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Development and Validation of a Stability-Indicating HPLC Method for Determination of Clorazepate Dipotassium and Its Main Impurities in Bulk Drug and Capsules

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Abstract

A simple isocratic stability-indicating HPLC method was developed and validated for determination of clorazepate dipotassium in the presence of its main impurities; nordiazepam and 2-amino-5-chlorobenzophenone, in bulk drug and capsules. The chromatographic analysis was performed on a Zorbax Eclipse XDB-C18 column (75 mm x 4.6 mm i.d., 3.5 μm) using a mobile phase consisting of 5 mM ammonium formate in methanol and 5 mM ammonium formate in water (65: 35, v/v) at a flow rate of 0.7 mL/min and UV detection at 230 nm. The forced degradation studies were performed under various conditions according to the ICH guidelines. The degradation products from the studies were investigated by HPLC and, later, by tandem LC-MS. The validation tests including specificity, linearity, accuracy, precision, LOD and LOQ were performed. The calibration curves of the drug and the two related substances were linear in the concentration of 2 to 100 μg/mL (r^2 = 0.9990), 2-50 μg/mL (r^2 = 0.9995) and 0.4-25 μg/mL (r^2 = 0.9993), respectively. The proposed method was proven to be suitable for the quantitative determination and stability studies of clorazepate dipotassium in bulk drug and capsules.

Keywords: clorazepate dipotassium, capsules, forced degradation, isocratic HPLC, LC-MS, stability-indicating

Introduction

Clorazepate dipotassium (CZP), (3*RS*)-7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepine-3-carboxylate compound with potassium hydroxide (1:1), is a soluble prodrug or drug precursor of long-acting benzodiazepine derivatives (Figure 1). It is utilized for the treatment of anxiety disorders, insomnia and alcohol withdrawal as well as adjunctive therapy in the management of epilepsy (partial seizures). Shortly after orally administrated, CZP is degraded into an active non-polar compound, *N*-desmethyldiazepam (NDZP) or nordiazepam (7-chloro-5-phenyl-1, 3-dihydro-2*H*-1, 4-benzodiazepin-2-one) by losing one water and one CO_2 molecule [1-3]. The acidic condition in the stomach makes CZP and NDZP further degraded into 2-amino-5-chlorobenzophenone ((2-amino-5-chlorophenyl) phenyl-methanone or ACB) and glycine [2]. The British Pharmacopoeia (BP) and the United States Pharmacopeia (USP) enlist ACB and NDZP (Figure 1) as impurities A and B in the monograph and require the limit testing for

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both of them [4,5]. Titration and TLC procedures are described in BP for the assay of an active pharmaceutical ingredient (API) and impurities only in bulk drug, respectively [4] whereas the USP employs titration and HPLC method for the determination of API in bulk form and pharmaceutical dosage form respectively. Even though HPLC is used for the determination of impurities; however, 3 different conditions are required for CZP and each impurity [5]. These are complicated, time-consuming and produced a lot of chemical waste.

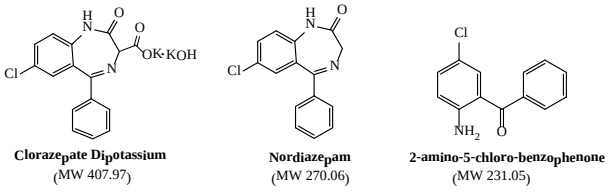

Figure 1 Chemical structures and the exact mass of clorazepate dipotassium and its impurities: nordiazepam and 2-amino-5-chlorobenzophenone.

The non-pharmacopoeial method for the assay of CZP in bulk powder and dosage form has been reported including HPLC [6-7] and differential pulse polarographic method [8,9]. Though a stability-indicating HPLC method has been reported, only ACB was studied [7]. The quantitative assay of CZP in the presence of its degradation products was performed by secondary derivative UV spectrophotometric [10-12] and spectrodensitometric method [13] which involved extraction and derivatization procedure. A-bio-analytical method for the determination of CZP and other benzodiazepines in whole blood, serum and urine by LC, LC-MS and GC-MS was reported as well [14-17]. However, these were non-stability-indicating techniques. This work was conducted in order to develop a selective and validate a stability-indicating HPLC-UV-MS method for the determination of CZP in bulk and capsule formulation after performing stress studies under a variety of ICH recommended test conditions [18]. In addition, the proposed method could be applied to monitor the presence of ACB and NDZP in CZP bulk and capsule formulation in one chromatographic run.

Materials and Methods

Chemicals and Reagents

Reference and working standard of CZP and its two impurities; NDZP and ACB, were kindly supported by Bureau of Drug and Narcotic, Department of Medical Sciences, Ministry of Public Health, Thailand. Two batches of a local commercially available capsule formulation used were labeled to contain 5 mg of CZP. The HPLC-grade methanol and ammonium formate (99.99%) were purchased from Merck (Darmstadt, Germany). Other chemicals used were of analytical grade. High purity water was prepared by using Milli-Q RO system (Millipore, Bedford, MA, USA).

