Comparative Evaluation Study on Different Brands of Lisinopril Tablet using Hplc and Uv Spectrophotometer

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Abstract
This study was undertaken with the objective of evaluating the chemical equivalence of different brands of lisinopril tablets with the use of two analytical methods which will be accurate and reproducible and to compare between the two methods. The chemical equivalence of nine (9) brands of lisinopril tablet were assessed through the use of HPLC and UV, where parameters such as the peak area and absorbance were used to determine the quantity of lisinopril. The result indicate that none of the samples contain less than 50% of the active principle (lisinopril). Also from the results obtained it can be seen that samples A, B, D and G failed the test in both UV and HPLC while samples E, F, H and I failed the test in HPLC, only sample C pass the test in both UV and HPLC. The result showed that only one brand (sample C) was within the specified official standard for both the HPLC and UV spectroscopy. While 5 brands fall within the limit for HPLC. The HPLC method is more suitable for assay of lisinopril tablet because it is more sensitive and has an added quality of reproducibility and accuracy while UV spectroscopy is less sensitive but has an added quality of simplicity of method thus, reducing the possibility of errors during the procedure.

Introduction
There has been an overwhelming rise in generic drug products in the pharmaceutical market today. The introduction of generic drug products from multiple sources into the health care system of developing countries was aimed at improving the overall health care delivery system in such countries. However, this has been accompanied by a variety of problems of which the most critical is the widespread distribution of fake and substandard drug products1.

Giving the authorizations of the flow of different generic drug products by the government into the market has made it paramount to the government to make sure that these drugs have the required specifications at all time. Recent reports indicate the availability of substandard and counterfeit drugs has reached a disturbing proportion in many low-income countries2.

The quality of pharmaceuticals is a global concern, and the lack of reliable drug quality assurances systems in many developing countries often contributes to the devastation diseases, particularly those that have build up resistance to traditional first-line medicines. USP DQI presently is working in four continents: Africa, Asia, Europe, and South America to facilitate and strengthen their drug quality systems to improve public health3. Research teams from pharmaceutical companies, universities, and research laboratories have made it their top priorities in development of new methods for quantitative evaluation of drugs, so as to study their stability in formulations, their biopharmaceutical equivalence as well as their chemical equivalence and this is as a result of the disturbing rise in several diseases such as hypertension, cancer, diabetic etc. another effort is being made by USP.DQI, by providing technical assistance and developing appropriate training programme based on rapid, low-cost, simple testing methods for detecting substandard drugs and monitoring drug quality throughout the supply chain especially in developing countries4.

In regard to all of this, therapeutic equivalences studies are carried out, where by evaluative studies on biopharmaceutical equivalence, bioequivalence, bioavailability (for determination of drug approvals into markets) and chemical equivalence of generic drug products in the markets are analyze in comparison to the specified standard or branded products. The first sage in ascertaining the therapeutic equivalence of any drug product involves ascertaining the chemical and biopharmaceutical equivalence of such drug product, in conducting these studies, pharmaceutical analytical processes are use: Pharmaceutical analyses that are carried out are base on...
those specified by official books, such as the B.P, N.P, or from new research methods developed by scientist which are simple, inexpensive and rapid for evaluating the quantity and quality of drugs.

CHEMICAL EQUIVALENCE
This means, a drug or chemical containing similar amount of the same ingredient as another drug or chemical.4

HISTORY OF LISINOPRIL

![Chemical structure of lisinopril](image)

Lisinopril is a synthetic peptide drug of the angiotensin-converting enzyme (ACE) inhibitor class that is primarily used in treatment of hypertension, congestive heart failure, and heart attacks and also in preventing renal and retinal complications of diabetes.5

Lisinopril is chemically described as (S)-1-[N2-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-lysyl]-L-proline dehydrate. Its empirical formula is C$_{21}$H$_{31}$N$_{3}$O$_{5}$.

PROPERTIES OF LISINOPRIL

Lisinopril is a white to off-white, crystalline powder, with a molecular weight of 441.52 and molar mass of 40.488g/mol. It is soluble in water and sparingly soluble in methanol and practically insoluble in ethanol, acetone, acetonitrile and chloroform.6

- Water soluble value: 13g/m
- Density: 1.21g/cm$^3$
- Log P: -0.9
- PKa: 3.85
- Melting point: 160$^\circ$C
- Boiling point: 666.4$^\circ$C at 760mmHg

EVALUATION OF LISINORIL TABLETS

The evaluation of tablets usually involves quantitative evaluation and assessment of the tablet’s physical, chemical and bioavailability properties as these attributes are considered in drug designing and in quality control.

