

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

Prathap B, Gobinath M, Haribaskar V, Kumar B, Kavitha M, Harika S, Bhavya C

Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Nellore, Andhra Pradesh, India

Abstract

Aim: The aim of the study was to develop a new, simple, sensitive, precise, accurate, and stability indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of bismuth subcitrate, tetracycline, and metronidazole in the combined capsule dosage form. **Materials and Methods:** The analysis with Inertsil C₁₈ (250 × 4.6 mm, 5 μ) column under ambient temperature and using mobile phase phosphate buffer pH = 3.5 and methanol in the ratio of 40:60 v/v. **Results and Discussion:** The retention time of metronidazole, tetracycline, and bismuth subcitrate was found to be 2.599 min, 3.805 min, and 4.661 min, respectively. The proposed method was validated according to the ICH guidelines. The linearity study of metronidazole, tetracycline, and bismuth subcitrate was found to be 125–625 μg/ml, 125–625 μg/ml, and 140–700 μg/ml and correlation coefficient (r²) was found to be 0.9994, 0.9993, and 0.9993, respectively. The percentage recovery was obtained as 99.95%, 99.86%, and 100.27% metronidazole, tetracycline, and bismuth subcitrate, respectively. The studies were carried out by conducting deliberate degradation of the sample with exposure to stress conditions such as acidic, alkaline, thermal, oxidizing agent, and light. **Conclusion:** This method was validated and meets the regulatory requirements for specificity, linearity, limit of detection, limit of quantification, precision, accuracy and stability for the determination of metronidazole, tetracycline, and bismuth subcitrate in bulk and capsule dosage form by RP-HPLC.

Key words: Metronidazole, Tetracycline, Bismuth subcitrate, Simultaneous Estimation Reversed-phase High-Performance Liquid Chromatography

INTRODUCTION

Bismuth subcitrate is chemically bismuth (3+) pentapotassium bis(2-oxidopropane-1,2,3-tricarboxylate) [Figure 1]. A bismuth compound used for peptic ulcer and gastro-oesophageal reflux disease (GORD).^[1] Colloidal bismuth subcitrate is very effective in the treatment of gastroduodenal disorders and appears to act through several mechanisms. It has a little acid-neutralizing effect and does not affect 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 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 proteases, lipases, glycosidases, and phospholipases.

Address for correspondence:

Dr. B. Prathap, Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Nellore - 524 346, Andhra Pradesh, India. Phone: +91-8125974910. E-mail: prathapnila@gmail.com

Received: 12-08-2018

Revised: 05-11-2018

Accepted: 26-11-2018

Tetracycline is chemically (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 [Figure 2]. It is a broad-spectrum polyketide antibiotic produced by the *Streptomyces* genus of Actinobacteria. It exerts a bacteriostatic effect on bacteria by binding reversible to the bacterial 30S ribosomal subunit and blocking incoming aminoacyl tRNA from binding to the ribosome acceptor site. It also binds to some extent to the bacterial 50S ribosomal subunit and may alter the cytoplasmic membrane causing intracellular components to leak from bacterial cells.^[2]

Metronidazole is chemically 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole. It is a prodrug [Figure 3]. It's a nitroimidazole used to treat amebiasis, vaginitis, trichomonas infections, giardiasis, anaerobic bacteria, and treponemal infections. It has also been proposed as a radiation sensitizer for hypoxic cells. According to the Fourth Annual Report on Carcinogens (NTP 85-002, 1985. p. 133), this substance may reasonably be anticipated to be a carcinogen 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, disrupts its helical structure, inhibiting bacterial nucleic acid synthesis and resulting in bacterial cell death.^[3] These research focus on the simultaneous estimation of bismuth subcitrate, tetracycline, and metronidazole in active pharmaceutical ingredient and combined capsule dosage form by reversed-phase high-performance liquid chromatography (RP-HPLC).

The extensive literature survey revealed that there were RP-HPLC, HPLC-mass spectrometry, and ultraviolet (UV) spectrophotometric and stability indicating RP-HPLC methods^[4-17] were available for the determination of bismuth subcitrate, tetracycline, and metronidazole individually or in combination with other drugs. However, no method was reported for the simultaneous estimation of bismuth subcitrate, tetracycline, and metronidazole in combined dosage form using the RP-HPLC method. The study was, thus, performed with an aim to develop a simple, economic, sensitive, rapid, accurate, and precise method for the determination of bismuth subcitrate, tetracycline, and metronidazole in the combined capsule dosage form.

MATERIALS AND METHODS

Chemicals and reagents

Bismuth subcitrate, tetracycline, and metronidazole were purchased from Pharmatrain Labs, Hyderabad. Orthophosphoric acid (OPA) purchased from standard deviation (SD) Fine Chemicals (Hyderabad, India), methanol and acetonitrile and HPLC grade water were obtained from Rankam. Bismuth subcitrate, tetracycline, and metronidazole are containing Pylera capsules manufactured by Allergan Pharmaceuticals Ltd.

