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Determination of cetirizine dihydrochloride, related impurities and preservatives in oral solution and tablet dosage forms using HPLC

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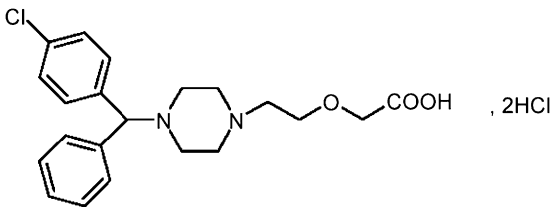
Abstract

An HPLC method was developed and validated for the determination of cetirizine dihydrochloride (CZ) as well as its related impurities in commercial oral solution and tablet formulations. Furthermore, two preservatives associated with the drug formulations, namely, propyl (PP) and butylparabens (BP) were successfully determined by this method. The chromatographic system used was equipped with a Hypersil BDS C18, 5 μm column (4.6 × 250 mm) and a detector set at 230 nm in conjunction with a mobile phase of 0.05 M dihydrogen phosphate:acetonitrile:methanol:tetrahydrofuran (12:5:2:1, v/v/v/v) at a pH of 5.5 and a flow rate of 1 ml min^{-1}. The calibration curves were linear within the target concentration ranges studied, namely, 2×10^2–8×10^2 μg ml^{-1} and 1–4 μg ml^{-1} for CZ, 20–100 μg ml^{-1} for preservatives and 1–4 μg ml^{-1} for CZ related impurities. The limits of detection (LOD) and quantitation (LOQ) for CZ were, respectively, 0.10 and 0.34 μg ml^{-1} and for CZ related impurities were in the ranges of 0.08–0.26 μg ml^{-1} and 0.28–0.86 μg ml^{-1}, respectively. The method proved to be specific, stability indicating, accurate, precise, robust and could be used as an alternative to the European pharmacopoeial method set for CZ and its related impurities.

Keywords: Cetirizine; HPLC; Preservatives; Stability indicting; Impurities

1. Introduction

Cetirizine dihydrochloride (CZ) is (RS)-2-[2-[4-[(4-chlorophenyl) phenyl methyl]piperazine-1-yl]ethoxy] acetic acid dihydrochloride whose structural formula is given below. It is described as a long acting non-sedating antihistamine with some mast-cell stabilizing activity. It is used for the symptomatic relief of allergic conditions including rhinitis and chronic urticaria [1].



As a bulk material, CZ was assayed by acid base titration [2,3] and HPLC [4,5] techniques.Various HPLC methods were used for CZ assay in various pharmaceutical formulations [6,7–10] where C18 columns of different brands were used. The mobile phases mainly consisted acetonitrile and phosphate buffer of different pH values. The detection was carried out by UV detection at 230 nm or 254 nm. CZ in pharmaceutical formulations was also determined by other techniques such as ultraviolet spectrophotometry [6,11,12], spectrofluorimetry [13,14], calorimetry [6,14-17] and ion-selective electrodes [18]. TLC and HPTLC methods were reported for the determination of CZ as a bulk material and in formulations, respectively [19,20].

Methods reported for the determination of CZ related impurities were limited [3,21]. The European Pharmacopoeial method [3] was used for the determination of CZ related impurities, namely: A, B, C, D, E and F whose formulas are given below (Scheme 1). The chromatographic system used in this method was equipped with a column of 250 × 4.6 mm

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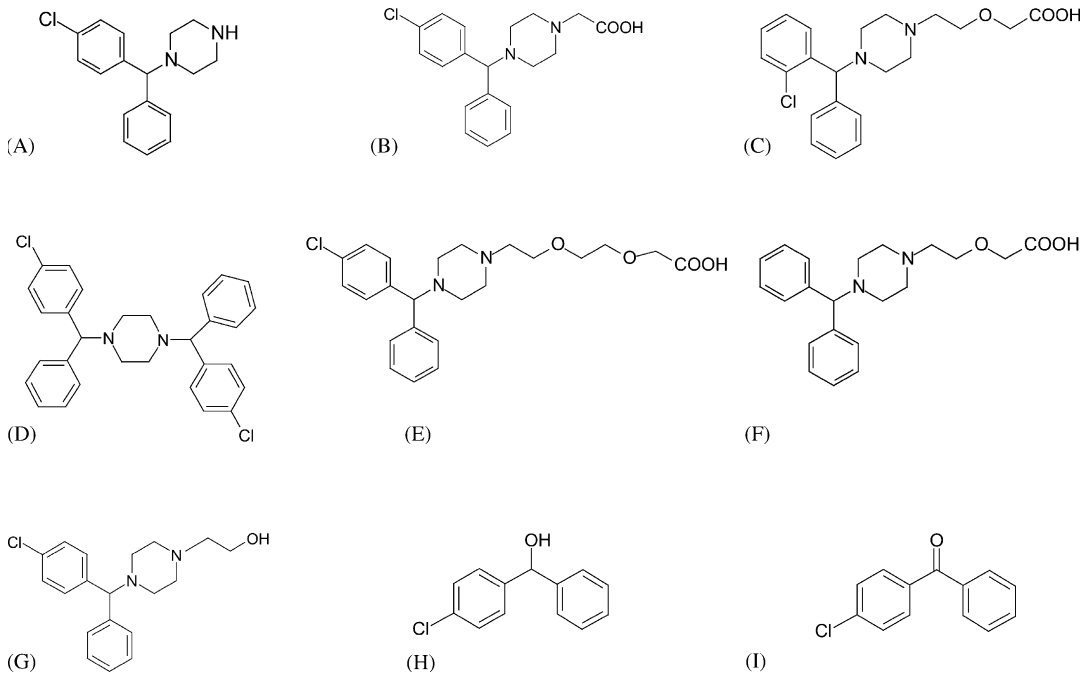

Scheme 1. CZ related impurities.

packed with 5 μm silica gel and the mobile phase was a mixture of dilute sulfuric acid: water: acetonitrile (0.4:6.6:93, v/v/v) with the flow rate set at 1 ml min^{-1} and the detection made at 230 nm. This method was tested here during the preliminary studies for the determination of CZ related impurities. It has been noticed that the column deteriorates when it is in use and has to be changed every two or three replicates to recover the normal performance. This behavior was attributed to the very low pH (<0.5) of the mobile phase. In the mean time, a considerable interference was noticed between impurities A and F and the non-pharmacopoeial impurities H and I.

