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Research Article
Determination of Fluconazole in Bulk Durg and its Solid Formulations by Acid-base Titration in Non-aqueous Medium

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Abstract

Background: Fluconazole (FLK) is a synthetic triazole derivative antifungal agent that has been shown to be effective against a wide range of systemic and superficial fungal infections. **Objective:** Two simple, rapid, reliable, precise and accurate and cost-effective non-aqueous titrimetric procedures have been developed for the determination of FLK in bulk drug and its pharmaceutical formulations. **Materials and Methods:** The methods are based on the titration of the drug with perchloric acid in glacial acetic acid with the visual end point detection using crystal violet as indicator or the potentiometric end point using a combined double junction pH electrode system. **Results:** The methods were applicable over the range of 2.0-15.0 mg FLK and the calculations are based on a 1:2 reaction stoichiometry. The procedures were also applied for the determination of FLK in its dosage forms and the results were found to be in a good agreement with those obtained by the reference method. The precision results expressed by intra-day and inter-day relative standard deviation values were good (RSD<0.75%). The accuracy was satisfactory as well (RE≤1.30%). Excipients used as additives in pharmaceutical formulations did not interfere in the proposed procedures as shown by the recovery study via standard addition technique recoveries in the range 99.23-101.03% with a standard deviation of ≤0.91%. **Conclusion:** The proposed methods were applied for determination of the FLK in tablets. Both the methods were validated and can be suggested for routine analysis in quality control department.

Key words: Fluconazole, non-aqueous titrimetry, pharmaceuticals, crystal violet, potentiometry

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fluconazole (FLK) chemically known as (2-(2,4-difluorophenyl)-1,3-bis (1h-1,2,4,-triazol-1-yl) propan-2-ol)[1]. Figure 1 is a synthetic triazole derivative antifungal agent that has been shown to be effective against a wide range of systemic and superficial fungal infections[2]. The FLK is almost completely absorbed (>90%) from the gastrointestinal tract after oral administration and about 11-12% of the drug in plasma is protein bound. The recommended dosage of FLK is generally 100-200 mg once daily and the time to attain peak concentration in plasma is 1.7-4.3 h after single dose drug administration. The FLK has a long half-life (32±5 h) and is excreted predominantly unchanged in urine[3,4] (75±9%).

A number of different analytical investigations have been done to determine the drug content in pharmaceuticals and body fluids; also for dissolution, bioequivalence and pharmacokinetic studies. Recently, Correa and Salgado[5] have reviewed the FLK properties and methods for its determination with emphasis on body fluids by HPLC. The drug in body biological fluids has been determined by liquid chromatography-tandem mass spectrometry (LCMS)[6-10], gas chromatography (GC)[11-17], GC-MS[18], micellar electrokinetic capillary chromatography[19], ISE-potentiometry[20] and microbiological assay[21-24].

According to the literature survey, the drug in bulk and dosage forms has been quantitated by UV-spectrophotometry[25-35], visible spectrophotometry[36-38], fluorospectrometry[39-41], proton NMR spectroscopy[42], ISE-potentiometry[20], thin layer chromatography[43], high performance thin layer chromatography[44,45], ultra performance liquid chromatography[46,47], microbiological assays[25,48-50] and high performance liquid chromatography[25,28,34,47,48,50-58]. Three non-aqeous titrametric methods in United states pharmacopeia[59], European pharmacopoeia[60] and British pharmacopoeia[61] are also reported for estimation of FLK in bulk drug in with perchloric acid used as titrant and the end point being located potentiometrically.

Inspite of its inherent simplicity, speed, cost effectiveness, excellent accuracy and precision, no visual titrimetric method has ever been reported for FLK. Three non-aqueous titrimetric procedures recommended by the pharmacopoeias[59-61] require large quantities of drug (about 200 mg for each titration) and 0.1 M $HClO_4$ with potentiometric end point detection which are slow and expensive interms of strength of $HClO_4$.

The present study describes two simple and accurate titrimetric procedures for the determination of FLK in pure as well as in tablet formulations. The methods involve the titration of FLK with 0.01 M perchloric acid in acetic acid medium and end point being detected either visually using crystal violet as indicator or potentiometrically using combined double junction pH electrode. The methods cut down the cost per analysis since they employ 0.01 M $HClO_4$ and a few milligram of FLK per analysis. Visual titration can be completed in less than 5 min there by realizing high throughput.

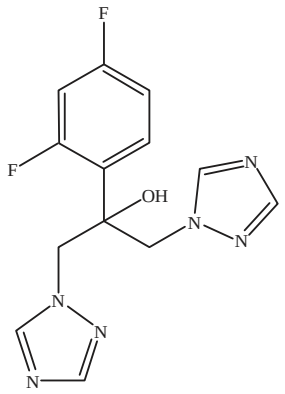


Fig. 1: Structure of fluconazole

MATERIALS AND METHODS

Apparatus: A Metrohm Swiss made 809 titrando auto titrator with combined double junction pH electrode system for non-aqueous acid-base titration was used for potentiometric titration. The KCl salt bridge was replaced with saturated solution of LiCl in ethanol.

