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Separation and estimation of Sulfamethoxazole using an improved RP-HPLC technique

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Abstract:

Sulfamethoxazole is a broad-spectrum antibiotic widely used in the treatment of bacterial infections, necessitating the development of simple and rapid analytical methods for its determination in pharmaceutical formulations. In this study, a simple and accurate analytical method was developed using High-Performance Liquid Chromatography (HPLC) for the determination of sulfamethoxazole in pharmaceutical tablets. A C18 column was used with a mobile phase consisting of methanol and water in a 50:50 (v/v) ratio, adjusted to pH 7.8, at a flow rate of 1.0 mL/min. The developed method showed good linearity in the concentration range of 5–100 µg/mL, with a correlation coefficient (R^2) of 0.9989. The resulting peak was symmetrical (tailing factor = 1.06) with a retention time of 1.5 minutes, and the number of theoretical plates exceeded 2000, indicating high column efficiency. The method also demonstrated good accuracy and precision, with recovery values ranging from 99.3% to 99.6% and a relative standard deviation (RSD) of less than 2%. Due to its simplicity, high sensitivity, and reliability, the method can be recommended for routine quality control analysis of pharmaceutical formulations containing sulfamethoxazole.

فصل وتقدير السلفاميثوكسازول
باستخدام تقنية كروماتوغرافيا RP-HPLC

آمال ياسين مناع ، محسن حمزة بكر
قسم الكيمياء ، كلية التربية للبنات ، جامعة تكريت ، تكريت ، العراق

مستخلص:

يعد السلفاميثوكسازول من المضادات الحيوية واسعة الطيف، ويُستخدم على نطاق واسع في علاج العدوى البكتيرية، مما يستدعي تطوير طرق تحليلية بسيطة وسريعة لتقديره في المستحضرات الصيدلانية. في هذا البحث، تم تطوير طريقة تحليلية دقيقة وسهلة باستخدام تقنية كروماتوغرافيا السائل عالي الأداء (HPLC) لتقدير السلفاميثوكسازول في المستحضرات الصيدلانية (الأقراص). استُخدم عمود من نوع C18، مع طور متحرك مكوّن من مزيج الميثانول والماء بنسبة 50:50 (v/v)، وضُبط الأس الهيدروجيني عند 7.8، بمعدل جريان 1.0 مل/ دقيقة. أظهرت الطريقة المطوّرة استجابة خطية جيدة ضمن مدى تركيز 5–100 ميكروغرام/ مل، بمعامل ارتباط بلغ 0.9989. كانت الذروة الناتجة متماثلة (عامل تماثل = 1.06)، وزمن الاحتباس 1.5 دقيقة، بينما تجاوز عدد الصفائح النظرية 2000، ما يشير إلى كفاءة نظام الفصل. كما أثبتت الطريقة دقة وتكرارية جيدة، إذ تراوحت نسبة الاسترجاع بين 99.3٪ و99.6٪، مع انحراف معياري نسبي (RSD) أقل من 2٪. وبفضل بساطتها، وحساسيتها العالية، ودقتها، يمكن اعتماد هذه الطريقة في مراقبة الجودة الروتينية للمستحضرات الصيدلانية التي تحتوي على السلفاميثوكسازول.

الكلمات المفتاحية: السلفاميثوكسازول ، الطور العكسي ، الدقة والتوافقية ، منحني المعايرة ، التطبيقات ، المستحضرات الصيدلانية .

1-Introduction

Sulfamethoxazole is one of the effective sulfa drugs, white in colour, and soluble in organic solvents. It is used in combination with trimethoprim in pharmaceutical preparations to treat a range of bacterial infections, urinary tract infections, and respiratory tract infections Due to the widespread use of this drug, there is a need to develop reliable methods to measure its concentration in pharmaceutical preparations to ensure its efficacy and safety. High-performance liquid chromatography (HPLC) is one of the most widely used techniques in the analysis of pharmaceutical compounds due to its precision and reliability. Studies have shown that this technique provides accurate results for estimating sulfamethoxazole in raw materials and their pharmaceutical forms. The study of the drug also shows various methods, including HPLC [4-10], spectroscopic methods, azotization and coupling [11,12] oxidative coupling [13] [14], and nanotechnology [15-20]. The study aims to develop and document a simple and accurate high-performance liquid chromatography method, with method validation in terms of accuracy, precision, linearity, limit of detection, and quantification.

2-meterial and method

The devices used were High-performance, liquid chromatography device with system consists of a P110 pump and a manual injector, UV-VIS-D-1100 detector,Sensitive balance, (150 cm×4.6mm1) , Ultrasound helps remove air from the organic solvents used in the mobile phase, aiding in the dissolution of solid chemicals and device aims to prevent bubble formation within the system during the flow of the mobile phase, ensuring smooth operation and improving performance efficiency.

2-1.Preparation of the experimental solutions;

1. Sulfamethoxazole solution at a concentration of 1000 µg/ml One hundred milligram of pure sulfamethoxazole were weighed and dissolved in an appropriate amount of 25% ethanol, an organic solvent. It was placed in a beaker glass to complete

the dissolution using an ultrasonic device for complete dissolution. It was then transferred to a 100 ml volumetric flask and the volume was completed with distilled water up to the specified mark.

