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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF METRONIDAZOLE, TETRACYCLINE AND BISMUTHSUBCITRATE BY USING RP-HPLC

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ABSTRACT: A novel stability indicating method is developed by using High performance liquid chromatography (HPLC) and evaluated for the simultaneous quantification of Metronidazole(MDZ), Tetracycline(TTC) and Bismuthsubcitrate(BMSC) in the capsule dosage form. Chromatographic Separation was achieved by using the instrument Waters made Alliance HPLC system equipped with auto sampler, UV detector and Phenominex C_{18} (4.6 mm x 250 mm) 5 µm column with a mobile phase composition of 0.1% TFA (pH 4.5) and the solvent Acetonitrile in the ratio 75:25 v/v delivered at a flow rate of 1.0ml/min and the detection was carried out using UV detector at the wavelength of 281 nm. The three active pharmaceutical ingredients were extracted from tablet dosage form using diluents (Mobile phase). The retention times for MDZ, TTC and BMSC were 2.287, 2.816 and 5.526 minutes respectively. Calibration curves obtained linearity over concentration graphs was linear and the proposed method showed very good recoveries for both bulk and tablet dosage forms. The correlation coefficient values of linearity for all three active ingredients were found to be 0.999 and the concentration range was 12.5-62.5µg/ml, 12.5-62.5µg/ml and 14-70µg/ml respectively. The results of the study indicated that the proposed RP-HPLC method is fast, simple, precise, accurate, robust, and reproducible, which can be useful for the routine estimation of MDZ, TTC and BMSC in pharmaceutical dosage form. The method developed was very selective for the simultaneous quantification of MDZ, TTC and BMSC, because effective separation of the drugs from their degradation products. This method can be employed as a stability-indicating one.

Keywords: Metronidazole(MDZ), Tetracycline(TTC), Bismuthsubcitrate(BMSC), RP-HPLC, Simultaneous estimation.

INTRODUCTION: Metronidazole is one of the frequently used antibiotic drugs, belonging to nitroimidazole category of antibiotics. It is commonly used for the treatment of gastrointestinal infections, giardiasis, trichomoniasis, and also for amebiasis which are parasitic infections. Metronidazole tablets have been using as antibiotic from several decades, due to the antiparasitic properties that set it compared to other drugs, using it to treat many number of infections. It is primarily available in tablet form, topical form, capsule form, and suppository preparations for the treatment of various infections.[1] The chemical structure of Metronidazole(MDZ) is shown in below figure 1.

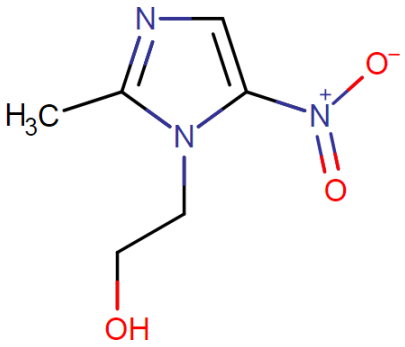

Figure 1: Chemical structure of Metronidazole (MDZ)

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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 is an antibiotic, submissively diffuses from end to end in the porin channels of a bacterial membrane along with reversibly binds to the 30S ribosomal subunit, prevents to binding of the tRNA to the mRNAribosome complex, and consequently interfering with protein synthesis.[2] The molecular structure of Tetracycline(TTC) is shown in the below figure 2.

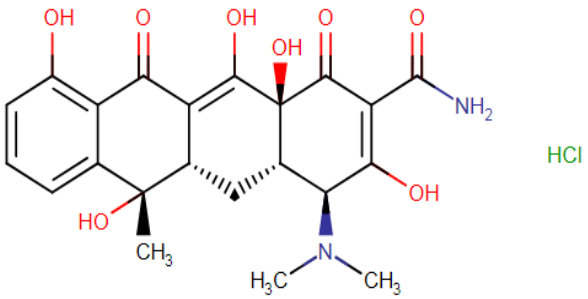

Figure 2: Chemical structure of Tetracycline (TTC)

Bismuth subcitrate is chemically bismuth (3+) pentapotassium bis(2-oxidopropane-1,2,3-tricarboxylate). The compound mainly used for the treatment of gastro-oesophageal reflux disease (GORD) and peptic ulcer. Colloidal bismuth subcitrate is tremendously useful for the treatment of gastroduodenal disorders and appears to act through numerous mechanisms due to the nature of little acid-neutralizing effect [1] and does not affect the acid secretion.[3] The chemical structure of Bismuthsubcitrate(BMSC) is shown in below figure 3.

