Determination of cilazapril in micrograms concentration using spectrophotometry

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Abstract

A simple, sensitive and economical spectrophotometric method was developed for the determination of cilazapril in bulk and tablet forms. The method is based on the bromination of cilazapril by the bromine generated by the action of the HCl on the bromate–bromide mixture followed by the reaction of unreacted bromine with a fixed concentration of methyl orange and measuring the absorbance at 530 nm. The absorbance-concentration plot was linear over the range 0.4–6 µg/ml with regression coefficient value of 0.9996. The limits of detection and quantitation were 0.0126 µg/ml and 0.0381 µg/ml, respectively. The method was successfully applied to tablet dosage forms. The recovery results obtained by the proposed method for the tablets dosage form were statistically compared with those of the official method by applying Student's *t*- and *F*-test. No significant difference was observed between the proposed and official methods

Keywords: Cilazapril, bromate–bromide mixture, methyl orange, tablet, analysis

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Introduction

Cilazapril, chemically described as (4S,7S)-7-[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]-6-oxo-1,2,3,4,7,8,9,10-octahydropyridazino[1,2-a]diazepine-4-carboxylic acid, is a antihypertensive drug and acts as inhibitor of angiotensin-converting enzyme [1]. After absorption, the prodrug cilazapril is hydrolyzed to its main metabolite cilazaprilat. The cilazaprilat competitively inhibits angiotensin-converting enzyme

[2,3]. Cilazapril is used in the treatment of hypertension [4] and congestive heart failure [5].

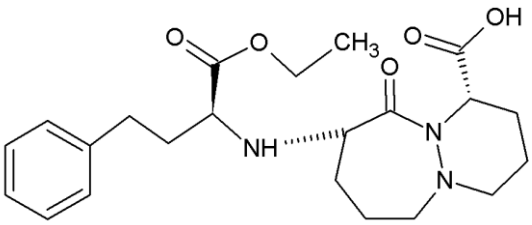

Figure 1: Structure of cilazapril

The safe and effective therapy with cilazapril depends on the quality of its pharmaceutical preparations and assessing the concentration of cilizapril in tablets for the purpose of quality control. The cilizapril is officially listed in European Pharmacopoeia and describes the potentiometric titration for its assay [6]. The analytical techniques that have been utilized for the determination of cilizapril and its active metabolite cilazaprilat in human urine, human plasma and pharmaceuticals include capillary zone electrophoresis [7], HPLC/MS/MS [8], HPLC with UV detection [9-11] and HPLC with amperometric detection [12]. For the estimation of cilazaparil alone in biological fluids and pharmaceuticals formulations different techniques have been reported. They include enzyme immune assay [13], gas chromatography–mass spectrometry [14], voltammetry [15,16], amperometric biosensor [17] and HPLC with UV detection methods [18, 19]. Most of the above reported methods are complicated, time consuming and require costly equipment.

Since the spectrophotometry technique is simple, economical and widely available it is considered as the most convenient technique. The literature survey revealed that there is only one report on the use of UV spectrophotometry [18]. In this report four methods (classic, first, second and third order derivative) were discussed for the estimation of cilazapril in pure and pharmaceutical formulation. The absorption maxima for classic, first, second and third order derivative methods were 212, 218, 222 and 224 nm, respectively. However, the UV spectrophotometric methods are limited in low precision, accuracy and selectivity. The literature is poor with regard to the visible spectrophotometric method of analysis. As much as our knowledge is concerned, no visible spectrophotometric method is reported for the assay of cilazapril.

In the present paper, one sensitive, precise and accurate visible spectrophotometric method for the determination of cilazapril has been described. The method is based on the bromination of cilazapril by the bromine generated *in situ* by the HCl on the $KBrO_3$-KBr mixture and the residual bromine was determined by reacting with a fixed amount of methyl orange. The proposed method has been successfully applied to the determination of cilazaparil in tablet dosage forms and results were compared statically with official method.

