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APPLICATION OF ACID-DYE COMPLEXATION METHOD FOR THE EXTRACTIVE-SPECTROPHOTOMETRIC DETERMINATION OF MOEXIPRIL-HCl

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ABSTRACT

New spectrophotometric procedures have been established for the assay of moexipril-HCl in bulk form, in pharmaceutical formulations. The procedures are based on the reaction between the examined drug and bromocresol purple (BCP), bromophenol blue (BPB), and bromothymol blue (BTB) in aqueous acidic medium producing an ion-pair complexes extracted in chloroform and measured at the optimum wavelengths (405, 410, and 415 nm for MOEX-BCP, MOEX-BTB, and MOEX-BPB, respectively). Reaction conditions were studied and optimized to obtain the maximum colour intensity. The reactions were extremely rapid at room temperature and the absorbance values remains unchanged for 48 h. The proposed methods have been applied successfully for the analysis of the drug in pure form and in its dosage forms. Statistical comparison of the results with those obtained by second derivatives spectrophotometric method shows excellent agreement and indicates no significant difference in accuracy and precision.

INTRODUCTION

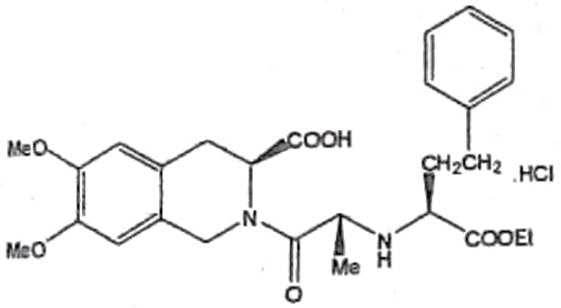


Fig. 1: Moexipril hydrochloride

Moexipril hydrochloride (MOEX), (3S)-2-[(2S)-2-{[(1S)-1-(ethoxycarbonyl)-3-phenyl-propyl]amino}-1-oxopropyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride (Fig. 1), is a new potent orally active non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor for the treatment of hypertension and congestive heart fail(1). Unlike captopril or lisinopril but similar to enalapril, quinapril, fosinopril, and ramipril, moexipril is a prodrug and has little pharmacologic activity until hydrolyzed in the liver to moexiprilat[2]. MOEX is administered alone or together with antihypertensive, diuretic hydrochlorothiazide (HCTZ). Combined therapy of MOEX and HCTZ had a significant greater blood pressure reduction than with the same dosage of the drug alone[1].

Reviewing the literature revealed that only a gas chromatographic-mass spectrometric method[3] has been reported for MOEX and its active metabolite moexiprilate in human plasma, HPLC and derivatives spectrophotometric method for determination of moexipril with hydrochlorothiazide in pharmaceutical formulation[4]. However, these methods are expensive and not available at most quality control laboratories. The spectrophotometric technique continues to be the most preferred method for assay of different classes of drug in pure and in biological samples because of its simplicity and reasonable sensitivity with significant economical advantages.

The weak UV absorption of moexipril-HCl in addition to small dose (7.5 or 15mg/tablet) means that the direct spectrophotometric assay is susceptible to interfere from formulation excipients and this necessitates the development of alternative method for the analysis. Many pharmaceutical compounds have been determined by the formation of an ion-pair complex[5-8]. This technique depends on the reaction of a drug that has basic cationic nitrogen and an anionic dye at a suitable pH, where a highly colored ion-pair complex is formed. This paper is concerned with the development of a new, simple, sensitive, inexpensive, extractive-spectrophotometric method describe for first time the determination of moexipril in bulk and in pharmaceutical formulation colorimetry. This method is based on ion-association complex formation of moexipril as basic drug with an acidic reagent, BCP, BPB, and BTB, and quantitative extraction of the product into chloroform solvent. No interference was observed when applied the proposed methods for the determination of MOEX in the presence of pharmaceutical additives.

EXPERIMENTAL

A) Instruments

A double-beam Shimadzu (Japan) 160 IPC UV-visible spectrophotometer connected to an IBM compatible fitted with UVPC Personal spectroscopy software version 3.7 (Shimadzu) was used.