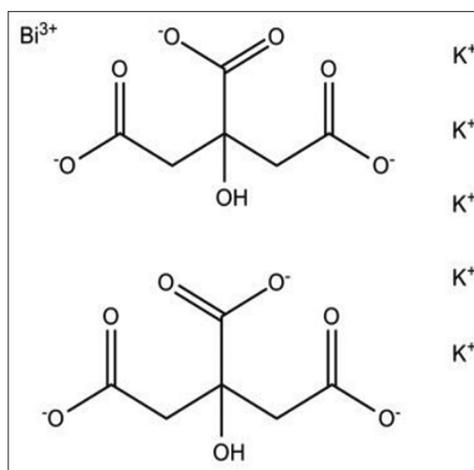


Figure 1: Structure of bismuth subcitrate

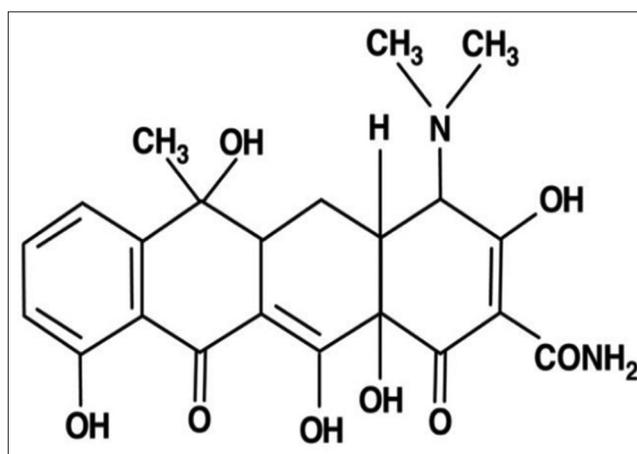


Figure 2: Structure of tetracycline

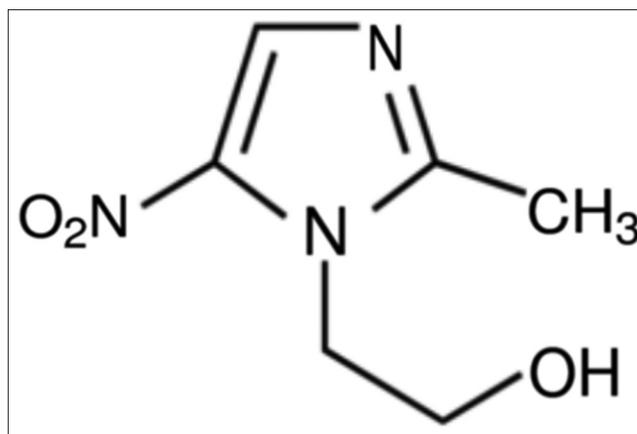


Figure 3: Structure of metronidazole

Instruments

Waters (2695) HPLC using the software Empower2 with PDA detector all the glass wares used were A Grade.

Preparation of standard solution

About 12.5 mg of metronidazole, 12.5 mg of tetracycline, and 14 mg of bismuth subcitrate stands were accurately weighed

and transferred into 10 ml clean, dry volumetric flask, 5 ml of diluent (methanol) was added, sonicated for 5 min and made up to the final volume with diluent. 3 ml of from the above stock solution was taken into a 10 ml volumetric flask and made up to 10 ml with diluent.

Preparation of sample solution

10 capsules were weighed and transfer the content. A power equivalent to 12.5 mg of metronidazole, 12.5 mg of tetracycline, and 14 mg of bismuth subcitrate stands were accurately weighed and transferred into 10 ml clean, dry volumetric flask, 5 ml of diluent was added, sonicated for 5 min and made up to the final volume with diluent. Then, it is filtered through 0.45 μ injection filter. 3 ml of from the above stock solution was taken into a 10 ml volumetric flask and made up to 10 ml with diluent.

Chromatographic conditions

Inertsil ODS C₁₈ (250 × 4.6 mm, 5 μ) column was used for the chromatographic separation at a detection wavelength at 280nm. The mobile phase of methanol and phosphate buffer pH 3.5 (adjusted with OPA) in the ratio of 60:40 v/v which was degassed under ultrasonication was selected for elution of components. Flow rate was optimized to 1.0 ml/min and injection volume 20μl was fixed on the satisfactory results of various system suitability parameters such as retention time, column efficiency, tailing factor, and asymmetry of the peaks. The results were shown in Figure 4 and Table 1.

METHOD VALIDATION^[18,19]

RP-HPLC method was validated according to the ICH guidelines for validation of analytical procedures for different validation parameters. The method was validated for its specificity, linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), and Limit of quantification LOQ.

System suitability

System suitability test was carried out to verify that the analytical system was working properly and can give accurate and precise results. The overall system suitability was evaluated for the system suitability of the proposed method. Data from six injections were utilized for calculating parameters such as theoretical plates, resolution, tailing factor, and percentage relative SD (RSD). The results were shown in Table 2.

Specificity

The specificity studies were carried out by varying specific conditions, that is, placebo study. A study conducted to demonstrate that diluent and placebo were not interfering with the analyte peak in the proposed method. Solutions of sample, placebo, and blank were prepared individually, and chromatograms were obtained. The results were shown in Figures 5 and 6.

