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Triiodide ion and alizarin red S as two new reagents for the determination of clotrimazole and ketoconazole

Khalil Farhadi *, Ramin Maleki

Department of Chemistry, Urmia University, Urmia, Iran

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Abstract

The reactions of a triiodide ion and alizarin red S with two important antifungal drugs containing an imidazole ring (ketoconazole (KC) and clotrimazole (CT)) have been studied for the development of two simple, rapid, sensitive and accurate indirect titrimetric and extractive-spectrophotometric methods for determining the concentration of these drugs. Spectroscopic studies and chemical analysis showed that the protonated forms of KC and CT react with triiodide ion forming highly stable and insoluble ion-pair products such as $(KCH_2)(I_3)_2$ and $(CTH)I_3$. Formation of ion-association complexes have been applied to the development of an indirect visual titrimetric method for the determination of KC and CT over the range 10^{-5}–10^{-2} M. The extractive-spectrophotometric method is based on the formation of (1:1) ion-association complexes between drugs and alizarin red S as chromogenic reagent in acidic citrate buffer that are extractable into chloroform with an absorption maximum at 425 nm. The system obeyed Beer's law in the concentration range 2.5–50 and 2.7–80 μg ml^{-1} for CT and KC, respectively. The proposed methods were applied for the analysis of the studied drugs in pure forms and their commercial preparations. Results are in good agreement with those obtained by official methods.

Keywords: Ketoconazole; Clotrimazole; Alizarin red S; Triiodide; Titrimetry; Spectrophotometry

1. Introduction

Ketoconazole (KC), *cis*-1-acetyl-4-[4-2-(2,4-dichlorophenyl)-2-(1H-imidazole-1-yl methyl)-1,3-dioxolan-4-yl] methoxy piperazine, is widely used as a typical antifungal drug in the treatment of tinea infections [1]. KC is a potent inhibitor of cytochrome P-450-dependent steroid hydroxylation in the adrenals [2]. In addition, KC enhances microsomal epoxid hydrolase activity with some epoxide substrates [3]. Clotrimazole (CT), 1-[(2-chlorophenyl) diphenyl methyl]-1H-imidazole, is an imidazole antifungal agent with similar action and activity to KC [4]. Most clinical studies indicate that systemic CT has little efficacy and considerable toxicity compared to other systemic imidazoles (miconazole and KC) [4]. Thus, due to the vital importance of determination of these drugs in pharmaceutical preparations and biological fluids, several spectroscopic [5–12], high per-

* Corresponding author. Fax: +98-441-3443442
E-mail address: kh.farhadi@mail.urmia.ac.ir (K. Farhadi).

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formance liquid chromatographic [13–17] and electrochemical [18–23] methods for assay of KC and also, various methods such as derivative spectrophotometry [24,25], colorimetry [5,6,26,27], HPLC [28–30], electrochemistry [18] and differential scanning calorimetry (DSC) [31] have been reported for the determination of CT in the literature. The official method normally involves titration in non-aqueous solvent [32,33]. To the best of our knowledge, despite the important ion-association complex formation and extractive-spectrophotometric methods, there are no previous reports based on the reaction of KC and CT with alizarin red S (ARS) and triiodide ion as ion pairing reagents.

We have recently studied the electrochemical behavior and chemical properties of various antifungal drugs containing piperazine or imidazole groups such as KC in aqueous and non-aqueous media [21–23]. In this study, we reported two simple, rapid, precise, accurate and inexpensive methods for the determination of KC and CT in pharmaceutical preparations. The indirect titrimetric method (method A) is based on the formation of stable and insoluble precipitated ion-pair complex between the protonated form of the drugs and triiodide ion in an acidic solution. Therefore, for quantitative purposes, a known excess triiodide ion was added to solution containing drugs and then the amount of unreacted triiodide ion was titrated with standardized sodium thiosulfate solution. The second method (method B) involves an extractive-spectrophotometric assay of KC and CT based on the formation of chloroform-soluble ion-association complexes between the protonated forms of drugs with ARS. The compositions of the complexes formed in two proposed methods have been established and after optimizing various analytical parameters, the obtained results were compared with those of official methods.

