Asian Journal of Chemistry Vol. 14, No. 2 (2002), 771–776

Role of Alkaloidal Precipitants for the Assay of Itraconazole in Capsules

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Four simple spectrophotometric methods (A–D) for the assay of itraconazole (ICZ), based on its ability to form water-insoluble complexes, are described. These methods involve the quantitative precipitation of ICZ with phosphomolybdic acid (PMA, method A), ammonium molybdate (AM, method B), iodine (I_2, method C) and tannic acid (TA, method D), followed by the second step of procedure, estimation of either released precipitant from the adduct (precipitate) through acetone addition (methods A, B or D) or the unreacted precipitant in the filtrate (method C) with the chromogenic reagents such as Co(II)-EDTA (for PMA), potassium thiocyanate (PTC, for AM), *p*-N-methyl aminophenol sulphate—sulphanilamide (PMAP-SA, for I_2) and PMAP-Cr(VI) (for TA). These methods obey Beer's law and give reproducible results. 'I'he percentage recoveries are 99.67%.

Key Words: Alkaloidal, Precipitants, Assay, Itraconazole.

INTRODUCTION

Itraconazole (ICZ), chemically known as 4-[-4-[4-[4-[(*cis*-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl)methoxy] phenyl] piperazin-1-yl] phenyl]-2-[(IRS)-1-methylpropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one, is an orally active antimycotic and potent inhibitor of human fungal pathogens. It is official in BP[1] and EP[2]. Few physico-chemical methods based on the techniques such as HPLC[3–8], NMR[9] and visible spectroscopy[10] appeared in the literature. In the present paper, we describe four visible spectrophotometric methods based on the precipitation of ICZ with the reagents (PMA, method A; AM, method B; I_2, method C and TA, method D) in the first step when used in excess. The second step was based on the color development reaction with either released precipitant from the precipitate with acetone (PMA, AM and TA) or unreacted precipitant in the filtrate (I_2) with chromogenic reagents such as

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Co(II)-EDTA (for PMA), potassium thiocyanate (for AM), PMAP-SA (for I_2) and PMAP- Cr (VI) (for TA).

EXPERIMENTAL

A Milton Roy Spectronic 1201 and Systronics 106 digital spectrophotometer with 1 cm matched quartz cells were used for spectral and absorbance measurements. An Elico LI-120 digital pH-meter was used for pH measurements.

All the chemicals and reagents used were of analytical grade and the solutions were prepared freshly. Aqueous solutions of PMA (Rea Chem, 1.094×10^{-2} M), cobalt nitrate (BDH, 1.03×10^{-1} M), EDTA disodium salt (S.D. Fine, 1.07×10^{-1} M), for method A; AM (E. Merck, 5.1×10^{-2} M), PTC (Ranbaxy, 5.14×10^{-4} M), for method B; I_2 (E. Merck, 3.93×10^{-3} M), PMAP (Loba, 8.71×10^{-3} M), SA (S.D. Fine, 1.16×10^{-2} M), phthalate buffer (pH 3.0) for method C and TA (Loba, 1.17×10^{-3} M), PMAP (Loba, 8.71×10^{-3} M), Cr(VI) (BDH, 1.01×10^{-2} M $K_2Cr_2O_7$), phthalate buffer (pH 3.0) for method D were prepared in triple distilled water.

Standard and sample drug solutions

A 1 mg/mL solution was prepared by dissolving 100 mg of pure ICZ or capsule powder equivalent, in 100 mL of dimethyl sulphoxide (DMSO) and used as it is, for methods A, C, D and further diluted to 500 μg/mL with same solvent for method B.

Recommended procedures

Method A: Aliquots of standard ICZ solution (0.2–1.2 mL, 1 mg/mL) were delivered into a series of centrifuge tubes containing 0.25 mL of conc. HCl and the volume in each tube was adjusted to 1.5 mL with DMSO. Then 4 mL of phosphomolybdic acid was added and centrifuged for 5 min. The precipitate was collected through filteration, followed by washing with distilled water (3 × 1 mL) until it was free from reagent. The precipitate in each tube was dissolved in 5 mL of acetone and transferred into a 25 mL graduated test tube. 1 mL each of cobalt nitrate and EDTA solutions were successively added and the tubes were heated for 10 min at 60–70°C. The test tubes were cooled and the solution in each tube was made up to the mark with distilled water. The absorbance was measured at 840 nm against a similar reagent blank. The amount of ICZ was calculated from Beer's law plot.