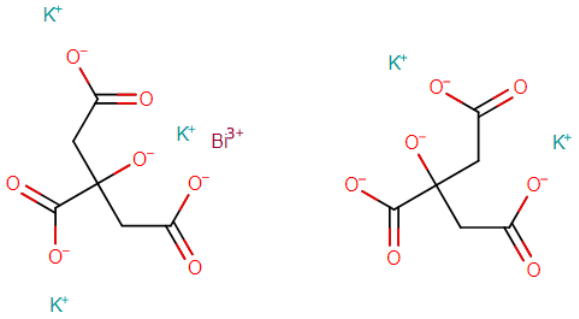

Figure 3: Chemical structure of Bismuthsubcitrate(BMSC)

During the literature survey it was revealed that, there are only a few of Ultra violet spectroscopic and HPLC methods available for the simultaneous determination and quantitation of Metronidazole(MDZ), Tetracycline(TTC) and Bismuthsubcitrate(BMSC) in pure active pharmaceutical ingredient and in combined tablet dosage forms. This current development study was designed to establish a new stability indicating HPLC method for the simultaneous determination and quantitation of Metronidazole(MDZ), Tetracycline(TTC) and Bismuthsubcitrate(BMSC) in combined pharmaceutical dosage form.

High pressure liquid chromatography (or) High performance liquid chromatography is a specific technique derived from column chromatography which was generally used for the separation and analysis of pharmaceutical industry to identify, and quantifying of the active compounds.[4]

Chromatographic separation techniques will be segregate according to whether the separation occurs on a column or planar surface. Those can be further classified into gas chromatography, liquid chromatography, paper and thin layer, and by the physical form, liquid or solid, of the stationary phase and the nature of the interactions of solutes with it, known as sorption mechanisms.[5, 6, 7]

HPLC principally requires a column which holds the stationary phase, a pump that passes the mobile phase(s) through the column, and a detector that shows signals of the compounds by retention times. Depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used the retention time can varies.[8]

Analytical validation can be mentioned as 'the establishment of the demonstrated experimental data that certifies the method executes in a systematic approach for which it was intended' and is the accountability of originating

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laboratory along with analytical method transfer (introducing of the validated method) to a selected manufacturing unit. So that, it can be applied and utilized on regular basis in the equal level of competence for which it was originally developed. [9,10]

EXPERIMENTAL WORK:

Chemicals and Reagents: Metronidazole(MDZ), Tetracycline(TTC) and Bismuthsubcitrate(BMSC) drugs and working standards were readily obtainable from the organization Pharmatrain, Balaji Nagar, Kukatpally, Hyderabad. Tri fluoro acitic acid used as mobile phase buffer and the solvent Acetonitrile were procured from Finar chemical limited. All the reagents and chemicals used were of HPLC grade, Milli-Q-water was used in the entire study.

INSTRUMENTS AND EQUIPMENTS: The Waters made HPLC systems equipped with PDA detector was used for the method development and validation. The output signal from the detector was processed, monitored and recorded by using Empower software.

Chromatographic Conditions: The eluent mobile phase combinations used were 0.1% TFA solution buffer pH 4.5 and solvent Acetonitrile were passed in a combination of (75:25), v/v in the isocratic mode of elution at a flow rate of 1.0 mL/min. The analytical HPLC column used was Phenominex C18 (4.6×250mm, 5µm). The wavelength 281 nm was opted for the detection; with a run time of 10min. The mobile phase solution was used as the diluent**.**

METHODOLOGY:

Preparation of solutions:

Preparation of 0.1 % TFA buffer: 1 mL of trifluoro acitic acid was transferred into a 1000mL glass beaker and adjusted the pH of the solution upto 4.5 with diluted TEA, finally the solution was filtered by using 0.45 Micron nylon membrane filter, sonicated it for 10 minutes.