HPLC-DAD and LC-MS Conditions

The HPLC system consisted of an Agilent 1100 series pump, an on-line solvent degasser, an autosampler, a photodiode-array detector (DAD) and a Chemstation software version A.08.01 (Agilent Technologies, USA). A reversed-phase column, 75 mm x 4.6 mm packed with 3.5 μm, Zorbax Eclipes XDB-C18 modified silica (Agilent Technologies, USA) and a guard column, 20 mm x 3.9 mm packed with 5 μm, C18 were used (Agilent Technologies, USA). The assay system was operated at ambient temperature. The separation was carried out under isocratic elution with 5 mM ammonium formate in methanol: 5 mM ammonium formate in water (pH 5) in the ratio of 65:35 (v/v). The flow rate was 0.7 mL/min and the wavelength was monitored at 230 nm. The injection volume was 5 μL. The forced degradation samples were analyzed using a DAD detector in scan mode covering the range of 200-400 nm.

To characterize the degradation products, all samples from the forced degradation studies were subjected to a HPLC system (as described above) coupled to an Agilent G2445D LC/MSD Trap SL mass spectrometer which operated using MSD Trap software version 4.0 LC-MS. The column and the mobile phase as described above were used, but the flow rate was reduced to 0.4 mL/min. Positive electron spray ionization (ESI) mode was used with mass/charge (*m/z*) ratio in the range of 50-3000 *m/z*. The probe voltage was set to 7.0 kV, the capillary voltage was at 3,500 V, the gas temperature was 325°C, and the nebulizer gas flow was 8.0 L/min.

Preparation of Stock and Standard Solutions

A stock solution of CZP in water was prepared at a concentration of 1 mg/mL. The solution vial was covered with aluminum foil to protect it from light and stored at 4°C and found to be stable over one week. Working aqueous standards were prepared from a stock by the appropriate dilution at 2, 5, 10, 25, 40, 50, 60 and 100 μg/mL. Two standard stock solutions of impurities; NDZP and ACB, were prepared individually at a concentration of 1.0 mg/mL using water as solvent. A combined standard solution containing 50 μg/mL of CZP, 25 μg/mL of NDZP and 10 μg/mL of ACB was prepared in water for a specificity test.

Preparation of Sample Solutions

The contents of twenty capsules were weighed, mixed well, and an aliquot of powder equivalent to the weight of one capsule was accurately weighed into a 100 mL volumetric flask and added a part of water. After sonication for 15 min, the mixture was made up to volume with water and then filtered. Suitable aliquot of the filtrate was further diluted to yield starting concentration of 50 μg/mL and filtered through a 0.22 μm nylon filter.

Forced Degradation Conditions

Stress degradation studies of CZP were carried out under the conditions of acid and base hydrolysis, oxidation and photolysis. Each degradation stress was performed in an aqueous solution of the drug at the

concentration of 1 mg/mL. The experimental vials were wrapped in aluminum foil during the incubation in order to avoid the effect of light except photolysis. The samples from each stress experiment were taken at designated time, diluted to yield stated CZP concentration of about 50 μg/mL, filtered through a 0.22 μm membrane and analyzed by HPLC along with a non-stressed sample. Each stress studies were performed in triplicates.

The hydrolytic degradation studies were performed in water, acidic and basic media at two different temperatures, at room temperature and 80 °C (in a heat block). The acidic hydrolysis was carried out in 0.01- 0.1 N HCl solution while the basic degradation study was performed in 0.01- 0.1 N NaOH media. The samples at a designed time were taken out from the experimental vial and adjusted the pH to 5 with 0.01-0.1 N NaOH or 0.01-0.1 N HCl (as desirable). For oxidative stress studies, the samples were treated with 0.3-3.0% (v/v) H_2O_2 at room temperature and 80 °C (in a heat block). Susceptibility of the drug to light was studied. Approximately 100 mg of active pharmaceutical ingredient powder of CZP was spread on a glass dish in a layer that was less than 2 mm in thickness. The samples were exposed to natural sunlight for 4 days, weighed and prepared as previously described.

Results and Discussion

Development and Optimization of the Method

One of the major aims for this study was to develop the HPLC method that could determine CZP and its main degradation products concomitantly. The condition from the drug assay monograph in the USP 29 was used as a starting point for the assay development. According to the chromatogram from the USP assay, peaks of both CZP and NDZP were broad and asymmetry. In addition, the phosphate buffer using in the USP assay could cause the ion suppression and salt precipitation in the MS ion source. To avoid the MS problem, a volatilable salt as ammonium formate was employed. Moreover, methanol was used as an alternative solvent since an ammonium formate was insoluble in acetonitrile. An isocratic system with a proportion of 35:65 (v/v) 5 mM ammonium formate in water: 5 mM ammonium formate in methanol provided a good resolution and a good peak shape. With the optimized system, the peaks of CZP, NDZP and ACB were observed at 1.8, 4.6 and 8.5 min, respectively (Figure 2). The overlaid DAD showed good UV absorbance of all monitored compounds at 230 nm and this wavelength has been chosen for detection. To verify the separation ability of the developed assay condition, it was tested on the bulk drug and capsules and the assay results are shown in Figure 2(B) and (C), respectively. A minor peak of NDZP was observed in the chromatograms of the drug and the commercial sample. Additionally, no excipient interference was observed under this assay condition.

