Spectrophotometric Estimation of Losartan Potassium with Methylene Blue by Ion-Pair Extraction Method

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Abstract
A selective and sensitive spectrophotometric extraction method was established and used to estimate antihypertensive drug, losartan potassium. The method is based on the formation of blue ion pair of the anionic drug, losartan, and the cationic dye, methylene blue, at adjusted pH 6.5 in aqueous solutions, followed by quantitative extraction to dichloromethane. The observed maximum absorbance was at λ 654.9 nm. With 4.53321 x 10⁻⁵ M⁻¹ cm⁻¹ molar absorptivity, Beer's law was obeyed within a concentration range of 0.03-1.5 μg / ml. The limit of detection and the limit of quantification were 0.01μg / ml and 0.03μg / ml, respectively. The method precision was estimated by a relative standard deviation (0.725%-1.64%), and the accuracy was validated at a recovery range (98.48-100.3%). The proposed method was successfully used for estimating losartan in the pharmaceutical formulation and in the urine sample.

Keywords: Losartan potassium, methylene blue, dichloromethane, and ion-pair complex.

التقدير الطيفي للوسارتان البوتاسيوم مع المثلين الأزرق بطريقة استخلاص الترابط الايوني

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الخلاصة
تم إنشاء طريقة استخلاص طيفية انتقائية حساسة ودقيقة لتقدير عقار اللوسارتان البوتاسيوم الخاص
لضغط الدم. تضمن الطريقة تكوين معقد الترابط الايوني الأزرق اللون النابض في الماء بين اللوسارتان الايوني السائل و صبغة المثلين الأزرق الايوني الموجود عند اس هيدروجيني 6.5. تم استخلاص معقد الترابط الايوني كمية بواسطة ثنائي كلوروميثان. تم قياس اقصى امتصاص لها عند طول موجي 654.9 نانومتر. وإن قانون بيير يطبق على مدى التركيز تراوحيث بحدود 0.03-1.5 مايكروغرام / ملتر وكان الامتصاصية المولارية 0.03125 x 4.53321 مولري 1 سم 1 م. وجد كشف وحد كمي قياسي 0.01 مايكروغرام / ملتر و 0.03 مايكروغرام / ملتر قطعي التوالي، قدرت دقة الطريقة بحسب الانحراف القياسي النسبي (0.725-6.4% ) وتوافق ب مدى الاسترجاع النسبي (98.48-100.3%). تم تطبيق الطريقة المترحة
بنجاح لتقدير اللوسارتان البوتاسيوم في المستحضر الصيدلاني وعينة الادار.

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1. Introduction
Losartan potassium; 2-butyl-4-chloro-1-[p-(o-1H-tetrazole-5-yl-phenyl)-benzyl]-imidazole-5-methanol potassium, has the chemical formula of C_{22}H_{22}ClN_{6}OK and molecular weight of 461.09 [1]. It is a white to off-white powder that is freely soluble in water, slightly soluble in acetonitrile, and soluble in isopropl alcohol [2]. The ionization constant of the compound is pKa = 5–6 [3]. Losartan is an imidazole moiety on biphenyl tetrazole Figure-1A with an antihypertensive activity that is used in the treatment of moderate and severe hypertension [1]. Several quantitative instrumental approaches were reported and developed for the estimation of losartan potassium, including colorimetric methods based on extraction using dyes such as calmagite and orange-II at pH 1.2 [4] and sulphophthalein bromothymol blue, bromphenol blue, and bromocresol green at pH 3-4 [5]. The charge-transfer reactions occur between the n-electron donor (losartan potassium) and the electron acceptors, namely iodine, 7,7,8,8-tetracyanoquinodimethane, 1,3,5-trinitrobenzene, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, p-chloranilic acid, tetracyanoethylene, 2,3,5,6-tetrabromo-1,4-benzoquinone, 2,3,5,6-tetrachloro-1,4-benzoquinone, and 2,4,7-trinitro-9-fluorenone. Different colored charge-transfer complexes and radical anions were obtained [6]. Derivative UV-spectroscopic methods were reported for the estimation of losartan potassium alone or formulated with other drugs [7-13]. HPLC chromatographic methods were established for the determination of losartan potassium in pure form or biological fluids [14-16], along with several electro-analytical [17-19], and spectrofluorimetric [20] methods. Ion-pair extractive spectrophotometry is a common and multipurpose method because of simplicity, low cost, sensitivity and rapidity for the determination of many pharmaceutical compounds [21; 22]. The current study was based on the formation of an ion-pair complex between losartan and the cationic dye methylene blue (MB), as shown in Figure-1B, which is extracted into an organic solvent. Losartan has no characteristic visible spectrum, though, when associated with organic dye MB, it can be extracted and detected in the visible region. This method was appropriate to be applied effectively for the quantitative determination of losartan in pharmaceuticals and spiked urine samples.

