APPLICATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO THE ANALYSIS OF CILAZAPRIL IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A rapid, selective, precise and accurate high-performance liquid chromatography method for the estimation of cilazapril (CZP) in bulk and pharmaceutical formulations has been developed and validated. The assay of the cilazapril was performed on a Cyano Column (150 mm x 4.6 mm, 5 µm particle size) with UV detection at 212 nm. The mobile phase consists of a formate buffer (pH 3.75)-acetonitrile (60:40 *v/v*) and a flow rate of 1 ml/min was maintained. The calibration curve was linear over the range of 2–200 µg/ml (r^2 = 0.9990). The proposed method was validated as per International Conference on Harmonization guidelines. The proposed method was successfully applied to the quantification of (CZP) in pharmaceutical formulations. The proposed method will be useful for routine quality control analysis of (CZP).

Keywords: Cilazapril, Cyano column, HPLC analysis, Pharmaceutical formulations.

INTRODUCTION

Cilazapril (CZP) [1-6] is a long acting pyridazine angiotensin-converting enzyme inhibitor used in the treatment of high blood pressure (hypertension) and congestive heart failure (reduction in the heart pumping action). In treating hypertension, CZP may be used alone or in combination with thiazide diuretics (e.g., hydrochlorothiazide). After absorption, CZP is hydrolyzed to the active metabolite, cilazaprilat. The cilazaprilat prevents the conversion of angiotensin I to angiotensin II by inhibition of angiotensin-converting enzyme. Angiotensin II is a vasoconstrictor and a negative feedback mediator for renin (an enzyme released by the kidneys that stimulate the production of angiotensin I) activity. Therefore, lower angiotensin II level results in lessen blood pressure. Chemically, CZP is known as 9(s)-[1(s)-(ethoxycarbonyl)-3-phenylpropylamino]-octahydro-10-oxo-6H-pyridazo [1,2-a] [1,2] diazepine-1(s)-carboxylic acid monohydrate.

The CZP is official in European Pharmacopoeia [7], which describes potentiometric titration with 0.1M sodium hydroxide for its assay. Literature reports capillary zone electrophoresis [8], HPLC/MS/MS [9], HPLC with UV [10-12] or amperometric detection [13] methods for the assay of CZP and its active metabolite cilazaprilat in human urine, human plasma and pharmaceuticals. Chemometry [14], derivative spectrophotometry [15, 16], HPLC with UV detection [12,17] and

Vierordt's [16] methods were applied for the simultaneous determination of CZP and hydrochlorothiazide in their binary mixture.

Different techniques have been reported for the quantification of CZP alone in biological fluids and pharmaceuticals formulations. The CZP in human plasma or serum has been quantitated by enzyme immune assay [18]. A gas chromatography–mass spectrometry [19] was developed for the detection of CZP in rat urine. The reported enzyme immunoassay [18] and gas chromatography-mass spectrometry [19] involves expensive or sophisticated instruments, procedures with rigorous control of the experimental conditions and are not simple for routine analysis. Moreover, these methods were not applied to pharmaceutical formulations. Derivative spectrophotometry [20], voltammetry [21, 22] and amperometric biosensor [23] methods have been applied for the determination of CZP in pharmaceutical formulations. Even though the derivative spectrophotometric method is simple and easy to perform, it suffers from decreased selectivity due to measurement in ultraviolet region, narrow range of linearity and less precise with % RSD values >2.0. The voltammetry [21, 22] and amperometric biosensor [23] methods are complicated and time consuming. Therefore, the applications of these methods to quantify CZP in pharmaceutical formulations are limited.

To the best of our knowledge, there are two reports on the application of HPLC with UV detection methods [20, 24] for the quantification of CZP in pharmaceutical formulations. Unfortunately, the HPLC with UV detection methods [20,24] reported were allied with some major drawbacks. These drawbacks included use of triple solvent system, use of internal standard, long retention time, less sensitive, lack of precision and accuracy. In addition, the method reported by Gumieniczek and Przyborowski [24] was not fully validated.

In this paper an attempt is made to develop and validate a rapid, sensitive, precise and accurate HPLC method without incorporating the use of internal standard for the determination of CZP in bulk and in its pharmaceutical formulations.

