Circular Dichroism Spectroscopy: A Facile Approach for Quantitative Analysis of Captopril and Study of Its Degradation

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* Supporting Information

ABSTRACT: Simple and selective zero- and second-order derivative circular dichroism (CD) spectroscopic methods have been designed for the assay of captopril in commercially available dosage forms. A normal CD spectroscopic scan (zero order) exhibits a negative band at 208 nm (method A) in distilled water. The calibration curve shows a linear response over the concentration range of 10−80 μg mL−1. The second-order derivative (D2) CD spectrum shows one positive band at 208 nm (method B) and one negative band at 225 nm (method C). Linear calibration curves were obtained in the concentration range of 10−70 μg mL−1 for both the methods (B and C). The detection limits were found to be 1.26, 1.48, and 2.38 μg mL−1 for methods A, B, and C, respectively. The study under stressed acidic, basic, and oxidative conditions showed the degradation of captopril. The proposed methods were validated as per ICH guidelines. All the proposed methods were compared with the reference method to demonstrate its suitability for quality control of captopril in its dosage forms.

INTRODUCTION

Captopril is chemically designated as (2S)-1-(3-mercaptop-2-methylpropionyl)-L-proline (Figure 1), which contains the carboxyl group with a pK₁ of 3.7 and a thiol group with a pK₂ of 9.8. It is the first angiotensin-converting enzyme inhibitor belonging to the sulphydryl group. It is helpful in reducing the peripheral resistance and maintaining the normal blood pressure.1−3 Furthermore, it is prescribed for the treatment of congestive heart failure, angina, Raynaud’s phenomenon, rheumatoid arthritis, and safeguards the kidney function in diabetic nephropathy.4−6 Captopril also found to be effective in the treatment of cancer as it restrains tumour angiogenesis by inhibiting endothelial cell matrix metalloproteinases and endothelial cell migration.7 Therapeutic effect of captopril may overturn to toxicity if taken in high doses.8 Because of its widespread consumption, it is mandatory to keep an eye toward the efficacy of drug, and hence quantitative analysis needs to be performed in order to control the quality of active pharmaceutical ingredients in pharmaceutical formulations.7

In view of quality assurance of pharmaceuticals, a variety of analytical techniques has been utilized to determine the active pharmaceutical ingredients and their degradation products and impurities in commercial dosage forms and bulk drugs.8−10 The United States of Pharmacopoeia11 and British Pharmacopoeia12 proposed high-performance liquid chromatography (HPLC) for quantitative determination of captopril. Literature survey showed that quantitative analysis of captopril in pharmaceutical formulations and biological samples has been done using different analytical techniques including thin-layer chromatography,13 HPLC,14−19 gas chromatography,20 NMR,21 spectrophotometry,22 spectrofluorimetry,23−25 FT-Raman,26 capillary electrophoresis,27,28 and electroanalytical methods.29−34

Circular dichroism (CD) spectroscopy has not been explored much in the field of quality assurance of drugs, although this analytical technique holds high selectivity toward direct quantitative determination of optically active drug and quiet sensitive to absolute configurations and conformational features.35 In recent years, CD has been employed for enantiopurity determination.36 The use of CD is not found in Chinese Pharmacopoeia and International Pharmacopoeia.37 However, CD has the ability to discriminate enantiomers and...
**RESULTS AND DISCUSSION**

**Spectral Studies.** The CD spectrum (zero order) of captopril dissolved in distilled water showed one negative band centered at 208 nm (Figure S1). The CD spectrum is considered to be the sum of the chirality of its individual components, (S)-proline and (2S)-3-mercapto-2-methylpropionic acid. The negative peak in the CD spectrum at 208 nm may be due to n → π* transition of the carboxylate group mixed with the n → σ* transition of the amino group of proline moiety. The quantitative determination of captopril was performed by measuring the ellipticity, θ, of the sample at 208 nm.

CD is the difference in absorption of left-handed polarized light versus right-handed polarized light, which can be expressed as

$$\Delta A(\lambda) = [\varepsilon_L(\lambda) - \varepsilon_R(\lambda)] \times d \times c \quad (1)$$

The ΔA can be related to ellipticity as

$$\Delta A = \frac{\theta}{32.98} \quad (2)$$

Equation 2 suggested that the measured ellipticity should obey Beer–Lambert’s law. The molar ellipticity [θ] was calculated using the equation

$$[\theta] = \frac{MW \times 100 \times \theta}{d \times c} \quad (3)$$

where MW is the molecular weight of captopril and d and c represent the path length (cm) and concentration (mg mL⁻¹). The molar ellipticity at 208 nm was found to be $-39383.81 \text{ cm}^2 \text{ dmol}^{-1}$.

