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# RP-HPLC ANALYTICAL METHOD OF DEVELOPMENT AND VALIDATION FOR SIMULANEOUS ESTIMATION OF TWO DRUGS LISINOPRIL, HYDROCHLOROTHIAZIDE AND ITS PHARMACEUTICAL DOSAGE

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## 1. ABSTRACT

*A new simple precise accurate RP-HPLC method was developed for the simultaneous estimation of LISINOPRIL and HYDROCHLORTHIAZIDE in solid dosage form ISOCRATIC MODE with a mixture of METHANOL AND AMMONIUM ACETATE BUFFER(PH:4.8)-30:70 wasselected as the mobile phase with a water – HYPERSIL C18 COLOUMN -250×4.6M,5µ equivalent . this mixture was found to be appropriate allowing good elution for lisinopril and hydrochlorthiazide and the retention time are found to be 1.991 min and 3.213 min respectively at flow rate of ml/min and detection wavelength of 212nm the linearity was found in concentration range of 20µg/ml to 80µg/ml for lisinopril and 25µg/ml to 100µg/ml for hydrochlorthiazide runtime of the method for elution of both lisinopril and hydrochlorthiazide was found to be 7min the method was extensively validated for linearity accuracy and precesion limit of detection and quantitation ruggedness and robustness all these analytical validation parameters were observed and the % RSD was determined which indicates that the RP-HPLC method for determnation of lisinopril and hydrochlorthiazide is useful.*

***KEY WORDS:Lisinopril , hydro chlorothiazide in HPLC ,LC Method developmeny and validation .***

## 2. INTRODUCTION

Analytical chemistry is often described as the area of chemistry involved in Characterization of the composition of matter both qualitatively (what is present) and quantitatively (how much is present) analytical chemistry is not a separate branch of chemistry but simply the application of chemical knowledge

PHARMACEUTICL ANALYSIS is the branch of analytical chemistry responsible for separating identifying and determining the relative amounts of the components making up a sample matter it is mainly involved in the qualitative identification or detection of compounds and quantitatively analysis of the substances present in bulk and p harmaceutical preparations

Quantitative analysis constitutes the largest part of analytica part of chemistry and is related to various methods and instruments employed in determining the concentration or amounts of constituents in samples it is also one of the basic criteria in the field of pharmacy where quality is t be critically maintained analytical chemistry maybe deined as the science and arts of determining the composition of materials in terms of elements of compounds contained analytical method is a specific applications of a technique to solve an analytical problems

Physico chemical methods are used to study the physical phenomenon that occurs as a result of chemical reaction among the physico chemical methods the most imp are optical (refracometry ,polarimetry . emission . fluorescence methods of analysis photometry including photo-colorimetry and spectrometry covering uv-visible and ir regions and nephlometry or turbidometry ) and chromatographic (coloumn paper tlcglc and hplc ) methods methods such as nmramd PNMR are becoming more and more popular . the chemical methods include the gravimetric and volumetric procedures which are based on the complex formation acid base precipitation and redox reactions. Titrations In non aqueous media and complexometry have also been used in pharmaceutical analysis .the number of new drugs is constantly growing . the requires new methods for controlling their quality moder pharmaceutical analysis must need the

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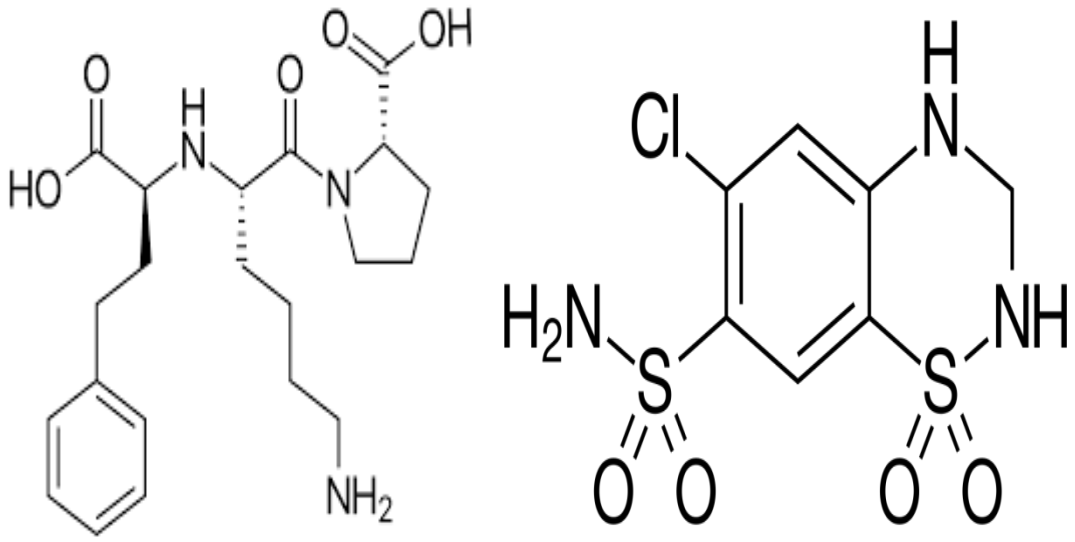


LISINOPRIL HYDROCHLOROTHIAZIDE

Figure1: structure of lisinopril and hyrochlorothiazide

## 3. Method of validation

system suitability :

A standard solution was preparedby using lisinopril and hydrochlothiazide working standard as per test method and was injected five times into the HPLC system . The system suitability parameterswere evaluated from standard chromatograms bycalculating the % RSD from five replicate injections for lisinopril and hydrochlorothiazide , retention times and peak areas .