B) Materials and Reagents

All chemicals and reagents were of analytical grade, distilled water was used through out the experimental

Chloroform, (Sigma-Aldrich, USA), BCP, BPB (Fluka, Switzerland), BTB (BDH, Pool, UK)

Moexipril-HCl and (primox®) tablet, batch No 031131. Labeled to contain 15 mg/tablet from Minapharm Co. (Egypt).

BCP, BPB, BTB prepared by Dissolve 0.1 g of each dye in 1.5 ml of 0.1M sodium hydroxide and 20

ml of ethanol (96%) and complete with distilled water to produce 100 ml.

Mcilvaine pH 2.2: prepared by mixing suitable volume (196 ml) of 0.1M of citric acid and (4 ml) of 0.2M of di-sodium hydrogen orthophosphate. Adjust to the required pH with 0.1M HCl or 0.1M NaOH[9].

Mcilvaine pH 4: prepared by mixing suitable volume (122 ml) of 0.1M of citric acid and (78 ml) of 0.2M of di-sodium hydrogen orthophosphate. Adjust to the required pH with 0.1M HCl or 0.1M NaOH[9].

Standard solution

Stock solution of moexipril-HCl containing 1mg ml^{-1} prepared in distilled water, working solution containing 200µg ml^{-1} was prepared by suitable dilution of stock solution by the same solvent.

Genera recommended procedures:

I - Procedure for the assay of bulk sample

Into a series of 50ml separating funnel, transfer Aliquot volume of the working standard solution, so that the final concentration is in the range stated in (Table 1), add 6 ml, 7 ml, and 3 ml of BCP, BPB, and BTB, respectively. Add 4 ml of Mcilvaine buffer pH 2.2 in case of BCP, and BPB and 5ml of pH 4 in case of BTB. The total volume was adjusted to 13 ml in case of BCP and BPB and to 10 ml in case of BTB and mix well. Chloroform (2×5 ml) was added to each separating funnel and the contents were shaking vigorously for 1min. The two phases were allowed to separate and the chloroformic layer was passed through anhydrous sodium sulphate. The absorbance was measured at specific wavelength against a reagent blank. A calibration graph was drawn or regression equation calculated.

II - Procedure for pharmaceutical formulation

The content of 10 tablets (primox®) was weighed, ground into a fine powder and mixed. An accurately weighed portion of the powder equivalent to one tablet was transferred into a 50mL volumetric flask. The volume was made up to the mark with distilled water. After 30 min of mechanical shaking, the solution was filtrated into a 50 ml calibrated flask through Whatman No. 42 filter paper. Serial dilutions were carried out using distilled water and the same procedure was applied as described under the procedure for bulk samples.

RESULTS AND DISCUSSION

A basic cationic nitrogen which present in MOEX-HCl reacts with an acid dyes, to form a colored product at a suitable pH. The yellow color of the resulting ion-pair complex was extracted with chloroform and measured at specific λ_{max} ranged from 405 to 415 nm. Moexipril can be transferred from the aqueous phase into the organic phase as an ion-pair formed with the anionic form of the acid dyes. The extraction equilibrium can be represented as follows:

$$MH^{+}_{(aq)} + D^{-}_{(aq)} \longleftrightarrow MH^{+}D^{-}_{(aq)} \longleftrightarrow MH^{+}D^{-}_{(org)}$$

Where MH^{+} and D^{-} represent the protonated moexipril and the anion of the dye, respectively, and the subscript (aq) and (org) referred to the aqueous and organic phases, respectively. The absorption spectra of the ion-pair complexes extracted into chloroform are shown in (Fig 2). The reagent blank under similar conditions showed no absorption. Then the linearity, accuracy, precision, sensitivity, stability of proposed methods were described and applied to pharmaceutical dosage forms as tablet and the results obtained were evaluated statistically.

Optimum Reaction Conditions for Complex Formation

Many investigations were carried out to establish the most favorable conditions to produce a highly intense colour and to achieve maximum color development in the quantitative determination of the drug. The influence of each of the following variables on the reaction was tasted.