![Losartan Potassium](image1a.png)

![Methylene Blue](image1b.png)

**Figure1** - Structural formulae of (A) losartan potassium and (B) methylene blue.

2. Experimental Part
2.1. Instruments
A double beam UV-visible spectrophotometer (Perkin Elmer Lambda 25, USA) was used to carry out spectral runs, with 1 cm quartz cells. A pH-meter (HANNA, Portugal) was used for fast and stable pH measurements.

2.2. Materials
Losartan potassium was kindly provided by Awa medica pharmaceutical company, Erbil, Iraq. Solvents including dichloromethane, chloroform, n-hexan, octan, carbon tetra chloride, and ethyl...
acetate were obtained from Chem-Lab company/Belgium. All other chemicals were of pure analytical reagent grade.

2.3 Reagents

2.3.1 Losartan potassium Stock Solution (100 µg/ml)

0.01 g of losartan potassium was dissolved in distilled water and diluted to 100 ml in a volumetric flask. A series of standard solutions was prepared by suitable dilution of a stock standard solution with distilled water. The stock solution was kept in a refrigerator.

2.3.2 MB Solution (0.01% W/V)

A stock solution of MB was prepared by dissolving 0.01 g of MB in 100 ml distilled water.

2.3.3 Buffer Solution

Different buffer solutions that ranged in pH between 2.2 and 7.5 were freshly prepared using standard methods [23]. The buffer systems included in this study are:

1- Glycine- HCl; pH 2.2–3.6, pKa = 2.35
2- Sodium acetate pH 3.6–5.6, pKa = 4.76
3- Phosphate buffer; pH 5.8–8.0, pKa = 7.20

Stock solutions: 0.2 mole/L of NaH₂PO₄ and 0.2 mole/L Na₂HPO₄. Place X ml of the 0.2 mol/L sodium dihydrogen phosphate NaH₂PO₄ solution in 100 ml volumetric flask, add the specific volume Y ml of disodium hydrogen phosphate Na₂HPO₄ solution, then mix.

| pH   | X/ml | Y/ml |
|------|------|------|
| 6.0  | 87.7 | 12.3 |
| 6.5  | 68.5 | 31.5 |
| 7.0  | 39.0 | 61.0 |
| 7.5  | 16.0 | 84.0 |

2.3.4 Preparation of Sample Stock Solution

Ten tablets of each commercial sample of losartan were accurately weighed and grounded to a fine powder, and the average weight of the tablet was calculated. A powder portion equivalent to 100 µg/ml of losartan was weighed accurately and dissolved in 50 ml of distilled water. The solution was filtered through a Whatman filter paper No.41, and the filtrate volume was made up to 100 ml with distilled water. An aliquot part of the solution was analyzed as in the later described procedure.

2.3.5 Deproteinization of samples

Five ml of urine samples taken from different healthy volunteers were spiked with a known amount of losartan potassium stock solution. The urine was collected in the beaker and the deprotonation process was performed by adding 3 ml of 0.15M Ba (OH)₂ to the urine sample, followed by 3 ml of 2.5% w/v ZnSO₄·7H₂O. The mixture was mixed well then filtered through a filter paper. The filtrate was collected and diluted to 100 ml with D.W in a volumetric flask [24].