For the quantitative determination of lisinopril substance, the USP XXIV pharmacopeia specifies HPLC method (USP XXIV, 2000) and in British pharmacopoeia it is potentiometry.7 There are different methods developed for the assay of lisinopril in single and multi component pharmaceutical dosage forms, which include a; spectrometry, fluorimetry, and HPTLC dentimetry.8

The result obtained from the study showed that more than 99% lisinopril was recovered in the presence of possible excipients and other active ingredient such as hydrochlorothiazide in lisinopril formulation, where by the proposed method compares favorably with other existing spectrophotometer methods in terms of reagent availability and that of Beer’s law limit.9

The results were then compared with reference method. The calculated student’s t-test values and F-test values did not exceed the theoretical values, which indicate the absence of any difference between methods when compared. This proposed method gives good results for lisinopril in raw and pharmaceutical formulations. However, it cannot be applied for the estimation of lisinopril in clinical samples, i.e. blood and urine because these samples usually contain amino acids and amino group containing metabolites may react with ninhydrin to produce Ruhemann’s purple color.9

MATERIALS AND METHOD

MATERIALS
Nine (9) different brands of lisinopril 10mg and 5mg tablets were used for the study.

Pure sample of lisinopril obtained from NAFDAC was used as standard

- 1000ml volumetric flasks
- 100ml volumetric flask
- 200ml volumetric flasks
- 50ml volumetric flasks
- Filter paper.
**Sonicator**  
**Beaker**  
**Pipettes**  
**Measuring cylinder**  
**Spatula**  
**Pestle and mortar**  
**Analytical weighing balance (Metler balance)**  
**UV visible spectrophotometer (Beckman)** which measured absorbance of each sample at 215nm.  
**High performance liquid chromatographic set up.**  
**Distilled water**  
**Monobasic potassium phosphate; phosphoric acid.**  
**Sodium – I – Hexosulfonate.**  
**Acetonitrile**  
**Methanol**  
**pH tester.**  
All reagent used were obtained from NAFDAC area office, Maiduguri Borno state.  

**SAMPLE COLLECTION**  
Nine (9) samples of lisinopril 10mg and 5mg tablets were obtained from various pharmacy shops within Maiduguri metropolitical council, the sample were obtained with their packs and were registered NAFDAC products.  

**PRACTICAL METHOD**  
The methodology used/employed for this study included UV visible spectrophotometry and high performance liquid chromatography.  

**PRACTICAL PROCEDURE**  
The tablets were assayed by high performance liquid chromatography using the following procedure:  
The average weight of tablets from each sample was determined by weighing 10 tablets and dividing the result by ten.  
then the tablets were crushed using a clean pestle and mortar this was done for each sample  
For each sample, powder containing 20mg of lisinopril was accurately weight and transferred into different 100ml volumetric flasks. All the nine samples were labeled using a pen and masking tape for differentiation. The reference standard USP lisinopril (lisinopril Dihydrate), was also weighed (20mg) and transferred into the 100ml volumetric flask and labeled as well.  
The diluents were then prepared (a mixture of 4:1 water and methanol) then 100ml of the diluent was added to each sample including the reference standard in order to obtain a concentration of 0.2mg/ml.  
The preparation was then sonicated for 5 minute after which, each was mechanically shacked for 20 minute.  
Then they were filtered through filter papers into clean beakers which were labeled as well and kept aside.  
The mobile phase was prepared; in a 1000ml volumetric flask, 1.0g of sodium 1-hexanesulfonate was dissolved in 800ml of phosphate solution, then 200ml of acetonitrile was added and mixed. After which, it was filtered through a membrane filter and degas. The phosphate solution is a mixture of monobasic potassium phosphate in water, where 4.1g of monobasic potassium phosphate was dissolve in 900ml of distilled water in a 1000ml volumetric flask and the pH was adjusted with phosphoric acid to 2.0, it was checked with a PH meter. The phosphate solution was then diluted to volume with distilled water. The prepared standard preparation and samples were put into vials which were put into the chromatographic machine separately.  

Enough of the mobile phase was put into the chromatographic tanks, the machine was put on, and then settings were made to select the vial to be run. A computer connected displayed the result of the analysis on the screen which is the chromatogram. It was then printed with the aid of a connected printer.  

