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Extractive Spectrophotometric Method for Determination of Meoxipril HCl and Perindopril in raw materials and tablets using ion pair formation

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A simple and rapid extraction spectrophotometric method has been developed for the determination of some angiotensin converting enzyme inhibitor drugs (ACE) such as perindopril (PRD) and meoxipril (MOEX) in pure and different dosage forms. The method involves the formation of tense yellow ion pairs between these drugs under investigation with methyl orange (MO) and bromocresol green (BCG) at pH 2.5 followed by their extraction with chloroform. The absorbance is measured at 414 and 416 nm for PRD and 414 and 413 nm for MOEX, using MO and BCG reagents, respectively. The analytical parameters and their effects on the proposed system are investigated. Experimental conditions for the method permits the determination of PRD and MOEX over the concentration ranges of 2 – 140 and 2 – 120 µg mL^{-1} for PRD and 2 – 90 and 2 – 140 µg mL^{-1} for MOEX using MO and BCG reagents , respectively. The sandell sensitivity is found to be 2.84 and 2.74 gcm^{-2} and 2.67 and 0.169 gcm^{-2} for PRD and MOEX using MO and BCG, respectively. The relative standard deviation and the limits of detection (LOD) were calculated. The proposed method has been applied successfully for the determination of the drugs under investigation in row materials and commercial tablets. No significant interference was observed from the excipients commonly used as pharmaceutical aids with assay procedure. The results are in good agreement with those obtained by the official method.

Key words: ACE**;** Angiotensin Extraction; Spectrophotometer; Converting Enzyme; Methyl Orange; Bromocresol Green

INTRODUCTION

Perindopril and meoxipril are ACE inhibitor and used in the treatment of hypertension and heart failure. [1] Perindopril is (2*S*,3*aS*,7*aS*)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxopentan-2-yl] amino} propanoyl]-octahydro-1*H*-indole-2-carboxylic acid and meoxipril is (3*S*)-2-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino}propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. Various methods are cited in the literature for the determination of perindopril such as amperometric biosensor [2-5], potentiometry [6], HPLC [7, 8] and spectrophotometry [9-12]. Meoxipril was determined by GC technique [13], carbon past electrode was used for the determination of meoxipril and it was enhanced by using sodium dodecyl sulphate [13]. It was also determined using PVC membrane electrode [15]. Simultaneous determination of meoxipril and hydrochlorothiazide in tablets by derivative spectrophotometric and high performance liquid chromatographic methods were also done [16]. An inspection of both the proposed method and the official ones for the cited drugs reveals that most of the official methods are cumbersome or involve the use of expensive equipments and reagents. Also, the proposed method is rapid and has very low detection limit which cannot be reached by most other methods. This paper reports simple, sensitive and accurate spectrophotometric method for the analysis of two antihypertensive agents namely perindopril and meoxipril in pure forms and pharmaceutical preparations.

MATERIALS AND METHODS

All reagents were of analytical grade and used without further purification, Water was always de- ionized. 0.02% (w/v) solutions of MO and BCG reagents were prepared by dissolving the accurate weighed amount of 0.02 mg in 100ml de-ionized water and Kept at 4°C in PVC containers. 100mg of the drugs under investigation was weighed in 100ml calibrated flask, dissolved in the least amount of methanol and diluted to the volume with de-ionized water. Universal buffer (Britton- Robinson): solution of pH (2-12) consisting of boric, phosphorus, acetic acids and sodium hydroxide [17].

A Perkin- Elmer model 601 with matched quarts cell of 1cm optical length was used for spectrophotometric measurements in the wavelength range from 200 to 800 nm. Adjustment of pH was done using HANNA pH-meter model ZH, Romania. Automatic Pipettes Scorex Swiss (50-200 and 200-1000µ) were used to measure very small volumes whereas glass micropipettes and burettes were used to measure large volumes.

Recommended procedure:

Determination of PRD and MOEX: In 50ml capacity separating funnel, 1 ml of 0.02% w/v MO or BCG were added to 0.5mL of PRD or MOEX (1mg/mL) solution and the volume was completed to 5 ml by universal buffer (pH=2.5). After 10 min, the ion- pairs were extracted twice with 5mL portions of chloroform after shaking for 1min. The ion pairs were collected in 10mL measuring flask and chloroform was dried over anhydrous sodium sulphate. The absorbance of the filtered extract was measured at 414 and 416nm for PRD and 414 and 413 nm for MOEX, using MO or BCG reagents, respectively, against a reagent blank, which was prepared similarly without drugs.