Acceptance criteria :

1 . The % RSD for the retention times of principal peak from 5 replicate injections of each standard solution should be not more than 2.0 %

2. The % RSD for the peak area responcesof principle peak from 5 replicate injections of each standard solution should be not more than 2.0 %

3. The number of theoretical plates ( N ) for the lisinopril and hydrochlorothiazide peaks are

NLT 3000.

4.The trailing factor ( T ) for the lisinopril and hydrochlorothiazide peaks is NMT 2.0

Observation

The % RSD for the retention times of principal peak areas were found to be within the limit . refer tab 6.9 as showing in fig: 6.13 -6.17 .

5.3.2 : specificity

Lisinopril and hydrochlorothiazide identification :

Solutions of standard and samples were prepared as per the test method are injected into chromatographic system .

Acceptance criteria :

1.

Chromatogram of standard and sample should be identical with near retention time .

Observation

The chromatograms of standard and sample were same identical with retention time .

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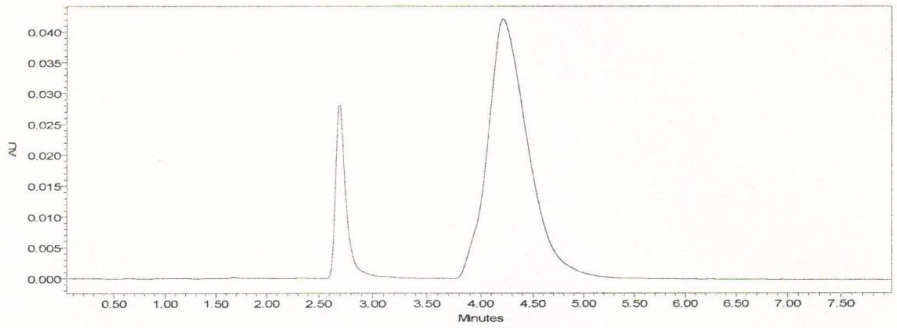
# 6. RESULTS AND DISCUSSION

## 6.1 METHOD DEVELOPMENT

### 6.1.1 Trail 1

**Fig 6.1 Chromatograph of trail 1**

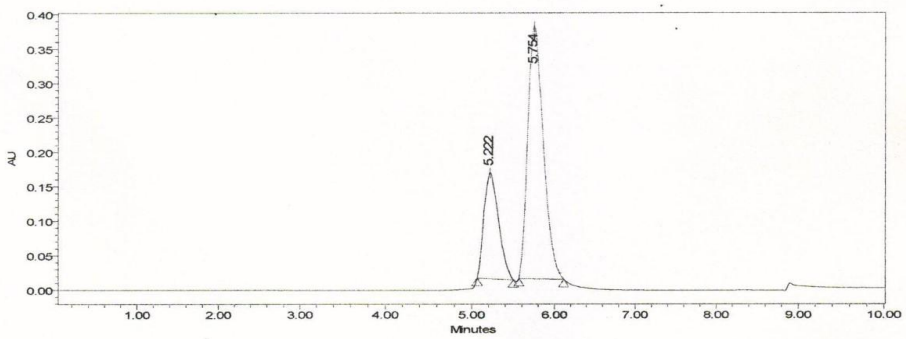



**Tab 6.1 Retention time of LIS and HCTZ in trail 1**

| S.no | Name of the peak | Retention time (min) | |
|---|---|---|---|
| | | LIS | HCTZ |
| 1 | LIS and HCTZ | -- | -- |

### 6.1.2 Trail 2

**Fig 6.2 chromatograph of trail 2**



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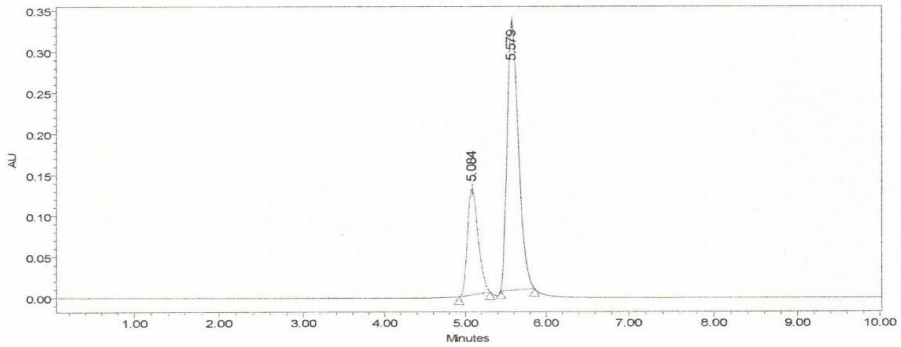
**Tab 6.2 Retention time of LIS and HCTZ in trail 2**

| S.no | Name of the peak | Retention time (min) | |
|---|---|---|---|
| | | LIS | HCTZ |
| 1 | LIS and HCTZ | 5.222 | 5.754 |