Preparation of the eluent (mobile phase solution): Accurately measured and transferred 750 mL (75%) of above prepared buffer solution and 250 mL (25%) of solvent Acetonitrile into a 1000mL mobile phase bottle, mixed and degassed by using ultra sonicator exactly for 10 minutes and followed by the filtration through 0.45 microns membrane filter paper under vacuum.

Preparation of the standard solution: Weighed accurately and transfer 62.5mg of MDZ, 62.5mg of TTC and 70mg of BMSC working standards into a cleaned and dried volumetric flask of capacity 100mL, added small amount of diluent and allowed to sonicated for dissolving of all the components completely and made up to the mark with the diluent. (Stock solution)

Further, accurately transferred 0.6 mL of the above prepared stock solution into a cleaned and dried volumetric flask of 10mL capacity, made up to the mark with diluent. (37.5ppm of MDZ, 37.5ppm of TTC and 42ppm of BMSC)

Assay of Pharmaceutical Dosage form: (Sample Solution Preparation): Accurately weighed and transfer equivalent to 62.5mg of MDZ, 62.5mg of TTC and 70mg of BMSC samples into clean and dried volumetric flask of 100mL capacity, added small amount of diluent and sonicated it up to 30 mins to dissolve all the components completely and made volume up to the mark with the diluent. Then it was filtered through 0.45 micron Injection filter paper. (Stock sample solution)

Further accurately transferred 0.6 mL of MDZ, TTC and BMSC from the above prepared stock sample solution into a volumetric flask of 10mL capacity and made up with diluent. (37.5ppm of MDZ, 37.5ppm of TTC and 42ppm of BMSC)

System suitability test parameters: To assess the system suitability test parameters such as theoretical plate count, tailing factor and retention time; equilibrated the column with the mobile phase at a flow rate of 1.0mL/min for 15 minutes. The chromatographic separation was achieved by passing the mobile phase in the combination of 0.1% TFA buffer of pH 4.5 and the solvent Acetonitrile in the ratio of 75:25v/v, by injecting 4.0 µL volume of standard solution into Phenominex C18 column (250 x 4.6mm, 5µm), at the flow rate of 1.0mL per minute. Theoretical plate count results, tailing factor value and retention time details of the developed method were mentioned in the table-1.

Assay of tablet dosage form: The proposed validated analytical HPLC method was applied successfully for the determination of MDZ, TTC and BMSC in synthetic mixture. The results obtained for MDZ, TTC and BMSC was equivalent to the corresponding respective labeled amounts and were mentioned in the table-2.

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METHOD VALIDATION:

Specificity:
For Specificity, Blank and Standard solution are injected into system. There was nothing interference in blank (diluent) chromatogram with the retention times of the analyte peaks.

Linearity:
Linearity I : (12.5ppm of MDZ, 12.5ppm of TTC & 14ppm of BMSC)
Linearity II : (25ppm of MDZ, 25ppm of TTC & 28ppm of BMSC)
Linearity III : (37.5ppm of MDZ, 37.5ppm of TTC & 42ppm of BMSC)
Linearity IV : (50ppm of MDZ, 50ppm of TTC & 56ppm of BMSC)
Linearity V : (62.5ppm of MDZ, 62.5ppm of TTC & 70ppm of BMSC)
Procedure: Injected each level of the above prepared solutions in the chromatographic (HPLC) system and measured the peak areas.
Plotted a graph between analyte concentrations (on X-axis) versus obtained peak area (on Y-axis) and calculated for the correlation coefficient. The obtained results were mentioned in the table-3.

Accuracy: Injected the standard solutions at three levels, Accuracy at 50%, Accuracy at 100% and Accuracy at 150%. Calculated the amount of obtained and amount of added for MDZ, TTC and BMSC, and calculated the individual and mean recovery values. The obtained results were mentioned in the table-4.

Precision and ID Precision: The above prepared standard solution was injected for six times and recorded the peak areas, for the evolution of precision parameter. The obtained %RSD results for the peak area of six injections was found in the accepted limits.
To assess the intermediate precision also known as Ruggedness of the proposed method, Precision parameter was executed on different day.
The obtained Precision parameter results data was mentioned in table-5 and ID Precision data was mentioned in table-6.

