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Area under curve UV spectrophotometric method for determination of captopril in bulk and chromatographic method development for the identification of captopril by TLC.

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ABSTRACT

The aim of this work is to establish simple economical, and rapid spectrophotometric and chromatographic identification method for qualification of captopril in Active Pharmaceutical Ingredient. The work was carried out to for estimation of captopril in bulk pharmaceutical from by utilizing area under curve (AUC)method using UV –Visible Spectrophotometry .For this purpose the wavelength range 200-400 nm was selected. Thin-Layer chromatography (TLC) is a chromatography used to separate non-volatile mixtures. TLC can be used for monitoring the progress of a reaction ,identification compounds present in a given mixture, and determination of active pharmaceutical ingredient .TLC analysis of captopril in pharmaceutical and for using this method of analysis in future for the development of bio analytical method .for TLC the mobile phase with different concentration was used .we have established that the most perfect RF observed using mobile phase .chloroform –methanol are used (9:1)and (8:2) ratio .the development method was found to be simple ,linear ,precise ,accurate and sensitive which can be used for routine analysis for spectrophotometric and Thin-layer chromatographic method estimation of active pharmaceutical Ingredient.

Keywords: Captopril, Thin layer chromatography, Spectroscopic, method development, AUC, Angiotensin.

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INTRODUCTION

Captopril is an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of hypertension and some type of congestive heart failure. captopril was the first ACE inhibitor developed and was considered a breakthrough both because of its novel mechanism of action and also because of the revolutionary development process. Adverse effect of captopril include cough due to increase in plasma level of bradykinin, angioedema, agranulocytosis, hyperkalemia, teratogenicity, postural hypotension, acute renal failure [1,2,3,10].

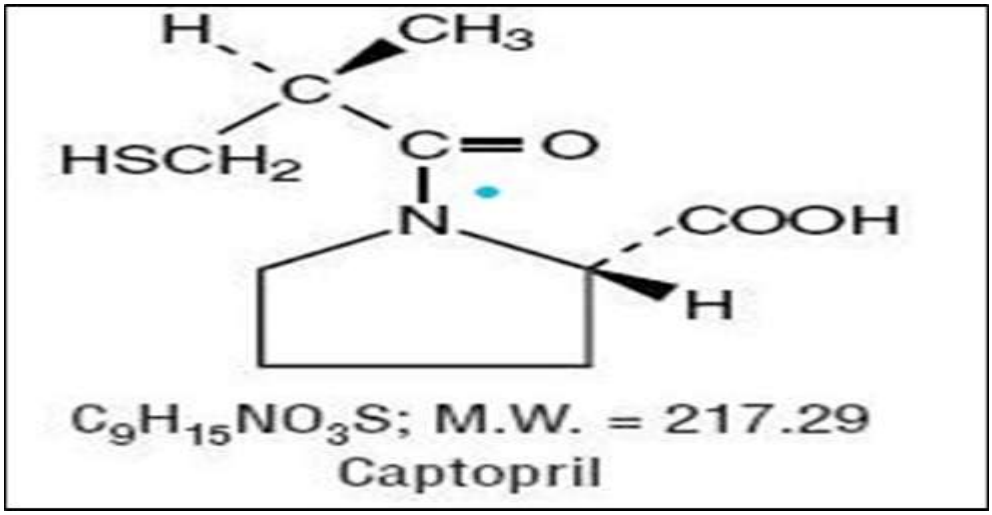

Figure 1: Chemical Structure of Captopril

MATERIALS AND METHOD

The entire chemicals used were of analytical grade. All solutions were freshly prepared with double distilled water.

Sample Preparation of Captopril for TLC:

Reference solution – About 10.0mg bulk drug of captopril dissolved in methanol and dilute with same solvent to 10.0ml.

Mobile phase –Chloroform-Methanol (9:1) and Chloroform-Methanol (8:2)

Sample that are applied 5ul applied the test solution and investigation solutions over a path of 10cm from starting line.