2. Experimental

2.1. Reagents

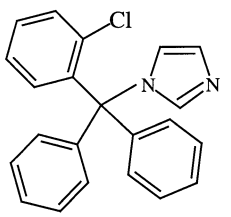
All chemicals used in this study were of highest purity available (Merck) and used without further purification. Doubly distilled deionized water was used throughout. The starch, thiosulfate and triiodide solutions were freshly prepared and standardized [34]. Reagent grade KC and its 200 mg tablet as well as 2% cream samples were obtained from Behvazan Pharmaceutical Company (Rasht, Iran). Jaber Ebne Hayyan Pharmaceutical Company (Tehran, Iran) supplied reagent grade CT, 100 mg vaginal tablet, 1% cream and 1% lotion samples.

2.2. Apparatus

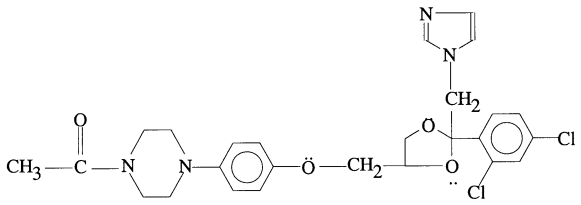
An LKB model 4054-UV recording spectrophotometer equipped with 10 mm matched quartz cells was used for spectral measurements. The pH-values were determined with a WTW mutilab 540 Ionalyzer (Germany) using a combined electrode. The infrared absorption spectra were recorded using a Shimadzu model IR-460. The ^{1}H NMR spectra were recorded by JEOL model EX 90A.

2.3. Study of reaction between triiodide ions and selected antifungal drugs (KC and CT)

KC has two basic piperazine and imidazole rings, while only an imidazole basic group exists in the chemical structure of CT.



Clotrimazole (CT)



Ketoconazole (KC)

Preliminary experiments showed that the proto-

nated forms of these compounds are immediately precipitated in the presence of excess I_3^- ions. The obtained results from the determination of unreacted triiodide ions in filtrate solutions clearly indicated that each mole of KC reacts with two moles of I_3^- ions as $(KCH_2)(I_3)_2$ ion-pair, while an ion-pair with a ratio 1:1 is formed as $(CTH)(I_3)$ in the case of CT. The possible processes occurred could be summarized according to the following reactions:

$$KC + 2H^+ \rightleftharpoons (KCH_2)^{2+}_{(aq)} \quad (1)$$

$$(KCH_2)^{2+}\ (aq) + 2I^-_{3\ (aq)} \rightleftharpoons (KCH_2)(I_3)_{2(s)} \quad (2)$$

and

$$CT + H^+ \rightleftharpoons CTH^+_{(aq)} \quad (3)$$

$$CTH^+\ (aq) + I^-_{3\ (aq)} \rightleftharpoons (CTH)(I_3)_{(s)} \quad (4)$$

where $(KCH_2)^{2+}$ and CTH^+ are protonated forms of KC and CT, respectively.

The molecular absorption spectroscopy was used to confirm the presence of triiodide ion in the structure of formed ion pairs. The obtained results from the UV–visible spectra of the chloroform solutions of KC, CT and corresponding triiodide ion pairs showed that, while KC and CT are colorless and have no absorption in visible region, the ion pairs are colored solutions possessing two sharp peaks at 292 and 364 nm, which are the characteristic of triiodide (free or solvent separated) ion [35,36].

In further investigations, the amount of triiodide ion in the structure of the chloroform-soluble ion pairs was determined potentiometrically using a standardized chloroform solution of tetrabutylammonium periodate (TBAP) [37]. The obtained results completely confirmed the compositions proposed in Eqs. (2) and (4).

The H NMR spectra of the two obtained ion pairs exhibit a sharp peak in acidic region ($\delta = 9$ versus TMS), probably due to pyridine-type proton of imidazole ring. Moreover, the appearance of multiple broad peaks in the range $\delta = 5.5$–7.0 in the spectrum of $(KCH_2)(I_3)_2$ confirmed that the nitrogen of piperazine ring attached to methoxy residue in KC can be considered as a suitable site for second protonation process.

