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A stability-indicating LC method for the assay estimation of itraconazole in pharmaceutical dosage form

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ABSTRACT

A stability- indicating HPLC method was developed for the quantitative determination of itraconazole in pharmaceutical dosage forms in the presence of degradation products. It involved a Hypersil BDS 100 mm × 4.6 mm, 3µm C18 column. The separation was achieved on simple isocratic method. The mobile phase contains a mixture of pH 7.5, 20mM K_2HPO_4 buffer: acetonitrile: tetrahydrofuran (50:49:12, *v/v/v*). The flow rate was 1.2 mL min^{-1} and the detection wavelength was 225 nm. The retention time of itraconazole is 3.3 min. The total runtime was 4 min within which drug and degradation products were separated. Itraconazole was subjected to different ICH prescribed stress conditions. Degradation was found to occur in oxidative condition, while drug was stable to hydrolytic, photolytic and thermal stress. The drug was particularly labile under oxidative stress condition. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The method developed was successfully applied to the determination of itraconazole in pharmaceutical preparations. The developed RP-HPLC method was validated with respect to linearity, accuracy, precision and ruggedness. © 2009 Trade Science Inc. - INDIA

KEYWORDS

Itraconazole;
Stability- indicating;
HPLC;
Validation.

INTRODUCTION

Present drug stability test guidance Q1A (R2) issued by international conference on harmonization (ICH)[1] suggest that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated.

Accordingly, the aim of the present study was to establish inherent stability of itraconazole through stress studies under a variety of ICH recommended test conditions[1-3] and to develop a stability-indicating assay[4-6].

Itraconazole (4-[4-[4-[4-[[cis-2-(2,4-dichloro phenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(1RS)-1-methylpropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one) is an antifungal medication which is available in 100 mg and 200 mg capsules for oral administration. It is like an antibiotic but is used to treat fungal infections. It is used to treat fungal infections in the lungs, in the central nervous system, in other parts of the body, as well as serious fungal infections of the skin and nails. The itraconazole oral solution is used to treat yeast infec-

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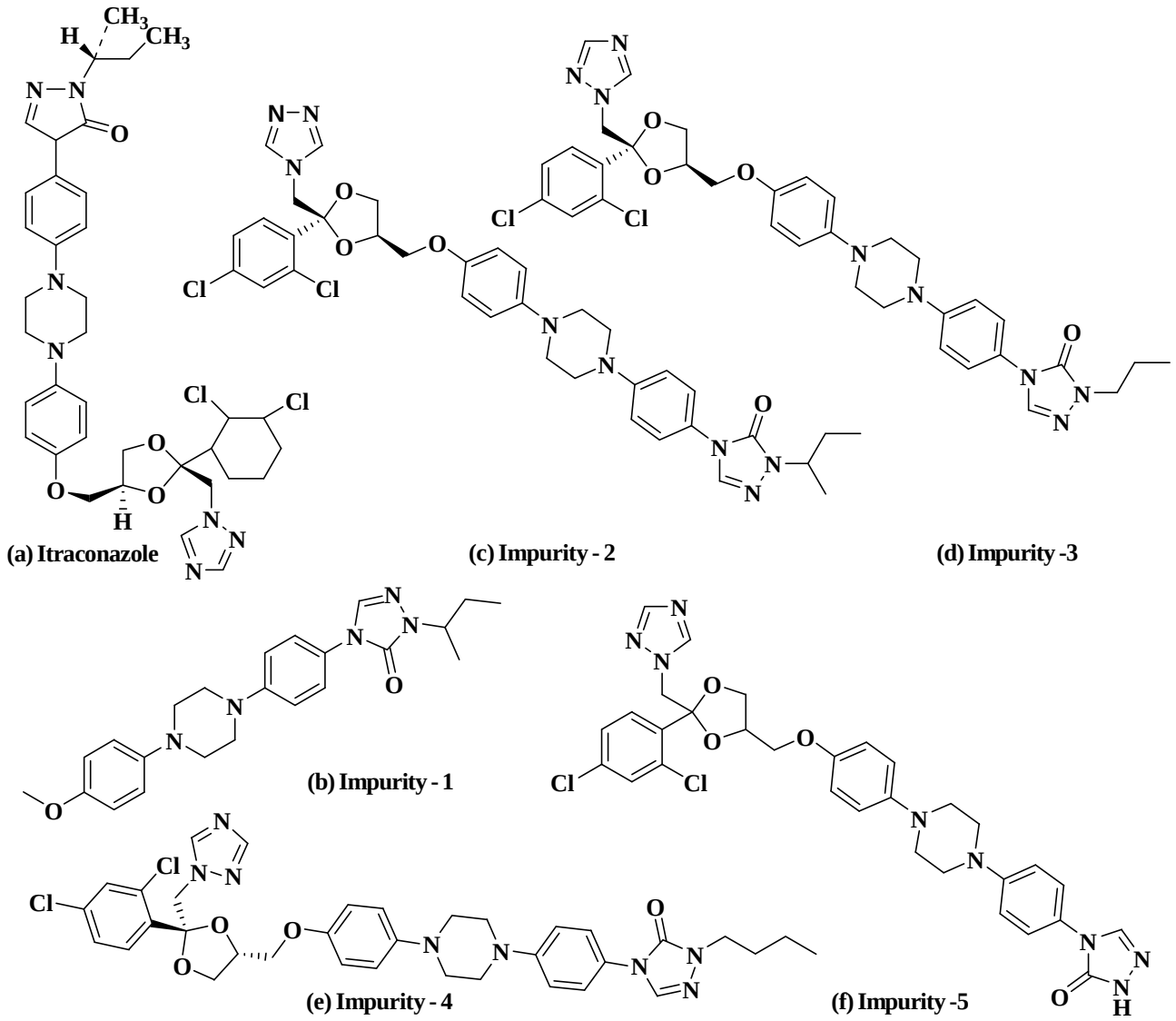

