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Simultaneous Estimation of Cefixime and Azithromycin in a Pharmaceutical Formulation by RP-HPLC

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Abstract:

This study fulfilled the preliminary research aims and proved that RP-HPLC could be used for the pharmaceutical analysis of Cefixime and Azithromycin in pharmaceutical formulations using UV detection. The medications belong to distinct classes. The proposed and verified approaches outperformed previously published methods in terms of sensitivity, selectivity, repeatability, stability, and recovery, all while exhibiting minor matrix effects. RP-HPLC has been shown to be an effective approach for the simultaneous measurement of azithromycin and cefixime.

Keywords: Simultaneous estimation, Cefixime, Azithromycin, Antibiotics, RP-HPLC, Chromatography.

INTRODUCTION:

A simple, accurate and precise RP-HPLC method was developed and validated for simultaneous estimation of Cefixime and Azithromycin in bulk and tablet dosage form. Azithromycin [9-de-oxy-9a-aza-9amethyl-9a-homoerythromycin A dihydrate] is an Azalide, a subclass of macrolide antibiotics. Cefixime (6R, 7R)-7-[2-(2-amino-4- thiazolyl) glyoxylamido]- 8-oxo-3-vinyl-5-1 –azabicyclo [4.2.0] ct-2- ene-2-carboxylicacid,7-9z)-[o carboxymethyl)- oxime] trihydrate is third generation cephalosporin antibiotic. As per literature survey, no analytical method has been reported for simultaneous estimation of Cefixime Trihydrate and Azithromycin Dihydrate in pharmaceutical dosage forms. [1] Therefore the present research work, our aim is to develop a novel, simple, accurate, sensitive, reproducible, economical analytical method

to estimate Cefixime Trihydrate & Azithromycin Dihydrate in their combined dosage form in routine analysis.

MATERIALS AND METHOD:

Materials:

Instruments:

1200 infinity LC isocratic pump, a Rheodyne injector with a 20 µL fixed volume loop, a variable wavelength programmable PDA detector, and EZICHROME ELITE Chromatographic Software, double beam UV-Visible spectrophotometer (Lab India -3120), UVWIN-5 software, ultrasonicator, shimadzu electronic analytical balance (AX-220), Systronics digital pH metre.

Chemicals

Cefixime and Azithromycin procured from Hetero laboratories, water methanol ortho phosphoric acid, potassium hydroxide, and potassium dihydrogen phosphate all sourced from Hi-media in Mumbai, India. The reagents and chemicals used are of the HPLC grade.

Preparation of standard stock solution

To make a standard stock solution of the pure drugs Cefixime and Azithromycin (1 mg/mL), 100 mg of the medication was transferred to a 100 mL volumetric flask, and the mixture was dissolved in methanol. The concentrations were adjusted to 1-5 µg/mL and 5-25 µg/mL, respectively, by further diluting the standard stock solution with mobile phase.

Preparation of buffer (pH-5.0)-20mM phosphate buffer

To make the buffer solution, 6.8 grams of potassium dihydrogen phosphate was weighed and dissolved in 1000 millilitres of HPLC-grade water. Before being filtered through 0.45 milipore size filter paper, the solution was brought to a pH of 5.0 using orthophosphoric acid and a 10M KOH solution. It was then degassed in an ultra bath sonicator for about 30 minutes.

Preparation of mobile phase solution

Before use, the mobile phase was sonicated and filtered through a 0.45 mili membrane filter. The mobile phase consisted of methanol, acetonitrile, and a newly made phosphate buffer solution (pH.5.0) in a proportion of 30:30:40 (v/v/v). It was subjected to processing for 15 minutes. [2]

METHOD DEVELOPMENT

When developing a method, it is necessary to choose the fixed and mobile phases as well as the wave length.

