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Determination of ketoconazole and trimetazidine hydrochloride through ion-pair formation with tetraiodobismuthate

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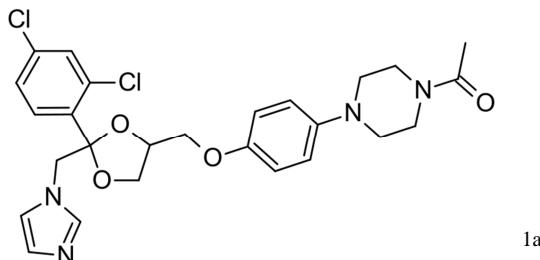
ABSTRACT

Ketoconazole (KC) and trimetazidine hydrochloride (TMH) react with tetraiodobismuthate to give ion-pair complexes. The ion pairs are readily extractable in 1, 2-dichloroethane to give a reddish orange colour with maximum absorption at 491nm that can be used for quantitative spectrophotometric analysis. By application of the methods of Sommer and Job involving non-equimolar solutions, the conditional stability constant ($\log K_c$) of the KC-BiI₄ ion pair (1:1) at the optimum pH of 2.5 and 0.075 M KI, was found to be 5.81. The mean recoveries for authentic samples of KC and TMH were 99.98 ± 0.39 and $100.05 \pm 0.51\%$, respectively. Alternatively, determination of the bismuth content of the ion pairs via atomic absorption spectrometry (AAS) provides an indirect method for the determination of these drugs. The mean recoveries were 99.83 ± 0.71 and $99.92 \pm 0.78\%$ for KC and TMH, respectively. Both methods were applied for the analysis of pharmaceutical preparations and the results obtained are comparable to the reported methods.

Keywords: Tetraiodobismuthate, Ketoconazole, Trimetazidine hydrochloride, Ion pairs, Spectrophotometry, Atomic absorption spectrometry.

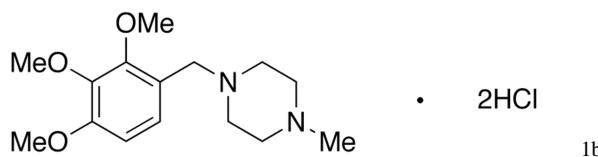
INTRODUCTION

Ketoconazole(KC) , [(\pm) -cis-1-acetyl-4- (4-{ [2-(2,4- dichlorophenyl) -2- (1H- imidazole - 1-ylmethyl) -1,3-dioxolan-4-yl]methoxy}phenyl) piperazine] (Scheme 1a), is an imidazole derivative with a wide antifungal spectrum and possesses some antibacterial activity is widely used in the treatment of dermal and systemic mycoses. Ketoconazole presents advantage over other imidazole derivatives in sustaining adequate blood levels following oral administration [1-3].



Several analytical methods have been developed for quantitative determination of ketoconazole, including potentiometry[4-5] ultraviolet (UV), visible spectrophotometry and colorimetry [6-14], spectrofluorimetry [14],HPTLC [15] chiral subcritical-fluid chromatography [16], ketoconazole ion selective electrode [17] capillary electrophoresis with diode array detection[18], high performance liquid chromatography (HPLC) using different detection modes [8,19-22] electrochemical detection [23-24] stripping voltammetric and polarographic techniques [25-26].

Trimetazidine hydrochloride (TMH); 1-[2,3,4-trimethoxyphenyl] methyl] piperazine dihydrochloride (Scheme 1b) is a clinically effective antianginal agent that has been used in the prophylaxis and management of angina pectoris, and in ischemia of neurosensorial tissues as in Meniere's disease [27]. The antianginal efficacy of TMH is comparable to propranolol but it does not reduce cardiac rate-pressure product or coronary blood flow [28].



Due to trimetazidine physiological significance, there is much interest in its determination of pharmaceutical quality control. Hence it attracted the attention of pharmaceutical analysts [29].Several methods have been reported for the determination of trimetazidine dihydrochloride in bulk and in pharmaceutical preparation; these methods include direct UV measurement, spectrophotometry [30-35]spectrofluorimetry [31]. Chromatographic methods were also reported, RP-HPLC assay methods [36-39], stability indicating HPTLC [40], cyclic and square wave voltammetry in bulk drug, tablet and urine [41] and slow injection chemiluminescence [42].The present work describes, for the first time, the analytical aspects of the reaction between KC and TMH with tetraiodobismuthate (BiI₄). The method was based on the formation of ion-pair complexes. The validation of a new spectrophotometric method for the determination of TMH assay in pharmaceutical forms is the aim of our study, thus selective and sensitive methods for the determination of these compounds have been devised.

