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Titrimetric and thin layer chromatographic fingerprint analysis of captopril solid dosage form – an Angiotensin-Converting Enzyme Inhibitor

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ABSTRACT

Captopril is an angiotensin-converting enzyme inhibitor (ACEI), that prevents the conversion of Angiotensin I to Angiotensin II, thereby decreasing blood pressure by lowering peripheral vascular pressure without increasing cardiac output or contractibility. 25 mg of 20 Captopril tablets from five brands (ACEI1-ACEI5) were weighed and pulverized into a fine powder. 100 mg of the powdered tablet active component was combined with 60 mL of water in a 100 mL measuring cylinder and thoroughly shaken for 20 minutes to extract the medicament. The solution will then be filtered. Titration was conducted by adding 2 mL of 5M HCl to 10 mL aliquots of captopril, 0.02 M Hexacyanoferrate (III), and 30% ZnSO4. For the titrimetric study, the excess oxidant was iodometrically measured in the presence of ZnSO4. Thin Layer Chromatographic Fingerprinting was done following pharmacopeia standards, compared with 5.0 mL of methanol and 10 mg captopril tablet powder. The blank titrimetric analysis of thiosulphate without the captopril samples gave 48.3 ml, and test samples ranging from 48.4 ml (ACEI2 and ACEI4), 48.5 ml (ACEI1) and 48.6 ml (ACEI3 and ACEI5), respectively. The TLC, for ACEI1, ACEI3, and ACEI6, under 254 nm, showed a violet color, and blue at 365nm, indicating the presence of captopril, with mean Rf values of 0.70, 0.71 and 0.70 cm respectively. Both methods showed the presence of captopril and an adequate amount of captopril in the tablet used for the analysis. Hence, these methods may be useful in routine captopril analysis.

Keywords: Captopril, TLC, Titrimetric, ACE Inhibitor, Chromatographic Fingerprint

1. INTRODUCTION

ACE inhibitors inhibit the angiotensin-converting enzyme, which converts the terminal two peptides of angiotensin-I to form the vasoconstrictor angiotensin-II,

lowering blood pressure by decreasing peripheral vascular pressure without increasing cardiac output or contractility. Angiotensin-II binds to the Angiotensin-I receptor on smooth muscles, inducing vasoconstriction and thereby raising blood pressure (Forrester et al., 2018; Mohapatra et al., 2021). Angiotensin II can also stimulate the adrenal cortex to release aldosterone, which causes the distal tubules and collecting ducts of the kidney to reabsorb water and salt in exchange for potassium, increasing extracellular volume and blood pressure (Gan et al., 2018). Inhibiting the angiotensin-converting enzyme (ACE) lowers plasma angiotensin II, causing vasodilation and lowering aldosterone secretion (Fountain and Lappin, 2021).

Captopril (1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline) is an antihypertensive agent that is also used in the management of kidney failure due to high blood pressure and diabetes. It is a sulfhydryl-containing proline analog of useful antineoplastic agents and an L-proline derivative with a (2S)-2-methyl-3-sulfanylpropanoyl group replaced on the nitrogen (Calaza and Carlos, 2008; Zheng et al., 2022). Captopril (Figure 1) can be synthesized by direct acylation of 1-proline with 3-acetylthio-2 methylpropionic acid chloride or acylation of protected tert-buthyl ester of 1-proline with 3-acetylthio-2-methylpropionic, respectively (Bartosz et al., 1997).