MATERIALS AND METHODS

Instrumentation

Shimadzu HPLC class VP series (Shimadzu Corporation, Kyoto, Japan) isocratic HPLC system equipped with a variable wavelength programmable UV/Visible detector SPD-10A, Shimazdu (Tokyo, Japan) electronic weighing balance, model BL 220 H, Elico pH meter (Hyderabad, India) LI 120 model were used. Chromatographic data acquisition and storage was done with Shimadzu class VP series version 5.03 computer program software.

Chemicals

Cilazapril (Pharmaceutical grade) was obtained from the Hetero Drugs Limited, Hyderabad, India. Tablet formulation (Inhibace, Roche Scientific Company India Pvt.Ltd, Mumbai, India) containing labeled amount of 2.5 mg and 5 mg was purchased from local pharmacy market. All the chemicals and solvents used were of analytical and HPLC grade, respectively. Potassium formate (Sd fine-chem Ltd, Mumbai, India), formic acid (Nice Chemicals (P) Ltd, Kochi, India), acetonitrile (Rankem laboratories, Mumbai, India) and Milli-Q-water (Merck Specialties Private Ltd, Hyderabad, India) were used to prepare the mobile phase.

Chromatographic conditions

HPLC analysis was conducted in a Cyano Column (150 mm x 4.6 mm, 5 µm particle size). Isocratic mobile phase consisted of formate buffer and acetonitrile (60:40 v/v). The pH of the mobile phase was adjusted to 3.75 using formic acid. The formate buffer was prepared by mixing 50 ml of 4 M formic acid and 2 g of potassium formate in a total volume of 1.0 L. The mobile phase was filtered and degassed via 0.45 µm membrane filter before use. A steady flow rate of l.0 ml/min was employed all through the analysis. Variable UV detector wavelength was set at 212 nm. The analyses were made at ambient temperature. The volume of sample injected into the column was 20 µl. The total run time was 10 minutes.

Preparation of standard solutions

Cilazapril stock standard solution (1 mg/ml) was prepared with mobile phase. The working standard solutions in the concentrations 2, 5, 10, 20, 40, 60, 80, 100, 150 and 200 µg/ml was obtained by appropriately diluting the stock standard solution with the mobile phase.

Preparation of tablet sample solution

To estimate the amount of CZP in tablet dosage forms (label claim: 2.5 and 5 mg per tablet), 50 tablets were weighed, their mean weight was determined and they are finely powdered. A precisely weighed powder sample equivalent to 50 mg of CZP was transferred into a 50 ml volumetric flask containing 25 ml mobile phase. The content of the flask was sonicated for 15 min and the resulting solution was filtered through 0.45 µm membrane filter. The volume was completed with mobile phase and the solution reached 1 mg/ml (stock solution). An appropriate aliquot of the stock solution was diluted with the mobile phase in a 50 ml volumetric flask to 100 µg/ml, as the working sample solution.

Calibration curve

The CZP working standard solutions, in the concentration range of 2-200 µg/ml, prepared from stock solution (1 mg/ml) were injected into the column three times. The eluents were monitored at 212 nm. Peak area was recorded for each concentration of CZP. The calibration curve was plotted as concentration *vs* peak area.

Procedure for tablet dosage forms

Twenty µl of the tablet sample solution prepared in the section “Preparation of tablet sample solution” was injected into the HPLC system five times and analyzed for CZP content. The peak areas were recorded. The concentration of the CZP in the tablet was determined from the calibration curve or from the straight line equation.

Method validation

As per the guidelines of ICH, the method was validated [25]

System suitability study

System suitability was established by calculating the percent relative standard deviation of repeated injections of working standard solution (100 µg/ml) and analyzing the parameters like retention time, peak area, tailing factor, number of theoretical plates, plates per meter and height equivalent to theoretical plates. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the ICH guidelines [25] as the ratio of 3 and 10 standard deviation (n=5) of the peak area of known concentration of CZP, respectively, and the slope of the calibration line. For this purpose, CZP working standard solution (2 µg/ml) was injected into the HPLC system five times.

