Stability Assurance: RP-HPLC Method Development and Validation for Novel Dental Abscess Bulk Drugs Combinations

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ABSTRACT

Background: This research focused on developing and validating a stability-indicating and RP-HPLC (Reverse Phase High-Performance Liquid Chromatography) method for the precise estimation of Metronidazole and Cefixime in bulk and formulation samples. The choice of Shim-pack C-18 column, with dimensions 250 X 4.6 mm then a particle size of 5 µm, was pivotal for achieving the desired chromatographic separation. The mobile phase used consisted of Methanol and Water in a ratio of 80:20 v/v.

Results: The chromatographic conditions yielded excellent results, with retention times of 3.127 minutes for Metronidazole and 2.096 minutes for Cefixime. The method demonstrated linearity across a concentration range of 5 to 30 µg/mL for both compounds. Furthermore, the Limit of Detection (LOD) and Limit of Quantification (LOQ) values were determined as 0.331/1.004 µg/mL for Metronidazole and 0.590/1.789 µg/mL for Cefixime, respectively.

Conclusion: In conclusion, the established RP-HPLC method underwent validation following ICH Q2 R1 guidelines to ascertain its accuracy, precision, and robustness. Its capacity to detect degradation products during stress testing, encompassing oxidation, photodegradation, acid, and base hydrolysis, validates it as a dependable stability-indicating technique. This method is suitable for the routine analysis of Metronidazole and Cefixime in pharmaceutical formulations, guaranteeing quality control and adherence to regulatory standards.

Keywords: Metronidazole, RP-HPLC, Method validation, Stability indicating method, Cefixime.

1. BACKGROUND

Dental Abscess is also known as Dentoalveolar Abscess, it is a localized collection of pus in the alveolar bone apically to the tooth. Necrotic dental pulp, periodontal tissue or peri coronal tissue are the primary cause of dentoalveolar infections. A necrotic pulp and inflammatory response of periapical connective tissue, result in acute dentoalveolar abscess [1]. When the cortical bone resorbs, a swelling may also occur. It may occur Secondary as outcome of dental cavities, trauma, deep filling, or botched Root Canal therapy. Bacteria and microorganism that enters to these microorganisms can induce acute inflammation and pus production through the apical foramen in periapical tissue. Dental or dentoalveolar infections, if left untreated then it can be highly painful as well as it represents a significant danger of descending into the deep neck area and ascending to the Cerebral Sinuses [2]. The primary treatment for these infections is local dental treatment that focuses on treating the source of the infection in order to establish drainage via the soft tissue, either therapy of root canal or extraction of the problematic tooth. Antibiotics are usually considered a last resort when an infection has progressed beyond the confines of the tooth and cannot be adequately treated surgically, or when the bacterial load exceeds the body's immune defences. A periapical abscess specifically forms at

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the apical region of a tooth's root within the surrounding bone tissue. Periapical abscess forms at the tip of a tooth's root in the bone. [3] It typically occurs as result untreated dental decay that has penetrated the pulp chamber, leading to infection and abscess formation at the tooth's apex (tip of the root). Periapical abscesses are commonly caused by bacterial invasion of dental pulp. Peri coronal Abscess develops above the crowned part of erupted tooth. It occurs when there isn't enough space for the fully emerge, creating a flap of gum tissue that can trap food particles and bacteria. Infection and abscess formation can cause pain, swelling, and discomfort. Periodontal Abscess occur in the gums, mostly in the periodontal pockets (space between the teeth and gums). This type of abscess usually associated with advanced gum disease (periodontitis). That are caused when the bacteria become trapped in these pockets, they can lead to infection, abscess formation, and localizing swelling. Gingival Abscess forms in the gum tissues without affecting the tooth or periodontal ligaments. It mostly occurred due to localized trauma, foreign bodies, or irritants becoming embedded in the gum tissue which causing to infection and abscess formation [4]. Dental caries was present in 91.1% of people between the ages of 20 and 63, according to data from the National Health and Nutrition Examination Survey (2010–2012) carried out by the National Centre for Health Statistics. [5]. When the rates are compared with non-Hispanic white adults, these rates were lower for Hispanic, non- Hispanic black Americans, non- Hispanic Asian people (Dye et al. 2012) [6]. Approx. 26.9% of adults between the ages of 20 to 63 have untreated tooth decay. Untreated tooth decay remained greater in Hispanic at 36.1% than non- Hispanics black Americans (42.2%). According to (Dye et al 2012), 19.1% of persons over 64.9% had untreated tooth decay. [6] Dental abscesses and Emergency room (ER)visits for dental related hospital are rather common. According to one study (Wang et al 2005), In the United State found that the oral infection admission in hospitals happened at an amount of 1.1 as per 2601 population. Rate for paediatric emergency room (ER) visits for dental abscess is much greater at 46.9% (Graham et al 2000). [7,8]. This evidence not only suggests an extremely high occurrence of poor oral health, which is a key risk factor to develops dentoalveolar abscess, however, the research suggests that socioeconomic and racial factors may also have a role. Because of the community's size, rapid spread, and socioeconomic demographics, provider practices may shift.[1.1] pathophysiology of dentoalveolar abscess it usually start with bacteria gaining access to the pulp chamber of a tooth or the periodontal tissue through cavities, cracks, or gum pockets. commonly bacteria involve streptococcus species and anaerobes Bacteria like Fusobacterium and Prevotella. The bacteria multiply rapidly, leading to an infection. The body's immune response triggered to combat the infection. Immune system leads white blood cell to the site of infection [9]. This leads to inflammation, causing swelling, redness, and pain. As per white blood cells & bacteria accumulate, pus, a thick fluid filled with bacteria, dead cells &tissue debris, starts to form. The pus creates a pressure within the tooth or the surrounding gum tissue. The accumulation of pus creates a confined space, that forms an abscess. In periapical abscess case, it is located at the tip off the tooth root. In periodontal abscess (in the gum disease), it forms within the periodontal pocket. Pressure forming the abscess causes intense pain, localized swelling, and can lead to fever and general discomfort. If left untreated, the disease spread to nearby tissues, including adjacent teeth, the jawbone, or into the bloodstream, that potentially cause the systemic infection [10]. To address, the discussed issue, the research work has been focused on the development of novel formulation consisting of Metronidazole and Cefixime as APIs. Furthermore, the novel method based on Reverse phase high performance liquid chromatography has been developed and validated. The observations and results are discussed in further sections. Moreover, the stability studies have been carried out using RP-HPLC method.

