Library Progress International
Vol.44 No. 3, Jul-Dec 2024: P.1832-1845

Print version ISSN 0970 1052
Online version ISSN 2320 317X

Original Article

Available online at www.bpasjournals.com

HPLC Analytical Method Development and Validation for Simultaneous Estimation of four Drugs: Sulfamethoxazole, Trimethoprim, Isoniazid and Pyridoxine hydrochloride Tablets

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How to cite this article: Rathinasamy M, Senthilkumar Palaniappan. (2024). HPLC Analytical Method Development and Validation for Simultaneous Estimation of four Drugs: Sulfamethoxazole, Trimethoprim, Isoniazid and Pyridoxine hydrochloride Tablets. *Library Progress International,* 44(3), 1832-1845

ABSTRACT

The objective of this work is to design and verify a high-performance liquid chromatography technique for the simultaneous measurement of isoniazid, pyridoxine hydrochloride, trimethoprim, and sulfamethoxazole in pharmaceutical dosage forms. These medicines are essential parts of fixed-dose combination regimens used to prevent infections in HIV/AIDS patients. Experiments were conducted to find the optimal UV detector wavelength, column, and mobile phase composition as part of the technique optimisation. A Hypersil BDS C18 column was used to provide the chromatographic conditions, while methanol and potassium dihydrogen phosphate were used as the mobile phase. The procedure showed quick elution of all four medications in less than six minutes, proving its effectiveness in comparison to previous research. The International Council for Harmonisation (ICH) guidelines were followed in the validation process, which covered specificity, system appropriateness, linearity, accuracy, precision, robustness, and ruggedness. The new method's accuracy, precision, linearity, robustness, and ruggedness are demonstrated by the findings, which make it appropriate for regular drug analysis in pharmaceutical formulations. The method's effectiveness was assessed using commercial tablet samples, which showed that it could reliably measure the amount of medication in marketed goods. In summary, the established HPLC technique contributes to effective quality control and pharmaceutical analysis in the context of HIV/AIDS prophylaxis by providing a quick and dependable method for the simultaneous estimation of sulfamethoxazole, trimethoprim, isoniazid, and pyridoxine hydrochloride.

Key Words: Trimethoprim, HPLC method, Isoniazid, ICH

INTRODUCTION

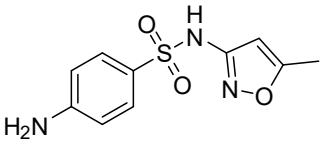
AIDS and the Human Immunodeficiency Virus (HIV) are among the most difficult illnesses to treat in the world. HIV/AIDS has been known to have widespread social, economic, and medical ramifications since it was first discovered in the early 1980s. The body's resistance to infections and illnesses is gradually weakened by this viral infection, which affects the immune system. Around 39 million individuals globally will be HIV positive in 2022, according to a WHO report. [1]

HIV infection involves a complex series of steps that ultimately lead to the depletion of the immune system's crucial CD4 and T cells, initially targets CD4 receptors, predominantly found on the surface of T cells, macrophages, and dendritic cells. The viral envelope glycoprotein, gp120, binds to the CD4 receptor and a co-receptor on the host cell membrane. This binding triggers conformational changes in the viral envelope, facilitating fusion with the host cell membrane and entry into the host cell. Once inside the host cell, the viral RNA is reverse transcribed into DNA by the enzyme reverse transcriptase. The resulting viral DNA forms a pre integration complex, which moves into the cell nucleus.[2] The viral DNA integrates into the host cell's genome and becomes a permanent part of the host cell's genetic material. The host cell's machinery is hijacked to transcribe and translate the integrated viral DNA into new viral RNA and proteins. Viral RNA and proteins are transported

to the cell surface, where new virions assemble.[3] The enzyme protease cleaves the long viral polyproteins into functional proteins, allowing for the formation of mature, infectious virions. New virions bud from the host cell, acquiring an envelope derived from the host cell membrane and maturation involves the final processing of viral proteins into their active forms.[4]

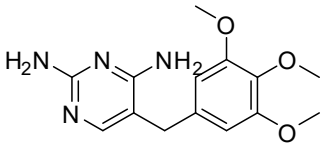
Throughout this process the continuous replication of HIV leads to progressive decline in CD4+ T cells, compromising the immune systems ability defence against infections. Additional, the activation of the immune system and the production of the inflammatrory mediators contribute to the overall immunodeficiency observed in HIV- infected individuals, rendering the infected individual susceptible to opportunistic infections.

