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A simple and green analytical method for determination of some angiotensin receptor antagonists by binary complex formation

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ABSTRACT

A Simple, rapid and sensitive analytical method was developed for the determination of eprosartan mesylate (EPS), irbesartan (IRS) and olmesartan medoxomil (OLS) in pure forms, pharmaceutical tablets, urine, and blood plasma samples. The method is based on formation of an ion association complex between studied drugs and eosin-Y (E*Y*) dye in aqueous buffered medium. Under the optimum conditions, the binary complexes showed absorption maxima at 547 nm. Standard calibration curves were linear in the range of 2-10, 2-8 and 2-12 μg/ml and detection limits of 0.148, 0.082 and 0.181 μg/ml for the three drugs respectively. The correlation coefficients were not less than 0.9999. The reaction mechanism was studied by using density functional theory method. The proposed green analytical method has the advantage of being applicable for the determination of the three drugs without prior extraction. It is recommended for quality control and routine analysis where time, cost effectiveness and high specificity of analytical techniques are of great importance.

KEYWORDS

Eosin-Y;
Ion-pair complex;
Spectrophotometry;
Angiotensin receptor antagonists;
Antihypertensive drugs.

INTRODUCTION

The development of rapid, simple and inexpensive analytical methods is of growing interest, especially since fast decisions are often needed in the environmental field. In addition, in the development of a new analytical method the amounts and toxicities of the reagents used, and the wastes produced, are as important as any other analytical feature. There is therefore a great need for the development of methods that are less harmful to humans and to the environment, and which are in accordance with the 12 principles of Green Chemistry[1].

Eprosartan mesylate EPS, irbesartan IRS and olmesartan medoxomil OLS, Figure 1, are belong to non-peptide, orally active angiotensin-II receptor antagonists (ARA II) used in the treatment of hypertension, congestive heart failure, and chronic renal failure[2,3]. According to the literature, many methods have been used to determine the studied drugs. By using UV- spectrophotometry[4-6], the manipulation is simple, but the linear rang is narrow and the sensitivity is low. VS-Spectrophotometric[7-11] and spectrofluorimetric[9,12,13] methods, have high sensitivity, but cumbersome, time-consuming manipulation and poor repeatability. Capillary electrophoresis[14,15], LC-MS[16-20], HPTLC and HPLC[21-40] methods need costly apparatus and harm-

ful organic solvents. Polarographic[7,41-44] methods need to use a lot of metal mercury which harms human health. The aim of the present work was to develop a simple and environmentally friendly method for the rapid determination of (OLS) in pure form, pharmaceutical tablet, urine, and blood plasma samples using VS-spectrophotometry. The proposed method is based on the formation of an ion association complex between OLS and eosin-Y (E*Y*) dye in aqueous, buffered medium.

The advantages that the suggested method, offers over other methods, include simplicity and minimize use of toxic reagents/solvents makes it very useful analytical method that address the notion of Green Chemistry, which aims to develop methods and techniques that

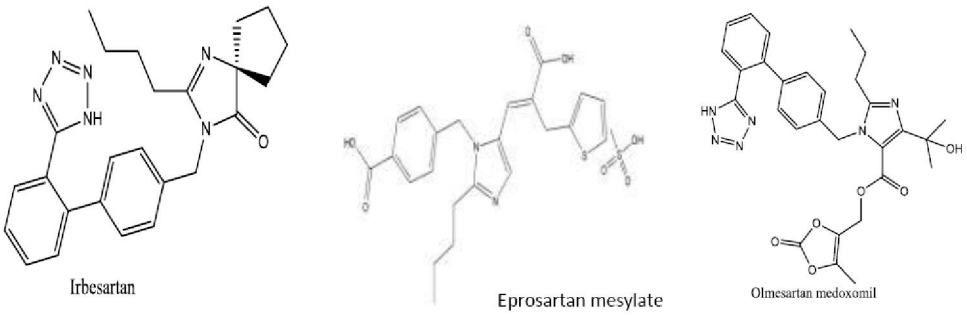

Figure 1 : Chemical structure of irbesartan, eprosartan mesylate and olmesartan medoxomil.

reduce or eliminate the use and generation of substances hazardous to human health or to the environment[1].

EXPERIMENTAL

Apparatus

An Optima UV-Visible spectrophotometer (Model SP-3000 plus, Optima Co., Japan), Equipped with 1 cm matched quartz cells were used for absorbance measurements. WTW 315i pH meter was used for pH measurements.

Materials and reagents

All chemicals and reagents used were of analytical or pharmaceutical grade.