The method of derivative spectroscopy offers alternative approaches to enhance the sensitivity and specificity in analysis. In this study, validation of three methods (A, B, and C) is considered for determination of captopril in pharmaceutical preparations. The shape of the derivative curve is highly dependent upon the smoothing factor (n). Therefore, different values of n in the range 1–5 were considered in recording the D² spectra of the drug. The best results were obtained with n = 4, scan rate = 50 nm/min, and band width = 2 nm.

**Effect of pH.** The CD spectra of captopril were recorded in acetate buffer of varying pH values (1–5) and water in order to obtain the best response (Figure 2). It was found that the maximum ellipticity was obtained in water as the solvent. In acetate buffer solutions, the ellipticity was decreased which may be because of degradation of captopril. Therefore, all further studies were performed by dissolving captopril in distilled water.

**Calibration Curve. Zero-Order Method.** Under the optimized experimental conditions, zero-order CD spectra of varying concentrations of captopril (10–80 μg mL⁻¹) were recorded (Figure 3a). The calibration curve (Figure 3b) was constructed by plotting ellipticity (at 208 nm) against the concentration of the drug. The linearity of the curve was obtained in the concentration range of 10–80 μg mL⁻¹. The high value of correlation coefficient (R²), that is, 0.9996 validates the linearity of regression line. The regression parameters are shown in Table 1. The limits of detection and quantitation were evaluated as per International Conference of Harmonization guidelines (ICH) using the following expression:

$$\text{LOD} = \frac{3.3SD}{b} \quad (4)$$

$$\text{LOQ} = \frac{10SD}{b} \quad (5)$$

where LOD is the limit of detection, LOQ is the limit of quantitation, SD is the standard deviation, and b is the slope of the regression line.

Figure 2. CD spectra of captopril (50 μg mL⁻¹) in acetate buffer of varying pH.

Figure 3. (a) CD spectra of varying concentrations of captopril (b) calibration plot for method A.
where SD and \( b \) are the standard deviation of intercept and the slope of regression line, respectively. The limit of detection equivalent to 1.26 \( \mu g \) mL\(^{-1}\) signifies good sensitivity of the proposed method.

**Second-Order Derivative Method.** Calibration curves were prepared for the determination of captopril using the CD spectroscopic method operated under the D\(^2\) mode in the wavelength range \( 200–300 \) nm. The D\(^2\) spectrum shows one positive band with maximum at 208 nm and one negative band peaking at 225 nm (Figure 4). Calibration plots (Figure 5) were obtained at 208 nm (method B) and 225 nm (method C) separately by plotting second-order derivative ellipticity against the concentration in the range of 10–70 \( \mu g \) mL\(^{-1}\). Statistical data were evaluated for both the calibration curves by using least squares method and are reported in Table 1. In methods B and C, the calibration curves were obtained by plotting the second-order derivative of ellipticity versus concentration at 208 and 225 nm, respectively, and found to be linear in the concentration range of 10–70 \( \mu g \) mL\(^{-1}\) for both the methods. In method A, the linearity of the calibration curve was 10–80 \( \mu g \) mL\(^{-1}\). This difference in the linear range may be due to the derivatization of ellipticity. The differences in detection limits for methods A, B, and C were due to the different standard deviation of intercepts and slopes of regression lines.

**Method Validation. Accuracy and Precision.** The accuracy (% relative error) and precision (% RSD) of the proposed methods under optimum experimental conditions were evaluated by performing analysis of captopril at three concentration levels (10, 40, and 60 \( \mu g \) mL\(^{-1}\)) in the same day (intraday) at different time intervals and in six different days (interday). Five replicate analyses were performed and the results are reported in Table 2. The percent relative error varies from 0.27 to 0.14%, 0.11 to 0.28%, and 0.20 to 0.03% for methods A, B, and C, respectively. The intraday precision was found to vary from 0.11 to 1.13%, 0.11 to 0.28%, and 0.10 to 1.20%, for methods A, B, and C, respectively. The interday precision varies from 0.15 to 0.51%, 0.10 to 0.50%, and 0.16 to 0.94%, for methods A, B, and C, respectively. The results demonstrated the good repeatability and reproducibility of the proposed methods.

**Selectivity.** Glucose, fructose, lactose, starch, and magnesium stearate, the commonly occurring excipients in the commercialdosages were examined in order to verify its interference. Monosaccharides and polysaccharides which are optically active did not interfere with the CD signal as they do not absorb in the UV region. The methods showed no interference from excipients.

**Effect of Forced Degradation.** To study the stability of drug under stress conditions, captopril was induced to oxidation, sunlight, alkaline, and acidic hydrolysis, leading to appreciable changes. Summary of results of stress degradation studies of captopril is reported in Table 3. Captopril disulphide is the main degradation product of captopril under all stressed conditions.