Effect of pH

The influence of pH on the ion-pairs formation of MOEX-HCl with various dyes has been studied using Walpole buffer (NaOAc/HCl), (NaOAc/AcOH), Clark and labs buffer (KCl/ HCl), (Pot hyd phthalate/ HCl), phosphate buffer, Mcilvaine buffers. It is evident that the maximum color intensity and maximum absorbance were found in Mcilvaine buffer of pH 2.2 with BCP, and BPB, and pH 4.0 with BTB (increasing of pH 5 in case of BTB increases the absorbance of both ion-pairs and also the blank, so that pH 4 is the most suitable one), respectively. The results are shown in (Fig. 3). Moexipril hydrochloride contains a secondary amino group, which is protonated in acid medium, while the sulphonic acid group present in sulphonphthalein-type of dye is the only group undergoing dissociation in the pH range of 1.0-5.0. Finally, the protonated MOEX-HCl forms ion-pairs with anionic dyes, which are quantitatively extracted into chloroform.

Effect of buffer volume

The influence of buffer volume on the ion-pairs formation of MOEX-HCl with various dyes has been studied, the results showing that 4ml for BCP, BPB and 5ml for BTB are adequate to achieve rapid and complete separation of the two phases and prevent the emulsion formation.

Effect of dye volume

The effect of the concentration of BCP, BPB, and BTB on the absorbance of the organic phase was studied for solutions containing a fixed amount of MOEX-HCl and prepared as described in the general procedure. It was found that increasing the concentration of either BCP or BPB causes a gradual increase in the absorbance up to a concentration of 6 ml in case of BCP and 7 ml in case of BPB (Fig. 4). Further increase in the dye concentration did not show any increase in the absorbance but it affected formation of an emulsion and subsequently, long time for the separation of the two phases was required. It

was found that In case of 3 ml is the most suitable volume to achieve reproducibility and to decrease the blank absorbance.

Effect of extracting solvent

The effect of the extracting solvent used for moexipril drug on extraction efficiency and color intensity was examined. A number of organic solvents such as dichloromethane, chloroform, carbon tetrachloride, benzene, and toluene were examined for extraction of the ion-pair complexes. Chloroform was selected because of its slightly higher efficiency and considerably lower extraction ability for the reagent blank than other extractive organic solvents.

Effect of equilibration Time and Temperature

The optimum reaction time was determined by following the color development at ambient temperature. Complete color intensity was attained simultaneously after mixing for all complexes. Raising the temperature up to 35°C has no effect on the absorbance of the formed complexes, whereas above 35°C, the absorbance starts to decay (Fig. 5).

Effect of time after extraction

The effect of time after separation of the organic phase on the absorbance was studied for both systems. The obtained results showed that maximum color intensity was attained simultaneously after separation of the organic phase and the intensity remained constant for at least 48 hours.

Phase ratio

The ratio of aqueous to organic phase was ineffective and the ratio 3:1 was chosen for extraction of the colored species. It was also noticed that the order of addition of the reagents had neither an effect on the absorbance nor on the color of the complexes.

Effect of shaking time

Shaking time of 0.5-3 min provided a constant absorbance and hence, 1.0 min was used as an optimum shaking time throughout the experiment.

Effect of excipients

No significant interference was observed from the excipients commonly used in the moexipril formulations, such as talc powder, starch, lactose, avisil, hydroxyl propyl cellulose and magnesium stearate. It was found that the above excipients at levels as high as 20-fold excess had no effect on the absorbance of the ion-pair complexes.

Composition of ion-pair complexes

The composition of the ion-pair complexes was established by Job's method[10] of continuous variations using variable dye and MOEX-HCl concentrations. The results indicated that 1:1 (drug: dye) ion-pairs are formed through the electrostatic attraction between the positive protonated drug and the anion of these dyes as shown in scheme 1.

