World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METRONIDAZOLE, TETRACYCLINE, BISMUTH SUBCITRATE IN ITS BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

Shareefa Asra Shoeb*[1] and Dr. R. Vani[1]

Department of Pharmaceutical Analysis and, Shadan Women's College of Pharmacy, Hyderabad.

***Corresponding Author: Shareefa Asra Shoeb**

Department of Pharmaceutical Analysis and, Shadan Women's College of Pharmacy, Hyderabad.

Article Received on 20/09/2019 **Article Revised on 10/10/2019** **Article Accepted on 30/10/2019**

ABSTRACT

A short selective, precise, accurate and sensitive for the estimation of Metronidazole, Tetracycline and Bismuth Subcitrate was done by RP-HPLC. The assay of Metronidazole, Tetracycline and Bismuth Subcitrate was performed with tablets and the % assay was found to be 100.99 and 100.70 and 100.50 which shows that the method is useful for routine analysis. The linearity of Metronidazole, Tetracycline and Bismuth Subcitrate was found to be linear with a correlation coefficient of 0.999 and 0.999 and 0.999 which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.5 and 0.7 for Metronidazole, Tetracycline and Bismuth Subcitrate which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.7 and 0.4 and 0.3 for Metronidazole, Tetracycline and Bismuth Subcitrate which shows that the method is repeatable when performed in different days also.

KEYWORDS: Of Metronidazole, Tetracycline and Bismuth Subcitrate, Validation, stability indicating method, degradation products.

INTRODUCTION

Metronidazole, 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethan-1-ol is a prodrug. Unionized metronidazole is selective for anaerobic bacteria due to their ability to intracellularly reduce metronidazole to its active form. This reduced metronidazole then covalently binds to DNA, disrupt its helical structure, inhibiting bacterial nucleic acid synthesis and resulting in bacterial cell death.

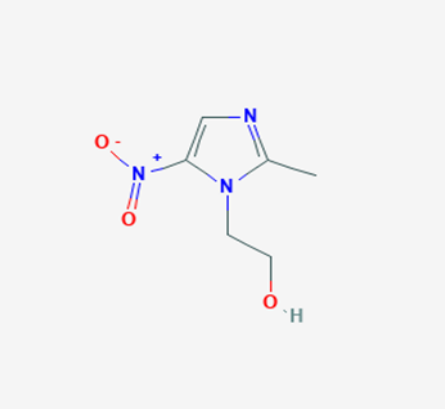

Fig. 1: Metronidazole, 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethan-1-ol.

Tetracycline,(4S,4aS,5aS,6S,12aS)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4, 4a,5, 5a, 6, 11,12a-octahydrotetracene -2-carboxamide passively diffuses through porin channels in the bacterial membrane and reversibly binds to the 30S ribosomal subunit, preventing binding of tRNA to the mRNA-ribosome complex, and thus interfering with protein synthesis.

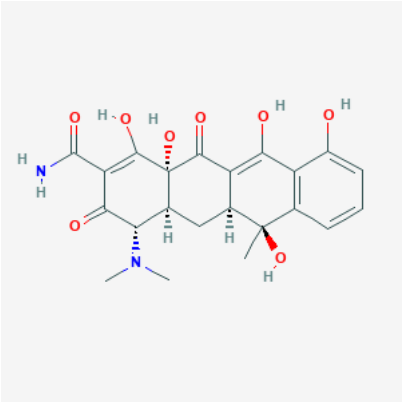

Fig. 2: Tetracycline,(4S,4aS,5aS,6S,12aS)-4-(dimethylamino)-3,6,10,12,12apentahydroxy-6-methyl-1,11-dioxo-1,4,4a, 5,5a,6,11,12a-octahydrotetracene -2-carboxamide

Experimental

Optimized Chromatographic Conditions

Instrument used	:	Waters HPLC with auto sampler and PDA detector.
Temperature	:	Ambient
Column	:	Inertsil ODS C_{18} (4.6 x 250mm, 5µm)
Buffer	:	0.1% Octa sulphonic acid buffer
P^H	:	3.0
Mobile phase	:	70% buffer 30% Acetonitrile
Flow rate	:	1 ml per min
Wavelength	:	277 nm
Injection volume	:	20 µl
Run time	:	15 min.