The other method [21] was developed and validated to determine all impurities mentioned in the European pharmacopoeial method [3] except impurity C in addition to other eight related impurities. The method was based on LC/MS technique equipped with a cyano-column coupled to an electrospray ionization mass spectrometer where a binary gradient elution system composed of 50 mM ammonium acetate solution (pH 7) and acetonitrile was used. This method was applied only for the tablet formulations using the expensive MS detection technique and a gradient elution system.

Thus, this work aimed at developing a simple HPLC method for simultaneous determination of CZ and its related impurities in the presence of formulation excipients either in solution or solid formulations based on isocratic system and a commonly used UV detector. Also the method was also validated to determine the two preservatives PP and BP used in solution formulations in conjunction with the other drug components.

2. Experimental

2.1. Materials and equipment

CZ, CZ related impurities (A–I), PP, BP, the commercial products: Cetolerg tablets (5 and 10 mg), Cetolerg solutions (1 and 10 mg ml^{-1}) and the formulation excipients were all provided by the Jordanian Pharmaceutical Manufacturing Company (JPM). Zyretic (UCB, Belgium) tablets (10 mg) and Zyretic solution (1 mg ml^{-1}) were obtained from the Jordan market. All other chemicals were of HPLC or analytical grade and obtained from Acros.

The chromatographic system was consisted of an HPLC (Thermoseparation products, with programmable solvent module P1000, and programmable detector module UV1000) equipped with a Hypersil BDS C18 (5 μm, 4.6 × 250 mm) column.

2.2. Analytical solutions

2.2.1. Mobile phase

A degassed and filtered mixture of 0.05 M potassium dihydrogen phosphate, acetonitrile, methanol and tetrahydrofuran (60:25:10:5, v/v/v/v) with a pH of about 5.5 was used as the mobile phase.

2.2.2. Standard reference solutions

50 mg of CZ were accurately weighed and dissolved in 100 ml of the mobile phase. Further, dilutions were carried out to obtain CZ solutions within the target concentrations. Standard solutions of preservatives were prepared by dissolving,

accurately weighed, 40 mg of PP and PB in 200 ml of the mobile phase followed by five-fold dilution with the mobile phase. For standard solutions of CZ related impurities, 5 mg of each impurity were accurately weighed and dissolved in 200 ml of the mobile phase followed by 10-fold dilution with the mobile phase.

2.2.3. Drug-matrix preparation

The materials used in the drug-matrix preparation used in solution formulations include: PP, BP, propylene glycol, glycerin, glacial acetic acid, sodium acetate, saccharin sodium, carboxymethyl cellulose sodium, apricot flavor, and purified water. When the preservatives were assayed, PP and PB were removed from the matrix and CZ was added. Drug-matrix for CZ tablets was made by mixing microcrystalline cellulose, lactose, magnesium stearate, and opadry coating material.

2.2.4. Solutions for testing degradation and specificity

0.1M HCl, 0.1M NaOH and 1% H_2O_2 solutions were used to induce degradation of CZ and the preservatives (PP and PB). 250 mg of CZ and 80 mg of each of PP and BP were separately dissolved in 100 ml of each of the degradation solutions and kept at 80 °C for 10 h. 10 ml of each solution were then diluted separately to 50 ml with the mobile phase.

The effect of light on the stability of CZ in its solid state or in solution was studied for samples of CZ powder (thin layer in petri dish) and in CZ solution (200 μg ml^{-1}) by exposing them to low intensity UV lamp and daylight for 65 and 2 days, respectively.

2.2.5. Solutions for testing linearity and range

Standard solutions used for testing the linearity of calibration plots for CZ were prepared by separately dissolving accurately weighed quantities of CZ (20–80 mg) in 100 ml of the mobile phase. Further, dilutions with the mobile phase were carried out to prepare another set of CZ concentrations in the range of 1–4 μg ml^{-1}. The solutions of CZ related impurities were prepared by dissolving accurately weighed 5 mg of each component in 200 ml mobile phase followed by further dilutions to obtain solutions having concentrations ranging from 1 to 4 μg ml^{-1}. Solutions of preservatives were prepared by dissolving accurately weighed 40 mg of PP or BP in 100 ml mobile phase, followed by further dilutions with the mobile phase to obtain solutions having concentrations ranging from 20 to 100 μg ml^{-1}.

2.2.6. Solutions for testing accuracy

Samples of CZ and its related impurities were prepared by transferring into three 100 ml volumetric flasks three quantities of CZ in the range of 25–75 mg and three quantities of impurities, respectively, in the range of 0.1–0.4 mg, followed by additions of 50 ml of the solution's drug-matrix or 1400 mg of the tablet's drug-matrix. Mobile phase was then added to make the volume up to the mark. The samples containing the tablet's drug-matrix were sonicated for 15 min centrifuged at 4000 rpm for 15 min and the supernatant was used for HPLC injections.

