SHORT COMMUNICATIONS

DEVELOPMENT OF AN ACCURATE UV SPECTROSCOPIC METHOD FOR TINIDAZOLE IN VAGINAL BUFFERS

ABSTRACT

A precise UV spectroscopic method was developed for accurately quantifying tinidazole (TNZ) using the Shimadzu 1900i UV spectrophotometer. Different sample solutions containing TNZ were scanned across a range of concentration 6 µg mL^{-1} to 26 µg mL^{-1} between 200-400 nm, generating overlay TNZ spectra showing an absorption maximum at 318nm in vaginal buffer which is made up of phosphate buffer (PB) 4.2 pH with 2% Tween 80® LR and 4 mL of methanol. The construction of a six-point calibration curve demonstrated the linearity of TNZ within the 6-26 µg mL^{-1} concentration range. The regression equation derived from this calibration curve was subsequently employed to accurately determine the concentration in accuracy studies. In the analysis of bulk TNZ, a recovery rate ranging from 98.41% to 102.28% was achieved. The analysis results were validated following ICH (International Council on Harmonization) guidelines and compiled with the required criteria and standards.

Keywords: Tinidazole, vaginal pH, International Council on Harmonization, buffer, validation, assay

INTRODUCTION

Tinidazole (TNZ), similar to metronidazole, has been widely used for over 20 years with demonstrated efficacy and safety against various infections. It was recently approved by the FDA to treat amebiasis, trichomoniasis, giardiasis and amoebic liver abscess. Tinidazole was first prescribed in 1967 to treat infections caused by Trichomonas vaginalis. It was subsequently used to treat anaerobic infections such as bacterial vaginosis, intraperitoneal infections, wound infections, skin infections and a variety of protozoal illnesses[1]. In microorganisms, it causes DNA strand breakage or inhibits DNA synthesis. In bacteria, tinidazole causes synthesis inhibition or damage to DNA. It interacts with the electron transport route of ferredoxin in trichomonas, causing nitro group reduction and the consequent production of nitro free radicals, which in turn causes DNA binding and cell death. TNZ is presently accessible in liquid suspension[2] and tablet forms, either as a standalone medication or combined with other antibiotics. Conventional dissolution techniques have been the use of methanol, phosphate buffer (pH 6.8)[3], HCl as the solvent. But because of the fluctuations in vaginal pH and the difficulties in soluble TNZ, a novel approach utilizing phosphate buffer (PB) 4.2 pH with 2% Tween® 80 LR and 4 mL of methanol has been created[4].

MATERIALS AND METHODS

Materials

Using the appropriate cuvettes, the Shimadzu 1900i UV-Visible spectrophotometer was operated. High-Performance Liquid Chromatography (HPLC) or analytical reagent (AR) grade Tween® 80 LR (S D Fine Chem. Limited, Mumbai) and Methanol (Advent Chembio Pvt. Ltd., Mumbai) were used.

Methods

Linearity/Calibration curve

Stock solution preparation

- **Standard stock solution (A):** 100 mg TNZ was accurately weighed and added to a 100 mL volumetric flask. Further, dissolved in phosphate buffer (pH 4.2) containing 2% Tween® 80 LR and 4 mL of methanol to mark[5].
- **Standard working solution (B):** - An aliquot of 10 mL was withdrawn from Solution A and diluted to 100 mL with PB 4.2 containing 2% Tween® 80 LR and 4 mL of methanol in a 100 mL volumetric flask[5].

Working solutions preparation

By placing specified volumes of a TNZ working solution into 10 mL volumetric flasks and modifying the volume with PB 4.2 containing 2% Tween® 80 LR and 4 mL of methanol, standard TNZ solutions with concentrations ranging from 6 µg mL^{-1} to 26 µg mL^{-1} were prepared. A calibration curve was generated using the

absorbance-concentration data, demonstrating strong linearity ($R^2 \approx 0.99$). The absorbance was measured at 318 nm (n = 3)[5].

Precision

Precision in analytical procedures assesses the level of concordance among measurements obtained through multiple analyses of a standardized sample, conducted under specific conditions, and confirmed through both intraday and inter-day sampling[6].

Intraday precision

To assess precision, the procedure was attempted on standard solutions containing 6, 10, 14, 18, 22, and 26 µg mL^{-1} of TNZ, with three replicates (n=3) conducted on the same day. The Relative Standard Deviation (%RSD) values for each concentration were all below 1.0, indicating a high degree of precision within the method[7].

Interday precision

Standard solutions of TNZ (6, 10, 14, 18, 22, and 26 µg mL^{-1}) were analysed in order to assess the method's precision. The precision within a day was assessed by analysing the samples at three different times, that is in morning, afternoon and evening, for Inter-day precision. For Intraday precision, the experiment was repeated thrice a day for 3 consecutive days[8].

Repeatability study

To assess repeatability, the sample was scanned six times in a row using any 1 concentration sample of TNZ ranging from 6-26 µg mL^{-1}. The standard deviation was then recorded[8].

Ruggedness and robustness

The analysts used two distinct UV spectrometers to scan a sample of same concentration thrice, and %RSD was determined[8].

The method's robustness was evaluated by subjecting it to minor variations in the experimental conditions specifically, the change analyst, and %RSD were determined to assess the efficiency of the method.

