WebLog Journal of Analytical and Pharmaceutical Chemistry
Research Article
Published: 11 Jun, 2026

Quantitative Analysis of Cilazapril and Alacepril in Their Bulk and Pharmaceutical Dosage Form Using 1,10-Phenanthroline

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Abstract

A new, simple and rapid spectrophotometric method with broad linear dynamic range was developed for the quantification of two angiotensin- converting enzyme inhibitors, namely cilazapril and alacepril. The method was based on the oxidation of the two drugs by Fe(III) in presence of 1.10 phenanthroline, the formed tris-(Fe-o-phen) complex in acetate buffer and optimum pH was measured at λ_{max} 510 and 512 nm for cilazapril and alacepril respectively with linear relationship over concentration range from 10-54 $\mu g ml^{-1}$. The results of the reported method of the analysis suggest that the developed approach is simple, sensitive, accurate and precise.

Keywords: Cilazapril; Alacepril; Spectrophotometry; Oxidation; 1,10-Phenanthroline

Introduction

The drugs, cilazapril CLZ: (4S,7S)-7-[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino]-6-oxo-1,2,3,4,7,8,9,10-octahydro pyridazino[1,2-a]diazepine-4-carboxylic acid and alacepril: (2S)-2-[[(2S)-1-[(2S)-3-acetylsulfanyl-2-methylpropanoyl] pyrrolidine-2-carbonyl]amino]-3- which are an angiotensin-converting enzyme inhibitors used for the ameliorative cause for hypertension and congestive heart failure. Congestive heart failure is the dependent variable of hypertension out of three-quarters of all the reported cases. Cilazapril is officially listed in European Pharmacopoeia [1]. Substantial literature based on the quantitative assay of the drug have been reported, but donor–acceptor phenomenon coupled with spectrophotometric determination sustains the larger part of research because of being cost-effective, rapid and sharp coloration, undemanding experimental setup [2]. Use of this phenomenon-based approach is a critical step in proposing cost-effective, rapid, and well-grounded methods for the determination, detection, and quantitative assay of drugs in bulk and pharmaceutical formulations and to study the potential mechanism of their action [3]. Donor species containing N, O, and S atoms have drawn marked importance in this phenomenon [4]. Previous researches have revealed the diverse independent methods for CLZ determination. There are many reports documented on high performance liquid chromatography [5], liquid chromatography–mass spectrometry [6], RP-LC [7], square wave voltammetry, stripping voltammetry, spectrofluorimetry [8], spectrophotometry [9, 10]. Alacepril, a new long-acting oral angiotensin-converting enzyme inhibitor, is elective in the treatment of essential hypertension [11,12]. It is deacetylated to form deacetyl alacepril and then converted to captopril. Alacepril was developed in Japan as a new angiotensin converting enzyme inhibitor characterized by longlasting antihypertensive activity [13]. Alaceprilis metabolized to captopril via a metabolite desacetyl-alacepril. It was reported that the duration of the anti-hypertensive effect lasted 1.5-2.0 times as long as thatof captopril [14].

In this paper for the quantitative determination of cilazapril and alacepril an economic, effective, rugged, and safe analytical methods which are simple, selective, and compliant too was developed and based on the reduction of Fe (III) by the two cited drugs and consequently Fe (II) ion produced is estimated by complexation with 1,10-phenanthroline. The proposed method was applied for determination of the studied drugs in their pharmaceutical preparations and was validated statically.

Experimental

Apparatus

Metertech Inc. SP-8001 UV-VIS Spectrophotometer (Taiwan, R.O.C) with 1cm quartz cells connected to an IBM computer loaded with software application. An Elico model Li-10 pH meter

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Received Date: *29 May 2026*
Accepted Date: *09 Jun 2026*
Published Date: *11 Jun 2026*

Citation:
Abdalaty N. Quantitative Analysis of Cilazapril and Alacepril in Their Bulk and Pharmaceutical Dosage Form Using 1,10-Phenanthroline. WebLog J Anal Pharm Chem. wjapc.2026.f1106. https://doi.org/10.5281/zenodo.20952706

was used for pH measurements.

Materials and reagents

Chemicals used were of the highest purity available from their sources and pure analytical grade.

Cilazapril pure drug: Manufactured by Dr. Reddy's laboratories, Batch No ED004A0L, LTD. Ameerpet, Hyderabad (India).

Inhibace tablet containing 5 mg cilazapril per tablet produced by Roche Pharm. Turkey.