Detection of wavelength

We recorded the spectra of Cefixime and Azithromycin in methanol solutions after diluting them. By scanning each sample independently on a UV spectrophotometer in the UV range (200-400 nm) in spectrum mode, we were able to determine that the drug's absorption spectra were most concentrated at the isobestic point 260 nm. The analysis was performed by setting the HPLC system's PDA detector to 260 nm.

Choice of stationary phase

Octadecyl columns of various forms, configurations, and manufacturers have been used in preliminary research studies. In the end, the analytical column Kromasil ODS C18 (250 x 4.6 mm, 5µ) was successful in achieving the desired separation.

Selection of the mobile phase

The mobile phase was optimised by several systematic testing. To achieve clean peak-to-base separation of the components and avoid excipient interference, we used a variety of solvents, including methanol, water, and acetonitrile, in varying ratios, along with mobile phase pH values and buffer solutions. In the mobile phase, using a 20 mM phosphate buffer (pH-5), we were able to achieve satisfactory peak symmetry, which

was resolved and free from tailing: isocratic condition methanol: acetonitrile 40:30:30 (v/v/v).

Selection of the mobile phase flow rate

Optimal separation was achieved by varying the mobile phase flow rates from 0.5 to 1.2 mL/min. To maximise solvent savings, maintain a minimal flow rate and run duration. The trials revealed that the analyte was most effectively eluted at a flow rate of 1 mL/min.

Optimized chromatographic conditions

An RP-HPLC technique that is sensitive, exact, and accurate was developed for the detection of Cefixime and Azithromycin in pharmaceutical dosage forms after many systematic experiments were completed to optimise the chromatographic conditions. [3]

Table 1: Optimized chromatographic conditions of Cefixime and Azithromycin

Standard Concentration	Cefixime3.0µg/mL	
	Azithromycin15.0µg/mL	
Pumpmode	Isocratic	
	20mMPhosphatebuffer(pH-5):	
Mobile phase	Methanol:Acetonitrile(40:30:30,v/v/v)	
Wavelength	260nm	
Column	KromasilC_{18}column(250x4.6mm,5µ)	
Column Temp	Ambient	
Diluent	MobilePhase	
Injector	Rheodyne	
Injection Volume	20µL	
Flowrate	1mL/min	
RetentionTime	Cefixime	3.46min
	Azithromycin	4.47min
Runtime	6min	
PeakArea	Cefixime	4392722
	Azithromycin	299946
Theoreticalplates	Cefixime	5926
	Azithromycin	20272
TailingFactor	Cefixime	1.21
	Azithromycin	1.42

