International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 299-308 www.ijprajournal.com ISSN: 2249-7781

Stability Indicating UV-Visible Spectroscopic Method For Simultaneous Estimation Of Bismuth Sub-Citrate, Metronidazole And Tetracycline In Their Formulation

[1]Dr.B.Prathap, [2]Sola Niharika,[3]CH.Sandhya, [4]K. Divya Charitha, [5]B.Manoja, [6]K.Swathi,

Principal, Dhanalakshmi Srinivasan College of Pharmacy, Perambalur,Tamilnadu.
Ratnam Institute of Pharmacy, Nellore, Andhra Pradesh
Corresponding Author: Sola Niharika

Date Of Submission: 05-05-2021 Date Of Acceptance: 22-05-2021

ABSTRACT: A simple accurate, precise method was developed for the simultaneous estimation of the bismuth sub-citrate, tetracycline and metronidazole in their formulation. The method involved solving simultaneous equations based on measurement of absorbance at three wavelengths, 217nm, 249nm, and 289nm i.e., λmax of the bismuth sub-citrate, tetracycline and metronidazole respectively. Beer's law was obeyed in the concentration range of 14-70µg/ml, 12.5-62.5µg/ml, and 1.60-4.80µg/ml for bismuth sub-citrate, tetracycline and metronidazole and correlation coefficient (r^2) values are 0.9999, 0.9999 and 0.9998 respectively. The proposed method was validated as per the ICH guidelines for parameters like linearity, specificity, precision, accuracy, robustness and ruggedness. %RSD of the tetracycline, bismuth sub-citrate and metronidazole were found to be 0.395, 0.232 and 0.601 respectively. The % mean recovery of bismuth sub-citrate, tetracycline and metronidazole was found to be 99.51%, 99.42% and 100.47% respectively. The method was accurate, specific, precise and found to be suitable for the quantitative analysis of the drug and dosage form.

KEYWORDS: Simultaneous Estimation, Metronidazole, Bismuth sub-citrate, Tetracycline, UV-visible spectroscopy[1-6].

I. INTRODUCTION

Simultaneous estimation method[7] (Vierodt's method) is used to determine the concentration of several components present in the same mixture even if their spectra overlap. If a sample contains two absorbing drugs (X and Y) each at which absorbs at the $\lambda_{max}$of the other, it may be possible to determine both drugs by technique of simultaneous equations that certain criteria apply.
The information required is

a) The absorptivities of X at λ_1 and λ_2, a_{x1} and a_{x2}, respectively
b) The absorptivities of Y at λ_1 and λ_2, a_{y1} and a_{y2}, respectively
c) The absorbances of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.

Let C_x and C_y be the concentrations of X and Y respectively in the diluted sample.

Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbances of X and Y.

At λ_1, $A_1 = a_{x1}bc_x + a_{y1}bc_y$. (1)

At λ_2, $A_2 = a_{x2}bc_x + a_{y2}bc_y$. (2)

Then the concentrations can be calculated as

$$C_x = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2} \;\&\; C_y = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

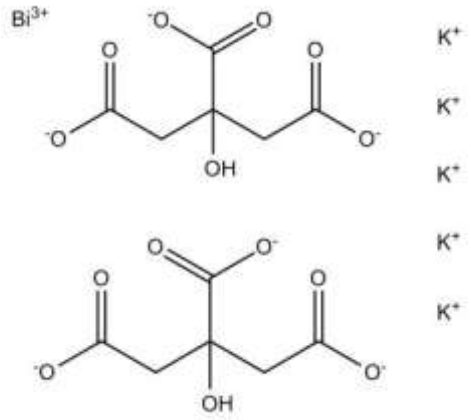
Bismuth sub-citrate[15-17] (bismuth; pentapotassium;2-hydroxypropane-1,2,3-tricarboxylate) is a mineral and a soluble complex, bismuth salt of citric acid used to treat stomach ulcers caused by Helicobacter pylori infections in combination of metronidazole and tetracycline. Bismuth sub-citrate is very effective in the treatment of gastro duodenal disorders and seems to act via several mechanisms.It has little acid neutralizing effect and does effect acid secretion.It is uncertain whether it affects pepsin secretion, but it does inhibit peptic activity. It causes an increase in mucus glycoprotein secretion and may also bind to the gastric mucus layer to act as a diffusion barrier to HCl.It accelerates ulcer healing and causes an accumulation of epidermal growth factor around the ulcer. In addition, it has a cytoprotective effect and increases mucosal secretion of prostaglandins and bicarbonate. It quickens ulcer recuperating and

