Journal of Pharmacreations

PharmaCreations

Pharmacreations |Vol.2 | Issue 1 | Jan-Mar-2015
Journal Home page: www.pharmacreations.com

Research article ***Open Access***

HPLC method development and validation for simultaneous estimation of Doxycycline and Tinidazole

Jeyabaskaran.M[1*], Prof.Rambabu.C[2], Dr.Sree Janardhanan V[3], Uma Maheswara Rao K[3], Lakshmi Maneka S[3] and Ramesh M[3]

[1] *Department of Chemistry, Dr. M.R. Appa rao Campus, Nuzvid, Krishna(Dist) Affiliated to ANU, Guntur, Andhra Pradesh, India.*

[2]*Professor and Head, Department of Chemistry, Dr.M.R.Appa rao Campus, Nuzvid. Krishna (Dist) Affiliated to ANU, Guntur, Andhra Pradesh, India.*

[3] *Browns College of Pharmacy, Ammapalem (V), Konijerla (M) Wyra road, Khammam (Dist), Telangana State, India.*

*** Corresponding author: Jeyabaskaran.M**
E-mail id: jeyabaskar2000@gmail.com

ABSTRACT

The aim of the present work was to develop a gradient RP-HPLC for simultaneous analysis of Doxycycline and Tinidazole in tablet dosage form. Method: chromatographic system was optimized using an agilent XDB C18 (150 x 4.6mm,5µm) column with potassium dihydrogen phosphate (pH 3) and acetonitrile in the ratio of 70;30%v/v, as a mobile phase, at a flow rate of 1.0 ml/min. Detection was carried out at 220 nm by a photodiode array detector. Result: doxycycline and tinidazole were eluted with retention times of 3.372 and 2.490 mins, respectively. Beer's lambert's law was obeyed over the concentration ranges of 50 -200µg/ml and 150 - 600µg/ml for doxycycline and tinidazole, respectively. Conclusion: the high recovery and low coefficients of variation confirm the suitability of the method for simultaneous analysis of both drugs in a tablet dosage form. Statistical analysis proves that the method is sensitive and significant for the analysis of doxycycline and tinidazole in pure and in pharmaceutical dosage form without any interference from the excipients. The method was validated in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible.

Keywords: Doxycycline, Tinidazole, HPLC, ICH guidelines.

INTRODUCTION

Doxycycline and Tinidazole combination of drugs belong to the Tetracycline derivative and Imidazole group respectively. Chemically Doxycycline is 2-(amino-hydroxy-methylidene)-4-dimethylamino-5, 10,11,12atetrahydroxy-6-methyl- 4a,5,5a, 6-tetra hydro-4H-tetracene-1, 3,12-trione is commonly used to treat variety of infections like chronic sinusitis, syphilis.[1-4]. The structure of Doxycycline was shown in the figure-1. Doxycycline is lipophilic and can passes through the lipid bilayer of the bacteria. It binds to the 30S and 50S ribosomal subunits and blocking the binding of aminoacyl tRNA to the mRNA and inhibiting bacterial protein synthesis. Tinidazole is 1-(3-chloro-2-hydroxy propyl) -2-

methyl-5 nitroimidazole is an antiamoebic drug. Tinidazole is a prodrug and antiprotozoal agent. A free nitro radical is generated from the nitro group of Tinidazole through ferrodoxin mediated electron transport system. This free radical covalently binds to DNA causing DNA damage which leads to cell death.[1-3] The structure of Tinidazole was shown in the figure-2.

The liquid chromatographic method for the determination of Doxycycline is the choice of some Pharmacopoeias [5-6]. Several techniques have been reported for the *in vitro* and *in vivo* determination of Doxycycline and include microbiology [7], fluorimetry [8], lanthanide sensitized luminescence [9], chemiluminescence [10], optical fibre sensor [11], solid surface phosphorescence [12], ion selective electrode (ISE)-potentiometry [13], cyclodextrin based fluorosensor [14], internal solid contact sensor based on a conducting polypyrrole [15], thin-layer chromatography (TLC) [16], TLC-fluorescence scanning densitometry (TLC-FSD) [17], derivative spectrophotometry [18] and HPLC for body fluids [19-25]. Tinidazole was estimated in body fluids and in pharmaceutical formulations by spectrophotometry [26-31], potentiometry [31], HPLC methods [31-33], polarography [34,35] and resonance light scattering technique [36]. It is necessary to develop a method to determine the combination both in pure and formulation. The present paper describes a simple, sensitive validated and economic method for the simultaneous determination of doxycycline and tinidazole by HPLC.

