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RESEARCH ARTICLE

# Simultaneous Determination and Validation of Hydrochlorothiazide & Eprosartan in bulk drug and Marketed Formulation by Rp-Hplc

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## ABSTRACT

Validation of developed and RP HPLC analytical method according to ICH Guidelines. A review of literature reveals that no analytical methods are available for these drugs. There are no methods in the literature for the estimation of drugs in combined dosage forms. Analytical methods have been report for hydrochlorothiazide and eprosartan using RP-HPLC in pharmaceutical dosage form. The development of simple and precise reverse phase high performance liquid chromatography of hydrochlorothizide and eprosartan using single method in bulk in bulk drug and market formulation.

**Key Words- Hydrochlorothizide, Eprosartan, RP- HPLC**

## INTRODUCTION

Chromatography is a non-destructive procedure for resolving a multi-component mixture of trace, minor, or major constituents into its individual fractions. Different variations may be applied to solids, liquids, and gases. While chromatography may be applied both qualitatively and quantitatively, it is primarily a separation tool. Quantitative analysis can be carried out by measuring the area of the chromatographic peak. Chromatography may be defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases.

**The chromatographic method of separation, in general, involves the following steps:**

- Adsorption or retention of a substance or substances on the stationary phase.
- Separation of the adsorbed substances by the mobile phase.
- Recovery of the separated substances by a continuous flow of the mobile phase; the method being called elution.
- Qualitative and quantitative analysis of the eluted substances[1].

## PRINCIPLE OF SEPARATION IN HPLC

The principle of separation in normal phase mode and reverse phase mode is adsorption. When mixtures of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationery phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated.

The most important component of HPLC in solvent delivery system is the pump, because its performance directly effects the retention time, reproducibility and detector sensitivity. Among the several solvent delivery systems (direct gas pressure, pneumatic intensifier, reciprocating etc.) reciprocating pump with twin or triple pistons is widely used, as this system gives less baseline noise, good flow rate reproducibility etc.

## MATERIALS

**Table No. 1. Chemicals and Solvents Used**

| S. No. | Chemicals | Manufacture |
|---|---|---|
| 01 | Eprosartan | Macleod's Pharmaceutical Ltd. |
| 02 | Hydrochlorothiazide | Kalindi Medicure Pvt. Ltd. |
| 03 | 3 Acetonitrile | Merck Ltd., India |

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| | | |
|---|---|---|
| 04 | Methenol | Merck Ltd., India |
| 05 | water | Merck Ltd., India |

## METHODS FTIR SPECTRUM

IR absorption spectra Eprosartan and Hydrochlorothiazide was obtained by KBr pellet method.

**Physiochemical characteristics**

- **Melting point**- M.P. of the Eprosartan and Hydrochlorothiazide was determined by melting point apparatus (lab hosp) and found to be 248-250ºC and 270-273 ºC respectively.

## ANALYTICAL METHOD DEVELOPMENT OF EPROSARTAN AND HYDROCHLOROTHIAZIDE BY HPLC:-

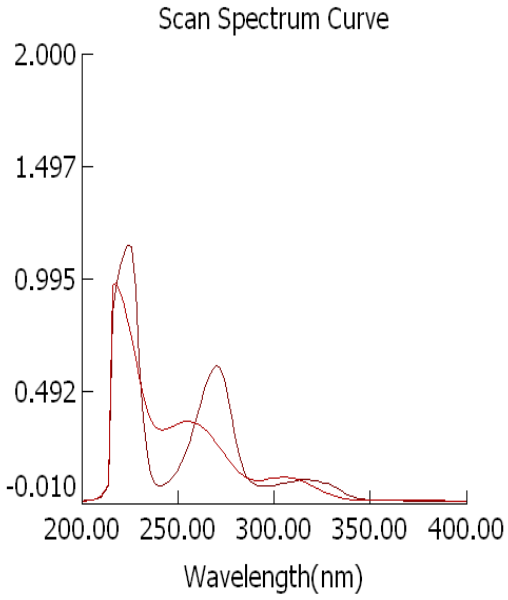



**Figure1. Wavelength of Eprosartan and Hydrochlorothiazide**

**I. Mobile Phase Selection:-**

Initially to estimate Eprosartan and Hydrochlorothiazide number of mobile phase in different ratio were tried. Results are shown in (Table no 02 & 03.). Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 50 mM KH2PO4 (pH4.0 with OPA): Acetonitrile in the ratio of 30**:**70 v/v. The mobile phase was filtered through 0.45 filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