Solutions of preservatives were made by separately dissolving three quantities of each of PP and BP in the range of 20–60 mg in 200 ml of the mobile phase. Five millilitre portions of the final solutions were separately transferred into 25 ml volumetric flasks followed by additions of 5 ml of the solution's drug-matrix into each flask and adjusting the volume up to the mark by the mobile phase.

2.2.7. Solutions for testing precision, stability and effects of method's parameters

Solutions of CZ and its related impurities were prepared by transferring accurately weighed portions of CZ (50 mg each) and related impurities (0.25 mg each) into 100 ml volumetric flasks followed by 50 ml additions of the solution's drug-matrix or 1400 mg of the tablet's drug-matrix. Mobile phase was then added to make the volumes up to 100 ml.

Solutions of preservatives were prepared by transferring 5 ml portions of the PP and PB standard stock solutions mentioned above into 25 ml volumetric flasks followed by 5 ml additions of the solution's drug-matrix; mobile phase was then added to make the volume up to 25 ml.

The above solutions were also used to study the effect of various method's parameters and their stability was tested by storing at ambient conditions for 24 h.

2.2.8. Chromatographic procedure and calculation

20 μl samples were injected into the chromatograph; the flow rate was set at about 1 ml/min and the HPLC chromatograms were recorded at a detector setting of 230 nm. The tailing factor for CZ peak found was not more than 1.5 and the relative standard deviation for replicate injections was not more than 2.0%.

3. Results and discussion

3.1. Development of the HPLC Method

During the method development, different parameters were manipulated to obtain an acceptable resolution between the analyte components with acceptable recoveries and to satisfy the HPLC system suitability and use it as a stability indicating method. These parameters include: flow rate (0.5–1.5 ml/min^{-1}), column temperature (25–45 °C), different types of C18 columns, sodium heptanesulfonate ion pair of different concentrations (0.5–1.5%), 0.05 M phosphate buffers of pH ranging from 3 to 8, and various organic modifiers including mixtures of methanol, acetonitrile and tetrahydrofuran with different ratios.

The preliminary work was conducted by using reversed phase C18 column and binary mobile phases consisting phosphate buffer and acetonitrile. The UV detection at 230 nm was found to be more sensitive where it gave high absorptivities for CZ and its related impurities. Binary mobile phase

systems of different ratios were found not suitable due to a bad resolution and low recoveries observed for some CZ impurities. No significant effect on resolution and recoveries was obtained by changing flow rate, column temperature or column trade names. The difficulty in developing a universal method for the determination of all CZ impurities might be ascribed to the wide range of polarity of the analytes. For example, impurities H and I were eluted slowly while impurities C, F, and E showed fast elution. The addition of an ion pair and changing the pH of the mobile phase did not significantly improve the resolution. When a mobile phase of multicomponents was used a better resolution was demonstrated. Various mixtures of phosphate buffer, acetonitrile and methanol of different ratios (60:30:10, 60:20:20, 40:40:20, 45:35:20 and 50:30:20, v/v/v) were tried. A significant improvement in resolution was achieved except for the separation of CZ from impurity G. Tetrahydrofuran was finally introduced into the mobile phase and the ratios of the phosphate buffer, acetonitrile, methanol and tetrahydrofuran were varied until the ratio of 60:25:10:5, v/v/v/v, respectively, was found to be the optimum in achieving good resolution for impurity G without affecting the resolution of other components. Thus, coupling of this mobile phase, a flow rate of 1 ml min^{-1} and a detection at 230 nm showed a significant resolution of CZ, impurities A, C, E, F, G, H, I, and the two preservatives PP and BP. Although these optimum conditions achieved significant resolution of several components, the impurity B showed an interference with the CZ peak, and impurity D was not detected. In case the determination of these two impurities is needed the European pharmacopoeial [3] method would be followed.

3.2. Specificity

The specificity was demonstrated by the HPLC chromatograms recorded for mixtures of CZ, preservatives and CZ related impurities dissolved in the mobile phase. Well-resolved peaks for CZ, PP, BP, impurities A, C, E, F, G, H and I were observed (Figs. 1 and 2) with relative retention times of 1.0, 1.4, 2.7, 1.3, 0.7, 0.8, 0.5, 1.2, 3.9 and 6.7, respectively. Only impurities B and D could not be detected by the proposed method, where impurity B and CZ showed overlapping and impurity D did not elute. Drug-matrices stored for 6 months at 40 °C and at 40 °C/75% RH showed zero response with respect to all analyte's components indicating matrix stability and a free matrix interference effect.