Conditional Stability Constants (K_f) of the Ion-Pair Complexes

The conditional stability constants (K_f) of the ion-pair associates for the moexipril-HCL under the experimental conditions described above were calculated from the continuous variation data using the following equation:

$$Kf = \frac{A/Am}{[1 - A/Am]^{n+1} CM(n)^n}$$

Where A and A_m the observed maximum absorbance and the absorbance value of all the drugs present is associated, respectively. C_M is the molar concentration of drug at the maximum and n is the stoichiometric constant with which dye ion associates with drug. Also the free energy changes were calculated using the following equation:

$$\Delta G = -2.303RTLogK_f$$

Where, R is the universal gas constant (8.314 J), T is the absolute temperature (273+25°C), K_f is conditional stability constants. The results of both K_f and ΔG are 4.19, 5.1, 4.75 and -23894, -29182, -27083 kJ, for BCP, BPB, BTB, respectively, indicate a spontaneous and stability nature of the reaction.

Validation data

Linearity of calibration graphs

Under the experimental conditions described, calibration graphs were obtained for each proposed methods. The regression equations, standard deviation of slopes and intercepts, standard deviation of residuals, correlation coefficients, and linear ranges, detection limit, and quantitation limit were given in (Table 1) for each spectrophotometric method. Regression analysis indicated linear relationship between absorbance and concentration; the calculated correlation coefficients showed good linearity and molar absorptivity showed high sensitivity

Quantification, accuracy and precision

In order to evaluate the accuracy and precision of the proposed method, solutions containing three different concentrations of the stated drugs were prepared and analyzed in five replicates. The analytical results obtained from this investigation are summarized in (Table 2). The low values of the relative standard deviation (RSD %) and percentage relative error (Er %) candidate the high precision and the good accuracy of the proposed methods

Application to pharmaceutical formulations

The proposed methods were successfully applied to determine MOEX-HCl in its dosage forms (primox®). The results obtained were compared statistically by Student's t-test (for accuracy), and variance ratio F-test (for precision) with the published method (4) that depend on Second derivative ultraviolet spectrophotometry with zero-crossing measurements at 215 nm.

The results in (Table 3) showed that the t- and F-values were smaller than the critical values indicating that there was no significant difference between the proposed and published methods.

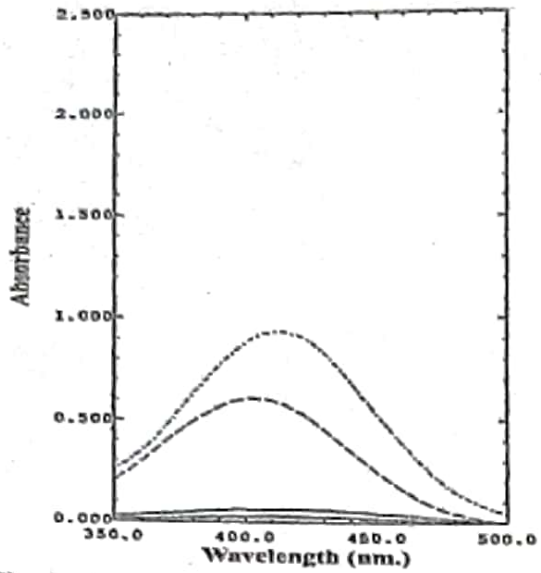


Fig. 2: Absorption spectra of moexipril-dye complex extracted into chloroform, Mox-BPB (._._.), Mox-BCP (——), and the reagent blank, BPB (.....), and BCP (—)

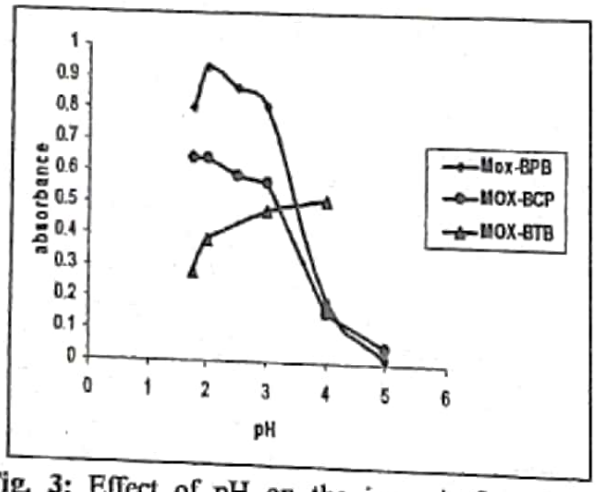


Fig. 3: Effect of pH on the ion-pair formation of Moexipril (20 µg mL-1) with sulphonphthalein dyes