Method Precision:
Prepared and injected six individual samples solutions to evaluate the method precision and calculate the % of Assay. The Method Precision results were mentioned in Table-7

Solution Stability:
The sample and standard solutions prepared under assay, has been kept in bench top for 24hours to perform solution stability and injected those standard and sample solutions after 24hours. The analysis performed for solution stability was with freshly prepared mobile phase. The obtained solution stability parameter results were mentioned in table-8

Robustness: Robustness of a method was evaluated by applying small changes in the chromatographic conditions such as flow rate (±10%), mobile phase ratio (±10%). In this experiment noticed that, there were no significant change in the results of system suitability test parameters, which confirmed that the proposing developed HPLC method, is robust.

Limit of detection and Limit of quantification: With the use of the slope and standard deviation of response method, the parameters limit of detection (LOD) and limit of quantitation (LOQ) of the method was established.

Degradation studies:

As per the International Conference on Harmonization (ICH) guideline, stability testing of a new drug substances and products requires that stress testing to be performed for revealing of the inherent stability characteristics. The objective of this study is to carry out the stress degradation studies on the MDZ, TTC and BMSC using the proposed method.
The Degradation results were mentioned in table-9

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Preparation of standard stock solution:
Accurately weighed 62.5mg of MDZ, 62.5mg of TTC and 70mg of BMSC working standards into a 100 mL of dried and cleaned flask and added small amount of diluent solution to dissolve the contents by sonicating and made up to the mark with the same solvent (Stock solution).

Hydrolytic degradation of the product under acidic condition: Transferred accurately 0.6 mL volume of the above prepared stock solution into a 10mL clean and dried flask and added 3 mL of 0.1M Hydrochloric acid to the flask. After that, the volumetric flask was allowed to keep at 60°C temperature in an oven for a period of 6 hours and then followed by neutralization by using 0.1 molar sodium hydroxide solution and finally diluted to the mark with diluent. Filtered the solution by using 0.22 micron syringe filters and placed the solution in HPLC vials.
Hydrolytic degradation of the product under alkaline condition: Transferred accurately 0.6mL of the above prepared stock solution into a clean and dried 10mL volumetric flask and added 3mL of 0.1M sodium hydroxide solution. After that, the volumetric flask was allowed to keep at 60°C temperature in an oven for a period of 6 hours and then neutralized it by using 0.1M Hydrochloric acid solution and finally dilueted to the mark with diluent. Filtered the solution by using 0.22 micron syringe filters and transferred the solution in HPLC vials.

Oxidative degradation
Transferred accurately 0.6mL above prepared stock solution into a clean and dried 10mL volumetric flask and added 1 mL of 3.0% v/v of peroxide (H_2O_2) solution into flask and diluted up to the mark with diluent. After that the flask was allowed to keep at ambient temperature for 20 minutes. Filtered the solution with 0.22 micron syringe filter and placed the same solution in HPLC vials.

Thermal degradation
MDZ, TTC and BMSC sample was transferred into a petridish and allowed to keep the same in an air oven at 110^0C for 24 hours. After completion of 24 hours, the treated sample was transferred and prepared the solution with diluent and placed in HPLC vials and the analysis was performed.
Photo degradation: Transferred 0.6 mL of above prepared stock into a clean and dried 10mL flask and exposed it onto direct sunlight over a time of 24hrs. After completion of 24 hours, the volume was made up to the mark by using diluent. Filtered the solution by using 0.22 micron syringe filter and placed the solution in HPLC vials.