Accuracy (% recovery)

Method accuracy was verified using the external standard addition method. Measurements were conducted thrice. A recovery experiment at 80%, 100%, and 120% levels assessed accuracy. About 10 mg of TNZ was added to four, 100 mL volumetric flasks, dissolved in 25 mL of PB 4.2 with 2% Tween® 80 LR and 4 mL of methanol, then sonicated for 15 minutes and filtered. 1 mL of the solution was transferred to a 10 mL volumetric flask and diluted to mark with PB 4.2 with 2% Tween 80®LR and 4 mL of methanol to yield a TNZ concentration of 10 µg mL^{-1}. Data from nine trials at three concentration levels were collected to calculate recovery percentage. Accuracy was confirmed within 98-102% range[9].

Limit of detection (LOD) and Limit of quantification (LOQ)

By testing 10 duplicates of standard solutions with a concentration of 6 µg mL^{-1} for TNZ, the method's LOD and LOQ were determined.

LOD

Formula for calculation of LOD[10]. $DL = 3.3 \sigma / S$

where, σ: The standard deviation of the response, S: The slope of the calibration curve

LOQ

Formula for calculation of LOQ[11]. $DL = 10 \sigma / S$

where, σ: The standard deviation of the response, S: The slope of the calibration curve

RESULTS

Linearity/calibration curve

Six data points ranging in concentration from 6 µg mL^{-1} to 26 µg mL^{-1}in PB (pH 4.2) with 2% Tween® 80 LR and 4 mL of methanol were used to evaluate the TNZ linearity. TNZ displayed 0.0309x + 0.0254 as its linear regression equation, demonstrating good linearity (R2 = 0.9962). According to the Indian Pharmacopoeia, the maximum absorption of TNZ in methanol was approximately at 318 nm, which is also the maximum absorption of TNZ in this solution.

Precision

The successful execution of precision testing for TNZ (6-26 µg mL^{-1}) at 318 nm in PB (pH 4.2) with 2% Tween® 80 LR and 4 mL of methanol was confirmed by consistent ratios below 2 for both intraday and interday precision, as mentioned in Table I.

Accuracy

An accuracy evaluation was performed using a three-tier spiking technique, in which standard amounts representing 80%, 100%, and 120% were added to the samples. The outcomes indicated outstanding retrieval,

with TNZ displaying a retrieval range of 100.49% to 100.74%, which falls in the limit. Table I represents the findings of the accuracy study.

Repeatability study

We evaluated repeatability by using a concentration of 18 µg mL^{-1} for TNZ at 318 nm, and the %RSD was determined to be not more than 2%, which is found to be suitable.

Ruggedness and robustness

The tests were performed to assess the ruggedness and robustness of the analysis. This involved changing the analyst, instrument, and wavelengths used. The results

Table I: Precision study of TNZ and % Recovery of TNZ

Type	Day	Conc (µg mL^{-1})	Sampling time			Avg	±SD	%RSD
			Morning					
			1	2	3			
Intraday	1	6	0.196	0.196	0.195	0.195556	0.000527046	0.269512
		10	0.343	0.342	0.343	0.342556	0.000527046	0.153257
		14	0.47	0.47	0.47	0.470333	0.0005	0.106308
		18	0.573	0.573	0.573	0.572667	0.0005	0.087311
		22	0.721	0.721	0.721	0.720778	0.000440959	0.061178
		26	0.813	0.811	0.813	0.812111	0.000927961	0.114265
Interday	2	6	0.196	0.196	0.196	0.192	0.002	0.201265
		10	0.345	0.345	0.346	0.345333	0.00057735	0.167186
		14	0.475	0.476	0.474	0.475	0.001	0.210256
		18	0.567	0.568	0.568	0.567667	0.00057735	0.101706
		22	0.727	0.727	0.729	0.727667	0.001154701	0.158685
		26	0.821	0.824	0.823	0.822667	0.001527525	0.18568
	3	6	0.196	0.195	0.196	0.195667	0.00057735	0.295068
		10	0.344	0.342	0.342	0.342667	0.001154701	0.336975
		14	0.475	0.475	0.473	0.474333	0.001154701	0.243437
		18	0.571	0.568	0.57	0.569667	0.001527525	0.268144
		22	0.724	0.724	0.727	0.725	0.001732051	0.238904
		26	0.819	0.823	0.824	0.822	0.002645751	0.321868

% Recovery of TNZ							
Drug	Spiked level	Amount in mixture (µg mL^{-1})	Amount added (µg mL^{-1})	% Recovery	Average % recovery	± SD	%RSD
TNZ	80%	10	8	98.41	98.416	0.005	0.005
				98.42			
				98.42			
	100%	10	10	100.81	100.81	0.01	0.00
				100.8			
				100.82			
	120%	10	12	102.28	102.286	0.005	0.005
				102.29			
				102.29			

indicated that the absorbance of the sample concentration (18 µg mL^{-1}) remained unaffected by deliberate and subtle variations in factors and levels. We calculated the %RSD for each measurement and found that the results met the required acceptance criteria.

LOD and LOQ

The results of LOD and LOQ for TNZ were 0.05 µg mL^{-1} and 0.15 µg mL^{-1}, respectively.

CONCLUSION

The developed and validated spectroscopic method of TNZ in PB 4.2 with 2% Tween 80® LR and 4 mL of methanol followed ICH recommendations, affirming its accuracy and precision for the vaginal formulations in the treatment of vaginal disorders, thereby exhibiting reliability and consistency. Based on the research findings regarding vaginal delivery, most articles showed that TNZ dissolves in simulated vaginal fluid. They revealed a lack of confirmed information on dissolution of TNZ in PB at a pH of 4.2. This highlighted the necessity to create a technique utilizing a modified PB of pH 4.2 with 2% Tween 80® LR and 4 mL of methanol. This established method is appropriate for accurately identifying and measuring TNZ in vaginal pharmaceutical formulations.

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