1. Eprosartan mesylate (EPS) was purchased from Dishman Pharmaceuticals (Ahmedabad, India) and Teveten™ tablets (Solvay Pharmaceuticals B.V., The Netherlands). Each tablet was labeled to contain 600 mg of eprosartan.
2. Irbesartan (IRS) was kindly provided by Medical Union Pharmaceuticals, Abu-sultan, Ismailia, Egypt. Approvel™ tablets (Sanofi-Aventis Egypt). Each tablet was labeled to contain 150 mg of irbesartan.
3. Olmesartan medoxomil (OLS) was purchased from Sigma-Aldrich, Germany and Olmetec® (SAJA Pharmaceuticals, KSA). Each tablet was labeled to contain 40 mg of olmesartan medoxomil.
4. Eosin-Y (E*Y*) (MP Biomedicals, Inc.France) was prepared as 4×10^{-3} *M* solution in distilled water.
5. Hcl-NaAc buffer solutions were prepared by mixing various volumes of 1.0 M HCl acid and 1.0 M NaAc with certain ratios.

Standard solutions

Into a 50-ml calibrated flask, 25 mg of each drug were accurately weighed, dissolved in 5 ml 0.1 N NaOH (EPS) or 5 mL methanol (IRS &OLS), and completed to volume with distilled water. This stock solution was diluted with distilled water to obtain the suitable concentrations that lie in the linear range of the assay.

Experimental method

Accurately measured aliquots of EPS, IRS or OLS solutions, in the concentration range, were transferred into a series of 10 ml volumetric flasks, E*Y* 4×10^{-3} *M* solution (0.5 ml for IRS, OLS or 0.7 ml for EPS) was then added to each flask followed by 1.0 ml of HCl-NaAc buffer solution (pH 3.0 for IRS or pH 3.5 for EPS and OLS). The mixtures were diluted to volume with distilled water and the absorbance was measured at 547 nm against an appropriate blank prepared simultaneously.

RESULTS AND DISCUSSION

Absorption spectra

According to the experimental method, the absorp-

tion spectra of EPS, IRS and OLS solutions, the reagent solution (the mixed solution of E*Y* and buffer solution), the reagent-drug complexes (against reagent blank) were obtained in the wavelength range of 200~600 nm. It can be seen, from Figure 2, that drugs solutions were achromatous. The absorption peak of the reagent solution was at 515 nm. When drug was added into the reagent solution, E*Y* reacted with studied drugs to form ion association complexes, the color of the solution changed and the maximum absorbance wavelength was 547 nm; compared with the maximum absorbance wavelength of the reagent solution, bathochromic shift was 32 nm.

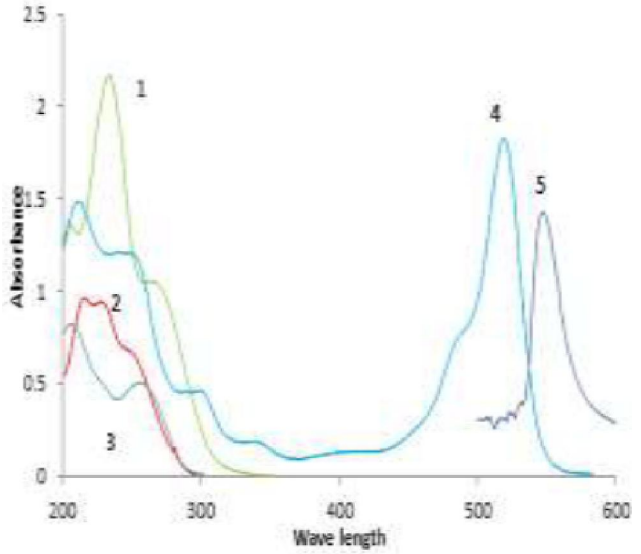

Figure 2 : Absorption spectra of studied drugs—eosin-*Y*. Against water: *1*—EPS; *2*—IRS; *3*—OLS; *4*—eosin-*Y*. Against reagent blank: *5*— complex

Optimum conditions for the reactions.

In this study, order of addition of reagents was found to be necessary for good precision. The concentration of reagent and the pH of the medium had been optimized to achieve the best sensitivity and linearity with maximum stability.

Effect of the pH

The effect of pH of HCl-NaAc buffer solution on the reaction was studied. It was evident, from figure 3, that over the range of pH 3.3–3.7 (EPS&OLS) and pH 2.7-3.3 (IRS), the absorbance was maximum and steady. If the pH was higher, it made against protonating of N in imidazole and tetrazol. If the pH was lower, it restrained the –ONa dissociation of E*Y* and was not propitious to the formation of association complex, the absorbance reduced. When pH was 3.5 (EPS&OLS), 3.0 (IRS) and the amount of buffer solution was 0.75-1.25 mL, figure 4, the sensitivity was maximal. Therefore all subsequent measurements were made at pH 3.0, 3.5 and the amount of HCl-NaAc buffer solution was 1.0 mL.