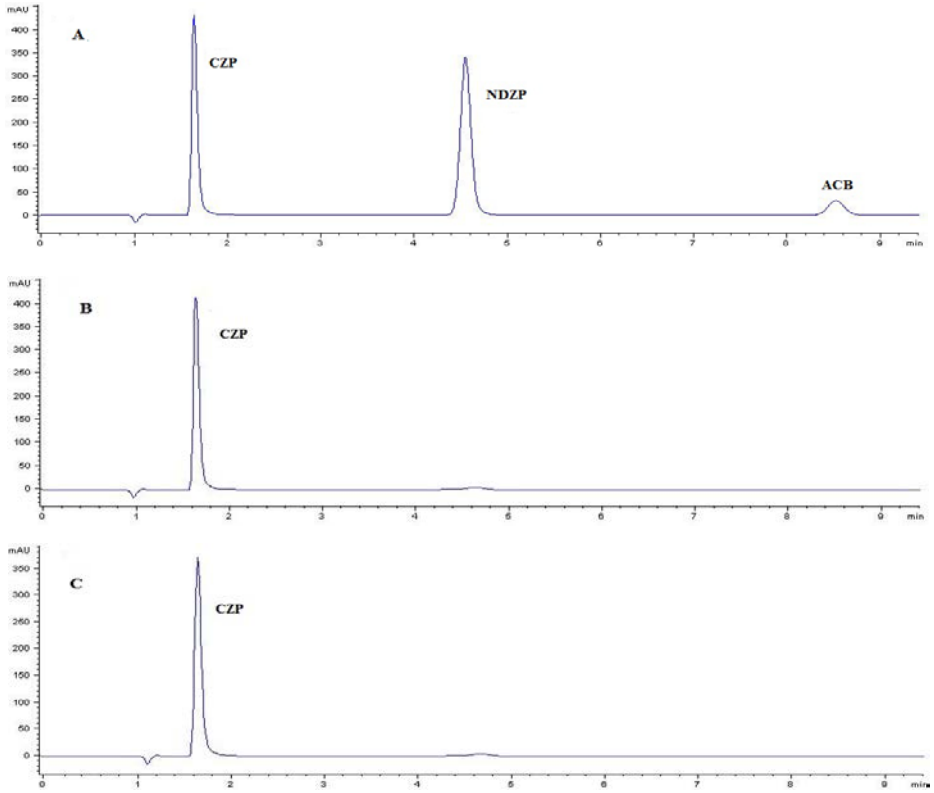

Figure 2 Typical HPLC chromatograms of (A) a standard mixture solution of CZP, NDZP and ACB (B) CZP bulk drug and (C) CZP capsules. All CZP solutions were prepared at a concentration of 50 μg/mL

Table 1 System suitability parameter.

Parameter	k′	α	Rs	N	USP tailing factor
clorazepate dipotassium	3.95			3,539	1.23
nordiazepam	12.23	3.10	17.78	7,835	1.11
2-amino-5-chlorobenzophenone	24.94	2.04	15.36	9,672	1.06
Preferable levels			> 2	> 2,500	< 1.5

The system suitability parameters listed in Table 1 was established by ten replicates. All parameters were satisfactory with good specificity for the stability assessment of CZP, NDZP and ACB. In addition, the drug was also completely separated from all forced gradation products with the satisfactory resolution greater than 2.

HPLC Results of Forced Degradation Studies

Forced degradation was performed to provide the stability-indicating properties. The chromatograms obtained from the stressed samples are illustrated in Figure 3. The degradation products were well separated from their parent drug. This result could verify the stability indicating power of the developed method. The retention times of CZP and its degradation products from each stress condition are summarized in Table 2.

Table 2 Forced degradation studies of CZP

Degradation mode	Conditions	% Area of CZP	Degradation products found	Retention time (min)
Control	none	99.90	NDZP	4.6
Hydrolysis				
Neutral hydrolysis	water , RT, 24 h	93.73	NDZP	4.6
	water , 80°C, 10 min	68.40	NDZP	4.6
	water , 80°C, 30 min	13.52	NDZP	4.6
Alkaline hydrolysis	0.1 N NaOH, RT, 24 h	95.21	NDZP	4.6
	0.1 N NaOH, 80°C, 10 min	72.71	NDZP	4.6
	0.1 N NaOH, 80°C, 30 min	34.49	Unknown I	2.8
			NDZP	4.6
			Unknown II	9.1
			ACB	8.5
Acid hydrolysis	0.01 N HCl, RT, 10 min	11.80	NDZP	4.6
	0.01 N HCl, RT, 30 min	0.00	NDZP	4.6
	0.01 N HCl, 80°C, 5 min	0.00	NDZP	4.6
	0.01 N HCl, 80°C, 30 min	0.00	Unknown I	2.8
			NDZP	4.6
	0.01 N HCl, 80°C, 120 min	0.00	Unknown I	2.8
			NDZP	4.6
			ACB	8.5
Oxidation	3% H_2O_2, RT, 24 h	87.44	NDZP	4.6
	3% H_2O_2, 80°C, 10 min	73.65	NDZP	4.6
	3% H_2O_2, 80°C, 30 min	14.80	NDZP	4.6
Photolysis (solid state)	Sunlight, 48 hr	0.00	NDZP	4.6

RT= room temperature

The data suggested that NDZP was the main degradation product from all of the stress conditions. The results were confirmed by the mass spectra on LC-MS. The retention time, ion mass and major fragments of CZP and its degradation products are listed in Table 3.

Table 3 LC-MS data for identification of CZP and its degradation products under m/z value for $[M+H]^+$.