Procedure for tablets: Ten tablets of PRD and MOEX were accurately weighed and the average weight of one tablet was calculated. The tablets were crushed well to a fine powder. A portion of the powder equivalent to 100mg drug was dissolved in the least amount of methanol. The resulting

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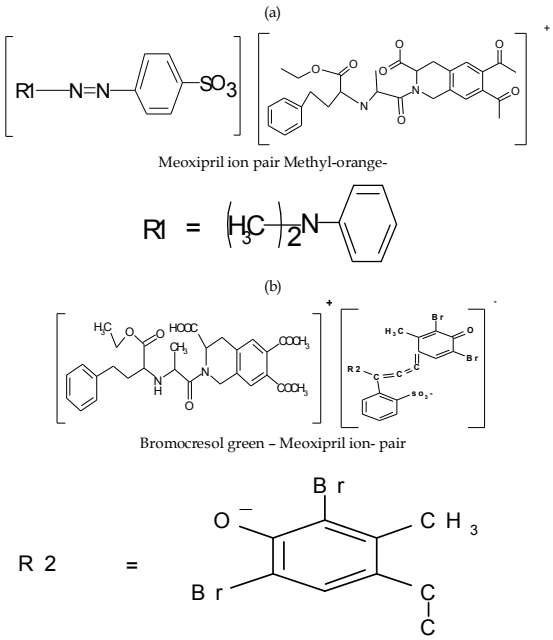
solutions were shacked well, filtered through whatman No. 1 filter paper and washed with the same solvent. The filtrate and washings of drugs were collected in 100ml measuring flask and completed to the mark with de-ionized water. An aliquot was used for the determination of each drug according to the procedure mentioned above.

RESULTS AND DISCUSSION

Mo and BCG reagents belong to the sulphonephthaline dyes. These dyes have the following ionic forms; the yellow spices HL^- are present in the aqueous solutions and on acidification they turn purple H_2L as an ampholyte. This colour change corresponds to protonation of quinoid oxygen. The second symmetric resonance structure L^{-2} arises by splitting of a proton from the hydroxyl group accompanied by bathochromic shift [18]. This work is undertaken in the view that, ion-pairs are formed between tertiary amino group of the cited drugs and MO or BCG reagents via the protonated nitrogen atom of the drugs (Scheme 1). On adding basic drugs solution, stable yellow ion-pairs are formed in acidic medium pH 2.5. The ion-pairs formed are soluble in chloroform and double extraction is necessary to extract the ion pairs quantitatively into organic phase.



Scheme 1: The proposed structures of (a) meoxipril with MO. (b) meoxipril with BCG.

Absorption spectra: The absorption spectra of the ion pairs (Figure 1) extracted in chloroform show maximum absorption at 414 and 416 nm for PRD and 414 and 413 nm for MOEX using BCG and MO reagents, respectively.

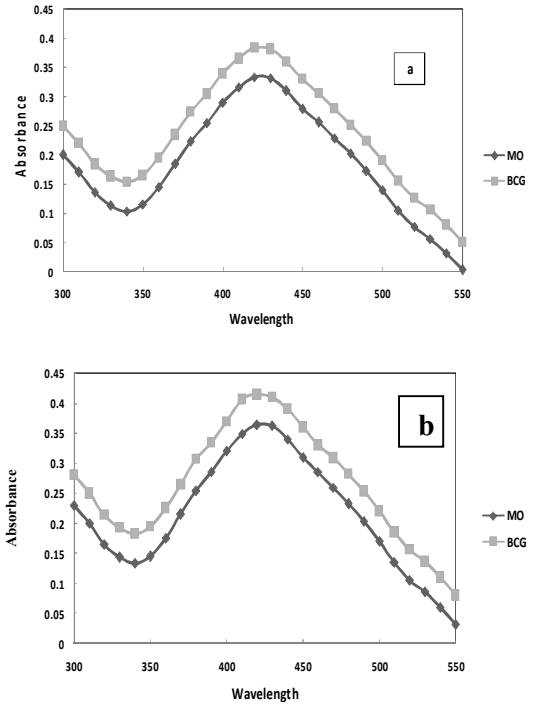


Figure (1): Absorption spectra of MOEX HCl (100 µg mL^{-1}) and PRD (100 µg mL^{-1}) drugs in chloroform
(a) PRD ion-pairs using MO and BCG reagents.
(b) MOEX HCl ion-pairs using MO and BCG reagents.