The fixed-dose combination medication Isoniazid/pyridoxine/sulfamethoxazole/trimethoprim (INH/B6/SMZ/TMP) is employed for the prophylaxis of opportunistic infections in individuals with HIV/AIDS.[6] This formulation incorporates isoniazid, pyridoxine, sulfamethoxazole, and trimethoprim, serving the purpose of preventing conditions such as tuberculosis, toxoplasmosis, pneumonia, malaria, and isosporiasis.[7]



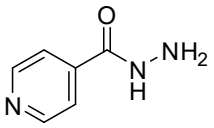
Sulfamethoxazole

(a)



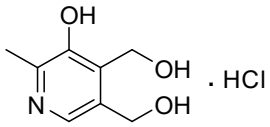
Trimethoprim

(b)



Isoniazid

(c)



Pyridoxine Hydrochloride

(d)

Fig. 1: Chemical Structures of the drugs

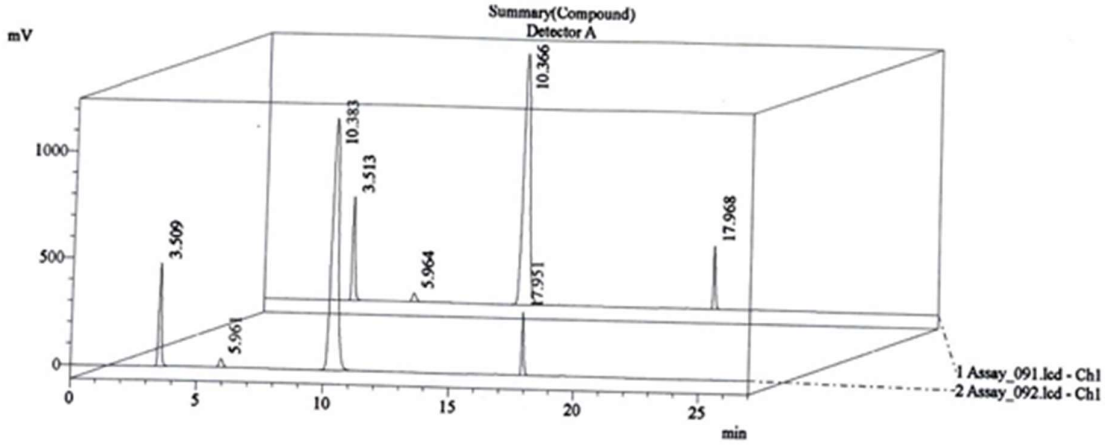


Fig2. Chromatogram of mixture of drugs

Isoniazid demonstrates potent activity against Mycobacterium tuberculosis, effectively acting as a bactericidal agent. Its mechanism of action involves the inhibition of the synthesis of long-chain mycolic acids, which are needed for mycobacterial cell wall synthesis. Notably, if isoniazid is employed as a sole treatment for clinical diseases caused by mycobacteria, resistance can quickly develop. While Pyridoxine, when converted to pyridoxal phosphate, serves as a co-enzyme essential for transamination and participates in numerous metabolic processes. Since metabolites of isoniazid bind to pyridoxine, rendering it inactive, supplementing with pyridoxine becomes crucial to counteract the inactivation induced by isoniazid. Sulfamethoxazole arrests the microbial production of dihydrofolic acid by competing with paraaminobenzoic acid (PABA). Concurrently, trimethoprim inhibits

dihydrofolate reductase (DHFR), the key enzyme for converting dihydrofolic acid into tetrahydrofolic acid. Under these conditions, this combined effect may lead to bacterial cell death. Notably, trimethoprim exhibits a significantly lower affinity for mammalian DHFR compared to its bacterial counterpart. Comprehensively, the combination of sulfamethoxazole and trimethoprim disrupts two successive stages in the synthesis of nucleic acids and proteins and thereby its inhibition.[8]