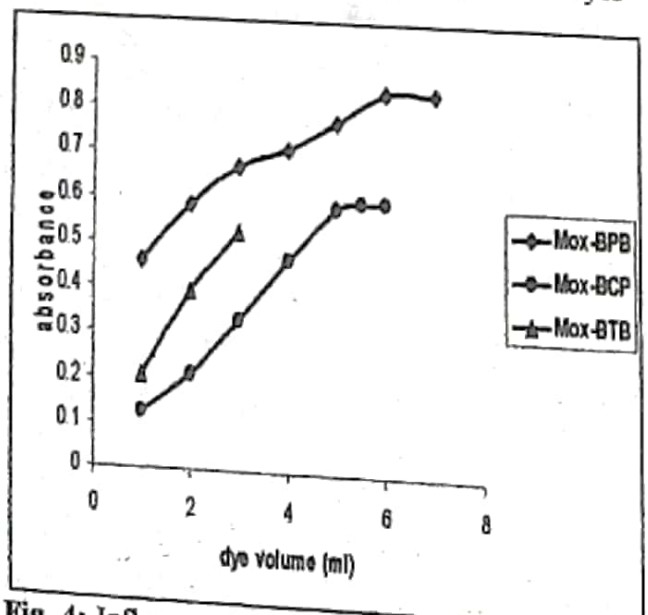


Fig. 4: Influence of volume of sulphonphthalein dyes on the absorbance of ion-pair complex of Moexipril (20 µg mL^{-1})

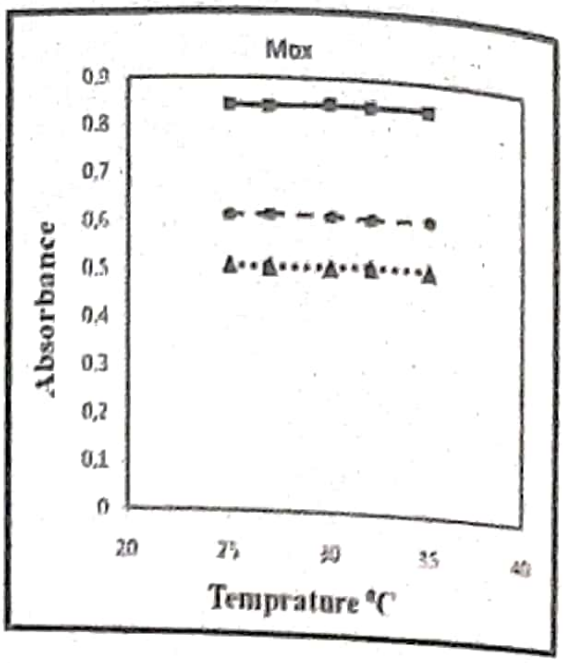


Fig. 5: Effect of temperature on the ion-pair complex formation of Moexipril with BCP (——), BPB (—), BTB (.....)

Table 1: Analytical parameters for determination of moexipril-HCl using the proposed methods

Parameters	BCP	BPB	BTB
λ_{max} (nm)	405	415	410
Beer's law limit (µg ml^{-1})	4-32	4-24	4-40
Molar absorptivity ($mol^{-1}cm^{-1}$)	1.7 ×104	2.1×10^{4}	1.5×10^{4}
Sandell's sensitivity (µg cm^{-2})	0.0323	0.0244	0.0382
Regression equation			
Intercept (a)	0.032	- 0.022	0.082
Slope (b)	0.029	0.043	0.022
Correlation coefficient (*r*)	0.9998	0.9998	0.9997
$S_{y/x}$	0.007	0.008	0.008
S_a	0.015	0.017	0.016
S_b	7.4×10^{-4}	1.1×10^{-3}	6.7×10^{-4}
Detection limit (µg ml^{-1})	0.064	0.065	0.077
Quantitation limit (µg ml^{-1})	0.212	0.213	0.257