**II. Selection of wavelength:-**

100 mg of Eprosartan and Hydrochlorothiazide was weighed accurately and transferred to a 100 ml volumetric flask, and the volume was adjusted to the mark with the mobile phase in the ratio of 30**:**70 v/v. From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200-400nm), maximum absorbance was found at **λ max** 270 nm.

**III. Preparation of Standard Stock Solution:-**

10 mg of Eprosartan and 10 mg of Hydrochlorothiazide was weighed accurately and transferred to separate 10 ml volumetric flask, and the volume was adjusted to the mark with the mobile phase (50 mm KH2PO4 (pH4.0 with OPA): Acetonitrile in the ratio of 30**:**70 v/v) to give a stock solution of 1000 g/ml.

**IV. Preparation of Working Standard Solution:-**

From stock solutions of Eprosartan 1 ml was taken and diluted up to 10 ml. from this solution 0.8, 1.6, 2.4, 3.2 and 4.0 ml solutions were transferred to 10 ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 8, 16, 24, 32, and 40 **µ**g/ ml concentration and From stock solutions of Hydrochlorothiazide 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0 and 2.5 ml solutions were transferred to 10 ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20 and 25 **µ**g/ ml concentration.

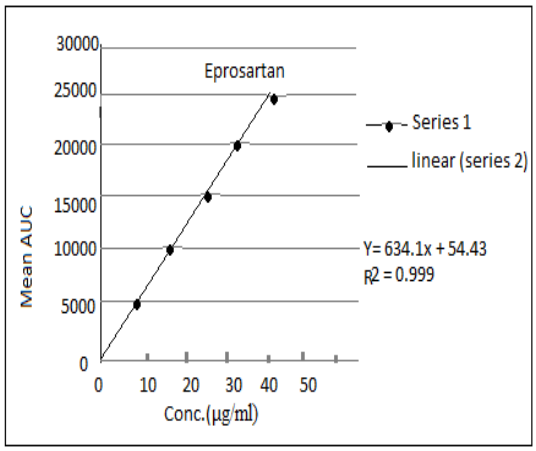



**Figure . Calibration Graph of Eprosartan**

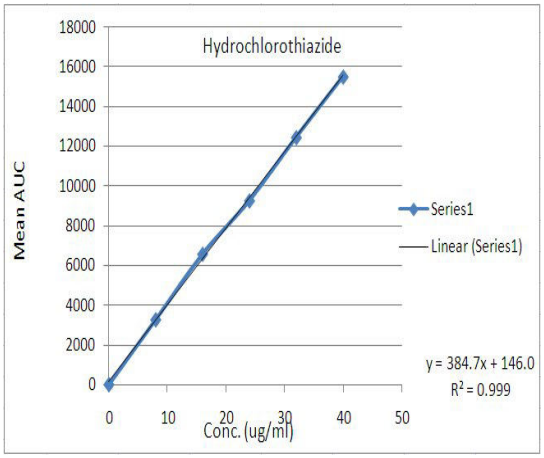



**Figure - Calibration Graph of Hydrochlorothiazide**

## V. Assay of tablet formulation

For analysis of the formulation, twenty tablets taken and determine the average weight. Powder equivalent to weight 32 mg of Eprosartan was transferred to 10 ml volumetric flask and dissolved in HPLC grade methanol. The solution was shaking vigorously for 10 mins and filtered through Whatman filter paper no.41, then volume was made up to mark with methanol. From the above solution 1 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 100 µg/ml. From the above solution 3.2 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 32 µg/ml. of Eprosartan and corresponding concentration of Hydrochlorothiazide 12.5 µg/ml. The solution contains Eprosartan and Hydrochlorothiazide in the proportions of 32:12.5. The amounts of Eprosartan and Hydrochlorothiazide calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation.