1.1 Metronidazole (MTZ)

Metronidazole (MTZ), 2-(2-methyl-5-nitro-1H-imidazol-

1-yl) ethanol Fig.1, it belongs nitroimidazole family, and class of antibiotics. MTZ is a crystalline powder that ranges in colour from white to pale yellow and has a faint smell, soluble in methanol and 158 to 160℃ .It is used to treat skin infection, mouth infection, dental abscess and infected gums [11,12]. MTZ is Approved FDA drug for treating protozoal infection, anaerobic bacterial infection and microaerophilic infection. Additionally, the FDA has approved in usage for the treatment of anaerobic bacterial infections brought on by the following species: Fusobacterium species, Prevotella species and Porphyromonas species [13]. MT Z's mechanism of action involves diffusing into the body, interfering with DNA to prevent protein synthesis, and breaking DNA strands and causing helical DNA loss. Thus, susceptible animals, it leads to cell death [14]. MTZ can be taken orally, topically, intravenously and it comes in capsule, tablet, topical foam. Combination of metronidazole and cefixime used in the treatment of dental abscess [15] MTZ is official in Indian Pharmacopeia (IP 2014) [16], British Pharmacopeia (BP 2016) [17] and United State Pharmacopeial Convention (USP 2010) [18]. Literature review reveals that various method are reported for the analysis of individual drug and in combination with other drugs but stability indicating RP-HPLC method has been reported for simultaneous estimation of metronidazole and cefixime. The current effort aims to establish a high-performance liquid chromatography technique for stability indication in the reverse phase, for the simultaneous quantification of metronidazole and cefixime.

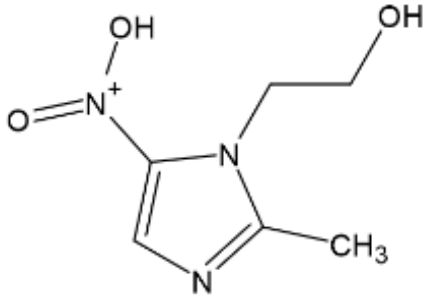

Fig.1. Chemical structure of Metronidazole

1.2 Cefixime

Cefixime (CEF), (6r, 7R)-7- {[2-(2-amino- 1,3-thiazol-4-yl)-2(carboxymethoxyiminio) acetyl] amino}-3 ethyenyl-8-oxo-5-thia-1 azabicyclo- [4.2.0] oct-2-ene-2 carboxylic acid (fig (2), is a crystalline powder that is white to light yellow in colour [19], soluble in methanol. It belongs to the third-generation oral cephalosporin class of antibacterial [20]. It is official in Indian Pharmacopeia [19], British Pharmacopeia [20] and United State of Pharmacopeia [21]. Cefixime is employed for addressing infections caused by susceptible gram-negative and gram-positive bacteria [21]. Its mechanism of action involves functioning as a cephalosporin through the utilization of its β-lactam ring to hinder the synthesis of bacterial cell walls by binding to the penicillin-binding protein transpeptidases located on bacteria. This inhibition of bacterial cell wall synthesis leads to lysis, particularly in rapidly proliferating bacterial species [22]. CEF can be taken orally, topically, intravenously and it available as capsule, tablet, topical foam [23].

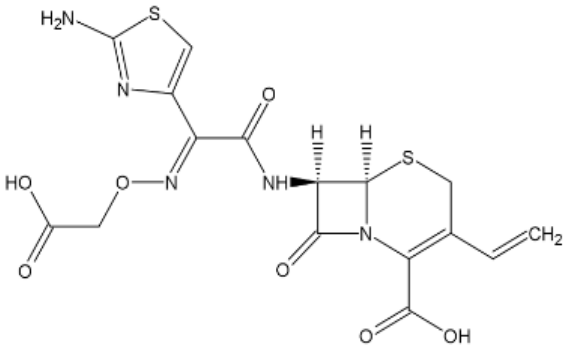

Fig. 2. Chemical structure of Cefixime

2. METHOD

2.1. Chemicals and reagents:

Metronidazole and Cefixime is a gift sample from Yarrow Chem product, Mumbai, India. HPLC grade chemicals: Methanol and Water were preferred for the development of the method, this was obtained from CDH (Central drug house) (P) Ltd.

2.2. Instruments:

Chromatography was carried out using a Shimadzu High Performance Liquid Chromatography system with a manual sampler, Win Chrome software, and UV-visible detector. The chromatographic separation was performed using Column C-18 (Shim-pack) 250 X 4.6 mm, particle size 5µm.

Selection of Wavelength: Wavelength was fixed at 298 nm by performing UV spectroscopy.

2.3. CHROMATOGRAPHIC CONDITION:

The method development for analyzing Metronidazole and Cefixime involved experimentation with various solvents. Ultimately, a mobile phase consisting of methanol and water in an 80:20 volume/volume ratio was chosen. This mobile phase was pumped through the system at a flow rate of 1 ml/min, and a Rheodyne injector equipped with a 20µl loop was used for sample injection. The eluent was detected using a UV detector with a 289 nm wavelength setting. The mobile phase was prepared through filtration using a 0.22 µm nylon membrane filter, then subjected to degassing in an ultrasonic bath.