Compound	LC Retention time (min)	LC-MS/MS Retention time (min)	MS	MS/MS fragment ions
CZP	1.8	2.8	315.0	271.1
			271.0	254.0, 243.0, 236.0, 226.0, 208.0, 193.0, 165.0 and 140.0*
NDZP	4.6	7.9	271.1	243.0, 226.0, 208.0, 193.0, 165.0 and 140.0*
ACB	8.5	14.9	232.0	185.9, 153.9* and 126.0
Unknown I	2.8	5.9	289.0	244.0, 232.0*, 216.1 and 154.0
Unknown II	9.1	15.3	271.2	253.9, 236.1 and 226.0*

* Most abundance MS/MS fragmentions

Due to the fragileness of the ester bond in the CZP structure, there were two ions at m/z value of 271.1 and 315.1 under the peak of CZP (MW 407.97) at 2.8 min. Regarding to the fragmentation outcome from the ms/ms experiment, the first ion was the $[M+H]^+$ of NDZP (MW 270.71) which was occurred from the thermal degradation of CZP within the ionization chamber. The other ion was a molecular ion peak of the acid form of CZP. Two potassium ions in the form of K and KOH in the CZP structure were replaced by proton (Figure 4(A)). According to the results from the stress studies, four degradation products of CZP were observed. The main degradation product found in every stress sample at 7.9 min was NDZP with 271.0 m/z value of $[M+H]^+$ (Figure 4(B)). ACB is shown at 14.9 min with 232.0 m/z value (Figure 4(C)). The other two unknown degradation products were appeared at 5.9 min with m/z 289.0 and 15.3 min with m/z 271.2, respectively (Figure 4(D) and (E).

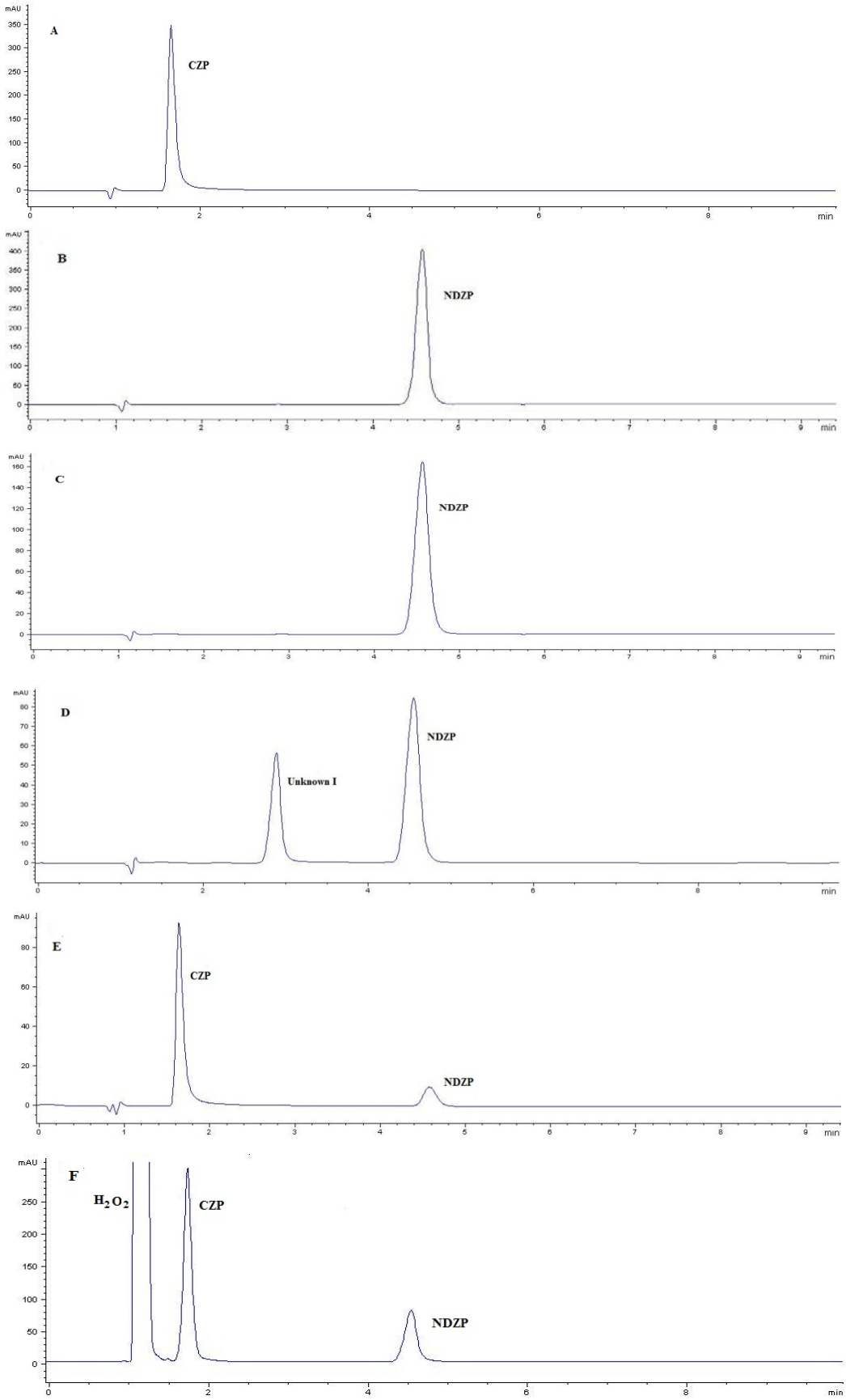

Figure 3 HPLC chromatograms of (A) CZP in water at room temperature at initial (B) acid hydrolysis-degraded CZP at room temperature for 30 min (C) acid hydrolysis-degraded CZP at 80°C for 5 min (D) acid hydrolysis-degraded CZP at 80°C for 30 min. (E) base hydrolysis-degraded CZP at 80°C for 5 min and (F) oxidative-degraded CZP at 80°C for 10 min.

