Chemical and biopharmaceutical assay of different brands of frusemide tablets

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ABSTRACT
Objective: To evaluate the chemical and biopharmaceutical equivalence of frusemide tablets from five different companies.
Methods: This study was conducted on five brands of frusemide tablets available in the pharmacies of Mosul city from different manufacturing companies. These brands have been evaluated using some quality control tests of uniformity of weight, hardness, disintegration time, dissolution rate determination for the tablets.
Results: The results obtained have been discussed in some detail using monographs in the United State Pharmacopeia (USP) and British Pharmacopeia (BP). All the brands content were within the acceptable range of USP (0.9-0.11%), the disintegration time of all the brands also within the range of the USP and BP. The dissolution profile also were within the acceptable range of USP not less than 0.8% of the labeled amount of frusemide is dissolved in 0.6 minutes.
Conclusion: All the brands tested in this study may be used interchangeably.
Keywords: Bioequivalence, dissolution, disintegration, frusemide, quality control.

Frusemide is one of the loop diuretics acts by inhibiting Na⁺-K⁺-Cl⁻ symport in the thick ascending loop of henle. It is used primarily in the treatment of acute pulmonary edema and other edematous conditions and is widely used for the treatment of chronic congestive heart failure. Frusemide is also used for the management of hypercalcaemia, hyperkalemia and in some anion over dose such as bromide, fluoride and iodide. Frusemide is also used to increase the rate of urine flow and enhance K⁺ excretion in acute renal failure.

Because of this multiple uses of frusemide and the increase in the number of prescriptions that contain this drug, which resulted in higher demand and the need to increase its supply has led to more importation while some indigenous pharmaceutical industries began to produce their own brands of frusemide.

Some of these brands or generic of frusemide is not bioequivalent to a reference product and need more investigation to assess their quality. For example, Elgindy et al evaluated five commercial brands of frusemide tablets using the official and non-official tests. These tests include: uniformity of weight, hardness, friability and disintegration time.
The results obtained showed that most of these brands failed to attain the USP requirements 3.

In vitro testing or quality control of drugs is a set of studies or experiments undertaken during production (in process) and occasionally ought to be undertaken post-production by regulatory agencies and researchers. Therefore, post-market surveillance or monitoring is very important for assessing the quality of drugs and to detect any substandard and counterfeit drug products.

Counterfeit and substandard medicines are a major cause of morbidity, mortality and loss of public confidence in drugs and health structures 4. China and India are known as the leading countries in counterfeit drugs production and also the bulk active ingredients they produce are used for counterfeiting worldwide 5.

Generic substitution could be considered when a generic copy of a reference drug contain identical amounts of the same active ingredients in the same dose formulation and route of administration as well as meet standards for strength, purity, quality and identity 6.

This study was undertaken to evaluate the chemical and biopharmaceuticals equivalence of five brands of frusemide tablets in the pharmacies of Mosul city.

**Materials and methods**

**Materials**

1. The generic products of frusemide used in this study are shown in table 1.

<table>
<thead>
<tr>
<th>Name of product</th>
<th>Strength in mg</th>
<th>Manufacturing company</th>
<th>Country of origin</th>
<th>Batch no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>lasimex</td>
<td>4</td>
<td>SDI</td>
<td>Iraq</td>
<td>B 2</td>
</tr>
<tr>
<td>Apix</td>
<td>4</td>
<td>Ajanta</td>
<td>India</td>
<td>AMO(^{3})VJ</td>
</tr>
<tr>
<td>Troge frusemide</td>
<td>4</td>
<td>Troge</td>
<td>Germany</td>
<td>2004-2001</td>
</tr>
<tr>
<td>Desal</td>
<td>4</td>
<td>Biofarma</td>
<td>Turkey</td>
<td>4040</td>
</tr>
<tr>
<td>Salex</td>
<td>4</td>
<td>ADCO</td>
<td>Egypt</td>
<td>81,249</td>
</tr>
</tbody>
</table>