Materials and methods:

Instrumentation

1. ELICO (Hyderabad, India) double beam model SL 159 digital spectrophotometer was used for measurements.
2. One cm matched quartz cells were used for absorbance measurements.

3. Samples were weighed by using Essae-Teraoka electronic weighing balance (Goa, India) PG1000 model.

Reagents

All the chemicals used were of analytical reagent grade and were obtained from sd fine-chem Ltd., Mumbai, India.

1. **Bromate–bromide mixture:** Stock standard solution of $KBrO_3$–KBr equivalent to 1 mg/ml $KBrO_3$ was prepared by dissolving accurately weighed 100 mg of $KBrO_3$ and 1 g of KBr in water and diluting to the mark in a 100 ml calibrated flask. Stock standard solution of $KBrO_3$–KBr (1 mg/ml $KBrO_3$) was appropriately diluted with water to get working bromate–bromide solution containing 10 µg/ml of $KBrO_3$.
2. **Methyl orange solution:** The stock standard solution (1 mg/ml) was prepared by dissolving accurately weighed 100 mg of methyl orange in water and diluting to the mark in a 100 ml calibrated flask. Working standard solution containing 50 µg/ml of methyl orange was prepared by further dilution of the stock standard solution with water.
3. **5 M Hydrochloric acid:** Prepared by diluting 42 ml of 12 N HCl to 100 ml with distilled water in a 100 ml volumetric flask.

Standard solutions of cilazapril

Reference standard cilazapril was obtained as gifted sample from Hetero drugs limited, Hyderabad, India and was used as received. Stock standard solution of cilazpril was prepared by dissolving 100 mg of cilazapril in 50 ml of distilled water in a 100 ml volumetric flask and then made up to the mark with distilled water (1 mg/ml). Working standard solution containing 20 µg/ml of cilazapril was prepared by apt dilution of stock standard solution with distilled water.

Recommended procedure

Aliquots (0.2–3 ml) of cilazpril working standard solution (20 µg/ml) were transferred into a series of 10 ml volumetric flasks to give final concentrations of 0.4-6 µg/ml. The total volume was adjusted to 3.0 ml by adding sufficient quantity of water. Two ml of 5 M HCl was added to each flask, followed by 1.0 ml of bromate–bromide (10 µg/ml in $KBrO_3$) solution. The contents of the flasks were mixed well and allowed to stand for 15 min with occasional shaking. Then, 1.0 ml of methyl orange solution (50 µg/ml) was added to each flask. After 5 min the contents of the flask were diluted to volume with water and mixed well. The absorbance of orange red colored solution was measured at 530 nm against a blank solution prepared in the same manner using water instead of cilazapril. The calibration curves were constructed by plotting the absorbance against the final concentration of cilazapril. The corresponding regression equation was derived. The concentration of cilazapril in the unknown samples were read from the calibration graph or computed from the regression equation.

Assay of cilazapril in tablet dosage forms

Inhibace tablets (Hoffmann-La Roche Limited, Switzerland) labeled to contain 2.5 mg/ 5 mg of cilazpril per tablet were purchased from the local pharmacy. Fifty tablets were powdered and mixed thoroughly. An amount equivalent to 50 mg of cilazapril was

weighed accurately and stirred well with 30 ml of distilled water. The resulting solution was filtered through Whatmann No. 1 filter paper. The filtrate was transferred to a 50 ml standard flask and diluted to volume with distilled water. This solution was appropriately diluted with water. Convenient aliquots were subjected to analysis by the following the recommended procedure. The percentage recovery of the cilazapril was calculated from the corresponding calibration curve or regression equation.