# RESULT AND DISCUSSION

**Figure- FTIR spectra of Eprosartan**

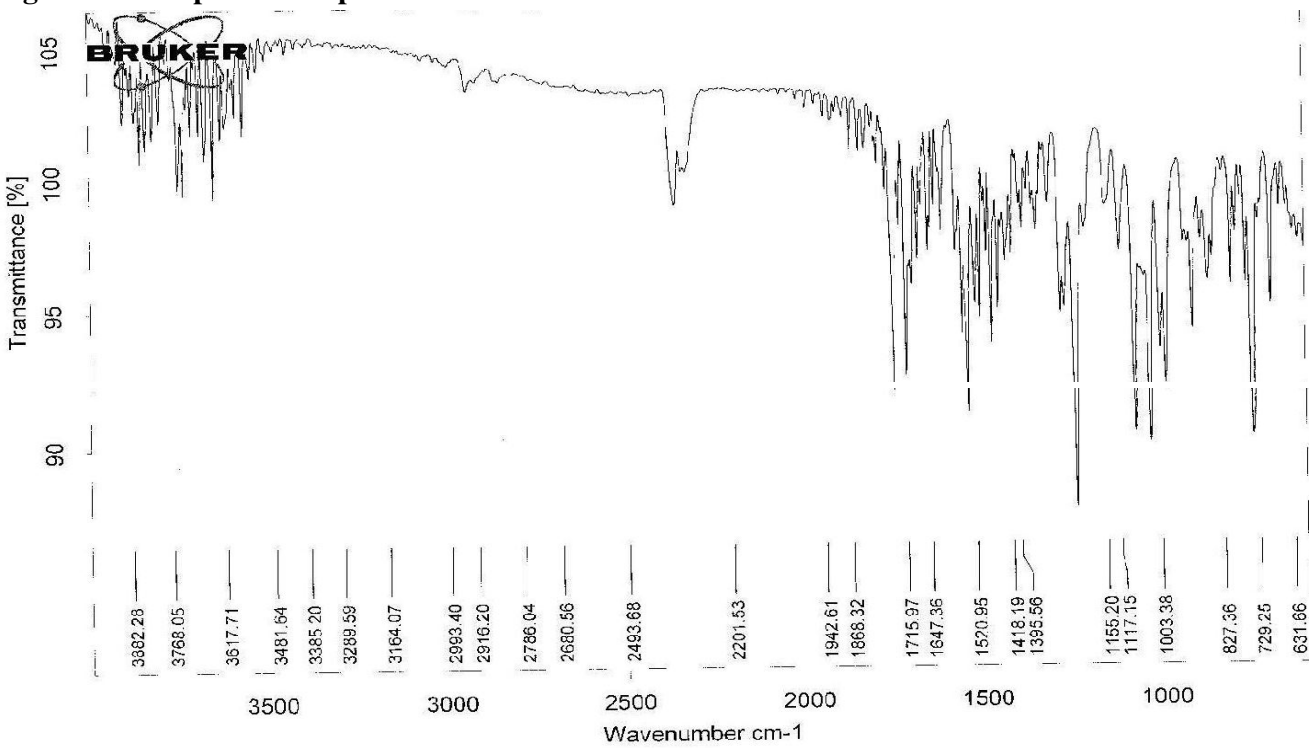
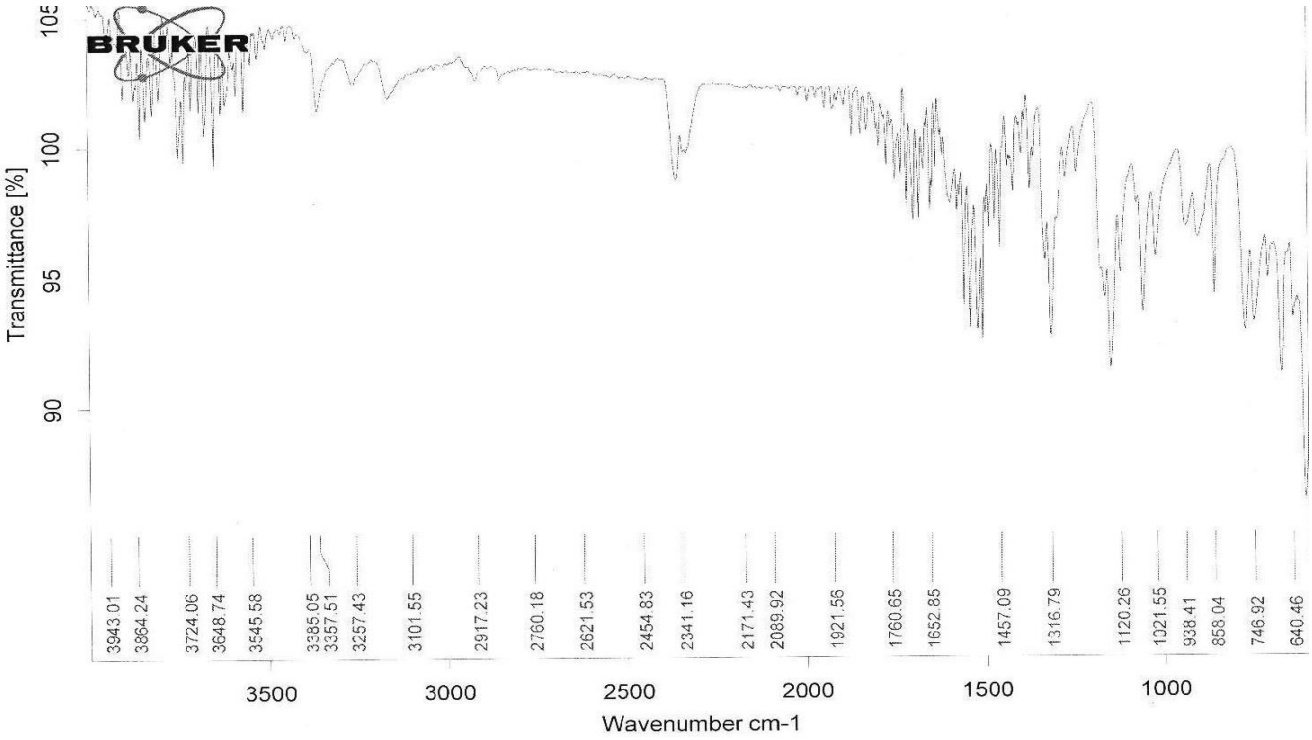