Accuracy

Accuracy was carried out by percentage recovery studies of bismuth subcitrate, tetracycline, and metronidazole at three different concentration levels (50%, 100%, and 150%). Percentage recovery was calculated from the amount added

Table 1: Optimized chromatographic parameters

Mode of separation	Isocratic
Mobile phase	Methanol: Phosphate buffer (60:40)
Column	Inertsil ODS C ₁₈ (250×4.6 mm, 5 μ)
Detection wavelength	280 nm
Runtime	15 min
Injection volume	20 μl
Flow rate	1.0 ml/min

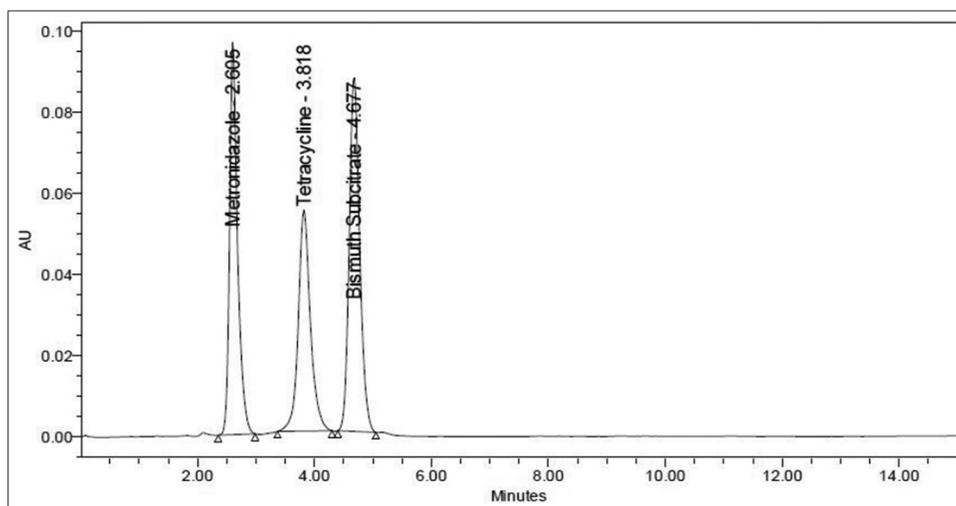


Figure 4: Optimized chromatogram of bismuth subcitrate, tetracycline, and metronidazole

and the amount recovered. The percentage recovery was within the acceptance criteria this indicates the accuracy of the method. (Acceptance criteria: Percentage recovery between 98% and 102%). The results were shown in Table 3.

Precision

The precision of an analytical procedure as the closeness of agreement between a series of measurements obtained from

Table 2: Result of system suitability parameters

Parameters	Metronidazole	Tetracycline	Bismuth subcitrate
Linearity range (µg/ml)	125–625 µg/ml	125–625 µg/ml	140–700 µg/ml
Retention time (Rt)	2.599	3.805	4.661
Number of theoretical plates (n)	3916.77	4845.78	3453.97
Correlation coefficient (r ²)	0.9994	0.9993	0.9993
Tailing factor (T)	1.34	1.12	1.18
LOD	3.00	2.96	3.02
LOQ	9.98	10.02	10.00