Figure 1: Chemical structures of (a) Itraconazole (b) Impurity-1 (c) Impurity-2 (d) Impurity-3 (e) Impurity-4 (f) Impurity-5

tions of the mouth, throat, and esophagus (Figure 1).

So far, few liquid chromatography procedures[7-13] have been described for the determination of itraconazole. But these procedures were developed to estimate the itraconazole alone or in combination with hydroxyitraconazole from serum and plasma. A LC method has been reported for 'development and validation of the methodology for evaluation of itraconazole in pharmaceutical products by HPLC'[14]. Whereas no single stability-indicating LC method has been reported for the quantitative estimation of itraconazole from pharmaceutical dosage form. Now it is necessary to develop a rapid, accurate and validated stability-indicating assay method for the quantitative determination of itraconazole in pharmaceutical dosage form.

Hence a rapid simple reproducible stability indicating RP liquid chromatography method was developed for the quantitative determination of itraconazole in pharmaceutical dosage forms in the presence of degradation products.

EXPERIMENTAL

Chemicals

Standards and capsules were supplied by Dr. Reddy's laboratories limited, Hyderabad, India. The HPLC grade methanol, acetonitrile and analytical grade

triethyl amine and ortho phosphoric acid were purchased from Merck, Darmstadt, Germany. Water was prepared by using Millipore MilliQ Plus water purification system.

Equipment

The Waters HPLC system we used consists of a binary solvent manager, a sample manager and a UV detector. The out put signal was monitored and processed using empower software, water baths equipped with MV controller (Julabo, Seelbach, Germany) were used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (MACK Pharmatech, Hyderabad, India).

Chromatographic conditions

The chromatographic column used was a Hypersil BDS C-18, 100 mm × 4.6 mm i.d with 3µ m particles. Mobile phase contains a mixture of pH 7.5 0.2 mM K_2HPO_4 buffer: acetonitrile: tetrahydrofuran (50:49:12, *v/v/v*). The flow rate of mobile phase was 1.2 mL min^{-1} and the detection was monitored at a wavelength 225 nm. The column temperature was maintained at 25 ºC. The injection volume was 10µ L.

Preparation of stock solutions

A stock solution of itraconazole standard and sample (0.5 mg mL^{-1}) was prepared by dissolving an appropriate amount in diluent (methanol: 0.1N HCl 70:30, *v/v*). Working solutions (0.1 mg mL^{-1}) were prepared from above stock solution in diluent for assay determination.

Preparation of sample solution

Twenty capsules were weighed and the content transferred into a clean and dry mortar, grinded well. Then an equivalent to 50 mg of itraconazole was transferred to a 100 mL volumetric flask, 70 mL of diluent added and sonicated for 20 min and diluted to 100 mL (0.5 mg mL^{-1}). About 5 mL of supernant solution was taken and diluted to 25 mL with diluent (0.1 mg mL^{-1}). This was filtered using 0.45 µ (Nylon 66- membrane) filter.

Stress studies

Specificity is the ability of the method to measure the analyte response in the presense of its potential impurities[4]. Stress testing of a drug substance can help to identify the likely degradation products, which can in tern help establish the degradation pathways and the intrinsic stability of the molecule.