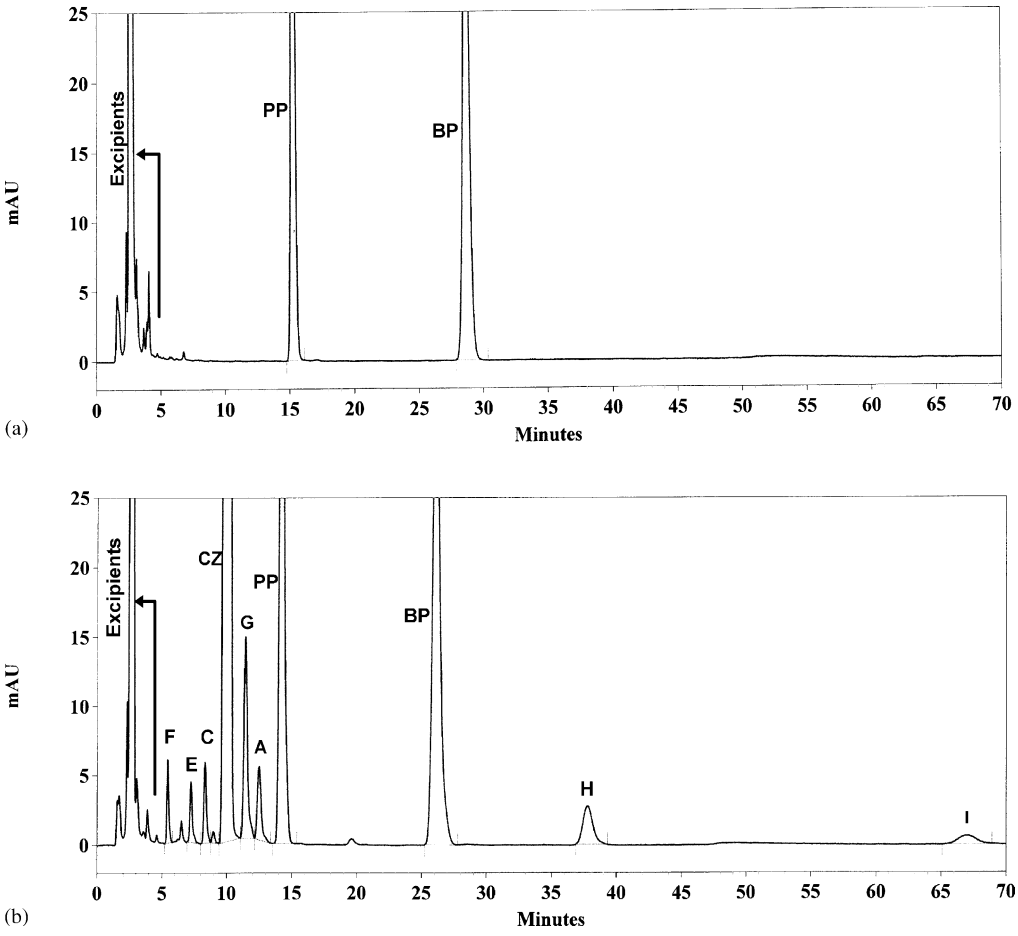

Fig. 1. HPLC chromatograms: (a) for solution's drug-matrix and (b) for a synthetic mixture of CZ (500 μg ml^{-1}) and CZ related impurities (2.5 μg each ml^{-1}) in the same drug-matrix.

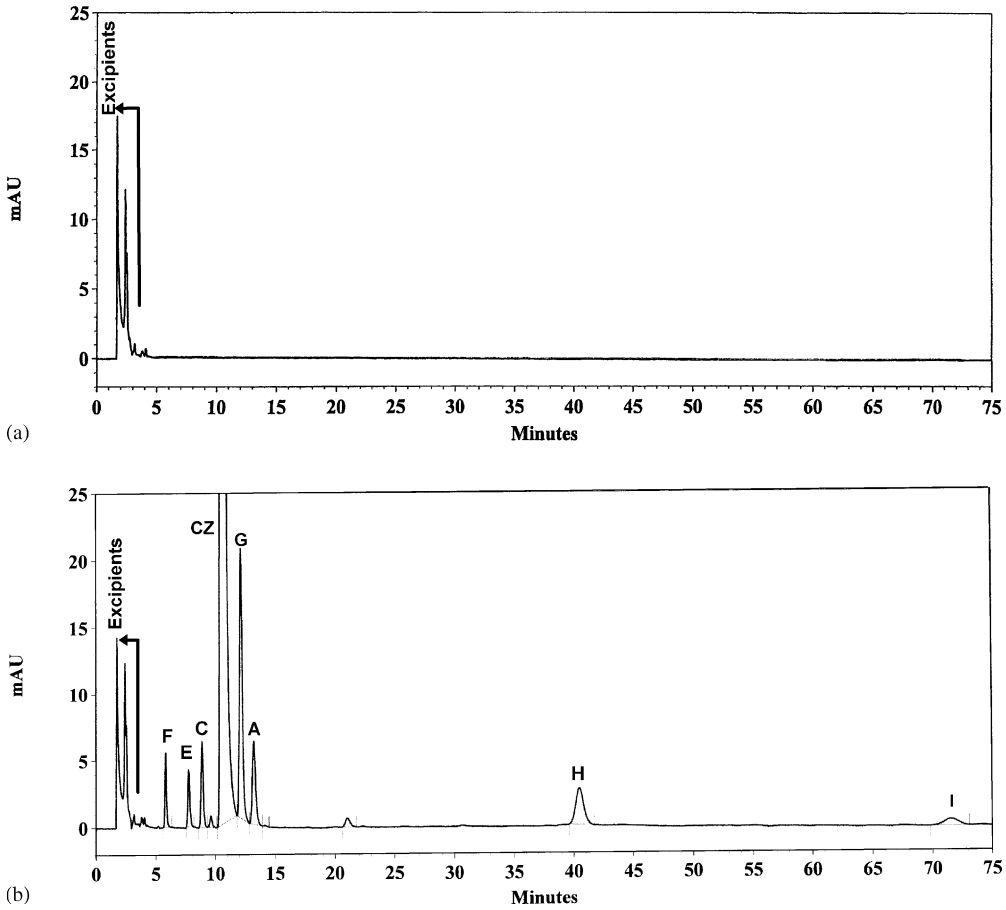

Fig. 2. HPLC chromatograms: (a) for tablet's drug-matrix and (b) for a synthetic mixture of CZ (500 μg ml^{-1}) and CZ related impurities (2.5 μg each ml^{-1}) in the same drug-matrix.