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 299-308 www.ijprajournal.com ISSN: 2249-7781

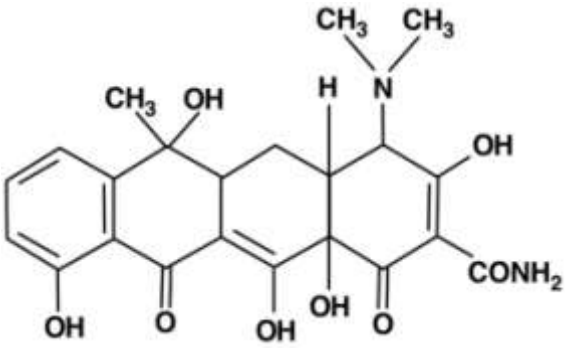
causes an amassing of epidermal development factor around the ulcer. It has bactericidal effects against Helicobacter pylori (which is associated with gastritis and peptic ulcers). It also prevents adhesion of H. pylori to epithelial cells and can inhibit enzymes secreted by H. pylori, such as protease, lipases, glycosidases and phospholipases.

Tetracyclines[18]((4S,4aS,5aS,6S,12aR)-4-(dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide) are called broad – spectrum antibiotics because they can be utilized to treat a wide variety of bacterial infections like skin, eye, intestines, respiratory tract, urinary tract, lymph nodes, and other body systems. Doctors may prescribe these medications to treat pneumonia, gonorrhoea, Rocky Mountain spotted fever, and other infections caused by microorganisms. Tetracycline is a short-acting antibiotic that inhibits bacterial growth by inhibiting translation. It binds to the 30S ribosomal subunit and prevents the amino-acyl t-RNA from binding to the A site of the ribosome.It also binds to the 50S ribosomal subunit. This binding is reversible in nature. During protein biosynthesis, the new t-RNA with the amino acid attempts to bind to A-site of the ribosome.

Metronidazole[19-21](1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) is used to treat bacterial infections in different areas of the body. The extended release tablets are used to treat women with vaginal infections (bacterial vaginosis). It works by killing the bacteria or preventing their growth. Metronidazole is primarily used to treat pelvic inflammatory diseases (along with anti-bacterial like ceftriaxone), pseudomembranous colitis, aspiration pneumonia, rosacea(topical), fungating wounds(topical), intra-abdominal infections, lung abscess, periodontitis, amoebiasis and oral infections. It is also often used to eradicate Helicobacter pylori along with other drugs and to prevent infection in people recovering from surgery.

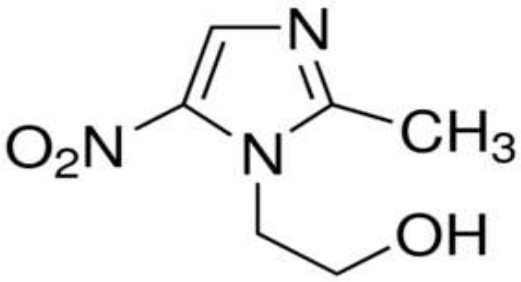


Structure of Bismuth sub-citrate



Structure of Tetracycline

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 299-308 www.ijprajournal.com ISSN: 2249-7781



Structure of metronidazole

II. MATERIALS AND METHODS

Instruments

A UV/VIS spectrophotometer, model LAB INDIA 3000+, with a spectral band width of 1.5nm with a pair of 10mm quartz cell was used for the experimental work.

Reagents and chemicals

All the chemicals used were of AR grade. Methanol and water was obtained from RANKEM, and the standards Bismuth sub-citrate, Tetracycline and Metronidazole was procured from KP LABS.