Results and discussion

Basis of the reaction

In acidic medium, the mixture of potassium bromate and potassium bromide produces bromine [20]. The reaction can be expressed as:

$$BrO_3^- + 5\,Br^- + 6\,H^+ \rightarrow 3\,Br_2 + 3\,H_2O$$

The bromine generated is utilized for the bromination/oxidation of organic and inorganic compounds. The residual bromine is determined by reacting it with fixed concentration of dyes. Bleaching action of bromine causes decoloration of dye by irreversible oxidative destruction. The bromate-bromide mixture and dyes are used as analytical reagents to quantify many compounds of pharmaceutical significance [21-25].

In the proposed method, a known excess of bromine, generated *in situ* by the action of the HCl on the bromate-bromide mixture, is used to brominate cilazapril in an acidic condition. The residual bromine is allowed to react with a fixed amount of methyl orange. The amount of bromine reacted with methyl orange corresponds to the amount of cilazapril. The reaction was followed spectrophotometrically at 530 nm. The probable reaction mechanism is given in Figure 2.

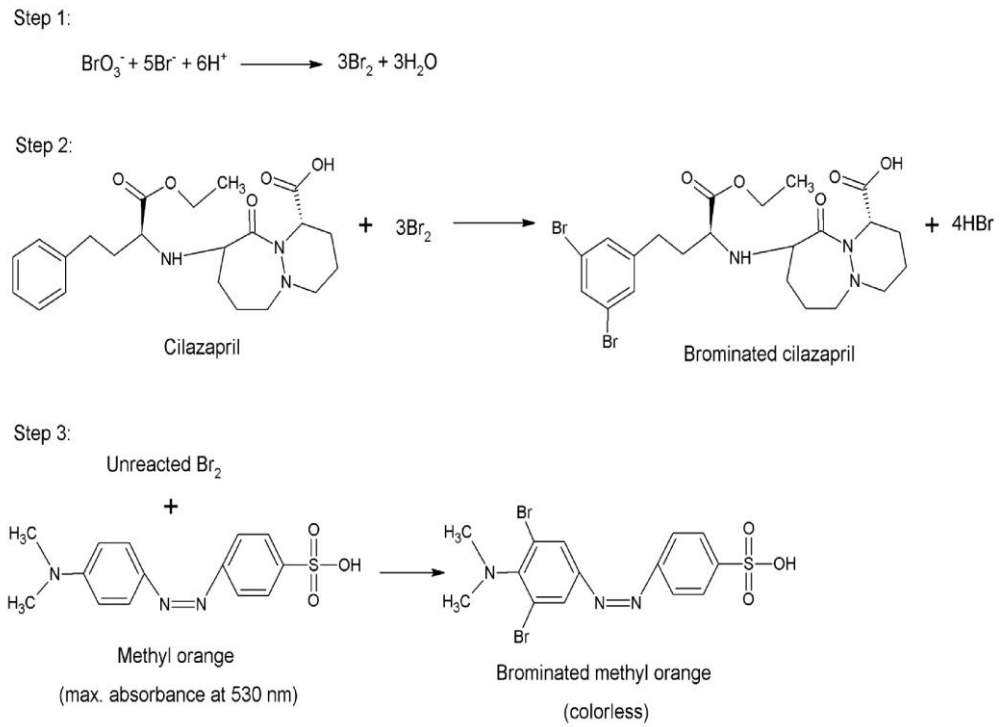

Figure 2: Reaction between cilazapril, bromate-bromide mixture and methyl orange

Optimization of experimental variables

The different experimental variables that affect the reaction were carefully studied and optimized. The optimum values were maintained all over the determination process.

Effect of $KBrO_3$ concentration

The effect of $KBrO_3$ concentration was analyzed using different $KBrO_3$–KBr mixture solutions equivalent to 5, 10, 15, 20, 25, 30 and 35 µg/ml $KBrO_3$. Increasing concentration of $KBrO_3$ produces an increase in absorbance upto 10 µg/ml. Beyond this value a gradual decrease in the absorbance is observed. Therefore, $KBrO_3$–KBr mixture solution equivalent to 10 µg/ml $KBrO_3$ and was chosen as the optimal concentration.