2. Preparation of the pharmaceutical formulation for sulfamethoxazole 4000 µg/ml

A solution of the pharmaceutical preparation sulfamethoxazole (400 mg/tablet) was prepared. The weight of ten tablets of the drug was taken, ground, and an amount equivalent to 400 mg was dissolved in 25% ethanol and placed in a beaker glass. After complete dissolution using an ultrasonic device for 10 minutes, the solution was filtered using filter paper to remove impurities. The solution was then transferred to a 100 ml volumetric flask, and the volume was completed with the solvent to the marked line. 2.5 mL of the pharmaceutical solution was drawn into a 100 mL volumetric flask and the volume was completed with the solvent to obtain a concentration of 100 µg/mL.

3-Results and Discussion

3-1 Optimal conditions

1- Column selection:

The C18 column was chosen, which is considered one of the most efficient columns for separation and is preferred for separating chemical compounds. The C18 column was selected with a length of 150 mm, an inner diameter of 4.6 mm, and a particle size of 5 microns, providing high separation efficiency, symmetrical peaks, and an acceptable analysis time.

2- Choosing the wavelength

A solution with a concentration of 10 µg/mL of sulfamethoxazole was prepared in a 25% ethanol solvent, and the absorption was measured in a 1 cm path length quartz cell. The UV absorption spectrum measurement showed a maximum wavelength of 270 nm, and it was used for subsequent experiments as shown in the figure below.

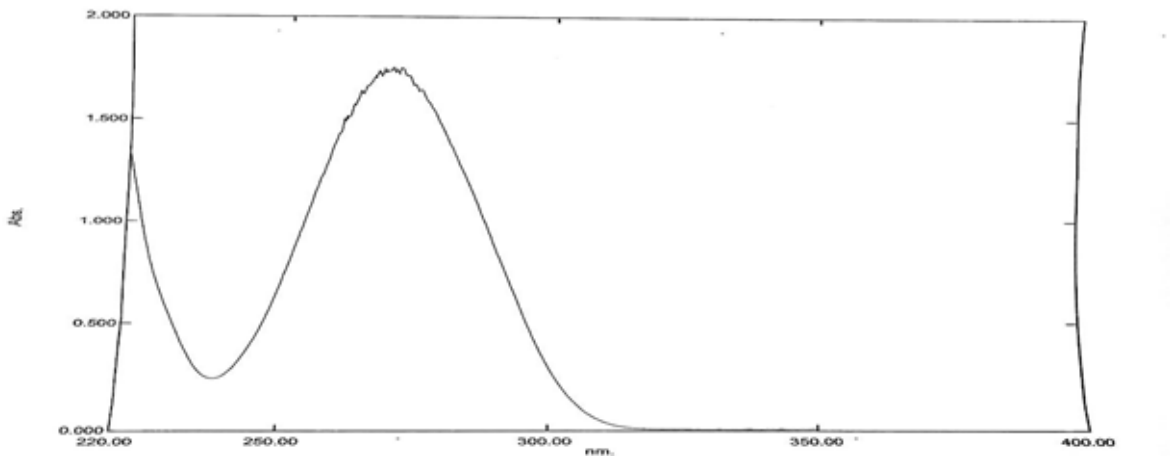


Figure (2) Absorption spectrum of sulfamethoxazole

4-Study of the Mobile phase

This study was conducted to select the best mobile phase for the separation of sulfamethoxazole, using deionised water and methanol. The pH was adjusted to 7.8 using a pH meter, the solution was filtered through a filter paper, and the study was conducted with five different ratios. 20 microlitres of a sample with a concentration of 400 micrograms/mL were injected at a flow rate of 0.8 and a wavelength of 270 nanometres. As shown in the figures below 3, 5, 4, 6, 7, table (1). Where the mobile phase at a 50:50 ratio of deionised water and methanol was optimal, a sharp peak appeared, indicating efficient separation for the chromatographic column.

Table(1): Study of the Mobile Phase

m.ph MeoH : H2O pH 7.8	R.t	Peak area	Peak high
90:10	2.4	4398.15	740.44
80 :20	3.3	8703.81	934.83
70:30	2.9	16462.72	981.51
60:40	3.3	15119.01	1641.89
50:50	3.6	14758.39	1185.72