Procedures

Preparation of standard solution

Accurately weigh about 10mg of bismuth sub-citrate and transfer it into a 10ml volumetric flask add methanol and shake it. Final volume of solution was obtained by adding methanol up to the mark. (1000µg/ml) (Stock-I).

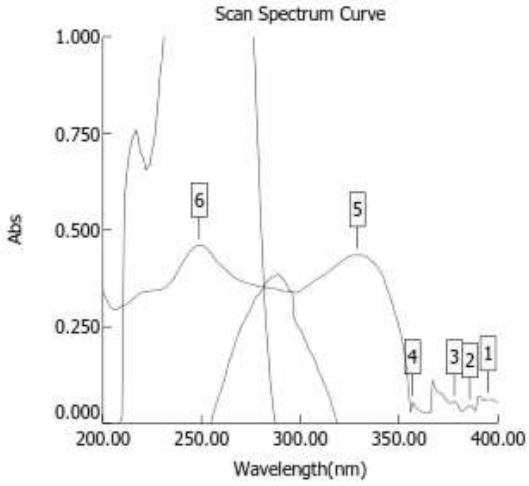
Preparation of sample solutions

Pipette out 1ml from the standard solution and transfer it into a 10ml volumetric flask and make upto the mark with methanol (stock-II). From this 10µg/ml concentrations were prepared and scanned in the entire UV range of 200-400nm.

III. METHOD DEVELOPMENT

Wavelength selection

The Standard solutions of Metronidazole 37.5µg/ml, Tetracycline 37.5µg/ml and 42 µg/ml of Bismuth Sub citrate are scanned in UV Spectroscopy in the range of 200 nm to 400 nm.



Overlain spectra of Metronidazole, Tetracycline and Bismuth sub-citrate

Assay

The sample and standard solutions were scanned and the absorbances are taken.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 299-308 www.ijprajournal.com ISSN: 2249-7781

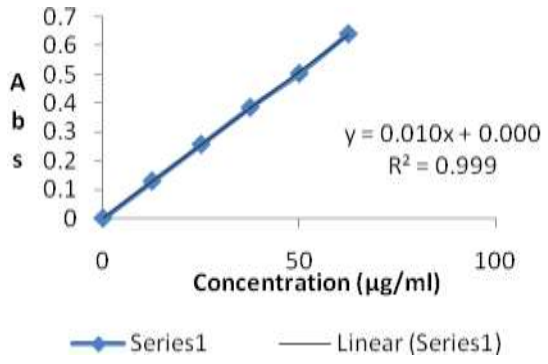
DRUG	LABEL CLAIM (MG)	% ASSAY
METRONIDAZOLE	125	96.96
TETRACYCLINE	125	95.63
BISMUTH SUB CITRATE	140	99.01

Assay results of Metronidazole, Tetracycline and Bismuth sub-citrate

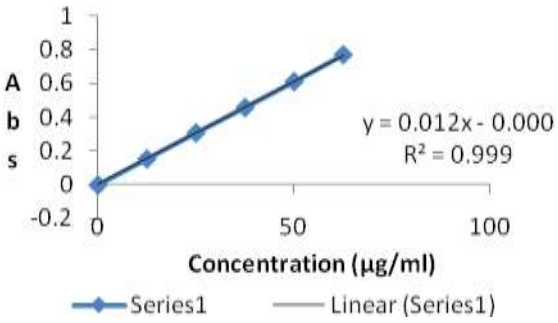
IV. VALIDATION PARAMETERS

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity was determined by series injections of whose concentrations span 50-150 of the expected concentration range. The linearity range was found to lie from 12.5µg/ml to 62.5µg/ml of Metronidazole, Tetracycline and 14 µg/ml to 70 µg/ml Bismuth Sub Citrate. The calibration curve of Metronidazole, Tetracycline and Bismuth sub-citrate are shown below.

