Bioequivalence evaluation of a triamterene–hydrochlorothiazide generic product: A new bioequivalence index for fixed-dose combinations

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A B S T R A C T
In this study, an open, double-blind, randomized, two-period, two-group crossover design was conducted in 14 healthy volunteers to study the bioequivalence of a fixed-dose generic product. After administration of test or reference products to each volunteer, both active ingredients were determined simultaneously in plasma samples using a developed and validated HPLC-UV method, and pharmacokinetic parameters, including $C_{\text{max}}$, $T_{\text{max}}$, $AUC_{0-\infty}$, $AUC_{0-\infty}$, terminal elimination rate constant ($k_{el}$), volume of distribution in steady state ($V_{d(\infty)}$), mean residence time (MRT), clearance (Cl), terminal elimination rate constant ($k_{el}$) were determined in each subject using the standard non-compartmental approach. Statistical comparison showed that the test and reference products were bioequivalent in terms of both the rate and extent of bioavailability of both active ingredients. Finally, a new parameter named range overlap index (ROI) was introduced for the first time in this study in order to judge about the overall bioequivalence of the combination products. This parameter indicates the extent in which the two CI90% ranges of each parameter for two active ingredients overlap with each other. The ROI is suggested to be equal or more than 50% for two combination products in order to be known as bioequivalent. The ROI values of the bioequivalence-indicating parameters were 61.90%, 84.6%, and 76.0% for $C_{\text{max}}$, $AUC_{0-12}$, and $AUC_{0-\infty}$, respectively, which are indicative for bioequivalence in all the cases.

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1. Introduction

Triamterene (2,4,7-triamino-6-phenylpteridine) (Fig. 1A) is a potassium-sparing diuretic commonly used in combination with hydrochlorothiazide (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide), a thiazide-type diuretic (Fig. 1B) in clinical management of edema and moderate hypertension (Materson, 1983; Mills et al., 1977). In fact, this combination is a diuretic/antihypertensive drug product that combines natriuretic and antikaliuretic effects in such a way that each component complements the action of the other. The hydrochlorothiazide component blocks the reabsorption of sodium and chloride ions, thereby increasing the quantity of sodium traversing the distal tubule and the volume of water excreted. The triamterene component exerts its diuretic effect on the distal renal tubule to inhibit the reabsorption of sodium in exchange for potassium and hydrogen ions. Its natriuretic activity is limited by the amount of sodium reaching its site of action.

Since the introduction of Dyazide by GlaxoSmithKline (GSK) in 1974 as the innovator brand of this preparation (Douglas et al., 1974), the product is marketed continuously as a part of routine antihypertension programs with the usual dosage of one tablet twice daily after meals.

Hydrochlorothiazide is incompletely but fairly rapidly absorbed from the gastrointestinal tract. Peak plasma concentrations occur between 60 and 120 min. It is excreted unchanged into urine, 50% being recovered within the first 12 h. The serum half-life is estimated to be in the range of 3–4 h (Hardman et al., 1996; Lacy et al., 2005).

Triamterene is rapidly absorbed from the gastrointestinal tract but to a variable extent (30–70% of the oral dose). The peak plasma concentration of triamterene is reached 2–4 h after an oral dose and the half-life of the medicine in plasma ranges from 1.5 to 2 h. Approximately 50% of the triamterene in plasma is protein bound. Triamterene undergoes metabolism in the liver. Triamterene and its metabolites are then excreted renally utilising the processes of filtration and tubular secretion. About 20% of an oral dose appears unchanged in the urine, 70% as the sulphate ester of hydroxytriamterene, and 10% as free hydroxytriamterene and triamterene glucuronide (Hardman et al., 1996; Lacy et al., 2005).