2.4 Recommended Procedure

To find the maximum wavelength of detection, 0.5 ml of 50 µg/ml standard stock solution of losartan potassium was pipetted into a 25 ml volumetric flask. Then 1.1 ml 100 µg/ml of MB solution was added, followed by 2 ml phosphate buffer solution pH 6.5. The mixture was diluted to the mark with distilled water, transferred to a separating funnel and 5ml of dichloromethane was added. The funnel was shaken for 2 min and allowed to stand for 3 min for clear separation of the two phases. The dichloromethane phase at the bottom of the funnel was transferred to the cuvette. This solution was scanned in the visible region between 400-800 nm. The same procedure was applied to the blank solution, i.e. drug-free solution.

2.5 Determination of losartan in spiked urine samples

The proposed method was applied to the determination of losartan in spiked urine provided by healthy volunteers. 5 ml of fresh urine was spiked by 5 ml and 10 ml of 100 µg/ml of losartan stock solution [25]. After deprotonization process, the mixture of urine was diluted to 100 ml by distilled water. A series of 1 ml of this urine was spiked with 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 µg/ml of 100 µg/ml losartan stock solution. The analysis was completed as in the above described recommended
3. Results and Discussion
3.1 Preliminary Tests - Absorption Spectra
Preliminary tests revealed that losartan potassium reacts with MB at room temperature in a neutral phosphate buffer medium. The blue colored product was extracted to an organic layer. A blank solution was prepared in a similar manner without losartan potassium. The absorbance / wave length spectra of the colored ion-pair complex were scanned in the 400-800 nm range against the blank. The blue colour product showed a maximum absorbance at 654.5 nm (Figure -2, before optimization).

![Absorption spectra of losartan MB ion-pair complex](image)

Figure 2 - Absorption spectra of (a) 1 µg/ml losartan MB ion-pair against reagent blank (b) reagent blank against dichloromethane.

3.2 Reaction Mechanism
Losartan potassium has a salt structure when dissolved in water, the anion losartan has been formed. Losartan anion attracts cationic MB due to the electrostatic attraction force formed between the negative and positive species in the solution, and the ion-pair neutral species is formed. The ion-pair neutral species was extracted into colorless dichloromethane phase. Consequently, colorless dichloromethane changes its color to blue, whereas the color of the dichloromethane layer remains unchanged in the blank experiment. Scheme 1 describes the formation of ion-pair complex.
3.3 Optimization of Variables

In order to achieve optimum conditions for this method, the following parameters were studied.

3.3.1 Effects of pH on Ion-Pair Formation

The influence of pH was studied by extracting the colored ion-pair in the presence of different types of buffers, such as glycine–HCl (pH 2.2–3.6), sodium acetate (pH 3.6–5.6), and phosphate buffer (pH 5.8–8.0). It was observed that the maximum color intensity and the highest absorbance values were observed in the phosphate buffer of pH 6.5. The results of buffer effects on the extraction process are shown in Figure 3a.

3.3.2 Effects of Volume of Buffer

The optimum volume of phosphate buffer at pH = 6.5 was also studied. It was concluded that 2.0 ml of phosphate buffer solution gives the best absorbance and results. Therefore, in the subsequent studies, pH adjustment in each sample was carried out using 2.0 ml of the phosphate buffer (Figure 3b).

3.3.3 Effects of MB Volume

The effects of MB volume on the color intensity of losartan MB ion-pair complex was investigated in the range of 0.1-1.5 ml of 0.01% of a solution of MB. The results (Figure 3c) showed that the
absorbance of losartan MB complex was directly proportional to the volume of MB up to 1.1 ml, which stayed roughly constant by further addition. Further addition makes the color of the aqueous solution more intense while the organic layer remains constant in color. Therefore, 1.1 ml of the MB was selected for the determination of losartan potassium.