2.4. Mobile phase preparation:80 ml of HPLC grade methanol are added to 20 ml of water in a mobile phase reservoir and the mixture is kept for sonication for 10 minutes.

2.5. Standard stock solution preparation:

Standard stock solution was equipped by dissolving 10mg of metronidazole and cefixime in 100mL methanol and water that gives the conc. of 100 µg/ml. this solution was diluted with mobile phase as needed to produce several standard solutions.

2.6. Method Validation:

Method validation in analytical chemistry encompasses the systematic evaluation and verification of a given analytical procedure to ascertain its appropriateness for the intended application, ensuring its capacity to deliver precise and dependable outcomes. This rigorous process entails a comprehensive array of examinations and trials aimed at confirming that the method satisfies predetermined standards, while also validating the consistency, replicability, and significance of the obtained results. [24].

2.6.1. Linearity and range:

The linearity of the analysis was assessed across six concentration levels spanning from 5 to 30µg/ml for Metronidazole and Cefixime each. A calibration curve was constructed by correlating concentration with the corresponding peak area, and linearity was determined using least squares regression analysis. Analytical range defined by the lowest and highest concentrations of the analyte demonstrated acceptable linearity as per the results obtained.

2.6.2. Precision

Precision, as defined by ICH rules, includes both repeatability and intermediate precision. Six replicates of the sample injection were used to assess repeatability. To determine intraday precision, three different doses of metronidazole and cefixime (10, 15, and 20 µg/mL) were tested three times on the same day. The three concentrations indicated above were tested on three repeated days for inter-day precision in order to assess day-to-day variability.

2.6.3. Accuracy:

Accuracy was determined by calculating recovery of the analyte of interest. A fixed amount of pre-analysed sample was taken and the standard drug was added and 80%, 100% and 120% levels. The standard conc. was fixed as 5µg/ml of metronidazole and 5 µg/ml for cefixime, and three conc. levels of 3, 5 and 7µg/mL for metronidazole and 3, 5 and 7µg/mL for cefixime were added to the standard concentration. Every level was carried out three times. The percentage recovery standard deviation [% RSD] were taken into consideration for testing accuracy.

2.6.4. Limit of detection (LOD):

LOD denotes the minimum concentration of an analyte within a sample that can be reliably identified, although it's not typically defined as an exact value. To determine LOD, the standard deviation of response and slope were

employed in calculations.

$$\text{LOD}= \frac{3.3\ X\ \sigma}{S}$$

The linearity curve was used to compute the slope and variability for Metronidazole and Cefixime conc. ranging from 5 to 30 µg/mL.

Limit of quantitation (LOQ):

LOQrepresents the minimum quantifiable quantity of an analyte in a sample that can be measured with the requisite precision and accuracy. LOQ determination relies on assessing the standard deviation of the response and the slope, with data sourced from the linearity curve.

$$\text{LOD}= \frac{10X\ \sigma}{S}$$

The linearity curve was used to compute the slope and variability for Metronidazole and Cefixime conc. ranging from 5 to 30 µg/mL.

2.6.5. Robustness:

The robustness of the study was examined by purposefully changing a few factors slightly, in accordance with ICH recommendations. The capacity of a drug to stay unaffected by minute variations in parameters such as temperature, detecting wavelength, flow rate, and mobile phase composition is mostly associated with resilience. Little adjustments to the chromatographic settings, such as changes to the mobile phase composition, detection wavelength, and flow rate, can be used to assess how resilient the approach is. At a concentration of 5µg/mL, robustness was evaluated.

3. Force degradation studies:

Forced degradation studies involve exposing the drug substance to different stress conditions to assess the level and speed of degradation that may occur during storage. The studied degradation pathways typically include acid hydrolysis, basic hydrolysis, oxidative degradation, and photolytic degradation [25-28].

3.1. Acid Hydrolysis: Acid hydrolysis was done by adding accurately weighed 10mg of metronidazole and cefixime into a clean and dry round bottom flask. 50ml of freshly prepared Methanolic 0.1 N HCl solution transfer to it and it was refluxed in a water bath for 6 hours by maintaining the temperature at 60℃. After refluxing the solution 1ml sample withdrew at different intervals for 6 h which was neutralized and diluted to 10ml with methanol. The 20 µL of the resultant solution (20 µg/ ml) was injected and analysed.

3.2. Basic Hydrolysis: To a clean and dry round bottom flask accurately weighed 10mg of metronidazole and cefixime was transferred. 50ml of freshly prepared Methanolic 0.1 NaOH was added to it and it was refluxed in a water bath for 6 hours by maintain the temperature at 60℃. After refluxing the solution 1ml sample withdrew at different intervals for 6 h which was neutralized and diluted to 10ml with methanol. The 20 µL of the resultant solution (20 µg/ ml) was injected and analysed.

3.3. Oxidation degradation: To a clean and dry round volumetric flask accurately weigh 10mg of metronidazole and cefixime drug was taken, to this 50ml of methanolic 5% (v/v) hydrogen peroxide solution was added & was kept in the dark for 6h. the solution (1ml) was diluted to 10mL with methanol. The 20 µL of resultant solutions (20 µg/ ml) was injected and analysed.

3.4. Photolytic degradation: Photolytic degradation was carried out by taking 10mg of Metronidazole and Cefixime drug into a clean and dry petri dish which is covered with a glass lid. The drugs are kept under sunlight for 12h and then, 10 mg of Metronidazole and Cefixime added in 50 mL of methanol. After extracting the solution (1 mL), methanol was added to dilute it to 10 mL. After 20 µL of the resultant solution (20 µg/ml) was injected and analysed.

4. RESULTS

A stability-indicating Reverse-phase High-Performance Liquid Chromatography (RP-HPLC) method was successfully developed and validated for the precise estimation of Metronidazole and Cefixime in bulk samples.