Reagents: All chemicals were used for analytical reagent grade. All solutions are made in glacial acetic acid (S.D. Fine chem., Mumbai, India) unless mentioned otherwise.

Perchloric acid (0.01 M): The stock solution of (0.1 M) perchloric acid (S.D. Fine chem., Mumbai, India) was diluted appropriately with glacial acetic acid to get a working solution of 0.01 M perchloric acid and standardized with pure potassium hydrogen phthalate and crystal violet as indicator[62].

Crystal violet indicator (0.1%): Prepared by dissolving 50 mg of dye (S.D. Fine chem., Mumbai, India) in 50 mL of glacial acetic acid.

Standard drug solution: Stock standard solution containing 2 mg mL^{-1} pure drug was prepared by dissolving the suitable amount of FLK (Dr. Reddy's laboratories limited, Hyderabad, India) in glacial acetic acid.

Visual titration: An aliquot of the standard drug solution containing 2.0-15.0 mg of FLK was measured accurately and transferred into a clean and dry 100 mL titration flask and the total volume was brought to 10 mL with glacial acetic acid. Two drops of crystal violet indicator were added and titrated with standard 0.01 M perchloric acid to a blue colour end point. An indicator blank titration was performed and corrections to the sample titration were applied. The amount of the drug in the measured aliquot was calculated from:

$$\text{Amount (mg)} = VM_wR/n$$

where, V is volume of perchloric acid consumed (mL), M_w is relative molecular mass of the drug, R is molarity of the perchloric acid and n is number of moles of perchloric acid reacting with each mole of FLK.

Potentiometric titration: An aliquot of the standard drug solution equivalent to 2.0-15.0 mg of FLK was measured accurately and transferred into a clean and dry 100 mL beaker and the solution was diluted to 25 mL by adding glacial acetic acid. The combined double junction pH electrode was dipped in the solution. The contents were stirred with magnetic stirrer and the titrant (0.01 M $HClO_4$) was added from a micro burette. Near the equivalence point, titrant was added in 0.01 mL increments. After each addition of titrant, the solution was stirred magnetically for 30 sec and the steady potential was noted. The addition of titrant was continued until there was non-significant change in potential on further addition of titrant. The equivalence point was determined by applying the graphical method. The amount of the drug in the measured aliquot was calculated as described under visual titration.

Procedure for formulations: Two brands of tablets, Nuforce-150 (from Pharma Force Lab, Himachal Pradesh, India) and AF-150 (from Ontop Pharmaceuticals pvt. Ltd., Bengalore, India) used in the investigation were purchased from commercial sources in the local market.

Twenty tablets were weighed and ground into a fine powder. An amount of tablet power equivalent ot 50 mg FLK was weighed accurately and transferred into a 25 mL calibrated flask and 10 mL of glacial acetic acid was added. The contents sonicated for 10 min, diluted to the mark with glacial acetic acid, mixed well and filtered using a Whatmann No. 42 filter paper. A suitable aliquot was next subjected to analysis by applying the general procedures as described earlier.

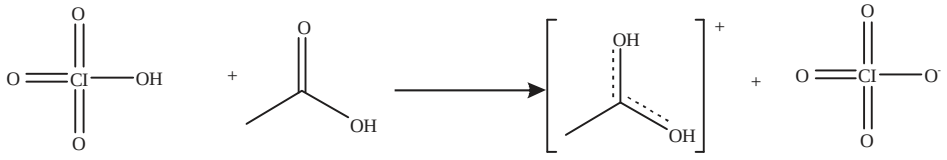
RESULTS AND DISCUSSION

Titration in non-aqueous medium is the most common method adopted by pharmacopoeias for weakly acidic or basic pharmaceuticals since it serves a double purpose: A solvent for weak acids/bases and tends to enhance the weakly basic properties of weak bases and weakly acidic property of weak acids due to the non-leveling effect of the medium.