Effect of HCl concentration

The influence of HCl concentration on the reaction was studied over the range of 1-7 M HCl. It is observed that the maximum absorbance was attained with 2 ml of 5M HCl. At higher molar concentration (>5M) the absorbance was decreased. Hence 2 ml of 5M HCl was used as an optimum HCl concentration.

Effect of methyl orange concentration

The influence of methyl orange concentration was investigated by carrying the reaction using different concentrations of methyl orange (10, 20, 30, 40, 50, 60, 70, 80, and 90 µg/ml). The results revealed that the color intensity was increased upto 50 µg/ml. Raising the concentration of methyl orange above 50 µg/ml, there is no change in the absorbance. Therefore, the optimum methyl orange concentration was fixed at 50 µg/ml.

Effect of time on bromination of drug

So as to determine the optimum time required for the bromination of cilazapril, the reaction was allowed to proceed at room temperature for varying periods of time (5,10, 15, 20 and 25 min). This study revealed that the maximum color intensity was attained at 15 min. Therefore, 15 min was found to be sufficient for bromination of cilazapril.

Effect of time on bleaching of dye

In order to determine the optimum time required for bleaching the methyl orange, the reaction was allowed to proceed at room temperature for varying periods of time (5, 10, 15, 20, 25 and 30 min). Bleaching of methyl orange completed within 5 min. So, 5 min was chosen as optimum time.

Method validation

The developed method was validated with respect to linearity, sensitivity, precision, accuracy, stability of colored species, selectivity and robustness as per the guidelines given by ICH [26].

Linearity

Under the optimized experimental conditions, a linear relationship was established by plotting the absorbance at 530 nm against the cilazapril concentration in µg/ml. The

linearity range was found to be 0.4-6 µg/ml. Linear regression analysis of the data gave the following equation:

$$A = 0.1249\ c + 0.0045\ (R^2 = 0.9996)$$

Where: A is the absorbance at 530 nm, c is the concentration of cilazapril in µg/ml and R^2 is the regression coefficient. The high values of the regression coefficient with small intercept indicate the good linearity of the calibration graph.

Sensitivity:

Molar absorptivity, Sandell's sensitivity, limits of detection (LOD) and limit of quantification (LOQ) are calculated to assess the sensitivity of the proposed method. The results are summarized in Table 1. The high values of molar absorptivity & low values of Sandell's sensitivity, LOD and LOQ point out the adequate sensitivity of the proposed method.

Table 1: Sensitivity data of the proposed method

Parameter	Value
Molar Absorbtivity (L/mole/cm)	5.824×10^5
Sandell's sensitivity (µg cm^{-2})	0.0074
LOD (µg/ml)	0.0126
LOQ (µg/ml)	0.0381

Precision and accuracy:

Intra-day precision and accuracy was evaluated by analyzing three concentrations (0.4, 3 and 6 µg/ml) and five replicates of each concentration in one day. The inter-day precision and accuracy was assessed by analyzing three concentrations (0.4, 3 and 6 µg/ml) and five replicates of each concentration over three successive days. The precision and accuracy were expressed as percentage relative standard deviation (%RSD) and percentage relative error (%RE), respectively. The %RSD and %RE were found to be small indicating reasonable repeatability and reproducibility of the proposed method (Table 2).

Table 2: Precision and accuracy of the proposed method

Type of analysis	Concentration of cilazapril (µg/ml)		% Recovery	% RSD	% RE
	Taken	Found (n=5)			
Intra-day	0.4	0.398	99.50	0.836	0.50
	3	2.989	99.63	0.503	0.37
	6	6.010	100.16	0.316	0.16
Inter-day	0.4	0.398	99.50	0.503	0.50
	3	2.986	99.53	0.871	0.47
	6	6.004	100.06	0.333	0.06

Recovery studies:

The method accuracy was further evaluated by recovery studies through standard addition technique. For this, fixed concentration of the pure cilazapril was spiked to the

preanalyzed dosage form at three different concentration levels (50%, 100% and 150%). The percent recovery values were calculated and are summarized in Table 3. The good recovery values indicating the accuracy of the proposed method and also established the absence of interference from excipients present in the tablet dosage form with the determination of cilazaparil by the proposed method.