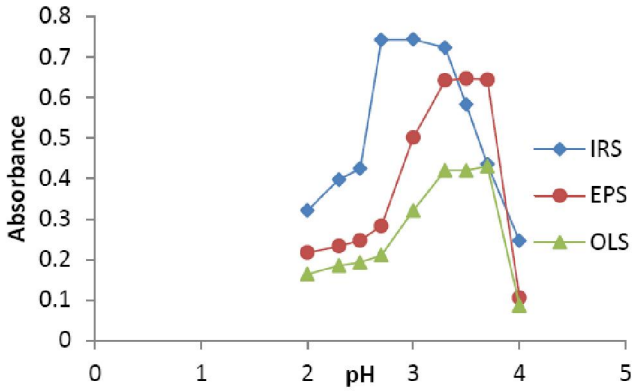

Figure 3 : Effect of pH on the absorption intensity of the ion-association complexes of ♦ IRS, ● EPS and ▲ OLS with eosin at λ=547 nm.

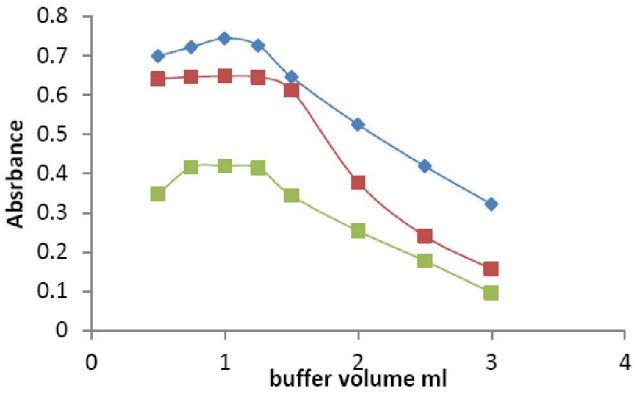

Figure 4: Effect of buffer volumes on the absorption intensity of the ion-association complexes of ♦ IRS, ● EPS and ▲OLS with eosin at λ=547 nm.

Effect of E*Y* concentration

The optimum reagent concentration was determined by adding various volumes of $4\times10^{-3}M$ E*Y* solution to a fixed concentration of the three drugs. Absorbance values obtained at 547 nm are shown in figure 5, from which it was found that 0.5 ml and 0.7 ml of E*Y* for IRS, OLS and EPS, respectively, were enough to develop the maximum absorbance intensity.

Effect of organic reagents

The effects of dissolvable organic reagents in water on the absorbance, such as methanol, ethanol, acetone,

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isopropyl alcohol etc., were examined. The experimental results, figure 6, showed that they had no the enhanced function, acetone and ether made the absorbance reduce contrarily. So, organic reagents were not chosen as the sensitizer in this experiment.

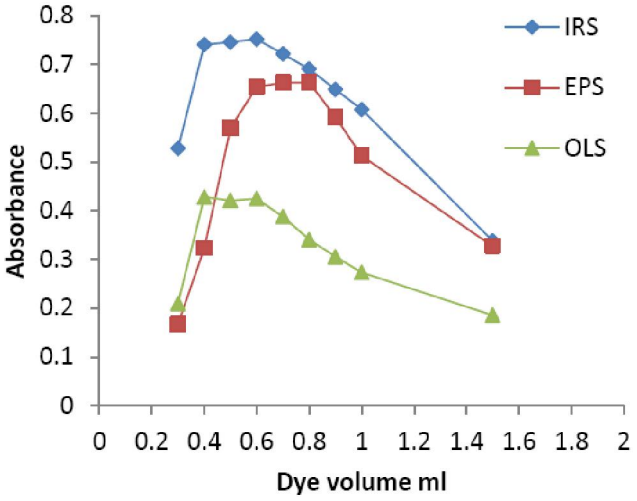

Figure 5 : Effect of 4×10^{-3}*M*dye volumes on the absorption intensity of the ion-association complexes of ♦ IRS, ● EPS and ▲OLS with eosin at λ=547 nm

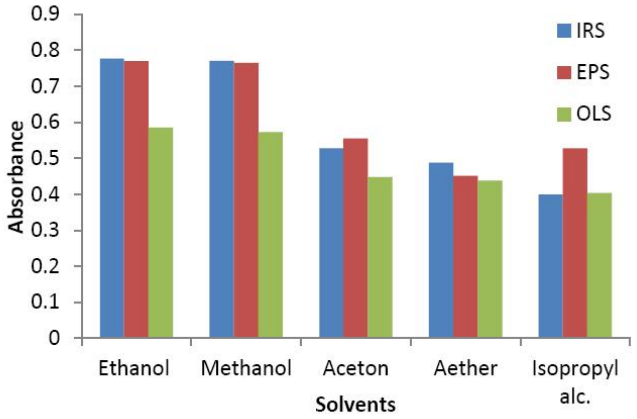

Figure 6: Effect of organic reagents on the absorption intensity of the ion-association complexes of ♦ IRS, ● EPS and ▲OLS with EY at λ=547 nm.

Effects of addition sequence, time and temperature on the system stability.