When the degradation of CZ, PP and BP was induced by 0.1 M HCl, 0.1 M NaOH or 1% H_2O_2 at 80 °C for 10 h, the HPLC chromatograms of the resulted solutions showed that the method is stability indicating for both CZ and the preservatives, PP and BP (Table 1). The HPLC chromatograms (Fig. 3) recorded after degradation showed well-resolved peaks for CZ, and some degradation products (DP1–DP3) other than the synthetic CZ impurities mentioned above. These degradation products showed also a significant resolution from CZ impurities, PP and BP. Furthermore, the expected hydrolysis degradation products of PP and BP did not show any peaks in the chromatograms indicating zero interference with the analyte's components.

Furthermore, the proposed method can be used to assess the photostability of CZ. Two photodegredants were detected at relative retention times of 0.76 and 0.90. And 99.5% of CZ was detected after exposure of CZ solution to daylight for 2 days. However, a significant decrease in the CZ potency was observed after exposing solid CZ to UV light for 65 days where 81.6% CZ was detected at the end of that period.

Table 1
Degradation of CZ and preservatives (PP and BP) stored in different media for 10 h at 80 °C.

Degradation medium	Compound	Initial concentration (μg ml^{-1})	Found concentration (μg ml^{-1})	Recovery (%)
0.1M HCl	CZ	491	339	69.0
	PP	101	0	0.0
	BP	99	0	0.0
0.1M NaOH	CZ	502	501	99.8
	PP	101	62	61.4
	BP	102	58	56.9
1% H_2O_2	CZ	501	26	5.2
	PP	96	37	38.5
	BP	101	37	36.6

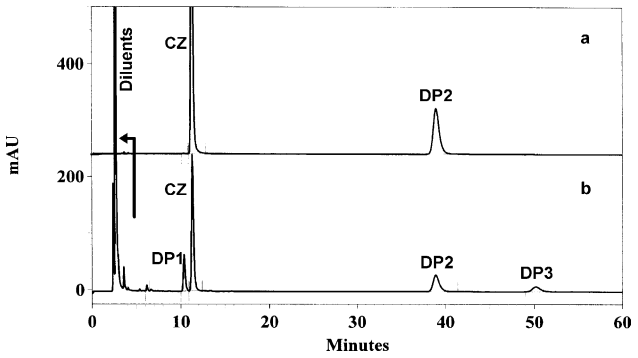

Fig. 3. HPLC chromatograms for CZ degradation in: (a) 0.1 M HCl and (b) 1% H_2O_2 solutions at 80 °C for 10 h.

3.3. Linearity and accuracy

The linearity of calibration curves was tested for the determination of CZ, CZ related impurities and preservatives at five concentration levels within the ranges of the target concentrations of each of them, namely, 2×10^2–8×10^2 μg ml^{-1}, 1–4 μg ml^{-1} and 20–100 μg ml^{-1}, respectively. The linear regression parameters (correlation coefficient, slope, intercept, 95% confidence intervals of the slope and of the intercept) were estimated and reported in Table 2. The linearity of the curves was better than 0.998.

The LOD and LOQ have been estimated from the calibration curves of CZ and its related compounds as three and ten times of the noise level for LOD and LOQ, respectively [22]. The values of LOD and LOQ for CZ were 0.10 and 0.34 μg ml^{-1}, respectively. However, the LOD and LOQ values for CZ impurities were in the ranges of 0.08–0.26 μg ml^{-1} and 0.28–0.86 μg ml^{-1}, respectively (Table 2).

The accuracy of the method was tested at three concentration levels within each analyte target concentration and each concentration level was analyzed by three different analysts. The average percent recoveries, R.S.D. and bias were, respectively, in the ranges of 98.0–99.9, 0.9–1.8 and −4.0 to 0.6 for CZ, 98.4–98.9, 0.9 and −2.4 to −0.2 for the PP and BP, and 92.1–108.3, 1.2–7.2 and −10.3 to 15.5 for CZ related impurities (Tables 3 and 4).

Table 3
Accuracy of the method for CZ and the preservatives.

Compound	Quantity (μg ml^{-1})		Recovery (%)	Bias[b] (%)
	Added	Found[a]		
CZ (solution's matrix)	250.0	247.2	98.9	−1.1
	500.0	496.0	99.2	−0.8
	750.0	720.3	96.0	−4.0
Average ± R.S.D.			98.0 ± 1.8	
CZ (tablet's matrix)	300.0	301.7	100.6	0.6
	500.0	500.7	100.1	0.1
	700.0	694.0	98.9	−1.1
Average ± R.S.D.			99.9 ± 0.9	
PP	20.0	19.6	98.2	−1.8
	40.0	39.6	98.9	−1.1
	60.0	59.9	99.8	-0.2
Average ± R.S.D.			98.9 ± 0.9	
BP	20.0	19.6	98.4	−1.6
	40.0	39.7	99.3	−0.7
	60.0	58.6	97.6	−2.4
Average ± R.S.D.			98.4 ± 0.9	

[a] Average of three individual results.
[b] Bias = %recovery − 100.

3.4. Repeatability and intermediate precision

The short term precision for each component was demonstrated as R.S.D. for six analyses made by each analyst for CZ, preservatives and CZ related impurities in the solution's and the tablet's drug-matrices. The R.S.D. values found were in the ranges of 0.7–1.2, 1.2–2.4 and 0.7–4.5 for CZ, preservatives and related impurities, respectively (Table 5). The intermediate precision was determined as the R.S.D. of 12 analyses made by two independent analysts. The overall R.S.D.s for CZ, preservatives and CZ related impurities were found to be in the ranges of 1.0, 1.7–1.8 and 1.7–4.4, respectively.

Table 2
Linearity of the calibration plots for CZ, preservatives (PP and BP) and CZ related impurities.

