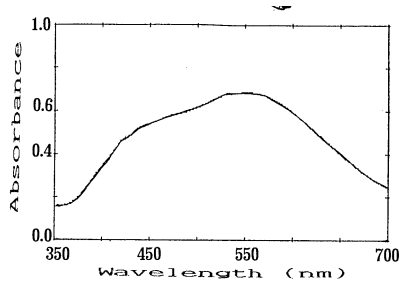


Fig. 2. Absorption spectrum of the coloured product formed through the reaction of 200 μg ml^{-1} of CZ with chloranil in DMF.

2.4. Standard solutions and calibration graphs for spectrophotometric measurements

2.4.1. Derivative procedure

A stock solution was prepared by dissolving CZ in water to obtain a concentration of 100 μg ml^{-1}. The standard solutions were prepared by dilution of the stock solution in water to reach concentration ranges of 1.2–10.0 and 0.8–10.0 μg ml^{-1} for ^{1}D and ^{2}D, respectively. The ^{1}D and ^{2}D curves of the working aqueous standard solutions were scanned in the range of 300–200 nm against water as a blank. The values of the ^{1}D and ^{2}D amplitudes at 239 (peak) and 243–233 nm (peak-to-trough), respectively, were measured, and the concentrations versus their absolute derivative amplitudes were plotted in order to obtain the calibration graphs. The notation for amplitude measurements in the derivative domain was made according to Fasanmade and Fell [9].

2.4.2. Colourimetric procedure

Stock solution was prepared by dissolving 100 mg of CZ in a 100 ml volumetric flask using 50 ml dimethyl formamide (DMF). The resulting solution was neutralized to phenolphthalein, using 0.1 M sodium hydroxide, and the volume was completed with the same solvent. Different volumes (1.2–2.5 ml) of the standard CZ solution were accurately transferred to a set of a 10 ml volumetric flasks. The volume in the flasks was brought to a constant volume using neutralized DMF. To each flask a 3

ml portion of chloranil solution was added. The reaction mixtures were left to stand at room temperature for 30 min and then the flasks were completed to volume with acetone. The absorbances of the resulting colored solutions were measured at 556 nm using a reagent blank simultaneously prepared.

2.5. Standard solutions and calibration graphs for chromatographic procedure (HPLC)

Standard solutions of CZ containing concentration range of 3–35 μg ml^{-1} and a fixed concentration (50 μg ml^{-1}) of salicylic acid (internal standard) were prepared in the mobile phase. Triplicate 20 μl injections were made for each concentration and the peak height ratio of each concentration to the internal standard were plotted against the corresponding concentrations to obtain the calibration graph.

2.6. Sample preparation

A total of 20 tablets containing CZ as the active ingredient were weighed and finely powdered. A portion of the powder equivalent to 10 mg CZ was weighed accurately, transferred to a 100 ml volumetric flask and suspended in 50 ml of water. The flask was placed in ultrasonic water bath for 15 min before completion to volume with the same solvent. For HPLC analysis, 10 ml aliquots of the resulting solution were transferred into a 50 ml volumetric flask, 1.0 ml of the internal solution (salicylic acid) was added and the volume was adjusted with

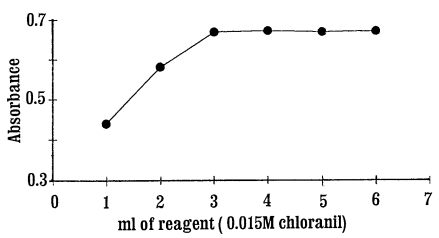


Fig. 3. Effect of chloranil concentration on the absorbance of (CZ-chloranil) complex measured at 556 nm (CZ = 200 μg ml^{-1}).

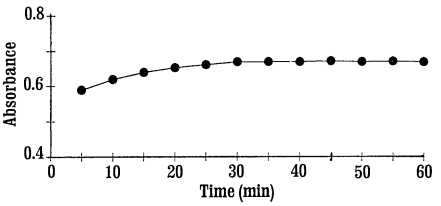


Fig. 4. Effect of reaction time on the absorbance of (CZ-chloranil) complex measured at 556 nm (CZ = 200 μg ml^{-1}).

the mobile phase. The solution was filtered through a 0.45 μm membrane filter. A volume of 20 μl was injected into the chromatograph concurrently with the appropriate standard solution (20 μg ml^{-1}) and the peak-height ratios (drug to internal standard) were used for the determination of CZ in each sample.

