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Research Article

CONDUCTOMETRIC DETERMINATION OF THE TWO ANGIOTENSIN-CONVERTING ENZYME INHIBITORS, RAMIPRIL AND ENALAPRIL MALEATE IN PURE FORM AND IN TABLETS USING PHOSPHOTUNGSTIC ACID

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ABSTRACT

Simple and sensitive conductometric method has been developed for the determination of ramipril and enalapril maleate using phosphotungstic acid (PTA) in pure form and in tablets. The proposed method is based on the conductometric determination of 5–20 mg and 7–20 mg of ramipril and enalapril maleate by titration with PTA in aqueous solution. All the reaction conditions for the proposed methods have been studied. The proposed methods were applied successfully for the determination of ramipril and enalapril maleate in tablets; the relative standard deviation values were not exceeding two for both drugs. The results obtained were compared statistically with those obtained by the reference method and showed no significant differences regarding accuracy and precision.

Keywords: Ramipril, Enalapril maleate, Conductometric titration, Phosphotungstic acid, Tablets dosage forms.

INTRODUCTION

Ramipril is (2S,3aS,6aS)-1-[(S)-2-[[(S)-1-(ethoxycarbonyl)-3-phenylpropyl] amino]propanoyl]octahydrocyclopenta[b]pyrrole-2-carboxylic acid [1], chemical structure is shown in Fig. 1. It is an angiotensin-converting enzyme (ACE) inhibitor used in the treatment of hypertension, heart failure, and after myocardial infarction to improve survival in patients with clinical evidence of heart failure [2].

The **B** P [1] proposes a potentiometric titration technique for the determination of Ramipril. Titration was done using 0.1 M sodium hydroxide. The volume added was recorded at the second point of inflection. The USP [3], however, describes a more complicated chromatographic procedure.

Many methods have been described for the determination of ramipril. Concerning the chromatographic methods, it has been determined in pharmaceutical preparations or biological fluids either alone or combination with other drugs by high-performance liquid chromatography (HPLC) [4-7]. Furthermore, HPTLC methods have been reported [8,9].

Literature described different spectroscopic methods for determination of ramipril including spectrophotometric methods [10-17], and also derivative spectrophotometric methods [18,19], and chemometric methods [20] have been reported.

Other reported techniques were voltammetry [21,22], potentiometry [23], conductometry [24], and capillary electrophoresis methods [25] have been applied for the determination of ramipril.

Enalapril maleate is ((S)-1-{N-[1-(ethoxy carbonyl)-3-phenylpropyl]-L-alanyl}-L-proline, (Z)-2-butenedioate), chemical structure is shown in Fig. 2. It is a prodrug that **is** hydrolyzed in the body to enalapril at which is an inhibitor of ACE.

The USP 24 describes HPLC method for its quantitative estimation [3]. Quantitative determination of enalapril maleate can be carried out by various reported methods such as HPLC [26-30], capillary zone **ele**ctrophoresis method [31], polarography [32], atomic absorption spectroscopy [12], and membrane selective electrodes [33]. Some spectrophotometric methods have also been reported for quantitative estimation of enalapril maleate in bulk and dosage forms [12,34-39]. Different spectrophotometric method [40] has also been reported.

Precipitimetry conductometric titrations using phosphotungstic acid (PTA) as titrant are commonly used for the quantitative determination of different compounds, e.g. reproterol HCl, pipazethate HCl, salbutamol sulfate [41], papaverine hydrochloride [42], and dextromethorphan [43].

Phosphotungstic acid was also used in the spectrophotometric estimation of mebikar [44].

In this study, a simple and accurate conductometric method **h**as been proposed for determining ramipril and enalapril maleate, based on the conductometric titration with PTA. The proposed methods have been applied to the assay of ramipril and enalapril maleate in tablets.

METHODS

Materials and reagents

- All solvents and reagents were of analytical grade, and double distilled water was used throughout the work.
- Ramipril and enalapril maleate (Egyptian group for pharmaceutical industries Co., El-Obour city, Egypt).
- PTA (scientific limited, Northampton, UK)

Pharmaceutical preparation

- Tritace protect tablets (Sanofi-aventis Egypt s.a.e., El Sawah El Amiriya, Egypt) labeled to contain 10 mg ramipril per tablet.
- Enalapril maleate tablets (October Pharma S.A.E, 6 October City, Egypt) labeled to contain 20 mg enalapril maleate per tablet.

Instrumentation

- JENWAY model 470 conductivity/TDS meter (470 201), with Conductivity/temperature probe (027 298) was used.

General procedures

Preparation of stock and standard working solutions

1) Ramipril

Working standard solution of 1 mg/ml (2.4×10^{-3} M) was prepared by dissolving 100 mg of the pure drug in the least amount of methanol then completing to 100 ml with double distilled water.

Enalapril maleate
Working standard solution of 1 mg/ml (2.03×10^{-3} M) was prepared by dissolving 100 mg of the pure drug in 100 ml double distilled water.

PTA acid
Working solution 1×10^{-3} M prepared by dissolving 0.288 g in double distilled water then completing to 100 ml with double distilled water.