Compound	Calibration range (μg ml^{-1})	r[a]	(Slope ± CI) ×10^{-4}	(Intercept ± CI) ×10^{-4}	Response factor, f[b]	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
CZ	1.2–3.8	0.9994	4.20 ± 0.25	−0.15 ± 0.65	1.0	0.10	0.34
CZ	194–795	0.9996	3.40 ± 0.10	−14.0 ± 66.0	–		
PP	21–100	0.9992	16.00 ± 1.18	3.3 ± 72.9	–		
BP	21–101	0.9991	14.90 ± 1.16	1.9 ± 72.6	–		
Impurities A	0.9–3.8	0.9996	5.09 ± 0.26	−0.08 ± 0.66	0.8	0.26	0.86
Impurities C	1.0–3.9	0.9978	2.45 ± 0.30	−0·32 ± 0.79	1.7	0.12	0.40
Impurities E	1.0–4.1	0.9996	3.28 ± 0.18	0.06 ± 0.50	1.2	0.08	0.28
Impurities F	1.0–3.9	0.9997	2.52 ± 0.10	0.03 ± 0.27	1.7	0.11	0.37
Impurities G	0.8–3.2	0.9994	15.10 ± 0.98	−0·53 ± 2.11	0.3	0.09	0.31
Impurities H	1.0–4.0	0.9997	6.45 ± 0.28	−0.25 ± 0.76	0.7	0.13	0.42
Impurities I	1.0–4.2	0.9995	2.32 ± 0.14	−0.6 ± 0.38	1.8	0.10	0.34

[a] r: Correlation coefficient, CI: 95% confidence interval
[b] f: slope (CZ)/slope (compound).

Table 4
Accuracy of the method for CZ related impurities.

Compound	Matrix for solutions				Matrix for tablets			
	Quantity (μg ml^{-1})		Recovery (%)	Bias (%)	Quantity (μg ml^{-1})		Recovery (%)	Bias[b] (%)
	Added	Found[a]			Added	Found[a]		
Impurities A	1.20	1.18	98.1	−1.9	1.25	1.24	98.9	−1.1
	2.40	2.40	100	0.0	2.40	2.41	100.3	0.3
	3.60	3.46	96.2	−3.8	3.35	3.24	96.8	−3.2
Average ± R.S.D.			98.1 ± 1.9	–	–	–	98.7 ± 1.8	
Impurities C	1.23	1.16	94.6	−5.4	1.25	1.44	115.5	15.5
	2.46	2.49	101.3	1.3	2.46	2.69	109.3	9.3
	3.69	3.59	97.2	−2.8	2.93	2.93	100.0	0.0
Average ± R.S.D.			97.7 ± 3.4				108.3 ± 7.2	
Impurities E	1.29	1.30	100.1	0.1	1.25	1.25	100.3	0.3
	2.58	2.62	101.7	1.7	2.58	2.59	100.3	0.3
	3.87	3.82	98.8	−1.2	2.86	2.81	98.0	−2.0
Average ± R.S.D.			100.2 ± 1.5				99.5 ± 1.3	
Impurities F	1.20	1.27	106.1	6.1	1.25	1.21	96.8	−3.2
	2.40	2.49	103.6	3.6	2.40	2.38	99.2	−0.8
	3.60	3.66	101.6	1.6	2.98	2.87	96.2	−3.8
Average ± R.S.D.			103.8 ± 2.2				97.4 ± 1.6	
Impurities G	1.21	1.19	98.1	−1.9	1.25	1.23	98.1	−1.9
	2.42	2.44	100.8	0.8	2.42	2.42	100.0	0.0
	3.63	3.53	97.2	−2.8	3.88	3.73	96.2	−3.8
Average ± R.S.D.			98.7 ± 1.9				98.1 ± 9	
Impurities H	1.25	1.17	93.6	−6.4	1.25	1.21	96.5	−3.5
	2.50	2.50	99.9	−0.1	2.50	2.47	98.8	−1.2
	3.75	3.49	93.2	−6.8	3.20	3.10	97.0	−3.0
Average ± R.S.D.			95.6 ± 3.9				97.4 ± 1.2	
Impurities I	1.30	1.21	93.1	−6.9	1.25	1.32	105.6	5.6
	2.60	2.43	93.5	−6.5	2.60	2.56	98.5	−1.5
	3.90	3.50	89.7	−10.3	3.23	3.47	101.2	1.2
Average ± R.S.D.			92.1 ± 2.3				101.8 ± 3.5	

[a] Average of three individual results.
[b] Bias = %recovery − 100.

3.5. Stability of solutions and robustness

The stability of the solutions of CZ, preservatives and CZ related impurities dissolved in the mobile phase and in the absence (standard preparation) or the presence of the drug-matrices (matrices for solutions and for tablets formulations) were tested over a period of 24 h. The freshly prepared and stored samples were analyzed and the results are reported in

Table 5
Precisions results for CZ, the preservatives (PP and BP), and CZ related impurities.