The presence of KC and CT as protonated forms in the structure of ion-association complexes was examined by IR spectroscopy (KBr disks). Infrared spectrum of $(KCH_2)(I_3)_2$ displayed a set of strong and broad N–H stretching vibration bands in the range 2700–2250 cm^{-1}, which is the characteristic of tertiary amine salts [38]. Furthermore, no considerable shift in stretching vibration band of C=O amide type in $(KCH_2)(I_3)_2$ in comparison to KC, once more confirmed that the nitrogen of piperazine ring attached to methoxy moiety was protonated in the ion-pair. It is quite clear that only possibility for the protonation of CT is pyridine-type nitrogen of imidazole ring.

2.4. Study of reaction between ARS and selected antifungal drugs (KC and CT)

Fig. 1 shows the electronic absorption spectra of KC (A) in chloroform and the extracted chloroform solution of KC in the presence of excess amount of acidic ARS solution (B). As it is seen, the overall feature of the resulting KC–ARS adducts is quite different from that of KC. The wavelengths of maximum absorptions observed are 290 and 425 nm for KC–ARS complex formed and 260 nm for KC. A large shift in maximum absorption of KC in the presence of ARS towards longer wavelengths illustrates a strong interaction between them.

It should be mentioned that ARS was not extracted into chloroform in the absence of KC even from high acidic solutions. These observations confirmed that ARS as an anionic reagent can only be extracted into chloroform as ion-associated complex in the presence of lipophilic protonated KC. Similar results were obtained from the interaction between CT and ARS. This interpretation is in good agreement with the previous results of the examination of KC and CT with other substances [7,10].

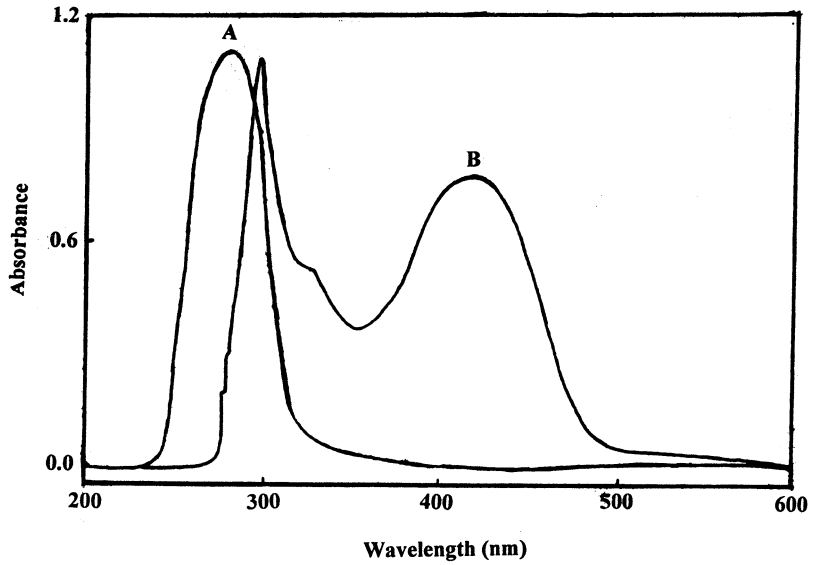
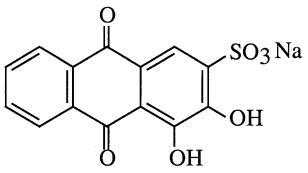


Fig. 1. UV–Vis spectra of 10^{-4} M KC (A) and KC in the presence of excess amount of Alizarin red S (B) in chloroform.



Alizarin Red S (ARS)

The composition of the formed complexes determined by Job's method indicates a 1:1 complex between the drug and ARS in chloroform (Fig. 2). It may be expected, in both drugs, the ion-pair formation is based on the interaction between protonated form of the pyridine-type nitrogen of the imidazole ring and ARS.