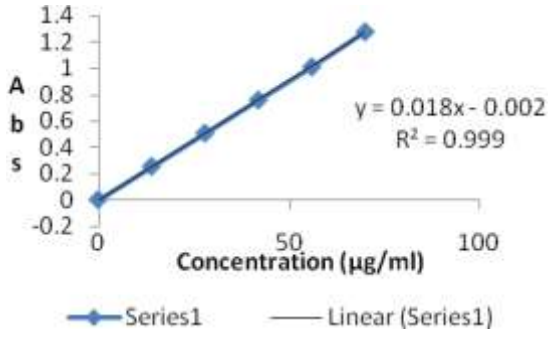


Calibration curve of Metronidazole



Calibration curve of Tetracycline

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 299-308 www.ijprajournal.com ISSN: 2249-7781



Calibration curve of Bismuth sub-citrate

Accuracy

The accuracy of an analytical method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value.Theaccuracystudywasperformed for50%, 100% and150 %for all drugs.Eachlevelwasscanned intriplicate intothesystem. The Absorbance of each level was usedforcalculationof%recovery.

% CONCENTRATION (AT SPECIFIC LEVEL)	ABSORBANCE	AMOUNT ADDED	AMOUNT FOUND	% RECOVERY	MEAN RECOVERY
METRONIDAZOLE					
ACCURACY 50%	0.197	6.25	6.52	104.30	100.47
ACCURACY 100%	0.377	12.5	12.18	97.47	
ACCURACY 150%	0.578	18.75	18.68	99.63	
TETRACYCLINE					
ACCURACY 50%	0.225	6.25	6.29	100.70	99.42
ACCURACY 100%	0.446	12.5	12.23	97.83	
ACCURACY 150%	0.682	18.75	3.03	99.73	
BISMUTH SUB CITRATE					
ACCURACY 50%	0.376	7	7.01	99.93	99.51
ACCURACY 100%	0.751	14	13.86	99.01	
ACCURACY 150%	1.133	21	20.91	99.58	

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 299-308 www.ijprajournal.com ISSN: 2249-7781

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 homogeneous sample under the prescribed conditions. Accurately weigh and transfer 10mg of formulation (3.205mg of Metronidazole, 3.205mg of Tetracycline and 3.58mg of Bismuth Sub-citrate) into a 10ml clean dry volumetric flask (STOCK-I) & add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Make

RESULTS	ABS OF METRONIDAZOLE AT 289 NM	ABS OF TETRACYCLINE AT 249 NM	ABS OF BISMUTH SUB-CITRATE AT 217 NM
ID Precision-1	0.388	0.458	0.754
ID Precision-2	0.386	0.454	0.752
ID Precision-3	0.384	0.452	0.753
ID Precision-4	0.387	0.453	0.756
ID Precision-5	0.385	0.454	0.754
ID Precision-6	0.385	0.452	0.757
AVERAGE	0.386	0.454	0.754
STANDARD DEVIATION	0.001	0.002	0.002
%RSD	0.382	0.491	0.247

Intermediate precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Accurately weigh and transfer 10mg of formulation (3.205mg of Metronidazole, 3.205mg of Tetracycline and 3.58mg of Bismuth Sub-citrate) into a 10ml clean dry volumetric flask (STOCK-I) & add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Make further dilutions and Scan the solution 3 timess in range of 200-400nm.

RESULTS	ABS OF METRONIDAZOLE AT 289 NM	ABS OF TETRACYCLINE AT 249 NM	ABS OF BISMUTH SUB-CITRATE AT 217 NM
Precision-1	0.385	0.452	0.755
Precision-2	0.382	0.454	0.752
Precision-3	0.386	0.451	0.754
Precision-4	0.389	0.452	0.755
Precision-5	0.384	0.456	0.753
Precision-6	0.385	0.453	0.757
AVERAGE	0.385	0.453	0.754
STANDARD DEVIATION	0.002	0.002	0.002
%RSD	0.601	0.395	0.232

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 299-308 www.ijprajournal.com ISSN: 2249-7781

Robustness

Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method's robustness, a number of method parameters, for example, pH, flow rate, column temperature, injection volume, detection wavelength or mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined.