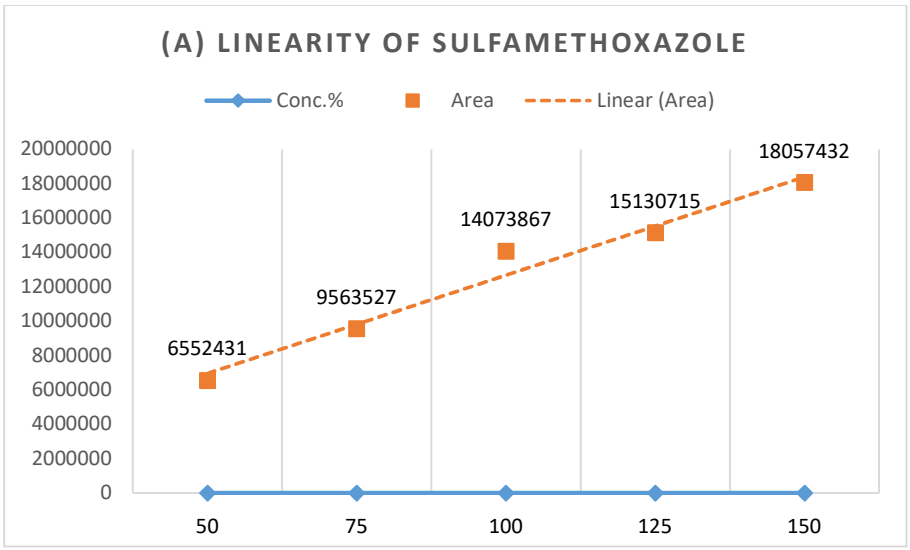
Table 1: System suitability results

Standard		Average	%RSD
Sulfamethoxazole	Retention time	5.95	0.14
	Area	14085486	0.09
	Resolution	5.01	
	Theoretical plates	8500	
	Asymmetry	0.85	
Trimethoprim	Retention time	4.21	0.11
	Area	13243564	0.2
	Resoresloution	9.08	
	Theoretical plates	7820	
	Asymmetry	0.92	
Isoniazid	Retention time	2.93	0.62
	Area	11754387	0.1
	Resolution	7.12	
	Theoretical plates	5257	
	Asymmetry	1.02	
Pyridoxine hydrochloride	Retention time	1.89	0.12
	Area	9985643	0.25
	Resolution	11.9	
	Theoretical plates	2090	
	Asymmetry	1.17	
Dosage form			
Sulfamethoxazole	Retention time	5.85	0.11
	Area	14154365	0.65
	Resolution	5.12	
	Theoretical plates	8350	
	Asymmetry	0.84	
Trimethoprim	Retention time	4.91	0.12
	Area	12995466	0.1
	Resolution	9.12	
	Theoretical plates	7880	
	Asymmetry	0.93	
Isoniazid	Retention time	2.84	0.55
	Area	12456745	0.3
	Resolution	7.24	
	Theoretical plates	5128	
	Asymmetry	1.04	
Pyridoxine hydrochloride	Retention time	1.7	0.11
	Area	10584245	0.12

	Resolution	11.86	
	Theoretical plates	2011	
	Asymmetry	1.16	

The drugs depicted in Figure 1 generally used for preventing opportunistic infections in HIV/AIDS patients. Isoniazid, pyridoxine, sulfamethoxazole, and trimethoprim constitute a potent combination, commonly prescribed either individually or in various combinations based on specific clinical requirements. Additionally, these four drugs are commercially accessible in the market as a combined formulation in different dosage forms. The development and validation of methods for these drugs have been undertaken, considering their crucial role in HIV/AIDS prophylaxis.

Various techniques for the separate quantification of each drug have been documented in existing literature. [9-10] Some methods even enable the concurrent estimation of two drugs. [11] However, there is a scarcity of research focused on the simultaneous estimation of all four drugs, such as, Isoniazid, pyridoxine, sulfamethoxazole, and trimethoprim.