Table 3: Recovery studies of the proposed method

Spiked level (%)	Concentration of cilazapril (µg/ml)			% Recovery	% RSD	% RE
	Taken	Spiked	Total found (n=3)			
50	1	0.5	1.502	100.13	0.514	0.13
100	1	1	1.995	99.72	0.621	0.28
150	1	1.5	2.511	100.44	0.482	0.44

Stability of the colored species

The stability of the colored species was studied by measuring the absorbance of the reaction solution (after dilution) at different time intervals. It was observed that the absorbance of the colored species remains stable for at least 1 hour. This allowed the processing of large number of samples and their comfortable measurements with ease in quality control laboratories.

Robustness

Method robustness was demonstrated by evaluating the influence of small and deliberate variation in the experimental variables on its analytical performance. Robustness of the method was performed at two different concentration levels (0.4 and 6 µg/ml). During these experiments, one parameter was changed while the others were kept unchanged. The recovery percentage and percentage RSD were calculated each time (Table 4). The results revealed that small variation in the experimental variables did not significantly affect the procedure. Therefore, the method can be inferred to be robust.

Table 4: Robustness of the proposed method

Parameter	Concentration of drug (µg/ml)		Recovery (%)	RSD (%)
	Taken	Found (n=3)		
Volume of $KBrO_3$–KBr solution (1.0 ± 0.1 ml)	0.4	0.392	98.00	0.510
	6	5.997	99.50	0.484
Volume of 5M HCl (2.0 ± 0.1 ml)	0.4	0.396	99.00	0.758
	6	6.012	100.20	0.665
Volume of methyl orange (1.0 ± 0.1 ml)	0.4	0.391	97.75	0.256
	6	6.009	100.15	0.766
Time for bromination (15 ± 2 min)	0.4	0.398	99.50	0.503
	6	6.006	100.10	0.799
Time for bleaching (5 ± 1 min)	0.4	0.395	98.75	0.759
	6	5.995	99.91	0.851

Application of the method to tablet dosage form

The developed and validated method was applied for the determination of cilazapril in their available commercial dosage forms, Inhibace tablets. The assay results obtained by developed method are summarized in Table 5. The percentage recovery and %RSD values suggesting that the proposed method has good accuracy and precision.

Comparison with the official method

The results of the proposed method were statistically compared with the results of the potentiometric titration method given by European Pharmacopoeia [6] by applying the Student's *t*-test and *F*-test for accuracy, precision respectively. The results are shown in Table 5. The calculated *t*-value and *F*-value at 95% confidence level did not exceed the tabulated values of 2.306 and 6.39, respectively. The results revealed no significant difference between the proposed and official methods regarding the accuracy and precision.

Table 4: Comparison of results of proposed method with official method

Method	Concentration of cilazapril (mg)		% Recovery	% RSD	*t*-Value	*F*-Value
	Tablet	Found (n=3)				
Proposed	2.5	2.496	99.84	0.721	-	-
	5	5.012	100.24	0.879	-	-
Official [6]	2.5	2.504	100.16	0.806	0.716	1.214
	5	4.993	99.86	0.582	0.824	1.218

Conclusion

The present study described the successful use of bromate–bromide mixture and methyl orange as analytical reagents in the development of a new visible spectrophotometric method for the precise and accurate quantification of cilazapril in bulk and tablet dosage forms. The developed method showed the advantages of being simple, sensitive, cost effective and does not need expensive sophisticated apparatus. The method can be employed as an alternative analytical method for the determination of cilazapril. Therefore, the proposed method is suggested for the routine analysis of cilazapril in quality control laboratories.

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