SET-I Accurately weigh and transfer 10mg of formulation (3.205mg of Metronidazole, 3.205mg of Tetracycline and 3.58mg of Bismuth Sub-citrate) into a 10ml clean dry volumetric flask (STOCK-I) & add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Make further dilutions and Scan the solution 3 times in range of 200-400nm.

SET-II repeat the same procedure and scan after 24hrs in the range of 200-400nm.

S.NO	WAVE LENGTH (NM)	ABSORBANCE
ROBUSTNESS OF METRONIDAZOLE		
1	284 nm	0.388
2	289nm	0.386
3	294nm	0.387
ROBUSTNESS OF TETRACYCLINE		
1	244nm	0.452
2	249nm	0.455
3	254nm	0.454
ROBUSTNESS OF BISMUTH SUB-CITRATE		
1	212nm	0.755
2	217nm	0.751
3	222nm	0.752

V. DEGRADATION STUDIES

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. Stress degradation studies are carried out on the Metronidazole, Tetracycline and Bismuth sub-citrate using the proposed method.

Forced degradation:

Under acidic condition: From the above prepared stock solution, pipette out 1ml and make up to 10ml with methanol in volumetric flask (stock-II). To this add 1ml of 0.1N HCl and keep it aside for an hour. Now the scan the solution in the range of 200-400nm.

Under basic condition: From the above prepared stock solution, pipette out 1ml and make up to 10ml with methanol in volumetric flask (stock-II). To this add 1ml of 0.1M NaOH and keep it aside for an hour. Now the scan the solution in the range of 200-400nm.

% DEGRADATION RESULTS	METRONIDAZOLE		TETRACYCLINE		BISMUTH SUBCITRATE	
	ABS	% DEGRADATION	ABS	% DEGRADATION	ABS	% DEGRADATION
STANDARD	0.386		0.455		0.757	
ACID	0.369	4.3	0.440	3.2	0.719	5.1
BASE	0.378	2.6	0.448	1.6	0.706	6.8

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 299-308 www.ijprajournal.com ISSN: 2249-7781

VI. CONCLUSION

An attempt was made to develop and validate a stability indicating UV-VISIBLE method for the simultaneous estimation of the bismuth sub-citrate, tetracycline and metronidazole in their formulation and the solvent used was methanol. The optimized wavelength for bismuth sub-citrate, tetracycline and metronidazole was at 281nm. The developed method is validated based on ICH guidelines.

The UV-Visible method was developed for the quantification of metronidazole, tetracycline and bismuth sub-citrate.

The linearity lies between 12.5-62.5 µg/ml for metronidazole and tetracycline, 14 –70 µg/ml for Bismuth sub-citrate.

The stress degradation studies was indicated for bismuth sub-citrate, tetracycline and metronidazole (below 10%), it shows no degradation was found in the proposed method.

The advantages present in the simplicity of sample preparation, the low costs of reagents used. The proposed method ensures sufficient and precise quantification of the compounds. Hence, the proposed method is rapid, accurate, precise, specific, robust, and economical. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, the proposed method can be used for routine drug analysis of Metronidazole, Tetracycline and Bismuth sub-citrate in their combination.