All chemicals were of AR-grade. All reagents were of HPLC grade and purchased from Merck pharmaceuticals. The formulation was purchased from the local pharmacy.

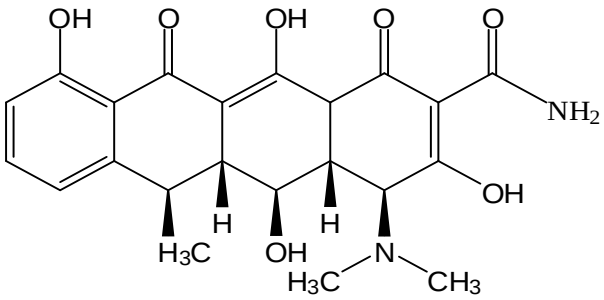

Figure 1: Structure of Doxycycline

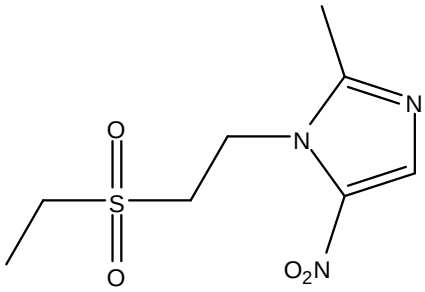

Figure 2: Structure of Tinidazole

INSTRUMENTATION

The analysis was performed on Waters2695 HPLC system with Waters2996 Photodiode Array detector. Data acquisition was performed by using Empower 2 software. Agilent XDB, C18 column (150 x 4.6mm, 5µ) was used as stationary phase. Injections were performed by the manual injector with 10µl. Different mobile phases were tested in order of their polarity to find out the best conditions for the separation of doxycycline and tinidazole. The selected mobile phase Potassium Dihydrogen Phosphate buffer of 0.1% (pH 3) and acetonitrile in the ratio of 70:30%v/v gave acceptable retention time (RT) and good resolution between doxycycline and tinidazole. The flow rate was maintained at 1.0 mL min^{-1}, with a run time of 10 min. The mobile phase was filtered by using 0.45µ filter and it was degassed by sonication prior to use. All determinations were made at ambient temperature.

STANDARD SOLUTION AND CALIBRATION CURVE PREPARATION

Doxycycline (10mg) and Tinidazole (30mg) were weighed and transferred separately to 10ml volumetric flask. Both the drugs were dissolved in 7ml of HPLC grade methanol and the volume was made up with the same to prepare 1000µg/ml. Pipette out 0.5ml of above and transfer in 10ml volumetric flask and make up the volume till the mark with methanol to make 50µg/ml standard solutions. Calibration standards were prepared taking aliquots and further diluted stock solutions in the

concentration ranges of 50 to 200µg/ml and 150 to 600µg/ml for doxycycline and tinidazole respectively and peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

SAMPLE PREPARATION

For the analysis of a tablet dosage form, 20 tablets were weighed individually and their average mass was determined. Then the tablets were crushed to fine powder. Transferred an accurately weighed portion of the powder, equivalent to 100mg of doxycycline and 300mg tinidazole to a 100ml volumetric flask and diluted with methanol till the mark and sonicated for 25 minutes. The solution was filtered through a Whatmann filter paper no.1.Filterate was then appropriately diluted with mobile phase to get a final concentration. Before the assay of the tablet formulations, five replicate aliquot of the appropriately diluted tablet stock solution were sonicated for 15minutes, then injected into the chromatographic system and analyzed quantitatively.