2.5. General procedure

2.5.1. Indirect titrimetric method (method A)

To a 10 ml aliquot of 10^{-4} M aqueous solution of each drug containing a few drops of HCl 1 M (pH 2), 5 ml of 0.01 M triiodide solution was slowly added with continuous stirring until precipitation was completed. The resulting ion-pair precipitate was separated by filtration and washed thoroughly with deionized water. The unreacted triiodide ions were titrated with standardized solution of 0.01 M sodium thiosulfate in the presence of 1 ml of 0.2% (w/v) starch solution.

2.5.2. Extractive-spectrophotometric method (method B)

Two milliliters of standard or sample solutions containing an appropriate amount of KC or CT was pipetted into a 50 ml separatory funnel containing 6 ml of ARS (5×10^{-4} M) and 2 ml of 0.05 M citrate buffer (pH 2) and the solution was mixed well. Then, two 5 ml portions of chloroform were added and the solution was shaken for 5 min. The solution was allowed to stand for clear separation of the two phases. The absorbance of chloroform layer was measured against a reagent blank at 425 nm.

2.5.3. Tablet sample solution

Twenty tablets of each drug after determining the average weight were powdered. Powder of each drug equivalent to 25 mg was accurately weighed and shaken with water containing a few drops of HCl (pH 2) and the volume was made up to 100 ml in a volumetric flask, then, the recommended procedures for the determination of KC and CT (method A or B) were followed.

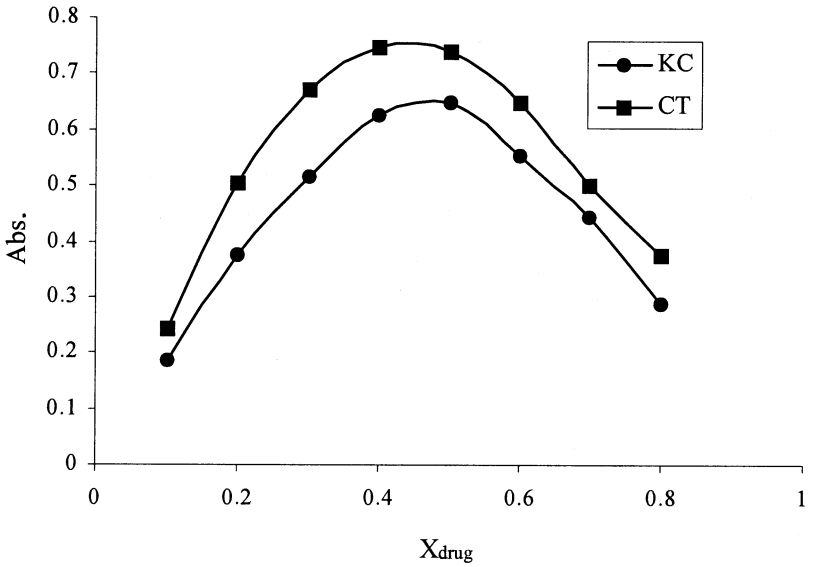


Fig. 2. Continuous variations plots for KC–ARS (●) and CT–ARS (■) systems in chloroform measured at room temperature and at $\lambda = 425$ nm. Total concentration of drug+ARS is 5×10^{-4} M.

2.5.4. Cream or lotion sample solution

An accurately weighed portion of the cream or typical solution samples equivalent to 25 mg of the drug was shaken in acidic medium until they were completely dissolved and then filtered. The resulting clear solution was diluted to 100 ml with distilled water and recommended procedures (method A or B) were performed. The standard addition method was also employed for the accurate determination of the drug contents.

3. Results and discussion

3.1. Determination of KC and CT

3.1.1. Method A

Due to the simple stoichiometry of the occurred reactions dealing with the formation of ion pairs and also, high stability and insolubility of obtained precipitate in aqueous media, we were interested in developing a new simple indirect titrimetric method for the quantitative determination of KC and CT in pharmaceutical preparations. Therefore, we used I_3^- ion as a suitable reagent for the precipitation of the protonated forms of KC and CT. Thus, a known excess I_3^- ion was added to an acidic solution of KC or CT and then, the unreacted I_3^- ions were determined using a standardized solution of sodium thiosulfate in the presence of starch solution.