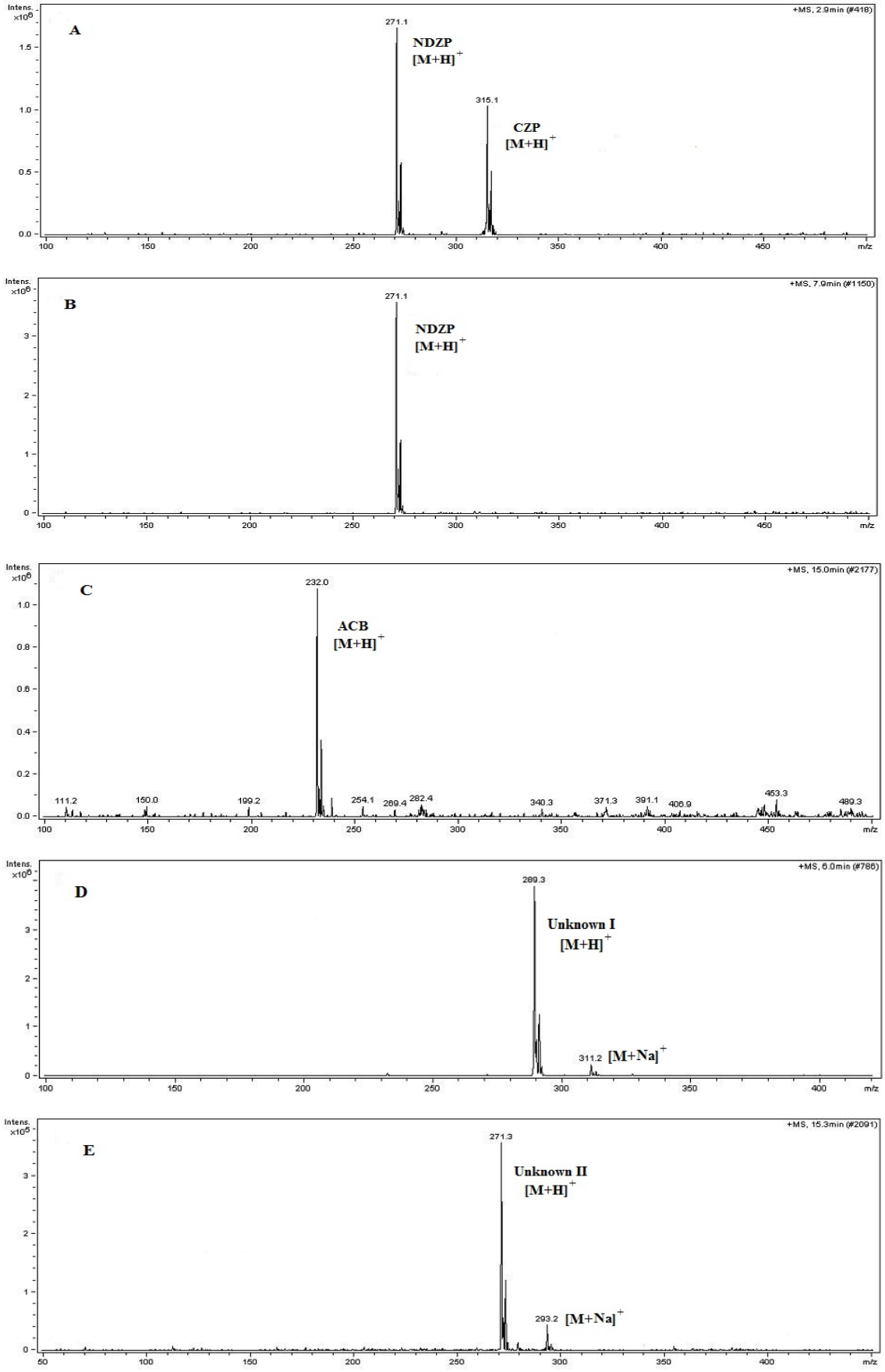

Figure 4 MS profiles of (A) CZP at Rt 2.8 min, (B) NDZP at Rt 7.8 min, (C) ACB at Rt 14.9 min, (D) unknown I at Rt 5.9 min and (E) unknown II at Rt 15.3 min.