**CHROMATOGRAPHIC PARAMETERS;**  
Mobile phase = 0.125% solution of sodium-1- hexasulfonate in phosphate solution (pH=2). Acetonitrile = 800:200ml  
Flow rate = 1.5ml/min  
Column temperature = 40 degrees  
Detection = at 215nm  
The results were then use to calculate the percentage content and the content in mg for each sample.  
The tablets were assayed spectrophotometrically using the following procedures  
From the powdered drug samples, powder containing 20mg of lisinopril was weighed from each sample including the reference standard USP-lisinopril RS (Lisinopril Dihydrate) and then transferred into a 100ml volumetric flask. All were then labeled using pen and masking tape.  
Diluent containing 4:1 water to methanol was prepared in a volumetric flask.  
100ml of the diluents was then added to the samples in the volumetric flask to obtain a concentration of 0.2mg/ml. Each was then mixed, sonicating it for 5minutes and then filtered through a filter paper into clean beakers.
5ml of each filtrate was taken and put into 50ml volumetric flask and then the diluents(4:1 water to methanol) was added to it and make up the volume. The UV spectrophotometer was put at zero by running a base line(between 200-400) using 4:1 water to methanol solution as blank. The absorbance of each sample was determined at 215nm by putting small amount of the sample into the cuvette of the spectrophotometer, then cuvette was put into the machine. The sample procedure was repeated for the reference standard USP lisinopril using 20mg of powdered standard and the absorbance determined. Thus, this was use to calculate the percentage content and content in mg of lisinopril from each brand. The concentration of each sample was also determined using Beer Lambert’s law\textsuperscript{12}.

### Result

**TABLE 1. SHOWING THE WEIGHT OF TABLETS FROM DIFFERENT BRANDS**

<table>
<thead>
<tr>
<th>CONTENT AND CONTENT (in mg)</th>
</tr>
</thead>
</table>

Formula for percentage content:
- For HPLC:
  \[
  \text{Content} \% = \left( \frac{\text{Peak area of sample}}{\text{Peak area of standard (32885389)}} \right) \times 100
  \]
- For UV:
  \[
  \% \text{ Content} = \left( \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \right) \times 100
  \]

Formula for mg content:
- For HPLC:
  \[
  \text{Mg Content} = \left( \frac{\text{Peak area of sample}}{\text{Peak area of sample (32885389)}} \right) \times 100 \times \text{stated claim}
  \]
- For UV:
  \[
  \text{Mg Content} = \left( \frac{\text{Absorbance of sample}}{\text{Absorbance of standard(0.484)}} \right) \times 100 \times \text{stated claim}
  \]

**TABLE SHOWING THE RESULTS OBTAINED USING HPLC METHOD**

The concentration used is 0.2mg/ml, for all samples and the standard.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Peak area</th>
<th>%Content (%)</th>
<th>Content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Lisiofil</td>
<td>39087772</td>
<td>118.8</td>
<td>11.8</td>
</tr>
<tr>
<td>B Zestril</td>
<td>39219683</td>
<td>119.2</td>
<td>11.9</td>
</tr>
<tr>
<td>C Teva</td>
<td>30971616</td>
<td>94.1</td>
<td>9.4</td>
</tr>
<tr>
<td>D Stripril</td>
<td>38695735</td>
<td>117.6</td>
<td>11.7</td>
</tr>
<tr>
<td>E Lizopril</td>
<td>36247497</td>
<td>110.2</td>
<td>11.0</td>
</tr>
<tr>
<td>F Ranopril</td>
<td>35070321</td>
<td>106.6</td>
<td>10.6</td>
</tr>
<tr>
<td>G Takapril</td>
<td>26785366</td>
<td>81.4</td>
<td>8.1</td>
</tr>
<tr>
<td>H Lisoril</td>
<td>331.98478</td>
<td>100.9</td>
<td>10.0</td>
</tr>
<tr>
<td>I Makinga</td>
<td>34408636</td>
<td>104.6</td>
<td>10.4</td>
</tr>
<tr>
<td>Standard</td>
<td>32885389</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table showing result obtained using uv spectrophotometer**

The concentration used is 0.2mg/ml for all samples and the standard.