3.3.4 Effects of the Extracting Solvent

The selection of a suitable extraction solvent is an important parameter to achieve the most efficient extraction procedure. The important property of the extraction solvent is that it should have low solubility in water and high extraction capability for the analytes. Different organic solvents, such as dichloromethane, carbon tetrachloride, chloroform, ethyl acetate anhydrous, n-hexane, and octane were examined for their capability to extract losartan MB ion-pairs for the proposed methods. Dichloromethane was selected as the most appropriate solvent which recorded the maximum absorption for the extraction of the ion-pair (Figure-4).

3.3.5 Effects of Shaking Time

Shaking time ranging from 0.5-3.5 min was investigated during the extraction of the ion-pair complex in dichloromethane to achieve the extraction of the complex. The maximum and constant
absorbance value was achieved when the complex was extracted after 2.0 min shaking.

3.3.6 Effects of Temperature and Stability of the Ion-Pair

The stability of the ion-pair complexes formed between losartan and MB was followed by measuring the absorbance against time. As shown in Figure 5, it was found that losartan MB complex was stable for at least 60 minutes after extraction. The effect of temperature on the formation of ion-pair complexes was also investigated. It was observed that the complexes were stable at room temperature (25°C) with negligible change in the absorbance values. Above this temperature, there was a slight increase in the absorbance of the complexes. This is due to the easy volatilization of the solvent.

![Figure 5](image)

**Figure 5** - Effects of time on the stability of the ion-pair complex.

3.3.7 Stoichiometry of Ion-pair

The stoichiometry of ion-pair complexes formed between losartan and the reagent dye was studied by Job’s continuous variation method, to determine the composition of ion-pair complexes at a wavelength of 654.9 nm. The results shown in Figure 6 indicate a 1:1 losartan MB ion-pair complex formation through the electrostatic attraction between the positive MB dye and the anion of losartan. The slope ratio method indicated the formation of a 1:1 ion-pair complex. It also showed that 1 mole of each of losartan potassium and dye reagent are participated in the ion-pair complex formation. Accordingly, the configuration of the ion-pair complexes was recommended and shown in Figure 6.

![Figure 6](image)

**Figure 6** - Job plot of losartan MB ion-pair in dichloromethane at 654.9 nm
3.3.8 Association constant

The Benesi – Hildebrand equation (or the double-reciprocal plot) was applied to calculate the association constant \( K_{IP} \) of losartan MB ion-pair complex. The association constants for ion-pair complexes formed between the drug and the dye were evaluated using the Benesi-Hildebrand equation [26], as shown below,

\[
\frac{[\text{cat.}]}{A_C} = \frac{1}{\varepsilon_C} + \frac{1}{K_{IP} \varepsilon_C} \times \frac{1}{[\text{Drug}]} \]

where \([\text{cat.}]\) and \([\text{Drug}]\) are the total concentrations of MB and losartan, respectively, \( A_C \) is the absorbance of the ion-pair complex, \( \varepsilon_C \) is the molar absorptivity of the ion-pair complex, and \( K_{IP} \) is the association constant of the ion-pair complex. According to the Benesi–Hildebrand equation, the plot of \( \frac{[\text{cat.}]}{A_C} \) versus \( \frac{1}{[\text{Drug}]} \) should be linear, with slope and intercept equal to \( \frac{1}{K_{IP} \varepsilon_C} \) and \( \frac{1}{\varepsilon_C} \), respectively (Figure 7).

From the linear equation \( y = 0.1981 \times 10^{-11} x + 0.398 \times 10^{-6} \), the intercept is \( \frac{1}{\varepsilon_C} = 0.398 \times 10^{-4} \), and the slope is \( \frac{1}{K_{IP} \varepsilon_C} = 1.981 \times 10^{11} \).

However, it should be noted that \( \varepsilon_C \), which is the molar absorptivity of the ion-pair itself, should not be confused with any stoichiometric values calculated regarding the amount of any analyte being determined. It was reported that the value of any analyte to be calculated was better defined by Beer's law, while the Benesi – Hildebrand equation estimates the ion-pair complex molar absorptivity [27]. The association constants and the molar absorptivity of the ion-pair are listed in Table 1.