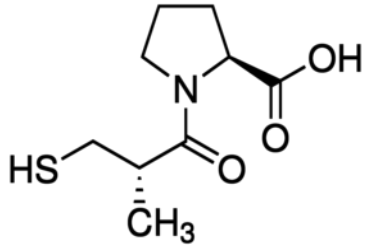

Figure 1 Structure of captopril

Captopril is readily absorbed after oral administration and found in the blood after 15 minutes (Dessì-Fulgheri et al., 1987). It has a short half-life in human plasma, ranging from 1.6 to 1.9 hours. About 70% of the oral dose is absorbed and has an absolute bioavailability of 60% in a healthy fasting human individual (Fujimura et al., 1986; Duchin et al., 1988). Captopril demonstrated fast absorption in a single-dose pharmacokinetic trial in which 25mg was administered to 12 volunteers. Its Cmax was 0.5 to 1.0 hour after oral administration, and when the dose was increased to 50mg, it had a Cmax50/Cmax25 of 1.914, indicating that it is not dose-dependent or possesses non-linear kinetics (Liao et al., 2003). Captopril blood levels decreased rapidly after reaching their peak and were undetectable 6 to 8 hours later (Jankowski et al., 1995). It binds to serum albumin and other plasma proteins fast (Busher, 1990). After intravenous injection to healthy subjects, it has a total body clearance and steady-state volume of distribution of about 0.7 L/h/kg and 0.8 L/kg, respectively.

Furthermore, it attained peak concentration in healthy subjects 45 to 60 minutes following oral administration (Lin et al., 2004). The human red blood cell methyltransferase enzyme is involved in captopril S-methylation. The average rate of methylation in the liver was found to be higher in women than in men, while there was no gender difference in kidney metabolism. The average methylation rate for all patients was 47 + 23 pmol/min/mg (Drummer et al., 1983). Captopril is primarily eliminated through the kidney (Duchin et al., 1988). Captopril administration reduces peripheral vascular resistance in hypertensive patients while increasing cardiac output (Ferrara et al., 1984). Following captopril administration, there is an increase in renal blood flow, although the glomerular filtration rate is usually unchanged. Blood pressure reductions are usually observed from 60 to 90 minutes after oral dosing (Lu and Liu, 1990).

Proteinuria, renal insufficiency, nephrotic syndrome, renal failure, polyuria, oliguria, neutropenia with myeloid hypoplasia, and a rash with pruritus are some of the side effects of captopril. Other symptoms include arthralgia, eosinophilia, angina pectoris, myocardial infarction, Raynaud syndrome, congestive heart failure, dysgeusia (loss of taste perception), anaphylactoid reactions, flushing or pallor, tachycardia, chest pain, and palpitations. Spectrophotometry measures the radiant energy transmitted or reflected by a sample at a specific wavelength Horncastle, (1973), while titrimetric analysis (titration) is a quantitative technique that measures the volume of a solution containing a titrant and a specific amount of an analyte (Bewick et al., 2009). The basis of captopril titration is the oxidation of the sample with excess Hexacyanoferrate (III) [Fe3+(CN)6] (El-Enany et al., 2008). The spectrophotometric analysis involves the oxidation reaction of captopril with Fe3+(CN)6 under acidic conditions and subsequent determination of Fe3+(CN)6, which results in red coloration, measured at 510 nm (El-Didamony and Erfan, 2010).

Captopril pills have been subjected to extensive pharmaceutical testing (Hillaert and Van-den-Bossche, 1999). Titration is a qualitative analytical procedure that involves measuring the volume of a solution containing a known concentration of a reagent (titrant) and an analyte. Titrations, such as iodometry, use a redox interaction between the analyte and titrant, while TLC uses a mobile phase with appropriate solvents and a stationary phase, which is usually silica gel. The high specificity of TLC has been employed for quantitative analysis, with spot elution, and spectrophotometric measurement using specific UV light wavelength. TLC is critical during the early stages of drug development when knowledge about contaminants and degradation products in drug components and drug products is limited. Therefore, the study intends to compare captopril pills using a simple titration approach and thin-layer chromatography fingerprinting using established protocols.

2. METHOD

Preparation of Solutions

Titrimetric analysis has been used to quantify active ingredients in food and pharmaceutical products (Ere et al., 2020). In this study, 20 captopril tablets from each of the five brands were weighed and ground into a fine powder. About 100 mg of the powered tablet (ACEI1) was added to 60 mL water in a 100 mL measuring cylinder and firmly shaken for 20 minutes. After that, the solution was filtered, resulting in the extraction of captopril active form from the powdered material. This was repeated for each brand (ACEI2, ACEI3, ACEI4, and ACEI5).