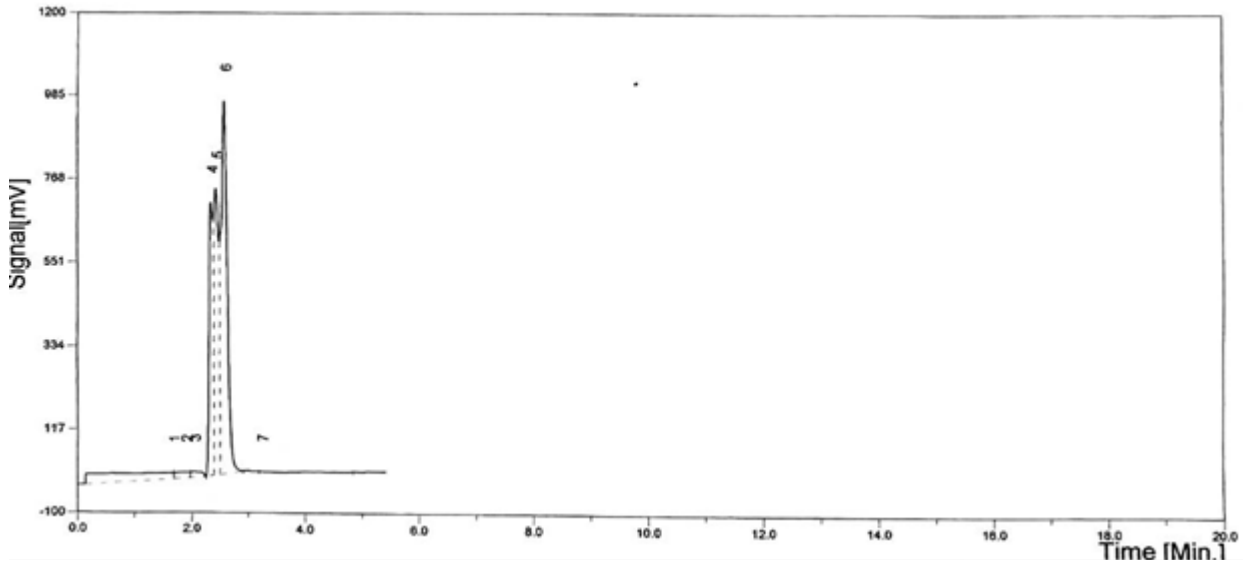


Figure (3) "A 20 µL aliquot of the sample was injected using a mobile phase composed of 90:10 (v/v) methanol:water."adjust PH=7.8

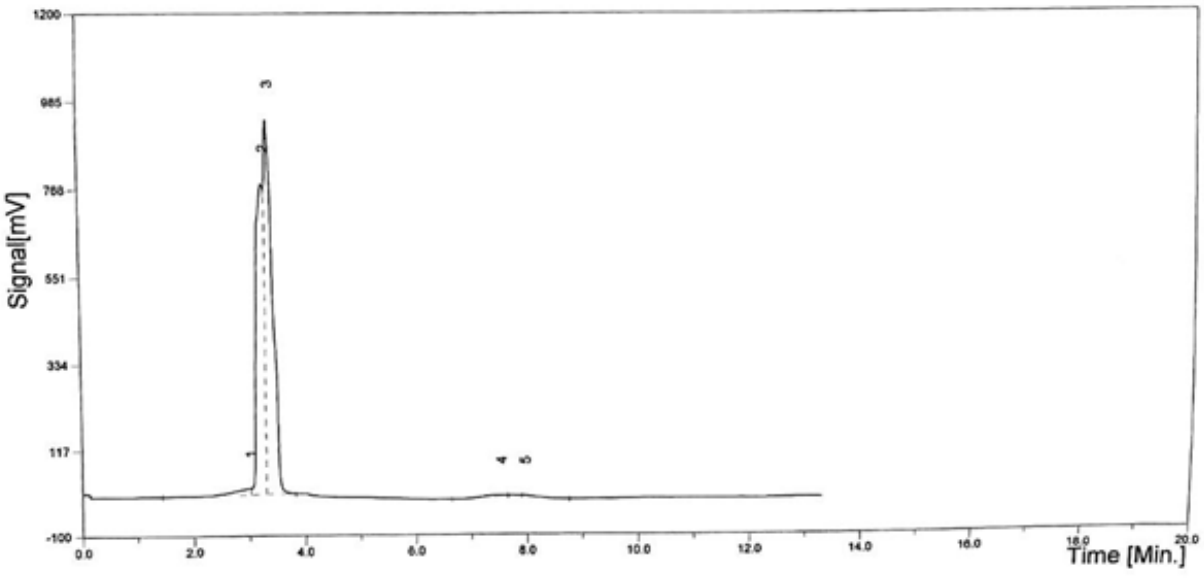


Figure (4) "B 20 µL aliquot of the sample was injected using a mobile phase composed of 80:20 (v/v) methanol:water."adjust PH=7.8