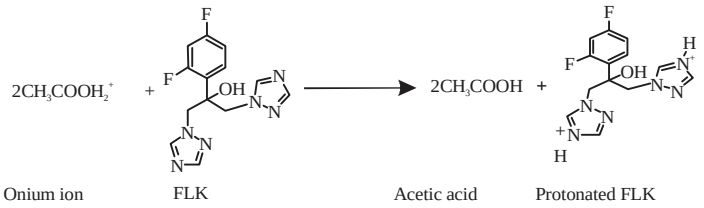
The present methods are based on the neutralization reaction involving the basic property of FLK and employ two techniques. The methods are based on the principle that substances which are weakly basic in aqueous medium, when dissolved in non-aqueous solvents exhibit enhanced basicity thus, allowing their easy determination. Acetic acid is the most commonly employed medium for titration of weak bases due to its protophillic and protogenic properties and its ability to donate protons and accept protons[63] as follows:

$$CH_3COOH \leftrightarrow H^+ + CH_3COO^-$$

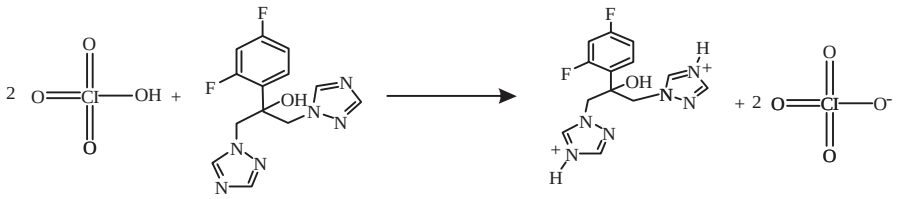
When a strong acid such as $HClO_4$ is dissolved in a weaker acid such as CH_3COOH, the latter is forced to act as a base and accepts a proton from $HClO_4$ to form an onium ion[64]. The onium ion formed ($CH_3COOH_2^+$) can very readily give up its proton to react with FLK so the basic properties of the drug is enhanced and hence, titration between FLK and $HClO_4$ becomes easy. The reactions occurring are as follows:



Perchloric acid Acetic acid Onium ion Perchlorate ion



The overall reaction is



Crystal violet gave satisfactory end point for the amounts of analyte and concentration of titrant employed. A steep raise in the potential was observed at the equivalence point with potentiometric end point detection (Fig. 2a, b). With both methods of equivalence point detection, a reaction stoichiometry of 1:2 (Drug:Titrant) was obtained which served as the basis for calculation. Using 0.01 M perchloric acid, 2.0-15.0 mg of FLK was conveniently determined. The relationship between the drug amount and the titration end point was examined. The linearity between two parameters is apparent from the correlation coefficients of 0.9997 and 0.99997 obtained by the method of least seqares for visual and potentiometric methods, respectively. From this it is implied that the reaction between FLK and perchloric acid proceeds stoichimetrically in the ratio 1:2 in the range studied.

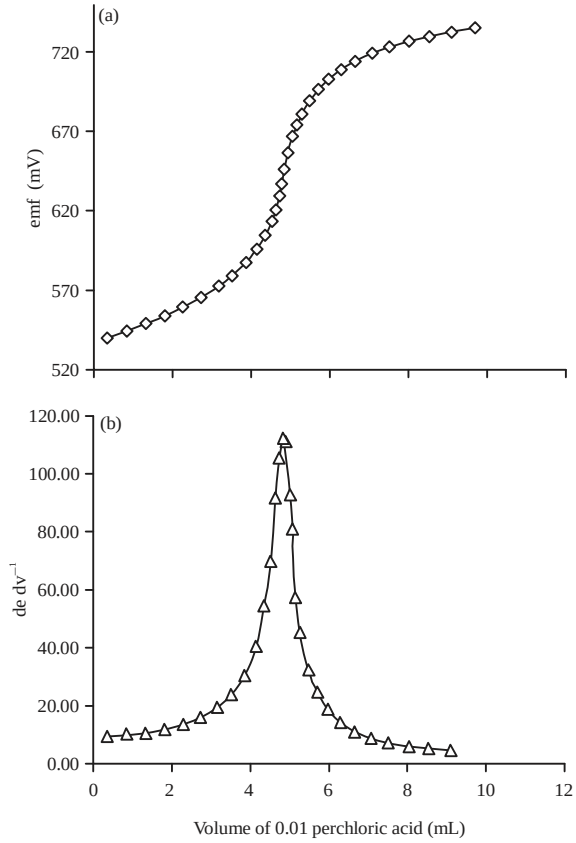


Fig. 2(a-b): Relationship between titrant and drug

Method validation

Intra-day and inter-day accuracy and precision: The precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different amounts of FLK within the range of study in each method were analysed in seven and five replicates in method A and method B, respectively, during the same day (intra-day precision) and five consecutive days (inter-day precision). For inter-day precision, each day analysis was performed in triplicate and pooled-standard deviation was calculated. The RSD values of intra-day and inter-day studies for FLK showed that the precision of the methods was good (Table 1). The accuracy of the methods was determined by the percent mean deviation from known concentration and results are presented in Table 1.

Ruggedness of the methods: Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using four different burettes. The inter-analysts RSD were less than 0.81% whereas the inter-burettes RSD for the same FLK amounts ranged from 0.16-0.80% suggesting that the developed method was rugged (Table 2).