Detection: -Examination in ultraviolet light at 254 nm.

Instrumentation

Shimadzu (Kyoto, Japan) model UV- 1800 double beam UV- Visible spectrophotometer attached with computer operated software UV probe 2.33 with spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was used to measure absorbance of the resulting

solutions. Analytical balance of make Mettler Toledo (Model JL 1503- C) was used for weighing purpose.

METHOD:

Experimental Work

A) To check the solubility of Captopril:

25 mg of Captopril was weighed and solubility of this sample was checked in 25 ml distilled water, methanol, ethanol, acetone, methyl acetate, acetonitrile, butyl acetate.

B) To identify the λ max of Captopril:

Weigh1gm of the pure drug and dissolve it in small portion of methanol and make up the volume up to 10 ml using distilled water to give a standard stock solution of 1000µm/ml. From above solution 2.5 ml of the standard solution was withdrawn in volumetric flask and diluted to 25 ml to prepare 100ppm solution. Suitable dilutions were made with distilled water to get standard solutions of concentrations: 5, 10, 15, 20, and 25 µm/ml. [7,8] Spectrum peak details are shown in Figure 2 Spectrum peak pick.

Table 1: Calibration curve of Captopril.

Concentration	Absorbance
5	0.322
10	0.428
15	0.531
20	0.627
25	0.726

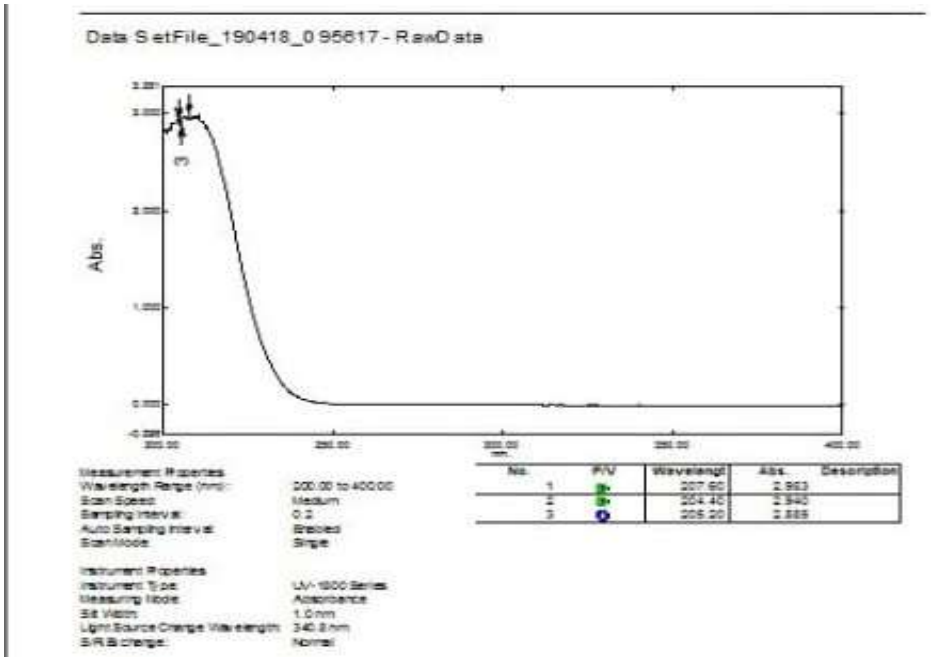

Figure 2: Spectrum Peak pick

C) Area Under Curve Method:

In case of AUC (Area under Curve) method is applicable where there is sharp peak or broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by the entering the wavelength ranges over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC. Thus, the calibration curve can be constructed by plotting concentration versus AUC. [4, 5]

D) Analytical Method Development and Validation:

Linearity:

The linearity of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample. For which demonstrated that the analytical procedure is of linearity. The standard solution of Captopril (5, 10, 15, 20, and 25 µm/ml) was pipette out in a separated series of 10ml volumetric flask. Make up the volume with distilled water and mixed well. The absorbance maxima and area under curve for the solutions was measured at 205 nm and range of 200 – 400 nm for two methods respectively against distilled water as blank. Calibration Curve table of Captopril is shown in Table. 1. Calibration curve of Captopril. [4, 6]

RESULTS AND DISCUSSION:

A) Calibration Curve for Drug:

Absorbance maxima method:

In the Experimental conditions described, the graph obtained for the absorbance maxima for pure drug showed linear relationship (Figure 3). Regression analysis was made for the slope, intercept and and correlation coefficient values. The regression equations of calibration curve were y = 0.1007x+0.2247 (r^2 = 0.996) at 205nm for absorption maxima the range was found to be 5 - 25 µm/ml for the UV spectrophotometric analysis. Calibration Curve is shown in Table. 1. Calibration Curve of Captopril. Calibration curve of Captopril is shown in Figure. 3. Calibration Curve of Captopril.

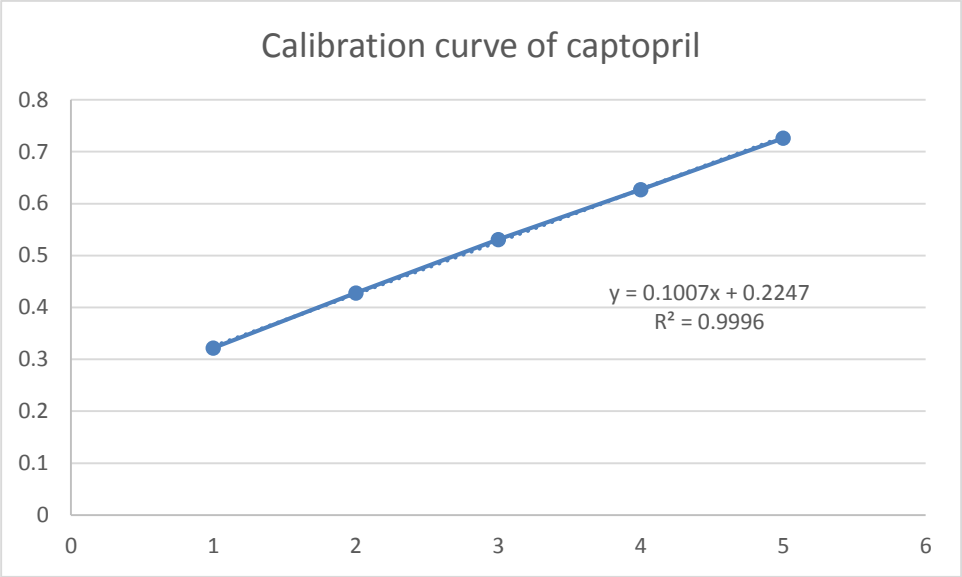

Figure 3: Calibration Curve of captopril.

A) Area under Curve Method:

In the Experimental conditions described, the graph obtained for the Area under Curve (AUC) spectra showed linear relationship (Figure 4). Regression analysis was made for the slope, intercept and correlation values. The equation is y = 0.1007x+0.2247 (r^2 = 0.996) at 200 – 400 nm for Area under Curve spectrophotometry analysis. The range was found to be 5 - 25 µm/ml for the Area Under Curve UV spectrophotometric analysis.