MATERIALS AND METHODS

Apparatus

A Shimadzu UV-1601 spectrophotometer with quartz cells of 1-cm optical path length and a Perkin-Elmer A Analyst 100 atomic-absorption spectrometer equipped with a bismuth hollow cathode lamp were used. A HI 9321 Hanna Microprocessor pH-meter with a combined glass-saturated calomel electrode was also used.

Materials and Reagents

All solutions were prepared with analytical-reagent grade chemicals and bidistilled water was used. The studied drugs were of pharmaceutical grade. Ketoconazole (Janssen Pharmaceutical, Beerse, Belgium), and trimetazidine hydrochloride (Servier Egypt Industries Limited, under Licence of les Laboratories Servier, France), were obtained from the manufacturer.

Standard bismuth (III) solution, 0.02 M, was prepared by dissolving 0.9702 g of $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ (Merck) in 2 mL of concentrated HNO_3 and adding distilled water to 100 mL and standardized complexometrically [43].

Potassium iodide solution 1 M was prepared by dissolving 16.66 g of KI (Merck) in 100 mL of water. Solutions of lower concentration were obtained by accurate dilution of this solution with water.

Preparation of standard sample solutions

Ketoconazole stock solution was prepared by dissolving 50 mg of the drug in 20 mL of 0.1 M HCl and completed to 100 mL with water.

Trimetazidine hydrochloride stock solution was prepared by dissolving 50 mg of the drug salt in 100 mL of water. Other dilute solution ($100 \mu\text{g mL}^{-1}$) of each drug was made by accurate dilution with water.

Recommended procedure**Spectrophotometric method**

The mixture of 3.0 mL (2×10^{-3} M) of Bi (III) nitrate and 2.5mL (0.3 M) of KI were placed in 75 mL separatory funnel. Three mL of the drug solution containing 25-300 μg were added and the pH was adjusted to 2.5 by adding an appropriate amount of KOH and HNO_3 (0.2M). The solution was completed to 10 mL with water, then 5 mL of 1,2- dichloroethane and 0.5 mL acetone were added and shaken for 1 min. The organic layer was separated and filtered through a filter paper (no. 4) and measured at 491 nm against a reagent blank prepared and treated similarly.

Atomic-absorption method

The organic layers of the above method were allowed to dryness on a water bath and the residues were dissolved in 1 mL of DMF and completed to 5 mL with water in calibrated flasks. The solutions were then aspirated into the flame using a hollow cathode lamp of bismuth at wavelength= 223.1 nm, lamp current: 10 mA and slit width = 0.2 mm with an air/ acetylene flame. The atomic absorption of bismuth in the DMF-aqueous solution is measured. The concentrations of the tested drugs were calculated from the relevant calibration graph of regression equation.

Assay of pharmaceutical preparation**Analysis of Nizoral tablets or cream (ketoconazole)**

An accurately weighed amount of cream or powdered tablets equivalent to 50 mg of drug was transferred to a 100 mL volumetric flask and extracted with 20 mL 0.1 M HCl for 10 min and diluted with water. The mixture was filtered through a filter paper (no. 4) and washed with water. The filtrate and washings were collected in a 100-mL calibrated flask and diluted to volume with water. A volume of the later solution was diluted with water to obtain a solution equivalent to $100 \mu\text{g mL}^{-1}$ of drug and then subjected to analysis by the recommended procedure.

Analysis of Vastarel tablets (trimetazidine hydrochloride)

An accurately weighed amount of the finely powdered tablets equivalent to 50 mg of the drug salt was transferred to a 100 mL volumetric flask and extracted with water, filtered through a filter paper, washed with water and then continued as above.

RESULTS AND DISCUSSION

Tetraiodobismuthate (BiI_4^-) compounds have been used as reagents for the determination of some nitrogenous drugs [43-47]. The formation of the ion pair between the tertiary amine of piperazine ring of the drug and BiI_4^- binary complex occurs via the protonated nitrogen atom of the drug.

