

Research Article

A Spectrophotometric Method for the Determination of Ramipril in Solid Dosage Forms

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Abstract

Purpose: To develop a simple and cost effective spectrophotometer method for the determination of ACE inhibitor ramipril in dosage forms.

Methods: UV spectrophotometry was used to develop and validate a simple method for the assay of ramipril in solid dosage form at λ_{max} of 210 nm, as per International Conference on Harmonization (ICH) guidelines. Aqueous methanol (5 %) was used as the blank solvent. The method was validated for linearity, recovery, accuracy, precision, specificity in the presence of excipients, and also for inter-day stability under laboratory conditions.

Results: Validation results showed linearity in the range 1 – 38 $\mu\text{g/ml}$; recovery accuracy 101.55%; regression equation $Y = 0.0256X + 0.0697$, R^2 of 0.9942; precision RSD < 2.00 %; and negligible interference from common excipients and colorants. The method was accurate (95 % confidence limit) compared to standard liquid chromatography (LC) method, with comparable reproducibility when used to assay a commercial product (Ramitace[®], 2 and 5 mg tablets).

Conclusion: The validated data were within allowable limits and therefore, the proposed method is recommended for routine quality control (QC) analysis

Keywords: Ramipril, Spectrophotometric assay, Validation, Solid dosage forms

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INTRODUCTION

Ramipril, a prodrug converted to the active metabolite, ramiprilat, by liver esterase enzymes [1], is (2S,3aS,6aS)-1-[(S)-2-[[[(S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]octahydrocyclopenta[b]pyrrole-2-carboxylic acid. It is an angiotensin-converting enzyme (ACE) inhibitor. ACE inhibitors are used to treat hypertension and congestive heart failure. They act by lowering the production of angiotensin II, thereby relaxing arterial muscles while at the same time enlarging the arteries, allowing the heart to pump blood more easily, and increasing blood flow due to more blood being pumped into and through larger passage ways [1-3].

Hypertension, a common cardiovascular disease, affects about 26 % of all adults worldwide [4-5]. It is a risk factor in patients with arteriosclerosis, stroke, myocardial infarction and end-stage renal disease.

Some colorimetric and kinetic spectrophotometric methods have been reported for the determination of ramipril in pure form, and dosage forms [6-9]. These are based on complexation, charge transfer or derivatization principles. The British Pharmacopoeia recommends a liquid chromatographic (LC) method for its determination in raw material and in dosage forms [10]. The literature on analytical procedures, based on simple spectrophotometry for the determination of ramipril in pharmaceutical formulations, is scanty.

Clinically, the desired goal of any drug is to achieve the desired therapeutic effect following administration. It is therefore imperative that the quality of the drug or drug product is assured. With the increasing incidence of hypertension among the productive age group [4,5], ascertaining the quality of the several brands of antihypertensives has become imperative. This would be facilitated if a simple, rapid and cost-effective UV spectrophotometric method

for the determination of active pharmaceutical ingredients (API) in formulated drug products is available.

In this study, a simple UV spectrophotometric method was developed and validated as per International Conference on Harmonization (ICH) guidelines [11,12]. The method was also used in the determination of the content of ramipril in solid dosage forms and compared with a pharmacopoeial method [10].

EXPERIMENTAL

Materials

Pure ramipril (99.98 %) was received as a gift from May & Baker Nigeria Plc, Ikeja, Nigeria. Methanol (analytical grade) was purchased from Sigma Aldrich. Distilled water was used for all analysis. A Cecil spectrophotometer model number CE7200 was used for the analysis. Excipients, including sodium starch glycolate (SSG), lactose, maize starch and microcrystalline cellulose (Avicel PH 101), were of pharmaceutical grade and obtained from May & Baker Plc, Nigeria.

Determination of wavelength of maximum absorption

Pure ramipril (0.01 g) was dissolved in 50 ml of methanol in 50 ml volumetric flask to give a 200 µg/ml ramipril stock solution (Ro). Ro (5 ml) was further diluted to 100 ml with distilled water to give a 10 µg/ml ramipril solution, and this was scanned in the wavelength region 190 to 800 nm to determine the wavelength of maximum absorption using 5 % aqueous methanol as reagent blank

Linearity study

The 200 µg/ml ramipril solution, Ro, used for the wavelength of maximum absorption determination was employed as stock solution for linearity study. Aliquots in the range of 0.5 to 19 ml of this solution were taken and diluted to 100 ml with distilled

water to obtain different concentrations within the range 1 – 38 µg/ml and used for the linearity calibration plot.

