Development And Validation of a Novel RP-HPLC Method For Simultaneous Estimation of Tinidazole and Diloxanide Furoate in Pharmaceutical Dosage Forms

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***Abstract*—A simple, rapid, precise, accurate, and reproducible Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Tinidazole and Diloxanide Furoate in pharmaceutical tablet dosage forms. Tinidazole is a nitroimidazole derivative widely used for the treatment of protozoal and anaerobic bacterial infections, while Diloxanide Furoate is a luminal amoebicide commonly prescribed in the management of intestinal amoebiasis. The increasing use of combination therapy necessitates the development of reliable analytical methods for routine quality control. Chromatographic separation was achieved using a Thermosil RP-C18 column (4.6 × 100 mm, 5 µm) with a mobile phase consisting of Sodium Acetate Buffer and Methanol in the ratio of 30:70 v/v. Detection was performed at 240 nm with a flow rate of 1.0 mL/min. The retention times of Tinidazole and Diloxanide Furoate were found to be 2.408 min and 3.016 min, respectively. The developed method was validated according to ICH Q2(R1) guidelines. The validation results demonstrated excellent linearity, precision, accuracy, robustness, specificity, and sensitivity. The proposed method was found suitable for routine quality control analysis of Tinidazole and Diloxanide Furoate in pharmaceutical dosage forms.**

***Index Terms*—RP-HPLC, Tinidazole, Diloxanide Furoate, Method Development, Method Validation, Pharmaceutical Analysis.**

I. INTRODUCTION

Analytical chemistry plays a vital role in pharmaceutical industries by ensuring the quality, safety, and efficacy of pharmaceutical products. Among various analytical techniques, High Performance Liquid Chromatography (HPLC) has emerged as one of the most widely employed analytical tools due to its high sensitivity, reproducibility, precision, and ability to separate complex mixtures[1]. HPLC is extensively utilized during drug discovery, formulation development, quality control, and stability studies[2]. The technique provides rapid and accurate quantitative estimation of active pharmaceutical ingredients and impurities, making it indispensable in pharmaceutical analysis[3]. The efficiency of HPLC depends on several chromatographic variables including stationary phase selection, mobile phase composition, detector wavelength, flow rate, and column characteristics[4]. These parameters significantly influence separation efficiency and analytical performance. The widespread application of RP-HPLC in pharmaceutical analysis is mainly attributed to its capability to analyze polar and non-polar compounds with excellent resolution and shorter run times[5].

Tinidazole is a synthetic nitroimidazole derivative possessing potent antiprotozoal and antibacterial activity. It is commonly used for the treatment of infections caused by Trichomonas vaginalis, Entamoeba histolytica, and Giardia lamblia[6]. The drug acts by generating toxic free radicals that damage microbial DNA, leading to cell death. Tinidazole exhibits excellent oral bioavailability and prolonged half-life, making it an effective therapeutic agent for parasitic infections[7].

Diloxanide Furoate is an antiamoebic drug used primarily for the treatment of asymptomatic intestinal amoebiasis[8]. It acts as a luminal amoebicide and is hydrolyzed in the gastrointestinal tract to release the active moiety, diloxanide. Combination therapy involving Tinidazole and Diloxanide Furoate provides

effective eradication of both tissue and luminal forms of amoebic infections and is therefore widely prescribed in clinical practice[9].

A review of the available literature revealed several RP-HPLC methods for Tinidazole alone or in combination with other drugs[10]. However, a simple and validated RP-HPLC method for simultaneous estimation of Tinidazole and Diloxanide Furoate suitable for routine pharmaceutical analysis was limited[11]. Therefore, the present investigation aimed to develop and validate a novel RP-HPLC method according to ICH guidelines[12-15].

II. MATERIALS AND METHODS

The study was conducted using HPLC-grade solvents and analytical-grade reagents. Tinidazole and Diloxanide Furoate working standards were obtained from in-house sources, while commercial tablet formulations were procured from the market. Chromatographic analysis was performed using a Waters HPLC system equipped with an autosampler, UV detector, and Empower software. Additional laboratory instruments included a UV-Visible spectrophotometer, digital balance, pH meter, and sonicator.