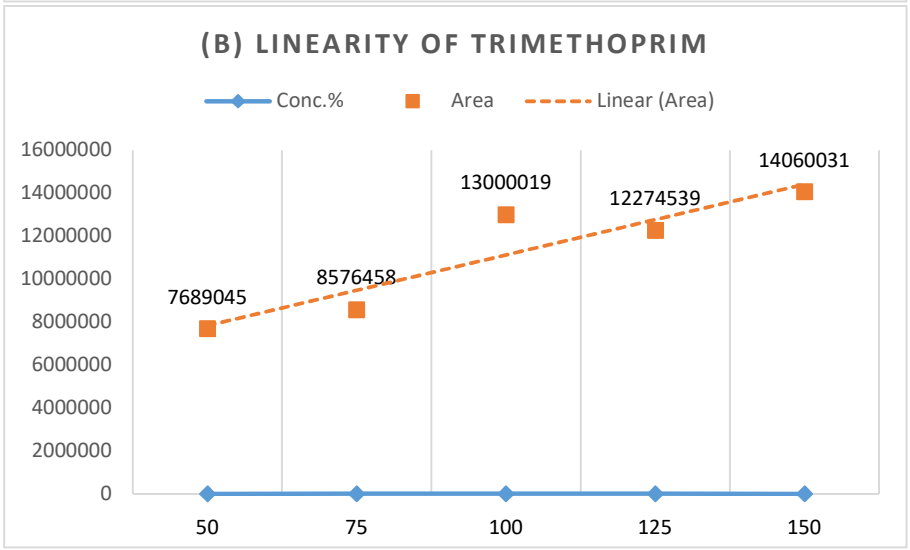
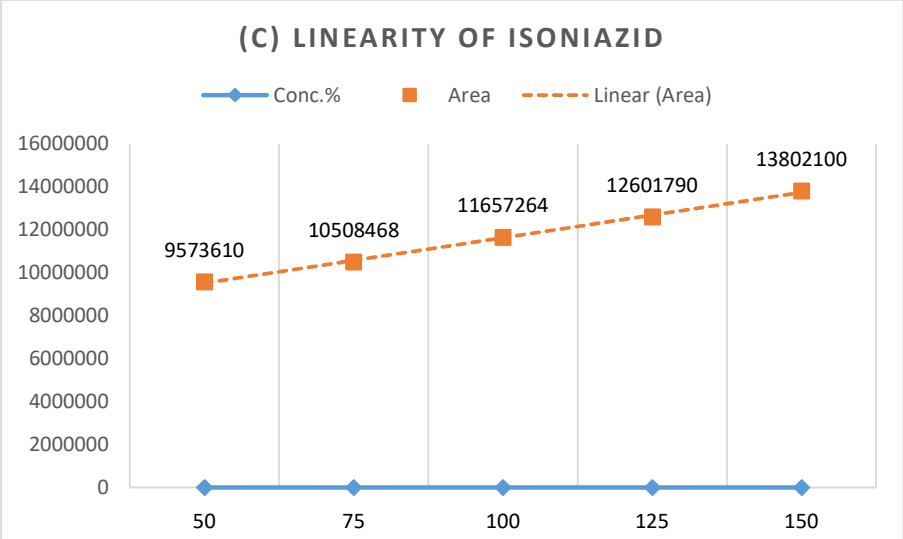


Fig.3 : Graphs for linearity of the drugs



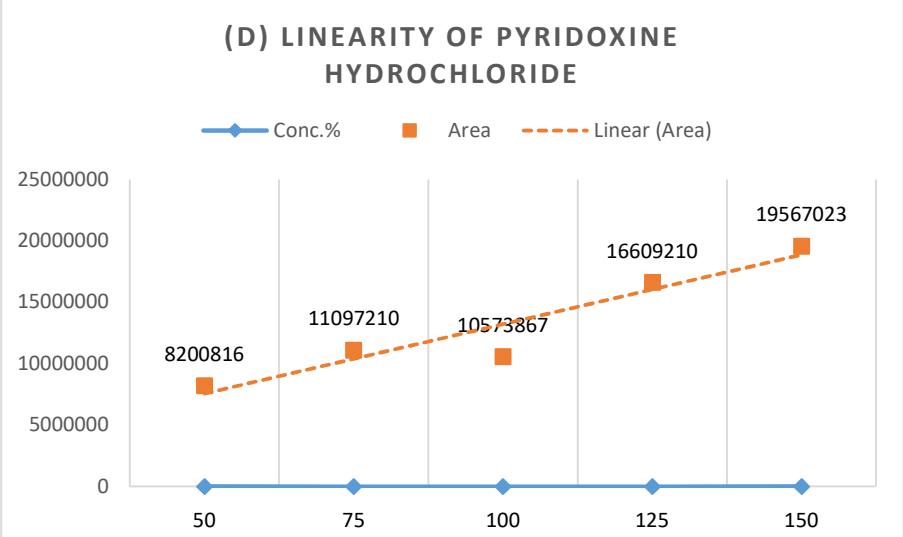


Fig.4: linearity graph

Table 2: Results on linear graph

Drugs	Linearity Range	Correlation coefficient
Sulfamethoxazole	50-150%	0.9853
Trimethoprim	50-150%	0.9226
Isoniazid	50-150%	0.9992
Pyridoxine hydrochloride	50-150%	0.9496

2. Section of Experiments

Our goal was to create a reliable technique for determining sulfamethoxazole, trimethoprim, isoniazid, and pyridoxine hydrochloride simultaneously in their pharmaceutical dose forms. To ensure a quick and efficient analytical process, a number of studies were carried out to optimize the chromatographic conditions, including the mobile phase, column selection, and detector settings.

