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Separation and determination of sulfamethoxazole, trimethoprim and metoclopramide hydrochloride by RP-HPLC method in pure and in Pharmaceutical formulations

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Abstract

A very accurate, fast and simple method for separating and determine pharmaceutical compounds sulfamethoxazole (Sulfa), trimethoprim (Tri), and metacalpermide hydrochloride (Meta). The separation process was performed by HPLC on a C18 stationary phase column. The method was developed and validated. The mobile phase is a mixture of acetonitrile buffer 60.40 under isocratic elution. The flow rate was 1.2 mL/min, and the optimum temperature for the separation was 45 °C. The analysis time is up to 4 minutes with retention times of 2.204, 2.873 and 4.983 minutes for the studied pharmaceutical compounds. The linearity was in the range of 0.1-100 µg/mL. The developed method was tested for the quantitative determination of pharmaceutical compounds in its pure form and in different commercial pharmaceutical formulations with less interference of additives.

Keywords
High Performance Liquid Chromatography, Sulfamethoxazole,Trimethoprim, and Metoclopramide HCl.

Many technological advances have appeared in use a good modern analytical method for quality control and analysis of pharmaceutical compounds[1]. One of these methods is HPLC as a method for analysis of multi-ingredient pharmaceutical preparations needs separation treatment and several injections during analysis [2]. In this paper, three pharmaceutical compounds, sulfamethoxazole (Sulfa), Trimethoprim (Tri), and metoclopramide hydrochloride (Meta). Sulfamethoxazole (Sulfa), is a white powder that is insoluble in water and soluble in methanol. It is usually used to treat the urinary tract infections. It is use as an alternative to antibiotics containing amoxicillin to treat sinusitis. It can also be used to treat toxoplasmosis and it is the drug of choice for pneumocystis pneumonia, which affects primarily patients with HIV [[3]. Sulfamethoxazole is benzene sulfonamide, 4-amino-N-(5-methyl-3-isoxazolyl)-; N1-(5-Methyl-3-isoxazolyl) sulfanilamide [4]. Trimethoprim It is an antibiotic primarily used to treat urinary treat infections, although it can be used to combat any symptoms of aerobic bacterial species[5]. Trimethoprim 2,4-Pyridinediamine,5- [3,4,5-trimethoxyphenyl methyl]-2,4-Diamino-5-(3,4,5-trimethoxybezyl)pyrimidine [6].

Metaclpoermide HCl it is an antiemetic and vasodilator drug. It is therefore primarily used to treat nausea and vomiting, and to facilitate gastric

emptying in patients with gastro paresis [7]. Metaclpermide hydrochloride is a benzamide, 4-amino-5-chloro-N-[2-(diethylamino) ethyl]-2-methoxy-, mono hydrochloride, monohydrate; 4-Amino-5-chloro-N-[2-(diethylamino)ethyl]-o-anisamide mono hydrochloride monohydrate[8]. Various analytical techniques have been reported in the literature for the determination of these pharmaceutical compounds [9]. The chromatographic methods are one of the best methods. High performance liquid chromatography (HPLC). It is used by different researchers [10]. Many researchers describe determination of dosage of pure tablets model and real sample using a range of analytical techniques. Analytical techniques including liquid chromatography (LC) and gas chromatography (GC) are commonly used in Compounds in mixtures are separated and identified, but mostly liquid chromatography. They are used to identify pharmaceuticals, as they are mostly non-volatile [11]. In the current study, the separation of these compounds by HPLC was developed to determine Sulfa Tri and Meta in pure and commercial forms. Separation and identification of these three compounds have been achieved together with short time analysis.

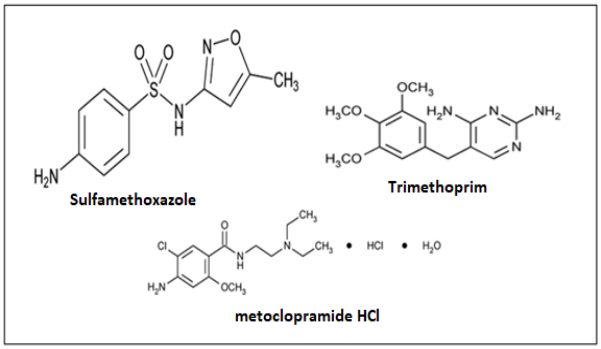

Figure 1. Chemical structures of the pharmaceutical compounds

Materials and Methods

Chemicals

The HPLC grade acetonitrile, and pure pharmaceutical (sulfamethoxazol, trimethoprim, Metoclopramide) were supplied from India and china. The commercial pharmaceutical were purchased from the local markets. Their names and specifications are given in Table 1.

Table 1. List of different commercial pharmaceutical formulations

Name	company	Active ingredient	dosage
Septrin tablets	GlaxoSmithKline United Kingdom	Sulfamethoxazole Trimethoprim	400 mg 80mg
Septrin suspension	GlaxoSmithKline United Kingdom	Sulfamethoxazole Trimethoprim	200mg 40mg
Kindiprim tablets	Al-kindi co. for pharma, Iraq	Sulfamethoxazole Trimethoprim	400 mg 80mg
Kindiprim suspension	Al-kindi co. for pharma, Iraq	Sulfamethoxazole Trimethoprim	200mg 40mg
Meclokindi tablets	Al-kindi co. for pharma, Iraq	Metoclopramide HCl	5mg
Premosan tablets	Julphar, The United Arab Emirates	Metoclopramide HCl	10mg
Neosil syrup	Al-Gadeed Amman-Jordan	Metoclopramide HCl	5mg
Meclokindi syrup	Al-kindi co. for pharma, Iraq	Metoclopramide HCl	5mg