We can see, from figure 7, that when the addition sequence was drug — buffer solution — E*Y*, the absorbance was the biggest. When the addition sequence was buffer solution — E*Y* — drug, the absorbance was less. Otherwise, the absorbance was monitored at different intervals of time. The absorbance remained basically unaltered for 2 h. The effect of the temperature on the absorbance was also examined in the range of room temperature to 50°C. The result indicated that the influence was little. So, we chose the addition sequence was drug — buffer solution — E*Y*, the absorbance was measured at room temperature.

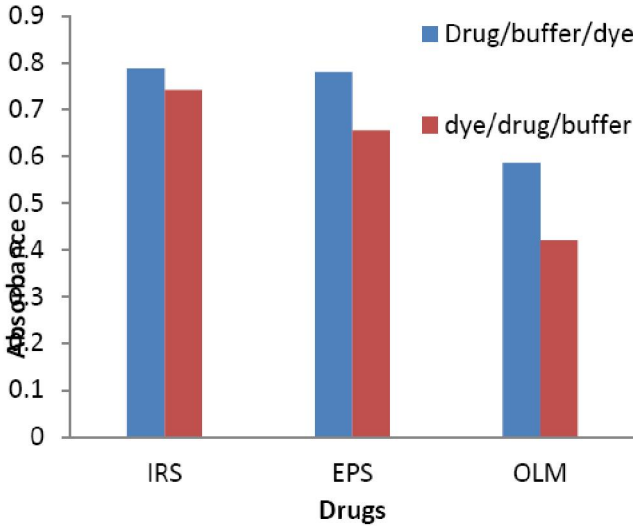

Figure 7 : Effect of the addition sequence on the absorption intensity of the ion-association complexes of IRS, EPS and OLS with EY at λ=547 nm.

Composition of the ion-associates complex

In suitable buffered pH, the studied drugs were protonized and existed as cations; E*Y* was ionized and was present as large anions. They formed an ion-association complex by electrostatic and hydrophobic interaction. The composition of the ion-association complex was determined by Job's method of continuous variation[45]. The results obtained are shown in figure 8

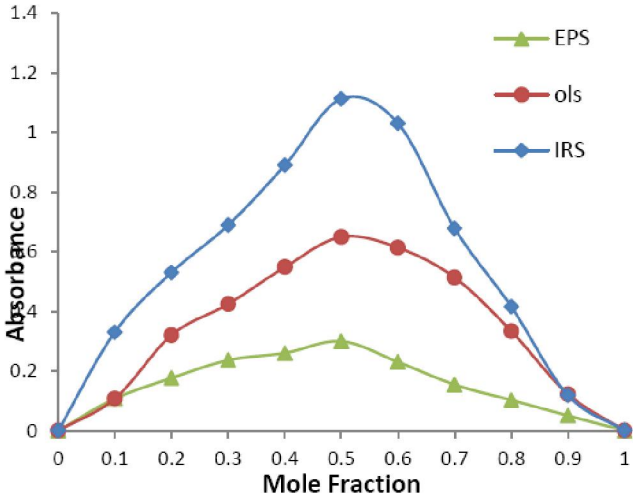

Figure 8: Continuous variation plots for the ion-association complexes of ♦ IRS, ● EPS and ▲OLS ($4x10^{-3}$ M) with EY at λ=547 nm

and indicated that the composition of the associates was equimolar (1:1) for the three drugs.

Quantum chemical calculations.

DFT calculations with a hybrid functional B3LYP (Becke's three parameter hybrid functional using the LYP correlation functional) at 6-31G(d,p) basis set were performed with the G09 software package, and Spartan 10 program in order to evaluate the atomic charge and the dipole moment of behavior of the studied drugs in water media (ε= 78.39). The methodology used in this investigation is conductor polarized continuum model (CPCM).

To predict reactive sites of electrophilic and nucleophilic attack for the investigated molecules, Molecular electrostatic potentials (MEP) at the B3LYP/6-31G(d,p) optimized geometry was calculated. MEP is related to the electronic density and is a very useful descriptor in understanding sites of electrophilic attack and nucleophilic reactions as well as hydrogen bonding interactions. The electrostatic potential is also well suited for analyzing processes based on the —recognition of one molecule by another, such as in drug–receptor, and enzyme–substrate interactions, because it is through their potentials that the two species first —see each other.

The negative (red) regions of MEP were related to electrophilic reactivity and the positive (blue) regions to nucleophilic reactivity. From figure 9, MEP of E*Y* molecule has several possible sites of nucleophilic attack. The positive regions are mainly over –ONa and –COONa. From the charge distribution of E*Y*, we can see from figure 10 that the maximal positive charge distribution of Mulliken atom, which on the Na atoms in the side of the molecule, were +0.556 and +0.708 under the consideration of the water effects. Other atomic charge distribution is even. The algebraic summation of the atomic charge in the -COONa charge was -0.169 and the -ONa side was +0.06. Therefore -ONa charge was the primary part to attract the negative charge of Mulliken atom

On the other hand, the charge distribution of EPS, IRS and OLS show the following:

As can be seen in MEP of EPS, Figure 11, the molecule has several possible sites of electrophilic attack. The negative regions are mainly over N3 in imidazole ring and over oxygen in the two carbonyl groups.