On mixing aqueous solutions of BiI_4^- complex and KC or TMH in an acidic medium, a reddish orange precipitate appears that is attributed to the ion pair formed in the reaction.

Spectral characteristics

The absorption spectrum of the ion- pair complex of ketoconazole or trimetazidine with tetraiodobismuthate, binary complex in 1, 2-dichloroethane exhibits absorption maximum at 491 nm, which can therefore be used as the wavelength for the analytical determinations. The reagent blank at this wavelength has a low absorbance; all measurements were performed against a reagent blank.

Effect of organic phase

Extraction of both tetraiodobismuthate binary complex and ion-pair complex has been investigated by using different solvents. Ketones and particularly methylisobutylketone which extract the salt but iodobismuthic acid interfered by being extracted as well. On the other hand, slightly polar solvents such as chloroform, dichloromethane and 1,2-dichloroethane only extract the ion pair of KC or TMH. Among these last solvents, 1,2-dichloroethane was chosen as the extraction solvent because of its higher efficiency and considerably lower extraction ability for the reagent blank. Addition of a small amount of acetone proved to be useful: the colour intensity of the analyte and the reagent blank increased with increase percentages of acetone in the aqueous phase, although the differences between them diminished. The ratio adopted in this study was always $\leq 5\% (\text{v/v})$ acetone-water.

Effect of pH

Fig. 1 shows the results obtained when varying pH for the aqueous phase within the range 1.5 to 4.5. At $\text{pH} > 3$, there is a decrease in the extraction yield with increasing pH, probably because of precipitation of bismuth as hydroxo-species. The absorbance at λ_{max} (491nm) remains constant in the pH range 2-3. The pH of the solution at 2.5 was selected for the drug determination.

As the shape of the absorption maximum does not vary with pH, so it is assumed that only one type of ion pair is formed at this pH range. The use of a suitable buffer solution is not useful due to the fact that the presence of any foreign substance in the buffer solution could interfere with the ion-pair complex, then 0.2 M of KOH or HNO_3 was used to adjust the pH of the solution.

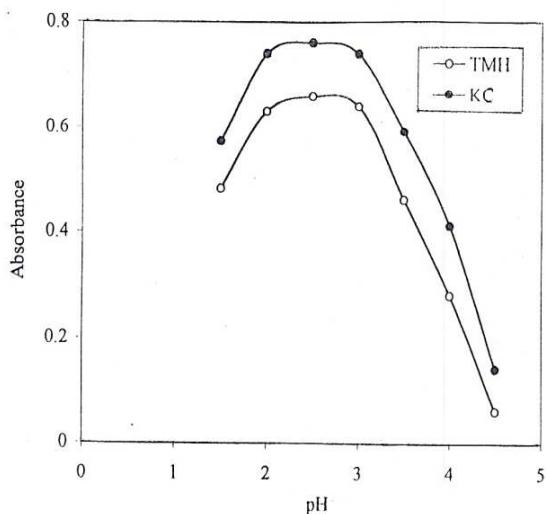


Fig. 1 Effect of pH on BiI4- ion-pair formation complexes with KC and TMH in 1,2-dichloroethane. [Drug]org. = 35 µg mL⁻¹, [Bi(III)]aq. = 6x10⁻⁴ M, [KI]aq. = 0.075 M, pH= 2.5, λ=491 nm.