**Application.** Captopril in two different pharmaceutical dosage forms was determined by the proposed methods and the reference method. The results of the proposed method were compared with those of the reference method using point and

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**Table 1. Optical and Statistical Results of Regression Analysis**

| Parameters | zero order | second order derivative |
|------------|------------|-------------------------|
|            | method A   | method B                | method C                |
| linear dynamic range (\( \mu g \) mL\(^{-1}\)) | 10–80 | 10–70 | 10–70 |
| regression equation | \( \Theta = -0.354 \) \[CAP\] – 0.429 | \( \Theta = 0.003 \) \[CAP\] + 0.011 | \( \Theta = -0.002 \) \[CAP\] – 0.002 |
| correlation coefficient \( (R^2) \) | 0.9996 | 0.9987 | 0.9994 |
| intercept | -0.4291 | 1.194 \times 10^{-2} | -2.40 \times 10^{-3} |
| \( S_a \) | 0.1357 | 2.37 \times 10^{-3} | 1.03 \times 10^{-3} |
| \( \pm S_a \) | 0.3209 | 5.82 \times 10^{-3} | 2.53 \times 10^{-3} |
| slope | -0.3546 | 3.29 \times 10^{-3} | 2.30 \times 10^{-3} |
| \( S_b \) | 2.68 \times 10^{-3} | 5.31 \times 10^{-3} | 2.32 \times 10^{-3} |
| \( \pm S_b \) | 6.36 \times 10^{-3} | 1.30 \times 10^{-4} | 5.67 \times 10^{-5} |
| detection limit (\( \mu g \) mL\(^{-1}\)) | 1.26 | 2.38 | 1.48 |
| quantitation limit (\( \mu g \) mL\(^{-1}\)) | 3.83 | 7.22 | 4.50 |

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**Figure 4.** Second-order derivative CD spectra of captopril (10–70 \( \mu g \) mL\(^{-1}\)).

**Figure 5.** Calibration plots for second-order derivative methods (method B at 208 nm and method C at 225 nm).
As the calculated \( t \) - (paired) and \( F \) -values at the 95% confidence level were less than the tabulated ones, it was concluded that no significant difference among the two methods was observed. In the interval hypothesis test, the true bias based on recovery experiments was evaluated from the following equation \[ \bar{X}_1 - \bar{X}_2 = \frac{S_p^2 \cdot t_{\alpha/2}}{n_1} + \frac{S_p^2 \cdot t_{\alpha/2}}{n_2} \] where \( \bar{X}_1 \) and \( \bar{X}_2 \) are mean values determined by the proposed and reference methods, respectively. \( S_p \) and \( t \) are the pooled standard deviation and one-sided \( t \)-value at the 95% confidence level, respectively. \( n_1 \) and \( n_2 \) are the number of measurements of the proposed and reference methods, respectively. The values of lower limit (\( \theta_L \)) and upper limit (\( \theta_U \)) are reported in Table 4.

**CONCLUSIONS**

Simple and selective CD spectroscopic methods were first designed for analysis of captopril in commercial dosages. This methodology is based on the measurement of ellipticity against captopril concentration. The adequate sensitivity (LOD = 1.26, 2.38, and 1.48 \( \mu \)gm L\(^{-1} \) for the respective methods) and high precision (RSD \( \leq \) 1.5%) over the calibration range made the proposed methods favorable over the existing methods for the
The ellipticities were measured at 208 and 225 nm for a series of solutions (10−70 μg mL⁻¹). Calibration graphs were ascertained by plotting second-order derivative ellipticity against concentrations of captopril. Alternatively, regression equations were also obtained.

**Degradation Studies.**

- **i. Alkaline- and acidic-forced degradation:** 10 mg of captopril in 100 mL of 0.1 mol L⁻¹ sodium hydroxide or hydrochloric acid was refluxed separately at 60 °C for 48 h. After cooling the solutions to room temperature, the individual samples were neutralized with 0.2 mol L⁻¹ sodium hydroxide or hydrochloric acid to avoid further decomposition. This forced degradation in alkaline and acidic media was performed in dark so as to prevent possible photodegradation. Suitable aliquots were taken from the stock-degraded samples for assay under the aforementioned conditions.

- **ii. Oxidative-forced degradation:** The stock solution of captopril was prepared by dissolving 10 mg of a pure drug in 10 mL of distilled water. To this solution, 1 mL of 30% hydrogen peroxide was added and the volume was completed to 100 mL with distilled water. The solution was kept for 7 days at room temperature. Suitable aliquots were taken for analysis.

- **iii. Effect of sunlight:** Captopril solution (100 μg mL⁻¹; 100 mL) was exposed to sunlight for 7 days and analysed using the proposed procedures.