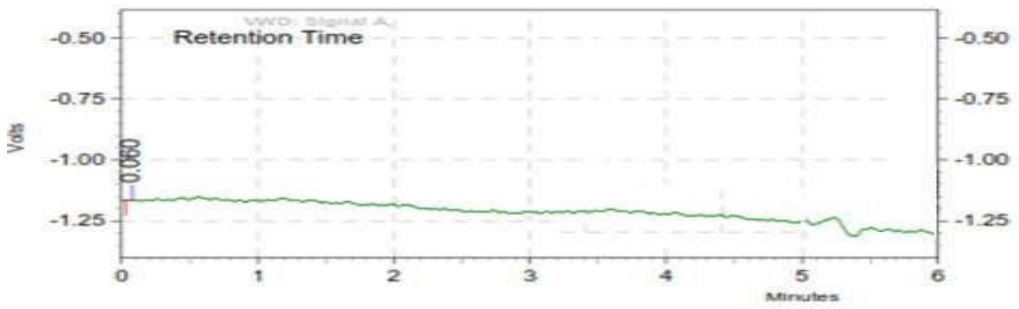

Fig.1:Blank chromatogram

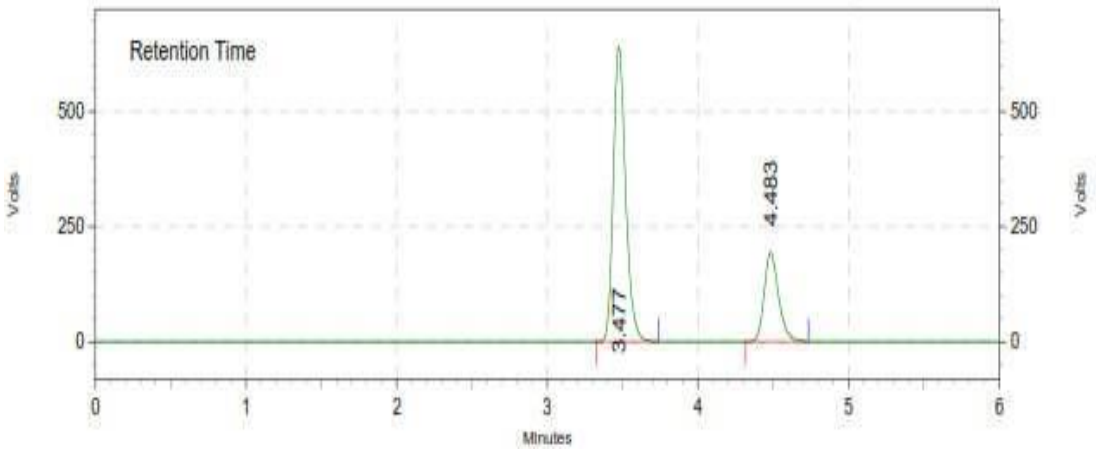

Fig.2:Standard chromatogram of Cefixime and Azithromycin

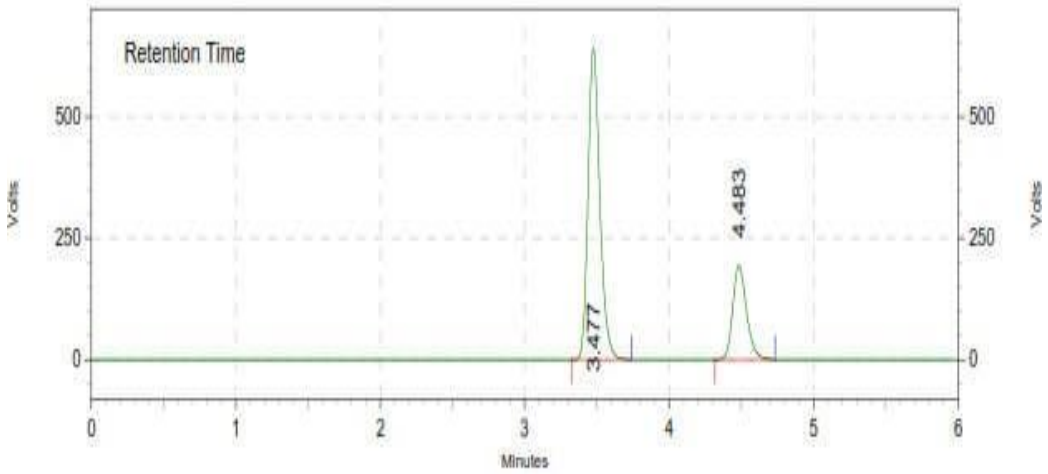

Fig.3:Test formulation chromatogram Cefixime and Azithromycin

METHOD VALIDATION

In accordance with ICH standards, the suggested procedure was verified. Filter validation, solution stability, robustness, specificity, linearity, precision, accuracy (recovery), system appropriateness, and robustness were the parameters examined for validation.[4]

Specificity

No diluent or placebo peaks were detected at the major peaks. Therefore, Cefixime and Azithromycin were both accurately and selectively measured using the chromatographic method. Research on excipient specificity has shown that they had no effect on the results. Cefixime and azithromycin both exhibited symmetric peaks in the standard solution, with retention durations of 3.47 and 4.48 minutes, respectively. Table 2 displays the findings.