**6.1.3 Trail 3**

**Fig 6.3 chromatograph of trail 3**




**Tab 6.3 Retention time of LIS and HCTZ in trail 3**

| S.no | Name of the peak | Retention time (min) | |
|---|---|---|---|
| | | LIS | HCTZ |
| 1 | LIS and HCTZ | 5.084 | 5.579 |

**6.1.4 Trail 4**

**Fig 6.4 chromatograph of trail 4**

## 4. SUMMARY AND CONCLUSION

Development of new analytical methods for the determination of drugs in pharmaceutical dosage forms is more important in pharmacokinetic, toxicological and biological studies. Today pharmaceutical analysis entails much more than the analysis of active pharmaceutical ingredients or the formulated product. The pharmaceutical industry under increased scrutiny from the government and the public interested groups to contain costs and at consistently deliver to market safe .Efficacious product that fulfill unmet medical needs, The pharmaceutical analyst plays a major rule in assuring identity, safety, efficacy, purity and quality of the drug product. The need for pharmaceutical analysis is driven largely by regulatory requirements. The commonly used tests of pharmaceutical analysis generally entail compendia testing method development, setting specifications, and method validation. Analytical testing is one of the more interesting ways for scientists to take part in quality process by providing actual data on the identity, content and purity of the drug products. New methods are now being development with a great deal of consideration to worldwide harmonization. As a result, new products can be assured to have comparable quality and can be brought to international markets faster.

Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, precision and accuracy in estimation of drugs.

**Chapter-1** Deals with the introduction to HPLC, analytical method development, analytical method validation and hypertension.

**Chapter-2** Deals with aim and plan of the work.

**Chapter-3** Deals with the literature survey of selected drugs (Lisinopril, hydrochlorothiazide) and Methods.

**Chapter-4** Deals introduction to the selected drugs, their description, mechanism of action, pharmacokinetics, adverse effects, uses and contraindications etc.

**Chapter-5** Deals with materials and methods of methods development and validation for the estimation of lisinopril& hydrochlorothiazide.

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**Chapter-6** Deals with results and discussions part of the method development and validation parameters.

**RP-HPLC method development**

A simple reverse phase HPLC method was developed for simultaneous estimation of Lisinopril and hydrochlorothiazide in bulk and their pharmaceutical formulations. Hypersil ODS C18 (250x4.6mm, 5u) column, along with Methanol: Ammonium acetate buffer (30:70) as mobile phase. The flow rate was 1.0 ml/min and effluent was monitored at 212nm. The retention times were 1.991min & 3.213 min for Lisinopril & hydrochlorothiazide respectively.

**Table 7.1 Summary report of HPLC validation**

| **Parameters** | **Acceptance criteria** | **HPLC Results** | |
|---|---|---|---|
| **System suitability** | For 5 replicate injections | 2.005 | 3.213 |
| | Retention time | | |
| | Theoretical plates NLT 3000 | 9919 | 10220. |
| | Asymmetry factor NMT 2.0% | 1.0908 | 1.6494 |
| **Specificity** | No interference with blank and placebo | No interference | |
| **PRECISION**<br><br>**A) System precision or Repeatability** | %RSD of six determinations: NMT 2.0% | 0.258 | 0.0135 |
| **B) System precision or Reproducibility** | %RSD of six determinations: NMT 2.0% | 0.2336 | 0.5497 |
| **Accuracy** | The % recovery at each level shall be NLT 98.0% and NMT 102.0% of the added amount. | 99.07 | 99.02 |

| | | | |
|---|---|---|---|
| **Linearity** | The Correlation coefficient shall be NLT 0.999 | 0.999 | 0.999 |
| **Robustness** | All the system suitability parameters should pass for all the conditions. | The system suitability parameters passed for all the Conditions. | |
| **Ruggedness** | All the system suitability parameters should pass for all the conditions. | The system suitability parameters passed for all the Conditions. | |

Analysis of drugs present in pharmaceutical dosage form is quite challenging problem and hence attempts were made to develop analytical methods for simultaneous estimation of LIsinopril& hydrochlorothiazide in Pharmaceutical dosage forms.

All the proposed methods are simple, selective, reproducible, sensitive and accurate with good precision. Some of the methods were proved to be superior to mist of the reported methods. All these proposed methods for estimation of Lisinopril & hydrochlorothiazide were successfully applied in pharmaceutical formulations.

The proposed method can be used as alternative methods to the reported ones for the simultaneous estimation of Lisinopril & hydrochlorothiazide.

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