7. Frusemide powder is a gift from SDI Company, Samara-Iraq.
8. 0.1N sodium hydroxide.

**Methods**

1. **Assay of contents:** Twenty tablets of frusemide were weighed and powdered. 0.5g of the powered frusemide was shaken with 7 ml of 0.1 N sodium hydroxide for 1 minute; sufficient 0.1 N sodium hydroxide was then added to produce 50ml. The suspension was then filtered. Five ml of the filtrate was pipetted and diluted to 70ml with 0.1N sodium hydroxide. The absorbance of the resulting solution was then measured at the maximum of 172nm and the percentage of contents is calculated from the following formula 7.

\[
\% \text{ content} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100
\]

2. **Determination of uniformity of weight:** Twenty tablets from each of the five brands of frusemide were weighed individually using a saturius balance. The average weights of the tablets were calculated as well as their percentage of deviation from the average weight 8.

3. **Hardness test.** The crushing strength (hardness) was determined with a tablet hardness tester (Pharma test Company). Four tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded 9.
 Disintegration test. Six tablets from each brand were employed for the test using disintegration apparatus (Pharma test-Germany) at \(73^\circ C\). The disintegration time was taken to be the time where no particle remained on the basket of the system.

 Dissolution rate determination. This was determined by using USP dissolution apparatus (paddle). Physiological buffer solution pH 6.8 was used as the dissolution medium and the temperature of the medium was maintained at \(73 \pm 0.5^\circ C\). One tablet was placed in a basket, which was lowered into the medium and the basket was rotated at 100 r.p.m. Samples (10 ml) were withdrawn at timed interval 5, 10, 15, 20, 45 minutes and replaced with 10 ml fresh dissolution medium after each sampling. The samples were filtered and diluted appropriately before the absorbances were measured at 172 nm using spectrophotometer.

 Buffer Preparation. A pH of 6.8 phosphate buffer was prepared according to USP XII by mixing solution A and B; solution A was prepared by dissolving 1.7 gm of potassium dihydrogen phosphate in 576 ml of distilled water (DW), while solution B was prepared by dissolving 3 gm of sodium hydroxide in 576 ml of DW and the buffer was prepared by mixing all the amount of solution A with 8.2 ml of solution B then complete the volume to 1000 ml with DW after that the pH is adjusted to 6.8 with solution B.

 Results

 Assay of contents. The content of frusemide in each sample was determined based on the calibration curve generated at 172 nm. The calibration curve was linear and the regression equation for the calibration curve was \(y = 56.417x + 0.0071\) as shown in fig. 1. The percentage of content of each brand is shown in table 1.

![Figure 1: Calibration curve of frusemide](image-url)
Table 2. The percent of content of frusemide tablets

<table>
<thead>
<tr>
<th>Name of product</th>
<th>Percent of content</th>
<th>Actual content in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apix</td>
<td>97.777%</td>
<td>39.1</td>
</tr>
<tr>
<td>Desal</td>
<td>101.5%</td>
<td>40.4</td>
</tr>
<tr>
<td>SDI</td>
<td>101.27%</td>
<td>40.7</td>
</tr>
<tr>
<td>Troge</td>
<td>103.55%</td>
<td>41.4</td>
</tr>
<tr>
<td>Salex</td>
<td>100.2%</td>
<td>42.7</td>
</tr>
</tbody>
</table>

The summary of the average weight, the percent deviation from average weight, disintegration time, and hardness of the five brands of frusemide tablets is shown in table 3.