For derivative spectrophotometric method, a 1 ml aliquot of the resulting solution was transferred to a 25 ml volumetric flask and the volume was adjusted with water. The ^{1}D and ^{2}D amplitudes were measured directly as described under the calibration graph and the concentration of CZ in each sample was determined by interpolating the appropriate calibration graph. Another set of solutions containing five times the concentrations used in the derivative mode were, analyzed by direct measurements of the absorbance at 230 nm and the concentrations of CZ were calculated by direct comparison with standard solutions.

For the colourimetric procedure, an amount of the tablets powder equivalent to 50 mg CZ was transferred to a 50 ml volumetric flask using 30 ml DMF (three times each of 10 ml). The resulting DMF solution was neutralize with 0.1 M sodium hydroxide using phenolphthalein as indicator and then completed to volume with DMF. The combined extract was filtered and 2 ml of the filtrate was transferred quantitatively to a 10 ml volumetric flask and the procedure followed as described under the calibration graph. The concentration of CZ in each sample was calculated by direct comparison with standard solutions.

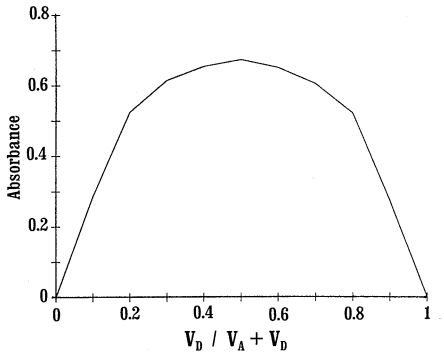


Fig. 5. Continuous variation plot for (CZ-chloranil, 1×10^{-3} M) complex ratio.

3. Results and discussion

3.1. Derivative ultraviolet spectrophotometry

Water was used for extracting CZ from tablets samples. Fig. 1A shows the absorption (zero-order) UV spectra of (a) CZ standard solution and (b) an extract of commercial tablets. There is a significant interference in the conventional spectrophotometric determination, as indicated by the upward displacement of the drug spectral band. However, the application of the derivative spectrophotometric technique allowed complete elimination of the back-ground absorption due to the excipients. In fact, the amplitude of the peak at $\lambda = 239$ nm to the zero line $^1D_{239}$ in the first derivative spectrum (Fig. 1B) and the peak-to-trough amplitude $^2D_{243,233}$ was the basis for the development of a specific and simple procedure for the analysis of CZ tablets. Linear calibration graphs, $^1D_{239} = 1.645 \times 10^{-2}C - 4.762 \times 10^{-4}$ ($r = 0.9998$; $n = 11$) and $^2D_{243,233} = 3.253 \times 10^{-2}C + 2.793 \times 10^{-4}$ ($r = 0.9999$; $n = 11$) were obtained between the measured amplitudes and the CZ concentrations ($C = 1.2$–10 and 0.8–10 μg ml^{-1} for ^{1}D and ^{2}D, respectively).

3.2. Colourimetric procedure

Cetirizine molecule contains a free carboxylic group and the availability of the electrons on the two oxygens makes them good electron donors. At the same time, the resonance stability of carboxylate anion increases their elerctron density and hence their electron donating characters [10]. The addition of a quinone (for e.g. chloranil) in polar solvent (dimethyl formamide) to CZ salt yielded highly coloured chromogen [11]. It was observed that CZ free acid did not produce any colour before neutralization, indicating that the drug anion was the site of interaction as electron donors to the acceptor (quinone-chloranil) [12–15]. Fig. 2 shows the absorption spectrum of the reaction between CZ and chloranil against reagent blank, with an absorption maximum at 556 nm, which was used for all the subsequent measurements.

Investigations of the effect of the chloranil concentration and the reaction time for the developed colour sensitivity and obedience to

Table 1
Comparative analytical data for the determination of cetirizine dihydrochloride

Analytical method	Slopes* $b \pm (tS_b)$ (cm^{-1} μg ml^{-1})	Intercept** $a \pm (tS_a)$	Linearity S_b rel (%)
$^1D_{239}$	1.65×10^{-2} (2.59×10^{-4})	-4.76×10^{-4} (1.45×10^{-3})	0.70
$^2D_{249,233}$	3.25×10^{-2} (2.66×10^{-4})	2.79×10^{-4} (1.48×10^{-3})	0.36
Colourimetric	3.35×10^{-3} (1.58×10^{-4})	-1.32×10^{-4} (2.84×10^{-2})	1.70
HPLC	4.41×10^{-2} (5.84×10^{-4})	1.30×10^{-3} (1.22×10^{-2})	0.54

* Confidence intervals of the slopes ($P < 0.05$).
** Confidence intervals of the intercept ($P < 0.05$).