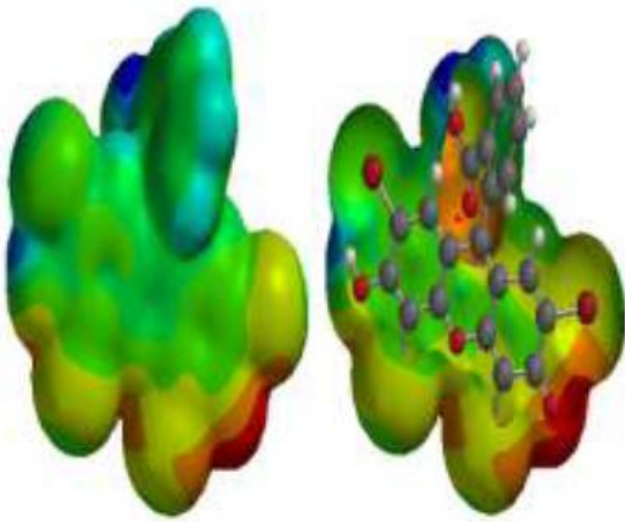

Figure 9 : E*Y*molecular electrostatic potential (MEP) map calculated at B3LYP/6-31G (d,p) level

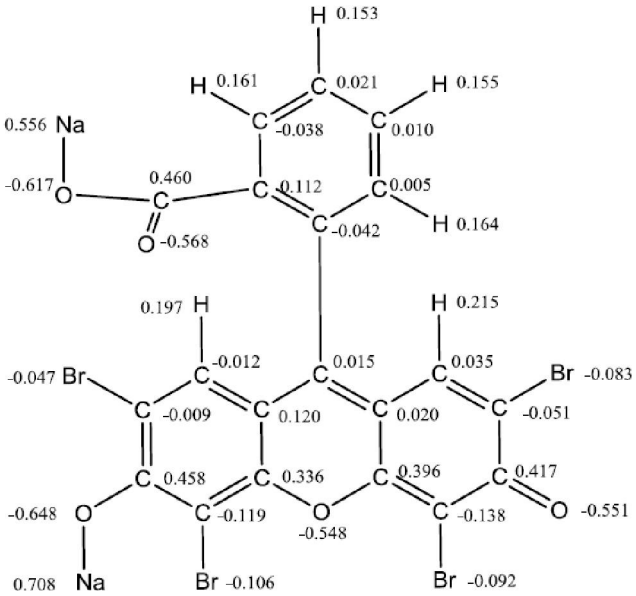

Figure 10 : EYMulliken charge distribution at ground

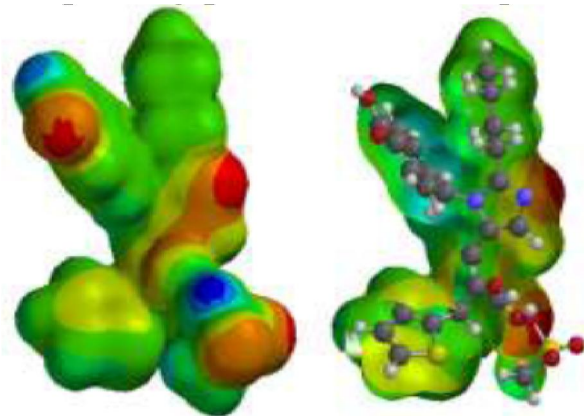

Figure 11 : EPS molecular electrostatic potential (MEP) map calculated at B3LYP/6-31G (d,p) level

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From figure 12, the maximal negative charge distribution of the Mulliken atom on N3 atom in imidazole is -0.521 and on the oxygen in the two carbonyl groups is -0.512, -0.514, respectively. The algebraic summation of the atomic charges on N3 and on oxygen of the two carbonyl groups are -0.144, 0.032 and -0.015, respectively. So N3 charge was the primary part to attract the positive charge of E*Y*Mulliken atom.

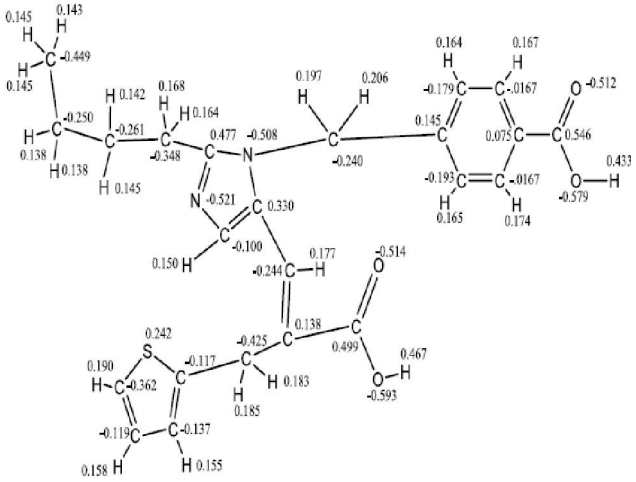

Figure 12: EPS Mulliken charge distribution at ground

In MEP of IRS, figure 13, the negative regions are mainly over N_1, oxygen atom attached to C_4 in daizaspironone moiety and over tetrazol moiety which is the most negative moiety. The algebraic summation of the atomic charges attached to N_2, N_3, $N_4$and N_5, in tetrazol moiety, are +0.372, -0.658, -0.595 and -0.042 respectively, therefore N_3 charge is the primary part to attract the positive charge of Mulliken atom, figure 14.