**Procedure for the Assay of Captopril in Pharmaceutical Formulations.** A single tablet containing 25 mg captopril from each brand, namely acetin and capotril, was weighed and crushed to fine powder. A portion of the fine powder corresponding to 10 mg of the captopril was weighed accurately and swirled in distilled water for some time in order to achieve complete dissolution of the active drug. The residue was then filtered on a Whatman no. 1 filter paper and the filtrate was transferred into a 100 mL volumetric flask and completed to volume with distilled water. Suitable aliquot was taken to perform quantitative analysis following the methods A, B, and C.

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**Table 4. Point and Interval Hypothesis Tests: Comparison of the Proposed Methods with the Reference Method at 95% Confidence Level**

| formulations | method A | method B | method C | reference method |
|--------------|----------|----------|----------|------------------|
| acetin       | % recovery | 100.64   | 99.86    | 99.52            | 100.30           |
| RSD (%)      | 1.28      | 0.84     | 1.01     | 1.11             |
| t-value      | 0.45      | 0.71     | 1.16     |                  |
| F-value      | 1.34      | 1.78     | 1.21     |                  |
| Θ_L          | 0.980     | 0.990    | 0.992    |                  |
| Θ_U          | 1.018     | 1.019    | 1.020    |                  |
| capotril     | % recovery | 99.92    | 99.38    | 99.36            | 99.60            |
| RSD (%)      | 1.07      | 0.83     | 1.30     | 1.01             |
| t-value      | 0.54      | 0.38     | 0.33     |                  |
| F-value      | 1.13      | 1.48     | 1.65     |                  |
| Θ_L          | 0.983     | 0.989    | 0.985    |                  |
| Θ_U          | 1.015     | 1.016    | 1.019    |                  |

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Experimental Section

Materials and Reagents. Captopril was purchased from Sigma-Aldrich (St. Louis, USA). Sodium hydroxide and hydrochloric acid were obtained from Qualigens Fine chemicals Ltd., India. Hydrogen peroxide (30%) was purchased from Avantor Performance Materials India Ltd. (Thane, India). The commercial dosages of captopril, such as Acetin (Wockhardt Ltd., India) and Capotril (Lupin Laboratories Ltd., India), were purchased from local pharmacy. Each tablet was labeled to contain 25 mg captopril.

**Equipment.** A JASCO J-815 CD spectrometer (Jasco Corporation, Tokyo, Japan) was used for recording CD spectra at ambient temperatures. This instrument is equipped with a 150 W Xenon lamp, 2.0 mm quartz cuvette, and temperature control unit. The following parameters were used for recording the spectra.

- Bandwidth = 2 nm
- Response time = 15 s
- Standard sensitivity
- Wavelength range = 200−300 nm
- Scanning rate = 50 nm/min
- Scan accumulations = 04 (averaged at the end)

The pH measurements were carried out using an Eutech digital pH meter (model: Cyberscan pH 2100).

**Preparation of Stock Solutions.**

- Captopril (0.1 mg mL⁻¹) standard solution was prepared by dissolving 10 mg of the pure drug in 100 mL distilled water.
- 0.1 and 0.2 mol L⁻¹ sodium hydroxide solutions were prepared in distilled water.
- 0.1 and 0.2 mol L⁻¹ hydrochloric acid solutions were also prepared in distilled water.

**Analytical Procedures. Method A (Zero Order).** Varying volumes of the stock solution of captopril (0.1 mg mL⁻¹) equivalent to 10−80 μg mL⁻¹ were pipetted into a 10 mL volumetric flask and diluted to volume with distilled water. The zero-order spectra of all the solutions were recorded against distilled water as blank. The ellipticity (θ) at 208 nm was plotted against the concentration of pure drug. Also, the regression equation was developed to determine the concentration of captopril in the samples.

**Methods B and C (Second-Order Derivative).** Zero-order spectra were derivatized in the wavelength range of 200−300 nm and the second-order derivative spectrum exhibited a positive band peaking at 208 nm and a negative band centered at 225 nm. The ellipticities were measured at 208 and 225 nm for a series of solutions (10−70 μg mL⁻¹). Calibration graphs were ascertained by plotting second-order derivative ellipticity against concentrations of captopril. Alternatively, regression equations were also obtained.

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Quantification of captopril. In addition, the results obtained in the analysis of captopril in commercial dosages demonstrated its successful applicability in the quality assurance of an active pharmaceutical ingredient.
The authors are grateful to the Chairman, Department of Chemistry, Aligarh Muslim University, Aligarh, for providing necessary research facilities. UGC (DRS-II) and DST (FIST and PURSE) are also acknowledged for providing necessary supports to carry out this work. One of the authors (Sumaiya Khan) is thankful to DST (Purse) for research associateship.

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