According to the requirements of the bioequivalence of the pharmaceuticals issued by US food and drug administration...
(FDA) and world health organization (WHO), two fixed-dose multi-active products are known as bioequivalent only if the bioequivalence requirements are met by both active ingredients independently. Therefore, the majority of the bioequivalence studies reported on triamterene–hydrochlorothiazide involve the evaluation of the bioequivalence of two active ingredients separately and independently (Blume et al., 1984; Sharoky et al., 1989; Williams et al., 1990). While this requirement is certainly necessary for overall bioequivalence, it is not enough, since it suffers from the lack of a criterion for decision making about the ‘combined bioequivalence’. In other words, if one evaluates each component separately, since the active ingredients are administered in combination, when the 90% confidence intervals of the active ingredients are far distant from each other, there may be some individual cases in statistical population in which one of the ingredients is bioequivalent with its reference while the other one is bioinequivalent. As a probable solution to this problem, we propose a new criterion, ROI, in the present study for statistical judgment about the bioequivalence of the two-in-one products using the typical case of triamterene-H as a model.

2. Materials and methods

2.1. Materials

Triamterene (CAS 396-01-0) and Hydrochlorothiazide (CAS 58-93-5) pure chemicals and triamterene-H test product (T: 50 mg, H: 25 mg) were provided by Iran Darou Pharmaceuticals (Tehran, Iran). Dyazide reference product (GlaxoSmithKline, UK; T: 50 mg, H: 25 mg) was purchased locally. Other Chemicals and solvents were from general lab or HPLC purity grades, as needed, and were purchased locally.

2.2. Preparation of calibration standards and quality control samples

For preparing stock standard solution of Triamterene-H, 10 mg of both triamterene and hydrochlorothiazide was dissolved in 100 ml deionized water to prepare a 100 μg/ml solution and stored in a refrigerator at 4 °C. The stock solution was further diluted with water to obtain the different working solutions ranging 25, 50, 100, 250, 500, 1000, 2000 ng/ml. Then, calibration curves of triamterene and hydrochlorothiazide was prepared by spiking blank plasma within the concentration range of 2.5–200 ng/ml. Quality control (QC) samples were prepared at low (10.0 ng/ml), medium (50.0 ng/ml) and high (100.0 ng/ml) concentrations in the same way as the plasma samples for calibration.

2.3. Samples preparation

A liquid–liquid extraction procedure was used for the isolation of triamterene-H from plasma samples. For this purpose, at first plasma samples that were stored at −20 °C, allowed to thaw at room temperature before processing and then 8 ml of diethyl ether was added to 2 ml plasma and, after vortex mixing for 1 min and centrifugation at 5000g for 10 min, the upper organic layer was transferred by aspiration to another tube and evaporated under nitrogen at 40 °C to dryness. The residue was, then, reconstituted in 200 μl mobile phase and following a brief mixing, a volume of 100 μl was injected to equilibrated HPLC system.

2.4. Chromatographic condition

A reversed-phase high performance liquid chromatography (HPLC) method was developed and validated for determination of the plasma concentrations of triamterene and hydrochlorothiazide simultaneously throughout the study. The analytical column was a fused silica-based Chromolith C18 column (Merck, Germany; particle size 5 μm; 100 mm × 4.6 mm) equipped by a guard column with the same packing (Merck, Germany; particle size 5 μm; 10 mm × 4.6 mm). A mixture of 0.04 M phosphate buffer (pH 6.5) adjusted with phosphoric acid 85%) and acetonitril (65:35 v/v) was used as the mobile phase, delivered with a double-reciprocating pump (Knauer, Germany, model Smartline 1000) at a flow rate of 1 ml/min. The column was kept at room temperature and a UV–Visible detector (Knauer, Germany, model Smartline 2500) was used for analyte detection at wavelength of 210 nm. A Rhodyne device was used for injection of 100 μl of sample. The total run time was set on 15 min with triamterene and hydrochlorothiazide retention time of 7.3 and 9.4 min, respectively.

2.5. Validation

A series of analytical method validation tests were carried out on the developed assay method, including linearity, within-run and between-run precision and accuracy, recovery, limit of detection (LOD) and limit of quantitation (LOQ).