**Figure - FTIR spectra of Hydrochlorothiazide**




## SOLUBILITY:-

**Table No.2 - Solubility of Eprosartan mesylate**

| S.No. | Solvent | Solubility |
|---|---|---|
| 01 | Water | Insoluble |
| 02 | 0.1N HCl | Insoluble |
| 03 | Methenol | Freely soluble |
| 04 | Ethenol | Freely soluble |
| 05 | Acetone | Freely soluble |
| 06 | Methenol:water (80:20) | Springly soluble |

**Table No. 3 -Solubility of Hydrochlorothiazide**

| S.No. | Solvent | Solubility |
|---|---|---|
| 01 | Water | Insoluble |
| 02 | 0.1N HCl | Insoluble |
| 03 | Methenol | Freely soluble |
| 04 | Ethenol | soluble |
| 05 | Acetone | soluble |
| 06 | Methenol:water (80:20) | soluble |

## Linearity and Calibration Graph:-

**Table No.4 - Result of Linearity of Eprosartan**

| Std. Conc. (µg/ml) | 0 | 8 | 16 | 24 | 32 | 40 |
|---|---|---|---|---|---|---|
| 1 | 0 | 5214.365 | 10454.236 | 14689.125 | 20578.365 | 25455.236 |
| 2 | 0 | 5245.145 | 10467.336 | 14685.256 | 20547.235 | 25469.365 |
| 3 | 0 | 5241.363 | 10468.225 | 14701.365 | 20569.325 | 25471.236 |
| Mean | 0 | 5233.624 | 10463.27 | 14691.92 | 20564.98 | 25465.28 |
| SD | 0.00 | 13.70565 | 6.395245 | 6.866073 | 13.07571 | 7.142434 |
| %RSD | 0.000 | 0.261877 | 0.061121 | 0.046734 | 0.063582 | 0.028048 |