![Figure 7- Benesi-Hildebrand plots for association constants of losartan MB ion-pair complexes](image)

3.3.9 Final Absorption Spectra

After obtaining the optimum conditions for the formation of the ion-pair complex, the final absorption spectra were tested. It was found that the colour system has the spectra shown in Figure -2 (after optimization). The colored ion-pair complex showed maximum absorbance at \( \lambda = 654.9 \text{ nm} \).

3.4 Justification of the Recommended Method

3.4.1 Analytical Parameters

A standard graph of the ion-pair complex was constructed by plotting absorbance against concentration under the optimum conditions, as shown in Figure-8.
Figure 8- Calibration graph of the losartan MB ion-pair complex.

The calibration curve indicates that the extracted ion-pair obeyed Beer’s Law within the concentration range of 0.03 – 1.5 µg/ml. The linearity of calibration curves was proved by the high correlation coefficient value ($r^2 = 0.9990$). At higher than 1.5 µg/ml, there was a deviation from Beer’s Law and the line lost its linearity. The apparent molar absorptivity ($\varepsilon$) was calculated and found to be $4.53 \times 10^5$ M$^{-1}$ cm$^{-1}$. The limits of detection and quantification were found to be 0.01 µg/ml and 0.03 µg/ml, respectively; these low values indicate the high sensitivity of the method. The analytical parameters of the calibration curve are shown in Table-1.

Table 1- Analytical parameters and precision of extraction of losartan MB ion-pair complex.

| Parameter                          | Value                |
|------------------------------------|----------------------|
| $\lambda_{nm}$                      | 654.9                |
| Linear range, µg/ml                | 0.03-1.5             |
| Detection limit,µg/ml              | 0.01                 |
| Quantification limit ,µg/ml        | 0.03                 |
| Correlation coefficient,$r^2$      | 0.9990               |
| Molar absorptivity, M$^{-1}$, Cm$^{-1}$ | $4.53 \times 10^5$ |
| $\varepsilon$, M$^{-1}$, cm$^{-1}$ | $2.5 \times 10^5$ (L mol$^{-1}$ cm$^{-1}$) |
| $K_{IP}$, M$^{-1}$                 | $2.009 \times 10^7$ |

3.4.2 Repeatability (Precision)

Repeatability is characterized as the closeness of agreement between independent test results, obtained by the same procedure, on the same test material, by the same operator, in the same laboratory, and using the same equipment within a short time interval. A series of six replicate measurements were performed at five different concentrations (0.03, 0.3, 0.4, 0.7, and 1.5 µg / ml) within the Beer law limit. RSD % was found to be below 2 %, indicating that the method was repeatable. The results obtained are summarized in Table-2.

3.4.3 Trueness (Accuracy)

To determine the accuracy of the proposed methods, their trueness was evaluated by analyzing 5 different concentrations (0.03, 0.3, 0.4, 0.7, and 1.5 µg/ml) of losartan potassium using the recommended procedure. The percentage recovery was calculated using the following equation: $Er\%=[\text{founded} - \text{true}/\text{true}] \times 100$. The mean recovery value lied within 98.48 - 100.3%, indicating that the method was accurate (Table-2).
Table 2- Accuracy and precision evaluation of the determination method of losartan MB ion – pair complex

| Actual concentration, μg/ml | Found concentration, μg/ml | % Recovery ± S.D | RSD% | E% |
|-----------------------------|---------------------------|-----------------|------|----|
| 0.03                        | 0.0299                    | 99.71±0.414     | 0.725 | 0.33 |
| 0.3                         | 0.295                     | 98.48±1.7934    | 0.953 | 1.66 |
| 0.4                         | 0.4007                    | 100.1±0.4825    | 1.22  | 0.17 |
| 0.7                         | 0.698                     | 100.3±0.3236    | 1.27  | 0.28 |
| 1.5                         | 1.528                     | 99.51±3.11      | 1.64  | 1.86 |

RSD%: Average of six determinations.