OPTIMISATION OF HPLC METHOD

The HPLC procedure was optimized with a view to develop a simultaneous assay method for doxycycline and tinidazole. Preliminary experiments were carried out to optimize the parameters affecting simultaneous estimation of two drugs. Reverse phase column [Agilent XDB C18 (150 x 4.6mm, 5µ) column] was selected on the basis of the polarity of drugs for analysis. Following parameters were optimized for the development of method i.e. column, wavelength, mobile phase concentration, solvent, flow rate, concentration of buffer. The solvent type, solvent strength, detection wavelength and flow rate were varied to determine the best chromatographic conditions for the separation of doxycycline and tinidazole in chromatogram. The mobile phase conditions were optimized to avoid interference from solvent and formulation excipients. Other criteria, for example, time required for analysis, flow rate of mobile phase, symmetry of eluted peaks, assay sensitivity and solvent noise during drug analysis were also considered. The spectra of the analytes were determined independently and in combination. It was observed that at wavelength 220nm both the drugs could be detected simultaneously with no mobile phase interference, good separation, sensitivity and consistent baseline. The feasibility of various combinations of solvents such as acetonitrile, methanol, buffer and water with altered flow rate (in the range 0.8 – 1.2 ml/min), was investigated for complete chromatographic resolution of above drugs with best sensitivity, efficiency, and peak shape.

METHOD VALIDATION

Validation of an analytical method is a necessary step in controlling the quality of quantitative analysis. Validation can be defined as the process by which it is established, by laboratory studies that the analytical parameters of the method should meet the requirements for the intended analytical applications. Thus, with the background knowledge of linearity, accuracy,

Precision and robustness of the analytical method, it is relatively easy to derive the confidence and the reliability of the analytical data obtained with it. Validated the developed method as per ICH and FDA [37-41] guidelines with parameters like specificity, precision, accuracy, linearity and range, ruggedness and robustness etc..

System Suitability

System suitability parameter was calculated before starting validation parameters. It was determined by taking the Coefficient of variation of peak area, peak asymmetry and theoretical plate of the six standards injections by using the same standard method which given assay method.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of the analyte in the sample.

The range of the analytical procedure is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Linearity established across the range of the analytical procedure. It was determined at seven levels over the range of 25% to 200% of test concentrations. A standard linearity solution was

prepared to attain concentration of 25%, 50%, 75%, 100%, 125%, 150% and 200% of the test concentration. The area at each level is calculated and a graph area versus concentration is plotted. The correlation co-efficient (r^2) was calculated and recorded.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical method was determined by assaying a sufficient number of aliquots of a homogenous sample to be able to calculate statistically valid estimates of standard deviation and relative standard deviation.

Repeatability

Repeatability expresses the precision under the same operating conditions. It was assessed by performing the determination of concentrations and three replicates of working standard solution in intraday and inter day study.

Reproducibility

Reproducibility expresses the precision between laboratories. The reproducibility of an analytical method was determined by analysis of aliquots from homogenous lots in different laboratory. It was assayed by performing six determinations and two replicator of each concentration in two laboratories.

Robustness

The robustness of analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness was studied by evaluating the effect of small but deliberate variations in chromatographic conditions. The conditions studied were flow rate (altered by ±0.2ml/min).

Accuracy and recovery study

The accuracy of an analytical procedure expresses the closeness of the agreement between value which is accepted either as conventional true value or an accepted reference value and value found. Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte (50%, 100% and 150%) in the sample. Accuracy assayed by using nine determinations over a minimum of three concentration levels, covering the specified range (i.e. three concentrations and three replicates of each concentration).

Specificity

In case of the assay, demonstration of specificity requires that it can be shown by the presence of impurities or excipients. It was done by spiking the drug substance or product with the appropriate levels of impurities or excipients and demonstrating that the assay result is unaffected by the presence of these extraneous materials. Placebo (sample without analyte) was prepared in the same way as the sample under the conditions prescribed in the assay method and duplicate injection was taken and observed any significant peak area (not more than 1%) at the analyte RT.

RESULTS

Development and optimization of HPLC method

The proposed method was optimized with a view to develop a suitable analytical method for the analysis of doxycycline and tinidazole in combined pharmaceutical dosage form. It was found that the mobile phase containing Potassium Dihydrogen Phosphate buffer of 0.1% and acetonitrile (pH 3) in the ratio of 70:30%v/v in gradient elution mode at a flow rate of 1.0ml/min gave optimum and adequate peak separation, with less tailing and resulted in the best resolution. All experiments were performed at ambient temperature. Run time was taken 10min for each run. Under the optimum chromatographic conditions, the retention times obtained.