Various validation parameters were explored, including stressed samples and adjustments to mobile phase compositions and flow rates. Optimal conditions were established through iterative testing. Metronidazole and Cefixime exhibited well-resolved peaks with excellent symmetry and a stable baseline using a mobile phase consisting of Methanol and Water (80:20 v/v) at a flow rate of 1.0 ml/min. Retention times for Metronidazole and Cefixime were determined to be 3.127 and 2.096, respectively, with distinct peaks observed at 298 nm (refer to Fig. 3).

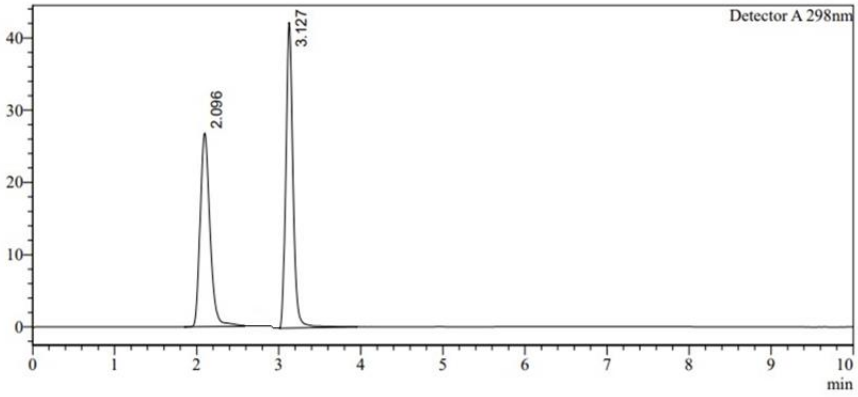

Fig.3. Chromatogram of Metronidazole and Cefixime

4.1. Linearity and range:

For linearity of six-point concentration curve was obtained in concentration ranges 5 µg/mL- 30 µg/mL for metronidazole and cefixime. The response of the drugs was originated to linear in the selected concentration range, the regression equation y=107373x – 23595, Y= 101639x + 8362 for metronidazole and cefixime and, the correlation coefficient (r^2) for metronidazole and cefixime were 0.9999 and 0.9997 respectively. (Fig.4,5). Result of linearity shown in table.1,2.

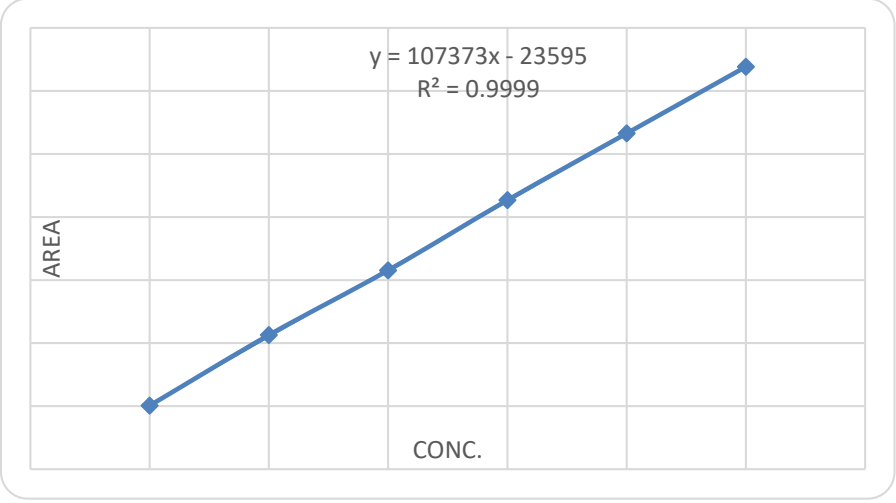

Fig.4. Calibration curve of Metronidazole. Y = 107373x – 23595 Slope = 107373, Intercept = 23595, Correlation coefficient = 0.9999.

Table 1. Linearity and Range data for Metronidazole

Sr no.	Concentration µg/mL	Peak Area (mv)
1.	5 µg/mL	503746
2.	10 µg/mL	1063894
3.	15 µg/mL	1576912
4.	20 µg/mL	2133997
5.	25 µg/mL	2663240
6.	30 µg/mL	3190766
Average Area		1855426
Slope		107373
Y – intercept		23595
Correlation Coefficient		0.9999

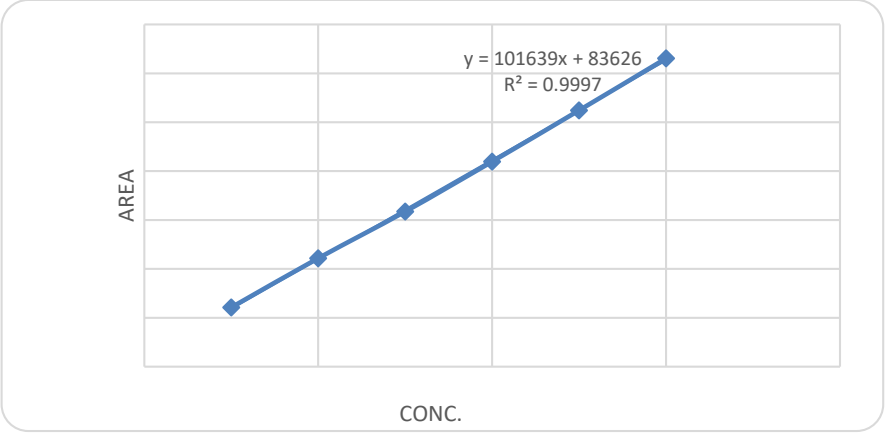

Fig.5. Calibration curve of Cefixime. Y= 101639x + 83626 Slope= 101639, Intercept = 83626, Correlation Coefficient = 0.9997

Table 2. Linearity and Range data for Cefixime

Sr. no.	Concentration µg/mL	Peak Area (mv)
1.	5 µg/mL	605746
2.	10 µg/mL	1108922
3.	15 µg/mL	1586645
4.	20 µg/mL	2097990
5.	25 µg/mL	2620909
6.	30 µg/mL	3153655
Average Area		1862311
Slope		101639
Y – intercept		8362
Correlation Coefficient		0.9997

4.2. Precision:

The analytical method's precision is characterized by the concordance level among individual test outcomes derived from applying the method across various samples. Precision assessments of the proposed method were conducted, encompassing evaluations for repeatability and intermediate precision (both within and between days). The performance of the HPLC instrument was assessed under chromatographic conditions by repetitively injecting 5 µg/mL of metronidazole and cefixime. The Relative Standard Deviation (RSD) was ascertained to be within acceptable limits, signifying the enhanced accuracy of the proposed methodology. Precision result are shown in Table 3,4 and 5.