Acidic Condition

CZP was found to be very labile in acid. It completely degraded to NDZP immediately after put in 0.1 N HCl. To be able to monitor the degradation behavior of the drug in acid media, the acid strength of the media was reduced for 10 times. At room temperature, less than 12% was left after kept in 0.01 N HCl for 10 min and total drug was disappeared within 30 min (Figure 3(B)). NDZP was the only degradation product produced. When the temperature was raised up to 80°C, the degradation of the drug increased dramatically. Total drug turned into NDZP within the first five min of incubation (Figure 3(C)). After 60 min of incubation, three peaks were observed. The first two peaks at 4.6 and 8.5 min corresponded to NDZP and ACB, respectively. The other peak appeared at 2.8 min (unknown I) (Figure 3(D)). Parallel LC-MS investigation, the loss of carbon dioxide from the molecule could be accelerated by heat and the proton in the acid medium. Therefore in 0.01 N HCl at 80°C at 5 min, only a peak of NDZP is shown (Figure 5(A) and (D)). A peak of unknown I with m/z value of 289.3 was observed after 30 min (Figure 5(C)). Increasing in the mass value for 18 amu (m/z 271.0→m/z 289.3) suggested an addition of a water molecule. With the longer exposure, the water adduct of NDZP was gradually turn into ACB (*m/z* value of 232.0) (Figure 5(B) and (E)). A loss of 57 amu suggested an addition of another water molecule and, concomitantly, an elimination of glycine (*m/z* value of 75) to get this degradation. The proposed acid hydrolysis degradation pathway is shown in Figure 6.

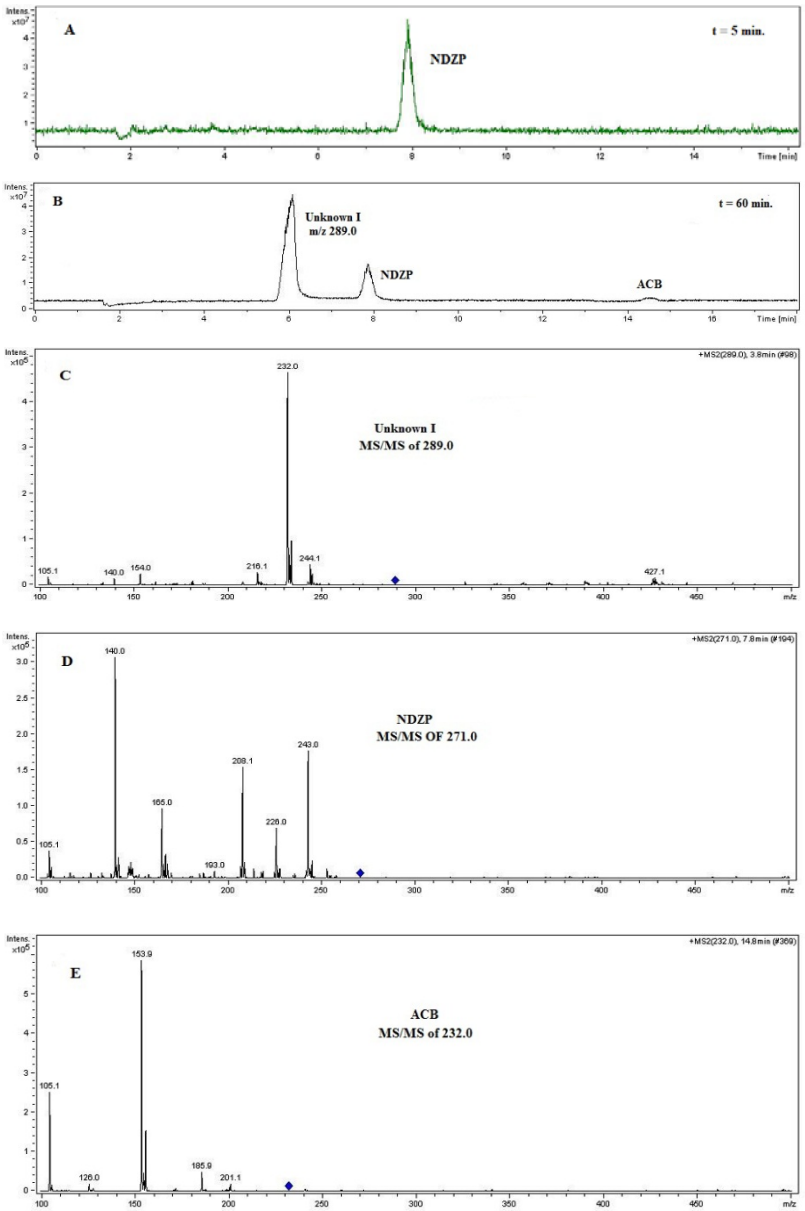

Figure 5 MS profiles for acid hydrolysis-degraded CZP at 80°C (A) at 5 min and (B) at 60 min and the MS/MS tandem fragmentation patterns for the daughter ions at (C) m/z 289 (unknown I), (D) m/z 271.0 NDZP and (E) m/z 232.0 of ACB

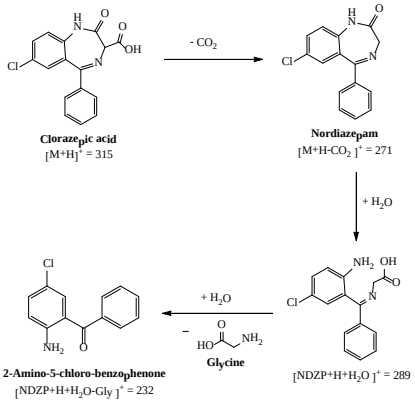

Figure 6 Proposed acid hydrolysis degradation pathway of CZP.