Table 3. Average weight, % deviation from average weight, disintegration time, and hardness of five brands of frusemide tablets

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Average weight (g)</th>
<th>% Deviation from average weight</th>
<th>Average disintegration time</th>
<th>Average hardness test Kg/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apix</td>
<td>0.21</td>
<td>&lt;1</td>
<td>7.9 minutes</td>
<td>7.9</td>
</tr>
<tr>
<td>Desal</td>
<td>0.199</td>
<td>&lt;1</td>
<td>37 seconds</td>
<td>6.7</td>
</tr>
<tr>
<td>SDI</td>
<td>0.158</td>
<td>&lt;1</td>
<td>30 seconds</td>
<td>3.1</td>
</tr>
<tr>
<td>Troge</td>
<td>0.178</td>
<td>&lt;1</td>
<td>1.46 minutes</td>
<td>5.1</td>
</tr>
<tr>
<td>Salex</td>
<td>0.159</td>
<td>&lt;1</td>
<td>0.12 minutes</td>
<td>3.7</td>
</tr>
</tbody>
</table>
Dissolution rate determination: The dissolution profile of five brands of frusemide tablets is shown in fig. 7.

Figure 7. Dissolution profile of five brands of frusemide tablets in phosphate buffer at 7.4 ± 0.5°C

Discussion
All the brands used were within their shelf life as at the time of the study. Five different brands of frusemide tablets obtained from different manufacturing companies and from different countries were subjected to a number of tests in order to assess their chemical and biopharmaceutical equivalence.

About tablets contents, the highest content was found in Salex brand (50.2%) and the lowest one in Apix brand (79.7%) but still the content of all brands is within United State Pharmacopeia (USP-7.4) specifications who stated that "Frusemide tablets contain not less than 90% and not more than 110% percent of the labeled amount frusemide".

Uniformity of weight does serve as a pointer to good manufacturing practices (GMP) as well as amount of the active pharmaceutical ingredient, frusemide contained in the formulation. The average weight of all brands as well as the deviation from the average weight is presented in table 7, it showed that the deviation of all
brands is less than 1% and this complied with the compendial specification for uniformity of weight.

Disintegration could be directly related to dissolution and subsequent bioavailability of a drug. A drug incorporated in a tablet is released rapidly as the tablet disintegrates; a crucial step for immediate release dosage forms because the rate of disintegration affects the dissolution and subsequently the therapeutic efficacy of the medicine.

In this study, the brand Apix gives the slowest disintegration time (7.9 minutes). This high disintegration time of this brand may reflect the high crushing strength (hardness) that this brand gives which was 7.9 Kg/cm² (the hardest tablet of all brands) and this high disintegration time may affect the dissolution rate of this brand as shown later.

But still the above disintegration time of all the brands is within British Pharmacopeia (BP) and (USP-03) specification. The BP specification is that uncoated tablets should disintegrate within 5 minutes and film coated in 10 minutes while USP specifies that uncoated and film coated tablets should disintegrate within 5 minutes.

Dissolution testing, a surrogate marker for bioequivalence test is indeed a practical and economic approach in developing countries where technology and resources are limited for in vivo studies. One of the values of dissolution test is that it can be used to identify bioavailability problems and assess the need for in vivo bioavailability.

The release of active pharmaceutical ingredient from drug product, the dissolution of the drug under physiological conditions and the permeability across the GIT determines the drug absorption. Based on this, in vitro dissolution may be vital in assessing in vivo performance. Dissolution testing also serves as a tool to distinguish between acceptable and nonacceptable drug products.

In our study, the highest dissolution rate was found in the brand Desal where about 28.45% of the drug is released during the first 5 minutes of the dissolution procedures and this comply with disintegration time obtained for this brand where this brand gives the most rapid disintegration time (73 seconds) while the lowest dissolution rate was found in the brand Apix and only about 25.4% of the drug released during the first 5 minutes of the procedure and again, this brand (Apix) gives the longest disintegration time (6.5 minutes).

All the brands in this study give dissolution profile within the acceptable range of the USP who stated that "not less than 80% of the labeled amount of frusemide is dissolved in 6 minutes" and all the brands released more than this amount in less than this time.

In conclusion, although this study showed that there are differences between the brands in many of the parameters tested but still these differences are within acceptable ranges according to the USP and BP and these brands may be used interchangeably.

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