Titrimetric analysis

An equivalent amount of 2 mL of 5M HCl was added to a 10 mL aliquot of ACEI1 in a conical flask, 10 mL of 0.02 M of Fe3+(CN)6, and 10 mL of 30% ZnSO4 solution, mixed thoroughly and left for 20 minutes with intermittent shaking. 5 g of iodine crystal was weighed and dissolved in 10% potassium solution, resulting in a volume of 1L for complete iodine crystal dissolution. In the presence of a starch indicator, 5 ml of iodine solution was added and titrated with 0.02 M thiosulphate solution. The following equation was used to calculate the amount of drug utilized in a blank titration: Drug (mg) = (V1 – V2) MR/n (parameters defined below).

The excess oxidant was iodometrically measured in the presence of zinc sulphate, after the oxidation process. The oxidation reaction was found to be quantitative in an HCL medium, with 2 mL of 5M acid in an overall volume of 25 mL producing a uniform molar ratio, whereas ZnSO4 was introduced to ensure quick and irreversible oxidation of iodide by Fe3+(CN)6 through the elimination of Fe3+(CN)6 formed as the slightly soluble potassium zinc Fe3+(CN)6. Under the given acid and oxidant concentrations, Fe3+(CN)6 oxidation was sluggish and required 20 minutes at room temperature. This was repeated for each brand.

Thin Layer Chromatographic Fingerprinting

An equivalent amount of 5.0 mL of methanol was added to 10 mg ACEI1 powder and diluted with methanol to 10 mL, stirred, and filtered. Solution as a reference: A 10 mg pharmacopeia standard captopril sample was dissolved in methanol and diluted to 10 ml with the same solvent. As a mobile phase, chloroform and methanol (9:1) were used. A 10 cm chromatographic plate was loaded with a drop of the test solution and tablet solutions. The chromatogram was analyzed in ultraviolet light at 254 and 365 nm after ascent by the drug samples. The retardation factor (Rf) was determined. The spectrophotometric analysis focused on the oxidation of captopril with Fe3+(CN)6 in acidic conditions and the subsequent determination of Fe3+(CN)6, as well as the reaction of captopril with 1,10-phenanthroline in mildly acidic conditions (at pH 3-4), which resulted in the formation of red color that could be measured at 510 nm (El-Didamony and Erfan, 2010).

The addition of an increasing amount of captopril to Fe3+(CN)6, also increased the concentration of Fe3+(CN)6 after the oxidation reaction. At room temperature, the oxidation reaction progressed gradually and was completed after 10 minutes of heating in a water bath with 5M HCL. In the studied range (2.5-120 mg), 1mL of 100 mg/mL Fe3+(CN)6 and 1.5 mL of 5M hydrochloric acid were sufficient to achieve oxidation. Because the color reaction between Fe3+(CN)6 and 1:10-phenanthroline occurs at pH 3-4, 1.5 mL of 0.5M sodium carbonate solution was required to elevate the pH to roughly 3.5 before adding the 1:10 phenanthroline solution. The hue produced remained constant for several weeks.

3. RESULTS

Titrimetric analysis

Table 1 Blank Titration of thiosulphate without captopril tablet

Volume of thiosulphate (mL)	Initial volume (mL)	Final volume (mL)
First titration	0.00	47.5
Second titration	0.00	48.5
Third titration	0.00	49.0
Titre volume	48.3	

Table 2 Titration of thiosulphate with captopril tablet

Volume of thiosulphate (mL)	Sample code									
	ACEI1		ACEI2		ACEI3		ACEI4		ACEI5	
	IV	FV	IV	FV	IV	FV	IV	FV	IV	FV
First titration	0.00	46.5	0.00	45.8	0.00	47.0	0.00	46.6	0.00	48.1
Second titration	0.00	49.0	0.00	48.8	0.00	48.9	0.00	47.8	0.00	48.9
Third titration	0.00	50.0	0.00	49.7	0.00	48.5	0.00	49.8	0.00	50.0
Titre volume	48.5		48.4		48.6		48.4		48.6	