2.1. Chemicals, Materials, and Reagents

Medopharm Laboratories provided standard samples of trimethoprim, isoniazid, pyridoxine hydrochloride, and sulfamethoxazole. Potassium dihydrogen phosphate, heptanesulfonic acid, orthophosphoric acid, and ethanol of

high-performance liquid chromatography (HPLC) quality were acquired from [source].

2.2. Tools

A Shimadzu UV-Visible spectrophotometer (Model-2450) and an HPLC (Shimadzu, Model-LC-2010 CHT) were used in the entire study.

2.3.Chromatographic Conditions

The stationary phase was a Hypersil BDS (C18 - 250 mm × 4.6 mm; 5 µm) column. 13.6 g of potassium dihydrogen phosphate were dissolved in 800 mL of filtered water in a 1000 mL beaker to create mobile phase A. 2.2 g of sodium salt heptanesulfonic acid was added to this solution, and 10% orthophosphoric acid was used to bring the pH down to 3.0 ± 0.05. The water was added up to 1000 mL. Methanol was the substance in mobile phase B. A injection volume 50 µL and a 1.0 mL/min flow rate were used with detection wavelength at 280 nm.

2.4. Standard Solution Preparation

The experimental protocol involved dissolving 10 mg of Sulfamethoxazole, Trimethoprim, Isoniazid, and Pyridoxine hydrochloride in separate 25 mL volumetric flasks to obtain a solution of 0.4 mg/mL (standard). Tetrahydrofuran (0.5 mL) was added to each flask, and the solutions were diluted with the mobile phase until the desired level was reached.

2.5. Sample Preparation for Linearity Measurement

Linearity solutions were created with concentrations of 125%, 100%, 75%, 50%, and 150%. To make the 150% solution, dissolve 15 mg of pyridoxine hydrochloride, trimethoprim, isoniazid, and sulfamethoxazole in 100 milliliters of water. The mobile phase was diluted appropriately to prepare subsequent solutions. [12, 13]

2.6. Sample Preparation for Accuracy measurement

For the accuracy investigations, five distinct solutions were produced up to volume using 50%, 75%, 100%, 125%, and 150% linearity solutions. Each solution contained 10 mg of sulfamethoxazole, trimethoprim, isoniazid, and pyridoxine hydrochloride dissolved in 25 mL volumetric flasks.

2.7. Sample Preparation for Batch Analysis

For the batch analysis, two commercial samples designated as Test Compound 1 and Test Compound 2 were used. Each batch consisted of ten tablets that were weighed, ground into a uniform powder, and then dissolved in 25 milliliters of the mobile phase. After being filtered and sonicated, the solutions were added to the HPLC apparatus.

2.8. Validation of Analytical Methods

2.8.1. Specificity

The specificity is generally carried out to precisely measure the drug in the presence of possible contaminants or matrix elements is known as specificity. In order to make sure that there were no interfering peaks from other chemicals present in the sample matrix, the retention durations of pyridoxine hydrochloride, trimethoprim, isoniazid, and sulfamethoxazole were closely examined throughout this validation stage. Analyte retention durations in the sample and standard solution were compared to identify and resolve any possible interactions or interferences.

2.8.2. System Suitability

System suitability tests are carried out to ascertain the chromatographic system is operating suitably and consistently throughout the analysis. To confirm the chromatographic system's resilience, parameters such peak asymmetry, theoretical plates, and resolution between peaks were assessed. [14, 15] The analytical method's reliability and reproducibility are guaranteed by verifying that these parameters satisfy preset acceptance standards.

2.8.3. Linearity

The method of analyzing the correlation between analyte concentration and response (peak area or height) is known as linearity evaluation.[16] For every analyte, concentration versus area plots were created using standard solutions at different concentrations.[17], the correlation coefficient (r) was computed to evaluate the linearity of the procedure across the designated concentration range.[18] A robust linear link between concentration and reaction is shown by a high correlation coefficient around 1, which validates the method's applicability for quantitative investigation.

