Determination of Lisinopril and Hydrochlorothiazide in Bulk and Multicomponent Pharmaceutical Dosage Form by RP-HPLC Method

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ABSTRACT

Objective: To develop a simple, accurate, linear and precise RP-HPLC method for determination of Lisinopril and Hydrochlorothiazide in bulk and combined pharmaceutical dosage form and validate as per the ICH guidelines.

Methods: In the methods used Phenomenex Luna C18 (2) (250 x 4.6 mm, 5 µ)column, mobile phase 0.1 % Formic acid: Methanol (70: 30%, v/v), flow rate of 1 ml/min and the detection wavelength of 215 nm using PDA detector.

Results: The calibration curves were linear $r^2 = 0.9989$ and 0.9974 in the concentration range of 4 to 6 ug/ml and 10 to 15 ug/ml for Lisinopril and Hydrochlorothiazide sequentially. The developed method resulted in elution of Lisinopril at 3.97 min and Hydrochlorothiazide at 4.53 min. The % recovery was found to be 99.31% to 99.83 % and 100.75% to 101.16% for Lisinopril.
and hydrochlorothiazide. The limit of detection was found to be 0.32 ug/ml and 1.24 ug/ml for Lisinopril and Hydrochlorothiazide. Limit of Quantification was found to be 0.97 ug/ml and 3.75 ug/ml for Lisinopril and Hydrochlorothiazide sequentially.

**Conclusion:** The present method of RP-HPLC was found to be an accurate, simple and easy, specific, precise, linear, quick and inexpensive. Within the concise analysis time this method gives a superior resolution between both the compounds. That’s why the method to support good for the routine analysis of Lisinopril and Hydrochlorothiazide in several pharmaceutical industries and also in academics.

**Keywords:** RP-HPLC, Lisinopril, Hydrochlorothiazide, Validation, Method development

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### INTRODUCTION

Lisinopril is a class of angiotensin converting enzyme i.e., ACE inhibitors. It is work by reducing a certain chemical that secure the blood vessels that’s why blood flow further smoothly. Its chemical name is N\textsubscript{2}-[(1S)-1-Carboxy-3-phenylpropyl]-L-lysyl-L-proline. The angiotensin converting enzyme is a peptidyl dipeptidase that work the conversion of angiotensin-I to vasoconstrictor material angiotensin-II. This ACE inhibitor is used in the therapy of heart failure and hypertension. The angiotensin-II stimulates the cortex obstruction of ACE which outcome in reduced plasma angiotensin-I and consequently show vasopressor activity and to reduced aldosterone secretion, in which finally reduce may results in portably enhancement of serum potassium\textsuperscript{[1,2]}. 

Fig. 1. Structure of Lisinopril

Hydrochlorothiazide is a class of diuretics. From the body into the urine hydrochlorothiazide works by causing the kidneys to get rid of unneeded water and salt. Its chemical name is 2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro 3,4-dihydro-, 1,1, -dioxide. The solubility of hydrochlorothiazide is slightly soluble in water and sparingly soluble in acetonitrile. It coheres to and impede the enzyme carbonic anhydrase. Hydrochlorothiazide is often used single or in combination with another medicament for treatment of various diseases like congestive heart failure, hypertension, diabetes insipidus, symptomatic edema, renal tubular acidosis, osteoporosis, hypoparathyroidism, edema and also used in the avoidance of kidney stones [3-5].

Fig. 2: Structure of Hydrochlorothiazide

The detailed literature survey disclosed that there was a spectrophotometric method for concurrent estimation of Lisinopril with another combination. In bulk and pharmaceutical dosage forms some spectroscopic, Spectrofluorometric, LC methods have also described earlier for the determination of lisinopril in bulk and pharmaceutical dosage form [6-12]. The substantial literature survey was achieved and found that there are some analytical techniques for the establishment of hydrochlorothiazide single or in its combination with other drugs in pharmaceutical preparations like spectrophotometry, TLC, flow injection and HPLC [13-18]. There was a several RP-HPLC
techniques have been developed for the resolution of Lisinopril in combination with other drugs. Although, there was no RP-HPLC technique has been described for concurrent estimation of Lisinopril and Hydrochlorothiazide in bulk and pharmaceutical dosage form. In this study we dispense easy, accurate, simple, rapid and particular HPLC technique for simultaneous estimation of RP-HPLC assay procedure for the analysis of Lisinopril and Hydrochlorothiazide in bulk and pharmaceutical dosage form. As per the ICH guidelines the developed method was validated[19-22].

MATERIALS AND METHODS

Materials: The pharmaceutical grade Lisinopril was supplied by Micro Labs Limited, Mumbai-400072 and Hydrochlorothiazide drug was obtained from Cadila Healthcare Ltd, Sanad (Gujarat), India. Commercial tablet of Lisinopril and Hydrochlorothiazide (100mg) was acquired from the local drug market. From Merck Methanol, Formic acid and HPLC grade water were obtained. The all solvents used in this work are HPLC grade. RP-HPLC is of Agilent1260 HPLC System equipped with quaternary pump (G7111A), Autosampler injector (G7129A), DAD detector (G7115A) operated with software Openlab Ezchrom, column is of Phenomenex,USA.