RESULTS:

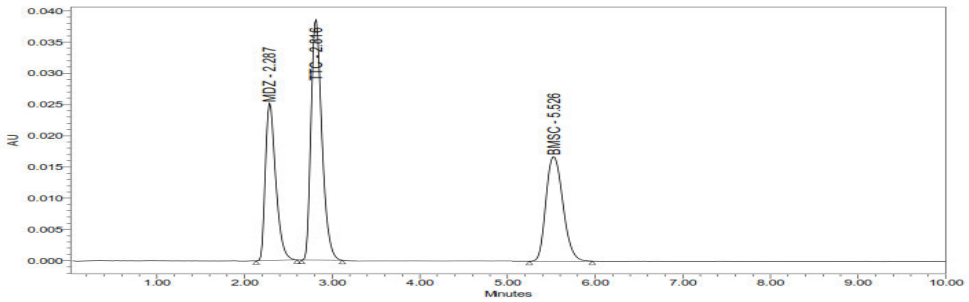

Figure 4: Chromatogram of Standard solution

Table 1: System suitability test parameter results

Parameter	MDZ	TTC	BMSC
USP Plate count	5288.57	6056.39	5739.57
Retention time	2.287	2.816	5.526
USP Tailing	1.46	1.38	1.14
USP Resolution	--	2.35	8.95

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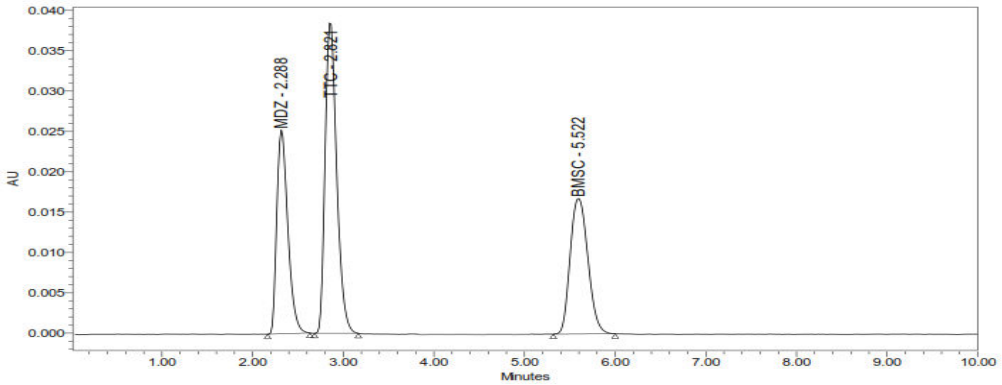

Figure 5: Sample chromatogram

Table 2: Assay Results

Drug Name	Label Claim (mg)	% Assay
Metronidazole(MDZ)	125	99.91
Tetracycline(TTC)	125	99.25
Bismuthsubcitrate(BMSC)	140	100.30

The assay of Metronidazole(MDZ), Tetracycline(TTC) and Bismuthsubcitrate (BMSC) was executed with the dosage tablets and the assay(% w/w) results was found to be 99.91, 99.25 and 100.30 respectively which indicates that the method can be useful for regular analysis.

Table 3: Linearity Results

Parameters	MDZ	TTC	BMSC
Concentration range (µg/mL)	12.5-62.5	12.5-62.5	14-70
Correlation coefficient (r)	0.999	0.999	0.999
Intercept	465.9	405.43	518.95
Slope	5257.5	5957.1	5876.7

The linearity results of MDZ, TTC and BMSC was found to be linear with a correlation coefficient of 0.999, 0.999 and 0.999 respectively, which is indicating that, the method is capable enough with good sensitivity.