LOD: Limit of detection; LOQ: Limit of Quantification

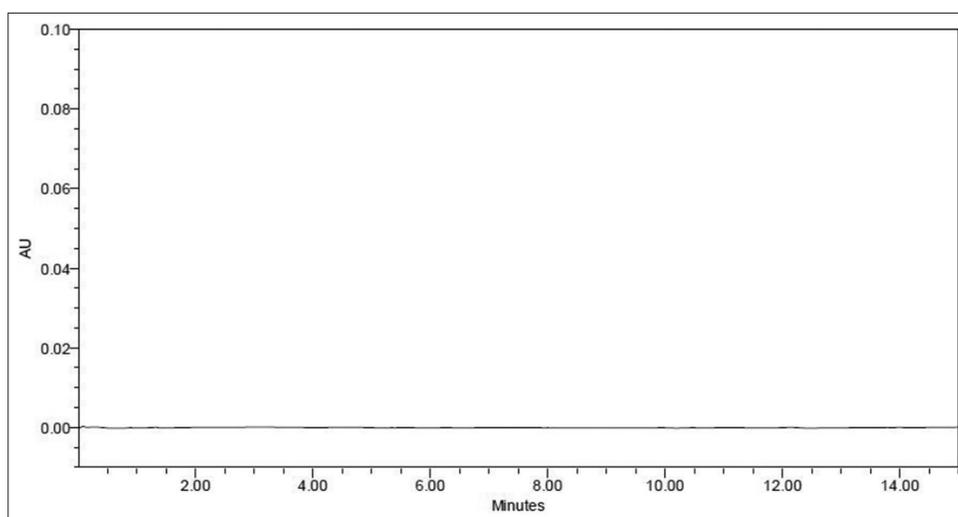


Figure 5: Chromatogram of blank

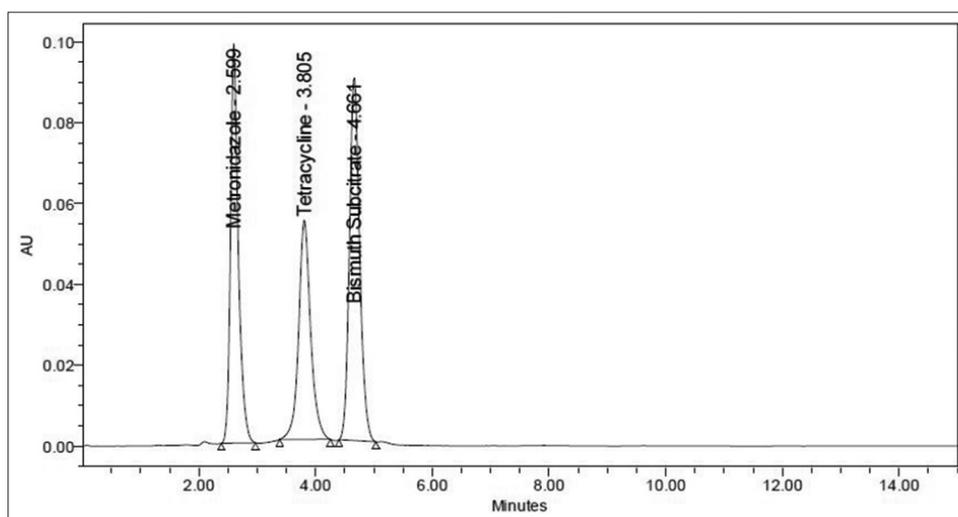


Figure 6: Chromatogram of standard

Table 3: Data for accuracy

Samples	Area	Amount added (mg)	Amount recovered (mg)	% Recovery	Mean recovery
Metronidazole	454265.0	6.25	6.21	99.36	99.95
	913613.7	12.5	12.49	99.92	
	1378454.7	18.75	18.85	100.53	
Tetracycline	376592.7	6.25	6.23	99.68	99.86
	751955.3	12.5	12.43	99.44	
	1139401.3	18.75	18.84	100.48	
Bismuth subcitrate	545426.7	7.0	7.04	100.57	100.27
	1085120.3	14.0	14.00	100.00	
	1630732.7	21.0	21.05	100.23	

multiple sampling of the same homogeneous sample under the prescribed conditions. The results were shown in Table 4.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. The linearity of the method was determined by preparing serial dilutions of minimum 5 concentrations of working standard solution in the range of 125–625 µg/ml for metronidazole and tetracycline and 140–700 µg/ml for bismuth subcitrate [Table 5]. The area of each injection was obtained, and the peak area was plotted against concentration. The regression coefficient (r^2), y-intercept and slope of the regression were calculated.

LOD and LOQ

The LOD is defined as the lowest concentration of an analyte in a sample that can be detected but not quantified. The LOQ is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy. The LOD and LOQ can be calculated based on the SD of the response and the slope of calibration curve. $LOD = 3.3 \sigma/S$ and $LOQ = 10\sigma/S$ where σ is the SD of the intercept of the regression lines and S is the slope of the calibration curve.

Robustness

The robustness of an analytical variation in method parameters such as flow rate, mobile phase was varied within a realistic range, and the quantitative influence of the variables was determined. The results were shown in Table 5.

Stability studies

Stability testing was established for estimating the allowing time span between sample collection and sample analysis.

Table 4: Precision results

Analytes	Mean area	SD	%RSD
Metronidazole	960417.7	8148.4	0.8
Tetracycline	733191.3	3655.2	0.5
Bismuth subcitrate	1108027.8	3722.7	0.3

SD: Standard deviation, RSD: Relative standard deviation

It is also important to evaluate an analytical methods ability to measure drug products in the presence of its degradation products. Forced degradation studies typically involve the exposure of samples of the drugs to the relevant stress conditions of acid, base, thermal, oxidative, and UV. The results were shown in Table 5.

Acid degradation

To 3.0 ml of stock solution into a 10 ml volumetric flask and 3 ml of 1N HCl was added. Then, the volumetric flask was kept at 60°C for 6 h and then neutralized with 1N NaOH and makeup to 10 ml with diluent. Filter the solution with 0.45 µ syringe filter and place in vials. The chromatogram was recorded to assess the stability of the drug substance. The results were shown in Figure 7.

Alkali degradation

To 3.0 ml of stock solution into a 10 ml volumetric flask and add 3 ml of 1N NaOH. Then, the volumetric flask was kept at 60°C for 6 h and then neutralized with 1N HCl and makeup to 10 ml with diluent. Filter the solution with 0.45 µ syringe filter and place in vials. The chromatogram was recorded to assess the stability of the drug substance. The results were shown in Figure 8.

Oxidative degradation

To 3.0 ml of stock solution into a 10 ml volumetric flask, 1.0 ml of 3.0% v/v hydrogen peroxide is added the volume was made up to the mark with diluent. The solutions were kept for 30 min at 60°C. The chromatogram was recorded to assess the stability of the drug substance. The results were shown in Figure 9.

Table 5: Forced degradation results for metronidazole, tetracycline, and bismuth subcitrate

Types of degradation	Metronidazole		Tetracycline		Bismuth subcitrate	
	Area	% degraded	Area	% degraded	Area	% degraded
Acid	882563	3.26	735622	2.50	964622	10.08
Base	897865	1.58	746565	1.05	974452	9.99
Peroxide	877382	3.83	715326	5.19	968643	10.05
Thermal	896677	1.71	725442	3.85	993645	8.21
Photo	870864	4.54	711078	5.75	978563	9.61