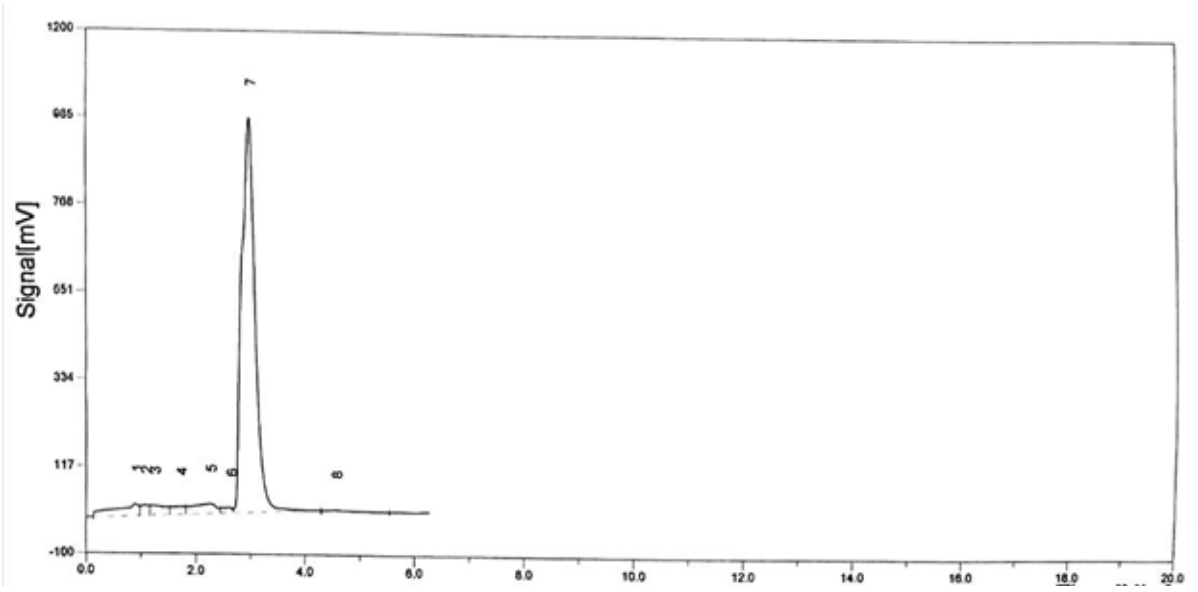


Figure (5) "C 20 µL aliquot of the sample was injected using a mobile phase composed of 70:30 (v/v) methanol:water."adjust PH=7.8

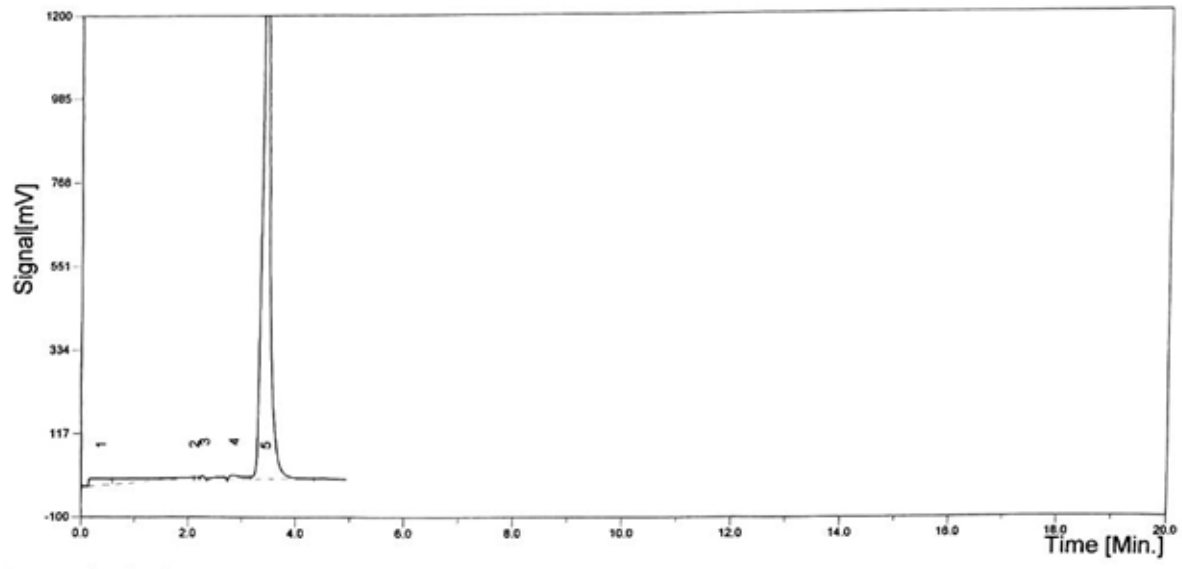


Figure (6) "D 20 µL aliquot of the sample was injected using a mobile phase composed of 60:40 (v/v) methanol:water."adjust PH=7.8