REFERENCES

[1]. Beckett A.H and StenlakeJ.B;text book of pharmaceutical chemistry 4th Edn,-part-2 CBS publishers and Distriburots,New Delhi,1998, 278-307
[2]. StinderAhuja, Stephen Scypinski, Hand book of modern pharmaceutical Analysis, vol 3(1)
[3]. https://www.omicsonline.org/pharmaceutical-sciences-journals.php
[4]. https://www.sciencecompany.com/UltravioletorUVLightAnIntroduction.Aspx.
[5]. Synder, L.R. Introduction to Modern chromatography, 2nd Edition, 1979.
[6]. Practical pharmaceutical chemistry 4th edition – part two, edited by A.H.Beckett, J.B.Stenlake, page no. 275-278.
[7]. Practical pharmaceutical chemistry 4th edition – part two, edited by A.H.Beckett, J.B.Stenlake, page no. 284-286.
[8]. Dr. Mohammed Alzuhairi. Introduction to High Performance Liquid Chromatography; Jun 06, 2016.
[9]. A WHO guide to cGMP; requirements, part 2: validation, Jan 2001.
[10]. A Guidelines on general principles of validation, CDER, May 1987.
[11]. LesblaneDA.Rinse sampling for cleaning validation studies. Pharma tech, vol-22(5); 1998, 66-74.
[12]. ICH harmonized Tripartite Guidelines, Validation of analytical procedures: Text and methodology Q2(R1), November 2005.
[13]. A guide to good manufacturing practice 1996, 1st edition, chapter 9.
[14]. K.Kathiresan and Kiran Krishnan. Basics of validation – pharmaceutical perspective, 1st edition, K.K. Publisher's Chidambaram, 2005, 1-122
[15]. https://www.drugbank.ca/drugs/DB09275
[16]. Material safety data sheet(MSDS), Bismuth subcitrate potassium, APExBIO, An apoptosis and epigenetics company, 6-30-2016, 1-5.
[17]. https://www.ndrugs.com/?s=bismuth%20subcitrate%20potassium/metronidazole/tetracycline%20hydrochloride&t=actions New Zealand data sheet, metronidazole, 25 february 2013, 1-14.
[18]. https://www.drugbank.ca/drugs/DB00759
[19]. https://www.drugbank.ca/drugs/DB00916
[20]. Evren H. Turgut, Mine Özyzici, Bioavailability File: Metronidazole, Journal of Pharmaceuitical Sciences, 29, 2004, 39-49.
[21]. YunqiWua, Reza Fassihib,Stability of metronidazole, tetracycline Hcl and famotidine alone and in combination,International Journal of Pharmaceutics, 2005, 1–13
[22]. Elena Gabriela Oltean, A. Nica, Development and Validation of A RP-HPLC Method for The Quantization Studies of Metronidazole in Tablets and Powders Dosage Forms, Vol. 5(2), December 2011, 71-73
[23]. PratibhaVerma, VarshaNamboodiry, Soni Mishra, Ashok Bhagwat, SuvarnaBhoir, A Stability Indicating HPLC Method for The Determination of Metronidazole Using Ecofriendly Solvent as Mobile Phase Component,International Journal of Pharmacy and Pharmaceutical Sciences, vol-5(2), 2013, 469-501.