Note: $S_{y/x}$= Standard deviation of residua; S_a= Standard deviation of intercept; S_b= Standard deviation of slope of regression line

Table 2: Evaluation of the accuracy and precision of the proposed method for moexipril-HCl determination

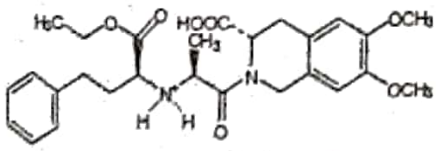
Dye	Add ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	percentage Recovey %	S.D[a]	(RSD %)	(ER %)
BCP	8	8,08	101	0,12	1,49	1,08
	24	23,84	99,33	0,4	1,69	-0,65
	32	32,26	100,81	0,098	0,3	0,82
BPB	8	7,99	99,87	0,07	0,88	-0,083
	12	12,61	101,33	0,26	2,16	1,36
	18	18,25	101,39	0,15	0,85	1,41
BTB	12	12,04	100,33	0,098	0,82	0,33
	24	24,15	100,62	0,14	0,58	0,625
	40	39,96	99,9	0,21	0,53	-0,09

[a] Mean of five determination

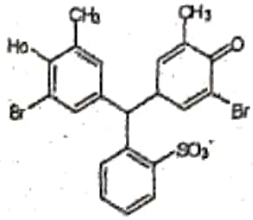
Table 3: Application of the proposed methods to determination of moexipril-HCL in pharmaceutical formulations

Commercial product	Recovery[a] ± SD			Reference method[4]
	BCP	BPB	BTB	
Primox® tablet (15mg/tab)	99.29 ±1.57	100.17 ± 1.35	100.11 ±1.57	99.43 ±0.721
t	0.19	1.18	0.96	
F	4.74	3.5	4.7	

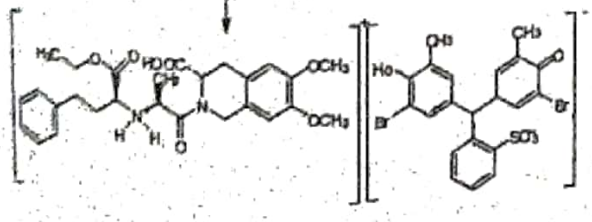
[a]: Mean of six determination
[b] The theoretical values of t and F at P = 0.05 are 2.57 and 5.05



Protonated Moexipril



BTB



Scheme 1: Ion-associated complex

CONCLUSION

This is the first time that spectrophotometric methods are being reported for the assay of moexipril-HCl in pure form and also in its dosage forms. The reported methods such as high performance liquid chromatography and gas chromatography methods in the literature involve more procedural steps and take more operator time and expertise. However, the proposed spectrophotometric methods provide economic procedures, less time consuming, and more sensitive and accurate compared with other reported methods. The proposed methods may be applied for routine analysis and in quality control laboratories for the quantitative determination of the studied drug in raw materials, in pharmaceutical formulations and in the presence of its degradation products.

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التعيين الطيفى لمادة الموكسيريل عن طريق تكوين الايون المزدوج باستخدام الصبغات الحمضية

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تم استنباط ثلاث طرق جديدة لتعين مادة الموكسيريل فى صورتها النقية وفى المستحضرات الصيدلية. وتعتمد هذه الطريقة على تكوين الأيون المزدوج واستخراجه فى مذيب عضوى وقياس الامتصاص الضوئى له عند طول موجى محدد باستخدام كلا من البروموكريزول البنفسجى والبروموفينوالأزرق والبروموثيمول الأزرق. وقد أعطت هذه الطرق نتائج جيدة عند مقارنتها بالطريقة المرجعية.