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METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF TINIDAZOLE BY REVERSE PHASE HPLC TECHNIQUE

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ABSTRACT: The aim of the current study is to develop a simple, specific, rapid and precise quantification technique for the estimation of tinidazole from tablet dosage form. Successful separation of the drug was carried out on a C_{18} column (particle size 5 µm, 250 mm length × 4 mm i.d.) using a mobile phase consisting of a 5.3 mM phosphate buffer solution and acetonitrile in the ratio of 60:40 (v/v). The detection wavelength is 318 nm. The method has been validated as per ICH (Q2) guidelines on the basis of accuracy, precession, linearity, sensitivity and robustness. The method is found to be linear with limit of detection and limit of quantitation 0.25µg/ml and 0.76µg/ml respectively. The average elution time is only 5.0 minutes with the analyte elution taking place at about 3.0 minutes making the method rapid and cost effective for routine analysis.

INTRODUCTION: Tinidazole (TZ) is 5-Nitro imidazole derivative, chemically related to metronidazole and is a drug of choice for the treatment of amoebic and parasitic infections [1]. TZ is a popular anti protozoal agent [2] with established efficacy and acceptable tolerability and is approved by FDA for the treatment of giardiasis, amebiasis, amoebic liver abscess and trichomoniasis [3-7]. It also found effective against a wide range of clinically significant anaerobic bacteria like Bacteroides and Clostridium difficile and microareophilic bacterium like *Helicobacter pylori* [7].

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In susceptible organisms tinidazole is reduced to cytotoxic intermediates that bind covalently with the protozal DNA causing an irreversible damage[8]. In adult human tinidazole has 100% bioavailability and is minimally bound to plasma proteins (12%). It has a plasma elimination half life of 12.3 hours and about 63% of this drug is eliminated through hepatic metabolism[9, 10].

Its dosage is independent of sex, race and hepatic metabolism however it is not recommended for patients with hepatic impairment due to lack of clinical data in such patients. Recent comparative studies present greater clinical efficacies of TZ over metronidazole (MTZ) in the treatment of trichomoniasis, giardiasis and amebiasis [11-14]. Due to its higher efficacy in a large number of disease conditions a large number of formulations containing TZ are available in the market. As a result suitable method for the quantification of the

same from available marketed formulations is necessary. Current literature survey presents several methods for the quantification of TZ which include either a spectrophotometric or a chromatographic technique. Most of these methods reported the use of HPLC coupled with MS for the exact quantification making the quantification technique costly and complicated[15-17]. In the current study we reported a simple, rapid and yet specific chromatographic technique for the exact quantification of TZ from marketed formulation. The simplicity of the method may encourage its regular application in the quantification of TZ from marketed formulations. The method has been validated as per ICH (Q2) guidelines.

MATERIALS AND METHODS:

Apparatus: Quantitative HPLC determination was performed on a Waters Alliance e 2695 separation module with double pump[18-22]. An equilibriated C_{18} column (particle size 5 µm, 250 mm × 4 mm ID) was used for chromatographic separation[23]. A rheodyne injector with a 10 µl loop was used for the injection of standard and sample solutions of tinidazole. Chromatographic detection was made with Waters 2489 dual lambda absorbance detector. For preparation of HPLC grade water Aurium 611 UV water purifier of Sartorius, Germany was used. Chromatograms were analysed using Empower-3 software.

Chemicals and reagents:

Standard TZ (99.8%) was kindly gifted by a local pharmaceutical industry and was used as reference standard without further purification. TZ tablets (Tiniba 300) were purchased from local pharmacy. All solvents were of HPLC grade and reagents were of analytical grade. Potassium di-hydrogen phosphate, dipotassium hydrogen phosphate of AR grade and acetonitrile, phosphoric acid of HPLC grade were purchased from Merck Ltd., Mumbai.

Chromatographic condition:

The mobile phase used in the chromatographic separation was of 5.3 mM phosphate buffer solution and acetonitrile in the ratio of 60:40 (v/v). The pH of the mobile phase was adjusted to 3.5 ± 0.1 with orthophosphoric acid. The injection volume was 10 µl with a pump flow rate 1 ml /min. The column temperature was maintained at room temperature (25 ± 2°C) and the eluent was detected at 318 nm.

Preparation of standard solution:

The standard solution of tinidazole was prepared by transferring 25 mg of the reference standard drug in 25 ml volumetric flask with 10 ml HPLC grade water and one drop of conc HCl followed by sonication for 15 minutes and the final volume was made by mobile phase. 1 ml of the above solution was taken in a 25 ml volumetric flask and diluted up to the mark by mobile phase in order to obtain the solution with final concentration of 0.04 mg/ml. The contents of standard solution were filtered through 0.45 µm syringe filter before making any injection.