Table.3. Interday precision of the developed method for Metronidazole and Cefixime

Interday precision										
Cefixime						Metronidazole				
Sr.no.	**Conc. (µg/ml)**	**Peak Area**	**Mean**	**S. D**	**%RSD**	**Conc. (µg/ml)**	**Peak Area**	**Mean**	**S. D**	**%RSD**
1.	**10**	1039941 1038742 1038423	1039035	800.3839	0.077031	**10**	1063894 1059893 1068722	1064170	4420.951	0.415437
2.	**15**	1486645 1485445 1476457	1482894	5568.056	0.357497	**15**	1576917 1568176 1566586	1570560	5562.714	0.354187
3.	**20**	1896880 1887760 1897564	1894068	5473.583	0.288986	**20**	2078651 2067541 2087341	2077844	9924.618	0.47764

Table.4. Intraday precision of the developed method for Metronidazole and Cefixime

Intraday precision										
Cefixime						Metronidazole				
Sr.no.	**Conc. (µg/ml)**	**Peak Area**	**Mean**	**S. D**	**%RSD**	**Conc. (µg/ml)**	**Peak Area**	**Mean**	**S. D**	**%RSD**
1.	**10**	1044513 1032714 1042081	1039769	6229.924	0.599164	**10**	1064083 1046601 1061801	1057495	9503.226	0.898654
2.	**15**	1475642 1476910 1454644	1469065	12505.32	0.851243	**15**	1567819 1549881 1565987	1561229	9870.252	0.63221
3.	**20**	1896780 1897651 1868542	1887658	16560.38	0.877298	**20**	2068741 2065813 2047659	2060738	11420.68	0.554203

Table.5. Repeatability of the developed method for Metronidazole and Cefixime Repeatability

Cefixime			**Metronidazole**	
Sr.no	**(Conc.(ug/ml)**	**Peak Area**	**(Conc.(ug/ml)**	**Peak Area**
1	5	726907	5	503685
2.	5	724305	5	503746
3.	5	724305	5	504367
4.	5	727905	5	502634
5.	5	726770	5	505412
6.	5	725640	5	503576
7.	**Mean**	726189.3	**Mean**	503903.3
8.	**S. D**	1263.724	**S. D**	925.4015
9.	**%RSD**	0.174021	**%RSD**	0.183647

4.3 Accuracy: 6

The percentage recovery of the spiked sample was with 100±2% which ensures the accuracy of the developed method. Result of accuracy shown in Table.6,7.

Table.6. Accuracy of the developed method for Metronidazole

Metronidazole							
Sr. no.	**Unfortified sample**			**Fortified sample**			**%Recovery**
	Conc. (ug/ml)	**Area**	**Mean (Area)**	**Conc. (ug/ml)**	Area	**Mean (Area.)**	
1.	**3**	247585	270123	**3+5**	690101	694045	**99.9%**
		266921			691021		
		298865			701013		
2.	**5**	417312	415222	**5+5**	818849	812419	**99.2%**
		414651			811976		
		413705			806434		
3.	**7**	560792	564875	**7+5**	1038396	1039139	**96.3%**
		565438			1000161		
		568395			1078860		

Table.7. Accuracy of the developed method for Cefixime

Cefixime							
Sr. no.	**Unfortified sample**			**Fortified sample**			**%Recovery**
	Conc. (ug/ml)	**Area**	**Mean (Area)**	**Conc. (ug/ml)**	Area	**Mean (Area.)**	
1.	**3**	451656	454744	**3+5**	1016632	1056745	**93.9%**
		458478			1029151		
		454100			1124452		
2.	**5**	618023	619811	**5+5**	1258303	1287130	**99.0%**
		629738			1299026		
		610873			1304061		
3.	**7**	907362	910655	**7+5**	1603697	1617327	**97.6%**
		917302			1655845		
		907302			1592440		

4.3. Limit of detection and Limit of Quantification:

LOD and LOQ were determine by using the standard deviation and calibration curve of the y-intercepts and slope of the metronidazole and cefixime. The LOD for metronidazole and cefixime has determined as 0.331 µg/ml, 0.590 µg/ml, and LOQ originate to be 1.004 µg/ml and 1.789 µg/ml for the same drug. Thus, the above result showed that was sensitive and other drug are less sensitive, the developed method can detect at low concentration and quantify.

4.4. Robustness:

The robustness of metronidazole and cefixime was assessed by the introduction of small changes in the chromatographic condition such as a change in flow rate of 1mL/ min, wavelength detection 1 nm, and a change in the mobile phase composition by 1%. The measured concentration was 5g/ ml. The standard deviation of response was calculated for each parameter, and the% RSD was less than 2%, demonstrating the method's robustness, as shown in Tables 8, 9.