The effect of nature of acids used and the optimum acidic pH for the quantitative precipitation of KC and CT with I_3^- ions were studied over the pH range 1.0–4.0. The recovery values of KC and CT using proposed indirect titration method versus pH of solution are shown in Fig. 3. As can be seen, at pH range 1.5–2.5 for KC and 1.5–2.0 for CT, the recovery values reach maximum; therefore, these ranges were selected as the optimum pH range for quantitative studies. For pH > 2.5 (KC) and pH > 2.0 (CT), the decrease in recovery values was possibly due to the decreased concentration of protonated form of drugs. At pH below 1.5, a slight decrease in recovery is probably due to the interference of the H_3O^+ on triiodide ions resulting in diminished activity of I_3^- requirement for the complete precipitation of drugs in solution. Among acetic, nitric, sulfuric and hydrochloric acids used for pH adjustment, the results obtained by using HCl were very reproducible.

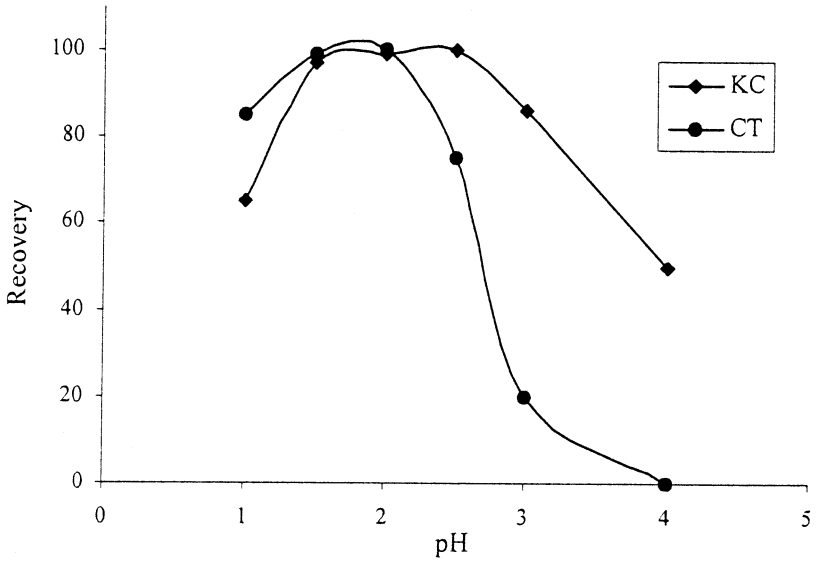


Fig. 3. Effect of pH of solution on the recovery values of 5×10^{-4} M KC (■) and 5×10^{-4} M CT (●) measured using method A.

Assays of KC and CT in pure form using the proposed method and the official method [32,33] were carried out and the results are summarized in Table 1. The official method for the determination of antifungal drugs containing imidazole rings is based on an acid–base potentiometric titration using a solution of standardized anhydrous perchloric acid in glacial acetic acid. The recovery and relative standard deviation results presented in Table 1 clearly indicate a good agreement between the KC and CT contents determined by the proposed and official methods. This was confirmed by statistical analysis based on the variance ratio test (F-test) and Student's t-test (see Table 1).

3.1.2. Method B

Ion-pair complex extractive-spectrophotometry has been frequently used for the quantitative analysis of many pharmaceutical compounds [39]. In this study, we used ARS as a very suitable anionic dye, to form an intense yellow color ion-pair complex with KC and CT, in acidic pH, which is soluble in chloroform and can be measured at 425 nm. In preliminary experiments, a number of immiscible organic solvents were examined in order to provide an applicable extraction procedure. Table 2 shows the absorbance of ion-pair complexes of KC and CT in organic solvents.

Table 1
Determination of pure KC and CT by method A and the official method

Drug	Taken (mg)	Method A			Official method			F[a]	t[a]
		Found[b]	Recovery (%)	RSD (%)	Found[b]	Recovery (%)	RSD (%)		
KC	10	9.9	99.0	2.0	10.1	101	1.9	1.09	1.62
	50	49.6	99.2	2.4	49.9	99.8	2.2	1.18	0.42
CT	10	9.7	97.0	3.1	9.8	98.0	3.0	1.05	0.53
	50	48.8	97.6	2.5	49.3	98.6	2.1	1.39	0.69

[a] Theoretical values of F and t are 6.39 and 2.31 respectively, at 95% confidence level.
[b] Means of five separate determinations.