All stress decomposition studies were performed at an initial drug concentration 0.1 mg mL^{-1}. Acid hydrolysis was performed in 5N HCl at 70^{0}C for 48 h. The study in basic solution was carried out in 5N NaOH at 70^{0}C for 48 h. For study in neutral solution, drugs dissolved in water was heated at 70^{0}C for 48 h. Oxidation studies were carried out at 40 ºC in 6% hydrogen peroxide for 48 h. Photo degradation studies were carried out at according to option 2 of Q1B in ICH guidelines[3]. Samples were exposed to light for an overall illumination of 1.2 million lux h and an integrated near ultraviolet energy of 200 watt hm^2. The drug product was exposed to dry heat at 80^{0}C for 24 h. Samples were withdrawn at appropriate time and subjected to HPLC analysis after suitable dilution (0.1 mg mL^{-1}).

Method validation

Precision

The precision of the assay method was evaluated by carrying out six independent assays of itraconazole (0.1 mg mL^{-1}) test samples against qualified reference standard. Different analyst from the same laboratory evaluated the intermediate precision of the method. The percentage of RSD of six assay values was calculated.

Linearity

Linearity solutions were prepared from stock solution at five concentration levels from 50% to 150% of analyte concentrations (from 50 to 150µg mL^{-1}). The slope, Y-intercept and correlation coefficient were calculated.

Accuracy

The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 50%, 100% and 150% of target test concentration in capsules. The percentages of recoveries were calculated.

Solution stability and mobile phase stability

The solution stability of itraconazole was carried out by leaving the test solution in tightly capped volu-

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metric flask at room temperature for 24 h. The same sample solution was assayed for a 6 h interval up to the study period against freshly prepared standard solution of itraconazole. The mobile phase stability was also carried out by assaying the freshly prepared standard solution for 24 h interval up to 48 h. The mobile phase preparation was kept constant during the study period. The percentage of RSD of assay of itraconazole was calculated for the study period during mobile phase and solution stability experiments.

Robustness

To determine the robustness of the method the experimental conditions were deliberately changed and the % RSD for five injectins area of standard was evaluated. The mobile phase flow rate was 1.2 mL min^{-1}; to study the effect of flow rate on resolution it was changed to 1.0 and 1.4 mL min^{-1}. The effect of pH was studied at pH 7.3 and 7.7 (instead of pH 7.5). The effect of column temperature was studied at 20 and 30^0C (instead of 25^0C). In all these experiments the mobile phase components were not changed.

RESULTS AND DISCUSSION

Stress studies

Degradation was not observed for itraconazole during stress conditions like hydrolytic, photo, thermal degradation and water hydrolysis. Degradation (38%) was observed at RRT 0.35, 0.44 and 0.81 in 6% hydrogen peroxide at 40^0C for 48 h. Peak purity test results confirm that the itraconazole peak is homogeneous and pure in all the analyzed stress samples.

Mass balance (%assay + % degradents + % impurities) is calculated for stress sample. The mass balance of stressed sample was > 99.0% for all stress conditions (TABLE 3).

Method development and optimization of stability indicating assay method

The method was optimized to separate major degradation products formed under varies stress conditions. The main target of the chromatographic method is to get the separation for closely eluting degradation products, mainly the degradation product at 0.81 RRT. The degradation samples were run using different stationary phases like C18, C8, Cyano and Mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2-8) and using organic modifiers like acetonitrile, tertahydrofuran and methanol in the mobile phase. But the separation was satisfactory in the adopted chromatographic conditions only. It indicated that the isocratic with 49% acetonitrile and 12% tetrahydrofuran as organic modifiers in mobile phase was successful in separating drugs and all chromatographic degradation products (Figure 3)

Validation of Developed Stability-Indicating method

Precision

The percentage RSD value for the precision study was 0.4% (inter-day precision) and 0.4% (intra-day precision). This is confirming good precision of the method (TABLE 1).

Linearity

Linearity calibration plots for this method was obtained over the calibration ranges tested; i.e. 50 to 150µg mL^{-1} itraconazole and the correlation coefficient obtained was greater than 0.999. The results show that an excellent correlation existed between the peak area and concentration of the analyte. The slope and Y-intercept of the calibration curve were calculated. The mean regressions equation was found as A =15761 C - 7602 (r^2 = 0.9999, n = 6). A = aC + b, where A is the peak area ratio of the drugs, a is the slope, b is the intercept