2.8.4. Accuracy.

The degree to which measured results resemble real or known values of the analyte concentration is evaluated through accuracy assessment. By adding known amounts of sulfamethoxazole, trimethoprim, isoniazid, and pyridoxine hydrochloride at various percentages (50%, 75%, 100%, 125%, and 150%) to the standard solution, recovery percentages were calculated. By comparing the measured concentrations to the spiked concentrations, the accuracy of the procedure was determined; larger recovery percentages suggested more accuracy and greater method reliability.

2.8.5. Method Precision

Also referred to as repeatability or intra-day precision, method precision assesses how consistently findings from repeated injections of the same sample under the same circumstances may be relied upon. [19, 20, 21] The chromatographic apparatus was filled with six duplicates of the sample solutions, and the assay's outcomes which included peak areas and retention times were noted. The relative standard deviation (RSD) was computed to evaluate the method's precision. A smaller RSD values correspond to greater precision and reliability.

2.8.6. Robustness

Testing for robustness involves determining how sensitive the procedure is to even minute changes in experimental factors like temperature, pH, or flow rate. The flow rate of the technique was intentionally varied in this validation stage, ranging from the predetermined values (1.7 mL/min to 1.5 mL/min and 1.9 mL/min. The method's robustness and resistance to small alterations were demonstrated by comparing the chromatograms produced under these modified circumstances to determine if there was any discernible influence on the method's performance.

2.8.7. Ruggedness

Testing for ruggedness assesses the method's reliability when used by various analysts or on various days. This validation process makes sure that the technique yields accurate and consistent findings with different persons and experimental setups. various analysts conducted separate analyses on various days, and the percentage RSD for peak areas and retention times was computed. RSD readings that are consistent over several days and analysts attest to the method's robustness and applicability for regular laboratory use.

2.8.8. Performance of the Method

In this scenario, real-world samples labelled as Test Compounds 1 and 2 are analyzed using the validated technique. This completes the validation process. The pharmaceutical dosage forms for isoniazid, trimethoprim, pyridoxine hydrochloride, and sulfamethoxazole are shown by these samples. By applying the validated analytical approach to these samples, the method's accuracy and applicability in measuring the target analytes in complex matrices were evaluated.

3. Results and Discussion

Following a thorough optimization process, the new approach has shown to be very effective. It surpasses current literature techniques, allowing for the elution of all four medications in just six minutes. The need for methods that save expensive equipment and chemist time is increasing in today's industrial environment, enabling faster reporting on product analyses. As a result, interest in ultra-fast liquid chromatography (UFLC) is growing. Our approach's effectiveness and speed fit these industry demands like a glove. The results of this method's validation,

which was carried out in compliance with ICH criteria, are described as follows.

3.1. Specificity

Carefully measured retention durations for each standard substance were used. The retention durations for isoniazid, trimethoprim, pyridoxine hydrochloride, and sulfamethoxazole were determined to be 6.000 min, 4.200 min, 2.950 min, and 1.900 min, respectively. All four medications were clearly resolved upon injection of the drug mixture; their relative retention durations in the standard mix were found to be 6.020 min, 4.230 min, 2.890 min, and 2.000 min. This lack of chromatographic interference indicates that the approach may be used for simultaneous estimation in dose forms and medication mixes.

3.2. system suitability

To ascertain the system was suitable for the planned analysis, its suitability was carefully assessed. This required a thorough evaluation of a number of factors, such as variations in peak asymmetry, retention durations, and separation efficiency. The system's performance was carefully examined by computing characteristics including resolution, theoretical plates, and peak asymmetry for both standard and sample . The results, reported in Table 1, offer important new information about how regularly and dependably the system can produce chromatographic results.

3.3. Linearity

Evaluation of linearity is essential to ascertain if the technique can reliably measure analytes over a range of concentrations. The correlation coefficient (r), which varied from 0.98 to 1.00 in our investigation, allowed us to determine that concentration and reaction had a strong linear connection. Figure 3 provided another graphic illustration of this, demonstrating a clear proportionality between the concentration and the area response. The method's dependability and adaptability for quantitative analysis across a broad range of concentrations are guaranteed by this careful examination.