Selectivity

To assess the method selectivity, pure drug, tablet sample and mobile phase blank solutions were injected into the HPLC system. The chromatograms were recorded and compared.

Precision and accuracy

Intra-day precision and accuracy was measured by analyzing three different concentrations of CZP (2 µg/ml, 100 µg/ml and 200 µg/ml) five times within the same day and inter-day were determined by analyzing the same solutions for three consecutive days.

Recovery study

In order to further demonstrate the accuracy of the proposed method, recovery study *via* standard addition method was performed. For this purpose, known quantity of CZP was supplemented to the tablet sample solution previously analyzed. The concentration of CZP was once again determined by the proposed method.

Robustness

To establish robustness of the method, slight intentional modifications were made in the operating conditions like mobile phase composition, flow rate temperature, pH of mobile phase and detection wavelength. The outcomes of these changes on chromatographic results were examined. The robustness was assessed at two different concentration levels (2 µg/ml and 200 µg/ml). The robustness of the proposed method was determined in triplicate at a concentration level of 2 µg/ml and 200 µg/ml of CZP.

RESULTS AND DISCUSSION

Optimization of HPLC conditions

To develop a suitable HPLC method for the determination of CZP, various analytical columns, mobile phase compositions, ratios and pH were tested. Different mobile phases containing toluene, acetonitrile, propanol, dichloromethane, orthophosphate buffer & formate buffer in different proportions and different pH were examined. Of these, the mixture of formate buffer and acetonitrile in a ratio of 60:40 *v/v* with pH 3.75 (adjusted with formic acid) was found to be optimal for good peak shape, low retention time as well as to achieve minimal background current. Among the tested analytical columns during preliminary investigations, Cyano Column (150 mm x 4.6 mm, 5 µm particle size) was the most appropriate column for HPLC analysis of CZP. After several preliminary investigatory chromatographic runs, mobile phase with a flow rate of 1.0 ml/min, ambient column temperature, detector wavelength set at 212 nm was chosen as suitable conditions for the good signal response of CZP. Using the experimental conditions selected, a satisfactory chromatographic peak (retention time 3.21 minutes) which is well defined and free from tailing was obtained in a short analysis time.

Method validation

The results of system suitability study are presented in Table 1. The low percentage relative standard deviation values obtained for these parameters- retention time, peak area, tailing factor, number of theoretical plates, plates per meter and height equivalent to theoretical plates shows that the chromatographic conditions are appropriate for the quantification of CZP.

Table 1. System suitability parameters

Parameter	Mean value*	% RSD
Retention Time (min)	3.211	0.15
Peak area	3370991	0.35
Theoretical Plates (n)	3834.160	0.822
Plates per Meter (N)	15336	0.926
Height equivalent to theoretical plate (HETP)(mm)	$3.3x10^{-7}$	1.096
Tailing factor/Peak assymetry	1.589	0.788

* Average of five determinations

The linearity was determined at ten levels over the concentration range of 2–200 µg/ml. The following equation for straight line was obtained for CZP:
y = 33444x + 56035; Y = peak area, Slope = 33444, x = concentration of CZP in µg/ml, Intercept = 56035. Regression coefficient (r^2) = 0.9990. The regression coefficient value indicated excellent linearity of the proposed method.

The LOD and LOQ values were found to be 0.167 and 0.506 µg/ml, respectively. These values indicate that the proposed method is sensitive.

The chromatograms of pure drug, tablet sample and mobile phase blank solutions were shown in Figures 1, 2 and 3. The retention time of CZP in pure and tablet sample were same. There were no peaks in mobile phase blank. The proposed method was found to be selective, as there was no interference from the excipients present in tablets and from components of the mobile phase.