Table 6: Results of robustness studies

Chromatographic conditions	Metronidazole		Resolution	Tetracycline		Resolution	Bismuth subcitrate	
	USP tailing	USP plate count		USP tailing	USP plate count		USP Tailing	USP plate count
Flow rate (0.8 ml/min)	1.36	3973.00	4.02	1.36	4675.00	2.92	1.19	3469.00
Flow rate (1.0 ml/min)	1.36	3883.53	4.08	1.13	4798.00	2.89	1.19	3308.40
Flow rate (1.2 ml/min)	1.29	3834.00	3.77	1.02	4549.00	2.80	1.17	3205.00
Mobile phase composition (10% less)	2.06	4080.00	5.30	0.94	4736.00	3.20	1.12	3702.00
Mobile phase composition (actual)	1.36	3883.53	4.08	1.13	4798.00	2.89	1.19	3308.40
Mobile phase composition (10% more)	1.68	4190.00	3.53	1.10	4765.00	2.68	1.34	3912.00

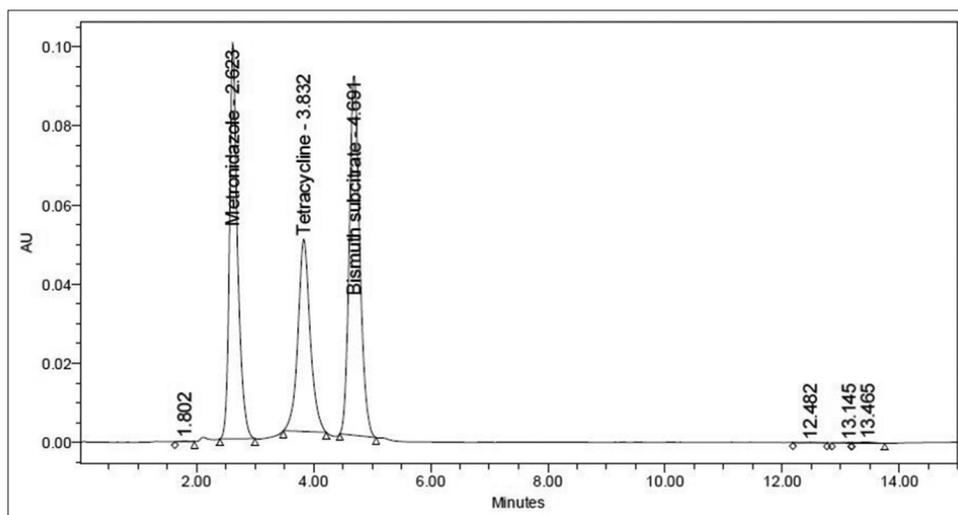


Figure 7: Typical chromatogram of acid degradation of metronidazole, tetracycline, and bismuth subcitrate

Thermal degradation

The standard drug solution was placed in an oven at 105°C for 6 h to study dry heat degradation. The chromatogram was recorded to assess the stability of the drug substance. The results were shown in Figure 10.

Photodegradation

To 3.0 ml of stock solution into a 10 ml volumetric flask and expose to sunlight for 24 hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45µ syringe filter and place in vials. The chromatogram was recorded to

assess the stability of the drug substance. The results were shown in Figure 11.

RESULTS AND DISCUSSION

Optimization of the method was carried out by performing various trials by changing in mobile phase composition and column, etc. The chromatographic conditions were achieved by methanol and phosphate buffer in a ratio of 60:40v/v adjusted pH 3.5 with OPA was selected as mobile phase and Inertsil ODS C₁₈ (250 × 4.6 mm, 5 µ) column as stationary

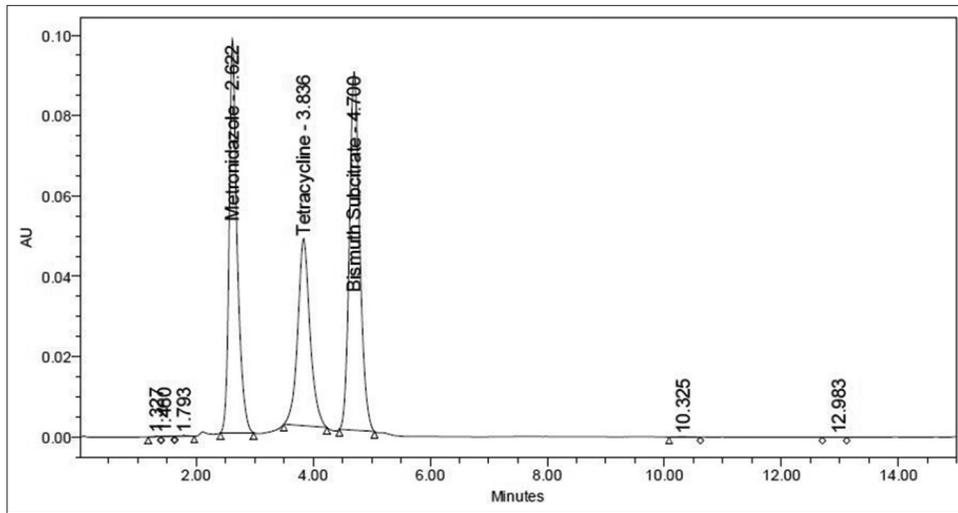


Figure 8: Typical chromatogram of base degradation of metronidazole, tetracycline, and bismuth subcitrate