Key: IV - Initial volume, FV- Final volume

The amount of active drug was determined using the formula;

Drug (mg) = (V1 – V2) MR/n; Where:

V1 = Volume of thiosulphate in blank titration;

V2 = Volume of thiosulphate in the sample titration;

M = Molar mass of drug (217.29 mg);

R = Morality of Fe3+(CN)6 (0.02);

n = Number of moles Fe3+(CN)6 reacting per mole of the drug (0.2);

Amount of Active Drug (mg) = (48.5 - 48.3) x 217.29 x 0.02/0.2 = 4.3458mg

Amount of ACEI1 = 4.35 mg

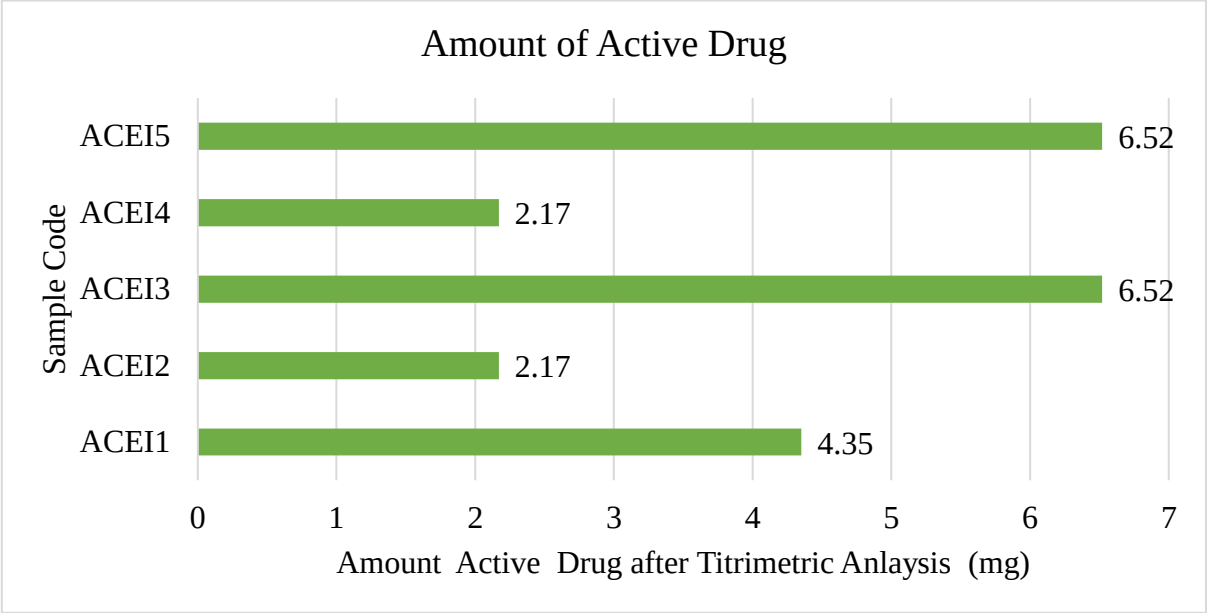

Figure 1 Amount of active drug quantified from each brand.

The linear regression equation: A = 0.01 + 0.04 C [A =Absorbance, C = Concentration]
r = 0.9982,
Apparent molar absorptivity = 9.14×103 L/mol/cm
Sandell sensitivity = 23.78 ng cm-2; Limit of detection = 0.0776 mg/Ml
Quantification limit = 0.2589 mg/mL (lowest standard concentration that could be determined with acceptable accuracy and precision).

Over a concentration range of 0.25-12 mg/mL, Beer's law was followed for experimental conditions. Absorbance = 0.01+ 0.04 concentration mg/mL (r = 0.9982) is the linear regression equation. Sandell sensitivity was 23.78 ng cm-2, and apparent molar absorptivity was 9.14103 L/mol/cm. The detection limit was 0.0776 mg/mL, while the quantification limit was the lowest standard concentration that could be established with acceptable accuracy and precision, which was 0.2589 mg/mL.