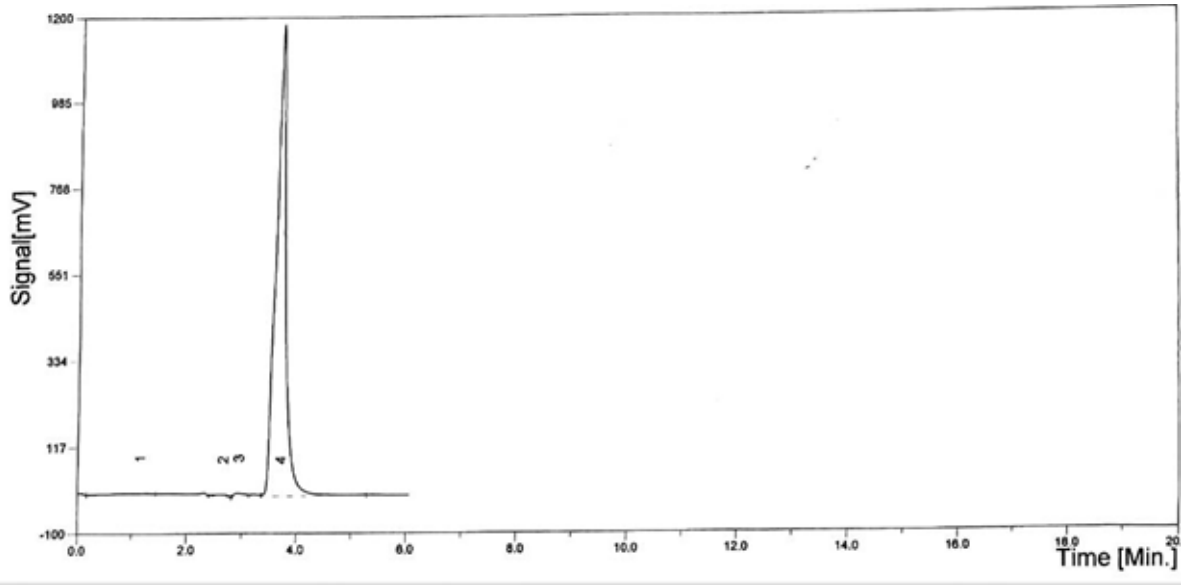


Figure (7) "E 20 µL aliquot of the sample was injected using a mobile phase composed of 50: 50 (v/v) methanol:water."adjust PH=7.8

3 -Effect of flow rate

The effect of the flow rate of the mobile phase on retention time, peak shape, and separation efficiency was studied by varying the flow rate within the range of 0.8-1.5 mL/min. It was observed that increasing the flow rate reduces the retention time. Therefore, a flow rate of 1.0 mL/min was chosen, providing an appropriate and acceptable analysis time.

4- Calibration curve

A series of 20 ml volumetric flasks were prepared with different and increasing concentrations of sulfamethoxazole solution (5, 25, 50, 75, 100) µg/ml, where 0.1, 0.5, 1, 1.5, and 2 ml were withdrawn from the stock solution and the volume was completed with the solvent up to the marked line. 20 microlitres were injected sequentially into a C18 column, using a mobile phase of 50:50 mL of methanol and water at a pH of 7.8 and a flow rate of 1 mL/min, with a wavelength of 270 nm. The response area under the curve was recorded, and the results are shown in Table (2) and Figures 8, 9, 10, 11, and 12. Table (2) below illustrates the calibration curve.

$$N=5.54\times(R.t^2/w_{1/2})^2$$

Table No. (2) Calibration curve concentrations

Con µg/ml	R.T	Peak area	N
5	1.5	200	1085.121
25	1.5	1101	1085.121
50	1.5	2019	1085.121
75	1.5	3217.45	1085.121
100	1.5	4191.22	1085.121

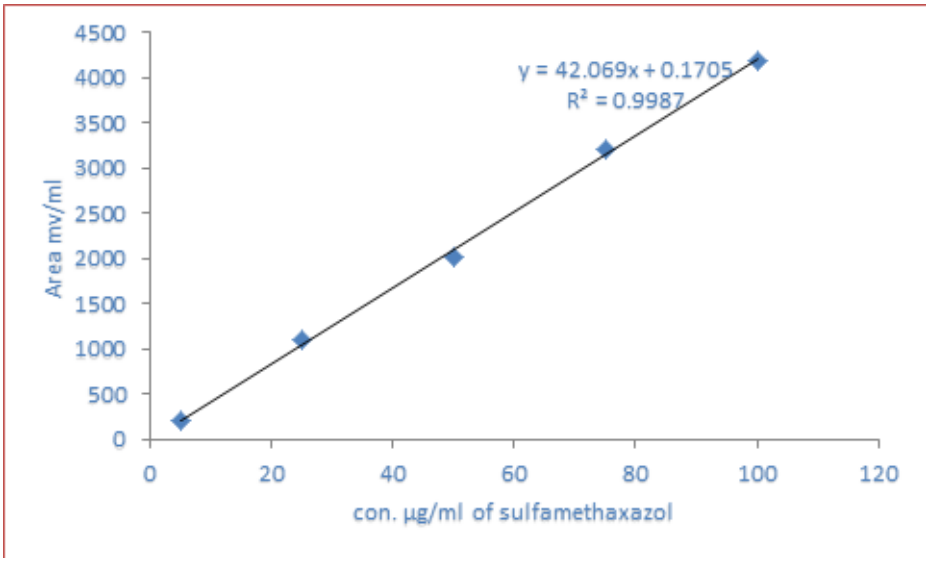


Figure (8). Calibration curve of sulfamethoxazole using HPLC. A linear correlation was observed between peak area and concentration over the range of 5–100 µg/mL with $R^2 = 0.9987$.