Validation

System suitability

System suitable parameters such as retention time, theoretical plates, peak area, resolution, and peak

asymmetry were determined. The results obtained were statistically analyzed and found within the range (table-1).

Linearity and range

The statistical data obtained are represented in table-2. The result shows that within the concentration range 50 to 200 µg/ml and 150 to 600µg/ml for doxycycline and tinidazole, respectively. There was an excellent correlation between peak area and concentration of each drug.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection and limit of quantification for Doxycycline and Tinidazole were calculated from the linearity data using relative standard deviation of the response and the slope of the calibration curve. The limit of detection of a compound is defined as the lowest concentration of analyte that can be detected. LOD value of Doxycycline and Tinidazole was found to be 0.419 and 2.228 µg/mL respectively. The limit of quantification is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. LOQ value of Doxycycline and Tinidazole was found to be 1.27 and 6.75µg/mL respectively.

Repeatability

The results of the intraday and inter day precision experiments are shown in table – 3. Separation of the drugs was found to be similar when analysis was performed on different time (intraday) and on different days (inter day). The developed method was found to be precise, with relative standard deviation (RSD) values less than 2%.

Reproducibility

The results of the reproducibility experiments (performed in different laboratories) are shown in table -4. The developed method was found to be precise, with RSD values less than 2%

Robustness

Minor change in chromatographic condition (change in flow rate, altered by 0.2ml/min) did not cause a significant change in, peak area, theoretical plates and RT of doxycycline and tinidazole.(table - 5).

Accuracy and recovery study

Good recoveries of the doxycycline (98.61 -100.88) and Tinidazole (94.966 – 100.87) were obtained at various added concentrations for the Doxycycline and Tinidazole (table - 6).

Specificity

Injections of placebo (sample without analyte) were performed to confirm specificity of method. Obtained results show that excipients mixture of the tablet shows no specific peak at the RT of the analyte peak. This shows that the excipients do not interfere with the analyte peak and the assay is specific for the simultaneous estimation of doxycycline and tinidazole tablets.

DISCUSSION

A suitable analytical procedure refers to the way of performing the analysis with accuracy and precision. This developed method is describes in detail the steps necessary to perform each parameter for validation. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The quality control laboratory requires analytical methods which are simple, robust, and rugged, interpretation of results of method is directly proportional to the concentration of analyte within a given range shows linearity of method. Different environmental condition doesn't cause any significant change in results shows stability and reproducibility of developed method. There was no interference by excipients with analyte peak shows proposed method is specific for analyte. As well as recovery study shows the developed method is highly accurate. Hence the proposed HPLC method has been evaluated and validated for the accuracy, precision, and linearity and found to be convenient, sensitive and specific for the quality control of doxycycline and tinidazole in tablet dosage form.

Table.1.: System suitability tests (S.D – Standard Deviation, R.S.D – Relative Standard Deviation)

Parameter	**Compound**	
	Doxycycline	**Tinidazole**
Retention Time	S.D – 0.033	S.D – 0.0102
	R.S.D – 1.049	R.S.D – 0.4121
Peak Area	S.D – 3504.5	S.D – 5027
	R.S.D – 0.6	R.S.D – 0.4
Peak Asymetry	S.D – 0.024	S.D – 0.0167
	R.S.D – 1.708	R.S.D – 1.415
Theortical Plates	S.D – 59.69	S.D – 142.75
	R.S.D – 1.259	R.S.D – 2.901

Table. 2.: Linearity parameters for calibration curves of DOXY and TINI

Compound	**Level of conc. In µg/ml**	**Slope**	**Intercept**	**Correlation coefficient (r^2)**
		Mean ± S.D	**Mean ± S.D**	
DOXY	50 - 200	4776.3 ± 6.658	4182.3 ± 606.6	0.999
TINI	150 - 600	4070.6 ± 19.65	3510.6 ± 2748.8	0.999

Table.3.: Precision of method. (S.D – Standard Deviation, R.S.D – Relative Standard Deviation)