Alacepril pure drug: Manufactured by Dr. Reddy's laboratories, Batch No. EMCPE24, LTD. Norsapur TQ. Medak DT, A.P, (India).

Alacepril Tablets containing 25mg offered by Nichi-iko Pharmaceutical Co., Ltd, Japan.

Ethanol: Adwic, Batch No. E002822, (HPLC grade).

1.10 phenanthroline monohydrate: Fine-Chem. ltd., Batch. No. 0797/679 /300 342.

Ferric ammonium sulphate: LAB-SCAN Analytical Sciences, Batch No7973.

Acetic acid: Adwic, Batch No. A0026322 (HPLC grade).

Sodium acetate: Adwic, Batch No. S0029322 (HPLC grade).

Stock standard solutions

Pure drugs: 1 mgml^{-1} solutions in ethanol of cilazapril and alacepril were prepared. The solutions were found to be stable for at least one week at 4°C. Working standard solutions were then prepared by suitable dilution of the standard stock solutions.

1.10-phenanthroline solution: prepared as 0.5% (w/v) in ethanol.

Ferric ammonium sulphate solution: prepared as 0.25%) w/v) in 0.08M H_2SO_4.

Acetate buffer: prepared by dissolving 5.4 gm. of sodium acetate in 50 ml of water, adjusted to pH 4.6 with glacial acetic acid and dilute to 100 ml with water. freshly prepared solutions were always employed.

General Procedures

For pure drugs

Accurately measured aliquots of pure standard cilazapril and alacepril solutions contains (0.25-1.35mg) in heating tubes, 2ml of ferric ammonium sulphate, 2.5ml of acetate buffer pH 4.6 and 1 ml of 0.5 1.10 phenanthroline were added successively in each tube. The contents were mixed well and placed in a water bath at 50°C for 20 minutes to develop the color. After heating, the tubes were cooled to the room temperature and the contents were quantitatively transferred to 25 ml volumetric flasks, completed to the volume with distilled water and the absorbance was measured at λ_{max} 510 and 512 nm for cilazapril and alacepril respectively against reagent blank prepared simultaneously.

For pharmaceutical preparation

Twenty tablets of each drug were weighed and finely powdered, the quantities of the powdered tablets equivalent to 25 mg cilazapril and alacepril were dissolved in 2×10 ml portions worm ethanol, filtered and washed with ethanol, the filtrate and washing were combined and the final volume was made up to a 25 ml with ethanol. An aliquot equivalent to 0.1 mg from each solution was used for the application of the proposed methods applying the standard addition technique by adding standard drugs to the pre analyzed tablets and the procedure was continued as mentioned under general procedures. The amount of drug was calculated either from the calibration graph or the regression equation.

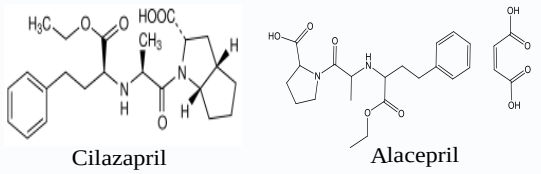

Figure:

Results and Discussion

It is well known that 1,10-phenanthroline form stable, intensely red colored, water soluble chelates with Fe^{+2} ion, the formed complex may be due to the fact that the unshared pair of electrons on each of the two nitrogen atoms of 1,10-phenanthroline complexes with Fe^{+2} ion formed by the reaction between the cited drugs and Fe^{+3} ion. The formed complexes showed intense red color measured at λ_{max} 510 and 512 nm for cilazapril and alacepril respectively (Figure 1).

Optimization of reaction conditions

The optimal reaction conditions affecting the reaction of the two studied drugs with the selected reagent were carefully studied. The experimental conditions were established by varying each parameter individually.

Effect of reagent concentration

The addition of 1 ml of 0.5% 1,10-phenanthroline was sufficient to give maximum and reproducible color intensity. Increased concentration up to 5 ml had no effect on the complex formation. While addition of 2.5 ml of 0.25% ferric ammonium sulphate gives maximum color intensity.

Effect of temperature and heating time

At room temperature the color was developed very slowly. Maximum absorbance was obtained on heating at 50°C for 20 minutes. Further heating caused no change in color. The obtained complexes were stable for more than 12 hours.

Effect of pH

Of the buffers investigated (universal, phosphate, borate and acetate) acetate buffer proved to be the optimal. A pH adjustment was necessary because the reaction was affected by change in the pH over a range of 2.5 to 6. The optimal pH for the proposed method was 4.6 and 2.5 ml of this buffer gave the highest color intensity (Figure 2).