Intra-day precision test

Aliquots (5, 10 and 15 ml) of 200 µg/ml ramipril stock solution were taken and diluted to 100 ml with distilled water to 10.0, 20.0 and 30.0 µg/ml, respectively. Triplicate absorbance measurements of each of these concentrations were taken and the mean, standard deviation and RSD of the replicate absorbance measurements calculated.

Inter-day precision test

The selected concentrations for the intra-day precision study were again analysed the following day and the mean, standard deviation and RSD calculated

Recovery study

This study was carried out using pre-formulated granules containing 4.717 and 2.358 %w/w pure ramipril, and well-known excipients such as sodium starch glycolate (SSG), maize starch, lactose and microcrystalline cellulose. The granulation (1 g) was then transferred into a 100 ml volumetric flask. Methanol (99.9 %, 50 ml) was then added, shaken for 15 min using a vortex mixer and diluted to the 100 ml mark with the same solvent. The mixture was filtered using Whatman no. 1 filter paper to obtain sample stock solution, Po. This stock solution (5 ml) was further diluted to 100 ml with distilled water and then assayed for content of ramipril using the proposed method at 210 nm, with a solution containing 12.5 µg/ml of pure ramipril as standard. All analyses were carried out in triplicate using 5 % aqueous methanol as blank

Recovery study was also carried out using the standard liquid chromatography (LC) procedure of British Pharmacopeia [10]. An Agilent 1200 series system was used for the analysis with the test samples and reference,

ramipril standard preparation analysed at 250 µg/mL in 0.1M HCl. An octadecylsilane ODS C18 stainless steel column (0.25 m x 4.0 mm x 5 micron) was used. Other chromatographic conditions were; detector wavelength (210 nm), column temperature (25 °C), flow rate (1.0 ml/min), injection volume (20 µl), and elution time (30 min). The mobile phase was prepared by adjusting the pH to 2.1 with concentrated orthophosphoric acid, a mixture containing 420 volumes of acetonitrile and 580 volumes of solvent A (prepared by dissolving 14 g of sodium perchlorate in 500 ml of water, adding 2 ml concentrated orthophosphoric, and diluting to 1000 ml with water. The pH of this solution was then adjusted to 2.5 with triethylamine.

Assessment of specificity

This test was carried out using well-known excipients as indicated above. Dummy granules (1g) devoid of the pure ramipril were prepared in triplicate as in the recovery study. Their absorbance at 210 nm was taken and compared with both that of the blank (5 % aqueous methanol) and that obtained for the recovery study.

Limit of detection (LOD) and of quantitation (LOQ)

LOD and LOQ were determined using the expression in Eqs 1 and 2.

$$\text{LOD} = 3(\text{SD}/a) \dots\dots\dots (1)$$
$$\text{LOQ} = 10(\text{SD}/a) \dots\dots\dots (2)$$

where SD = standard deviation of the intercept and a = mean slope obtained from the calibration plot.

Assay of ramipril in commercial brands

Accurately weighed amount of crushed tablet powder, equivalent to 25 mg ramipril, was transferred into a 100 ml volumetric flask, 50 ml of absolute methanol was added, shaken for 15 min in a vortex mixer and diluted to the 100 ml mark with the same solvent. It was

then filtered using Whatman number. 1 filter paper to obtain stock solution, Po. Five milliliters of Po was further diluted to 100 ml with distilled water and then assayed for content of ramipril using the proposed method. A solution containing 12.5 µg/ml of pure ramipril was used as standard for comparison. All analyses were carried out in triplicate.