Compound	**Interday**		**Intraday**	
	Mean area ± S.D	**R.S.D**	**Mean area ± S.D**	**R.S.D**
DOXY	542501 ± 1949.4	0.4	555289 ± 3504.5	0.6
TINI	1420592 ± 6538	0.5	1383458 ± 5027	0.4

Table.4.: Ruggudness study. (S.D – Standard Deviation, R.S.D – Relative Standard Deviation)

Compound	**Analyst - 1**		**Analyst – 2**	
	Mean area ± S.D	**R.S.D**	**Mean area ± S.D**	**R.S.D**
DOXY	555289 ± 3504.5	0.6	568496 ± 51107.6	9.0
TINI	1383458 ± 5027	0.4	1436252 ± 110282.3	7.7

Table.5.: Robustness study. (S.D – Standard Deviation, R.S.D – Relative Standard Deviation)

Parameter		**Doxycycline**		**Tinidazole**	
		Mean area ± S.D	**R.S.D**	**Mean area ± S.D**	**R.S.D**
Flow Rate	0.8ml/min	609739 ± 2135.7	0.4	1560916 ± 903.6	0.1
	1.2ml/min	519783 ± 2501.7	0.5	1266861 ± 1748.6	0.1
Mobile Phase	65:35	544005 ± 7434.4	1.4	1385404 ± 10568.4	0.8
	75:25	555521 ± 1684.4	0.3	1401547 ± 2316.8	0.2
Temperature	- 5 □ C	553059 ± 159.9	0.0289	1389294 ± 2454.9	0.2
	+ 5 □ C	548788 ± 2628.7	0.5	1385268 ± 2628.7	0.1

Table.6.: Accuracy and recovery study. (S.D – Standard Deviation, R.S.D – Relative Standard Deviation)

Compound	Amount of drug added (%)	Theoretical cotent (µg/ml)	Concentration found (µg/ml) ±SD	RSD (%)	Recovery (%)
Doxycycline	50	50	49.86 ± 0.57	1.143	99.74
	100	100	100.82 ± 0.78	0.7736	100.82
	150	150	148.67 ± 0.59	0.3968	99.11
Tinidazole	50	150	147.37 ± 4.54	3.08	98.25
	100	300	300.91 ± 1.071	0.3559	100.29
	150	450	445.96 ± 3.07	0.6907	98.92

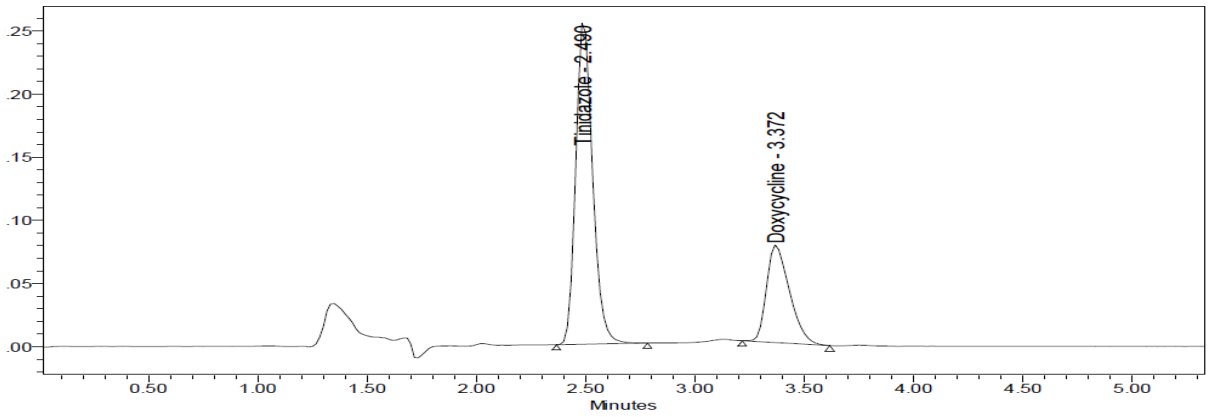

Figure.3 chromatogram of assay of doxycycline and tinidazole

REFERENCE

[1] O' Neil M.J. The Merck Index: An Encyclopedia of Chemicals, Drug and Biologicals. 14thedn. Merck& Co. Inc. 2006. 3440 and 9447.