3.4. Accuracy

By calculating the % recovery of each medication, the accuracy of the procedure was carefully assessed. The obtained results proved that the recoveries were well within the acceptable limit and recorded in Table 3. This indicated that the procedure was accurate and appropriate for estimating the four medications at the same time. A thorough validation process like this guarantee trust in the analytical outcomes and the method's suitability for practical pharmaceutical analysis.

Table3: Results for accuracy

Sol	Drugs	Initial conc area	Sol 1 area	50% area	sol 1-50% area	% recovery
Sol 1	Sulfamethoxazole	14073867	20597356	6552431	14044925	99.79
	Trimethoprim	13000019	20609452	7689045	12920407	99.39
	Isoniazid	11657264	21182741	9573610	11609131	99.59
	Pyridoxine hydrochloride	10573867	18630982	8200816	10430166	98.64
		Initial conc area	**Sol 2 area**	**75% area**	**Sol 1-75% area**	**% recovery**
Sol 2	Sulfamethoxazole	14073867	23404578	9563527	13841051	98.35
	Trimethoprim	13000019	20909379	8076458	12832921	98.71
	Isoniazid	11657264	22149878	10508468	11641410	99.86
	Pyridoxine hydrochloride	10573867	21559072	11097210	10461862	98.94
		Initial conc area	**Sol 3 area**	**100% area**	**Sol 1-100% area**	**% recovery**
Sol 3	Sulfamethoxazole	14073867	27903464	14073867	13829597	98.26
	Trimethoprim	13000019	25835629	13000019	12835610	98.74
	Isoniazid	11657264	23165397	11657264	11508133	98.72
	Pyridoxine hydrochloride	10573867	21100190	10573867	10526323	99.55
		Initial conc area	**Sol 4 area**	**125% area**	**Sol 1-125% area**	**% recovery**
Sol 4	Sulfamethoxazole	14073867	29016345	15130715	13885630	98.66
	Trimethoprim	13000019	25298503	12274539	13023964	100.18
	Isoniazid	11657264	23929857	12601790	11328067	97.18
	Pyridoxine hydrochloride	10573867	27000874	16609210	10391664	98.28
		Initial conc area	**Sol 5 area**	**150%area**	**Sol 1-150% area**	**% recovery**
Sol 5	Sulfamethoxazole	14073867	32098464	18057432	14041032	99.77
	Trimethoprim	13000019	26893025	14060031	12832994	98.72
	Isoniazid	11657264	25302789	13802100	11500689	98.66
	Pyridoxine hydrochloride	10573867	29993235	19567023	10426212	98.60

Table 4: results of method precision

Drug	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Average	SD	% RSD
Sulfamethoxazole	99.45 %	98.95 %	99.11 %	99.05 %	99.01 %	99.22 %	99.13 %	0.001812	0.18
Trimethoprim	99.47 %	99.34 %	99.21 %	100.00 %	99.09 %	99.53 %	99.44 %	0.003187	0.32
Isoniazid	98.02 %	98.05 %	98.27 %	99.60 %	98.26 %	99.55 %	98.63 %	0.007433	0.75
Pyridoxine hydrochloride	99.27 %	99.45 %	99.23 %	98.44 %	99.32 %	99.16 %	99.15 %	0.003588	0.36

Table 5: % RSD at different flow rates

	Retention time % RSD	Area % RSD	Sample area % RSD
1.5ml/min			
Sulfamethoxazole	0.15	0.08	0.31
Trimethoprim	0.1	0.3	0.24
Isoniazid	0.6	0.65	0.28
Pyridoxine hydrochloride	0.11	0.23	0.16
1.9 mL/min			
Sulfamethoxazole	0.13	0.07	0.3
Trimethoprim	0.09	0.32	0.22
Isoniazid	0.49	0.63	0.27
Pyridoxine hydrochloride	0.1	0.2	0.15

3.5. Method Precision

A crucial factor that indicates the repeatability and reproducibility of the procedure is precision. We proved that the procedure is accurate and repeatable by computing the percentage relative standard deviation (% RSD) values for assay precision, which are shown in Table 4. This thorough assessment offers certainty about the method's consistency and dependability in producing accurate analytical findings.

3.6. Robustness

By purposefully introducing differences in the experimental settings, the robustness of the approach was assessed. The chromatogram showed no appreciable alterations in spite of these intentional modifications, highlighting the method's resilience. The comprehensive results, shown in Table 5, offer insightful information about how well the approach can tolerate changes in experimental conditions without compromising analytical performance.