Preparation of Buffer and Mobile Phase

Preparation of 0.1% Octa sulphonic acid buffer

0.1g of octa sulphonic acid was weighed and taken in a 1000ml volumetric flask and adjust the P^H with Diluted OPA upto 3, finally the solution was filtered by using 0.45 Micron membrane filter, sonicate it for 10 mins.

Preparation of mobile phase

Accurately measured 700 ml (70%) of above buffer and 300 ml of Acetonitrile HPLC (30%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Preparation of the Metronidazole, Tetracycline and Bismuth Subcitrate Standard & Sample Solution

Standard Solution Preparation

Accurately weigh and transfer 25mg of Metronidazole, 25mg of Tetracycline and 28mg of Bismuth Subcitrate working standard into a 100 ml clean dry volumetric flask add small amount of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 25mg of Metronidazole, 25mg of Tetracycline and 28mg of Bismuth Subcitrate in (marketed formulation=177.1 mg of tablet Powder) sample into a 100mL clean dry volumetric flask add small amount of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is Filtered through 0.45 micron Injection filter. (Stock solution)

Further pipette 3 ml of Metronidazole, Tetracycline and Bismuth Subcitrate from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Metronidazole, Tetracycline and Bismuth Subcitrate peaks and calculate the % Assay by using the formulae.

RESULTS AND DISCUSSION

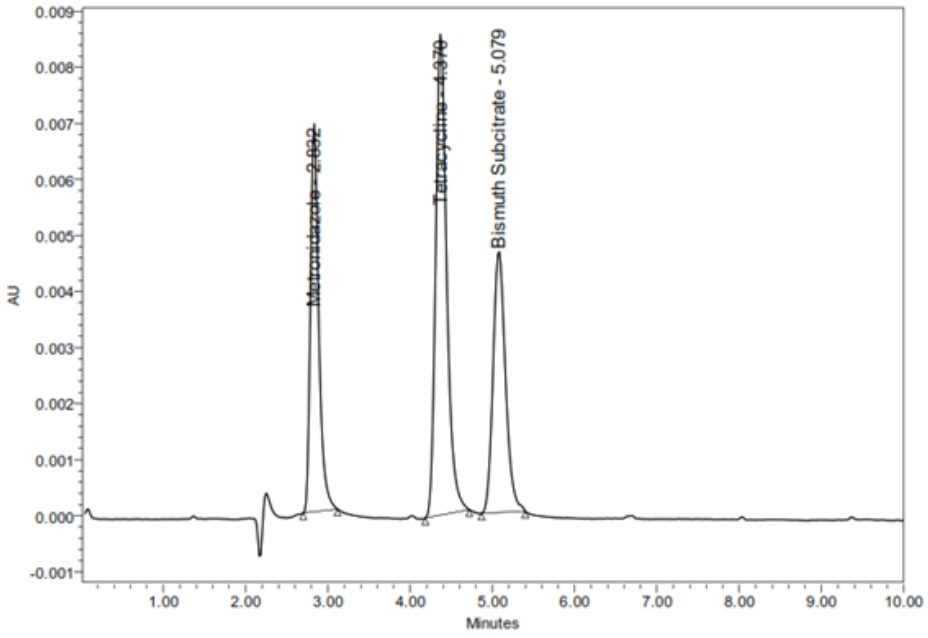

Fig. 3: Standard Chromatogram of Metronidazole and Tetracycline and Bismuth Subcitrate.

Table 1: Results of system suitability parameters.

S. No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Metronidazole	2.832	48315	6884		1.37	3795.08
2	Tetracycline	4.370	79273	8577	7.23	1.26	5232.61
3	Bismuth Subcitrate	5.079	49106	4657	2.72	1.18	5321.81

Table 2: Linearity Results: (for Metronidazole).