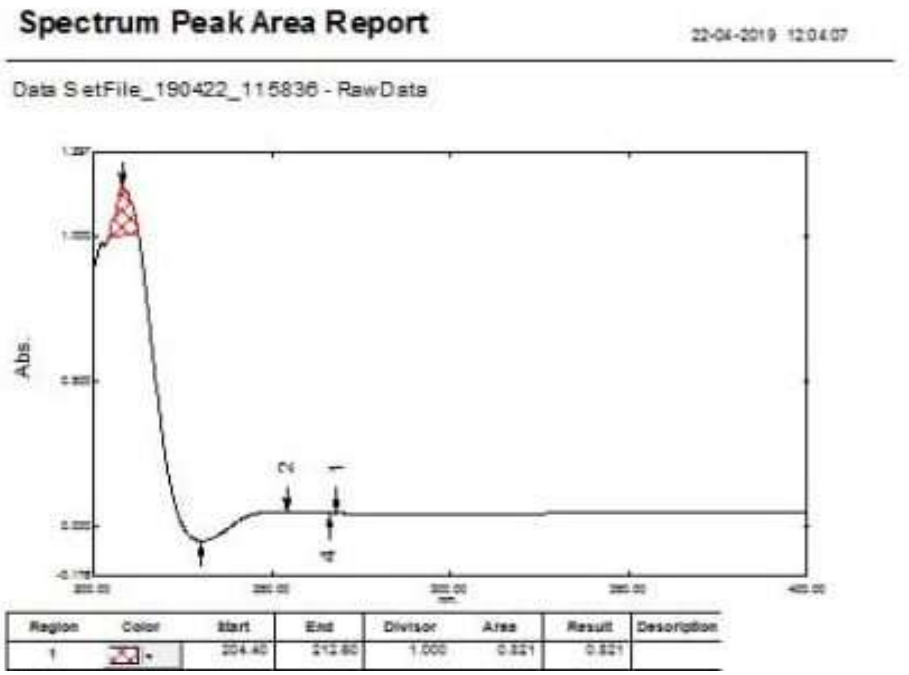

Figure 4: Area Under Curve of Captopril.

Table 2: Area Under curve of Captopril.

Parameter	AUC
Wavelength Range (nm)	200-400
Concentration Range (um/ml)	5-25
Slope (m)	0.1007
Intercept (c)	0.2247
Correlation Coefficient ((r^2)	0.996

Development of methods for the chromatographic identification of API from group of angiotensin-converting enzyme inhibitors in pharmaceutical