Table 1: Chemicals and Reagents Used

S. No	Chemical/Reagent	Grade
1	Water	HPLC Grade
2	Methanol	HPLC Grade
3	Acetonitrile	HPLC Grade
4	Orthophosphoric Acid	GR
5	KH_2PO_4	GR
6	K_2HPO_4	GR
7	Nylon Filter (0.22 µm)	HPLC Grade
8	Filter Paper (0.45 µm)	HPLC Grade

Table 2: Instruments Used

Instrument	Manufacturer
HPLC System	Waters
UV Spectrophotometer	Lab India
Digital Balance	Ascoset
pH Meter	ADWA
Sonicator	Enertech

The method development process involved systematic optimization of chromatographic conditions such as mobile phase composition, detection wavelength, column selection, flow rate, injection volume, and column temperature. Several chromatographic trials were conducted to obtain adequate separation with acceptable peak symmetry and resolution. A Thermosil RP-C18 column (4.6 × 100 mm, 5 µm) was selected because of its superior chromatographic performance and reproducibility. The optimized chromatographic conditions consisted of Sodium Acetate Buffer and Methanol (30:70 v/v) as mobile phase, flow rate of 1.0 mL/min, detection wavelength of 240 nm, and ambient column temperature. Under these conditions, excellent separation was achieved within a run time of five minutes.

Table 3: Optimized Chromatographic Conditions

Parameter	Condition
Column	Thermosil RP-C18 (4.6 ×100 mm, 5 µm)
Mobile Phase	Sodium Acetate Buffer: Methanol
Ratio	30:70 (v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	240 nm
Injection Volume	20 µL
Run Time	5 min

III. METHOD DEVELOPMENT

During method optimization, eight chromatographic trials were conducted using different combinations of methanol, acetonitrile, water, and buffer systems. Initial trials failed to provide satisfactory peak resolution and peak symmetry. Modifications in mobile phase composition and pH significantly improved chromatographic behavior. Among all investigated conditions, the optimized mobile phase consisting of Sodium Acetate Buffer and Methanol (30:70 v/v) provided sharp peaks with excellent resolution and acceptable retention times. The final method resulted in retention times of 2.408 minutes for Tinidazole and 3.016 minutes for Diloxanide Furoate. The shorter analysis time enhanced laboratory productivity and reduced solvent consumption.

Table 4: Optimized Retention Times

Drug	Retention Time (min)
Tinidazole	2.408
Diloxanide Furoate	3.016

IV. METHOD VALIDATION

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines. Validation parameters included specificity, linearity, accuracy, precision, robustness, limit of detection, and limit of quantification. The method demonstrated excellent specificity with no interference from excipients present in the tablet formulation.

Linearity studies showed a direct relationship between concentration and peak area over the selected analytical range. Correlation coefficients were found to be greater than 0.999, indicating excellent linearity. Accuracy studies were performed using recovery experiments at different concentration levels, and percentage recoveries were found within acceptable limits. Precision studies showed low percentage relative standard deviation values, confirming method reproducibility.

Robustness evaluation demonstrated that small deliberate variations in chromatographic conditions did not significantly affect analytical performance. The developed method therefore exhibited adequate robustness for routine laboratory use.

Table 5: Validation Summary

Parameter	Observation
Specificity	No interference
Linearity	Excellent ($R^2 > 0.999$)
Accuracy	98–102% Recovery
Precision	%RSD < 2
Robustness	Acceptable
Sensitivity	Satisfactory

V. RESULTS AND DISCUSSION

The developed RP-HPLC method successfully achieved simultaneous estimation of Tinidazole and Diloxanide Furoate within a short chromatographic run. The optimized chromatographic conditions produced symmetrical peaks with good resolution and acceptable system suitability parameters. The method exhibited excellent analytical performance throughout validation studies.

The retention times obtained for Tinidazole and Diloxanide Furoate indicated efficient separation without overlap or interference. Validation results confirmed compliance with ICH guidelines. Recovery studies demonstrated high accuracy, while precision studies established reproducibility of the method. The robustness study further confirmed the reliability of the developed analytical procedure.

Compared with previously reported methods, the present method offers significant advantages including shorter run time, simple mobile phase composition, reduced solvent consumption, and improved analytical efficiency. Therefore, the method is highly suitable for routine quality control applications in pharmaceutical industries.

VI. ADVANTAGES OF THE DEVELOPED METHOD

The developed RP-HPLC method possesses several advantages including simplicity, rapidity, accuracy, precision, reproducibility, and cost-effectiveness. The use of a simple mobile phase composition reduces preparation time and operational complexity. Short retention times minimize solvent consumption and increase sample throughput. Compliance with ICH validation requirements further support its application in routine quality control laboratories.

VII. CONCLUSION

A novel RP-HPLC method was successfully developed and validated for the simultaneous estimation of Tinidazole and Diloxanide Furoate in pharmaceutical dosage forms. The optimized chromatographic conditions provided excellent separation with retention times below four minutes. Validation studies demonstrated that the method is accurate, precise, robust, specific, and reproducible. The developed analytical procedure satisfies ICH guideline requirements and can be effectively applied for routine quality control analysis of Tinidazole and Diloxanide Furoate formulations in pharmaceutical industries.

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