Table 2
Concentration range, detection limit and relative sensitivity of the different method

Analytical method	Concentration range (μg ml^{-1})	Detection limit (μg ml^{-1})	Relative sensitivity[a]
$^{1}D_{239}$	1.20–10.0	0.135	0.36
$^{2}D_{249,233}$	0.80–10.0	0.072	0.19
Colourimetric	120.0–250.0	4.377	11.55
HPLC	3.0–35.0	0.379	1.00

[a] Calculated relative to the HPLC method.

Beer's law were carried out. After the study of the above mentioned factors, it was found that 3 ml of 0.015 M chloranil solution (Fig. 3) for 30 min at room temperature (Fig. 4) represented the optimum conditions for developing the colour. The reaction stoichiometry between CZ and chloranil have been determined by applying Job's method of continuous variation [16]. The obtained results in Fig. 5 indicates a molar ratio of 1:1 between CZ:chloranil. The stability constant of the formed ion-paired complex was calculated to be 1.93×10^{8}. Under the above mentioned conditions, the graph obtained by plotting the absorbence at 556 nm against concentration was found to be linear in the range 120–250 μg ml^{-1} (Table 2). The intercept, slope and detection limit obtained by the least squares treatment of the data are given in Table 1 and Table 2. The molar absorptivity (l M^{-1} cm^{-1}) and Sandall's sensitivity (μg cm^{-3}/0.001 A) were found to 1.55×10^{3} and 0.259, respectively.

3.3. Chromatographic procedure (HPLC)

A reversed phase HPLC method was developed to provide a specific procedure suitable for rapid quality control of CZ tablet dosage form and as reference method for both the derivative and the colourimetric procedures. A mobile phase consisting of acetonitrile/0.01 M aqueous ammonium dihydrogen phosphate (32:68), with the addition of 1 g of tetrabutyl ammonium hydrogen sulphate to every litre, was chosen after several trials with acetonitrile/water and methanol/water. The apparent pH of the aqueous phase was adjusted to 3 using phosphoric acid. To optimize the assay parameters, the effect of acetonitrile and the apparent pH on the capacity factor (k') values were studied. The capacity factor (k') values for CZ, HZ and the internal standard (salicylic acid) were substantially affected by the variation in the acetonitrile percentage in the mobile phase (Fig. 6). At high concentration of acetonitrile ($>40\%$), all the three peaks of CZ, HZ and the internal standard over-

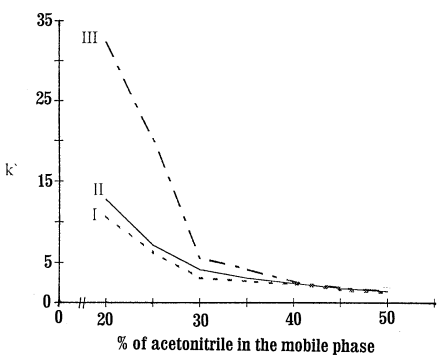


Fig. 6. Plot of the capacity factor (k') versus the acetonitrile concentration in the mobile phase (**I**-HZ; **II**-IS and **III**-CZ).

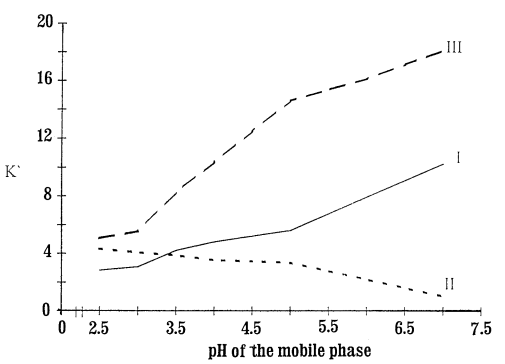


Fig. 7. Plot of the capacity factor (k') versus the apparent pH of the mobile phase (**I**-HZ; **II**-IS and **III**-CZ).

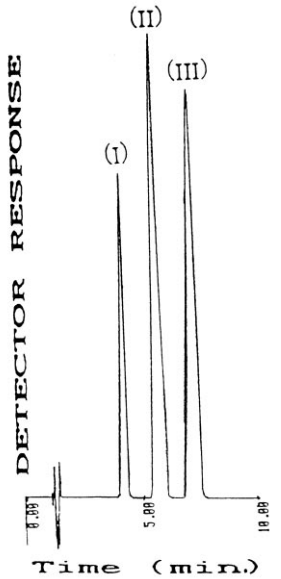


Fig. 8. A typical chromatogram of a 20 μl injection of synthetic mixture of HZ (**I**) (20 μg ml^{-1}); IS (**II**) (20 μg ml^{-1}) and CZ (**III**) (20 μg ml^{-1}).