<table>
<thead>
<tr>
<th>Sample Solution</th>
<th>Absorbance</th>
<th>%Content (%)</th>
<th>Content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Lisiofil</td>
<td>0.621</td>
<td>128.3</td>
<td>12.8</td>
</tr>
<tr>
<td>B Zestril</td>
<td>0.591</td>
<td>122.1</td>
<td>12.2</td>
</tr>
<tr>
<td>C Teva</td>
<td>0.493</td>
<td>101.8</td>
<td>10.1</td>
</tr>
<tr>
<td>D Stripril</td>
<td>0.690</td>
<td>142.5</td>
<td>14.2</td>
</tr>
<tr>
<td>E Lizopril</td>
<td>0.912</td>
<td>118.4</td>
<td>18.3</td>
</tr>
<tr>
<td>F Ranopril</td>
<td>0.617</td>
<td>127.4</td>
<td>12.7</td>
</tr>
<tr>
<td>G Takapril</td>
<td>0.272</td>
<td>56.1</td>
<td>5.61</td>
</tr>
<tr>
<td>H Lisoril</td>
<td>0.569</td>
<td>117.5</td>
<td>11.7</td>
</tr>
<tr>
<td>I Makinga</td>
<td>0.556</td>
<td>114.8</td>
<td>11.4</td>
</tr>
<tr>
<td>Standard</td>
<td>0.484</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The result obtained using the UV shows that only sample C Pass the test for the specified limit having 101.8% while for UV it was 119.2%.

Thus indicating that none of the samples contain less than 50% of the active principle (lisinopril) from the table of percentage content of the analyzed sample results using HPLC and UV spectroscopy methods. All samples failed the test except sample C.

It can be concluded that samples A, B, D and G failed the test in both UV and HPLC while samples E, F, H and I failed the test in HPLC.

V spectroscopy method is more suitable for the assay of lisinopril tablet than HPLC because its procedure is simple, rapid and less expensive thus reducing the possibility of error in terms of procedure. While HPLC method is more accurate and sensitive than UV method, this can be seen with standard deviation (SD) and coefficient of variation (CV).

Discussion
Lisinopril is a prescription only medication used to treat several conditions involving the heart and blood vessels such as congestive heart failure and light blood pressure. The drug comes in tablet form. Lisinopril is taken by mouth, and is typically taken once a day.

Although most people tolerate lisinopril tablets well, the medication is not suitable for everyone that is why health care providers dwell on patient’s medical condition before prescribing lisinopril. Some common side effects involve diarrhea, coughing and dizziness.

Lisinopril is part of the angiotensin converting enzyme (ACE) inhibitors class which is mostly used to help control the progression of diabetes and kidney diseases as well. According to the United State Pharmacopeia (USP), a lisinopril tablet should contain not less than 90% and not more than 110% of lisinopril.

The percentage content of the analyzed sample tablets using HPLC ranges from 81.4-119.2% while for UV ranges from 56.1-188.4% .The content (in mg) for HPLC showed a range of 5.5-11.9 mg and for UV is 5.61-142.5mg.

Thus indicating that none of the samples contain less than 50% of the active principle (lisinopril) from the results obtained using HPLC method, it can be seen that 5 sample C, E, F, H, and I passed, while samples A, B, D, and G failed because A, B, and G were all above the limit specified by USP while sample D Is below the limit (81.4% and 8.1mg).

The result obtained using the UV shows that only sample C Pass the test for the specified limit having 101.8.1% and 10.1 mg while the remaining samples (A B D E F G H I) fail the test for the specified limit of lisinopril.

Conclusion
It can be concluded that samples A, B, D and G failed the test in both UV and HPLC while samples E, F, H and I failed the test in HPLC.

Only sample C pass the test in both UV and HPLC.

The U-V spectroscopy method is more suitable for the assay of lisinopril tablet than HPLC because its procedure is simple, rapid and less expensive thus reducing the possibility of error in terms of procedure. While HPLC method is more accurate and sensitive than UV method, this can be seen with standard deviation (SD) and

### Table for the calculation of standard deviation and coefficient of variation of HPLC method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mg content</th>
<th>X-X</th>
<th>(X-X)^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Lisiofil</td>
<td>11.8</td>
<td>1.867</td>
<td>3.4856</td>
</tr>
<tr>
<td>B Zestril</td>
<td>11.9</td>
<td>1.967</td>
<td>3.8690</td>
</tr>
<tr>
<td>C Teva</td>
<td>9.4</td>
<td>-0.533</td>
<td>0.2840</td>
</tr>
<tr>
<td>D Striplil</td>
<td>11.7</td>
<td>1.767</td>
<td>3.1222</td>
</tr>
<tr>
<td>E Lizopril</td>
<td>5.5</td>
<td>-4.433</td>
<td>19.6514</td>
</tr>
<tr>
<td>F Ranopril</td>
<td>10.6</td>
<td>0.667</td>
<td>0.4448</td>
</tr>
<tr>
<td>G Takapril</td>
<td>8.1</td>
<td>-1.833</td>
<td>3.3598</td>
</tr>
<tr>
<td>H Lisoril</td>
<td>10.0</td>
<td>0.067</td>
<td>0.0044</td>
</tr>
<tr>
<td>I Makinga</td>
<td>10.4</td>
<td>0.467</td>
<td>0.2180</td>
</tr>
</tbody>
</table>