Method B: Aliquots of standard ICZ solution (0.25–1.5 mL, 500 μg/mL) were delivered into a series of centrifuge tubes containing 0.25 mL of conc. HCl and the volume in each tube was adjusted to 1.5 mL with DMSO. Then 1 mL of ammonium molybdate solution was added and centrifuged for 5 min. The precipitate was collected through filtration followed by washing with 50% aqueous alcohol (3 × 1 mL) until it was free from the reagent. The precipitate of each tube was dissolved in 5 mL of acetone and transferred into a 25 mL graduated tube. 3 mL each of conc. HCl and potassium thiocyanate solutions were successively added and the tubes were heated for 10 min at 60–70°C. The test

tubes were cooled and the solution in each tube was made up to the mark with distilled water. The absorbance was measured at 480 nm against a similar reagent blank. The amount of ICZ was calculated from Beer's law plot.

Method C: Aliquots of standard ICZ solution (0.25–1.5, 1 mg/mL) were delivered into a series of centrifuge tubes containing 1.0 mL of 0.1 M HCl and the volume in each tube was adjusted to 3.0 mL with DMSO. Then 1.5 mL of iodine solution was added and centrifuged for 5 min. The precipitate was collected through filtration and subsequently washed with distilled water (2 × 1 mL). The filtrate and washings were collected in a 25 mL graduated test tube. Then 15 mL of pH 3.0 buffer and 1.5 mL of PMAP solution were successively added. After 2 min, 2.0 mL of SA solution was added. The absorbance was measured after 10 min at 520 nm against distilled water. A blank experiment was also carried out without the drug. The decrease in absorbance and in turn the drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of ICZ was calculated from Beer's law plot.

Method D: Aliquots ot standard ICZ solution (0.2–1.2 mL, 1 mg/mL) were delivered into a series of centrifuge tubes containing 0.25 mL of conc. HCl and the volume in each tube was adjusted to 1.5 mL with DMSO. Then 1.0 mL of tannic acid solution was added and centrifuged for 5 min. The precipitate was collected through filtration, followed by washing it with distilled water (2 × 1 mL) until it was free trom the reagent. The precipitate in each tube was dissolved in 5 mL of acetone and transferred into a series of 25 mL graduated test tubes. Then 15 mL of pH 3.0 buffer and 1.5 mL of PMAP solutions were successively added. After 2 min, 2.0 mL of Cr(VI) solution was added and the volume was made up to the mark with distilled water. The absorbance was measured after 5 min at 580 nm against a similar reagent blank. The amount of ICZ was calculated from Beer's law plot.

RESULTS AND DISCUSSION

In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were undertaken by varying one parameter at a time and controlling all other parameters to get maximum colour development, minimum blank colour, reproducibility and reasonable period of stability of final coloured species formed. The conditions so obtained were incorporated in the recommended procedures. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity are given in Table-1. The precisions of the methods were found by measuring absorbances of six replicate samples containing known amounts of drug and the results obtained are given in Table-1. Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and are presented in Table-1 .

The accuracy of the methods was ascertained by comparing the results[11] obtained for pharmaceutical formulations by proposed methods and reference method (UV, developed in the laboratory using ICZ solution in dichloromethane, λ_{max} 282 nm) statistically by the t- and F-tests and the results are summarized in

Table-2. The results of the recovery experiments by the proposed methods are also listed in Table-2. Recoveries were determined by adding standard drug to the pre-analysed pharmaceutical formulations. The ingredients usually present in pharmaceutical formulations did not interfere in the proposed methods.