Application: The described titrimetric procedures were successfully applied to the determination of FLK in its pharmaceutical formulations (Nuforce-150 and AF-150). The results obtained from Table 3 were statistically compared with the reference method[28]. The reference UV-spectrophotometric method involved the measurement of the absorbance of FLK tablet solution at 260 nm in HCl media. The results obtained by the proposed methods agreed well with those of reference method and with the label claim. The results were also compared statistically by a Student's t-test for accuracy and by a variance F-test for precision with those of the reference method at 95% confidence level as summarized in Table 3. The results showed that the calculated t and F-values did not exceed the tabulated values inferring that proposed methods are as accurate and precise as the reference method.

Recovery study: Accuracy and the reliability of the methods were further ascertained by performing recovery experiments. To a fixed amount of drug in formulation (pre-analysed): Pure drug at three different levels was added and the total was found by the proposed methods. Each test was repeated three times. Table 4 shows that recoveries

Table 1: Intra-day and inter-day accuracy and precision

		Intra-day accuracy and precision			Inter-day accuracy and precision		
Method	FLK (mg)	FLK found	RE (%)	RSD (%)	FLK found	RE (%)	RSD (%)
Visual titrimetry (n = 7)	5	5.02	0.40	0.75	4.99	-0.20	0.53
	10	10.13	1.30	0.34	9.98	-0.20	0.43
	15	15.12	0.80	0.25	14.88	-0.80	0.23
Potentiometric titrimetry (n = 5)	5	5.02	0.40	0.44	4.96	-0.80	0.18
	10	10.09	0.90	0.05	9.96	-0.40	0.38
	15	15.11	0.73	0.09	14.95	-0.33	0.12

RE: Relative error and RSD: Relative standard deviation

Table 2: Method ruggedness expressed as intermediate precision (RSD %)

		Ruggedness	
Method	FLK (mg)	Inter-analysts (RSD %) (n = 4)	Inter-burettes (RSD %) (n = 4)
Visual titrimetry	5	0.81	0.80
	10	0.46	0.40
	15	0.31	0.27
Potentiometric titrimetry	5	0.35	0.23
	10	0.12	0.34
	15	0.14	0.16

Table 3: Results of assay in tablets and comparison with the reference method

		Found* (Percent of label claim±SD)		
			Proposed method	
Tablet brand name**	Nominal amount (mg)	Reference method	Visual titrimetry	Potentiometric titrimetry
AF-150	150	99.2±0.91	100.2±1.04	100.1±0.64
			t = 1.6	t = 1.88
			F = 1.29	F = 2.0
Nuforce-150	150	98.9±0.95	99.78±0.44	99.9±0.46
			t = 1.88	t = 2.11
			F = 4.76	F = 4.3

*Average of five determinations, tabulated t-value at the 95% confidence level is 2.77 and tabulated F-value at the 95% confidence level is 6.39

Table 4: Results of recovery study by standard addition method

		Visual titrimetry				Potentiometric titrimetry		
Tablet	FLK tablet (mg)	Pure FLK (mg)	Total (mg)	Pure FLK recovered* Percent±SD	FLK tablet (mg)	Pure FLK (mg)	Total (mg)	Pure FLK recovered* Percent±SD
AF-150	2.06	5.0	7.07	100.10±0.65	2.06	5.0	7.02	99.39±0.17
	2.06	7.5	9.55	99.89±0.91	2.06	7.5	9.53	99.72±0.06
	2.06	10.0	12.18	100.97±0.63	2.06	10.0	12.02	99.70±0.12
Nuforce-150	2.10	5.0	7.17	101.03±0.65	2.10	5.0	7.06	99.44±0.29
	2.10	7.5	9.62	100.24±0.48	2.10	7.5	9.56	99.55±0.16
	2.10	10.0	12.15	100.44±0.38	2.10	10.0	12.01	99.23±0.13

*Mean value of three determinations

were in the range from 99.23-101.03% indicating that commonly added excipients to tablets did not interfere in the determination.

CONCLUSION

Many of the reported methods suffer from such drawbacks as high cost, multiple steps and also several clean-up steps (HPLC). They are time consuming and often poorly reproducible, some require toxic organic solvents. Any method chosen for routine analysis should be reasonably simple, used materials should readily available in the laboratory or readily obtainable and require a minimum amount of equipment. These objectives have been fulfilled by the two titrimetric procedures developed. The methods provide two simple procedures for the determination of FLK in pure form and its dosage form. The present methods have the distinct advantages over the previously reported methods in terms of speed, cost effectiveness, simplicity, sensitivity, accuracy and ease of performance does not need expensive and highly sophisticated equipment or high-cost organic solvents which are required for HPLC technique. Therefore, the proposed method can be used in laboratories where modern and expensive instruments are not available.

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