2.5.1. Linearity and range

Linearity was evaluated using freshly prepared spiked plasma samples and calibration curves were constructed using seven non-zero standard points covering the range of 2.5–200 ng/ml. In addition, a blank (non-spiked sample) was run to discard the presence of interferences. Plasma samples were spiked at concentrations of 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, 200.0 ng/ml. The samples were extracted as described in item 2.3. The standard calibration curves for triamterene and hydrochlorothiazide were constructed using ratio of peak-height versus nominal concentrations of the analytes. The calibration curves for triamterene and hydrochlorothiazide were calculated by the equation: \( y = ax + b \), where \( y \) is the peak-height ratio, \( x \) the concentration of drugs, \( a \) the slope and \( b \) the intercept of the regression line. By successive decreasing of concentration, the limits of detection (LOD) and the limit of quantification (LOQ) were evaluated as a concentration giving a signal-to-noise ratio of 3:1 and the lowest concentration of the drugs capable being evaluated with precision and accuracy under the stated experimental conditions, respectively. Biological samples spiked with triamterene and hydrochlorothiazide were injected in decreasing concentrations until the lowest
2.5.2. Precision and accuracy

For determination of within-run and between-run precision and accuracy, three different series of samples at concentrations of 5.0, 25.0 and 100.0 ng/ml of triamterene-H were analyzed within a single instrument run and in different runs, respectively. The accuracy was calculated from ratio of measured concentration, based on calibration curve, to the nominal added concentration; whereas, precision was evaluated by calculating the within-run and between-run coefficients of variations of the measured concentrations at each level (CV%).

2.5.3. Relative recovery (matrix effect)

To assess the extraction recovery, three series of samples at concentration levels of 10.0, 50.0 and 100.0 ng/ml of triamterene-H, were prepared in two medium; one with human plasma and the other without plasma. To the set without plasma, deionized water was added instead of plasma. All the samples were processed as described in Section 2.3. The recovery (extraction efficiency) was calculated from the ratio of peak height of plasma standard to the peak height of water standard.

2.6. Bioequivalence study

Fourteen healthy, non-smoking, non-alcoholic, male volunteers, aged within the range of 19–28 years (23.15 ± 2.73 years) enrolled and participated in the study after a brief physical examination, history and routine clinical laboratory tests, including hematocrit value, hemoglobin concentration, red blood cells, white blood cells, and platelets counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCHC), and serum concentrations of SGOT, SGPT, alkaline phosphatase, and creatinine, in order to exclude the possibility of having familiar history of or active disease(s) influencing drugs pharmacokinetic as well as to make sure about the hemodynamic, renal, or hepatic function of the volunteers. The results of clinical laboratory tests showed that all the volunteers were healthy and had no history of kidneys and metabolic diseases. The demographic data of the volunteers are shown in Table 1. The whole study protocol was in accordance with the Ethical Guidelines of Iranian Ministry of Health for Clinical Studies and reviewed and approved by the Institutional Ethics Committee. Also, the clinical protocol of the study was reviewed and approved by the Ethics Committee of the Iranian Food and Drug Organization and, accordingly, all the volunteers completed written informed consent form after they had receive detailed instructions about the aims, restrictions and possible adverse effect which could be experienced as a result of taking the drug. Subjects did not receive any medication during the 2 weeks period prior to the start and also were not undergoing any pharmacological treatment during the study period. The study was an open, randomized, two-period, two-group cross-over design with a 7 days washout period between doses. During the first period, volunteers from group A received a single dose of triamterene-H tablets (50 mg Triamterene and 25 mg Hydrochlorothiazide) manufactured by Iran Darou (test), while volunteers from group B received a single dose of Dyazide tablets manufactured in GSK (reference). During the second period, the procedure was repeated on the groups in reverse. The tablets were administered to the volunteers in the next morning after an overnight fast, with 200 ml of water. Volunteers received standard breakfast, lunch and snack, respectively, 3, 6 and 10 h after drug administration. No other food was permitted for consumption during the blood sampling period. Blood samples (5 ml) from a suitable venous were collected by indwelling catheter into heparin-containing tubes at 0 (baseline), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.0, 9.0, and 12.0 h post-dosing. The blood samples were centrifuged at 3000g for 10 min and the plasma fraction was separated by aspiration and stored at –20 °C until being assessed for triamterene-H content. Volunteers did not ingest any alcoholic drink, coffee or other xanthine-containing drinks like tea during the trial.