Preparation of sample solution:

Twenty tablets (each containing 300 mg of TZ) were weighed accurately and ground to fine powder. The powdered mass equivalent to 25 mg of TZ was accurately weighed and transferred to a volumetric flask. About 10 ml of HPLC grade water and 1 drop of conc. HCl were added to it and sonicated for 15 minutes. The volume was then made up to the mark by mobile phase and filtered through Whatman filter paper no. 1. 1 ml of the filtered solution were taken in a 25 ml volumetric flask and diluted with mobile phase in order to obtain solution with final concentration of 0.04 mg/ml of TZ. The contents of sample solution were filtered through 0.45 µm syringe filter before making any injection.

Analysis of formulations:

10 µl sample solution was injected on HPLC system in an optimized chromatographic conditions and chromatographed in triplicate. A representative chromatogram has been given in **Fig. 1**. Content of TZ in tablet was calculated by comparing mean peak area of sample with that of standard. Results of analysis of tablet formulation were shown in **Table 1**.

Method validation:

The proposed analytical method was validated as per recommendation of USP and ICH guidelines in terms of system suitability parameters, linearity, intraday and interday precision, accuracy, robustness, ruggedness, LOD and LOQ[24, 25].

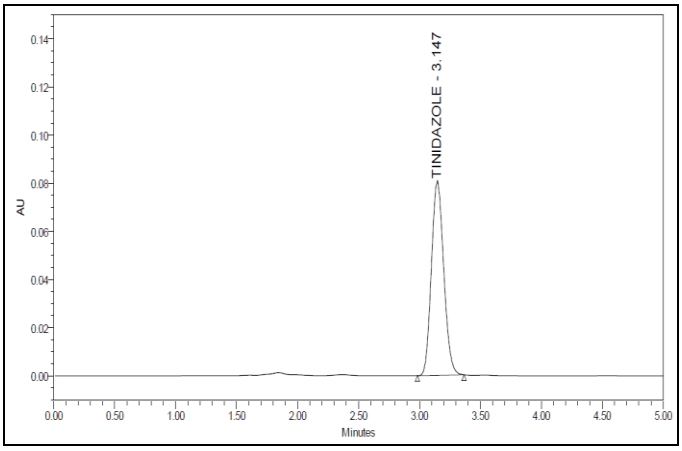

FIG. 1: CHROMATOGRAM OF TINIDAZOLE

TABLE 1: SAMPLE FORMULATION

Formulation	Drug	Amount of Drug (mg/tab)		% of Label Claim	% RSD
		Labelled	Estimated*		
Tiniba 300mg/Tab Zydus Healthcare; Rangpo, Sikkim. Batch no. ZHN3780	Tinidazole	300	299.54	99.84	0.19

* Mean from three replicate analyses.

Method validation:

The proposed analytical method was validated as per recommendation of USP and ICH guidelines in terms of system suitability parameters, linearity, intraday and interday precision, accuracy, robustness, ruggedness, LOD and LOQ[24, 25].

System suitability:

To establish the validity of the proposed analytical procedure, a system suitability test was done. The standard solution of TZ was scanned in the UV range of 200–400nm and its wavelength of maximum absorbance was found to be 318nm (**Table 2**). Data from six injection of 10µl of the working standard solution of tinidazole was used for evaluation of the system suitability parameters like retention time, tailing factor, the number of theoretical plates. The results obtained were shown in **Table 2**.

TABLE 2: SYSTEM SUITABILITY PARAMETER

Parameters	Tinidazole
Wavelength maxima (nm)	318
Retention Time (mins)	3.147
Tailing factor	0.2019
Theoretical Plate	301196
LOD (µg/ml)	0.25
LOQ (µg/ml)	0.76

Linearity:

The linearity for developed HPLC method was determined at five concentration levels of TZ ranging from 10.72 – 85.70 µg/ml. The calibration curve was calculated by plotting response factor against concentrations of TZ (**Fig.2**). The regression equation was found to by Y=13060x +995.5, where y is the peak area and x is the concentration of TZ. The linearity parameters were summarized in **Table 3**.

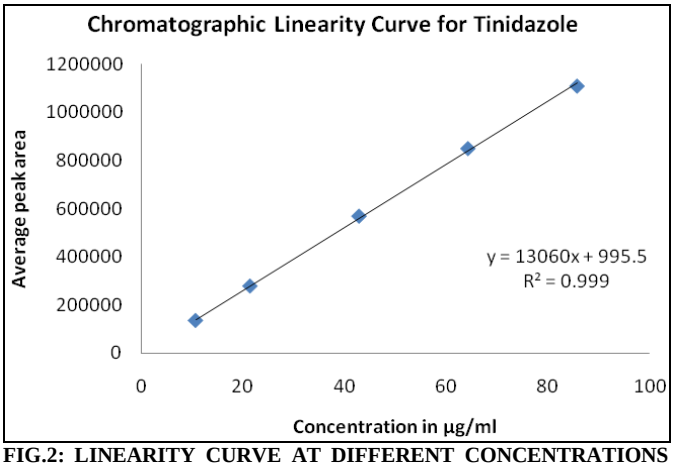

FIG.2: LINEARITY CURVE AT DIFFERENT CONCENTRATIONS OF TINIDAZOLE.