Methods:

Chromatographic conditions: The developed method used a Phenomenex Luna C18 (2) (250 ×4.6 mm, 5u) , a mobile phase 0.1 % Formic acid: Methanol (70:30 %, v/v) , flow rate of 1 ml/min and a detection wavelength of 215 nm using a DAD detector.

Mobile phase preparation: The mixture of 70 volumes of Formic acid and 30 volumes of Methanol was prepared. To remove the all gasses mobile phase was sonicated for 10 min.

Diluent: The mobile phase was used as a diluent.

Standard solution preparation:

Lisinopril Standard Stock Solution-I (LSSS-I): Initially Prepare a Standard Stock Solution (SSS-I) of by adding 5 mg of Lisinopril in 100 ml volumetric flask & add 50 ml diluent, mix for 2 minutes and make up the volume to 100 ml with diluent. (Conc. of Lisinopril = 50 µg/ml).

Hydrochlorothiazide Standard Stock Solution-I (HSSS-I): Then prepare a Standard Stock Solution (SSS-I) of Hydrochlorothiazide by adding 12.5 mg in 100 ml volumetric flask & add 50
ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Hydrochlorothiazide = 125 µg/ml).

Then add 1.0 ml of LSSS-I & 1.0 ml HSSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Lisinopril = 5 µg/ml & Hydrochlorothiazide = 12.5 µg/ml).

**Lisinopril Ion Pair & Hydrochlorothiazide Sample:** Lisinopril Ion-pair Standard Stock Solution-II (LSSS-II): Initially Prepare a Standard Stock Solution (SSS-II) of by adding 5 mg of Lisinopril in 100 ml volumetric flask & add 50 ml diluent, mix for 2 minutes and make the volume to 100 ml with diluent. (Conc. of Lisinopril = 50 µg/ml).

Hydrochlorothiazide Standard Stock Solution-II (HSSS-II): Then prepare a Standard Stock Solution (SSS-II) of Hydrochlorothiazide by adding 12.5 mg in 100 ml volumetric flask & add 50 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Hydrochlorothiazide = 125 µg/ml).

Then add 1.0 ml of LSSS-II & 1.0 ml HSSS-II in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Lisinopril = 5 µg/ml & Hydrochlorothiazide = 12.5 µg/ml).

**Drug Product Sample Preparation for Assay:**

**Tablet Sample Solution (TSS):** 10 Tablets were weighed and average weight was calculated. And tablets were crushed & mixed in mortar and pestle. Powder weight equivalent to 0.5 mg Lisinopril and 1.25 mg Hydrochlorothiazide was weighed into 10 ml volumetric flask & add 5 ml diluent, sonicate for 10 minutes and make the volume to 10 ml with diluent. (Conc. of LIS = 50 µg/ml, HTZ = 125 µg/ml). Pipette out of 1 ml of above solution in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Lisinopril = 5 µg/ml & Hydrochlorothiazide = 12.5 µg/ml).

**RESULTS AND DISCUSSION**

**Method development:** For better separation and resolution, the various chromatographic conditions were tried. Phenomenex Luna C18 (2) (250 x 4.6 mm, 5 µ) column was found adequately. Peak purity of Lisinopril and hydrochlorothiazide was checked using PDA detector
and 215 nm was examine satisfactory for detecting both the drugs with sufficient sensitivity. In the several ratios a number of solvents over an extensive range of pH weretried, yet likewise peak shape was wide or resolution was not good. Frequent trials to get superior, sharp peak with a systematic resolution between two peaks of Lisinopril and Hydrochlorothiazide complete on a C18 column in isocratic HPLC gave acceptable results. The run time was superior in an isocratic trial with mobile phase contain 0.1 % Formic acid: Methanol (70:30 %, v/v) and Phenomenex Luna C18(2) (250 x 4.6 mm, 5 µ) column, flow rate 1 ml/min and detection wavelength 215 nm gave the acceptable results in terms of retention time, resolution, sensitivity and symmetry.

**Method Validation:** Afterwards method development the validation of the advanced method was accomplish in period of the following variables like accuracy, precision, linearity and range, percentage recovery, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

**System suitability:** The standard solution was prepared by the test technique and injected into the chromatographic system. The system suitability variables such as resolution, theoretical plates and asymmetric factors was evaluated. The all variables were found to be within a limit. The parameters of system suitability were shown in the table 1.

### Table 1: System suitability parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acceptance limits</th>
<th>Lisinopril</th>
<th>Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time</td>
<td>-</td>
<td>3.91</td>
<td>4.44</td>
</tr>
<tr>
<td>Resolution</td>
<td>NLT 2</td>
<td>0.00</td>
<td>2.74</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>NLT 2000</td>
<td>6631</td>
<td>9941</td>
</tr>
</tbody>
</table>

**Precision**

**Method precision:** By precision method studies the precision of the technique was confirmed. At working concentration, the sample solution was prepared and analysis was accomplished at
replicating. The sample solution of Lisinopril and Hydrochlorothiazide was prepared as per the test method and injected 5 times into the column. The results of precision were shown in table 2. The average was taken and the percent RSD was calculated and described. The percent RSD values were within the limits and the technique was found to be precise.