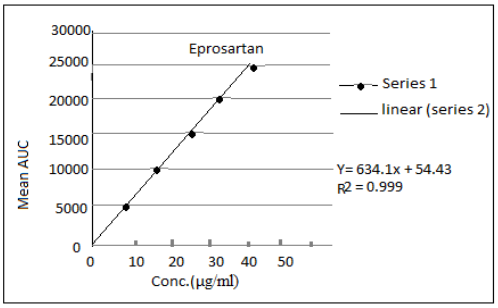



**Figure - Calibration Graph of Eprosartan**

### Regression Equation

Y= mx +c,

Y= AUC
m= slope =634.1
X= Conc. in g/ml
c= Intercept = 54.43

r2= 0.999

**Table No.5 - Result for Linearity of Hydrochlorothiazide**

| Std. Conc. (µg/ml) | 0 | 5 | 10 | 15 | 20 | 25 |
|---|---|---|---|---|---|---|
| 1 | 0 | 3257.359 | 6574.236 | 9245.365 | 12458.365 | 15478.369 |
| 2 | 0 | 3267.89 | 6547.236 | 9285.658 | 12468.365 | 15489.365 |
| 3 | 0 | 3285.589 | 6589.236 | 9241.369 | 12425.365 | 15539.659 |
| Mean | 0 | 3270.279 | 6570.236 | 9257.464 | 12450.7 | 15502.46 |
| SD | 0.00 | 11.64803 | 17.37815 | 20.0028 | 18.37269 | 26.68096 |
| %RSD | 0.000 | 0.356178 | 0.264498 | 0.216072 | 0.147563 | 0.172108 |

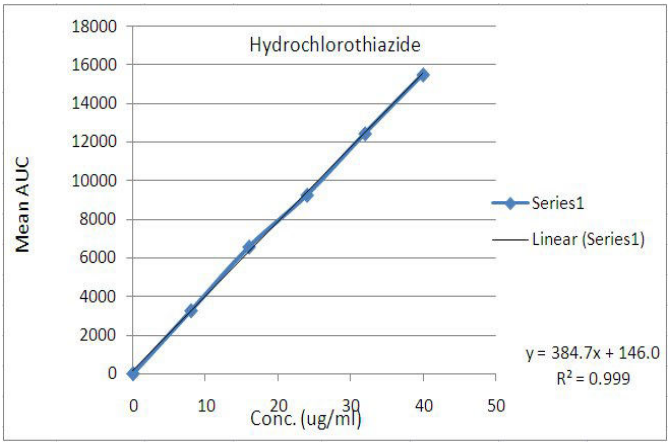



**Figure 9- Calibration Graph of Hydrochlorothiazide**

## Regression Equation

Y= mx +c,

m= slope = 384.7

X= Conc. in g/ml

c= Intercept 146.0

r2 = 0.999

**TableNo.6-Result of Analysis for Eprosartan and Hydrochlorothiazide in Tablet Formulation**

| µg/ml | EPROSARTAN | HYDROCHLOROTHIAZIDE |
|---|---|---|
| | 32 | 12.5 |
| Rep-1 | 31.99 | 12.5 |
| Rep-2 | 31.99 | 12.45 |
| Rep-3 | 32.00 | 12.48 |
| % found* | | |
| Rep-1 | 99.96875 | 100 |
| Rep-2 | 100 | 99.6 |
| Rep-3 | 100.0313 | 100.241 |
| SD | 0.31255 | 0.323754 |
| %RSD | 0.031255 | 0.323925 |

## CONCLUSION-

The method can be used for the estimation of Eprosartan and Hydrochlorothiazide simultaneously from its Tablet dosage form shows the Recovery results of the two different formulations on addition of Standard drug by HPLC. The Mean percentage Recovery and RSD were found to be less than 2 so the method was found to be more accurate without any interference.

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