Method Validation

Linearity

Under the optimum conditions a linear relationship existed between the absorbance and concentration of the drugs and beer's law was obeyed in the concentration ranges of 10-54 µg ml^{-1} cilazapril and alacepril by 1, 10-phenanthroline.

The main molar absorptivity, Sandell's sensitivity, correlation coefficients, limit of detection, limit of quantification, slops and intercepts were listed in Table 1.

The good linearity of the calibration graph and the negligible

Table 1: Assay parameter and spectral data for spectrophotometric determination cilazapril and alacepril by the proposed methods.

Parameters	Cilazapril	Alacepril
	1,10- phenanthroline	1,10- phenanthroline
Reaction time (mint.)	20	20
Temperature (°C)	50	50
Reagent concentration.	1%	1%
λmax for pure drug in ethanol (nm)	Lower than 200	Lower than 200
λmax for pure drug in methanol (nm)	210 280	210 280
λmax of reagent in ethanol (nm)	510	512
λmax for the product (nm)	10-54	10-54
Linearity range µg ml^{-1}	0.025	0.025
Slope (b)	0.15	0.05
Intercept (a)	0.89	0.353
variance	0.9999	0.9998
Correlation coefficient	$1.57x10^{4}$	$1.6x10^{4}$
Molar absorpitivity ($Lmol^{-1}cm^{-1}$)	3.2×10^{-2}	$3x10^{-2}$
Sandel l's sensitivity (µg cm^{-2})	0.744	0.124
Limit of detection (µg ml^{-1})	2.48	0.413
Limit of quantification (µg ml^{-1})		

Table 2: Statistical data for determination of cilazapril and alacepril by the proposed methods compared with the reference method.

Statistic	Cilazapril		Alacepril	
	Reference method	1,10-phenanthroline method	Reference method	1,10-phenanthroline method
Mean recovery* ±S.D.	100.14±1.18	99.7± 0.94	99.62±1.0137	99.34±0.594
N	5	5	5	5
Variance	1.383	0.89	1.0276	0.353
S.E	0.526	0.422	0.453	0.266
t-test[b]		0.652		0.533
F-test[b]		1.554		2..91

*Average of three experiments.
[b]Theoretical *t*- and *F* – value are 2.77 and 6.39 respectively for 4 degree of freedom at 95% confidence level.

scatter of the experimental points were clearly evident from the value of the correlation coefficient and variance.

The performance of the proposed methods was assessed by comparing the calculated t and F values with the reference methods indicates that the results are considered to be very satisfactory (Table 2).

Sensitivity

The detection limit (LOD) for the two spectrophotometric methods was calculated using the following equation [32].

$$LOD = 3S/K$$

Where S is the standard deviation of the replicate determination values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance were calculated and listed in Table 1.

The limit of quantification, LOQ defined by the following equation [15].

$$LOQ = 10S/K$$

According to this equation, the limit of quantification was calculated and listed in Table 1.

Precision and accuracy

Precision and accuracy studies of the proposed methods were done by carrying out five independent determinations at three concentration levels. The low values of the calculated relative standard deviations RSD% and SAE indicate the excellent precision and accuracy. The confidence limits at 95% were calculated and the results showed that there is no significant difference between the three sets results (Table 3).

Effect of interference

The effect of the presence of some common additives and excipients such as starch, talk, titanium dioxide and magnesium stearate was studied. It was found that there was no interference from additives or excipients for the proposed methods (Table 4).

Analytical applications

The proposed method was successfully applied for determination of cilazapril and alacepril in their tablet dosage forms applying the standard addition technique. The methods performance was assessed using the student's *t*-test and variance ratio *F*-test at 95% confidence level with five degrees of freedom with the reference method as shown in Table 5. Statistical analysis of the results indicates that the *t*- and *F*-values were less than the tabulated ones indicating that there was no significant difference between the proposed and reference method.

Table 4: Determination of cilazapril and alacepril by the proposed methods in presence of additives and excipients.

Another ingredient added	Drug taken	Cilazapril	Alacepril
	µgml-1	%*Recovery	%*Recovery
		1,10-phenanthroline	1,10-phenanthroline
–	8	99.65	99.2
Titanium dioxide	8	99.8	99.1
Talk	8	100.9	100.3
Starch	8	100.3	99.15
Mg stearate	8	100	100.3

*Average of three experiments

Table 3: Evaluation of the precision and accuracy of the proposed methods.