S. No	Linearity Level	Concentration	Area
1	I	0	0
2	II	25	16195
3	III	50	32747
4	IV	75	48679
5	V	100	64042
6	VI	125	81639
Correlation Coefficient			0.999

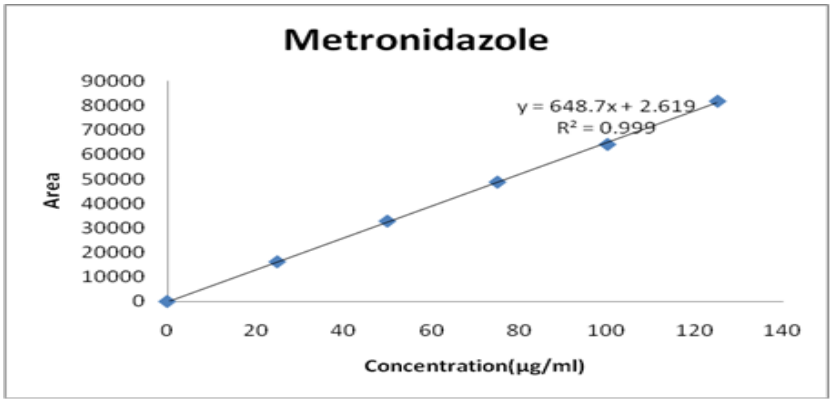

Figure 4: Calibration graph for Metronidazole.

Table 3: Linearity Results: (for Tetracycline).

S. No	Linearity Level	Concentration	Area
1	I	0	0
2	II	25	26761
3	III	50	51643
4	IV	75	78766
5	V	100	108870
6	VI	125	136478
Correlation Coefficient			0.999

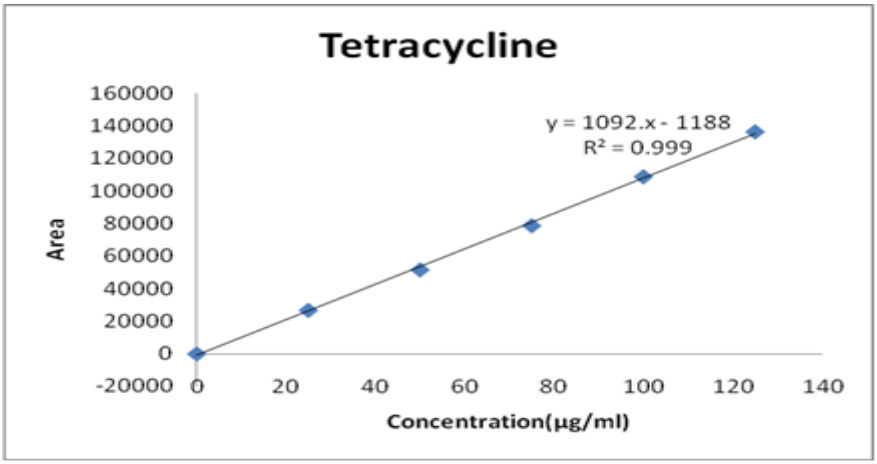

Fig. 5 Calibration graph for Tetracycline.

Table 4: Linearity Results: (for Bismuth Subcitrate).

S. No.	Linearity Level	Concentration	Area
1	I	0	0
2	II	28	16480
3	III	56	32798
4	IV	84	49192
5	V	112	64201
6	VI	140	80868
Correlation Coefficient			0.999

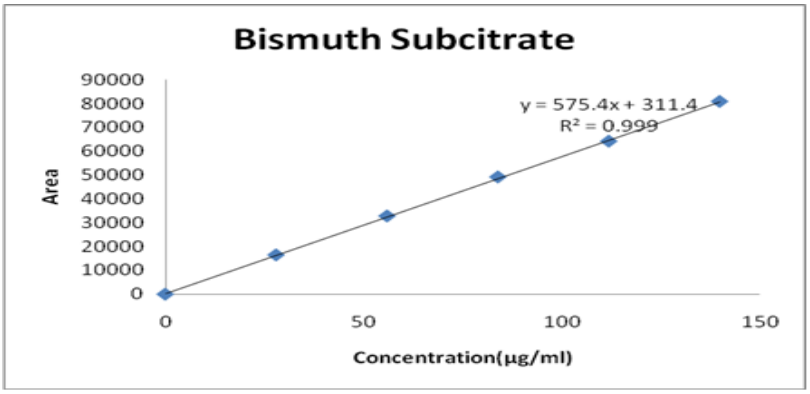

Figure 6: Calibration graph for Bismuth Subcitrate

Table 5: Results of Precision for Metronidazole, Tetracycline & Bismuth Subcitrate.