Effect of pH: The effect of pH on the formation of ion pairs in the presence of 100 µgmL^{-1} of the cited drugs has been studied using universal buffer (pH =1-6). It is evident that the maximum colour intensity and maximum absorbance were found at pH 2.5 in chloroform solvent. MOEX and PRD contain a secondary amino group which is protonated in acidic medium, while the sulfonic acid group present in sulfonphthalein- type of dyes is the only group undergoing dissociation in the pH range of 1.0-6.0. Finally, the protonated drugs form ion-pairs with ionic dyes, which are quantitatively extracted into chloroform (Fig. 2).

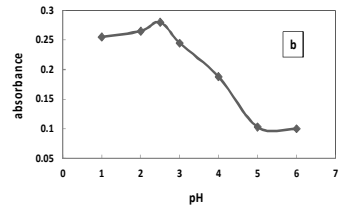


Figure (2): Effect of acidity of buffer on (a) [100] µg mL $^{-1}$ MOEX-MO ion pair using borax buffer. (b) [100] µg mL $^{-1}$ PRD –BCG ion pair using universal buffer

Effect of time: The effect of time on the formation of the ion pairs was studied carefully and it was found that the complete formation of the ion pairs needs 5 and 10 min for PRD and MOEX drugs, respectively, and 5 and 15 min for PRD and MOEX, respectively, before extraction with chloroform at 30± 1°C with MO and BCG reagents, respectively (Fig. 3).

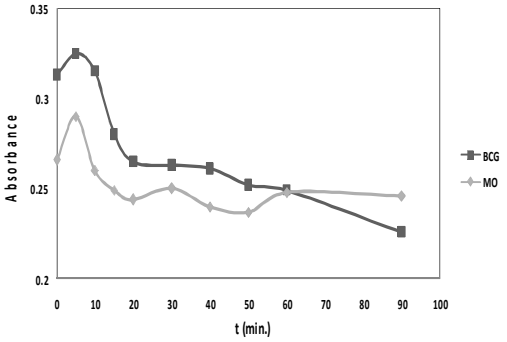


Figure (3): Effect of time on the spectra of the ion pairs of PRD drug (100 µg mL^{-1}) at 416 and 414 nm with MO and BCG reagents, respectively

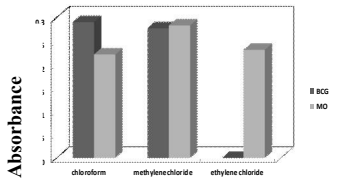


Figure (5): Effect of different types of solvents on the spectra of the ion pairs of PRD drug (100 µg mL^{-1}) at 416 and 414 nm with MO and BCG reagents, respectively

Effect of temperature: Absorbance- temperature curve of the reaction of PRD and MOEX drugs with MO or BCG is constructed at λ_{max}. The absorbance of the extracted ion- pairs is measured within the temperature range from 10-to 60°C. It is clear From the curve that, the absorbance is generally increased by increasing the temperature till reached a maximum value at 34 and 28°C for PRD and 40 and 22°C for MOEX using MO and BCG reagents, respectively, as shown in Fig.(4). The temperature is slightly increased or decreased above this temperature. Therefore, the optimum temperature for microdetermination of the drugs under study in pure and pharmaceutical formulation was at 34 and 28°C for PRD and 40 and 22°C for MOEX using MO and BCG reagents, respectively.