2.6.1. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated from plasma levels at different time points, applying a standard non-compartmental method. The maximum plasma concentration (Cmax) and time point of maximum plasma concentration (Tmax) values were obtained directly by visual inspection of the plasma triamterene and hydrochlorothiazide concentration–time data. The area under the plasma concentration–time curve from zero to the final sampling time (AUC0–12) for each subject was obtained using the trapezoidal method with the general formula:

\[
\text{AUC}_{0–12} = \sum \left( \frac{(C_n + C_{n-1})}{2} \times (t_n - t_{n-1}) \right); n : \text{non-zero sampling time points}
\]

where Cn is the plasma concentration at time tn.

The total area under the plasma concentration–time curve (AUC0–∞) was calculated using the basic equation:

\[
\text{AUC}_{0–\infty} = \text{AUC}_{0–12} + C_{12}/2 z
\]

where C12 is the plasma concentration measured at time 12 h and z is the terminal elimination rate constant. The terminal elimination phase was detected by visual inspection of the data, validated by linear regression analysis. The rate constant corresponding to this phase, z, was determined from the slope of the log-normal curve of plasma concentration–time data using the basic formula:

\[
\text{Slope} = -\frac{z}{2.303}; t_{1/2} = \ln 2 / z
\]

In addition to the primary bioavailability-indicating pharmacokinetic parameters described, in order to have a better insight into the pharmacokinetic profile of the drugs following administration of two products to the subjects participated in the study, we calculated a series of parameters, including mean residence time (MRT), total clearance (Cl), volume of distribution in steady state (Vd(50)) and terminal elimination rate constant (kel);

Table 1

<table>
<thead>
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<th>Volunteer code</th>
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<th>Gender</th>
<th>TBWa (kg)</th>
<th>IBWa (kg)</th>
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<td>M</td>
<td>76</td>
<td>71</td>
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<td>69</td>
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<td>SD</td>
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<td>CV%</td>
<td>11.35</td>
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<td>7.64</td>
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<tr>
<td>Max</td>
<td>28.00</td>
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<td>79.00</td>
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<tr>
<td>Min</td>
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<tr>
<td>Median</td>
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<td>70.5</td>
<td></td>
</tr>
</tbody>
</table>

a True body weight.

b Ideal body weight.

c Male.

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\[ MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}} \]  

Where \( AUMC_{0-\infty} \), the area under the first statistical moment–time curve, is the area under the curve of \( C_n \times t_n \) vs. \( t_n \), calculated using the trapezoidal method until the last time, i.e., 12 h plus the contribution of the time period beyond the last point using the formula:

\[ AUMC_{0-\infty} = AUMC_{0-12} + \left[ \frac{C_{12} \times 12}{2} \right] + \left[ \frac{C_{12}}{\lambda D} \right] \]  

The total clearance of the drugs was calculated from:

\[ Cl = \frac{(F \times Dose)}{AUC_{0-\infty}} \]  

Where \( F \) is the fraction of the oral dose reached to systemic blood circulation. Finally, the volume of distribution of the drug at steady state of the distribution equilibrium was obtained as:

\[ V_d(ss) = Cl \times MRT \]  

The terminal elimination rate constant, \( K_e \), was estimated from the slope of the terminal exponential phase of the plasma of the drugs concentration–time curve (by means of the linear regression method).