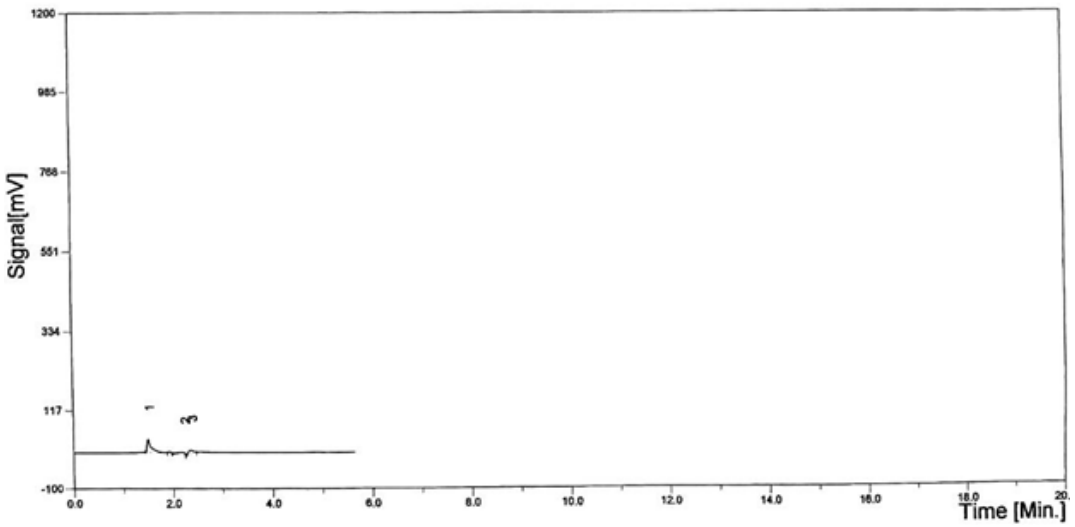
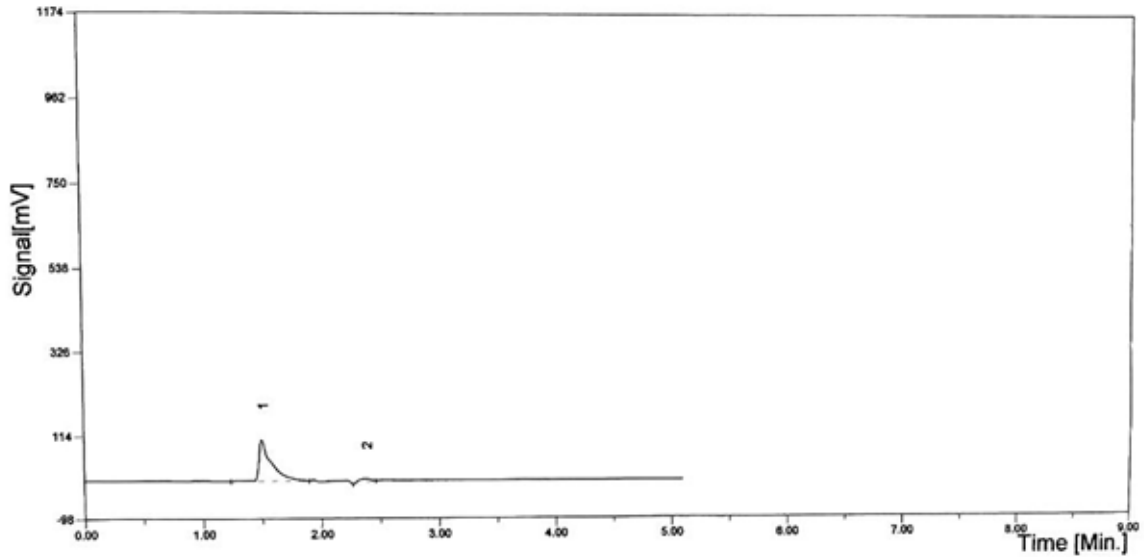


Figure (9). "A 20 µL volume was injected at a concentration of 5 µg/mL."



”Figure (10). B 20 µL volume was injected at a concentration of 25 µg/mL“.