<table>
<thead>
<tr>
<th>n</th>
<th>Lisinopril</th>
<th>Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep 1</td>
<td>113956</td>
<td>880223</td>
</tr>
<tr>
<td>Rep 2</td>
<td>112317</td>
<td>886114</td>
</tr>
<tr>
<td>Rep 3</td>
<td>113357</td>
<td>869037</td>
</tr>
<tr>
<td>Rep 4</td>
<td>111228</td>
<td>875486</td>
</tr>
<tr>
<td>Rep 5</td>
<td>113026</td>
<td>871974</td>
</tr>
<tr>
<td>Avg</td>
<td>112777</td>
<td>876567</td>
</tr>
<tr>
<td>STDEV</td>
<td>1048.742</td>
<td>6772.678</td>
</tr>
<tr>
<td>RSD</td>
<td>0.93</td>
<td>0.77</td>
</tr>
</tbody>
</table>

**Table 2: Precision data**

**Linearity:** The linearity of the test solution for the assay technique was prepared from Lisinopril and hydrochlorothiazide standard stock solution at five concentration levels 80% to 120% of assay concentration. The peak area compared to concentration data was treated by least squares linear regression analysis (fig. 3 and 4). The results have manifested a magnificent correlation between peak areas and concentration within the concentration range 4-6 ug/ml for Lisinopril and 10-15 ug/ml for Hydrochlorothiazide (table 3). For both drugs, the correlation coefficient was found to be 0.9989 for Lisinopril and 0.9974 for Hydrochlorothiazide which meet the technique validation acquisiense criteria and that’s why the method was said to be linear.
Fig. 3: Linearity chart of Lisinopril

\[ y = 23169x + 2885.4 \]
\[ R^2 = 0.9989 \]

Fig. 4: Linearity chart of Hydrochlorothiazide

\[ y = 66975x + 42997 \]
\[ R^2 = 0.9974 \]

Table 3: Linearity data of both drugs

<table>
<thead>
<tr>
<th>% Level</th>
<th>Lisinopril concentration (ug/ml)</th>
<th>Lisinopril peak area</th>
<th>Hydrochlorothiazide concentration (ug/ml)</th>
<th>Hydrochlorothiazide peak area</th>
</tr>
</thead>
</table>
Accuracy: The accuracy of the technique was resolute by recovery studies by the determination of percent mean recovery of Lisinopril and Hydrochlorothiazide at three dissimilar levels (80%, 100%, 120%). At individual level, three determinations were performed. For the drug percent recovery and mean percent recovery was shown in table 4. The perceive data were within the essential range, which shows superior recovery value and that’s why the accuracy of the method developed.

<table>
<thead>
<tr>
<th>Level (%)</th>
<th>Lisinopril % recovery</th>
<th>% Mean</th>
<th>Hydrochlorothiazide % recovery</th>
<th>% Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>99.20</td>
<td>99.31%</td>
<td>101.43</td>
<td>101.16%</td>
</tr>
<tr>
<td>80</td>
<td>99.42</td>
<td>100.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>101.05</td>
<td>100.32%</td>
<td>100.42</td>
<td>100.75%</td>
</tr>
<tr>
<td>100</td>
<td>99.59</td>
<td>101.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>100.59</td>
<td>99.83%</td>
<td>100.22</td>
<td>100.00%</td>
</tr>
<tr>
<td>120</td>
<td>99.08</td>
<td>99.79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Detection limit and Quantification limit: The Limit of detection (LOD) which constitute a concentration of the analyte at S/N ratio of 3.3 and Limit of Quantification (LOQ) at which S/N was 10 were decided analytically for the suggested technique. Therefore, the detection limit and quantification limit of both drugs were given S/N ratio of 3.3 and 10 sequentially. The results of LOD and LOQ are shown in table 5.
Table 5: Results of LOD and LOQ

<table>
<thead>
<tr>
<th>Sample name</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lisinopril</td>
<td>0.32 (ug/ml)</td>
<td>0.97 (ug/ml)</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>1.24 (ug/ml)</td>
<td>3.75 (ug/ml)</td>
</tr>
</tbody>
</table>

Assay of Lisinopril and Hydrochlorothiazide in tablet formulation: Behind prosperous development and validation of all these methods, it was working for analysis of the Lisinopril and Hydrochlorothiazide in composite tablet formulation. Between the two analytes the method results in exemplary separation with superior resolution. Furthermore, the elevated percentage of recovery and non-interference of the formulation excipients in retention time of the drugs manifest the selectivity of the technique for assessment of the both drug in their combined dosage form. The mean percent approximate for Lisinopril 101.18 % and Hydrochlorothiazide 99.30 % were in superior concurrence with the label claimed.

CONCLUSION

The present method of RP-HPLC was found to be an accurate, simple and easy, specific, precise, linear, quick and inexpensive. Within the concise analysis time this method gives a superior resolution between both the compounds. That’s why the method to support good for the routine analysis of Lisinopril and Hydrochlorothiazide in several pharmaceutical industries and also in academics.
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