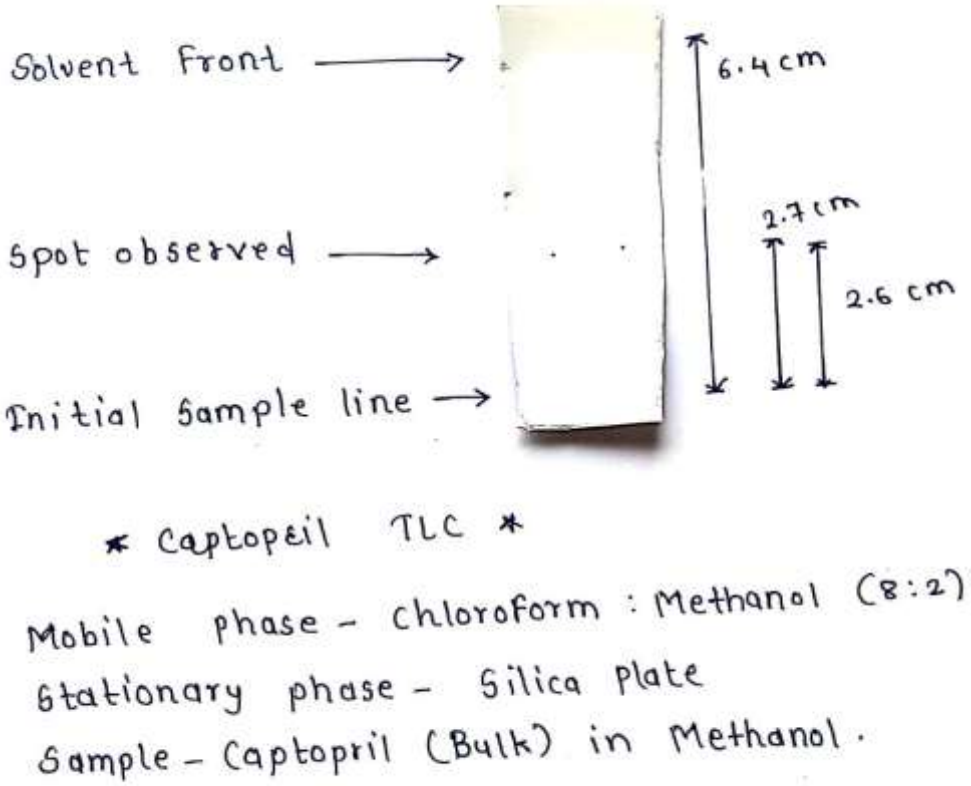

Figure 1: Representative chromatogram of captopril using ultraviolet detection at 254nm, mobile phase –chloroform-methanol (8:2).

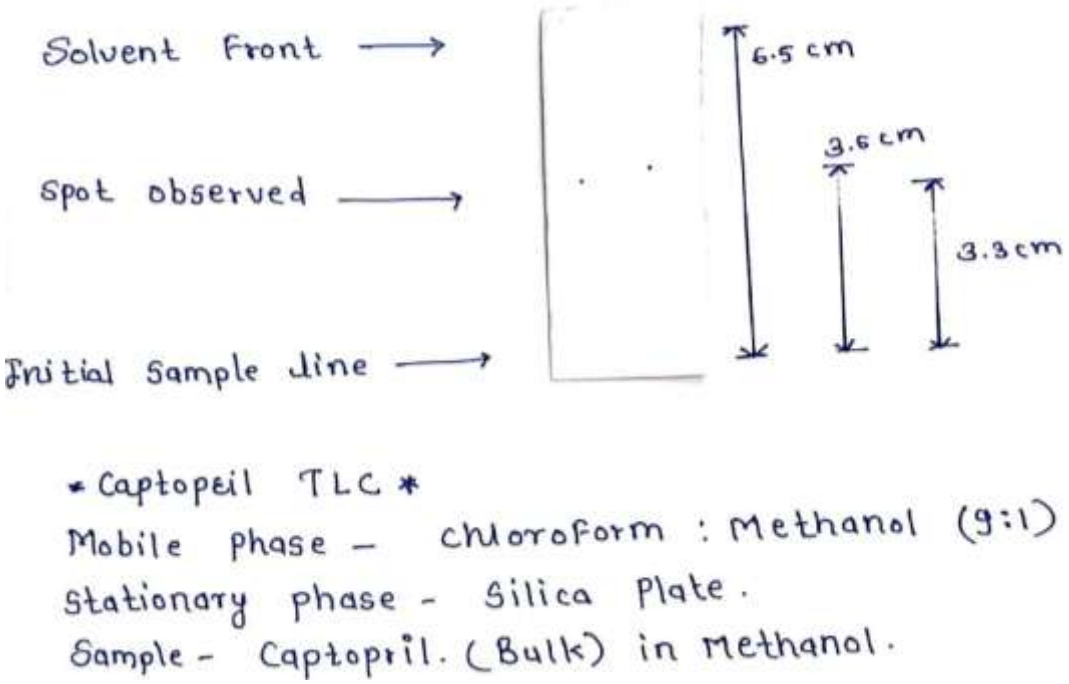

Figure 2: Representative chromatogram of captopril using ultraviolet detection at 254nm, mobile phase –chloroform-methanol (9:1).

CONCLUSION:

The simple and economic UV spectrophotometric AUC methods have been developed for the determination of Captopril. Because of cost – effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industries and successfully applied and suggested for the qualitative analysis of Captopril in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy. The results show the UV spectrophotometric method was found to be accurate, precise and sensitive. A chromatographic method is developed for identification of captopril by using mobile phase Chloroform-methanol as (9:1) and (8:2) ratio. these method can also give excellent result and can be employed for the routine analysis. Prospects for future research will be aimed at developing of bio analytical methods of determination of captopril in medicines and for analysis of their metabolites.

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