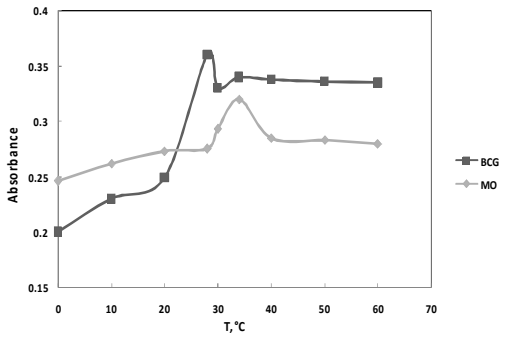


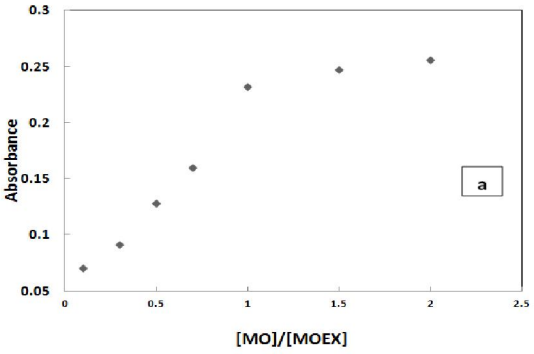
Figure (4): Effect of temperature on the spectra of the ion pairs of PRD drug (100 µg mL^{-1}) at 416 and 414 nm with MO and BCG reagents, respectively

Effect of organic solvents: Solvents like benzene, n-butanol, toluene and carbon tetrachloride cannot be used for the extraction of the ion-pairs formed, while chlororoform, 1,2-dichloroethane and methylene chloride can extract the ion-pairs quantitatively. The molar absorptivity values for the ion- pairs in chloroform using MO are ϵ= 955.6 and 1800 L mol^{-1} cm^{-1} for PRD and MOEX, respectively, at λ_{max} 416 and 413 nm. While ϵ = 989.6 and 11077 L mol^{-1} cm^{-1} for PRD and MOEX, respectively, at λ_{max} 414 and 414 nm, respectively, using BCG reagent. The results make chloroform solvents selected as the best media for extraction as well as its slightly higher efficiency and considerably lower extraction ability for the reagent blank (Fig. 5).

Effect of phase ratio of organic to aqueous layers: Reproducible absorbance readings were obtained after double extraction (5 mL for each one) with 10mL of chloroform, and 1min shaking time. The intensity of the colour formed after extraction by chloroform is stable for at least 24h.

Effect of reagent concentration: The drugs concentrations were kept constant at 100µgmL^{-1} while the concentration of MO and BCG (v/v) was varied from 0.1 - 3mL of 20µgmL^{-1} and prepared as described in the general procedure. It was found that increasing the concentration of either MO or BCG reagents affect a gradual increase in the absorbance up to a concentration of 1mL for MO and BCG reagents. Any further increase in the dye concentration did not show any increase in the absorbance but it showed formation of an emulsion and subsequently longer time for the two phases to separate was required.

Stoichiometry: The nature of the binding of the cited drugs with respect to the reagents which were presents in excess was determined by continuous variation and molar ratio methods [19]. The results indicate that a 1:1 [MO]:[drug] and [BCG]:[drug] ion-pairs are formed through the electrostatic attraction between positive protonated drugs and MO^{-} or BCG^{-} anion as shown by the proposed structure of PRD^{+} with MO^{-} and BCG^{-} reagents [20] and shown in Fig. (6).



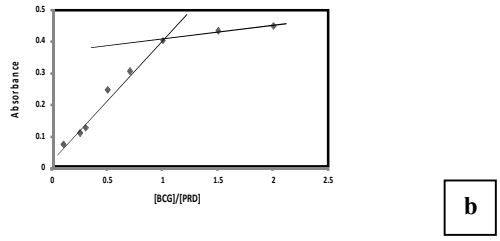


Figure (6): Stoichiometric ratio of the reaction of (a) MOEX with MO reagent at 413 nm and (b) PRD with BCG reagent at 414 nm using molar ratio method.