[24]. Marina Silva, Simone Schramm, Eunice Kano, Eunice Koono, ValentinaPorta, and Cristina Serra, Development and Validation of a HPLC–MS–MS Method for Quantification of Metronidazole in Human Plasma, journal of chromatographic science, vol-47, October- 2009, 781-784.
[25]. Amit J. Kasabe, Vikram V. Shitole, Vikram V. Waghmare, Vijay Mohite, Simultaneous Estimation of Metronidazole and Ofloxacin in Combined dosage form by Reverse Phase High Performance Liquid Chromatography Method, International Journal of Chemistry and Technology Research, vol-1(4); Oct-Dec 2009, 1244-1250,
[26]. KauserFathima, Dr. Osman Ahmed, Syed Vakeeluddin, Analytical Method Development and Validation for The Simultaneous Estimation of Ofloxacin and Metronidazole in Bulk and Dosage Form using RP-HPLC, indo American journal of pharmaceutical sciences, vol-4(7), 2017, 2017-2018.
[27]. A. B. Patel, N. J. Shah and N. M. Patel, Simultaneous Estimation of Metronidazole and Ciprofloxacin by RP-HPLC Method in Bulk Drug and Suspension, International Journal of Chemical Sciences, vol-7(3), 2009, 2115-2121
[28]. S.S. Khadabadi and Meenakshi G. Devkar, A Validated Rp-Hplc Method for Simultaneous Estimation of Metronidazole and Ciprofloxacin Hydrochloride in Pharmaceutical Dosage Form, International Journal of pharmaceutical sciences and Research, vol-4(12), 2013, 4736-4740
[29]. Minal R. Ghante, Harpreet K. Pannu, AmrutaLoni, TejashreeShivsharan, Development and Validation of A RP-HPLC Method for Simultaneous Estimation of Metronidazole and Norfloxacin In Bulk and Tablet Dosage Form, International Journal of Advances in Pharmaceutics, vol-4, 11 May 2012, 241-245.
[30]. Kuldip D Trivedi, Ankit B Chaudhary and Sellapan Mohan, Devlopment and validation of RP-HPLC Method for estimation of Metronidazole and Norfloxacin in suspension form, International Journal of Advances in Pharmaceutics, vol-2 (1), 2013, 5-11
[31]. SK AsmaParveen, ChandanaNalla, Development and Validation of a Simple and Rapid RP- HPLC Method for The Determination of Metronidazole and Norfloxacin In Combined Dosage Form, Indian Journal of Research in Pharmacy and Biotechnology,September – October 2013, 686- 691
[32]. Danao K.R, Hiradeve S.M, Moon R.S, Kasture A.V, Yeole P.G, RP-HPLC simultaneous estimation of metronidazole and diloxanide furoate in combination, International Journal of Pharmacy & Life Sciences, vol-1(2), June 2010, 82-85
[33]. Kalpana MJ, Naga sowjanya G, Ajitha A, Uma Maheshwara Rao V, Analytical Method Development and Validation of Simultaneous Estimation Of Metronidazole And Diloxanide Furoate in Tablet Dosage Form By RP-HPLC, International Journal of Pharmaceutical Research & Analysis, Vol-4(7), 2014, 415-420.
[34]. NiharRanjan Das, Development of a RP-HPLC Method for the Estimation of Metronidazole Benzoate Oral Suspension, Pharmaceutical and Biological Evaluations, vol-1, September 2014, 41-43.
[35]. Sivannarayana P., K. Rambabu, Simultaneous assay of Mupirocin and Metronidazole in formulations using Reverse Phase-High Performance Liquid Chromatography, International Journal of Bioassays, vol-12, 2016, 5151-5155.
[36]. M. Satish Kumar, A. Anjaneyulu, T. Mangilal, T. Vijaya Kumari, T. KavithaValidated Stability Indicating RP – HPLC Method for Simultaneous Estimation of Furazolidone And Metronidazole in Combined Pharmaceutical Dosage Forms, World Journal of Pharmacy and Pharmaceutical Sciences, Vol-4(1); 05, 2005, 1944-1961.
[37]. S. Yaswanth Kumar, P. Sivaprasad, A. Ashok Kumar, RP-HPLC, Method Development and Validation for Simultaneous Quantitative Estimation of Metronidazole and Nalidixic Acid in Tablets, International Journal of pharmacy and pharmaceutical Sciences, vol-7(2); 2015, 367-371.
[38]. Emad M. Hussien, HPLC Method Validation for Modernization of The Tetracycline Hydrochloride Capsule USP Monograph, Elsevier Publications, 26 September 2014, 239-244
[39]. FusaoKondo, Seiji Morikawa, And Susumu Tateyama, Simultaneous Determination of Six Tetracyclines in Bovine Tissue, Plasma

and Urine by Reverse-Phase High-Performance Liquid Chromatography, Journal of Food Protection, vol-52(I), 1988, 41-44.

[40]. Karno Ng, and Sean W. Linder, HPLC Separation of Tetracycline Analogues: Comparison Study of Laser-Based Polarimetric Detection with UV Detection, Journal of Chromatographic Science, vol-41(3), 2003, 460-466

[41]. Nabila Perveen and NaeemHasan Khan, Quantitative Analysis of Raw Material of Tetracycline and Its Related Substances by High Performance Liquid Chromatography, Malaysian Journal of Pharmaceutical Sciences, Vol-4(2), 2006, 49-61.

[42]. Hai-Tao Lu, Yue Jiang, Hua-Bin Li, Feng Chen, Ming-Hung Wong, Simultaneous Determination of Oxytetracycline, Doxycycline, Tetracycline and Chlortetracycline in Tetracycline Antibiotics by High-Performance Liquid Chromatography with Fluorescence Detection, Chromatographia, September 2004, 259-264.