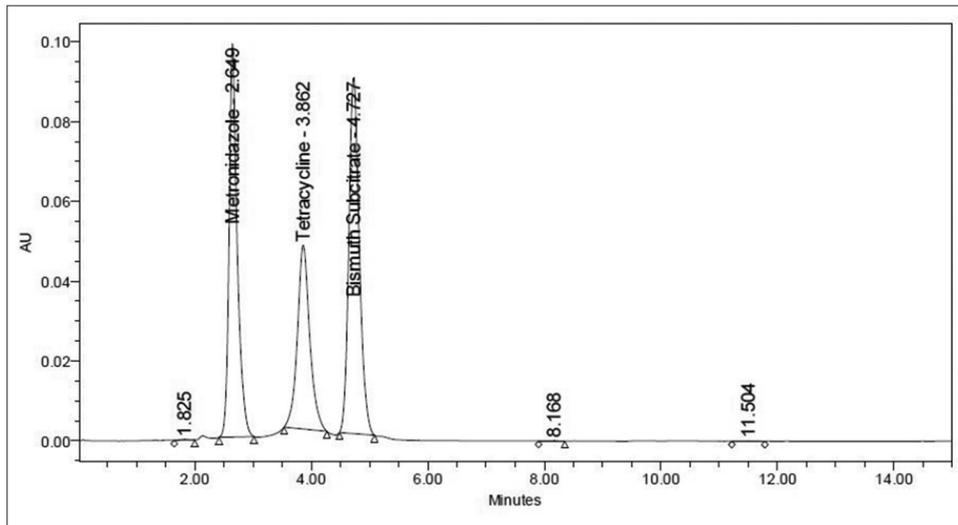


Figure 9: Typical chromatogram of peroxide degradation of metronidazole, tetracycline, and bismuth subcitrate

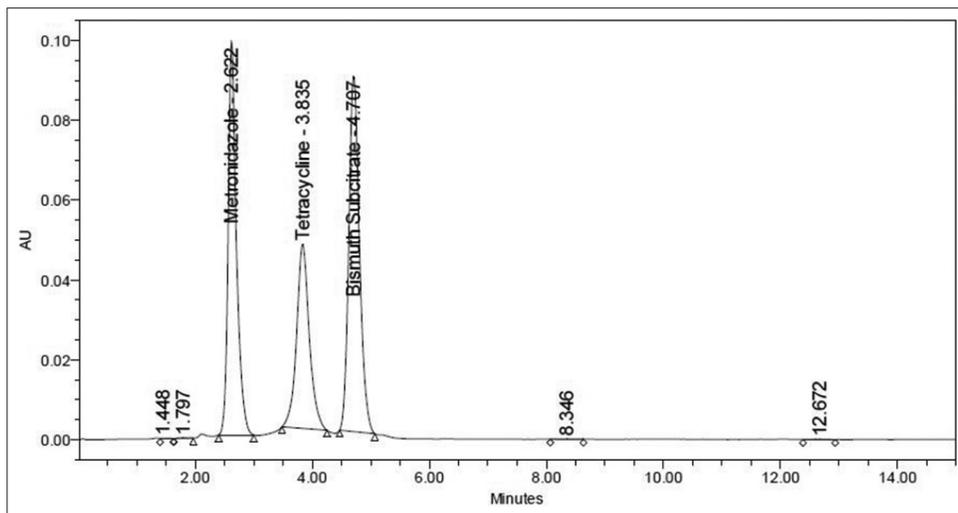


Figure 10: Typical chromatogram of thermal degradation of metronidazole, tetracycline, and bismuth subcitrate

phase because of better resolution, number of theoretical plates and symmetric peaks. Bismuth subcitrate, tetracycline, and metronidazole were found to show appreciable absorbance at 280nm determined by spectrophotometry, and

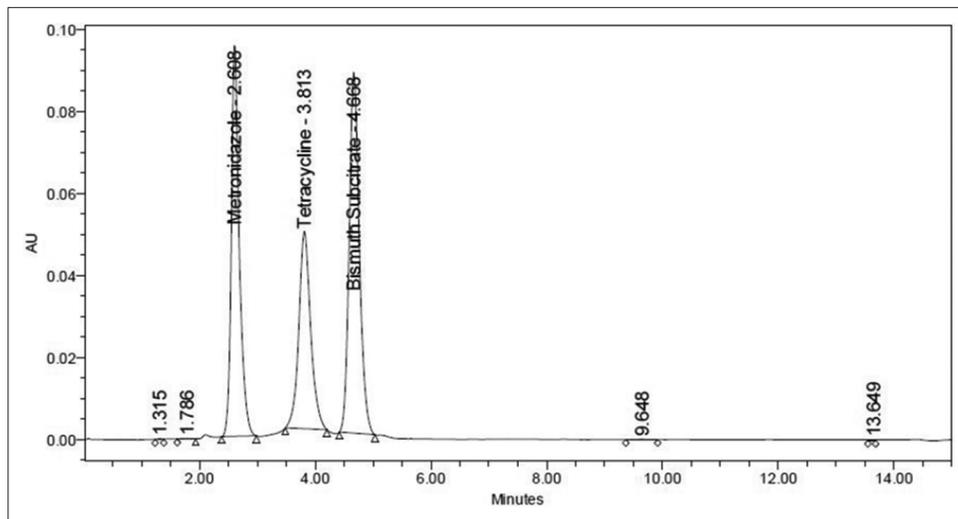


Figure 11: Typical chromatogram of photodegradation of metronidazole, tetracycline, and bismuth subcitrate

hence it was selected as the detection wavelength. Figure 3 represents chromatogram of a mixture of standard solutions.