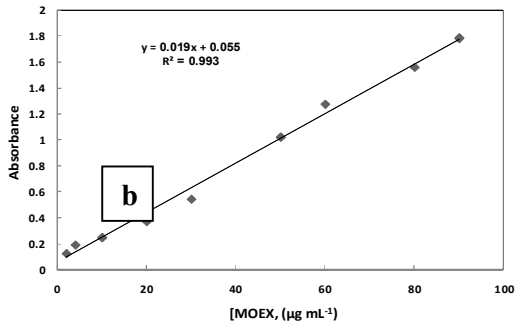


Figure (7): Beer's law plot for the determination of MOEX drug using BCG at 414 nm

Effect of excipients :No significant interference was observed from the excipients commonly used in PRD and MOEX drugs formulation such as talc powder, starch, lactose, avisil, hydroxyl propyl cellulose and magnesium stearate. It was found that the above excipients at level as high as 20 fold excess have no effect on the absorbance of the ion pair complexes.

Analytical performance:

Obeyence of Beer's law: Under the optimum conditions described above, the calibration graphs were constructed for the investigated drugs. The molar absorbtivity, Sandell sensitivity (S), concentration ranges, standard deviation, relative standard deviation and regression equation for each drug was tabulated in Table 1. Beer's law was obeyed over the concentration range of 2– 140 and 2 - 140µg mL^{-1} of PRD and MOEX, respectively, using MO reagent and 2 – 120 and 2 - 90 µg mL^{-1} of PRD and MOEX, respectively, using BCG reagent as shown in Fig. (7). The mean recovery values obtained in the range of 99.89 and 99.1% for PRD and MOEX using MO reagent and 100.5 and 98.3% using BCG reagent , respectively. This is supported also by the calculated valued of sandell sensitivity of 2.84 and 2.67 gm cm^{-2} for PRD and MOEX using MO and 2.74 and 0.169µg cm^{-2} for PRD and MOEX, respectively, using BCG reagent . It indicates the high sensitivity of the method. The correlation coefficients obtained are found to be 0.967 and 0.993 and 0.985 and 0.993 For PRD and MOEX using MO and BCG reagents, respectively. The SD are found to be 0.023 and 0.011 and 0.013 and 0.017 for PRD and MOEX using MO and BCG reagents, respectively. The relative standard deviation (RSD %) 0.790 and 0.112% and 0.129 and 1.199% for PRD and MOEX using MO and BCG reagents, respectively. The low values of the relative standard deviation indicate the high accuracy and precision of the method.

Table 1: Analytical parameters for the determination of Meoxipril HCl and Perindipril drugs using BCG and MO reagents

Parameters		BCG		MO	
		MOEX	PRD	MOEX	PRD
λ_{max} (nm)		414	414	413	416
Time (min.)		15	5	10	5
T(°C)		22°	28°	40°	34°
Conc. Range (µg mL^{-1})		2 - 90	2 - 120	2 - 140	2 – 140
ε ($L.mol^{-1}.cm^{-1}$)		11077	989.6	1800	955.6
Sandell Senstivity (µg cm^{-2})		0.169	2.74	2.67	2.84
A=mC+Z	m	0.019	0.014	0.001	0.024
	Z	0.055	0.117	0.123	0.066
Correlation coefficient (r)		0.993	0.985	0.993	0.967
SD		0.017	0.013	0.011	0.023
RSD (%)		1.199	0.129	0.112	0.790
LOD (µg mL^{-1})		0.34	0.641	0.72	0.81
LOQ (µg mL^{-1})		1.12	2.12	2.38	2.67
Percentage recovery (%)		98.30-99.20	97.50-109.00	98.40-99.05	100.00-102.00

Between day measurements: The validity and applicability of the proposed method and the reproducibility of the results obtained are studied. Five replicate experiments at three concentrations of PRD and MOEX were carried out. Table 2 shows the values of between – day relative standard deviations for different concentrations of the drugs obtained from experiments carried out over a period of four days. It was found that, the between- day relative standard deviations were less than 1%, which indicates that the proposed method is highly reproducible and MO or BCG reagents are successfully applied to determine PRD and MOEX via ion-pair formation [21].