Table.8. Robustnessof the developed RP- HPLC method for Metronidazole

Sr.no	Parameters			Peak Area	Retention Time (RT)	USP	
	Optimized		Used			Plate Count	Tailing factor
1.	Flow Rate (±1)	1ml/min	0.9ml/min	417081	3.423	5336	1.387
			1.1ml/min	551091	2.894	5013	1.374
2.	Wavelength detection (±1)	298nm	296 nm	625856	3.120	5088	1.380
			300 nm	604752	3.115	5120	1.375
3.	Mobile composition ±1)	80:20	79:21	697123	3.083	5170	1.381
			81:19	694133	3.013	5136	1.383

Table.9. Robustness of the developed RP- HPLC method for Cefixime

Sr.no	Parameters			Peak Area	Retention Time (RT)	USP	
	Optimized		Used			Plate Count	Tailing factor
1.	Flow Rate (±1)	1ml/min	0.9ml/min	715069	2.357	974	0.863
			1.1ml/min	849161	1.994	969	0.871
2.	Wavelength detection (±1)	298nm	296 nm	737037	2.104	1103	0.898
			300 nm	863406	2.127	1005	0.871
3.	Mobile composition (±1)	80:20	79:21	705669	2.086	1155	0.897
			81:19	682908	2.083	1146	0.872

4.5. Force Degradation:

The chromatograms from samples exposed to acidic, basic, oxidative, and photodegradation conditions exhibited distinct peaks corresponding to metronidazole and cefixime, with retention times of 3.127 and 2.096, respectively, alongside additional peaks at different retention times. Acid-induced degradation of metronidazole showed no new peaks, while cefixime displayed one additional peak. Base-induced degradation did not result in additional peaks for metronidazole but

showed one additional peak for cefixime. Both oxidative and photodegradation processes produced one additional peak each for both drugs. The percentage of degradation products is detailed in Table 11, and the results of forced degradation are graphically presented in Figures 6 to 13.

5. DISCUSSION

The RP-HPLC method developed for the analysis of Metronidazole and Cefixime employs a mobile phase consisting of Methanol: Water (80:20 v/v) at a flow rate of 1.0 ml/min. This method demonstrates robustness and reliability, particularly notable for its stability-indicating capability under stressed conditions. The observed distinct peaks at 298 nm signify optimal separation and peak resolution, ensuring selectivity in compound detection. Retention times of 3.127 minutes for Metronidazole and 2.096 minutes for Cefixime, coupled with a linear response range of 5 to 30 µg/mL, highlight its analytical efficiency. The determined LOD and LOQ values (0.331/1.004 µg/mL for Metronidazole and 0.590/1.789 µg/mL for Cefixime) underscore its sensitivity. A forced degradation study was conducted by subjecting the sample to diverse stress conditions, aiming to assess the robustness and stability-indicating characteristics of the developed analytical method. During acid degradation, Metronidazole did not show any new peaks, while Cefixime displayed one additional peak. Conversely, in base-induced degradation, Metronidazole remained without additional peaks, while Cefixime exhibited one new peak. Both oxidative and photodegradation analyses resulted in one additional peak for both compounds.

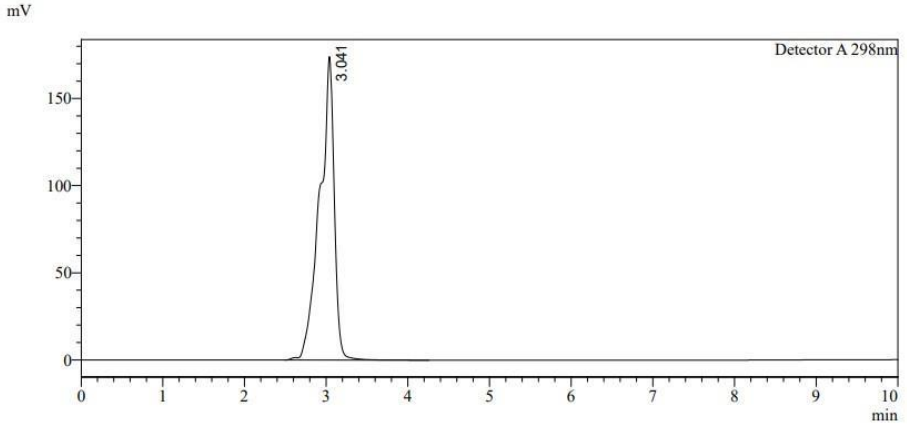

Fig. 6 Acid degradation in which Metronidazole undergoes in presence of methanolic 0.1 N HCl at 60℃

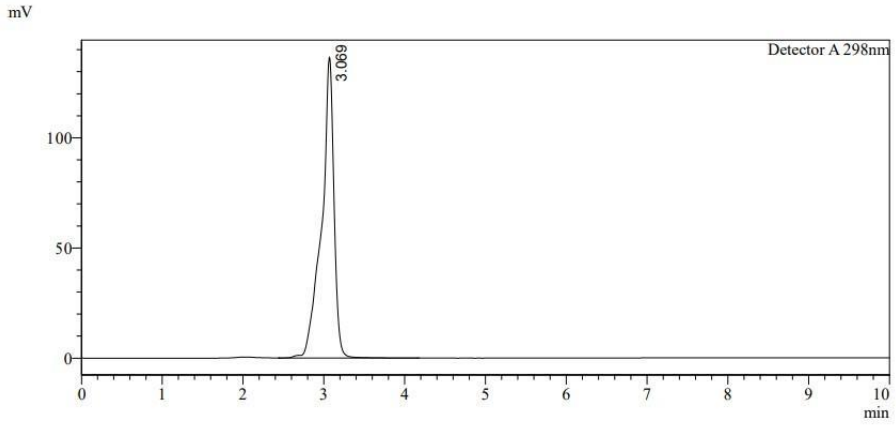

Fig. 7 Base degradation in which Metronidazole undergoes in presence of methanolic 0.1 NaOH at 60℃