Alkali Condition

CZP was found to be more stable in alkali. 95% of the drug was still unchanged after storage at room temperature for 24 h. Even at 80°C, less than 30% of the drug turned into NDZP (Figure 3(E)). At 30 min of incubation, 43.2 % of the drug still left but four degradation peaks were observed. On the TIC chromatogram (Figure 7), three peaks at 5.9 min (m/z 289.3) of unknown I, 7.9 min (m/z 271.1) of NDZP, and 14.9 min (m/z 232.0) of ACB were already found in the chromatogram under the acid stress condition. An additional peak (unknown II) was arisen at 15.3 min (m/z 271.2) and had the same mass as NDZP dose. When they were fragmentized in the MS/MS experiment, NDZP (Figure 7(C)) and unknown II (Figure 7(E)) showed a different mass profile. NDZP produced six daughter ions at m/z 243.0 ($[MH-CO]^+$), 226.0 ($[MH-NHCO-H_2]^+$), 208.0 ($[MH-CO-Cl]^+$), 193.0 ($[MH-NHCO-Cl]^+$), 165.0 ($[MH-CO-Ph]^+$) and 140.00 ($[MH-CO-PhCN]^+$), while, unknown II had only three major ions at m/z 253.0 ($[MH-NH_2]^+$), 236.1 ($[MH-Cl]^+$) and 226.0 ($[MH-NHCO-H_2]^+$).

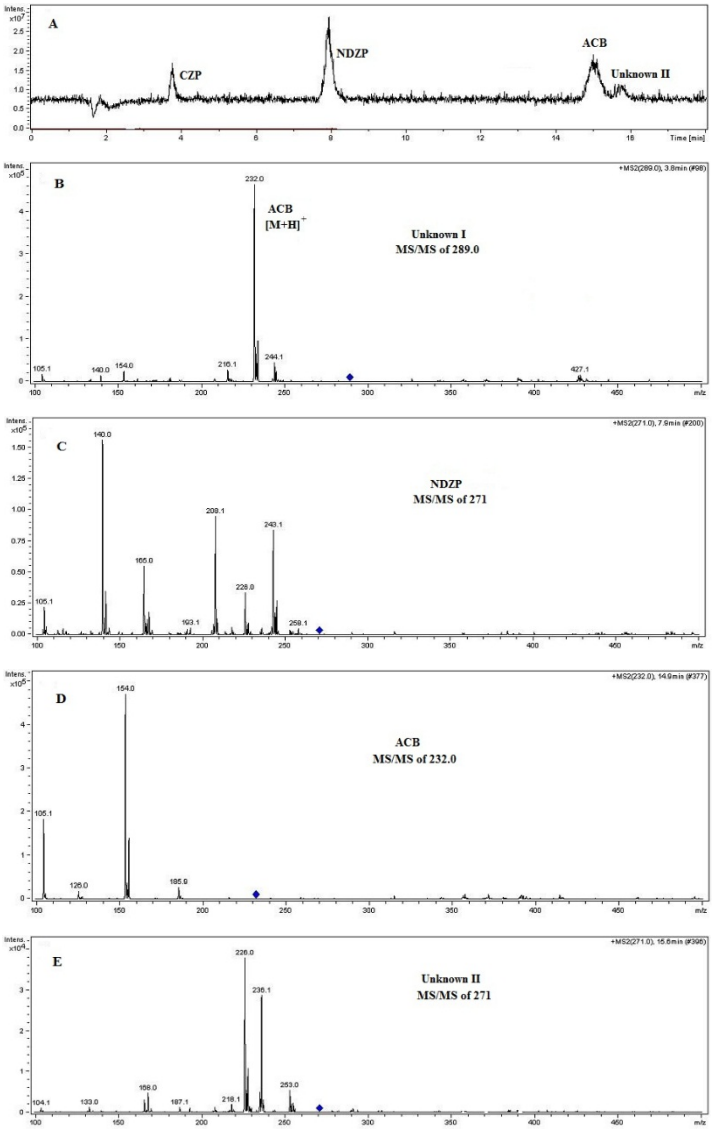

Figure 7 MS/MS tandem profiles for basic hydrolysis-degraded of CZP at 80°C for 60 min (A). Fragmentation patterns for the daughter ions; (B) m/z 289 (unknown I), (C) m/z 271.0 NDZP, (D) m/z 232.0 of ACB and (E) m/z 271.0 (unknown II).

Neutral Condition

10% of drug degraded to NDZP after 24 hours of storage at room temperature. At 80 °C, 70% and 10% of the drug were left after 10 and 30 min of incubation, respectively, leading to the formation of NDZP.

Oxidative Degradation Studies

The drug showed 12% degradation in 3% H_2O_2 at room temperature for 24 h, forming only NDZP and found 90% degradation of the drug at 80°C for 20 min (Figure 3(F)).

Photolytic Degradation Studies

Color of the drug powder was changed from white to off-white after exposed to sunlight over 4 days. The HPLC result showed that the drug was completely degraded to NDZP.

Validation of the HPLC Method

Specificity

The specificity of the method was established through the study of resolution factor of CZP peak from the nearest resolving peak. Peaks were identified with retention times compared with standards and confirmed the characteristic spectra by DAD in both sample and standard solutions. The peak purity value was greater than the threshold value of 995 and thus establishing the selectivity of the assay method. The mass detector also proved the mass purity for CZP, NDZP and ACB and thus confirmed the stability-indicating capacity of the developed method.

Linearity and Range

Linearity of system was established by analysis of eight different concentrations ranging from 2 to 100 µg/mL. Linear calibration plot for the related substances, NDZP and ACB were obtained at concentration range of 2-50 and 0.4-25 µg/mL, respectively. Triplicate injections were performed for each concentration and the peak area versus concentration data was calculated by least-squares regression method. The correlation coefficient for CZP, NDZP and ACB was 0.9990, 0.9995 and 0.9993, respectively, indicating good linearity. The linearity studies data are summarized in Table 4.