[2] Sweetman S C. Martindale: The Complete Drug Reference. 35thedn, Pharmaceutical press. London (U.K).2007. 238 and 764.

[3] British pharmacopoeia. Medicines and Health care Products regulatory agency (MHRA). London. 2008(1): 2655 and 2148.

[4] Quarterman MJ, Johnson DW, Abele DC, Lesher JL, Hull DS, Davis LS. Ocular rosacea. Signs, symptoms, and tear studies before and after treatment with doxycycline. Arch Dermatol 1997, 133, 49–54.

[5] Chinese Pharmacopoeia, Commission of the Ministry of Health, Beijing, 2000, 593.

[6] US Pharmacopoeia, The United States Pharmacopoeia Convention Inc., Rockville, MD, 1995, vol. XXIII, 557-559.

[7] Hojer H, Nilsson L: Rapid determination of doxycycline based on luciferase assay of bacterial adenosine triphosphate. J Antimicro Chemo,1978, 4, 503-508.

[8] Van den Bogert C, Kroon A M: Fluorometric determination of tetracyclines in small blood and tissue samples. J Pharm Sci, 1981, 70, 186-189.

[9] Izquierdo P, Gomez Hens A, Perez Bendito D: Simultaneous stopped flow determination of tetracycline and doxycycline in serum based on lanthanide-sensitized luminescence. Anal Lett, 1994, 27, 2303-2316.

[10] Salinas F, Muñoz de la Peña A, Duran MI: Indirect flow injection chemiluminescence method for the determination of tetracyclines using Cu (II) as a probe ion. Anal Lett 1990, 23, 863-876.

[11] Liu WH, Wang Y, Tang JH, Shen, GL, Yu RQ: Optical fiber sensor for tetracycline antibiotics based on fluorescence quenching of covalently immobilized anthracene. Analyst 1998, 123, 365-369.

[12] Xie HZ, Dong CA, Jin WJ, Wei YS, Liu CS, Zhang SS, Zhou BL: Solid surface room temperature phosphorescence of tetracycline antibiotics. Anal Chim Acta, 1996, 319, 239-247.

[13] Shoukry AF, Badawy SS: Determination of tetracycline and related compounds using plastic membrane ion-selective electrodes. Microchem J, 1987, 36, 107-112.

[14] Gong ZL, Zhang ZJ: Determination of tetracyclines with a modified β-cyclodextrin based fluorosensor. Anal Chim Acta 1997, 351, 205-210.

[15] Xian XS, Hassan YA. Internal solid contact sensor for the determination of doxycycline hydrochloride in pharmaceutical formulation. Talanta, 2002, 58, 387-396.

[16] Naidong W, Greelen S, Roets E, Hoogmartens J: Assay and purity control of oxytetracycline and doxycycline by thin-layer chromatography– a comparison with liquid chromatography. J Pharm Biomed Anal, 1990, 8, 891-896.

[17] Xie HZ, Dong C, Fen Y, Liu CS: Determination of doxycycline, tetracycline and oxy tetracycline simultaneously by TLC-fluorescence scanning densitometry. Anal Lett , 1997, 30, 79-90.

[18] Salinas F, Berzas Nevado JJ, Espinosa A: Determination of oxytetracycline and doxycycline in pharmaceutical compounds, urine and honey by derivative spectrophotometry. Analyst, 1989, 114, 1141-1145.

[19] Farin D, Piva G, Gozlan I, Kitzes R: High performance liquid chromatographic method for the determination of doxycycline in human plasma. Chromatographia, 1998, 47, 547-549.

[20] Ruz N, Zabala M, Kramer MG, Campanero MA, Dios-Viéitez MC, Blanco-Príeto MJ: Rapid and simple determination of doxycycline in serum by high-performance liquid chromatography: Application to particulate drug delivery systems. J Chromatogra, 2004, 1031, 295-301.

[21] Riond JL, Hedeen KM, Tyczkowska K, Riviere JE: Determination of doxycycline in bovine tissues and body fluids by high-performance liquid chromatography using photodiode array ultraviolet-visible detection. J Pharm Sci, 2006, 78, 44-47.