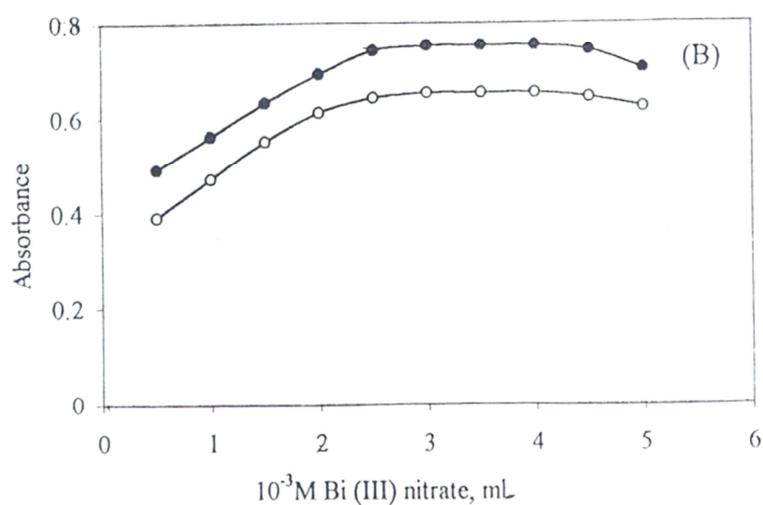
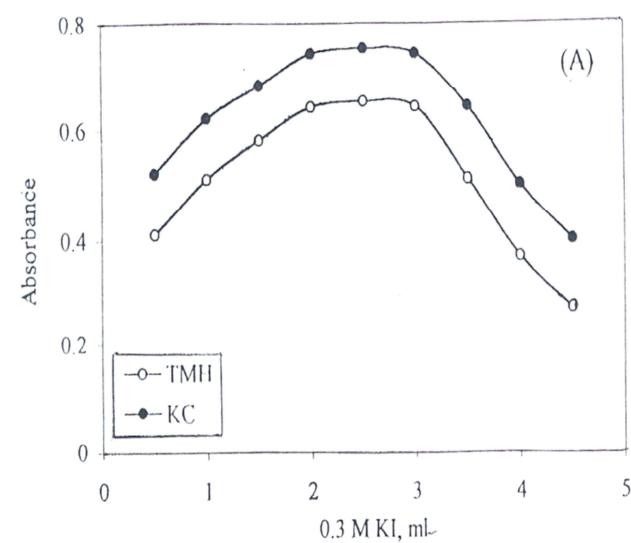


Fig.2 Effect of KI (A) and Bi(III) nitrate (B) concentrations on BiI4- ion-pair formation complexes with KC and TMH in 1,2-dichloroethane. [Drug]org. = 35 µg mL⁻¹, [Bi(III)]aq.=6x10⁻⁴ M (A), [KI]aq. = 0.075 M (B), pH= 2.5, λ=491 nm.

Optimum conditions for drug determination

Fig.2 (A) shows the influence of the iodide concentration on the ion-pair extraction at a constant concentration of bismuth. At the bismuth concentration used, 6×10^{-4} M, the iodide concentration of 0.06 to 0.09 M in the aqueous phase (10mL), was required to obtain the maximum absorption values, then 0.075 M KI was chosen. As shown in Fig. 2 (B), the ion pair was optimally extracted at a bismuth concentration of 6×10^{-4} M, above which the extraction efficiency slightly decreased. Dragendorf reagent solutions should not be used due to the liberation of iodine after a short time, which interferes with the determination of the drug. The favorable sequence addition of the reagents is bismuth (III)-KI-drug for the highest colour intensity.

The formation of ion pairs is rapid and the absorbance readings of the 1, 2-dichloroethane extracts of the associates were constant after 10 min and were stable for at least 4 h. Shaking time ranging from 0.5 to 5.0 min did not produce any change in colour intensity, and so 1 min shaking time was selected.

Consequently, the yield of a single extraction with 5 mL of 1,2-dichloroethane in optimal conditions with an organic: aqueous phase of 1:2 is practically 100%. Acetone was considered to be an ideal diluent for the extraction process, as it increases the extraction efficiency. The intensity of ion-pair extract was stable within the temperature range 20–40°C, then room temperature, $25 \pm 1^\circ\text{C}$ was used.

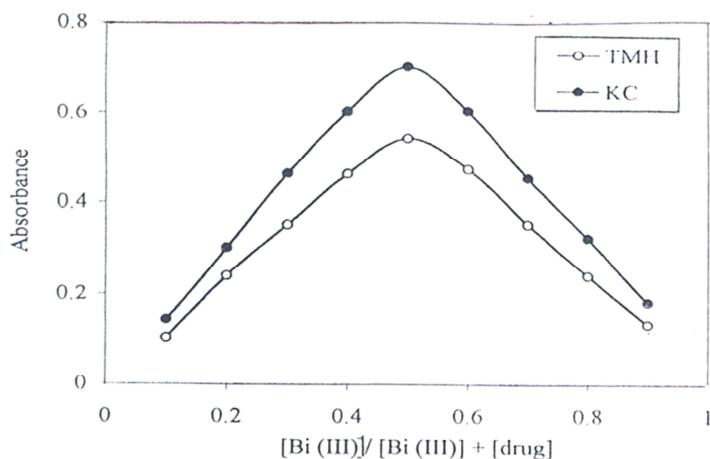


Fig. 3 Job's curve plots of equimolar solutions for BiI_4^- ion-pairs with KC and TMH in 1,2-dichloroethane. Total molar concentration = 2×10^{-4} M, pH=2.5, $[\text{KI}] = 0.075$ M, $\lambda = 491$ nm