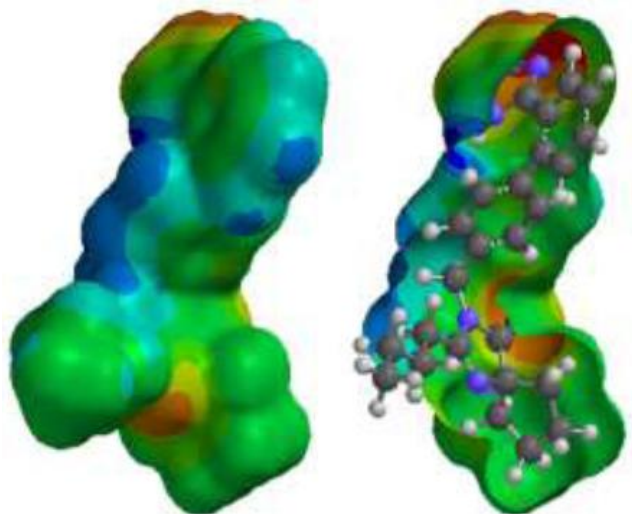

Figure 13 : IRS molecular electrostatic potential (MEP) map calculated atB3LYP/6-31G(d,p) level

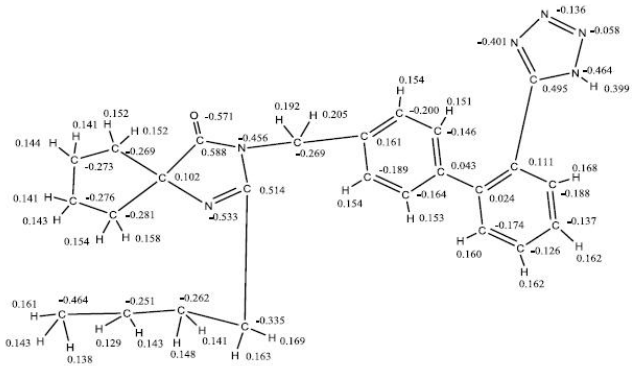

Figure 14 : IRSMulliken charge distribution at ground

As can be seen in the MEP of OLS molecule, figure 15, OLS has several negative regions; the most negative one is mainly over tetrazol moiety. The charge summations of the atoms attached to N2, N3 N4 and N5 in tetrazol moiety, figure 16, are +0.31,-0.651, -0.588 and -0.062 respectively, therefore N3 charge was the primary part to attract the positive charge of Mulliken atom.

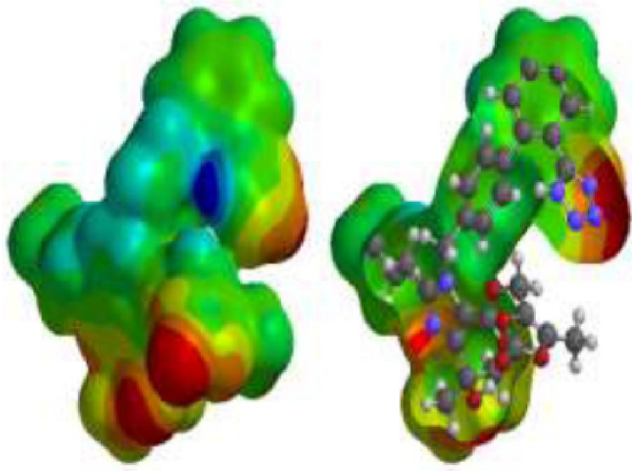

Figure 15 : OLS molecular electrostatic potential (MEP) map calculated at B3LYP/6-31G(d,p) level

When E*Y* and any of the studied drugs mixed, the charges would redistribute to reduce the energy of the system and make system steady. The total calculated dipole moment of E*Y* was 16.2886 and that of the studied drugs were and 9.8084, 8.4574 and 15.0823 Debye for EPS, IRS and OLS respectively, which indicated that their structure was asymmetric and the molecule had polarity. From the experimental results described above, there was a combining possibility in theory. Based on the charge distribution of Mulliken atom, the O atom in the side of the E*Y* molecule could combine the N atom which in imidazole (for EPS) or in tetrazol mol-

ecule (for IRS and OLS), and they combined to 1:1, which was consistent with the experimental facts. Therefore, the form of the ion-association complex not only based on electrostatic and hydrophobic interaction, but also on the charge transfer interaction of Mulliken atom. The possible molecular formula of the association complex between E*Y* and OLS was showed in Figure 17.