Construction of calibration curves

Aliquots of sample solution containing 5–20 mg and 7–20 mg of ramipril and enalapril maleate respectively were transferred to a 50 ml calibrated flask; volume was made up to the mark using double distilled water. The contents of the calibrated flask were transferred to a beaker, and the conductivity cell was immersed.

PTA was used as titrant; the conductance was measured subsequent to each reagent solution addition, and after stirring for 2 min, the conductance was corrected for dilution [45] by means of the following equation, assuming that conductivity is a linear function of dilution.

A graph of corrected conductivity versus the volume of added titrant was constructed, and end-point was determined conductometrically.

The amount of drugs under study was calculated according to the following equation:

Amount of drug =VMR/N

Where V is volume of titrant, M is molecular weight of drug, R is molar concentration of titrant, and N is no of moles of titrant consumed by one mole of drug.

Determination of stoichiometric balance using Job's method [46]

6 mL of 10^{-3}M ramipril and enalapril maleate were transferred to 50 mL volumetric flasks, and the volumes were made up to the mark with double distilled water. The contents were transferred to a beaker, and the conductivity cell was immersed.

10^{-3} M PTA was used for titration. The conductance was measured subsequent to each reagent solution addition and after thorough stirring for 2 min. A graph of conductivity versus volume was constructed.

Curve break is observed at drug-reagent molar ratio of 3:1.

Procedure for tablets

The contents of 10 tablets were pulverized, an accurately weighed amount equivalent to 100 mg of the studied drugs were extracted by shaking with 50 ml double distilled water for enalapril maleate while for ramipril, it was extracted with, least amount of methanol then these contents were transferred to a 100 ml volumetric flask, completed to the mark using double distilled water then filtered.

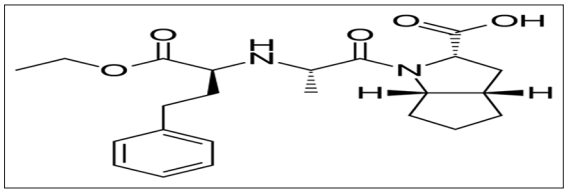

Fig. 1: Chemical structure of ramipril

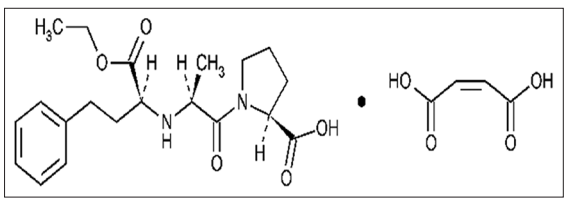

Fig. 2: Chemical structure of enalapril maleate

RESULTS AND DISCUSSION

On using PTA as a titrant for the determination of ramipril and enalapril maleate, ion associate is formed leading to a regular rise in conductance up to the equivalence point where a sudden change in the slope occurs.

Representative titration curve is shown in Figs. 3 and 4. Two straight lines are obtained, intersecting at the end-point. The conductance measured before the addition of the titrant (volume of PTA equals zero) is mainly due to the presence of ramipril and enalapril maleate. On adding PTA, an ion associate is formed between both drugs and PTA and the conductivity increases [47]. After the endpoint, more reagent acid is added, and the conductivity increases more rapidly. The reaction may be represented by the following equations:

3 Ramipril + $[PTA]^{-3}\rightarrow[Ramipril]^{+3}\ [PTA]^{-3}$

3 Enalapril maleate+$[PTA]^{-3}\rightarrow[Enalapril\ maleate]^{+3}\ [PTA]^{-3}$

The results from the conductometic titrations are summarized in Table 1. The data show that accurate results were obtained with good recoveries and low standard deviation (SD) values. The optimum concentration ranges for determination of the cited drug were in the range of 5–20 mg and 7–20 for ramipril and enalapril maleate, respectively. At such ranges, sharp inflections and stable conductance reading were obtained.

Investigations were carried out to establish the most favorable conditions for the reaction to attain endpoint. The influence of some variables on the reaction has been tested, and the optimum conditions for performing the titration in a quantitative manner were elucidated as described below.

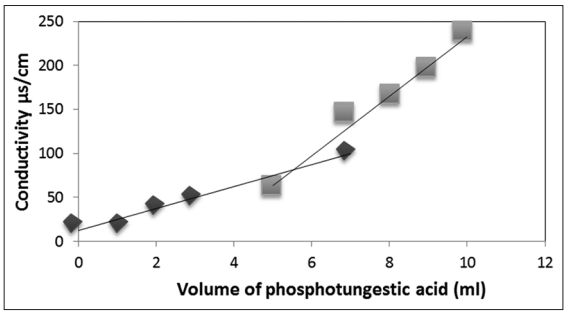

Fig. 3: Conductometric titration of 10 mg of ramipril using 1×10^{-3} M phosphotungstic acid