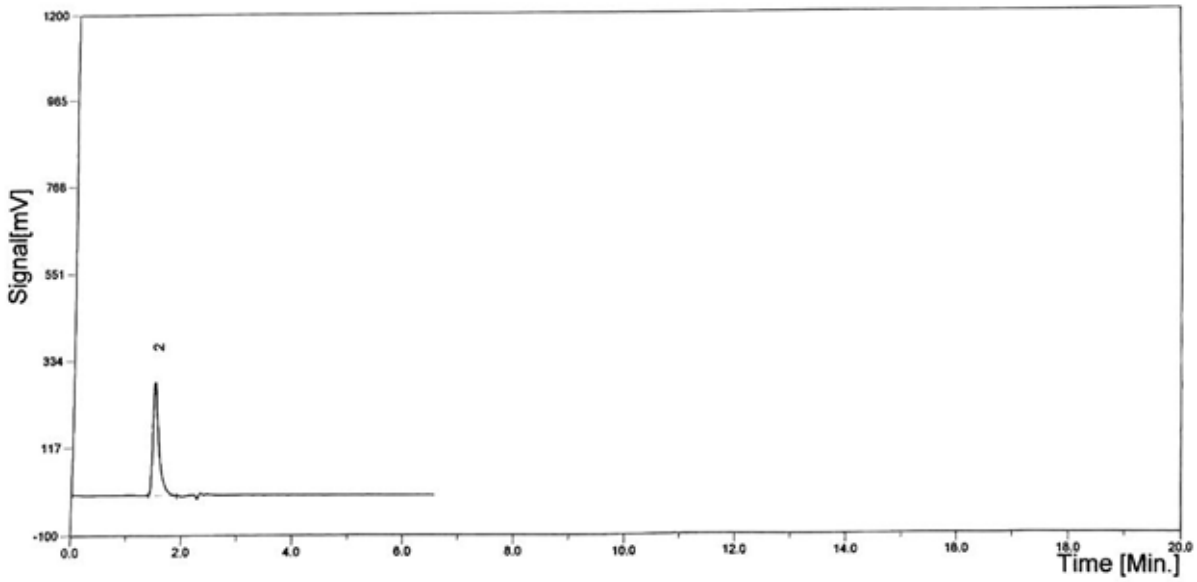


Figure (11). “C 20 µL volume was injected at a concentration of 50 µg/mL.”

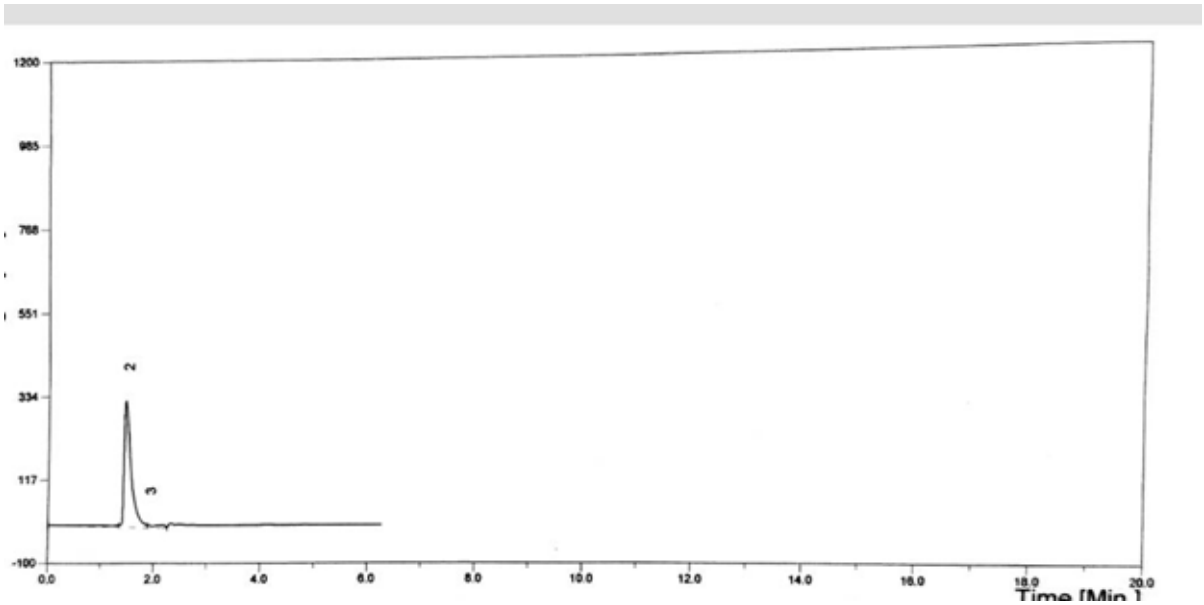
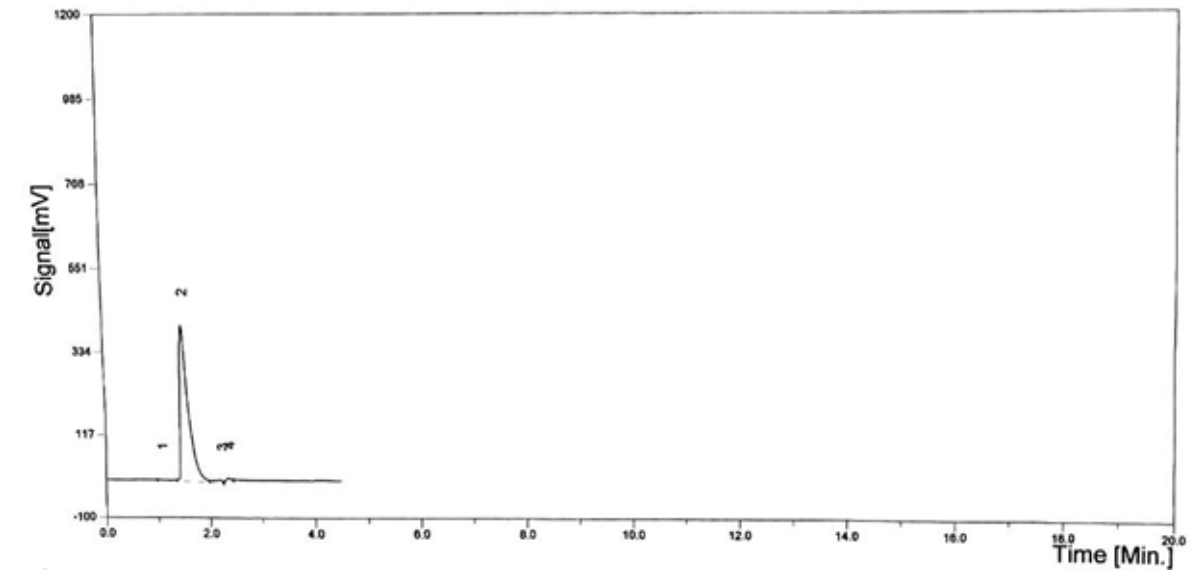