3.4.4 Interference

To study selectivity and the efficiency of the proposed method, a procedure of the analysis under the optimum conditions was carried out to evaluate the effects of excipient types (disintegrates, binders, and diluents) existing in a tablet dosage of microcrystalline cellulose, cellulose, starch, sodium starch glycol, and lactose monohydrate. In this study, the solutions containing 0.6 μg/ml of losartan potassium and 25 μg/ml and 50 μg/ml of excipients were used, following the recommended procedure (Table 3).

Table 3- Determination of 0.6 μg/ml of losartan potassium in the presence of excipients

| Excipients               | 0.6 μg/ml of losartan per 25 μg/ml foreign added | 0.6 μg/ml of losartan per 50 μg/ml foreign added |
|--------------------------|-----------------------------------------------|-----------------------------------------------|
|                          | Found* Error % Recovery %                     | Found* Error % Recovery %                     |
| Cellulose- microcrystalline | 0.588 1.92 98.08 | 0.590 1.6 98.40 |
| Cellulose                | 0.608 -1.47 101.47 | 0.628 -4.7 104.7 |
| Starch                   | 0.599 0.07 99.93 | 0.602 -0.34 100.34 |
| Sodium starch glycol     | 0.597 0.44 99.56 | 0.595 0.84 99.16 |
| lactose monohydrate      | 0.602 -0.46 100.46 | 0.595 0.78 99.22 |

* Mean value of three repeated measurements.

4. Application of the methods for real samples

4.1 Determination of losartan in tablets

The proposed method was applied for the estimation of losartan potassium in commercial tablets. The analysis outcomes were compared with those obtained from the reference HPLC quantification method [3], by using a stainless steel column C18 (5 μm particle size, L × I.D. 25 cm × 4.6 mm) at column temperature of 25°C, flow rate of 1.5 ml/min, injection volume of 10 μl, and spectrophotometric detector at λ 250 nm. Table 4 shows that the results obtained by the two methods were in close agreement. This is an indication that the suggested methods are as efficient as the reference method and are dependable for the determination of losartan potassium in commercial tablets.

Table 4-Determination of losartan potassium in a pharmaceutical preparation

| Sample     | Label claim | Official method | Proposed method |
|------------|-------------|----------------|-----------------|
| Losartan 100 | 100 mg     | 100.32          | 99.66 ± 1.14    |

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4.2 Determination of losartan potassium in spiked urine sample by standard addition

The recommended methods were also applied for the estimation of losartan potassium in urine samples under optimum conditions after deproteinization process. The standard addition method was performed on urine samples (Figure -9). The % recovery was calculated and showed values of 98.25% and 99.86 % for each 0.2 µg/ml and 0.4 µg/ml concentration of losartan potassium respectively (Table-5), implying that the standard addition method has enriched the accuracy.

Table 5-Accuracy and precision values of the standard addition method.

| Losartan add µg/ml | Found ± SD n=4 | % Recovery | %Error | RSD |
|--------------------|----------------|------------|--------|-----|
| 0.2                | 0.196± 0.0105  | %98.25     | %1.75  | 5.360413 |
| 0.4                | 0.399 ±0.0049  | %99.86     | %0.14  | 0.123914 |

Figure 9 - Standard addition for the determination of losartan potassium in the urine sample after deproteinization of 0.4μg /ml spiked urine.

5. Comparison with another extraction-spectrophotometric method

The proposed method was compared with a previously described method that has comparable procedures (4). It was observed that the present extractive spectrophotometric method with MB was of higher sensitivity (high ε and low detection limit) as compared to the other method (Table - 6). The ion-pair complex was stable for a longer time and the amount of extraction solvent (ml) was minimum. It also allows losartan to be determined successfully in tablet and urine samples.