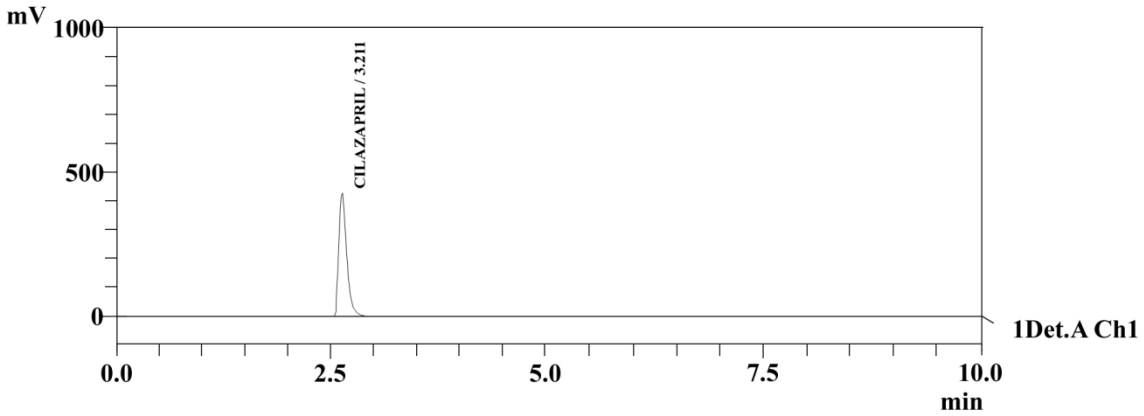

Figure 1. Chromatogram of pure drug

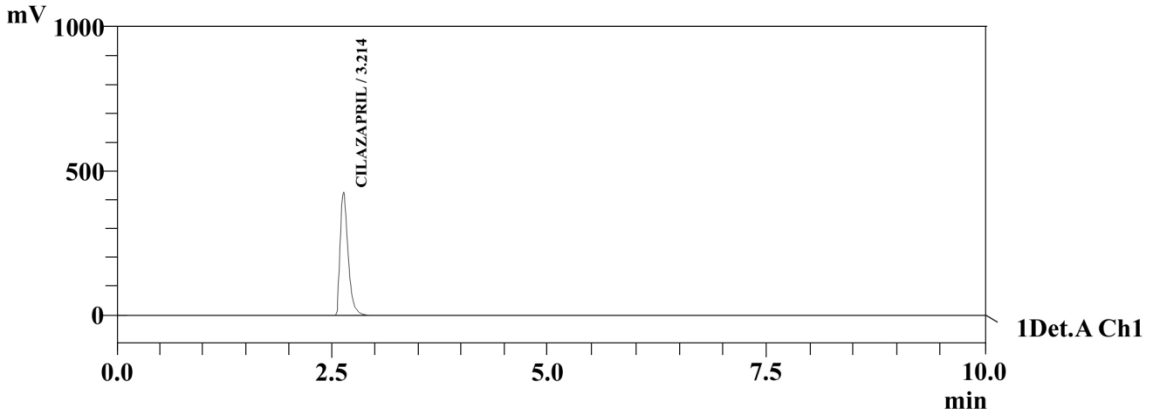

Figure 2. Chromatogram of tablet sample

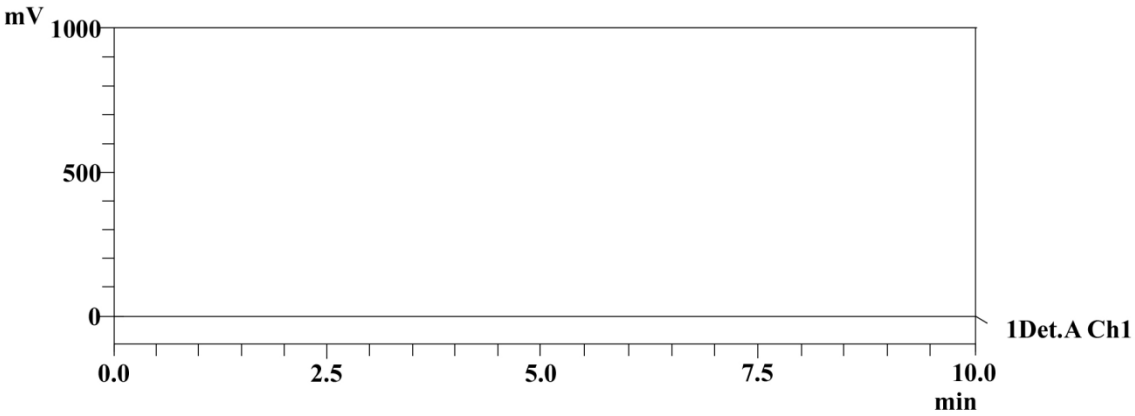

Figure 3. Chromatogram of mobile phase blank

The standard deviation and percentage relative standard deviation was calculated to represent precision. Percentage recovery and percent error was calculated to represent accuracy. The results of intra-day and inter-day analysis (Table 2) indicated good precision (% RSD < 1.0) and accuracy (% recovery in the range of 98.50-100.06) of the proposed method.