[22] Gastearena I, Dios-Vieitez MC, Segura E, Goni MM, Renedo MJ, Fos D: Determination of doxycycline in small serum samples by liquid chromatography. Application to pharmacokinetical studies on small laboratory animals. Chromatographia, 1993, 35, 524-526.

[23] Zarghi A, Kebriaeezadeh A, Ahmadkhaniha R: Rapid high-performance liquid chromatographic method for determination of doxycycline in human plasma. Bull Chim Farm, 2001, 140, 112-114.

[24] Prévosto JM, Béraud B, Cheminel V, Gaillard Y, Mounier C, Chaulet JF: Determination of doxycycline in human plasma and urine samples by high performance liquid chromatography. Application for drug monitoring in malaria chemoprophylaxis. Annal Biolog Clin, 1995, 53,29-32.

[25] Santos MD, Vermeersch H, Remon JP, Schelkens M, De Backer P, Ducatelle R, Haesebrouck F: Validation of a high-performance liquid chromatographic method for the determination of doxycycline in turkey plasma. J Chromatogra B Biomed Appl,1996, 682, 301-308.

[26] Singh L: Validated method for determination of tinidazole using direct colorimetry, UV-visible spectrophotometry and difference spectrophotometry in pure and tablet dosage form. J Pharmacy Research, 2010, 3: 1211

[27] Okunrobo LO: Titrimetric and spectrophotometric determination of tinidazole tablets, World J Chem, 2007, 2: 63-66.

[28] Prasad CV, Sripriya V, Saha RN, Parimoo P: Simultaneous determination of tinidazole, furazolidone and diloxanide furoate in a combined tablet preparation by second-derivative spectrophotometry. J Pharm Biomed Anal, 1999, 21:961-968.

[29] Adegoke OA, Umoh OE: A new approach to the spectrophotometric determination of metronidazole and tinidazole using p-dimethyl amino benzaldehyde. Acta Pharm, 2009, 59: 407-419.

[30] Prasad CV, Parihar C, Sunil K, Parimoo P: Simultaneous determination of tinidazole-clotrimazole and tinidazole-norfloxacin in combined tablet preparations by derivative spectroscopy. J Pharm Pharmacol Commun,1997, 3: 337-341.

[31] Basavaiah K, Nagegowda P, Chandrashekar U: Determination of tinidazole by potentiometry, spectrophotometry and high performance liquid chromatography. Indian J Chemical Technology, 2005,12: 273-280.

[32] Feng MX, Gao H, Yu FY: Determination of tinidazole and its degradation products in tinidazole injections by reverse-phase HPLC. Fenxi Zazhi, 1997, 17: 247-249.

[33] Pai PN, Rao GK, Srinivas B, Puranik S: RPLC Determination of Tinidazole and Diloxanide Furoate in Tablets. Indian J Pharm Sci, 2008, 70: 670-672.

[34] Abu Zuhri AZ, Al-Khalil S, Shubietah RM, El-Hroub I: Electrochemical study on the determination of tinidazole in tablets. J Pharm Biomed Anal, 1999 21:881-886.

[35] Rege PV, Sathe PA, Salvi VS, Trivedi ST: Simultaneous determination of norfloxacin and tinidazole in combined drug formulation by a simple electroanalytical technique. Int J Pharm Research Development, 2011, 3, 271-279.

[36] Jiang XY, Chen XQ, Dong Z, Xu M : The application of resonance light scattering technique for the determination of tinidazole in drugs. J Autom Methods Manag Chem,2007, 2007: 86857.

[37] International Conference on Harmonization, Q2A: Text on Validation of Analytical Procedures, Federal Register, 1995, 60(40), 11260–11262.

[38] International Conference on Harmonization, Q2B: Validation of Analytical Procedures: Methodology and Availability, Federal Register, 1997, 62(96), 27463–27467.

[39] FDA, Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation, Availability, Federal Register (Notices), 2000, 65(169), 52776–52777.

[40] www.fda.gov/cder/guidance/cmc3.pdf

[41] USP 25–NF 20, Validation of Compendial Methods Section (1225) (United States Pharmacopoeal Convention, Rockville, Maryland, USA, 2002), 2256.