Table 2: Between–day precision for the determination of MOEX and PRD drugs using BCG and MO reagents

Compound	[Drug] Taken µg mL⁻¹	[Drug] Found* µg mL⁻¹	% Recovery	SD	RSD (%)
1- Using BCG reagent					
PRD	10.00	10.09	109.00	0.013	0.129
	40.00	38.99	97.50	0.022	0.056
MOEX	10.00	9.92	99.20	0.119	1.199
	40.00	39.45	98.60	0.017	0.043
2- Using MO reagent					
PRD	10.00	10.12	101.20	0.08	0.790
	40.00	40.00	100.00	0.023	0.058
MOEX	10.00	9.84	98.40	0.011	0.112
	40.00	39.62	99.05	0.011	0.028

*Average of four determination

Limit of detection and quantification: According to ICH recommendation [22] the approach based on the S.D of the response and slope was used for determination of the detection (DL) and quantification limits (QL) by means of the following equation

$$DL= t/b\ [s^2\ (n-2/n-1)]^{1/2}$$

Where n is the number of standard samples (n = 4),t is the value of students t for n = 2 degree of freedom at 95% confidence level, b is the slope of regression line and S^2 is the variance characterizing the scatter of the experimental data points with respect to the line of regression. The quantification limit QL was calculated by the following equation

$$QL=(\ DL/3)\ x\ 10$$

Specificity and interference studies: The ion association spectrophotometric method based on the reaction of basic center of the drug with anionic dye, so any drug lack basic center couldn't be interfered with this method. The result revealed that there is no interference from hydrochlorothiazide the most commonly diuretics co-formulated and indomethacin the commonly co administered drug with PRD or MOEX due to the two drugs lack the basic center. It was also clear that the proposed method is specific and selective for determination of the drugs in the presence of their derivatives and the diacid form of MOEX (meoxi-prilate), the major basic- and acidic induced degradation products of meoxipril. These compounds lack the presence of basic amino group responsible for ion- pair formation [23].

Robustness: Robustness is the measure of capacity of analytical methods to remain unaffected by small variations of the operation parameters. Variations of the dye volume, temperature, and shaking time during extraction process did not have significant effect on the color intensity in the proposed method.

Stability: Solutions of studied drugs in distilled water exhibited no absorbance change for 12hour when kept at room temperature and for 24hour when stored in refrigerated at 4°C.

Application to pharmaceutical formulation: The validity of the proposed method was tested by determination of PRD and MOEX drugs in dosage forms. The concentration of the drugs in the dosage forms was calculated from the appropriate calibration graphs. There was no shift in the absorption maximum due to the presence of other concentrations of the dosage forms. Table 3 shows the results obtained for the determination of PRD and MOEX in the dosage forms and the results obtained are compared with those obtained applying the official method [25]. The results obtained were compared statistically by t- test and variance ratio F- test with those obtained by official method on the sample of the same batch [24]. The t- test values obtained at the 95% confidence level and degree of freedom did not exceed the theoretical tabulated value indicating that there is no significant difference between accuracy of the proposed and the official method. The F- values also showed that there is no significant difference between precision of the proposed and the official methods.

Table 3: Spectrophotometric determination of MOEX, PRD drugs in pharmaceutical preparation using MO and BCG reagents and official method

Reagent	Sample	Proposed [Drug] µg mL⁻¹		Official [Drug] µg mL⁻¹		% Recovery		SD*	SD**	F-test	t-test
		Taken	Found	Taken	Found	Proposed	Official				
BCG	Primox (15 mg / tablet)	10.00	10.00	10.00	10.05	100.00	100.5	0.09	0.356	0.06	2.5
		40.00	39.78	40.00	40.08	96.95	100.2	0.08	0.673	0.01	1.5
MO		10.00	9.90			99.00		0.04		0.03	0.8
		40.00	39.30			98.25		0.07		0.05	2.3
BCG	Coversyl (5 mg / tablet)	10.00	10.05	10.00	9.80	100.50	98.00	0.03	0.298	0.01	1.7
		40.00	39.15	40.00	39.70	97.88	99.25	0.06	0.577	0.04	1.9
MO		10.00	9.49			94.90		0.08		0.07	2.0
		40.00	40.00			100.00		0.02		0.02	1.0

* Proposed method. ** Official method.

CONCLUSION

The proposed method is rapid, accurate, sensitive and providing a simple solution for the problem of low absorptivity of the drugs in the UV region. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in pharmaceutical formulations. The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as pH, reagent concentration or temperature. The wide applicability of the new procedure for routine quality control is well established by the assay of the cited drugs in pure forms and pharmaceutical preparations.

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