For each drug, the pharmacokinetic parameters described were calculated based on the individual plasma concentration profiles for each volunteer and, then, the test-to-reference ratio of numerical as well as log-transformed data. In this study, to consider the combined statistical situations in real bioequivalence estimation, we defined a new arbitrary index named range overlap index (ROI) as:

\[ ROI = \left( \frac{\text{CI90\% ranges for two active ingredients/the whole CI90\% range for the drug with wider range}}{100} \right) \]  

Where CI90\% represents the 90\% confidence interval of the ratio of log-transformed parameters.

Statistically, the bioequivalence of two formulations was assessed by means of an analysis of variance (ANOVA) for crossover design and calculating 90\% confidence interval of the ratio of test/reference using numerical as well as log-transformed data. The formulations were considered bioequivalent if: (i) the difference between each of the respective primary parameters for test and reference products was found statistically non-significant for each active ingredient \( p < 0.05 \); (ii) the 90\% confidence intervals of the ratios of log-transformed data would be within 80–125\% for \( AUC_{0-12} \), \( AUC_{0-\infty} \), and \( C_{\text{max}} \) for each active ingredient, and (iii) the ROI was \( \geq 50\% \) (arbitrarily defined in this study).

### 3. Result and discussion

#### 3.1. Method development

In the way to develop a simple and popular method for triamterene/hydrochlorothiazide assay in human plasma for pharmacokinetic studies, HPLC with UV detection was selected as the method of choice. For best results, we tested a wide spectrum of organic solvents from different physiochemical categories with different volume fractions as well as combinations. In terms of the analysis condition, various mobile phases, in different proportions, buffered and non-buffered at various pH were attempted to provide concomitantly the best peak resolution and retention times.

After considering all the data, the optimum method condition described earlier was selected to be followed up. The number of theoretical plates \( (N) \), peak symmetry, and relative retention \( (k') \) of the method all were in the desirable range as can be judged from the chromatograms shown in Fig. 2; where, the retention times of triamterene and hydrochlorothiazide is shown in 7.3 and 9.4 min, respectively. Briefly, the notable advantages of the developed method over the history of Triamterene-H assay can be mentioned as follows:

- Available and popular instrumentation
- Available reagents and chemicals
- Simple and fast sample preparation procedure
- Sensitivity high enough for pharmacokinetic studies
- Acceptable accuracy and precision

These advantages of the method allowed it to be used successfully for the analysis of the large number of the plasma samples throughout the study.

#### 3.2. Method validation

The method validation tests showed the method linearity within the concentration range of 5–100 ng/ml for triamterene and 2.5–100 ng/ml for hydrochlorothiazide with the corresponding regression coefficients of more than 0.99 for both drugs. A typical regression equation of the method was \( y = 0.0185 \times x + 0.24 \) \( (r^2 = 0.9996, n = 7) \) for triamterene and \( y = 0.0215 \times x + 0.194 \) \( (r^2 = 0.9997, n = 7) \) for hydrochlorothiazide. For investigation of specificity, a blank (drug-free) human plasma as well as different concentrations of spiked human plasma were screened and no endogenous interference was observed at the retention time of triamterene and Hydrochlorothiazide. The average within-run and between-run percent coefficient of variation (CV\%) of the method for triamterene and hydrochlorothiazide were all below 12 percent, indicating acceptable degree of precision, both repeatability and reproducibility. Also, all the accuracy values of the method both within- and between-runs were acceptable (Table 2). For relative recovery (matrix effect) evaluation, varieties of extractions were tested and the best recovery was achieved by the liquid–liquid extraction with ether. The recovery was calculated by comparing the peak height ratio of both drugs in plasma samples to the peak height ratios in water as standard. As can be seen in Table 3, the data indicate an acceptable degree of recovery for both drugs throughout the linear ranges with more than 90 percent. In addition, the method showed very good sensitivity with respect to the range expected in the current study, with limit(s) of detection (LOD) and limit(s) of quantification (LOQ) of 2.5 and 5 ng/ml for triamterene and 1.25 and 2.5 ng/ml for hydrochlorothiazide, respectively.