Thin Layer Chromatographic analysis of captopril tablet

Table 3 Thin Layer Chromatographic analysis of captopril

Tablet number	ACEI 1		ACEI2		ACEI3		ACEI4		ACEI5	
	Rf value [CHF: MeOH] (9:1) cm	UV Lamb (254 nm, 365nm)	Rf value [CHF: MeOH] (9:1) cm	UV Lamb (254 nm, 365nm)	Rf value [CHF: MeOH] (9:1) cm	UV Lamb (254 nm, 365nm)	Rf value [CHF: MeOH] (9:1) cm	UV Lamb (254 nm, 365nm)	Rf value [CHF: MeOH] (9:1) cm	UV Lamb (254 nm, 365nm)
1.	0.71	v, b	0.70	v, lb	0.71	v, b	0.70	v, lb	0.69	v, b
2.	0.69	v, b	0.69	v, lb	0.70	v, b	0.67	v, lb	0.68	v, b
3.	0.70	v, b	0.70	v, lb	0.68	v, b	0.70	v, lb	0.72	v, b
4.	0.69	v, b	0.68	v, lb	0.69	v, b	0.70	v, lb	0.69	v, b
5.	0.71	v, b	0.71	v, lb	0.72	v, b	0.69	v, lb	0.69	v, b
MEAN±SD	0.70 ± 0.01		0.69 ± 0.01		0.70 ± 0.01		0.70 ± 0.01		0.70 ± 0.01	

Keys: CHF – Chloroform, MeOH – Methanol, UV – Ultraviolet, nm – nanometer, cm – centimeter, v-violet, b -blue, lb - light blue, SD - Standard deviation

4. DISCUSSION

The amount of drug contained in the tablet and the presence of the active ingredient were assayed using titration and TLC fingerprint analysis. From the titrimetric analysis, using both blank test and actual test the volume of thiosulphate used was obtained. The blank titrimetric analysis of thiosulphate without the captopril samples gave a titration volume of 48.3 ml, as the volume of the solvent expended for the reaction between captopril and thiosulphate (Table 1). This was slightly lower than the volume of the thiosulphate obtained from the actual titration with Captopril samples ranging from 48.4 ml (ACEI2 and ACEI4), 48.5 ml (ACEI1), and 48.6 ml (ACEI3 and ACEI5), respectively, indicating that higher quantity (volume) of thiosulphate reacted with captopril (Table 2).

Using the formula; Drug (mg) = (V1 – V2) MR/n, the actual amount of drug concentration after the titration was quantified for all five brands, as presented in (Figure 2). This method is also useful in quantifying the rate of drug metabolism or possible metabolites from drug products (Bunu et al., 2020). From the Thin layer chromatographic analysis, for ACEI1, ACEI3, and ACEI6, under 254 nm ultraviolet light, these samples showed a violet color indicating the presence of the test sample, and also under 365 nm, a blue color was observed, thus indicating the presence of captopril, with mean Rf values of 0.70, 0.71 and 0.70 cm respectively. A violet and light blue were observed for ACEI2, and ACEI4 at 254 and 365 nm, with Rf values of 0.69 and 0.70 cm, respectively (Table 3). These results are comparable to the expected captopril absorptive colors outlined in the United States Pharmacopoeia.

5. CONCLUSION

The study compared the titrimetric and thin-layer chromatographic analysis of captopril solid dosage formulations. Both methods showed the presence of captopril and an adequate amount of captopril in the tablet used for the analysis. Hence, titrimetric analysis and thin-layer chromatographic analysis may be employed as simple, cost-effective, and precise forms of captopril analysis in pharmaceutical formulations and also in the manufacturing process.

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Authors contribution

Samuel J Bunu: Data analysis, manuscript writing, critical review, and validation; Somtochukwu Kela–Eke: Laboratory investigation, data curation; Benjamin U Ebeshi: Conception, supervision, manuscript approval.

Ethical approval: Not applicable

Informed consent: Not applicable.

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Data and materials availability

All data associated with this study are present in the paper.

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