Effect of foreign materials

No significant interference was observed from the excipients commonly used in the drug formulations, such as talc powder, starch, lactose, glucose, cellulose and magnesium stearate. It was found that the above excipients at levels as high as 100-fold excess had no effect on the absorbance of the ion-pair complexes. Each drug for this study was dispensed and used as a single component (tablets or cream). So the assay in presence of other drugs is not necessary.

Stoichiometry of the ion-pair

The composition of the (BiI_4^-) drug ion pair, was investigated by applying Job's method of continuous variation [48]. The aqueous drug concentration and Bi(III) nitrate solutions were 1×10^{-3} M. Nine solutions were prepared containing drug and Bi(III) nitrate in various molar ratios so that their volume always amounted to 2 mL with addition of 2.5 mL (0.3 M) KI and adjusting pH to 2.5. The mixture was completed to 10 mL with water and 0.5mL of acetone. The extraction was performed with 10 mL, 1,2-dichloroethane and the absorbance was measured at 491 nm. The plot reaches maximum value at a mole fraction $X_{\max} = 0.5$ (Fig. 3) indicating the formation of 1: 1 ion-pair complex for the two drugs (KC and TMH).

In order to determine the stoichiometry of the ion pair of TMH (as example) within the aqueous phase, the solid precipitate was obtained, then filtered, washed and dried to constant weight, it shows a reddish orange colour and melting point at 162°C (m.p. of TMH = $225\text{--}228^\circ\text{C}$). The content of C, H, N and Cl are directly obtained through an elementary analyzer. The content of bismuth is determined by AAS from its dissolution in 20% (v/v) DMF-water; a calibration line prepared from a standard solution of bismuth is used for this aim. The results of analysis were the following: calculated: 17.09% C, 2.36% H, 2.85% N and 21.24% Bi, found: 17.20% C, 2.50% H, 3.10% N and 21.00% Bi, there is no evidence on the presence of Cl in the TMH ion-pair salt. The agreements between the analytical and calculated results indicate that the suggested formula as follow: $\text{C}_{14}\text{H}_{23}\text{N}_2\text{O}_3 \cdot \text{BiI}_4$ and molecular weight = 983.95.

Infrared spectra of trimetazidine hydrochloride and trimetazidine-tetraiodobismuthate within the range $4000\text{--}400$ cm^{-1} were recorded. The IR spectra showed that the trimetazidine - tetraiodobismuthate complex has been formed by exchange of chloride ion in trimetazidine hydrochloride for BiI_4^- ion. The broad bands at 3600 and 3490cm^{-1} due to the stretching frequency of $-\text{NH}_2^+$ attached with chloride ion of piperazine ring, became one band at 3430cm^{-1} and a new band appeared at 3230 cm^{-1} (NH_2^+ ion), due to formation of an ion association. A broad band at 2450 cm^{-1} due to the stretching frequency of $-\text{NH}^+$ combined with Cl^- ion of tertiary amine of piperazine ring, has been nearly disappeared in the spectra of ion-pair complex due to the absence of HC1, which was confirmed by elemental analysis.

Conditional stability constant of the ion-pair complex

The conditional stability constant of the KC- BiI_4 or TMH- BiI_4 ion-pair has been determined by applying the method of Sommer et al. [49], on the basis of results obtained by Job's method for the composition of the ion pair. The following equation for 1:1 complex can be used for calculation of the conditional stability constant of the ion pair,

$$K = \frac{(A/A_m)}{\left[1 - \left(\frac{A}{A_m}\right)\right]^2 C}$$

where A_m is a limiting absorbance at full colour development and A corresponds to the concentration of the ion-pair complex present at equilibrium, A and A_m can be calculated from Fig. 3, where C is concentration of Bi(III) is equal to 1×10^{-4} M, the $\log K \pm SD$ ($n = 4$) of the studied drugs are: 5.85 ± 0.06 and 5.39 ± 0.11 for KC and TMH, respectively.