TABLE 3: LINEARITY PARAMETERS

Parameters	Tinidazole
Linearity range (µg/ml)	(10.72- 85.70)µg/ml
Regression coefficient	0.999
Intercept	995.5
Slope	13060

Precession:

The precession of the proposed method was examined by intraday and interday studies. In the intraday studies, six repeated injections of standard solution were made whereas six repeated injections of standard solution were made for three consecutive days in case of interday variation studies. The percentage RSD with respect to the peak area, peak retention time and the amount were calculated for each case and presented in **Table 4**.

Accuracy:

Recovery studies by the standard addition method at three different levels (80%, 110% and 120% of final concentration) were performed with a view to justify the accuracy the accuracy of the proposed method. A known amount standard solution of pure drug was added to pre-analysed sample solution. These solutions were subjected for analysis by the

proposed method. Results of recovery studies were reported in **Table 5**.

TABLE 4: PRECISION PARAMETERS

Parameters	Intra-day	% RSD	Inter-day			
			Day1	Day2	Day3	% RSD
Peak Area	569132	0.12	569011	568945	568959	0.01
Peak RT	3.147	0.08	3.141	3.144	3.149	0.13
Amount (mg/Tab)	299.54	0.12	299.45	299.44	299.45	0.01

TABLE 5: ACCURACY PARAMETERS (RECOVERY STUDY)

Formulation	Drug	Labeled Amt. (mg/tab)	Assay amount (mg/tab)	% label claim (n =3)	Recovery Studies (n = 3)				
					Total Amt. after spiking (mg)	Amt recovered (mg) Mean ± SD	% Recovery	% Mean Recover	% RSD
Tiniba 300mg/Tab Zydus Healthcare; Rangpo, Sikkim. Batch no. ZHN3780	TZ	300.00	299.54	99.84	240	240.99±1.09	100.41	100.18	0.28
					330	329.56±1.44	99.87		
					360	360.98±0.99	100.27		

Robustness:

Robustness of the analytical method was checked by obtaining chromatogram with slight changes in the parameters like flow rate (± 0.1 ml/min), pH of the mobile phase (± 2%) and mobile phase composition (± 5%).

Ruggedness:

The ruggedness of the developed method was assessed by carrying out the experiment on different instrument by different operators and also different days[25].

LOD and LOQ:

The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method. The LOD with signal/noise ratio of 3:1 and the LOQ with signal/noise ratio of 10:1 were found to be 0.25 mg/ml and 0.76 mg/ml respectively (**Table 2**).

RESULTS AND DISCUSSION:

Preliminary experiments were carried out to achieve the chromatographic conditions for the determination of tinidazole. Several column type and lengths were tried. Other chromatographic parameters, chromatographic conditions were optimized by changing mobile phase composition and its pH. Eventually the optimum mobile phase containing 5.3 mM phosphate buffer: acetonitrile (60:40 v/v) was selected because it was found ideal to give a well resolved, sharp peak for tinidazole with retention time of 3.147 min (**Fig. 1**). System suitability studies were carried out by using freshly prepared standard solution of tinidazole. Various parameters obtained are summarized in **Table 2**. Linearity was assessed by plotting concentration *versus* area (**Fig.2**) which was linear in the range of 10.72 – 85.70 µg/ml for tinidazole with correlation co-efficient 0.999. The mean recovery and RSD of recovery were 100.18% and 0.28% respectively (**Table 5**). The lower values showed that there was no interference due to excepients and mobile phase and hence the method was found to be specific[24]. Precision studies were carried out using parameters like intraday and interday analysis precision. The study showed the results were within the acceptable limit and indicating that the method was reproducible (**Table 4**).

The method was robust and rugged as observed from insignificant variation in the results of analysis by changing in flow rate, pH and composition of mobile phase and analysis being performed by different analyst with different instruments. The LOD and LOQ values were calculated based on the standard deviation of the response and the slope of the calibration curve. The values of LOD and LOQ were found to be 0.25 mg/ml and 0.76mg/ml respectively (**Table 2**) which showed that the method was very sensitive[24].

CONCLUSION: The results of the above studies indicate that the developed method was found to be simple, accurate, linear, sensitive and reproducible and have short run time which makes the method rapid and economical. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of tinidazole in bulk drug and pharmaceutical formulations.

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