Table 4 Linearity, LOD and LOQ data.

Parameter	CZP	NDZP	ACB
Linearity			
Calibration range (µg/mL)	2-100	2-50	0.4-25
Regression equation			
Slope (*S*)	40.6206	69.7757	43.9667
Standard deviation of slope	0.1086	0.3972	0.2650
%RSD of slope	0.2673	0.5693	0.6028
Intercept	-35.2680	8.3809	-10.5870
Correlation coefficients (R^2)	0.9990	0.9995	0.9993
LOD (µg/mL)	0.18	0.07	0.05
LOQ (µg/mL)	0.62	0.24	0.18

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

The LOD and LOQ of CZP, NDZP and ACB were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The results given in Table 4 suggested that this method could be used for monitoring CZP's stability. The results also indicated sensitivity of the developed method for the degradation product determination at the low concentration.

Accuracy

The accuracy of the method was tested by determination of CZP in the solution prepared by standard addition method. Three known amounts of CZP ranging from 50 to 150% of the specification level were spiked in sample solutions. Three samples were prepared at each concentration. The recovery of added drug was calculated by comparing the peak area of the test samples with that of the standard solutions. The data obtained from 9 samples is summarized in Table 5. The result indicated that the proposed method was accurate and precise. Method accuracy or linearity of method, determined by plotting the amount of CZP found against the amount added over the range of 50-150 % of label amount, showed good linearity (y = 0.970x–0.85) with r^2 = 0.9990. The result was found to be satisfactory for intended purpose and was adequate for routine analysis.

Table 5 Accuracy results: recovery data of CZP.

Spiked concentration (µg/mL)	Measured concentration* (µg/mL)	%Recovery	%RSD
22.8	23.1	101.32	0.11
45.6	44.7	98.30	0.07
91.2	89.4	98.05	0.11
	Mean recovery (n=9)	99.38	1.79

* Mean value for three replicates of three different concentrations (n=9), and three injections for each replicate.

Precision

Precision was evaluated in term of repeatability (system precision), method precision and intermediate precision. To measure the repeatability, ten replicates (n=10) of the CZP standard solution and a sample solution at 100% of the target level were analyzed. The RSD% of peak area response and retention time was calculated and showed the satisfactory repeatability of the system (< 1%). Ten replicates (n = 10) of sample solutions were analyzed in the same day to determine method precision and intermediate precision was performed on three different days by preparing and analyzing in six replicates of separated sample solution at the same concentration level. The %RSD values for method precision and intermediate precision were 0.13 and 1.31, respectively. The low RSD (< 2%) showed the suitability of the method for the determination of CZP in capsules. The precision data is summarized in Table 6.

Table 6 Precision data.

		CZP	NDZP	ACB
System precision	(n = 10)	RSD (%)	RSD (%)	RSD (%)
Standard	Peak area	0.1069	0.0790	0.4506
	Retention time	0.0607	0.0548	0.0180
Sample	Peak area	0.0192	0.5157	Not found
	Retention time	0.0156	0.0240	Not found
		Actual Concentration (µg/mL)	Measured Concentration (µg/mL)	RSD (%)
Method precision	(n = 10)	50	49.17	0.13
Intermediate precision				
Day 1	(n = 6)	47.41	47.91	0.62
Day 2	(n = 6)	47.03	46.46	0.28
Day 3	(n = 6)	47.56	46.73	0.50
Total	(n = 18)	47.33	46.33	1.31

Robustness

The SST parameters kept on unaffected over deliberate changes in the chromatographic conditions (variation of the ratio of methanol in mobile phase by $\pm$2%, the flow rate of mobile phase by $\pm$0.05% and the wavelength of 230 nm by $\pm$2%), illustrating the robustness of the method.

Assays in Pharmaceutical Preparation

The proposed validated method was applied to determine CZP in two difference batches of CZP capsules. Satisfactory results were obtained from batch I as the mean percentage found in good agreement with label claimed. Batch II was found to be expired before the expiration date. Also, the discoloration of CZP in batch II was observed. NDZP could be investigated while ACB was non-detected in both batches. The results indicated that this method could be adopted for determination of CZP and monitored NDZP and ACB in capsules (Table **7**).

Table 7 Determination of CZP in capsules.

	CZP		NDZP	
	% label amount*	% RSD	µg per capsule found*	% RSD
Batch I	99.25	0.18	0.64	7.89
Batch II	88.82	0.19	15.21	0.56

* Mean value for three replicates (n=3), and two injections for each replicate.

Conclusion

A simple isocratic RP-HPLC method was successfully developed for the determination of CZP in the active ingredient and its pharmaceutical formulations. The complete separation of the analytes were accomplished within 10 min and found to be specific, linear, precise and accurate. All validation parameters were within the acceptance range. The *m/z* values and fragmentation patterns obtained for the degradation products through LC-MS studies helped to confirm the know degradation products; NDZP and ACB. The advantage of the method was the mobile phase developed could be used with both DAD and MS. This proposed method might be applied for the routine quantitative analysis of CZP in bulk drug and capsules as well as the content uniformity test. It also has been used for the stability study of CZP and monitored the related compounds as involved in both BP and USP.

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