Table 6- Comparison of the present study with other extraction- spectrophotometric method.

| Method                  | Reference 4 | Present       |
|-------------------------|-------------|---------------|
| Reagent                 | Calmagite   | Orange-II     | MB     |
| pH                      | 1.2         | 1.2           | 6.5    |
| Amount of buffer (ml)   | 2.0         | 2.0           |        |
| Amount of reagent       | 0.1% (1.0ml)| 2.0           | 0.01% (1.1ml) |
| Amount of extraction solvent (ml) | 10         | 10            | 5      |
| Stability               | up to 5 min after extraction | up to 5 min after extraction | 60 min |
| λ<sub>max</sub>, nm     | 491         | 486           | 654.9  |
| Linear range, µg/ml | 10–100 | 10–100 | 0.03-1.5 |
|---------------------|---------|---------|-----------|
| Detection limit, µg/ml | -       | -       | 0.01      |
| Quantification limit, µg/ml | -       | -       | 0.03      |
| Correlation coefficient, r² | 0.9998  | 0.9999  | 0.9990    |
| Molar absorptivity, M⁻¹ Cm⁻¹ | 1.74×10³ | 1.75×10³ | 4.53×10⁻³ |
| RSD%                | 0.119%–0.575 | 0.109%-0.219 | 0.725%-1.64% |
| Application         | synthetic mixture | synthetic mixture | tablet, and spiked urine |

6. Conclusions
The proposed method provides an accurate, sensitive, simple, and inexpensive method for the determination of losartan potassium in pure form, tablet, and urine sample. Under optimized extraction conditions, losartan MB ion-pair was extracted to a dichloromethane organic layer. The presence of excipients in the tablet did not cause any interference because the solution undergoes dissolution and filtration processes and most of them were removed. The suggested method was also successively applied for the estimation of losartan potassium in a spiked urine sample.