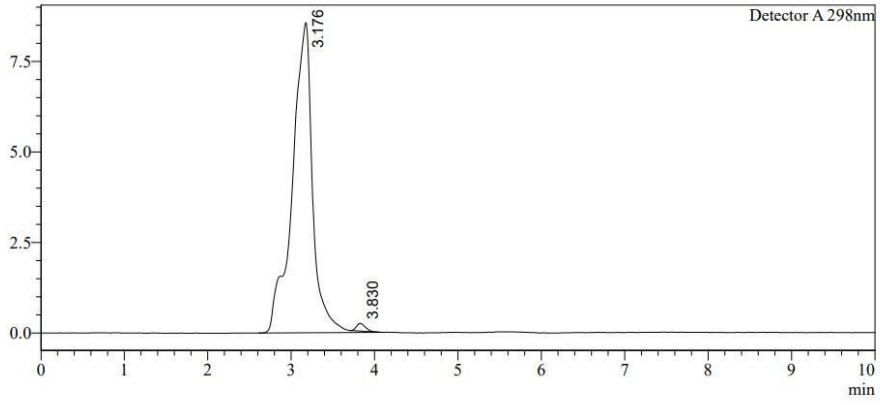

Fig. 8 Photodegradation in which Metronidazole undergoes in presence of sunlight 12 h.

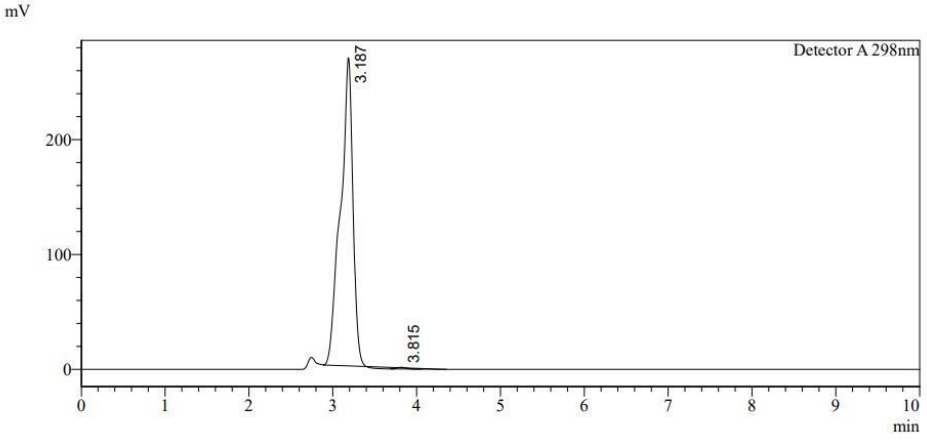

Fig. 9 Oxidative degradation in which Metronidazole undergoes in presence of methanolic 5%

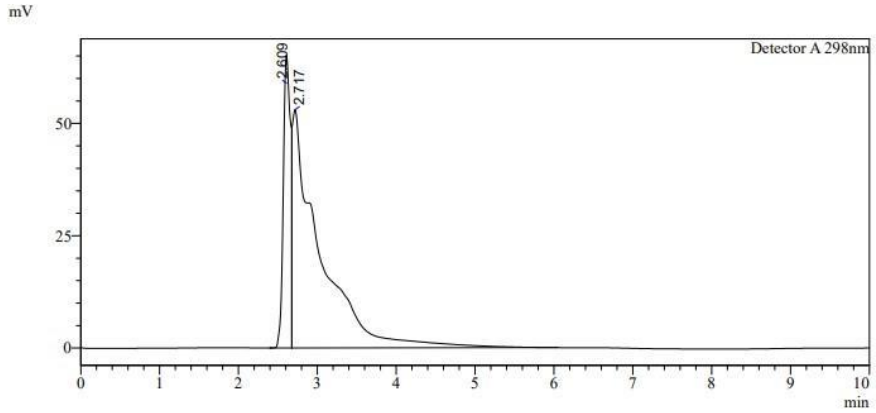

Fig. 10 Acid degradation in which Cefixime undergoes in presence of methanolic 0.1 N HCl at 60℃

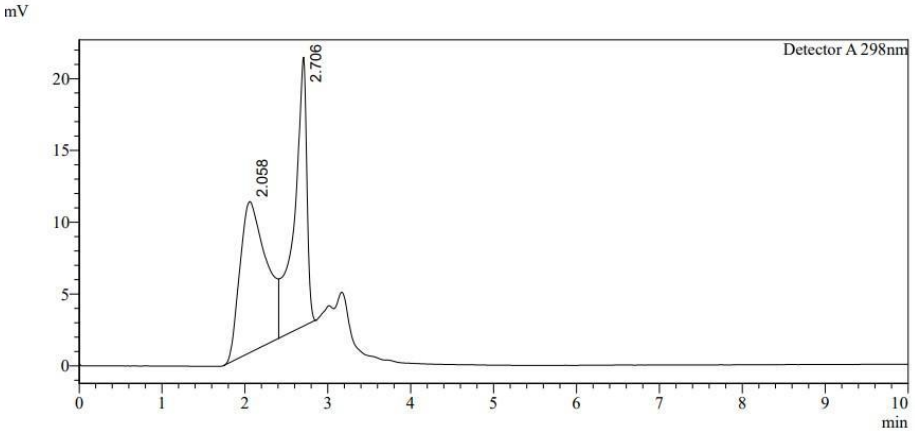

Fig. 11 Base degradation in which Cefixime undergoes in presence of methanolic 0.1 N NaOH at 60℃

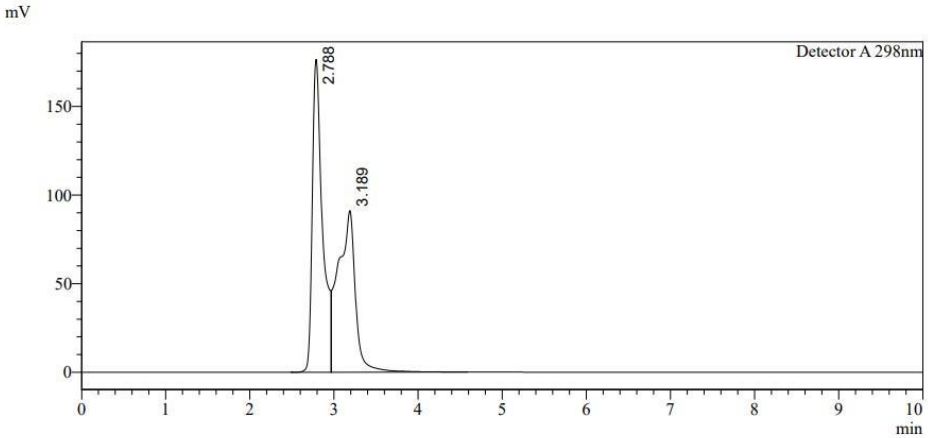

Fig. 12 Oxidative degradation in which Metronidazole undergoes in presence of methanolic 5% H2O2