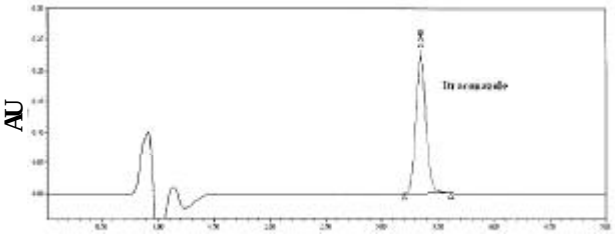

Figure 2 : Chromatogram of Itraconazole from capsules

TABLE 1: Precision results of itraconazole from capsules

Precision	% Assay						% RSD	% Mean
	1	2	3	4	5	6		
Intra-day	100.1	100.1	100.2	99.7	99.3	99.1	0.4	99.8
Inter-day	100.8	101.1	101.3	100.2	100.5	100.5	0.4	100.7

TABLE 2: Percentage recovery of itraconazole from capsules

Added (mg)	Recovered (mg)	Recovery (%)	RSD (%)
25.01	24.76	99.0	0.33
50.01	49.56	99.1	0.56
75.02	74.94	99.9	0.43

TABLE 3: Summary of results from forced degradation experiments

Stress condition	Time	Assay of active substance (%)	Mass balance (% assay + % impurities + % degradation products)	Remarks (Major degradation products)
Acidic hydrolysis (5 N HCl)	48 h	99.1	99.4	No degradation products formed
Basic hydrolysis (5 N NaOH)	48 h	99.2	99.6	No degradation products formed
Oxidation (6% H_2O_2)	48 h	68.2	99.1	Degraded to Impurity-3
Aqueous hydrolysis	48 h	99.5	99.8	No degradation products formed
Thermal treatment (60 °C)	10 days	99.1	99.5	No degradation products formed
Light (photolytic degradation)	10 days	99.2	99.6	No degradation products formed

TABLE 4: Results from study of robustness

Condition	Variation	%RSD for five injections area for standard
Temperature (± 5 ºC of optimum temperature)	20 ºC	0.5
	30 ºC	0.3
Flow rate (± 0.2 mL/min of optimum flow rate)	1.0 mL min^{-1}	0.4
	1.4 mL min^{-1}	0.3
pH (± 0.2 unit of set pH)	7.3	0.2
	7.7	0.3

and C is concentration of the measured solution in μg mL^{-1}. The results show that an excellent correlation existed between the peak area and concentration of the analyte.

Recovery

The percentage recovery of itraconazole in pharmaceutical dosage forms ranged from 99.0 to 99.9.

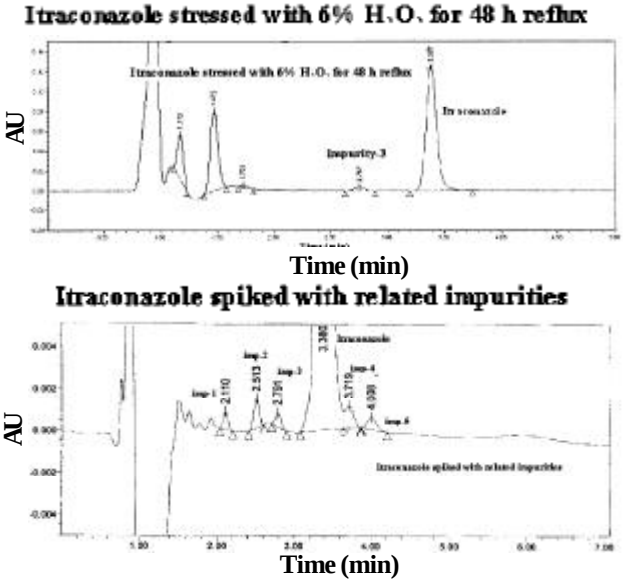

Figure 3: Chromatograms indicating the degradation of Itraconazole in peroxide and spiked with related impurities

Excellent recoveries were made at each added concentration (TABLE 2).

Solution stability and mobile phase stability

The solution stability and mobile phase stability experiment data confirms that sample solutions and mobile phase used during the assay were stable up to 48 h.

Robustness

When mobile phase flow rate, pH and column temperature were deliberately varied % RSD for area of five replicate injections of standard was less than 1.0, illustrating the robustness of the method (TABLE 4).

Capsules application

Analysis was performed for commercially available itraconazole capsules. The Mean assay (n = 6) for itraconazole was 98.9%. The percentage RSD value for the six assay values was 0.3%.

CONCLUSIONS

A novel isocratic HPLC method prove to be simple, linear, precise, accurate and specific. The total runtime was 4 min within which active drug and their degradation products were separated. The method was completely validated showing satisfactory data for all the

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method validation parameters tested. The Developed method is stability indicating and can be used for the quantitative determination of the itraconazole in presence of degradation products in stability by the industry.

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