Injection	Metronidazole	Tetracycline	Bismuth Subcitrate
Injection-1	48083	79068	47720
Injection-2	48572	78692	48505
Injection-3	48768	78039	48573
Injection-4	48335	79279	47978
Injection-5	48742	79015	48156
Injection-6	48653	78836	48327
Average	48525.5	78821.5	48209.8
Standard Deviation	266.9	432.9	325.4
%RSD	0.6	0.5	0.7

Table 6: Accuracy (recovery) data for Metronidazole.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	24519.7	50	50.41	100.81	100.62
100%	48838.3	100	100.40	100.40	
150%	73448	150	150.99	100.66	

Table 7: Accuracy (recovery) data for Tetracycline.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	39991	6.25	6.27	100.34	100.61
100%	80118.3	12.5	12.56	100.51	
150%	120725	18.75	18.93	100.97	

Table 8: Accuracy (recovery) data for Bismuth Subcitrate.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	24552	12.5	12.53	100.24	100.19
100%	49050.3	25	25.03	100.13	
150%	1631406	37.5	37.58	100.21	

Table 9: Results of LOD.

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Metronidazole	51	153	3.00
Tetracycline	51	151	2.96
Bismuth Subcitrate	51	154	3.02

Table 10: Results of LOQ.

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Metronidazole	51	509	9.98
Tetracycline	51	511	10.02
Bismuth Subcitrate	51	510	10.00

Degradation

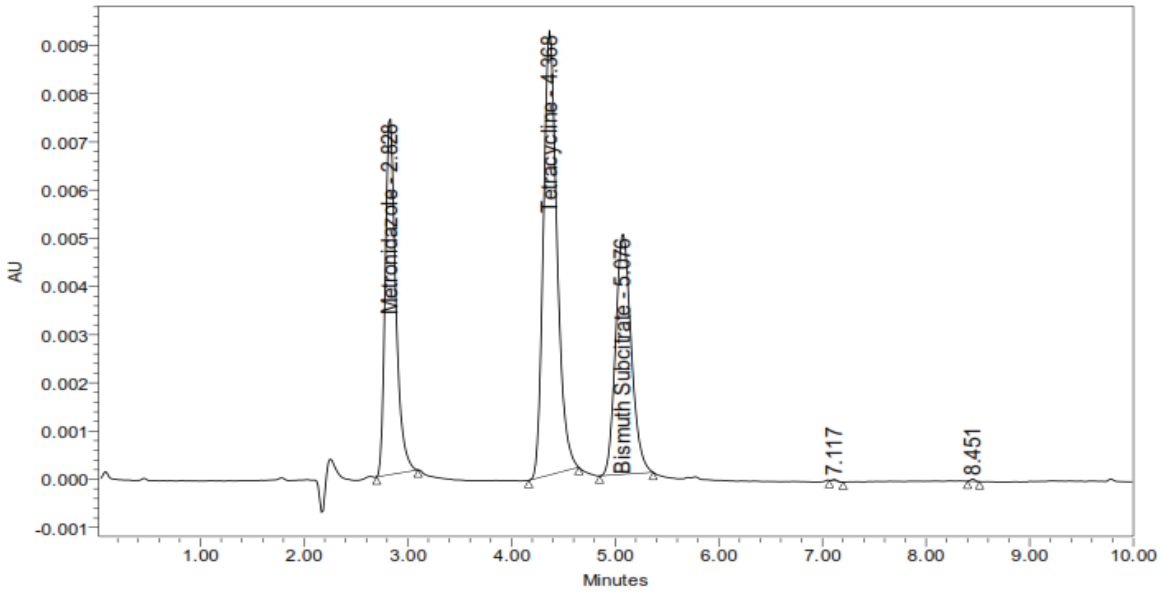

Figure 7: Chromatogram showing acid degradation.