References
1. Sean, C.S. 2007. Martindale: The complete drug reference. 35th Edition, vol 1. Pharmaceutical press.UK.
2. United States Pharmacopeial Convention. 2009. The United States pharmacopeia: USP 29, Rockville, MD. United States Pharmacopeial Convention 24.
3. Al-Majed, A.R., Assiri, E., Khalil, NY. and Abdel-Aziz, HA. 2015. Losartan: comprehensive profile. Profiles of Drug Substances, Excipients and Related Methodology. 40: 159-94.
4. Prabhakar, AH. and Giridhar, R. 2002. A rapid colorimetric method for the determination of Losartan potassium in bulk and in synthetic mixture for solid dosage form. Journal of pharmaceutical and biomedical analysis. 27(6): 861-866.
5. Mahmoud A. O., Osama H. A., Ahmed A. A. and Ahmed M. A. 2011. Spectrophotometric and spectrofluorimetric determination of certain angiotensin receptor blockers through complex formation. Journal of Pharmaceutical Sciences and Research. 3(10): 1499-1510
6. Darwish, I.A. 2005. Analytical study for the charge-transfer complexes of losartan potassium. Analytica Chimica Acta. 549: 212-220.
7. Stolarczyk, M., Maslanka, A., Apola, A. and Krzek, J. 2013. Determination of losartan potassium, quinapril hydrochloride and hydrochlorothiazide in pharmaceutical preparations using derivative spectrophotometry and chromatographic-densitometric method. Acta Pol Pharm. 70(6): 967-76.
8. Tarkase, K., Suryawanshi, S. and Joshi, R. 2012. Simultaneous derivative spectrophotometric determination and validation of losartan potassium in pharmaceutical dosage forms. International Journal of Pharmaceutical Sciences Review and Research. 13(02): 31-34.
9. Thomas, A., Chavan, U., Nanda, R., Kothapalli, L. and Deshpande, A. 2009. Simultaneous spectrophotometric estimation of Hydrochlorothiazide, Atenolol and Losartan potassium in tablet dosage form. Journal of Pharmaceutical Research, 8(3): 139-141.
10. Sevgi, T. and Serap, S. 2004. Comparison of UV-and second derivative spectrophotometric and high-performance liquid chromatographic methods for the determination of losartan in tablets. Turk J Pharm Sci. 1: 165-175.
11. Bonfilio, R., Favoretto, L.B., Pereira, G.R., Azevedo, R.C.P. and Araújo, M.B. 2010. Comparative study of analytical methods by direct and first-derivative UV spectrophotometry for evaluation of losartan potassium in capsules. Brazilian journal of pharmaceutical sciences. Brazilian Journal of Pharmaceutical Sciences.46(1): 147-155.
12. Ansari, M, Kazemipour, M., Baradaran, M. and Jalalizadeh, H. 2004. Derivative spectrophotometric method for determination of losartan in pharmaceutical formulations. Iranian Journal of Pharmacology and Therapeutics. 3(1): 21-0.
13. Rao P., Venugopal, V., Anil, K.G., Rajesh, B., Prasad, G. and Ravindergoud D. 2011. Quantitative estimation of losartan potassium in pharmaceutical dosage forms by UV spectrophotometry. International Journal of Research in Pharmacy and Chemistry. 1(3): 295-302.
14. Jalalizadeh, H., Souri, E., Farsam, H. and Ansari, M. **2003**. A high-performance liquid chromatographic assay for the determination of losartan in plasma. *Iranian Journal of Pharmacology and Therapeutics*. **2**(1): 18.
15. Ahmad, T.J.; Raj, A., Radhika, R.T., Ananda, S., Gowda, N.M. and Venkatesha, B.M. **2014**. Rapid ultra-performance liquid chromatography assay of losartan potassium in bulk and formulations. *Journal of Analytical Science and Technology*. **5**(1): 33.
16. Rao, K.S. and Srinivas, K. **2010**. RP-HPLC method for the determination of losartan potassium andراميريل in combined dosage form. *Indian Journal of Pharmaceutical Sciences*. **72**(1): 108.
17. Ali, S.A. and Hassan, A. **2014**. Cyclic Voltammetric Study of Losartan Potassium. *International Research Journal of Pure and Applied Chemistry*. **4**(1): 128.
18. Ensafi. A.A. and Hajian, R. **2008**. Determination of losartan and triamterene in pharmaceutical compounds and urine using cathodic adsorptive stripping voltammetry. *Analytical sciences*. **24**(11): 1449-1454.
19. Megied, A.M., Gaber, A.A., Abdelmageed. O.H. and Omar, M.A. **2014**. Determination of Losartan in tablet dosage form and some biological body fluids via copmlexation with Cu (II) using Cathodic Adsorptive Stripping Voltammetry. *Scientific Journal of October 6 University*. **2**(1): 8-13.
20. Attaa, B. N., Saad S. Elshabrawy Y. and Eid M. **2020**. First-derivative synchronous spectrofluorimetric method for estimation of losartan potassium and atorvastatin in their pure forms and in tablets. *Luminescence. Jan*, **19**: 1-11.
21. Omer, L.S. and Ali, R.J. **2018**. Assay of Orphenadrine Citrate in Pharmaceuticals via Extraction-Spectrophotometric Method. *Iraqi Journal of Science*. **59**(3A): 1152-1161.
22. Florea, M. and Ilie, M. **2017**. Ion-pair Spectrophotometry in Pharmaceutical and Biomedical Analysis: Challenges and Perspectives. *Spectroscopic Analyses–Developments and Applications. InTech, Croatia*:173-92.
23. Ruzin, S.E. **1999**. *Plant microtechnique and microscopy*. Oxford University Press New York
24. Najib, F.M. and Aziz, K.H. **2013**. Spectrophotometric determination of lamotrigine in pharmaceutical preparations and urine samples using bromothymol blue and bromophenol blue. *Malaysian Journal of Analytical Sciences*. **17**(2): 310-325.
25. Israa, M. J. A. and Sadeem, S. A. **2018**. Spectrophotometric methods for the determination of Naringenin in supplements and urine samples using diazotization coupling reactions. *Iraqi Journal of Science*. **59**(2A): 635-644.
26. Benesi, H.A and Hildebrant, J. **1949**. A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons. *Journal of the American Chemical Society*. **71**(8): 2703-2707.
27. Sabrein, H. M., Alyaa, I. M. and Ashour, A. A. **2018**. Exploring the nature of the clopidogrel–bromocresol green interaction via spectrophotometric measurements and quantum chemical calculations.,*RSC Adv.*, **8**: 29104-29114