By using Job's method of non-equimolar solutions [48] (for KC as example) the curves obtained for a five-fold and a ten-fold excess of reagent (Fig. 4), gave a value of X_{\max} (Table 1); this value obtained by projecting the peak maximum onto the abscissa and dividing it by the total volume used in each case (10 mL). The conditional stability constant was then calculated from the equation:

$$K = \frac{(P-1)(1-2X_{\max})}{C_{\text{drug}}[(1-P)X_{\max}-1]^2}$$

Where $P = 5$ or 10 and $C_{\text{Bi(III)}} = 10^{-3} \text{ M}$. The values of $\log K$ are presented in Table 1. The values of $\log K$ from both methods are in good agreement (Table 1).

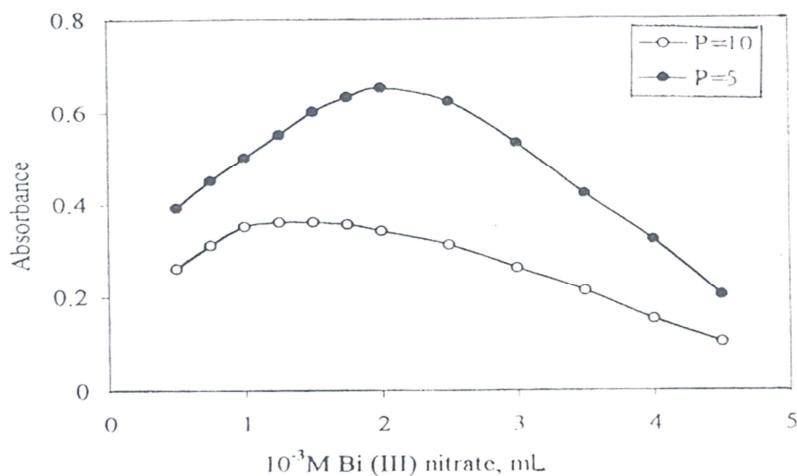


Fig. 4 Job's curve plots of non-equimolar solutions for KC-BiI₄-ion-pair in 1,2-dichloroethane. [Bi(III)] = 1x10⁻³ M, [KI] = 0.075 M, pH=2.5, λ =491 nm

Table 1 Conditional stability constant of the ketoconazole-BiI₄ ion pair

Sommer method*				
$\log K^{\circ}$	$\log K^{\circ}_{\min}$	$\log K^{\circ}_{\max}$	SD**	RSD (%)
5.85	5.78	5.91	0.06	1.02

Job's method of non-equimolar solution*

[Drug]	P	X _{max}	$\log K^{\circ}$
$2 \times 10^{-4} \text{ M}$	5	0.19	5.80
$1 \times 10^{-4} \text{ M}$	10	0.12	5.82
		Mean	5.81

*Conditions: pH = 2.5; [KI] = 0.075 M; temperature = 25 ± 0.5°C

** n = 4

Indirect AAS Determination of Drug

The reaction under investigation is suitable for indirect determination of KC and TMH by atomic absorption spectrometry (AAS). The drug associate with BiI₄ in 20% (v/v) DMF-water was directly aspirated and atomized in the instrument. The absorbance recorded was due to the bismuth that reacted with drug. The use DMF allows for best dissolution of the ion-pair and gives more sensitivity for the determination of drug at the microgram level by AAS.

Whereas, 1,2-dichloroethane is an unsuitable solvent for AAS determination due to smoky flame. The 20% (v/v) DMF-water is found to be suitable solvent mixture for this method.

Table 2 Analytical parameters for BiI₄ ion-pair formation with ketoconazole (KC) and trimetazidine hydrochloride (TMH).