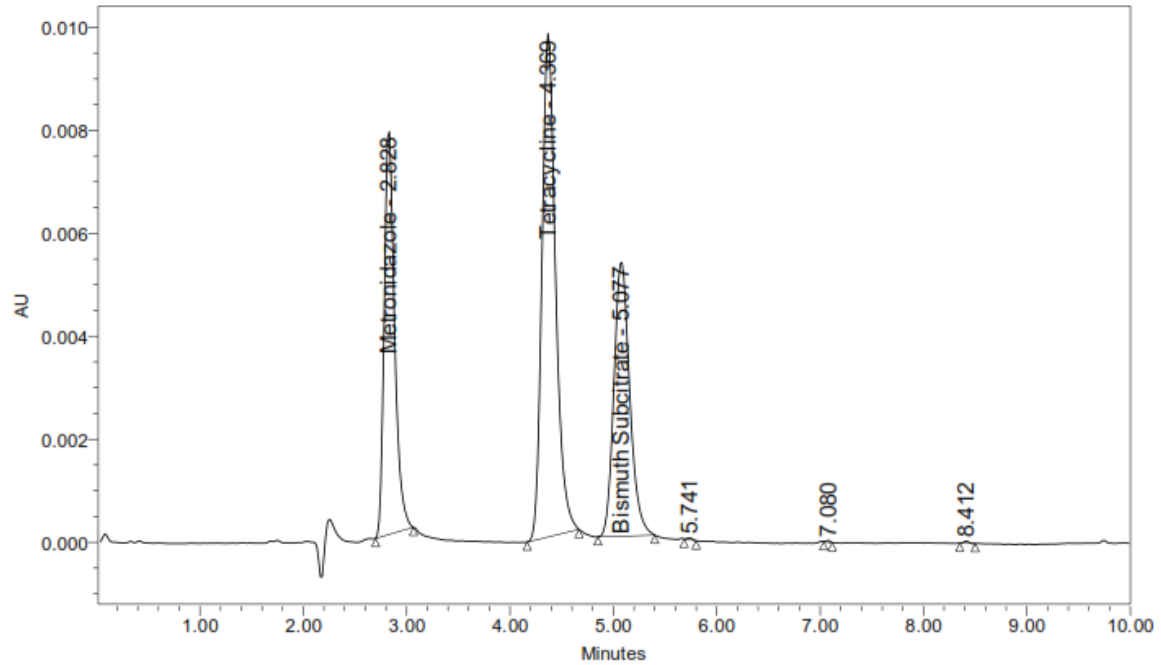

Figure 8: Chromatogram showing Base degradation.

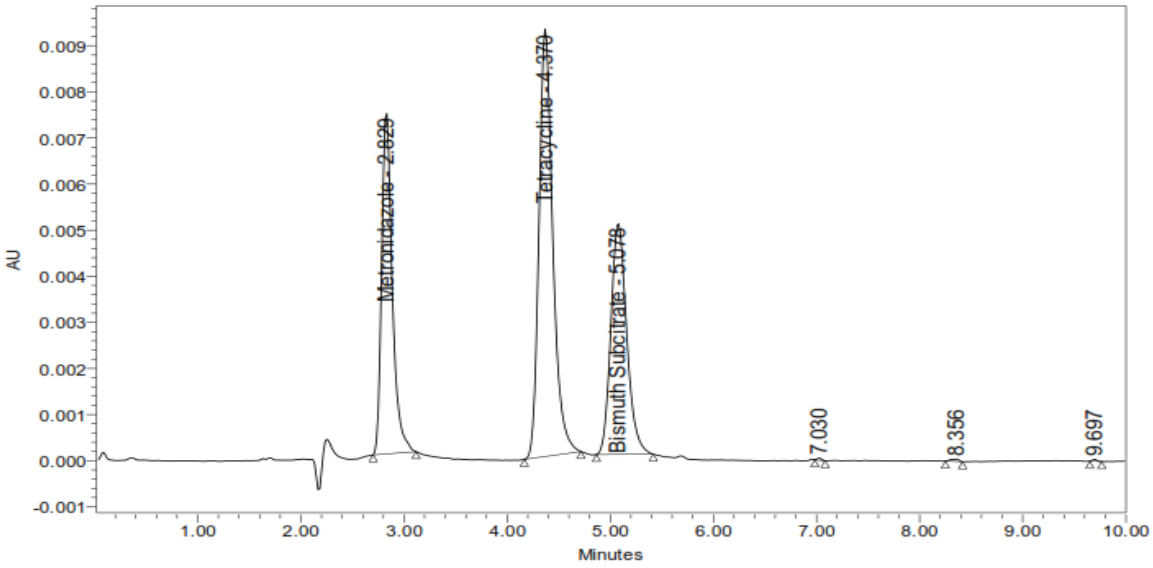

Figure 9: Chromatogram showing Peroxide degradation.