	Amount taken (μg ml^{-1})	Matrix for solution			Matrix for tablets		
		Analyst I	Analyst II	Overall %R.S.D. (n = 12)	Analyst I	Analyst II	Overall %R.S.D. (n = 12)
		Amount found[a] (μg ml^{-1})	Amount found[a] (μg ml^{-1})		Amount found[a] (μg ml^{-1})	Amount found[a] (μg ml^{-1})	
CZ	500.0	498.5 (0.7)	493.2 (1.1)	0.97	496.8 (1.2)	499.0 (0.8)	1.0
PP	40.0	39.9 (2.4)	39.9 (1.2)	1.8	–	–	–
BP	40.0	39.9 (2.3)	39.9 (1.3)	1.7	–	–	–
Impurities A	2.40	2.32 (3.8)	2.35 (2.2)	3.0	2.34 (2.3)	2.41 (0.7)	2.3
Impurities C	2.46	2.60 (4.1)	2.46 (2.6)	4.4	2.59 (2.7)	2.73 (0.8)	3.3
Impurities E	2.58	2.46 (4.2)	2.52 (1.7)	3.3	2.53 (1.7)	2.58 (0.7)	1.7
Impurities F	2.40	2.41 (1.6)	2.29 (4.3)	4.0	2.33 (1.7)	2.39 (0.8)	1.9
Impurities G	2.42	2.35 (2.3)	2.38 (1.9)	2.1	2.37 (1.9)	2.42 (0.7)	1.8
Impurities H	2.50	2.41 (3.3)	2.50 (1.7)	3.2	2.41 (3.7)	2.47 (3.1)	3.5
Impurities I	2.60	2.43 (2.8)	2.53 (4.5)	4.2	2.56 (2.4)	2.46 (3.7)	3.6

[a] Each reported quantity is the average of 6 measurements and the values in parenthesis are the percentage R.S.D. for the six measurements.

Table 6
Stability of solutions of CZ, preservatives (PP and BP) and CZ related impurities.

Compound	Quantity added ($\mu g\ ml^{-1}$)	Standard preparations			Matrix for solution			Matrix for tablets		
	Quantity found[a] ($\mu g\ ml^{-1}$)			%D	Quantity found[a] ($\mu g\ ml^{-1}$)		%D	Quantity found[a] ($\mu g\ ml^{-1}$)		%D
		Fresh solution	Stored solution		Fresh solution	Stored solution		Fresh solution	Stored solution	
CZ	500.0	495.2	498.9	−0.7	492.6	496.7	−0.8	498.3	495.0	0.7
PP	40.4	40.4	40.7	−0.7	40.8	41.3	−1.2			
BP	40.9	40.9	41.2	−0.7	41.4	42.2	−1.9			
Impurities A	2.40	2.40	2.29	4.6	2.40	2.2	5.4	2.40	2.36	1.7
Impurities C	2.46	2.47	2.35	4.9	2.55	2.31	9.4	2.54	2.43	4.3
Impurities E	2.58	2.58	2.49	3.5	2.63	2.54	3.4	2.57	2.55	0.8
Impurities F	2.42	2.35	2.19	6.8	2.55	2.49	2.4	2.38	2.37	0.4
Impurities G	2.42	2.42	2.34	3.3	2.44	2.32	4.9	2.41	2.39	0.8
Impurities H	2.50	2.50	2.38	4.8	2.49	2.38	4.4	2.52	2.38	5.6
Impurities I	2.60	2.57	2.56	0.4	2.46	2.45	0.4	2.42	2.40	0.8

[a] Each quantity is the average of 3 measurements.

Table 6. The percent differences in concentrations observed for CZ, preservatives and CZ related impurities were in the ranges of −0.8 to 0.7, −1.9 to −0.7 and 0.4 to 9.4, respectively. This indicates the possibility of using all analyte solutions in either standard or synthetic drug-matrices over a period of 24 h without degradation.

The optimum HPLC parameters set for this method were slightly changed for samples of CZ ($500\ \mu g ml^{-1}$), preservatives ($40\ \mu g ml^{-1}$ each), and CZ related impurities ($2.5\ \mu g ml^{-1}$ each) prepared in the presence of the drug-matrix for solution's formulations. The parameters include: flow rate, mobile phase ratio, pH, column age (old or new), wavelength of detection, filtration system and sonication time. Percent recoveries of CZ, preservatives and CZ related impurities obtained (Table 7) under the various conditions were within 95–101%, 93–100% and 88–112%, respectively. These results indicate the ability of the method to remain unaffected by small changes in the method's parameters, thus the method is considered robust.

Table 7
Robustness results for CZ, preservatives (PP and BP) and CZ related impurities

Parameter	Condition	CZ	PP	BP	Impurities A	Impurities C	Impurities E	Impurities F	Impurities G	ImpuritiesH	Impurities I
Flow rate	0.8 ml/min	98.9	97.8	97.6	88.9	91.6	95.0	89.0	90.5	100.8	101.0
	1.0 ml/min	98.8	99.8	98.5	99.0	95.5	97.4	99.0	97.9	102.0	103.0
	1.2 ml/min	98.4	98.6	97.9	87.1	92.2	94.8	88.0	90.5	103.3	99.8
Mobile phase Ratio	550:300:105:45	94.8	95.8	98.0	94.6	97.3	112.3	90.3	95.6	92.6	99.1
	600:250:100:50	98.8	99.9	98.6	99.0	95.5	97.4	99.0	97.9	102.0	103.0
	650:200:95:55	95.1	94.9	94.7	92.7	95.1	96.1	94.4	96.7	90.3	89.3
pH	5.3	94.3	95.3	92.7	95.3	97.6	93.3	92.0	98.0	92.4	99.7
	5.5	98.8	99.8	98.6	99.0	95.5	97.4	99.0	97.9	102.0	103.0
	5.7	96.2	96.9	95.5	96.1	101.7	96.4	99.5	96.4	98.0	101.3
Column type	New	99.1	98.9	99.5	99.0	95.5	97.7	99.0	97.9	102.0	103.0
	Old	99.5	98.6	98.2	91.0	102.6	93.1	91.7	104.3	92.8	89.3
Wavelength (nm)	225	95.7	97.3	96.0	100.5	91.0	95.9	100.7	96.7	95.2	107.7
	230	98.8	99.8	98.6	99.0	95.5	97.4	99.0	97.9	102.0	103.0
	235	98.4	99.7	97.1	97.6	93.3	96.7	96.4	97.9	98.1	101.6
Filtration system	Centrifuge	100.1	–	–	101.0	111.0	100.8	100.2	100.8	95.4	98.3
	Nylon filter	100.4	–	–	100.4	111.9	101.4	101.2	100.8	95.7	101.0
Sonication time (min)	10	100.4	–	–	100.8	110.1	100.6	99.8	100.3	96.6	97.7
	15	100.1	–	–	101.0	111.0	100.8	100.2	100.8	95.4	98.3
	20	100.8	–	–	99.4	108.7	99.4	100.0	99.4	96.2	95.7

Table 8
Analysis of various CZ commercial pharmaceutical preparations.