#### 3.3. Pharmacokinetic and bioequivalence study

The developed analytical method was applied to the determination of triamterene and hydrochlorothiazide in plasma samples generated during the bioequivalence study in 14 healthy Iranian male volunteers who were orally administered one tablet of Triamterene-H. The average plasma concentration profiles of triamterene and hydrochlorothiazide in the volunteers completed the study are shown in Figs. 3 and 4, respectively. These figures clearly show the applicability of the method, judged by comparing the generated data with the pharmacokinetic profile indexed for the drugs. Based on the plasma concentration–time data, the mean of primary and secondary pharmacokinetic parameters for triamterene and hydrochlorothiazide are listed in Table 4. The data show that there is no statistically significant difference between the two products. Also, for better comparing of test and reference drugs, numerical and log
transformed data of relative bioavailability of triamterene and hydrochlorothiazide are shown in Table 5. Finally, 90% confidence intervals of the active ingredients and the range overlap index (ROI) of the primary pharmacokinetic parameters of the two active ingredients, i.e., triamterene and hydrochlorothiazide are shown in Table 6 to approve bioequivalence of two formulations. The ROI values of the bioequivalence-indicating parameters were 61.90%, 84.6%, and 76.0% for $C_{\text{max}}$, $\text{AUC}_{0-12}$, and $\text{AUC}_{0-\infty}$, respectively, which are indicative for bioequivalence in all the cases.

In fact, the current study aimed at the bioequivalence evaluation of a generic triamterene–hydrochlorothiazide product manufactured in Iran with reference to the innovator brand Dyazide (GSK). The first important factor in the way to establish the study, as usual, was the availability of a reliable assay method capable of,
pharmacokinetic profiles shown in Figs. 3 and 4, it is evident that to be used throughout the study for plasma analysis. From the poses showed such a sensitivity, accuracy, and precision satisfactory tests, the simple and rapid HPLC method developed for this pur-

In the biological samples obtained from the volunteers taking part preferably, simultaneous determination of two active ingredients in the biological samples obtained from the volunteers taking part in the study, reflects the inherent variability of the population used in the study, reveals the inherent variability of the pharmacokinetic of the drugs. There is a remarkable difference in systemic clearance of the two drugs (Table 4), which can be attributed to the differences in the volume of distribution of two drugs, with the Vd(ss) values for triamterene being about 60% higher than that of hydrochlorothiazide, which, in turn, is a result of the higher lipid/water partition coefficient of the former drug. As the first measure of bioequivalence of two drug products, no statistically significant differences between the two products were found in terms of both the primary, and also secondary, pharmacokinetic parameters of both the drugs. In addition, the data on the relative bioavailability of both drugs in each subject, taking into account the statistical estimation of the confidence intervals of the primary parameters, showed that not only the numerical data (Table 5) but also the log-transformed calculations lie within the acceptable ranges of the regulatory authorities, i.e., 0.80–1.20 for the numerical and 0.80–1.25 for log-transformed data. In other words, the test product is bioequivalent with the reference innovator brand in terms of both the rate and extent of bioavailability and, therefore, can be regarded as pharmaceutically interchangeable.

Besides the standard approach and conclusion made on bio-
equivalence of two products, as described, there is always a general concern about the concept of the ‘combined bioequivalence’ of the two active ingredients contained in a dosage unit when it is admin-
istered as a fixed-dose product. In other words, considering that the bioequivalence is basically a statistical definition, for an individual patient who takes the test drug product, we expect with a significance level of 0.05 that the primary pharmacokinetic parameters obtained will be within the ratio interval of 0.8–1.25 to the results the same patient might obtain from the reference product. This scenario becomes somehow complex when we consider a two-in-one preparation, in which two different active ingredients have two different ratio ranges compared to the reference product. In more clear words, with one single dosage unit, one active ingre-
dient can be within the safe ratio range while the other one be out of the range. This case becomes more probable when the CI90% ranges of two actives are far distant from each other, for example one with CI90% of 0.8–0.90 and the other with CI90% of 1.10–1.25.