Parameters	Specrophotometric method		AAS method	
	KC	TMH	KC	TMH
$\lambda_{\text{max}}(\text{nm})$	491	491	223.1 (Bilamp)	223.1
Beer's law limits ($\mu\text{g mL}^{-1}$)	5-40	5-45	8-60	6-50
Detection limit ($\mu\text{g mL}^{-1}$)	0.53	0.56	2.20	1.65
Quantitation limit ($\mu\text{g mL}^{-1}$)	1.62	1.68	6.67	5.00
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	1.17×10^4	6.46×10^3		
Sandell sensitivity ($\mu\text{g cm}^{-2}$)	0.046	0.053	0.337	0.221
Slope (b) ^a	0.0222	0.0190	0.0030	0.0046
Intercept (a) ^a	-0.014	-0.001	-0.0015	-0.0040
Correlation coefficient (r)	0.9999	1.0001	0.9996	0.9993
Relative standard deviation (%), n=7	0.36	0.32	0.20	0.23
Recovery=RSD(%)(n=15)	99.87±0.86	100.01±0.81	99.85±0.45	99.91±0.61
Intraday precision (n=15) ^b	0.73	0.55	0.63	0.69
Interday precision (n=15) ^b	0.65	0.79	0.58	0.71

^aRegression equation: $A = a + b C$, where C is the drug concentration ($\mu\text{g mL}^{-1}$) and A is the absorbance.

^bRelative standard deviation

Method validation

Method validation was carried out under International Conference on Harmonization (ICH) guidelines for validation of an analytical procedure [50]. The assay was validated with respect to linearity, accuracy, precision, specificity, LOD, LOQ, robustness and ruggedness.

Linearity

Under the experimental conditions described for drug determination, standard calibration curves for KC and TMH were constructed by plotting absorbance versus concentration of drug. Conformity with Beer's law was evident in the concentration range of the final dilution cited in Table 2. The molar absorptivity, Sandell sensitivity and the regression equation for each drug are tabulated in Table 2. The correlation coefficients (*r*) were between 0.9993 and 1.0001, indicating good linearity.

Accuracy

The previously mentioned methods under linearity were repeated three times for five different concentrations of pure samples. The concentrations were calculated from the regression equation; the recovery and RSD (%) were calculated and grouped in Table 2.

Precision

Precision is defined as the closeness or agreement between independent test results obtained under optimum conditions and is normally expressed as the RSD (%). To measure the degree of method repeatability (intraday precision), three different concentrations of drug were prepared and analyzed each five times within the same day and in five successive days (interday precision).

The results obtained in Table 2, show that no significant difference for the assay, which tested within day (repeatability) and between -day (reproducibility). The relative standard deviation (RSD) was less than 1% which indicates high degree of precision of the proposed methods.

Specificity

The specificity of the methods were investigated by observing any interference encountered from the common tablet excipients such as talc, lactose, glucose, sucrose, starch and, magnesium stearate. These excipients did not interfere with the proposed methods. This fact indicates good selectivity of the methods for determination of these drugs both in raw materials and in tablets.

Detection and quantitation limits

According to ICH recommendation [50] the approach based on the standard deviation (SD) of the response and the slope (b) of Beer's law was used for determination the limit of detection (LOD = $3.3 \times SD/b$) and limit of quantitation (LOQ = $10 \times SD/b$). The results are illustrated in Table 2.

Table 3 Determination of ketoconazole and trimetazidine hydrochloride in pharmaceutical preparations using spectrophotometric and AAS methods

Drug	Dosage form	% Recovery \pm SD ^a		Official Method [4, 41]
		Spectrophotometric method tric method	AAS method	
Ketoconazole	Nizoral tablets ^b (200 mg/tab.)	99.93 \pm 0.63 <i>t</i> = 0.34 <i>F</i> = 2.13	99.85 \pm 0.74 0.47 1.55	100.10 \pm 0.92 [4] (2.306)d (6.390)d
	Nizoral cream ^b (20 mg/g)	99.98 \pm 0.58 <i>t</i> = 0.25 <i>F</i> = 2.52	99.73 \pm 0.69 0.72 1.78	
	Trimetazidine hydrochloride	Vastarel tablets ^c (20 mg/tab.)	99.91 \pm 0.52 <i>t</i> = 0.17 <i>F</i> = 2.25	99.88 \pm 0.61 0.23 1.64
				99.98 \pm 0.78 [41]

^a Mean \pm standard deviations of five determinations.

^b Nizoral tablets or cream (Jansen Pharmaceutical, Beersel, Belgium).