Table. 2:Specificity of AZT and CFX

Nameofthesolution	RetentionTime(min)
Blank	Nopeaks
Cefixime	3.46min
Azithromycin	4.47min

System suitability

Azithromycin and Cefixime standard stock solutions were tested for system applicability using recently manufactured solutions. Thoroughly combine the standard concentration in an equal volume. The suggested method's system appropriateness was expressed using the results obtained after injecting 20 µL of the sample into the HPLC system from the produced solution. The findings are shown in Table 3. [5]

Table. 3:System suitability results of CFX and AZT

RetentionTime	Cefixime	3.46min
	Azithromycin	4.47min
PeakArea	Cefixime	439283
	Azithromycin	299938
Theoreticalplates	Cefixime	85697
	Azithromycin	95771
TailingFactor	Cefixime	1.21
	Azithromycin	1.42
Resolution	Cefixime	-
	Azithromycin	3.9

Linearity & Range

Between fifty percent and one hundred fifty percent of the AZT and CFX target concentrations were manufactured as standard solutions. At concentrations of 5, 10, 15, 20, and 25 µg/mL of AZT and 1, 2, 3, 4, and 5 µg/mL of CFX, linearity was evaluated by doing individual measurements using different amounts of the stock standard solution diluted with the mobile phase. The injections were spaced out by ten minutes. For CFX, the linearity ranged from 1 to 5 µg/mL, whereas for AZT it was shown to exist between 5 and 25 µg/mL. In order to determine the linearity range, the chromatograms were recorded and a linearity graph was created by comparing the drug's peak area to the concentrations. [6]

Table. 4: Linearity and range of AZT and CFX

S.NO	**Conc.µg/mL**	**Area of CFX**	**Conc. µg/mL**	**Area of AZT**
1	1	152805	5	117047
2	2	283275	10	194015
3	3	439272	15	299945
4	4	588872	20	389025
5	5	771860	25	498014
Concentrationrange		1.0-5.0µg/mL	5-25µg/mL	
Slope(m)		152100	19496	
Correlationcoefficient		0.9978	0.998	