**Table 2**
Within-run and between-run precision and accuracy of the developed method for triamterene and hydrochlorothiazide assay in human plasma (n = 5).

<table>
<thead>
<tr>
<th>Nominal concentration (ng/ml)</th>
<th>Triamterene</th>
<th>Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precision (CV%)</td>
<td>Accuracy (Mean ± SD)</td>
</tr>
<tr>
<td></td>
<td>Within-run</td>
<td>Between-run</td>
</tr>
<tr>
<td>5</td>
<td>8.31</td>
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</tr>
<tr>
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<td>9.34</td>
</tr>
<tr>
<td>50</td>
<td>3.49</td>
<td>10.90</td>
</tr>
<tr>
<td>Mean</td>
<td>6.8 ± 2.87</td>
<td>9.95 ± 0.83</td>
</tr>
</tbody>
</table>

**Table 3**
Relative recovery (matrix effect) of the developed method for triamterene and hydrochlorothiazide in human plasma.

<table>
<thead>
<tr>
<th>QC samples</th>
<th>Triamterene</th>
<th>Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration added (ng/ml)</td>
<td>Recovery (%) (Mean ± S.D)</td>
</tr>
<tr>
<td>Low</td>
<td>10</td>
<td>91.07±1.64</td>
</tr>
<tr>
<td>Mid</td>
<td>50</td>
<td>92.79±0.84</td>
</tr>
<tr>
<td>High</td>
<td>100</td>
<td>95.89±3.04</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>93.25±2.44</td>
</tr>
</tbody>
</table>

**Fig. 3.** The mean plasma concentration–time profile of triamterene following a single oral dose of 50 mg as the test and reference formulations to 14 healthy male volunteers.

**Fig. 4.** The mean plasma concentration–time profile of hydrochlorothiazide following a single oral dose of 25 mg as the test and reference formulations to 14 healthy male volunteers.
To address the above-mentioned issue of ‘combined bioequivalence’, we propose in the current study a new index named range overlap index (ROI) indicating the extent by which the CI90% ranges of each parameter for two active ingredients overlap each other. We suggest that this index would be calculated in reference to the active ingredient with the wider range for each parameter in order to be more sensitive to the upper and lower limits of the overall data. Based on our literature search, consults with different clinicians, and also our personal experiences with bioequivalence studies, we propose a limit of ≥50% for a two-in-one product in order to be approved. Considering the data obtained in the current study, our test product can be regarded as bioequivalent with respect to this new index along with the general bioequivalence criteria discussed earlier in this section.

4. Conclusion

A generic triamterene–hydrochlorothiazide combination product manufactured locally in Iran was tested for bioequivalence with the innovator brand Dyazide (GSK). A rapid and simple HPLC method was developed and validated, which showed acceptable sensitivity, accuracy, and precision with respect to the purposes of this study. The study was well-conducted with no notable unexpected issues, using 14 healthy male volunteers. The overall data obtained in the volunteers showed that the two products are bioequivalent in terms of both the rate and extent of the bioavailability of both the active ingredients taken separately. To address the concern of ‘combined bioequivalence’, i.e., the bioequivalence of each active in presence of the other one, we proposed a new index, range overlap index (ROI), which reflects the extent by which the CI90% ranges of two actives for each parameter overlap each other. The test product was regarded as bioequivalent with respect to this index as well.

Conflict of interest

There was no conflict of interest for any of the authors of this paper.

Acknowledgments

The authors wish to thank Iran Darou Pharmaceuticals (Tehran, Iran) for kindly providing us with the test products and drugs raw materials.

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