TABLE 1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY

Parameters	Method A (PMA/Co(II)-EDTA)	Method B (AM/PTC)	Method C (I_2/PMAP)	Method D TA/PMAP-Cr(VI)
Beer's law limits ($\mu g\ mL^{-1}$)	8.0–48.0	5–30	10–60	8–48
Molar absorptivity (L mole cm^{-1})	7.413×10^3	1.384×10^4	5.295×10^3	6.596×10^3
Sandell's sensitivity ($\mu g\ cm^{-2}$/0.001 absorbance unit)	0.0952	0.051	0.1333	0.1081
Slope (b)	1.05×10^{-2}	1.948×10^{-2}	5.966×10^{-3}	9.396×10^{-3}
Intercept (a)	-4.0×10^{-4}	1.267×10^{-3}	6.29×10^{-2}	2.667×10^{-4}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Relative standard deviation (%)*	0.6840	0.5510	0.6230	1.0260
% Range of error (confidence limits)*				
0.05 level	0.7170	0.5780	0.6540	1.0770
0.01 level	1.1250	0.9060	1.0250	1.6900

*Six replicate samples.

Colored species formation

All the four methods involve two steps. First step is the quantitative precipitation of ICZ. The precipitation[12] is ascribed to the formation of a molecular complex resulting from the interaction of the unshared pair of electrons on nitrogen in heterocyclic moiety of ICZ with an unoccupied molecular orbital of the precipitant molecule (PMA, AM, I2 or TA). The second step varies in each method. In method A, the PMA released from the adduct with acetone on reduction by Co(II)-EDTA forms molybdenum blue. In method B, the molybdate released from the precipitate with acetone, when treated with PTC in strong acid medium produces orange-yellow coloured complex $[Mo[NCS]^{3-}]$.[13] In method C, the unreacted I_2 in the filtrate oxidises PMAP to produce highly reactive *in situ* *p*-N-methyl benzoquinone monoimine (PMBQMI), which in turn involves in coloured charge-transfer complex formation with SA[14]. In method D, the TA released from adduct with acetone forms coloured charge-transfer complex with PMBQMI (formed *in situ* from PMAP-Cr(VI)).[15]

The results indicate that the proposed methods are simple, sensitive and can be used for analysis, with speed at low cost without losing accuracy; suitable for the estimation of pharmaceutical dosage forms for ICZ.

TABLE 2
ASSAY OF ICZ IN PHARMACEUTICAL FORMULATIONS

Sample*	Labelled amount (mg)	Amount found by proposed methods†				Amount found by reference methods	% Recovery by proposed method‡			
		A	B	C	D		A	B	C	D
Capsules I	100	99.83 ± 0.68 t = 0.21 F = 1.32	99.69 ± 0.46 t = 0.15 F = 2.93	99.85 ± 0.38 t = 0.18 F = 4.32	100.22 ± 0.39 t = 0.99 F = 4.01	99.76 ± 0.78	99.83	99.69	99.85	100.22
Capsules II	100	100.25 ± 0.43 t = 1.03 F = 4.41	100.40 ± 0.53 t = 0.16 F = 2.94	100.27 ± 0.48 t = 0.10 F = 2.94	99.76 ± 0.45 t = 1.44 F = 4.16	100.33 ± 0.91	100.25	100.40	100.27	99.76
Capsules III	100	100.28 ± 0.67 t = 0.28 F = 1.26	100.12 ± 0.29 t = 0.67 F = 4.27	100.06 ± 0.42 t = 0.71 F = 2.08	100.13 ± 0.41 t = 0.66 F = 2.15	100.42 ± 0.60	100.25	100.40	100.27	99.76
Capsules IV	100	100.13 ± 0.46 t = 0.79 F = 1.80	100.12 ± 0.39 t = 0.84 F = 2.50	100.16 ± 0.38 t = 1.63 F = 2.66	100.42 ± 0.56 t = 1.89 F = 1.23	99.64 ± 0.62	100.13	100.12	100.16	100.42

*Two different batches of capsules from two different pharmaceutical companies.

†Average ± standard deviation of six determinations, the t- and F-test values refer to the comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, t = 2.57, F = 5.05.

‡After adding 10 mg pure drug to the pre-analysed pharmaceutical formulations, each value is an average of three determinations.

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(*Received:* 19 November 2001; *Accepted:* 5 Feoruary 2002) AJC-2585