Statistical parameter / Compared method	Taken µg ml^{-1}	Found ± SD[a]	RSD (%)	SAE [b]	Confidence limit [c]
Cilazapril	10	9.92±0.023	0.047	0.010	0.020
1,10-Phenanthroline	30	29.62±0.016	0.200	0.01	0.020
	50	49.46±0.023	0.054	0.007	0.014
	10	9.91±0.015	0.050	0.012	0.024
Alacepril	30	29.7±0.036	0.150	0.006	0.013
1,10-Phenanthroline	50	49.6±0.027	0.120	0.016	0.032

[a] Mean ± standard deviation for five determinations.
[b] Standard analytical error.
[c] Confidence limits at P = 0.95 and 4 degrees of freedom

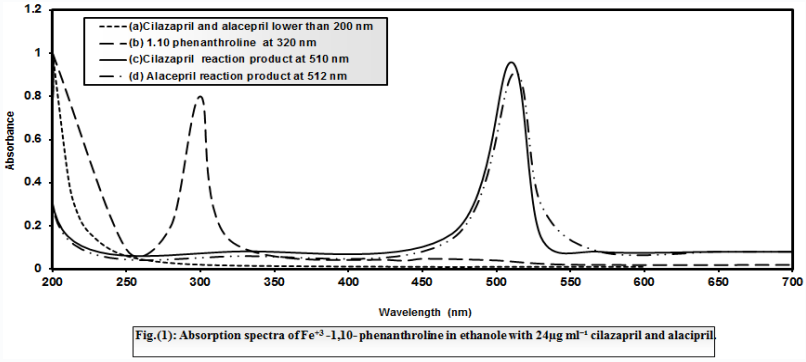

Fig.(1): Absorption spectra of Fe^{+3}-1,10- phenanthroline in ethanole with 24µg ml^{-1} cilazapril and alacipril.

Figure 1:

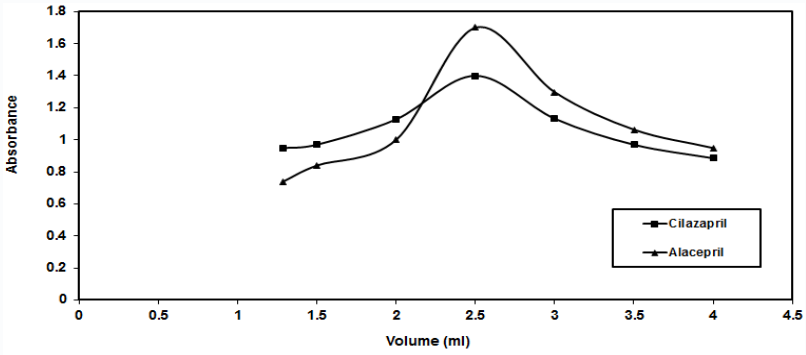

Fig. (2) :Effect of volume of acetate buffer PH 4.6 on the oxidation of (a) Cilazapril and (b) Alacepril in 1,10- phenanthroline method.

Figure 2:

Table 5: Statistical data for determination of commercial tablets of cilazapril and alacepril by the proposed methods compared with the reference method, applying the standard addition technique.

Statistic	Cilazapril (Inhibace 5 mg.)		Alacepril (Alacepril 25 mg.)	
	Reference method	1,10-phenanthroline method	Reference method	1,10- phenanthroline method
Mean recovery* ±S.D.	99.52±1.09	99.32±0.507	99.7±1.1	99.42±0.497
N	5	5	5	5
Variance		0.257		0.247
S.E	1.177	0.227	1.28	0.222
t-test[b]	0.48	0.38	0.504	0.51
F-test[b]		4..58		5.18

*Average of three experiments

[b] Theoretical *t*-value and *F* – value are 2.77 and 6.39 respectively for 4 degrees of freedom at 95% confidence level

Conclusion

The developed methods are economical, simple, sensitive and accurate and can be used for determination of cilazapril and alacepril in their pharmaceutical formulations. The described method was based the reduction of Fe^{+3} with consequence complexation of Fe^{+2} with 1,10-phenanthroline and validation showed them to be suitable for routine analysis of the studied drugs.

Also, the results showed that the proposed procedure more sensitive than another spectrophotometric assay of cilazapril [8] and alacepril [16].

In addition to simplicity, sensitivity and good precision and accuracy, these methods are rapid, and do not require specific sample treatments or any stringent experimental condition.

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