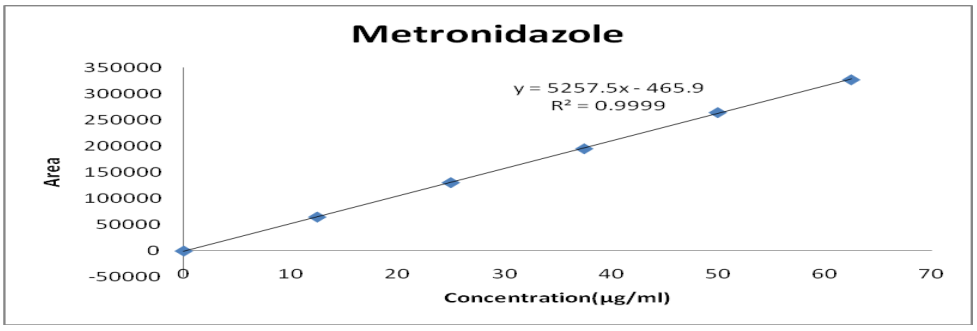

Figure 6: Linearity graph for MDZ

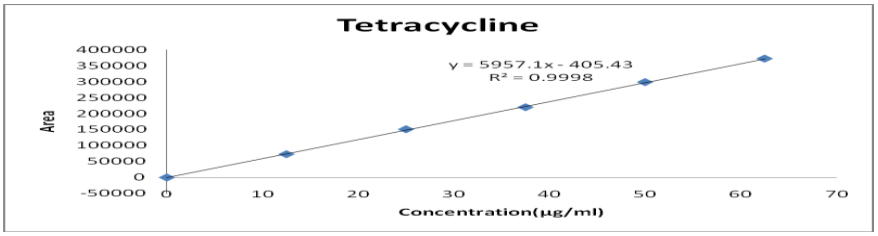

Figure 7: Linearity graph for TTC

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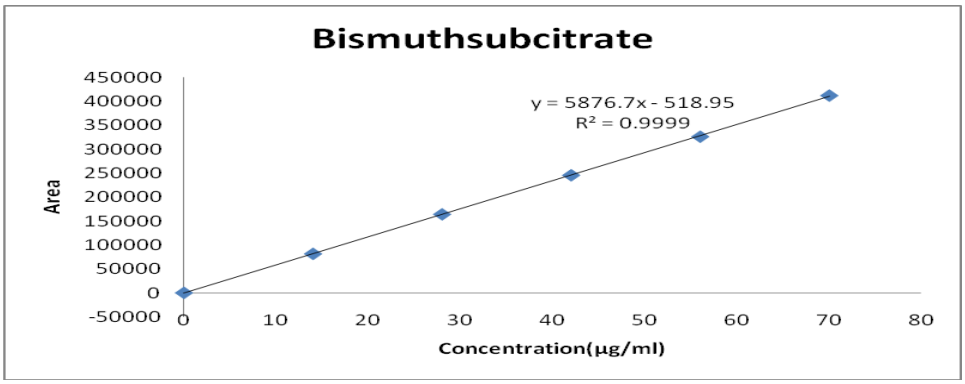

Figure 8: Linearity graph for BMSC

Table 4: Accuracy Results

Drug Name	% Concentration (Level)	Peak Area	Amount Added (in mg)	Amount Obtained (in mg)	% Recovery	Mean Recovery
MDZ	50%	97827.3	31.25	31.20	99.84	99.96
	100%	194511	62.5	62.04	99.26	
	150%	296232.3	93.75	94.48	100.78	
TTC	50%	107881.3	31.25	31.05	99.37	100.27
	100%	218507.0	62.5	62.90	100.64	
	150%	328291.0	93.75	94.50	100.80	
BMSC	50%	124985.3	35	35.26	100.75	100.49
	100%	248378.3	70	70.08	100.11	
	150%	374505.3	105	105.66	100.63	

For accuracy (recovery) parameter, acceptable limit for the percentage of recovery should be in between 98.0% - 102.0% range. The mean recoveries were found to be 99.96%, 100.27 and 100.49% for MDZ, TTC and BMSC respectively. The accuracy parameter results of the developed method showing that, the recoveries were well within the in the limits, which indicates that the proposed developed method is capable enough of showing good accuracy with reproducibility.

Table 5: Precision Results

Injection	MDZ	TTC	BMSC
Injection 1	194837	218464	249658
Injection 2	195736	219783	249566
Injection 3	196836	214753	247488
Injection 4	195424	218563	246358
Injection 5	193634	215846	242753
Injection 6	195429	217564	246323
Average	195316.0	217495.5	247024.3
Standard Deviation	1055.4	1873.9	2560.9
% RSD	0.5	0.9	1.0

The acceptable criteria for precision parameter is the %RSD should be less than 2.0, and the developed method showed the precision values as 0.5, 0.9 and 1.0 for MDZ, TTC and BMSC respectively, which indicating that the proposed developed method is precise.