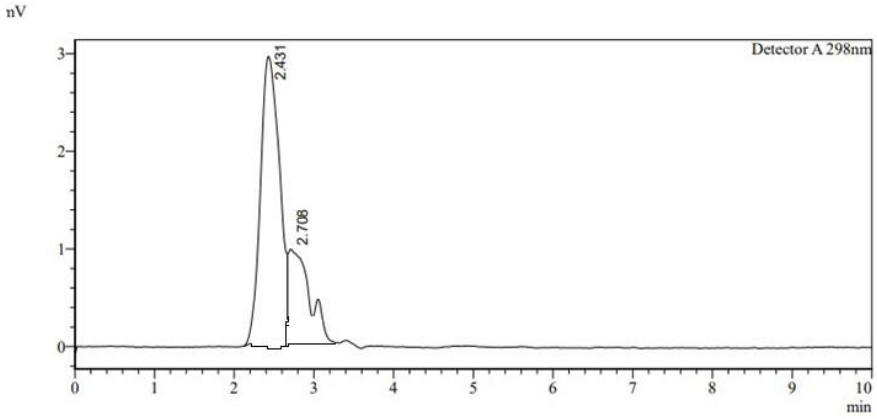

Fig. 13 Photodegradation in which Cefixime undergoes in presence of sunlight 12 h.

Table.11. Force Degradation study data of Metronidazole and Cefixime

Metronidazole	**Cefixime**					
Type of degradation	**Area**	**% Recovered**	**%Degradation**	**Area**	**% Recovered**	**%Degradation**
Acid	3427695	100%	0%	1712317	76.2%	23.8%
Base	1453599	100%	0%	428415	54.1%	45.9%
Oxidation	2725414	98.9%	1.1%	2807006	53.4%	46.6%
Photodegradation	146886	99.5%	0.5%	57022	81.7 %	18.3%

6. CONCLUSION

The study demonstrates that the developed RP-HPLC method is characterized by its rapidity, precision, accuracy, specificity, and stability. It was employed for the simultaneous quantification of both drugs in their bulk form, demonstrating short analysis times and minimal % RSD values, indicative of high precision. Adhering to ICH guidelines, a stability-indicating method was formulated and validated for the concurrent estimation of Metronidazole and Cefixime in bulk drugs using RP-HPLC. This method is deemed appropriate for routine quality control analysis of these compounds in active pharmaceutical ingredients.

7. ABBREVIATION:

RP-HPLC: Reversed-Phase High-Performance Liquid Chromatography

UV: Ultraviolet.

MTZ: Metronidazole

CEF: Cefixime

ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

LOD: Limit of Detection

LOQ: Limit of Quantification

RSD: Relative Standard Deviation

NaOH: Sodium Hydroxide

HCl: Hydrochloric Acid

Conc.- concentration

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ضمان الاستقرار: تطوير وتحقق طريقة RP–HPLC لتركيبات جديدة من المواد الدوائية المستخدمة في خراج الأسنان

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ملخص

الخلفية :ركز هذا البحث على تطوير والتحقق من طريقة RP-HPLC) الكروماتوغرافيا السائلة عالية الأداء ذات الطور العكسي) لتقدير ميترونيدازول وسيفيكسيم بدقة في العينات الخام والمستحضرات الصيدلانية. وقد كان اختيار عمود Shim-pack C-18بأبعاد 250 × 4.6 مم وحجم جسيمات 5 ميكرومتر أمرًا حاسمًا لتحقيق الفصل الكروماتوغرافي المطلوب. تألف الطور المتحرك من مزيج من الميثانول والماء بنسبة.(v/v) 80:20

النتائج :أسفرت الظروف الكروماتوغرافية عن نتائج ممتازة، إذ بلغ زمن الاحتجاز 3.127 دقيقة للميترونيدازول و2.096 دقيقة للسيفيكسيم. أظهرت الطريقة تدرجًا خطيًا في نطاق التركيز من 5 إلى 30 ميكروغرام/مل لكلا المركبين. بالإضافة إلى ذلك، تم تحديد قيم حد الكشف (LOD) وحد التقدير (LOQ) بـ 1.004/0.331 ميكروغرام/مل للميترونيدازول و1.789/0.590 ميكروغرام/مل للسيفيكسيم على التوالي.

الاستنتاج :خضعت طريقة RP-HPLCالمعتمدة للتحقق وفقًا لإرشادات ICH Q2 R1لتأكيد دقتها وتكراريتها وموثوقيتها. وقد أثبتت قدرتها على الكشف عن نواتج التحلل خلال اختبارات الإجهاد، بما في ذلك الأكسدة والتحلل الضوئي والتحلل في الأوساط الحمضية والقاعدية، مما يؤهلها كطريقة فعالة للدلالة على الاستقرار. تعتبر هذه الطريقة مناسبة للتحليل الروتيني لميترونيدازول وسيفيكسيم في المستحضرات الصيدلانية، مما يضمن مراقبة الجودة والامتثال للمعايير التنظيمية.

الكلمات الدالة: ميترونيدازول، طريقة RP-HPLC، التحقق من المنهجية، طريقة مؤشِّرة للاستقرار، سيفيكسيم.

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