### Table for the calculation of standard deviation and coefficient of variation of UV method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mg content</th>
<th>X-X</th>
<th>(X-X)^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Lisiofil</td>
<td>12.8</td>
<td>0.633</td>
<td>0.4006</td>
</tr>
<tr>
<td>B Zestril</td>
<td>12.2</td>
<td>0.033</td>
<td>0.0010</td>
</tr>
<tr>
<td>C Teva</td>
<td>10.1</td>
<td>-2.067</td>
<td>4.2724</td>
</tr>
<tr>
<td>D Striplil</td>
<td>14.2</td>
<td>2.033</td>
<td>4.1330</td>
</tr>
<tr>
<td>E Lizopril</td>
<td>18.8</td>
<td>6.633</td>
<td>43.9966</td>
</tr>
<tr>
<td>F Ranopril</td>
<td>12.7</td>
<td>0.533</td>
<td>0.2840</td>
</tr>
<tr>
<td>G Takapril</td>
<td>5.61</td>
<td>-6.557</td>
<td>42.9942</td>
</tr>
<tr>
<td>H Lisoril</td>
<td>11.7</td>
<td>-0.467</td>
<td>0.2180</td>
</tr>
<tr>
<td>I Makinga</td>
<td>11.4</td>
<td>-0.767</td>
<td>0.3791</td>
</tr>
</tbody>
</table>

### Table showing the mean, variance, standard deviation (sd) and coefficient of variance (cv) of the two method

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean</th>
<th>Variance (S^2) = [\sum(x-\bar{x})^2/n-1]</th>
<th>SD = [\sqrt{S^2}]</th>
<th>CV = [SD\times100%/X]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLC</td>
<td>9.933</td>
<td>4.309</td>
<td>2.0748</td>
<td>20.88</td>
</tr>
<tr>
<td>UV</td>
<td>12.167</td>
<td>96.8789</td>
<td>9.8427</td>
<td>80.89</td>
</tr>
</tbody>
</table>
coefficient of variation (CV) which is 2.0748 and 20.88% respectively for HPLC and 9.8427 and 80.89% respectively for UV method. Thus serving as evidence for the difference between the two methods.

**Recommendation**
There is a great association between dose and response, when the specified amount of active principle in a drug is less than it should be, the desired response required for therapeutic activity will not be obtained thus treatment failure occurs and this can lead to many unsuitable drug therapy problems.

In other to avoid drug therapy problems associated with therapeutic failure of drugs and considering the crucial need of combating hypertensive, kidney and liver diseases with lisinopril. It should be recommended that each batch of lisinopril tablets produce by every company undergoes quantitative analysis to ensure that right amount of lisinopril as specified by the official books.

It should also be recommended that pharmaceutical stores selling lisinopril should be able to carry out simple and rapid quantitative test on products they buy from companies to ensure their authenticity and should adhere to good storage practices specified by official books.
## Appendix

### Area Percent Report

Data File: C:\Documents and Settings\Lab Manager\Desktop\LISIOFIL-10.02.221211.dat  
Acquired: 12/22/2011 1:44:59 PM  
12:16:35 PM  
Printed: 2/2/2012

Analyst: LAB MANAGER  
Sample ID: LISIOFIL-10.02.221211  
Vial: 160  
Injection

![Graph of Retention Time vs. Area Percent](image)

<table>
<thead>
<tr>
<th>UV-VIS Results</th>
<th>Retention Time</th>
<th>Area</th>
<th>Area Percent</th>
<th>Integration Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>1.667</td>
<td>39087772</td>
<td>100.000</td>
<td>MM</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>39087772</td>
<td>100.000</td>
<td></td>
</tr>
</tbody>
</table>

Instrument Name: HPLC UV-VIS  
Acquisition Method: ASSAY 15311L.net  
Software Version: Version LaChrom  
Sequence: METHOD DEV.seq  
C:\EZChrom Elite\Enterprise\Projects\Default\Method\DICLOFENAC SR  
C:\EZChrom Elite\Enterprise\Projects\Default\Sequence\CIPROFLOXACIN

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