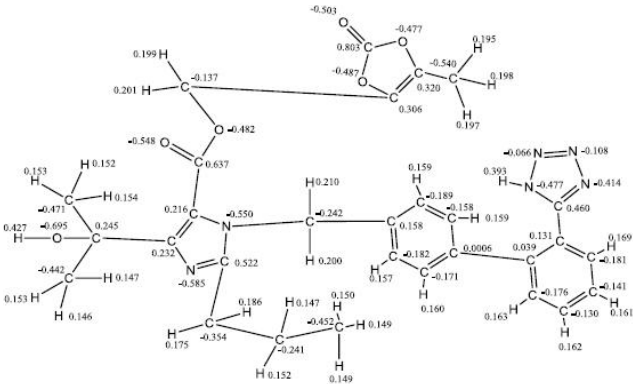

Figure 16 : OLS Mulliken charge distribution at ground

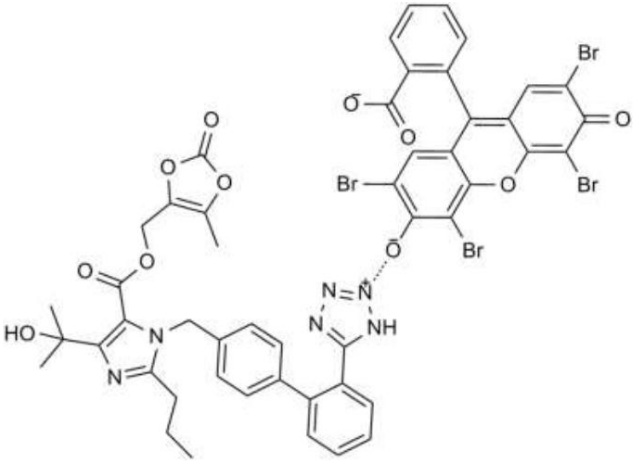

Figure 17 : Diagram sketch of ion association complex of EY-OLS

Method validation.

The proposed method was validated for linearity, precision, accuracy, recovery, limit of detection, and quantitation. Beer's law limits, molar absorptivity, regression equations and correlation coefficients, intercepts and slopes obtained by linear least squares treatment of the results were given in TABLE 1.

The accuracy and precision of the proposed method was established by five replicate samples, within the Beer's law limits, at three different concentration levels within 1 day (Intraday precision) as well as for 5 consecutive days (Inter day precision). The standard deviations (S.D.), relative standard deviations, %RSD were calculated by standard methods. The relative standard deviation was found to be less than 2%, indicating reasonable repeatability of the selected methods TABLE 2, 3.

TABLE 1 : Spectral and statistical data for the analysis of the studied drugs by the proposed method

Method	Eosin-Y		
Drug	**Eprosartan**	**Irbesartan**	Olmesartan
λ_{max} (nm)	547		
Beer's law limits (µg ml^{-1})	2-10	2-8	2-12
Molar absorptivity ($mol^{-1}cm^{-1}$)	4.2479×10^4	4.1322×10^4	4.4033×10^4
Limit of detection (µg ml^{-1})	0.148	0.082	0.181
Regression equation			
Slope *(b)*	0.095	0.099	0.070
Intercept *(a)*	0.22	-0.012	0.024
Correlation coefficient *(r)*	0.9999	0.9999	0.9999

TABLE 2 : Intra day precision of the proposed method for the determination of the studied drugs

Drugs	Concentration (µg/mL)		Recovery± R.S.D.
	Theoretical	Nominal ± S.D.	
EPS	2	1.97±0.77	98.88±0.77
	4	3.98±0.88	99.66±0.88
	8	7.95±1.03	99.46±1.03
IRS	2	1.99±0.96	99.68±0.97
	4	3.98±0.88	99.64±0.88
	8	7.86±0.82	98.32±0.84
OLS	2	1.98±0.90	99.38±0.90
	4	3.98±1.56	99.62±1.56
	8	7.92±0.80	99.00±0.81

TABLE 3 : Inter day precision of the proposed method for the determination of the studied drugs

Drugs	Concentration (µg/mL)		Recovery± R.S.D.
	Theoretical	Nominal ± S.D.	
EPS	2	1.98±0.020	99.26±1.022
	4	3.93±0.301	98.44±0.765
	8	7.92±0.087	99.04±1.098
IRS	2	1.98±0.012	99.04±0.609
	4	3.97±0.038	99.47±0.973
	8	7.90±0.052	98.82±0.668
OLS	2	1.99±0.011	99.74±0.582
	4	3.94±0.029	98.70±0.747
	8	7.98±0.066	99.80±0.838

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The recovery experiments were presented by using the standard addition method. For this purpose, a known amount of reference drug was spiked to formulated tablets at two different concentration levels and the nominal value of drug was estimated by the proposed method. Each level was repeated five times. The results, TABLE 4, were reproducible with low S.D. and R.S.D. No interference from the common excipients was observed.