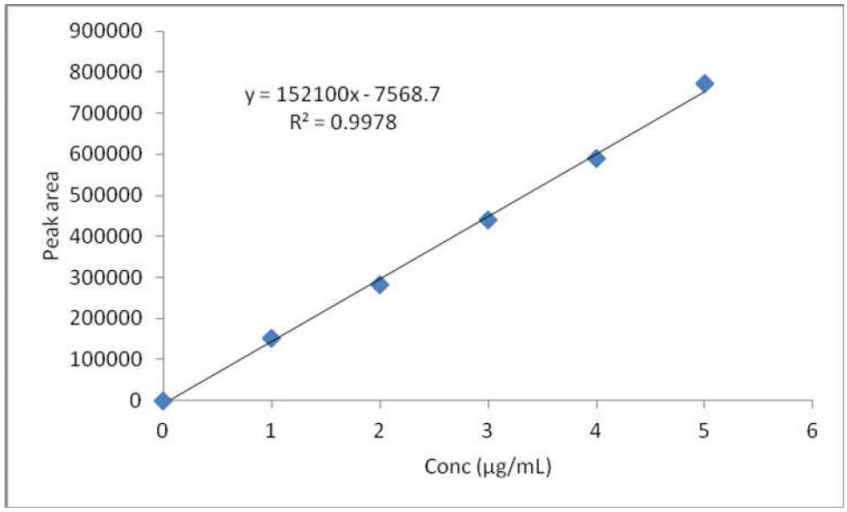

Fig4: Linearity of CFX

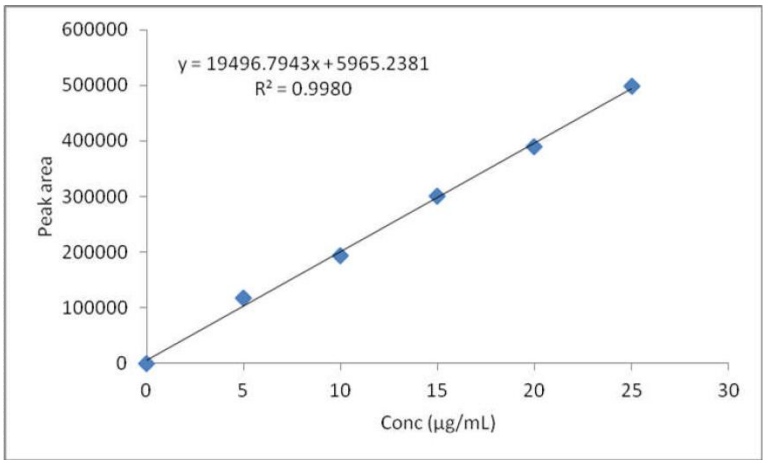

Fig5: Linearity of AZT

Precision

The intra-day and inter-day precision studies were carried out using a test sample assay method with six replicates on the same day and different days.

Table. 5.:Intra day precision data for CFX and AZT

Sample.No	Area ofCFX	%Assay	Area ofAZT	%Assay
1.	452282	99.12	293845	100.47
2.	449151	98.42	293545	100.38
3.	449623	98.54	293356	100.31
4.	459577	100.72	294620	100.74
5.	449635	98.53	293107	99.33
6.	449457	98.50	283762	98.66
Mean	451620	98.96	292040	99.99
SD	4061.34	0.89	4088.74	0.81
%RSD	0.91	0.90	1.40	0.81

Table.6.: Inter day precision data for CFX and AZT

Sample.No	Area ofCFX	%Assay	Area ofAZT	%Assay
1.	453273	99.34	293845	100.48
2.	456537	100.05	294776	100.80
3.	459272	100.65	288446	98.63
4.	456537	100.05	292447	100.00
5.	453724	99.44	292776	100.11
6.	451723	99.00	293846	100.48
Mean	455177	99.75	292689	100.09
SD	2761.74	0.61	2240.65	0.77
%RSD	0.61	0.61	0.77	0.77

Accuracy (Recovery)

Calculating AZT and CFX recoveries using the approach of standard adds allowed us to assess the correctness of the procedure. To get an idea of how much AZT and CFX were in the sample, we measured their peak areas and fitted them to the straight-line equation of the calibration curve. The sample solution already had a known quantity of CFX in it, at 2 µg/mL, and AZT at 10 µg/mL.

Table 7.Accuracy of CFX

Levelof % recovery	TargetConc.(µg/mL)	Amountof drugSpiked (µg/mL)	Nominalconc(µg/mL)	Amountfound(µg/mL)	% Recovery	Mean	SD	%RSD
80	2.0	1.60	3.60	3.61	100.27	100.15	0.41	0.41
				3.58	99.71			
				3.61	100.57			
100	2.0	2.00	4.00	3.98	99.74	100.08	0.29	0.29
				4.01	100.26			
				4.02	100.25			
120	2.0	2.40	4.40	4.38	99.76	100.29	0.46	0.46
				4.42	100.43			
				4.42	100.68			

Table8..Accuracy ofAZT

Levelof % recovery	TargetConc.(µg/mL)	Amountof drugSpiked (µg/mL)	Nominalconc (µg/mL)	Amountfound(µg/mL)	% Recovery	Mean	SD	%RSD
80	10.0	8.0	18.0	18.12	100.71	100.28	1.35	1.35
				17.77	98.77			
				18.24	101.38			

100	10.0	10.0	20.0	20.12	100.61	100.20	0.87	0.87
				20.15	100.79			
				19.82	99.21			
120	10.0	12.0	22.0	21.81	99.17	99.37	0.94	0.94
				21.67	98.54			
				22.07	100.40			

Ruggedness

The purpose of this is to demonstrate that the method's use does not introduce any bias into the test findings due to operational or environmental factors. Test findings should be reproducible under the typical variance in circumstances predicted from system to system and from analyst to analyst. Ruggedness is a measure of this repeatability. Six independent runs, each with a unique analyst, column, and system, were used to conduct the test. [7]