Figure (12). "D 20 µL volume was injected at a concentration of 75µg/mL."



"Figure (13). E 20 µL volume was injected at a concentration of 100 µg/mL."

5- Precision and compatibility of pure materials

Accuracy is the degree to which the measured result is close to the true or accepted value.

Precision - it refers to a measure of the repeatability and closeness of results when measuring the sample under the same conditions. Five measurements of the solution at concentrations of 50 and 75 µg/mL were conducted, and the results were as follows. Table (3).

Table(3). Accuracy and precision of sulfamethoxazole determination at concentrations of 50 and 75 µg/mL

µg/ml sulfa	. peak .area mV	RE%	recovery%
75	3196.55	0.4	100.4
	3182.22	-0.02	99.9
	3221.12	1.19	101.1
	3144.32	-1.2	98.7
	3171.1	-0.3	99.6
Average٪ SD RSD٪ N	3183.06	-	99.94% 0.8 0.8 3133.55
50	2008.89	-0.5	99.4
	2127.22	1.7	101.7
	2021.33	-3.3	96.6
	2107.33	0.7	100.7
	2053.57	-1.8	98.1
Average٪ SD RSD٪ N	2091.66	-	99.3% 2.02 2.04 3133.55

6- Descriptive analysis of the chromatogram

The chromatography results of the drug using high-performance liquid chromatography with the developed method showed a sharp and symmetrical peak, indicating good separation efficiency and effectiveness at a retention time of 1.5 minutes. The symmetry factor was 1.06, indicating an acceptable slight deviation in the peak shape. The number of theoretical plates exceeded 2000, reflecting high column efficiency and indicating no interference from impurities. The baseline also showed good stability throughout the experimental period, enhancing the reliability of the results. The developed method can be considered suitable and accurate for the estimation of sulfamethoxazole in pharmaceutical preparations.

7- Applications

The pharmaceutical drug solution was prepared at concentrations of 50 and 100 micrograms/mL from the previously prepared stock solution using the dilution law.

20 microlitres were injected into the high-performance liquid chromatography device under the same optimal conditions used in the calibration curve. The area under the peak response was recorded three times as shown in Table (4). It has been shown that the developed method has proven its efficiency in pharmaceutical preparations in the form of tablets.

Table(4). Results of pharmaceutical formulation analysis in three replicates using HPLC technique

R.t	Con.µg/ml	Peak Area MV	RE%	Recovery%	RSD%
1.5	50	2110.99	0.15	100.1	-
1.5	50	2089.22	-0.8	100.1	-
1.5	50	2122.90	0.72	100.7	-
Average N	3107.70 3107.70			-	0.33
1.5	100	4282.1	1.7	101.7	-
1.5	100	4221.33	0.27	100.2	-
1.5	100	4125.55	-1.9	98	-
Average N	4209.66 3107.70		-	99.7	1.59

8- Conclusion

In this study, an effective analytical method was developed using High-Performance Liquid Chromatography (HPLC) to estimate sulfamethoxazole in pharmaceutical preparations. The method demonstrated high accuracy and precision, in addition to a clear linear response within a concentration range of 5–100 µg/mL, with low detection and quantification limits, confirming its analytical efficiency. The verification results have shown that the method is compliant with the guidelines for the quality of analytical methods in terms of specificity, stability, accuracy, and reproducibility. Based on that, the method can be adopted as a reliable tool for estimating sulfamethoxazole in pharmaceutical preparations, as shown in Table (5) which summaries the results of the method validation.

The developed method was compared with another method, and the results were acceptable and efficient as shown in [23] . Table (5)

Table (5). The developed method was compared with another method, and the results were acceptable and efficient as shown in

parameter	Develop method	Azeez et al. 2023 method
Mobil phase	Methanol :water pH8.2 (50 : 50)	Acetonitrile:phosphate buffer 60:40
max λ	270 nm	254 nm
Column	C18 (150cm ×4.6mm) particle 5µm	C18 (250cm ×4.6mm) particle 5µm
Flow rate ml/min	1	1.2
Column Temperatur	25 c◦	45 c◦
IOQ	0.013	0.06
LOD	0.04	0.022
Linearity range µg/ml	5-100	1-100
PH	7.8	3.5-4
Coefficient of correlation	0.9987	0.9973
Pharmaceutical formulation	Tablet-made in India	Septrin tablet

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