TABLE 4 : Application of the proposed method for the analysis of the studied drugs in their tablets

Drug	Concentration (μg ml^{-1}) Taken	Spiked	Found±S.D.	Recovery± R.S.D.
Teveten™ tablets (600 mg EPS/tablet)				
EPS	3	3	5.93±0.078	98.88±1.326
	5	5	9.96±0.170	99.65±1.707
Approvel™ tablets (150 mg IRS/tablet)				
IRS	2	2	3.99±0.021	99.89±0.549
	4	4	7.98±0.066	99.80±0.838
Olmetec® tablets (40 mg OLS/tablet)				
OLS	4	4	7.99±0.054	99.97±0.679
	6	6	11.97±0.050	99.76±0.424

According to International Conference on Harmonization (ICH) guidelines[46], the limit of detection (LOD) and limit of quantification (LOQ) of the procedure were calculated according to the following expressions.

LOD= 3.3 *s/m* and LOQ= 10 *s/m*

Where *s*, is the Standard deviation of the absorbances (n = 5) of the sample and *m* is the slope of the corresponding calibration curve.

Applications

Determination of the studied drugs in pharmaceuticals

Five tablets of EPS, IRS and OLS were powdered separately and quantity of the powder equivalent to 50 mg, of each drug, was sonicated for 5 minutes with 5 ml 0.1N NaOH (EPS) or 5 ml methanol (IRS&OLS). The solution was filtered through filter paper into 100 ml volumetric flask and then diluted to volume with distilled water. The assay of studied drugs content was completed as described above.

The validity of the proposed method was evaluated by statistical analysis[47] between the results obtained and that of reference methods[6,8,12] table 4. The reference methods recommended are Two Simple and Rapid Spectrophotometric Methods for the Determination of a New Antihypertensive Drug Olmesartan in Tablets[8], Spectrophotometric determination of eprosartan mesylate in raw material and experimental tablets[6] and Spectrophotometric and Spectrofluorimetric determination of certain angiotensin blockers through complex formation[12]. Regarding the calculated student's *t*-test and variance ratio F-test (TABLE 4), there is no significant difference between the proposed and the reference methods regarding accuracy and precision.

Determination of OLS in spiked serum sample

In order to check, the possibility of applying the method to the human serum samples, the proposed method was applied to the determination of EPS, IRS and OLS in spiked serum sample. Proteins and endogenous substances were removed from the serum samples via precipitation by the addition of acetonitrile and centrifugation at 4000 rpm. The supernatants were then taken and diluted with deionized water. Stability of the fortified serum samples was tested by making five consecutive analyses of the sample over a period of approximately 24 h.

No significant changes were observed in the peak currents and potentials between the first and last measurements. The serum sample with standard EPS, IRS and OLS was spiked by OLS EPS, IRS and OLS to confirm the peak position and corresponding response for the added concentration. Linear response was observed by adding EPS, IRS and OLS to the previously fortified serum sample, which confirmed the applicability of the proposed method to human serum. The recovery results are given in the TABLE 5.

TABLE 5 : Applications of the proposed method for the studied drugs analysis in blood plasma and urine

Drug	Taken (μg ml^{-1})	Plasma sample Found	%Recovery	R.S.D.	Urine sample Found	%Recovery	R.S.D.
EPS	6	5.89	98.16	2.041	5.93	98.93	1.648
	8	7.86	98.32	1.042	7.91	98.92	1.177
IRS	6	5.92	98.80	0.927	5.95	99.16	1.251
	8	7.95	99.45	0.662	7.98	99.75	0.886
OLS	6	5.94	99.10	0.999	5.94	99.03	1.186
	8	7.95	99.4	0.836	7.93	99.12	0.955

Determination of OLS in urine sample

The applicability of the studied method for the determination of EPS, IRS and OLS in spiked urine was

investigated. The direct determination of EPS, IRS and OLS in urine was found to be possible by employing a high dilution of the sample. The experiment was performed for urine samples with five replicates for each sample. The results are given in table 5. From the recovery data it was observed that EPS, IRS and OLS can be determined in urine matrix with reliable results.

CONCLUSION

The data given above reveal that the proposed methodsare simple, rapid, accurate and sensitive with good precision and accuracy in determining EPS, IRS and OLS in their pharmaceutical tablet, urine and blood plasma without interference from common excipients or biological matrix. Moreover, it is less time-consuming and do not require various elaboration treatment and tedious extraction procedures required in chromatographic and other traditional extractive spectrophotometric methods. These, in addition to satisfactory sensitivity and reproducibility compared to many other methods as well as the convenience and simplicity, make the method applicable for routine analysis of the drug in pure form, tablet, urine and plasma

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