Method validation

System suitability

A RP-HPLC method was developed by monitoring the system suitability parameters, that is, Tailing factor (T), the number of theoretical plates (N), the runtime, and the cost-effectiveness. System suitability method acceptance criteria set in each validation run were tailing factor ≤ 2.0 and theoretical plates > 2000 . In all cases, the RSD for the analytical peak area for consecutive injections was $< 2.0\%$. A chromatogram obtained from reference substance solution was presented. System suitability parameters were shown in Table 2. All the system suitability parameters are found to be satisfactory. The peak is reasonably symmetrical. High numbers of theoretical plates indicate the efficient performance of the column with reasonable retention times.

Specificity

The blank chromatogram showed no interference peaks at the retention time of bismuth subcitrate, tetracycline, and metronidazole. This indicates that diluent solution used in sample preparation does not interfere in the estimation of bismuth subcitrate, tetracycline, and metronidazole which indicates the specificity of the proposed method. The results were given in Figure 5.

Linearity

The concentration range of 125–625 $\mu\text{g/ml}$ for metronidazole and tetracycline and 140 to 700 $\mu\text{g/ml}$ for bismuth subcitrate were found to be linear with correlation coefficients of 0.9994, 0.9993, and 0.9993 for metronidazole, tetracycline, and bismuth subcitrate, respectively. The results were given in Figures 12-14.

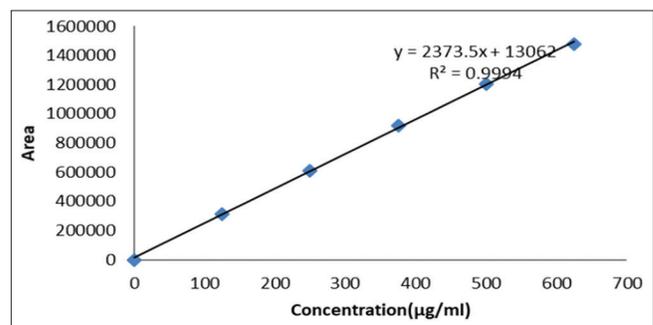


Figure 12: Linearity curve of metronidazole

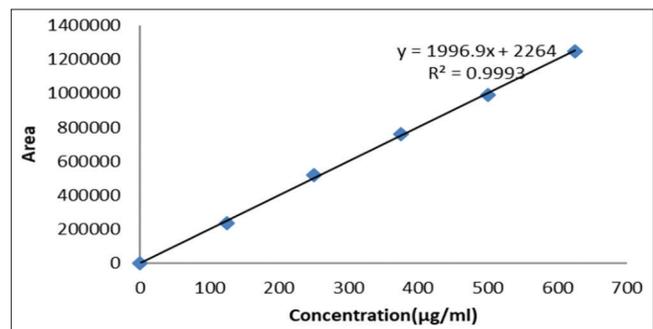


Figure 13: Linearity curve of tetracycline

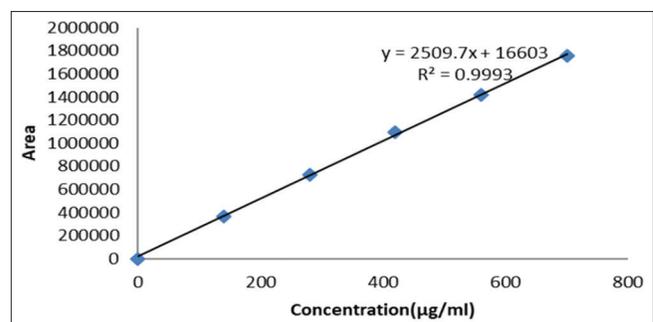


Figure 14: Linearity curve of bismuth subcitrate

Accuracy

The accuracy of an analytical method is the closeness of that results obtained by that method to the true value. Accuracy may often be expressed as percentage recovery by the assay of the known added amount of analyte (50%, 100%, and 150%). Percentage recovery should be in the range of 98.0% to 102.0%. The observed data were within the required range which indicates good recovery values, and hence the method was accurate. The results were shown in Table 3.

Precision

The percentage RSD was calculated for the peak areas of the drug, and it was found to be 0.8%, 0.3%, and 0.5% the %RSD for the metronidazole, tetracycline, and bismuth subcitrate, respectively. The %RSD for the area of six standard injections was should be not be more than 2%, and the method was found to be precise. The results were shown in Table 4.

Linearity

Linearity of detector response shows the linear relationship between the concentration and the detector response. The coefficient of bismuth subcitrate, tetracycline, and metronidazole was found to be 0.9993, 0.9993, and 0.9994, respectively. The linearity was found in the concentration range of 125–625 µg/ml for metronidazole, tetracycline, and 140–700 µg/ml for bismuth subcitrate. Regression equation of the metronidazole, tetracycline, and bismuth subcitrate is found to be $y=2373.5x+13062$, $y=1996.9x+2264$, and $y=2509.7x+16603$. The correlation coefficient value was <1 shows that the method was linear.

LOD

LPD of target assay concentration of metronidazole, tetracycline, and bismuth subcitrate using the formula method was found to be 3.0, 2.96, and 3.02 µg/ml, respectively.

LOQ

LOQ of the target assay concentration of metronidazole, tetracycline, and bismuth subcitrate using the formula method was found to be 9.98, 10.02, and 10.00 µg/ml, respectively.