Preparation of reference standard

Pure ramipril (25 mg) was accurately weighed and dissolved in 100 ml of absolute methanol. Out of this solution, 5 ml was further diluted to 100 ml with deionised water to obtain a 12.5 µg/ml ramipril standard solution. The absorbances of the test and reference standard solutions were taken using 5 % aqueous methanol as blank. The content of anhydrous ramipril in the commercial brands was determined using Eqs 3 - 5.

$$LH (\%) = 100(A_p \times W_s)/(A_s \times W_p) \dots\dots\dots (3)$$

where LH = content of ramipril (%w/w), Ap = absorbance of generic sample solution, As = absorbance of reference ramipril standard solution, Ws = weight of reference ramipril powder, and Wp = weight of generic powder sample.

$$\% \text{ Labeled dose of 2.5 mg dose formulation} = (LH \times W_{20})/50 \dots\dots\dots (4)$$

$$\% \text{ Labeled dose of 5 mg dose formulation} = (LH \times W_{20})/100 \dots\dots\dots (5)$$

where W20 = weight (mg) of 20 tablets of generic sample, the factor 50 and 100 are due to the labeled claim of 2.5 and 5.0 mg ramipril per tablet.

Statistical analysis

Where applicable, results were expressed as mean ± SD and analysed statistically using student *t*-test with the aid of Microsoft Excel 2003 software. Data were considered significantly different at 95 % confidence limit.

RESULTS

The wavelength of maximum absorption (λmax) was 210 nm. The linearity parameter (Table 1) and the corresponding regression data, indicated excellent linear relationship (R² = 0.994) over the working concentration range of 1.0 - 38.0 µg/ml. The limits of detection (LOD) and quantitation (LOQ) were 0.008 and 0,02 µg/mL, respectively

Table 1: Linearity data

Parameter	Result
λ _{max} (nm)	210
Beer's law linearity range	1– 38 µg/mL
Regression equation	y=0.0256x + 0.0697
Intercept (a)	0.0697
Slope (b)	0.0256
Correlation coefficient (r)	R ² = 0.9942.

Table 2: Intra- and inter-day precision data (n = 3, p = 0.05)

Conc (µg/ml)	Mean absorbance ± SD		Relative standard deviation (RSD, %)		Calculated t-value
	Day 1	Day 2	Day 1	Day 2	
10.0	0.337± 0.003	0.331± 0.004	0.914	1.2817	4.1181 *
20.0	0.609± 0.004	0.607± 0.003	0.581	0.4560	1.7889 *
30.0	0.869± 0.005	0.873± 0.011	0.527	1.2992	3.3333 *

No significant difference between day 1 and day 2 values at 95 % confidence level for t-value of 4.3000 at 4 degree of freedom

Table 2 presents the intra- and inter-day precision data for the proposed method. They indicate adequate sample stability and method reliability over a period of 24 h. This is because for the three selected concentrations within the linearity range, the observed RSDs were all < 7 %. Mean analyte recovery of 101.55 ± 0.82 % with RSD of 0.82 %, which is not significantly different from that obtained (100.63 %) using the pharmacopoeial (LC) method. The fact that

the calculated value of t (1.5880) was substantially less than the tabulated value of 2.9200 at 95 % confidence showed that the proposed method can be satisfactorily utilized for the assay of ramipril in dosage forms. This was confirmed when the new method was used to determine the absolute drug content of two dose levels of generic ramipril; the results gave % label claim of 101.73 ± 0.82 and 102.03 ± 0.08 % for 2.5 and 5.0 mg dose levels, respectively.

DISCUSSION

Both the proposed and pharmacopeial (LC) methods showed comparable accuracy and precision, though the proposed would be less costly to carry out. The proposed method has been validated as per ICH guideline and the results of the validation parameters were within acceptable limits. Good linearity was observed in the concentration range employed in the test with a regression coefficient (R^2) of 0.994, thus indicating a high degree of sensitivity. Recovery accuracy was insignificantly different from the theoretical value. Intra- and inter-day precision data also indicate good precision with $RSD < 7$ % which is within the allowable limit of ≤ 15 % [7]. When the developed method was applied to determine the content of ramipril in two commercial brands the results showed excellent reproducibility of the recovery and precision; furthermore, the assay data were well within pharmacopoeial limits for the tablets [10].

CONCLUSION

Considering the observed good validation results, the method may be used for routine quality control assay of ramipril where non-interfering species and/or excipients at the λ_{max} of 210nm are used in their formulation

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