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Table 6: ID Precision Results

Injection	MDZ	TTC	BMSC
Injection 1	192765	215873	243176
Injection 2	193375	216433	242896
Injection 3	193428	214863	244722
Injection 4	194672	217536	241564
Injection 5	192396	213854	243744
Injection 6	193459	214653	241865
Average	193349.2	215535.3	242994.5
Standard Deviation	777.0	1340.1	1175.6
% RSD	1.5	1.6	1.4

The acceptable criteria for intermediate precision parameter is the %RSD should be less than 2.0, and the developed method showed intermediate precision values as 1.5, 1.6 and 1.4 for MDZ, TTC and BMSC respectively, which indicating the proposed developed method is repeatable when executed in different day also.

Table 7: Method Precision Results

Sample Name	% Assay for MDZ	% Assay for TTC	% Assay for BMSC
Method precision-1	100.47	100.87	100.75
Method precision-2	100.46	100.35	100.18
Method precision-3	100.73	100.78	100.30
Method precision-4	100.32	100.65	100.45
Method precision-5	100.74	100.67	100.32
Method precision-6	100.77	100.80	100.29
Average	100.58	100.69	100.38
Standard Deviation	0.19	0.18	0.20
% RSD	0.19	0.18	0.20

Table 8: Solution Stability Results

S. No.	Standard Area (Mean*3)	Standard peak area after 24hrs (Mean*3)	Sample peak area after 24hrs (Mean*3)	% Of variation for standard & sample	% Assay
MDZ					
1	195568.3	195743.0	196378.3	0.09 & 0.41	100.21
TTC					
2	216685.7	217370.0	218401.3	0.32 & 0.79	100.59
BMSC					
3	247616.0	248040.0	247893.0	0.17 & 0.11	99.91

Table 9: Degradation Results

	MDZ		TTC		BMSC	
	Area	% Degraded	Area	% Degraded	Area	% Degraded
Standard	195568.3		216685.7		247616.0	
Acid	168356	13.91	184733	14.75	213825	13.65
Base	165831	15.21	181693	16.15	210583	14.96
Peroxide	167642	14.28	187326	13.55	215324	13.04
Thermal	169538	13.31	186356	14.00	211963	14.40
Photo	165342	15.46	185436	14.42	208486	15.80

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CONCLUSION:

The proposed method was found to be rapid, simple, precise, linear and accurate for the determination of MDZ, TTC and BMSC in the pharmaceutical dosage form. The method was successfully validated for the parameters specificity, LOD, LOQ, linearity, precision, robustness, accuracy, solution stability, and system suitability test values were found to be within the acceptance limits. The method was completely validated as per ICH guidelines. The method has significant advantages, in terms of selectivity, shorter run time and accurate than previously reported. The method validation data showed satisfactory results for all tested method parameters. This method can be useful for the regular analysis for the determination of the MDZ, TTC and BMSC in combined drug dosage tablet form with no interference of any excipients.

REFERENCES:

[1]. https://www.drugbank.ca/drugs/db00916

[2]. Shareefa Asra Shoeb And Dr. R. Vani, Method Development And Validation For Simultaneous Estimation Of Metronidazole, Tetracycline, Bismuth Subcitrate In Its Bulk And Pharmaceutical Dosage Form By Rp-Hplc, World Journal Of Pharmaceutical And Life Sciences, 2019, Vol. 5, Issue 11, 190-196.

[3]. Prathap 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, Asian Journal Of Pharmaceutics, Oct-Dec 2018 (Suppl), 12 (4), S1311.

[4]. Martin M., Guiochon, G. Effects of high pressures in liquid chromatography. J. Chromatogr. A, 2005; (1-2)7: 16-38.

[5]. Becket and Stenlake, Practical pharmaceutical chemistry, part 24th edition CBS publications and distributors, (2005).

[6].P. .D. Sethi, HPLC quantitative analysis of pharmaceutical formulations CBS publications and distributors, 1st edition, (2001).

[7]. B.K Sharma, instrumental method of chemical analysis, 23rd edition, goal publishers (2004).

[8]. Liu Y., Lee M.L. Ultrahigh pressure liquid chromatography using elevated temperature. Journal of Chromatography. 2006; 1104 (1-2):198–202.

[9]. Validating chromatographic methods, David M.Bliesner.

[10]. International conference on harmonization: ICH Q 2 (R1) Validation of Analytical Procedures: Text and Methodology (1995).