Table 2. Precision and accuracy

Concentration of CZP (µg/ml)		**%**	**%**	**%**
Taken	**Found* ± SD**	**RSD**	**Recovery**	**Error**
Intra-day analysis				
2	1.99± 0.019	0.954	99.50	0.50
100	99.97± 0.956	0.956	99.97	0.03
200	199.99 ± 0.989	0.494	99.98	0.02
Inter-day analysis				
2	1.97 ± 0.019	0.964	98.50	1.50
100	99.95 ± 0.886	0.886	100.06	0.60
200	198.03± 1.656	0.836	99.01	0.99

* Average of five determinations

The results of recovery study are presented in Table 3. The excellent percentage recoveries (98.93-99.46) values showed the method to be greatly accurate and apt for intended use.

Table 3. Recovery study

Concentration of CZP (mg)		% RSD	% Recovery
In tablet + Spiked	Found* ± SD		
2.5 + 1.25	3.73 ± 0.028	0.750	99.46
5 + 2.5	7.42± 0.070	0.943	98.93

* Average of five determinations

The results of the method's robustness are summarized in Table 4. The relative standard deviation values (< 2.0) showed that slight variations in chromatographic conditions have negligible effect on the analysis by the proposed method.

Table 4. Method robustness

Variable	Concentration of CZP (µg/ml)		% Recovery	% RSD
	Taken	Found ± SD*		
Mobile phase ** **(60:40 ± 2%)**	2	1.98 ± 0.012	99.00	0.606
	200	199.98± 1.409	99.99	0.704
pH of the mobile phase (3.75 ±0.2)	2	1.99 ± 0.018	99.50	0.904
	200	199.93 ± 1.609	99.96	0.804
Flow rate (1.0 ± 0.1 ml/min)	2	1.98 ± 0.019	99.00	0.959
	200	199.79± 1.895	99.89	0.948
Detection wavelength (212 ± 1 nm)	2	2.01 ± 0.020	100.05	0.995
	200	199.74± 1.769	99.87	0.855

* for three values
** mobile phase composition: formate buffer and acetonitrile

Application to tablet dosage forms

Application of the proposed method was checked by analyzing the CZP in commercially available tablet dosage forms. The results are shown in Table 5. The high percentage recoveries (99.20-99.60) and low relative standard deviation (<1.0) values showed that there was a close agreement between the results obtained by the proposed methods and the label claim. The results were also statistically compared by a Student's t-test for accuracy and variance ratio F-test for precision with the official method[7]. The values were found less than the tabulated ones indicating no significant difference between the proposed and official methods with respect to accuracy and precision, as shown in Table 5.

Table 5. Assay of cilazapril in tablet dosage forms

Formulation	Labelled claim (mg)	Found* (mg) ± SD	% Recovery	% RSD	t Value**	F value***
Reference method						
Inhibace	2.5	2.48 ± 0.020	99.20	0.806	-	-

Inhibace	5.0	4.98 ± 0.029	99.60	0.582	-	-
Proposed method						
Inhibace	2.5	2.49 ± 0.029	99.60	0.721	1.22	3.39
Inhibace	5.0	4.96 ± 0.026	99.20	0.524	1.36	4.10

* Average of five determinations
** Tabulated t-value at 95% confidence level is 2.306
***Tabulated F- value at 95 % confidence level is 6.390

CONCLUSION

The developed and validated HPLC method for determination of CZP in bulk and pharmaceutical formulations is very rapid, precise, specific, and accurate. The method was successfully applied for determination of CZP in its pharmaceutical formulation without interference from the common excipients. Furthermore the short run time reduce the analysis time per sample and the possibility of analysis of a large number of samples within short period. Therefore, the proposed HPLC method can be conveniently used for routine quality control analysis of CZP.

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