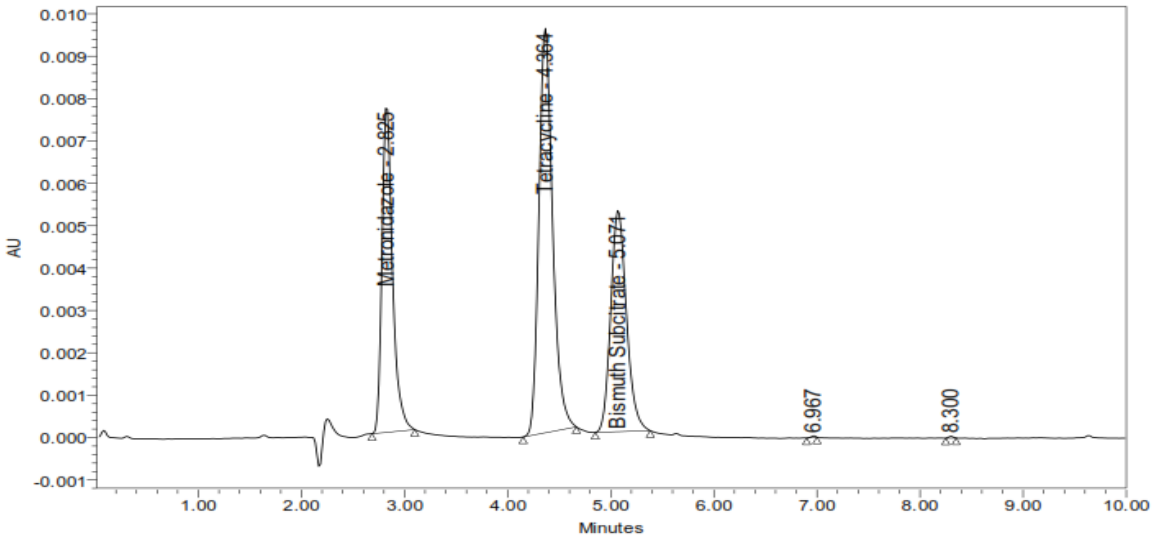

Figure 10: Chromatogram showing Thermal degradation.

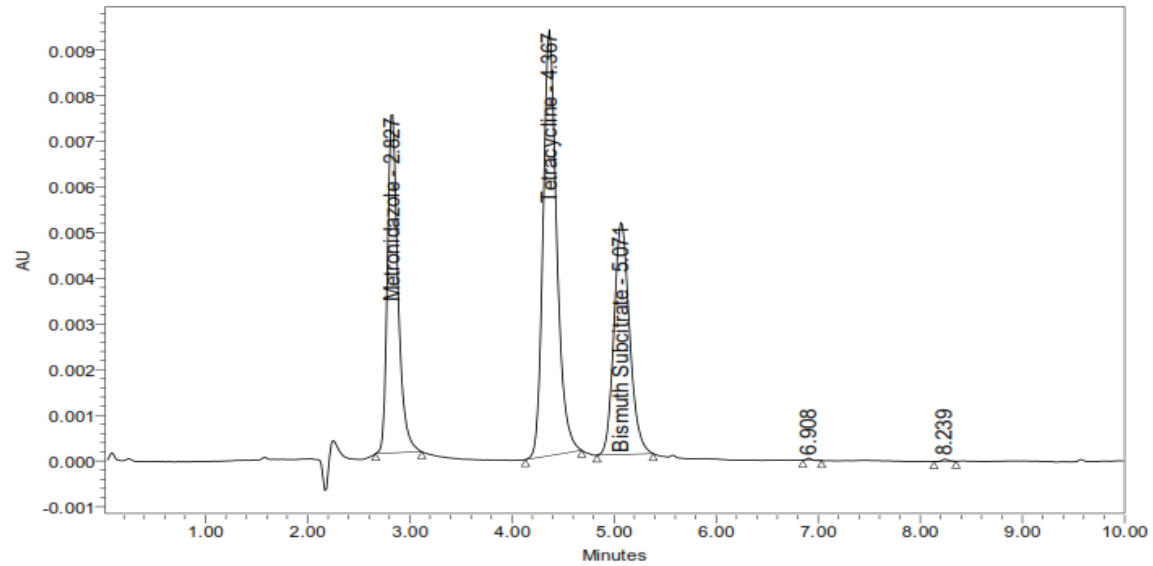

Figure 11: Chromatogram showing Photo degradation.