Sample	%Assay					
	CZ	PP	BP	Impurities E	Impurities F	Impurities G
Cetolerg 5 mg tablets	100.3	–	–	0.03	0.02	0.11
	104.4	–	–	0.03	0.02	0.12
	103.0	–	–	0.03	0.02	0.11
Cetolerg 1 mg ml^{-1} solution	99.5	98.5	100.1	0.07	–	–
	99.6	98.8	99.1	0.06	–	–
	97.3	99.2	99.1	0.06	–	–
Zyertic 10 mg tablets	99.3	–	–	0.05	–	0.13
Zyertic 1 mg ml^{-1} solution	99.1	–	–	–	–	0.13

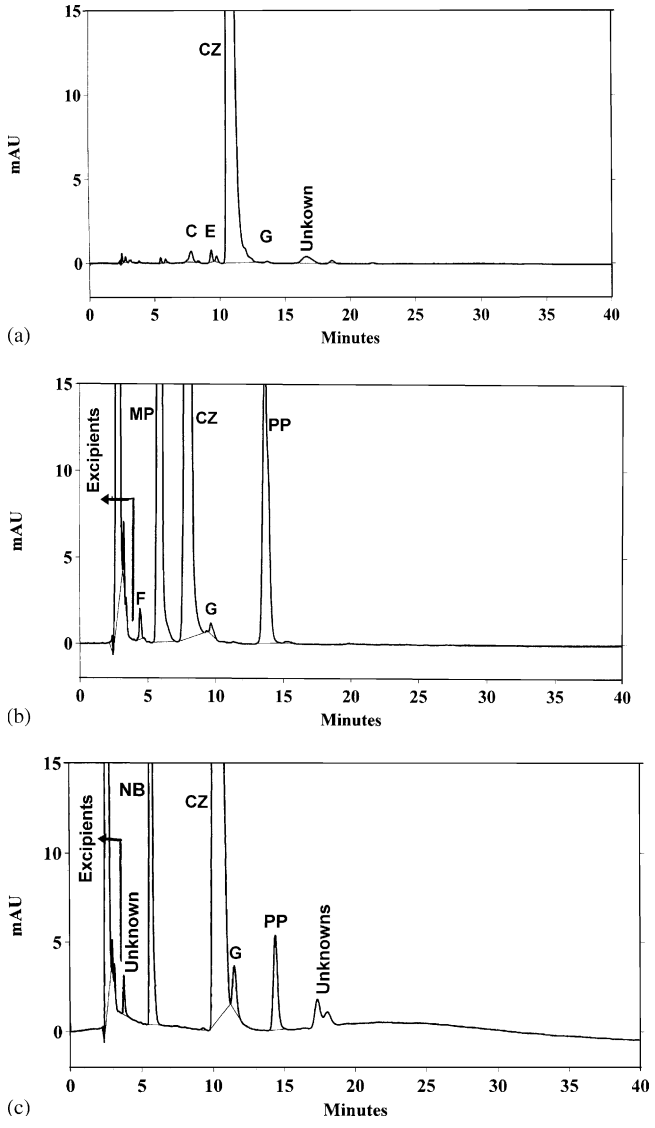

Fig. 4. HPLC chromatograms for: (a) Zyrtec 10 tablet, (b) Zyrtec 1 mg ml^{-1} solution and (c) Zyrtec 10 mg ml^{-1} solution.

3.6. Application

The JPM commercial dosage forms (Cetolerg solutions and tablets) and the originator products (Zyertic) were tested using the proposed method. Table 8 shows that the interference of excipients in the tablets dosage form is insignificant. For the JPM solution product, the method was capable (Table 8) to differentiate between CZ, its related impurities and the preservatives (PP and BP). Furthermore, the method successfully resolved two more preservatives, methlyparaben (MP) and sodium benzoate (NB) used in Zyertic solutions (Fig. 4b,c). These two preservatives can be quantified if their quantities are labelled. Further optimization is required prior the usage of the method, for determination of impurities E and F in Zyertic solutions as the two preservatives may interfere with these two impurities.

4. Conclusion

A new HPLC method is proposed for simultaneous determination of CZ, the preservatives (PP and BP) and seven of the synthetic impurities in solution and solid dosage forms. This method would be an alternative to the European pharmacopoeial method where the mobile phase of pH <0.5 would cause column deterioration after few runs leading to irreproducible results. Also, the European method showed relatively low resolution between impurities A, F, H, and I; in the meantime, impurities B and D were obscured here. The method was robust and showed good selectivity thus it could be used as a stability indicating for the assay of CZ. All statistical values (percentage recoveries, R.S.D., percentage difference, confidence limits of the slope and intercept, LOD and LOQ) were within the acceptable limits. Due to the presence of different interferents in solution formulations such as colors, flavors, sweetening agents (e.g. saccharin) and preservatives other than PP and BP, the proposed method should be re-evaluated prior usage for commercial solution products containing excipients other than those used in this work.

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