lapped. Lowering the ace-tonitrile percentage below 30%, the peaks became some what broad, but gave reasonable resolution between the three peaks. Variation of the apparent pH (Fig. 7) yielded good k' values at the apparent pH of 3, with reasonable retention times for all the three peaks. At the same time, due to the basic nature of CZ and HZ, the addition of the tetrabutyl ammonium hydrogen sulphate (as paired-ion) was essential to prevent tailing and to reduce the retention time of the drug. The above described chromatographic system allowed an adequate resolution ($R_s = 2.15$) between CZ ($t_r = 7.17$) and the internal standard, salicylic acid ($t_r = 5.62$) in a reasonable time (Fig. 8) (R_s, resolution; t_r, retention time).

For quantitative determinations a linear calibration graph ($Y = 4.406 \times 10^{-2}C + 1.300 \times 10^{-3}$; $r = 0.9999$; $n = 8$ where Y and C were the peak height ratios and concentrations, respectively) was obtained over the working concentration range 3–35 μg ml^{-1}. The relative standard deviation (0.34%) of the peak height ratio of CZ to the internal standard, derived from replicate ($n = 8$) analyses of a CZ solution, illustrate the precision of the chromatographic procedure. The specificity and selectivity of the HPLC system were ascertained by a separate chromatographic analysis of either the excipient mixtures or HZ without CZ; no interfering peaks at the retention times of CZ and salicylic acid (internal standard) peaks were observed.

3.4. Statistical evaluation of the developed procedures

The HPLC method was chosen as the analytical reference method. First and second derivative spectrophotometric and colourimetric procedures were compared with HPLC. The slopes, intercepts and linearity of each calibration graph were calculated and summarized in Table 1. The negative and positive intercept values for derivative, colourimetry and HPLC were not statistically ($P < 0.05$) different from zero. The order of linearity for the calibration graphs in the ranges stated in Table 2 for the different analytical method was: ^{2}D > HPLC > ^{1}D > colourimetry. The concentration ranges, detection limits and relative sensitivities are summarized in Table 2. The lowest detection limit calculated was obtained for ^{2}D method (0.072 μg ml^{-1}) indicating the highest sensitivity. The colourimetric method was the least sensitive (4.38 μg ml^{-1}). Relative sensitivities, based on detection limits, were calculated with respect to the chromatographic method. The order of sensitivity for these method was: ^{2}D > ^{1}D > HPLC > colourimetry. Commercially available tablets were analyzed using the HPLC, the derivative and colourimetric spectrophotometric methods. The results obtained were summarized in Table 3. No significant differences were found between the results obtained by the HPLC and the derivative procedures, for the same batch at the 95% confidence level (Student's t-test and F-variance ratio test).

4. Conclusion

The HPLC method and the spectrophotometric (^{1}D, ^{2}D and colourimetric) methods were found to be reproducible and accurate in the analysis of cetirizine dihydrchloride in pharmaceutical tablets. Under the experimental conditions, men-

Table 3
Assay results for the determination of cetirizine dihydrochloride in commercial tablets

	Recovery (mean ± S.D.)[a]				
	A_{max}	$^1D_{239}$	$^2D_{243,233}$	Color	HPLC
Commercial tablets[c]	106.87 (1.12)	100.26 (0.62)	100.06 (0.72)	99.68 (0.44)	100.18 (0.44)
*t**	13.617	0.258	0.348	1.968	
*F**	6.479	1.986	2.678	1.000	
Recovery[b]	99.80 (0.51)	100.08 (0.69)	100.00 (0.69)	100.00 (0.48)	100.00 (0.52)

[a] Mean of six determination, the values in parentheses are the standard deviations.
[b] For standard addition of 50% on the nominal content ($n = 6$).
[c] Zyretic tablets were manufactured by Glaxo Egypt S.A.E. (ElSalam, Cairo) under license from UCB Pharma (Brussels) and labeled to contain 10 mg CZ per tablet (Batch no. 60833A).
* The theoretical *t*- and *F*-values at 0.05 confidence limit are equal to 2.23 and 5.05, respectively.

tioned above, the second (^2D) derivative procedure was the most sensitive method; however, better selectivity was obtained with the colorimetric and the HPLC methods. All the proposed methods were linear with good reproducibility and sensitivity. In stability studies, in which CZ may exist with other decomposition or related substances, the preferred method is the HPLC. The latter avoids interference from the other components. In general, all the proposed methods can be used for the routine analysis of cetirizine dihydrochloride in bulk and tablet dosage form.

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