Robustness

Robustness of the method tested by keeping the column temperature as a constant and the chromatograms of drug solution was recorded with different flow rates such as 1.0 ml/min, 0.8 ml/min, and 1.2 ml/min and different and different ratio of mobile phase such as $\pm 10\%$. The peak was observed as sharp with good resolution and all system suitability parameters indicating the method was robust. The results were reported in Table 6.

Degradation studies

The degradation was determined by analyzing the drug solutions in the presence of acid, base, hydrogen peroxide, thermal, and light. The results of the peak area of bismuth subcitrate, tetracycline, and metronidazole were changed; hence, the drug was degraded, but the percentage degradation was $<10\%w/v$. The results were within the limit as per the International Conference on Harmonization (ICH) guidelines shown in Table 5.

CONCLUSION

The study was undertaken to develop and validate the stability indicating an RP-HPLC method for estimation of bismuth subcitrate, tetracycline, and metronidazole in pharmaceutical formulations. The method was developed and validated by means of accuracy, precision, linearity, LOD, and LOQ and robustness as per the ICH guidelines. The results of the study indicate that the proposed RP-HPLC method can be used in quality control departments with respect to routine analysis for the assay of the capsules containing bismuth subcitrate, tetracycline, and metronidazole.

ACKNOWLEDGMENT

An author expresses sincere thanks to the Ratnam Institute of Pharmacy for providing facility to carry out this work.

REFERENCES

1. Available from: <https://www.drugbank.ca/drugs/DB09275>. [Last accessed on 2018 Jan 02].
2. Available from: <https://www.drugbank.ca/drugs/DB00759>. [Last accessed on 2018 Jan 02].
3. Available from: <https://www.drugbank.ca/drugs/DB00916>. [Last accessed on 2018 Jan 02].
4. Silva M, Schramm S, Kano E, Koono E, Porta V, Serra C, *et al.* Development and validation of a HPLC-MS-MS method for quantification of metronidazole in human plasma. *J Chromatogr Sci* 2009;47:781-4.
5. Kasabe AJ, Shitole VV, Waghmare VV, Mohite V. Simultaneous estimation of metronidazole and ofloxacin in combined dosage form by reverse phase high performance liquid chromatography method. *Int J Chem Tech Res* 2009;1:1244-50.
6. Fathima K, Ahmed O, Vakeeluddin S. Analytical method development and validation for the simultaneous estimation of ofloxacin and metronidazole in bulk and dosage form using RP-HPLC. *Indo Am J Pharm Sci* 2017; 4:2017-8.
7. Patel AB, Shah NJ, Patel NM. Simultaneous estimation of metronidazole and ciprofloxacin by RP-HPLC method

- in bulk drug and suspension. *Int J Chem Sci* 2009; 7:2115-21.
8. Khadabadi SS, Devkar MG. A validated RP-HPLC method for simultaneous estimation of metronidazole and ciprofloxacin hydrochloride in pharmaceutical dosage form. *Int J Pharm Sci Res* 2013;4:4736-40.
 9. Ghante MR, Pannu HK, Loni A, Shivsharan T. Development and validation of a RP-HPLC method for simultaneous estimation of metronidazole and norfloxacin in bulk and tablet dosage form. *Int J Pharm Pharm Sci* 2012;4:241-5.
 10. Trivedi KD, Chaudhary AB, Mohan S. Development and validation of RP-HPLC method for estimation of metronidazole and norfloxacin in suspension form. *Int J Adv Pharm* 2013;2:5-11.
 11. Parveen SK, Nalla C. Development and validation of a simple and rapid RP-HPLC method for the determination of metronidazole and norfloxacin in combined dosage form. *Indian J Res Pharm Biotechnol* 2013;1:686-91.
 12. Danao KR, Hiradeve SM, Moon RS, Kasture AV, Yeole PG. RP-HPLC simultaneous estimation of metronidazole and diloxanide furoate in combination. *Int J Pharm Life Sci* 2010;1:82-5.
 13. Kalpana MJ, Sowjanya GN, Ajitha A, Rao VU. Analytical method development and validation of simultaneous estimation of metronidazole and diloxanide furoate in tablet dosage form by RP-HPLC. *Int J Pharm Res Anal* 2014; 4:415-20.
 14. Das NR. Development of a RP-HPLC method for the estimation of metronidazole benzoate oral suspension. *Pharm Biol Eval* 2014;1:41-3.
 15. Sivannarayana P, Rambabu K. Simultaneous assay of mupirocin and metronidazole in formulations using reverse phase-high performance liquid chromatography. *Int J Bioassays* 2016;5:5151-5.
 16. Kumar SY, Sivaprasad P, Kumar AA. RP-HPLC, method development and validation for simultaneous quantitative estimation of metronidazole and nalidixic acid in tablets. *Int J Pharm Pharm Sci* 2015;7:367-71.
 17. Hussien EM. Development and validation of an HPLC method for tetracycline related USP monograph. *Biomed Chromatogr* 2014;28:239-44.
 18. ICH. Validation of Analytical Procedures; Methodology, Q2 (R1). Geneva: International Conference on Harmonization, IFPMA; 1996.
 19. ICH Q1A (R2) Guidelines, Stability Testing of New Drug Substances and Products; 2003.

Source of Support: Nil. **Conflict of Interest:** None declared.