Table 11: Degradation results.

	Metronidazole		Tetracycline		Bismuth Subcitrate	
	Area	% Degradation	Area	% Degradation	Area	% Degradation
Standard	48547.7		79551.3		48888	
Acid	47042	3.10	77642	2.40	47324	3.20
Base	46528	4.16	76345	4.03	45925	6.06
Peroxide	46186	4.86	76674	3.62	46389	5.11
Thermal	45356	6.57	75623	4.94	45686	6.55
Photo	47464	2.23	77357	2.76	47697	2.44

Table 12: Results of Assay for Metronidazole and Tetracycline and Bismuth Subcitrate.

	Label Claim (mg)	% Assay
Metronidazole	125	100.99
Tetracycline	125	100.70
Bismuth Subcitrate	140	100.50

SUMMARY AND CONCLUSION

The estimation of Metronidazole, Tetracycline and Bismuth Subcitrate was done by RP-HPLC.

The assay of Metronidazole, Tetracyclineand Bismuth Subcitrate was performed with tablets and the % assay was found to be 100.99 and 100.70 and 100.50 which shows that the method is useful for routine analysis.

The linearity of Metronidazole, Tetracyclineand Bismuth Subcitrate was found to be linear with a correlation coefficient of 0.999 and 0.999 and 0.999 which shows that the method is capable of producing good sensitivity.

The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.5 and 0.7 for Metronidazole, Tetracycline and Bismuth Subcitrate which shows that the method is precise.

The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.7 and 0.4 and 0.3 for Metronidazole, Tetracycline and Bismuth Subcitrate which shows that the method is repeatable when performed in different days also.

The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.62% and 100.61% and 100.19% for Metronidazole, Tetracycline and Bismuth Subcitrate. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

The acceptance criteria for LOD and LOQ is 3 and 10. The LOD and LOQ for Metronidazole was found to be 3.00 and 9.98 and LOD and LOQ for Tetracycline was found to be 2.96 and 10.02 and LOD and LOQ for Bismuth Subcitrate was found to be 3.02 and 10.00.

The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

BIBLIOGRAPHY

1. Douglas A.Skoog, F. James Holler & Stanley R. Crouch. Instrumental analysis, India edition, 2007; 13-14.
2. Gurdeep R. Chatwal & Sham K. Anand. Instrumental Methods Of Chemical Analysis (Analytical Chemistry), 2.566-2.567.
3. Ahuja S & Dong MW. Handbook of Pharmaceutical Analysis by HPLC. 1st edition, Academic Press Publisher. UK, 2005.
4. Satinder Ahuja & Neil Jespersen. Modern Instrumental Analysis 47 (Comprehensive Analytical Chemistry), 47: 7-8.
5. Willard HH, Merrit LL, Dean JA, Settle FA. Instrumental methods of analysis, CBS Publishers and Distributors, New Delhi, 6th edition, 1986; 1-15.
6. Douglas A. Skoog, F. James Holler, Timothy A. Nieman. Principles of instrumental analysis, Saunders Golden Sun burst Series, Philadelphia, 2nd edition, 1980, 725-760.
7. From drugs.com.
8. Prathab B, Gobinath M, Haribaskar V, Kumar B, Kavitha M, Harika S, Bhavya C, Stability Indicating Reversed-phase High-Performance Liquid Chromatography Method Development and Validation for Simultaneous Estimation of Bismuth Subcitrate, Tetracycline, and Metronidazole in Bulk and Capsule Dosage Form.
9. Yunqi Wua, Reza Fassihi, Stability of metronidazole, tetracycline HCl and famotidine alone and in combination, Received 25 June 2004; received in revised form 22 October 2004; accepted, 22 October 2004.
10. Veldhuyzen Van Zanten S, Farley A, Marcon N, Lahaie R, Archambault A, Hunt R, Bailey R, Owen D, Spénard J, Stiglick A, Aimola N, Colin P, Bismuth-based triple therapy with bismuth subcitrate, metronidazole and tetracycline in the eradication of Helicobacter pylori: a randomized, placebo controlled, double-blind study. Can J Gastroenterol, 2000 Jul-Aug; 14(7): 599-602.