^c Vastarel tablets (Servier Egypt Industries Limited, under Licence of Les laboratoires Servier-France)

^d Values of *t* and *F* in parentheses are the theoretical values at *p* = 0.05

Table 4 Recovery data obtained by standard addition method for ketoconazole and trimetazidine hydrochloride in pharmaceutical preparations using spectrophotometric and AAS methods

Drug	Dosage form	Taken ($\mu\text{g mL}^{-1}$)	Added ($\mu\text{g mL}^{-1}$)	Spectrophotometric method		AAS method	
				Found \pm SD ^a	Recovery \pm RSD%	Found \pm SD ^a	Recovery \pm RSD%
Ketoconazole	Nizoral tablets ^b (200 mg/tab.)	20	10	29.82 \pm 0.10	99.40 \pm 0.34	29.73 \pm 0.18	99.10 \pm 0.61
		20	15	35.05 \pm 0.18	100.14 \pm 0.51	34.80 \pm 0.23	99.40 \pm 0.66
		20	20	39.80 \pm 0.31	99.50 \pm 0.78	40.04 \pm 0.21	100.10 \pm 0.52
Trimetazidine hydrochloride	Nizoral cream ^b (20 mg/g)	20	10	30.03 \pm 0.21	100.10 \pm 0.70	29.91 \pm 0.16	99.70 \pm 0.53
		20	15	34.70 \pm 0.32	99.14 \pm 0.92	35.05 \pm 0.20	100.14 \pm 0.57
		20	20	40.02 \pm 0.27	100.05 \pm 0.67	39.87 \pm 0.33	99.68 \pm 0.83
	Vastarel tablets ^c (20 mg/tab.)	20	10	30.08 \pm 0.16	100.27 \pm 0.53	29.85 \pm 0.13	99.50 \pm 0.43
		20	15	34.78 \pm 0.19	99.37 \pm 0.54	35.06 \pm 0.27	100.17 \pm 0.77
		20	20	40.08 \pm 0.24	100.20 \pm 0.60	39.82 \pm 0.33	99.55 \pm 0.83

^a Mean \pm standard deviations of five determinations.

^b Nizoral tablets or cream (Jansen Pharmaceutical, Beersel, Belgium).

^c Vastarel tablets (Servier Egypt Industries Limited, under Licence of Les laboratoires Servier-France)

Robustness and ruggedness of the methods

The robustness of the spectrophotometric or atomic spectrometry method is demonstrated by the constancy of the absorption intensity with deliberated minor change in the experimental parameters such as the change in the volume of Bi(III)nitrate and KI (± 0.2 mL) and pH of solution (± 0.2). These minor changes that may take place during the experimental operation did not affect the absorption intensity of the reaction product, illustrating the robustness of the method.

The ruggedness of the proposed methods was evaluated by applying the developed method to the assay of KC or TMH using the same instrument by two different analysts under optimized conditions on different days. Since there were no significant differences between the results obtained by the two

analysts, the proposed methods may be considered rugged.

Analytical application

Both methods were further applied to the determination of the investigated drugs in their pharmaceutical preparations. The results were compared with those obtained by the reported methods [22, 30]. Statistical comparison for the results of the proposed and reported methods (Table 3) was performed with regard to accuracy and precision using the Student's-t and F-ratio tests. At 95% confidence level, the calculated t-and F-values do not exceed the theoretical values, indicating that there is no significant difference between the proposed and the reported methods with regard to accuracy and precision.

Recoveries tested were determined by adding standard drug to the pre-analyzed mixture of tablets or cream. Spiked drug tablets or cream assays were used to determine accuracy and precision of the proposed methods for determination of drug. The results of analysis of the commercial tablets or cream and the recovery study (standard addition) of drug (Table 4) suggested that there is no interference from any excipients, which are presented in tablets or cream.

CONCLUSION

The ion-pair complexes of BiL_4^- with drug formed under the above mentioned conditions and measured spectrophotometrically or AAS can offer a sensitive, simple, cheap and rapid procedure for the determination of KC and TMH in bulk, tablets and creams dosage forms, without fear of interferences caused by the excipients expected to be present in tablets or cream. From the calculation of stability constants of the ion-pair complexes, were found to be stable. The methods had been validated for the determination of these drugs. The statistical analysis of the results confirmed that the developed methods were accurate and precise and could be recommended for use in quality control labs.

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