Table 2
Effect of different organic solvents on the extraction efficiency of CT–ARS ion-pair complex

Solvent	Absorbance[a]	
	CT	KC
Chloroform	0.550	0.235
Methylene chloride	0.618	0.319
Carbon tetrachloride	0.063	0.082
Toluene	0.122	0.058
Benzene	0.137	0.052

[a] Final concentration of CT and KC = 1×10^{-4} M.

Despite the higher molar absorptivity of the complexes in methylene chloride, chloroform was used as an organic phase throughout this work, mainly due to the lower volatility in comparison with methylene chloride. It should be mentioned that the colored ion-pair complex in the chloroform phase is quite stable for several days.

The effect of pH for the quantitative extraction of KC and CT with ARS in chloroform was studied over the range 1–5, as it was established by using a proper citrate buffer. The obtained results for KC and CT are shown in Fig. 4. The resulting data at higher pH values show that the extent of ion-pair extraction decreases drastically, most probably due to the decreased protonated form of drugs. At pH below 1.5, the decrease of ion-pair extraction may be due to the interference of the H_3O^+ on ARS, resulting in a diminished complexation power. Thus, pH 2 was chosen for further studies. Moreover, the optimum volume of citrate buffer (0.1 M) at pH 2 was investigated and 2 ml of citrate buffer was used for further studies.

The influence of the volume of the ARS (4×10^{-4} M) solution on the extraction of KC and CT was studied. The obtained results for CT and KC are shown in Fig. 5. As seen, the absorbance of ion-pair complexes in organic phase increases with increasing the amount of ARS in aqueous phase. Maximum extraction of ion-pair complexes occurs when the volume of reagent reaches 6 ml. A further excess of the reagent has no considerable effect on the fraction of the complex extracted.

The optimum volume of the organic phase and the number of extractions required were also studied. Maximum absorbance values were ob-

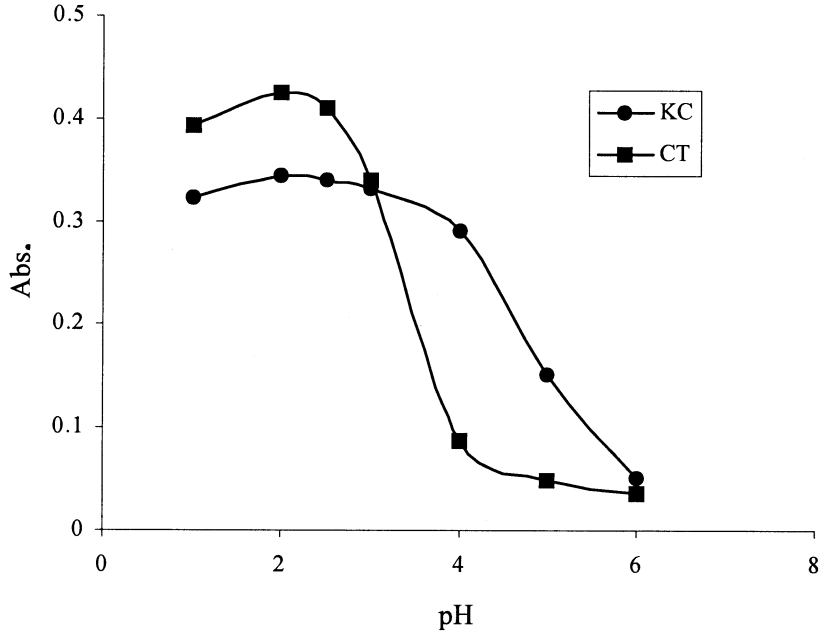


Fig. 4. Effect of pH of solution on the extraction of 0.35 mmol KC (●) and 0.35 mmol CT (■) using 6 ml of 5×10^{-4} M ARS as ion-pair complex adducts into 10 ml chloroform.