Table9.Ruggedness of CFX & AZT

Sr.No.	CFX(%Assay)			AZT(%Assay)		
	SETI	SETII	SETIII	SET I	SETII	SETIII
1	99.51	101.61	101.76	99.69	98.60	98.39
2	101.90	101.42	99.60	98.71	98.20	99.71
3	99.60	99.50	101.89	98.10	99.76	99.87
4	100.88	100.60	101.40	98.11	99.23	99.60
5	101.41	99.90	101.61	99.21	99.65	99.21
6	101.60	98.91	99.50	99.59	98.53	98.01
Average	100.81	100.32	100.95	98.91	99.00	99.12
SD	1.03	1.07	1.11	0.71	0.64	0.76
%RSD	1.03	1.06	1.09	0.72	0.65	0.77
Overall Average	100.70			99.01		
Overall %RSD	1.06			0.71		

SET–I: Variability due to HPLC system SET–II: Variability due to HPLC column, SET–III:Variability due to analyst

Robustness

Robustness was performed by change in mobile phase ratio, mobile phase flow rate andwavelengthofthedetector.Thetestwascarriedoutbysmallvariationinthechromatographic conditions at a concentration equal to standard concentrations 3 µg/mLfor CFX and 15 µg/mL for AZT and % change was calculated. % change in the results was calculated. [8]

Table 10.Robustness of CFX & AZT

S.No	Parameter	Condition	Cefixime		Azithromycin	
			Area(n=3)	% change	Area(n=3)	%change
1	Standard	Standardconditions	439272	0.000	299945	0.000
2	Mobile Phasecomposition(±2%)	20mMPhosphatebuffer (pH-5): Methanol: Acetonitrile(44:28:28,v/v/v)	433272	1.366	299725	0.073
		20mMPhosphatebuffer(pH-5):Methanol: Acetonitrile(36:32:32,v/v/v)	439171	-1.361	299827	-0.033
3	MobilephasepH (±0.2units)	4.8	439269	-0.022	297933	0.631
		5.2	439262	0.002	294921	1.011
4	Wavelength (nm)(±2%)	258	437262	0.455	294920	0.000
		262	432620	1.061	296345	-0.483
5	Flowrate(mL) ± 0.2mL	1.2	438262	-1.304	294947	0.472
		0.8	433242	1.145	295641	-0.235

Limit of detection and Limit of quantification

The LOD is the lowest concentration of an analyte that can be consistently distinguished from background values, and it is a scientific term. An analytical procedure's limit of quantification (LOQ) is the smallest quantity of analyte that can be quantitatively quantified with an acceptable level of accuracy and precision. In accordance with ICH rules, the following equation was used to compute the LOD and LOQ. The equations for LOD and L OQ are, respectively, 3.3 × σ / S and 1.0 × σ / S, where ғ represents the standard deviation of the y-intercepts of the regression lines and S is the slope of the calibration curve. [9]

Table.11.LOD and LOQ of CFX &AZT

Parameter	CFX	AZT
LOD(µg/mL)	0.087	0.691
LOQ(µg/mL)	0.266	2.096

Solution Stability

Standard and test stock solutions were used to assess the stability of the solution. After preparing and storing these stocks at room temperature and under refrigeration (2-8°C) for 36 hours, the percentage differences were determined.

Table 12 .Solution Stability of CFX at room temperature

Time	Standardstock			Teststock		
	Fresh	**StabilityStock**	**% Diff.**	**Fresh**	**StabilityStock**	**% Diff.**
Initial	439272	439272	NA	427182	427182	NA
6h	437262	434272	0.684	427181	421411	1.351
12h	435251	435272	-0.005	437153	437150	0.001
20h	439272	439272	0.000	443551	437151	1.443
26h	439272	446252	-1.589	427181	423230	0.925
30h	432732	439251	-1.506	427182	427150	0.007
36h	439232	436262	0.676	407181	412212	-1.235