3.7. Ruggedness

Testing a method's robustness entails assessing its performance over several days and by several analysts. We verified the robustness of the technique by comparing data from many sources and computing the % RSD for area and retention time (Table 6). This thorough evaluation guarantees the repeatability and dependability of the procedure in practical analytical environments.

3.8. Performance of the Method

A method's effectiveness is ultimately evaluated by how well it performs in actual situations. Test Compounds 1 and 2 were commercial samples analyzed to show the applicability and accuracy of the approach in determining the pharmaceuticals in commercial tablets. The comprehensive findings, displayed in Table 7, offer insightful information on the effectiveness of the technique and its appropriateness for standard pharmaceutical analysis.

Table 6: RSD of the drugs calculated based on different days and analysts

Day1 Analyst 1			
Drugs	**Retention time % RSD**	**Areas % RSD**	**Sample area % RSD**
Sulfamethoxazole	0.12	0.08	0.04
Trimethoprim	0.1	0.1	0.06
Isoniazid	0.58	0.2	0.04
Pyridoxine hydrochloride	0.13	0.23	0.02
Day 2—Analyst 2			
Drugs	**Retention time % RSD**	**AREAS % RSD**	**sample area % RSD**
Sulfamethoxazole	0.13	0.1	0.05
Trimethoprim	0.91	0.3	0.05
Isoniazid	0.59	0.1	0.03
Pyridoxine hydrochloride	0.11	0.21	0.04

Table 7: Examination of drugs in commercial samples

Test Compound 1			
Drug	**Label claim (mg/tab)**	**Acquired data (mg/tab)**	**Assay%**
Sulfamethoxazole	800	801.2	100.15
Trimethoprim	160	159.8	99.86
Isoniazid	300	299.2	99.73
Pyridoxine hydrochloride	25	25.1	100.4
Test Compound 2			
Drug	**Label claim (mg/tab)**	**Acquired data (mg/tab)**	**Assay%**
Sulfamethoxazole	800	798.2	99.78
Trimethoprim	160	160.9	100.56
Isoniazid	300	299.4	99.8
Pyridoxine hydrochloride	25	25.2	100.8

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4. Conclusion

This work has been developed and validated for the simultaneous determination of pyridoxine hydrochloride, trimethoprim, sulfamethoxazole, and isoniazid in pharmaceutical dosage forms.

The created approach has a number of advantages over current methods, such as simplicity, repeatability, and quick analytical times. The technique allows for the elution of all four medications in less than six minutes by optimizing the composition of the mobile phase, columns, and UV detector wavelength. This efficiency allows for the quick provision of product analysis findings, which is especially helpful in businesses where time-saving techniques are highly prized.

Following ICH requirements, the technique's validation was carried out, taking into account a number of factors including specificity, system applicability, linearity, accuracy, precision of the method, robustness, and ruggedness. The outcomes validate the method's applicability for regular analysis of these medications in pharmaceutical formulations by showing that it is exact, robust, linear, accurate, and resilient.

The lack of chromatographic interference between the analytes was verified by specificity testing, suggesting that the technique can assess medication responses effectively even when there are possible contaminants present. Tests for system appropriateness confirmed that the chromatographic system was suitable for the analysis, guaranteeing accurate separation and repeatability of findings.

According to linearity studies, the detector's response is proportionate to each drug's concentration across the measured range, and the correlation coefficients are within allowable bounds. Further supporting the method's reliability, accuracy testing showed that all four medicines had good recovery rates.

The created technique's consistency and reliability were confirmed by method precision tests, based on the repeatability of retention times and peak areas. The method's stability at varying flow rates was validated by robustness testing, so bolstering its appropriateness for regular analysis.

Furthermore, evaluations of ruggedness revealed comparable outcomes between days and analysts, demonstrating the method's resilience and reliability in practical situations. The usefulness of the approach for pharmaceutical quality control was further established by performance testing on commercial samples.

To sum up, the technique that was created provides a quick, accurate, and dependable way to estimate sulfamethoxazole, trimethoprim, isoniazid, and pyridoxine hydrochloride simultaneously in pharmaceutical dose forms. Its successful validation in accordance with global norms highlights its applicability for regular quality control analysis in the pharmaceutical sector, assisting in the prompt and effective assessment of HIV/AIDS preventive drugs.

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