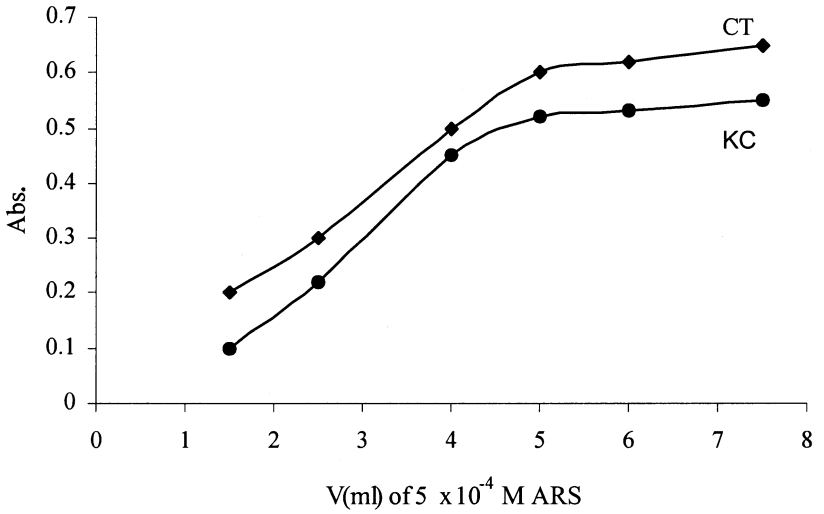


Fig. 5. Effect of volume of ARS (5×10^{-4} M) on the extraction of 0.5 mmol CT and 0.5 mmol KC at pH 2 (citrate buffer) into 10 ml chloroform.

tained by using 10 ml of chloroform during two portions of 5 ml extraction. In addition, the extraction of ion-pair complexes with ARS under the recommended conditions was found to be rapid. A shaking time of 3–5 min is sufficient for the complete extraction of the resulting 1:1 ion-pair complexes.

After the optimization of all the variables, Beer's law was obeyed over the concentration range 2.5–80 μg ml^{-1} for KC and 2.0–50 μg ml^{-1} for CT at 425 nm. The following equations correspond to the linear ranges for KC and CT at pH 2:

$$\text{KC}: \quad A = 0.0099C \ (\text{mg ml}^{-1}) + 0.1044, \quad r = 0.999, \quad n = 6,$$

$$\text{CT}: \quad A = 0.0129C \ (\text{mg ml}^{-1}) + 0.0114, \quad r = 0.999, \quad n = 6.$$

The detection limit, calculated following the expression $a+3S_{xy}$ [40], where a = intercept and S_{xy} = error standard deviation, was 1.6 μg ml^{-1} for KC and 1.4 μg ml^{-1} for CT.

3.1.3. Application to dosage forms

In order to establish the validity of the proposed analytical methods, the assay of KC and CT in their formulations were carried out on proprietary drugs. The same samples were analyzed by the official non-aqueous potentiometric method (Table 3) [32,33]. A sample potentiometric curve for the titration of CT lotion dissolved in glacial acetic acid with standardized perchloric acid is given in Fig. 6. It should be noted that, in performing of official methods on CT tablet and cream samples, at first step, the drug was completely extracted into chloroform and after evaporation of the solvent, the residue dissolved in glacial acetic acid and then, titrated with perchloric acid. On the other hand, the reaction medium in non-aqueous titrimetry must be scrupulously anhydrous and in practice even a trace amount of water will affect the results. Therefore, due to absence of such errors in proposed procedures, convenient and fast performing of method A in aqueous media using inexpensive reagents, and selective extraction of drugs into chloroform and direct application of spectrophotometry in wide concentration range on extracts by method B suggest their application to the analyses and quality control of the studied antifungal compounds in comparison to tedious time-consuming official methods.