Table.13.Solution Stability of AZT at room temperature

Time	Standardstock			Teststock		
	Fresh	**StabilityStock**	**% Diff.**	**Fresh**	**StabilityStock**	**% Diff.**
Initial	243846	243841	NA	226754	226752	NA
6h	243241	243211	0.012	246756	246746	0.003
12h	231354	231321	0.014	246642	245746	0.363
20h	223843	223443	0.179	246720	245267	0.589
26h	243821	243210	0.250	246742	242321	1.792
30h	243846	243241	0.248	246422	245441	0.397
36h	243456	240221	1.328	246720	245761	0.389

Table 14.Solution Stability of CFX at refrigerated temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	435122	435121	NA	424182	424181	NA
6h	433162	431242	0.443	497182	491410	1.161
12h	441131	445224	-0.928	432154	435150	-0.693
20h	438272	439242	-0.221	446551	444150	0.538
26h	436272	444252	-1.829	420182	420230	-0.011
30h	432722	438241	-1.275	427142	427150	-0.002
36h	437231	436242	0.226	405182	411210	-1.488

Table 15.SolutionStabilityofAZTatrefrigeratedtemperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	233743	233742	NA	226755	226752	NA
6h	242230	243110	-0.363	246723	246711	0.004
12h	231322	231312	0.005	246643	245611	0.418
20h	223822	223422	0.178	246711	245246	0.593
26h	243820	243210	0.250	245345	242144	1.304
30h	243835	243241	0.244	246410	245263	0.465
36h	243442	240232	1.319	246252	245755	0.201

Filter validation

The impact of the filter on the assay, dissolution, and impurities were the subjects of a research. The test solution was made according to the test protocol. After passing it through three separate filters (a 0.45µm PVDF filter, a 0.45µm PTFE filter, and a 0.45µm nylon filter), a small amount of the solution was centrifuged and then added to the HPLC system. We computed the percentage differences between the filtered sample and the one that had been centrifuged.

Table 16.Filter Interference Results for CFX & AZT

CFX				
Filtration Method	**Centrifuged**	**Nylon**	**PTFE**	**PVDF**
Area(Inj.1)	435273	439091	428262	438173
Area(Inj.2)	436936	434242	435272	435253
Avg. Area	436104.5	436666	431768	436712
%Difference		-0.129	1.122	-1.145

AZT				
Filtration Method	**Centrifuged**	**Nylon**	**PTFE**	**PVDF**
Area(Inj.1)	297962	297833	298634	299545
Area(Inj.2)	299946	297845	299835	288925
Avg.Area	298954	297839.5	299233	294236
%Difference		0.373	-0.469	1.671

ANALYSIS OF MARKETED FORMULATION

Preparation test solution

Twenty pills, each precisely measured, were ground to a powder in a mortar. The contents of a 25 mL volumetric flask were ultrasonicated for 10 minutes after a dosage corresponding to one tablet (5 mg of CFX and 1.5 mg of AZT) was transferred to it. 10 mL of methanol was then added to the flask. Applying whatmann filter paper No. 41 allowed the solution to pass through. The CFX and AZT solutions, which were produced in the same way, were diluted with methanol to obtain a concentration of 3 and 15 µg/mL, respectively.

Table 17. Analysis of Commercial Formulation

Tablet	**Labelclaimed(mg)**		**Conc.found(mg)**		**%Assay**	
ARIFIX-AZ	CFX	AZT	CFX	AZT	CFX	AZT
Tablets	5.00	1.50	4.98	1.48	99.81	99.32

CONCLUSION

This study fulfilled the preliminary research aims and proved that RP-HPLC could be used for the pharmaceutical analysis of Cefixime and Azithromycin in pharmaceutical formulations using UV detection. The medications belong to distinct classes. The proposed and verified approaches outperformed previously published methods in terms of sensitivity, selectivity, repeatability, stability, and recovery, all while exhibiting minor matrix effects

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