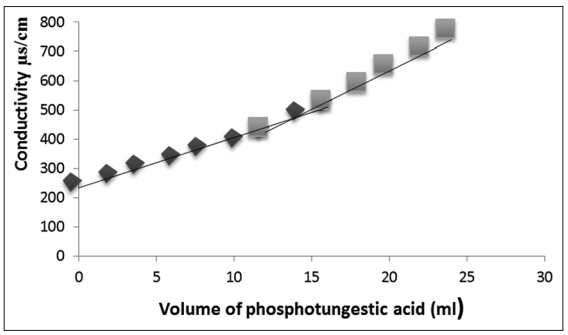

Fig. 4: Conductometric titration of 20 mg of enalapril maleate using 1×10^{-3} M phosphotungstic acid

Titrations in different media were attempted to obtain the best results. Preliminary experiments in

1. Aqueous solutions of both drug and reagent,
2. Drug and reagent solutions in ethanol-water (50%, v/v) mixture,
3. Methanolic solutions of both drug and reagent,
4. Drug and reagent solutions in methanol-water (50% v/v) mixture,
5. Drug and reagent solution in acetone-water (50% v/v) mixture.

Preliminary experiments showed that procedure in aqueous media was the most suitable for successful results for the cited drug (higher conductance and most sharp endpoint.).

Reagent's concentration

The optimum concentration of PTA was 10^{-3} M to achieve a constant and highly stable conductance reading after 2 min mixing. Concentrations less than these gave unstable readings and more time was needed to obtain constant conductance values.

Method validation

The developed methods were validated according to ICH guidelines [48]. The linearity range of conductivity as a function of drug concentration (Tables 1 and 2) provides an accurate measure of sensitivity of reagents used. Calibration curves have determination coefficients (R^2) higher than 0.999 indicating good linearity. According to ICH guidelines, the obtained values indicated a high sensitivity of the proposed methods. Statistical comparison of the results obtained from the analysis of the studied drug by the proposed method to those of reference method [1] using t- and F-tests, showed no significant difference between them (Table 3). Accuracy of the methods was expressed as the mean recovery percentage (average of four replicates within Beer's law limits) while the precision was expressed as the relative SD percentage (Table 4).

CONCLUSION

The simple and rapid procedure described in this chapter can be an alternative to the more complex and expensive methods for assay of

Table 1: Conductometric determination of ramipril and enalapril maleate in their pure forms using 1×10^{-3}M PTA

Ramipril		Enalapril maleate	
Taken (mg)	**Found %***	**Taken (mg)**	**Found %***
5	100.00	7	101.26
7	98.21	10	100.47
10	100.00	12	99.73
12	99.96	15	98.52
15	98.33	17	100.87
20	99.96	20	98.99
Mean±SD	99.41±0.884	99.92±1.08	
n	6	6	
V	0.78	1.17	
SE	0.36	0.44	
RSD	0.88	1.08	

SD: Standard deviation, RSD: Relative standard deviation, SE: Standard error, PTA: Phosphotungstic acid

Table 2: Conductometric determination of ramipril and enalapril maleate in their tablets dosage forms using 1×10^{-3} M PTA

Ramipril		Enalapril maleate	
Tritace protect tablet		**Enalapril maleate tablet**	
Conc (mg)	**Recovery %***	**Conc. (mg)**	**Recovery %***
5	100.00	10	100.44
7	99.10	12	99.75
15	99.96	15	99.01
17	100.42	17	99.13
20	99.96	20	100.47
Mean±SD	99.89±0.481	99.76±0.694	
n	5	5	
V	0.231	0.481	
SE	0.196	0.310	
RSD	0.48	0.692	

SD: Standard deviation, RSD: Relative standard deviation, SE: Standard error, PTA: Phosphotungstic acid

Table 3: Statistical analysis of results obtained by the proposed method, applied on Tritace® protect tablets compared with the reference method

Parameters	Ramipril		Enalapril maleate	
	Reference method [1]	**Proposed method**	**Reported method [39]**	**Proposed method**
N	3	5	6	5
Mean recovery	101.00	100.14	100.215	99.76
Variance	1.08	0.614	1.807	0.481
±SD	1.04	0.78	1.344	0.694
±RSD	1.03	0.78	1.347	0.692
±SE	0.60	0.32	0.549	0.310
Student-t [49]		1.346 (2.447)		0.681 (2.262)[a]
F-test [49]		1.77 (2.417)		3.756 (5.19)[b]

a and b are the theoretical student t-values and F-ratios at *P* 0.05. SD: Standard deviation, RSD: Relative standard deviation, SE: Standard error

Table 4: Evaluation of the interday and intraday precisions and accuracy for ramipril and enalapril maleate obtained by the proposed method

Drug	Taken conc (µg/ml)	Interday			Intraday		
		Recovery (%)*	Precision (RSD%)*	Accuracy (Er%)	Recovery (%)*	Precision (RSD %)*	Accuracy (Er%)
Ramipril	10	99.69	1.2	-0.31	100.31	0.72	0.31
Enalapril maleate	10	100.08	1.41	0.08	100.44	1.2	0.44

RSD%: Percentage relative standard deviation. Er%: Percentage relative error. *Mean of five determination

ramipril and enalapril maleate. The proposed method is easy and very useful for the determination of the studied drugs in pharmaceutical formulations and can be applied in laboratories for routine analysis.

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