The recovery experiments shown in Table 3 indicated a good agreement between the KC and CT contents determined by the proposed and

Table 3
Results of assay of studied drugs in pharmaceutical formulations by the proposed and official methods

Formulation	Labeled	Official method (%recovery ± S.D.)[a]	Method A			Method B		
			%Recovery ± S.D.[a]	t[b]	F[b]	%Recovery ± S.D.[a]	t[b]	F[b]
Tablet (KC)	200 (mg per tablet)	101.0 ± 0.6	100.5 ± 1.0	0.97	2.36	100.0 ± 1.2	1.68	4.00
Cream (KC)	2 (wt.% drug)	99.5 ± 1.0	101.0 ± 1.2	1.94	1.44	100.5 ± 1.2	1.30	1.44
Tablet (CT)	100 (mg per tablet)	102.0 ± 0.9	101.0 ± 1.4	1.34	2.42	101.5 ± 0.8	1.10	1.26
Cream (CT)	1 (wt.% drug)	102.8 ± 1.1	102.0 ± 1.2	0.95	1.19	102.2 ± 1.1	1.51	1.00
Lotion (CT)	1 (wt.% drug)	99.5 ± 0.7	100.1 ± 0.9	1.19	2.02	99.8 ± 0.8	0.84	1.31

[a] Average of five determinations ± S.D.
[b] Theoretical values of F and t are 6.39 and 2.31 respectively, at 95% confidence level.

official methods as well as the declared amount of drug in the preparations used. This is indicative of non-interference of the other ingredients and the excipients, which are present in the formulations. In addition, the obtained results show comparable accuracy (t-test) and precision (F-test) since the calculated values of t and F are less than the theoretical values.

We have also evaluated the accuracy of the proposed methods by performing experiments on

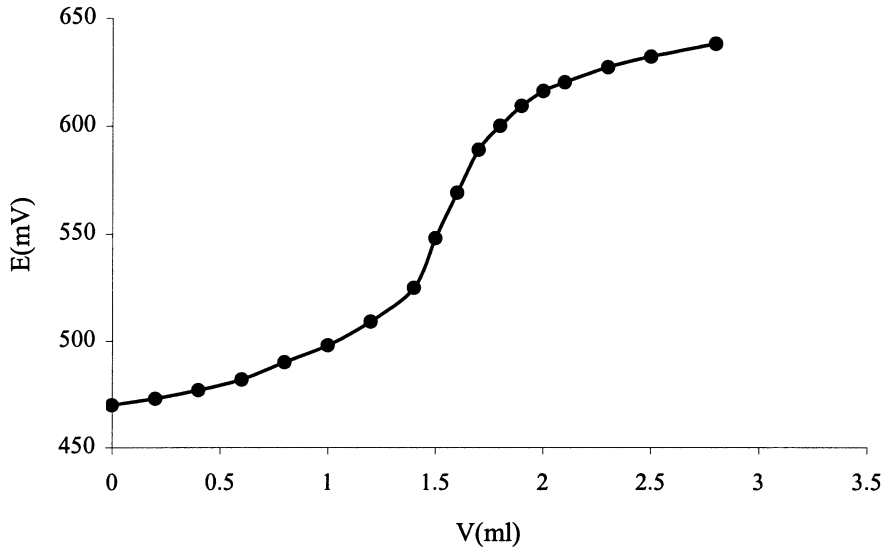


Fig. 6. Potentiometric titration of CT lotion sample (equivalent to 52 mg of the drug) dissolved in 20 ml glacial acetic acid with standardized 0.1 M perchloric acid.

the samples prepared from dosage forms and pure drugs. The percent recoveries obtained from three replicate measurements were found to be 100.5 and 101.0% for methods A and B, respectively. The relative standard deviations obtained from different samples were between 0.8 and 2.1%, reflecting repeatability and precision of the results.

4. Conclusions

This paper described the two methods for the determination of KC and CT in pure forms and pharmaceutical formulations. In terms of simplicity and expense, the proposed indirect titration method (method A) could be considered superior to the methods reported previously, especially with those based on chromatography and potentiometric titration in non-aqueous media. This method is suitable for the routine analysis of common antifungal drugs (e.g. CT and KC) in various commercial formulations (cream, tablet or lotion) of various amounts of drugs ranging from 10^{-5} to 10^{-2} M without any interference. On the other hand, a significant advantage of an extractive-spectrophotometric determination (method B) is that it can be applied to the determination of particular component in a complex dosage formulation, which is of major interest in analytical pharmacy. These advantages encourage its application in the analysis and quality control of these drugs in their dosage forms and in drug control laboratories without interferences of any ingredients.

Acknowledgements

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