HPLC system

1-Pumps LC-20AD
2-Manual injector
3-Degasser DGU-20A5
4-Column oven
5-Column for separation C18 (250 cm x 4.6 mm particle size 5 µm, Germany)
6-guard column (4 mm x 3 mm)
7-Detector UV-VIS
8-Data recording using Shimadzu software

Separation and mobile phase

Acetonitrile and buffer (60:40) were used in the separation process as the mobile phase. Buffer is 800 ml of deionized water, to which 2.15 g of sodium acetate is added, then 1 ml of triethylamine is added to it finally, pH is adjusted to 6.00 by glacial acetic acid. All solvents were filtered and degassed by ultra-sonication before use. Separation was achieved by flow rate of 1.2 mL min^{-1} and a temperature of 45 °C. The injection volume is 20 µl. isocratic of flow mode was use.

Preparation of stock solution for pharmaceutical compounds

Tablets for Sulfamethoxazole and Trimethoprim

Standard solution: 0.032 mg/mL of USP Trimethoprim RS per mL and 0.032J mg/mL

of USP Sulfamethoxazole RS per mL in Mobile phase from Standard stock solution
Sample stock solution: Transfer from finely powdered Tablets (NLT 20), an equivalent to 160 mg of sulfamethoxazole, to a 100-mL volumetric flask. Add 50 mL of methanol and sonicate, with intermittent shaking, for 5 min. Allow to equilibrate to room temperature, dilute with methanol to volume, and filter. Use the filtrate in the preparation of the Sample solution.
Sample solution: Nominally 0.16 mg/mL of sulfamethoxazole in Mobile phase from Sample stock solution

Suspension for Sulfamethoxazole and Trimethoprim

Standard solution: 0.032 mg/mL of USP Trimethoprim RS per mL and 0.032J mg/mL of USP Sulfamethoxazole RS per mL in Mobile phase from Standard stock solution
Sample stock solution: Transfer a volume of Oral Suspension, equivalent to 80 mg of sulfamethoxazole, to a 50-mL volumetric flask with the aid of 30 mL of methanol. Sonicate the mixture for 10 min with occasional shaking. Allow to equilibrate to room temperature, dilute with methanol to volume, and centrifuge. Use the filtrate in the preparation of the Sample solution.
Sample solution: Nominally 0.16 mg/mL of sulfamethoxazole in Mobile from Sample stock solution. Filter the solution.

Tablets for Metoclopramide HCl

Standard solution: 45 µg/mL of USP Metoclopramide Hydrochloride (equivalent to 40 µg/mL of metoclopramide) from Standard stock solution diluted with water.
Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of metoclopramide, to a 100-mL volumetric flask, add about 50 mL of water, and sonicate for 5 min, dilute with water to volume, and mix. Pass the solution through a filter of 0.45-µm pore size. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with Mobile phase.

Syrup for Metoclopramide HCl

Standard solution: 180 µg/mL of USP Metoclopramide Hydrochloride (equivalent to 160 µg/mL of metoclopramide) from Standard stock solution. Dilute with 0.01 M phosphoric acid. dilute with water.
Sample solution: Transfer a volume of Oral Solution, equivalent to about 4 mg of metoclopramide, to a 25-mL volumetric flask, and dilute with water.

Validation method

Validation of the developed method by linearity, accuracy, limit of detection and estimation limit of quantitation were used in the validation study. Linearity (r square) is expressed was calculated by recording the peak area of the different concentrations of the studied pharmaceutical compounds. The data was recorded and it is calculated using statistical analysis and linear regression method [12]. Method accuracy estimated percentage error (Er%), By injection three different levels: 12, 15, 18 $\mu g ml^{-1}$. Similarly, recovery ratios were calculated for the three pharmaceutical compounds (n = 3) with three different concentrations of pharmaceutical compounds [13]. LOD and LOQ they were represented as the concentrations of the analyzes where the observed signal-to-noise ratios were equal or greater than 3 for LOD and 10 for LOQ [14].

Results and Discussion

Through this study, method was developed for identification of Sulfa Tri and Meta is in its pure form and in different commercial pharmaceutical formulations. HPLC Terms optimized by studying the effects of organic modifier, flow rate and others.

Organic modifier

The isocratic mode was used, and the C_{18} (250mm Ч 4.6mm) column was maintained at 40 єC for all organic modifier experiments. In this study, acetonitrile was used, instead of methanol because of It has a weak ability to form hydrogen bonds and also has a high dipole moment that enables it to selectively dipole with a certain solution [15]. The organic ratio was improved by using a mixture of buffer: acetonitrile with a difference the ratios of the organic solvents (45:55, 40:60, 35:65, 30:70, 25:75, 20:80 and 15:85) as the mobile phase. The ratio of 40:60% is the best

separation of pharmaceutical compounds and high resolution (Table 2).

Table 2. The organic modifier percentage on retention time.

Mobile phase (v/v Buffer: acetonitrile	pharmaceutical	Parameter		
		t_R	k	N
15:85	Sulfa	2.569	0.327	969.6619
	Tri	3.564	0.842	1209.004
	Meta	8.457	3.371	3401.705
20:80	Sulfa	2.437	0.259	1405.673
	Tri	3.302	0.706	1601.94
	Meta	6.879	2.555	2800.038
25:75	Sulfa	2.412	0.246	1187.295
	Tri	3.250	0.679	1379.592
	Meta	6.851	2.541	2394.704
30:70	Sulfa	2.411	0.246	827.3468
	Tri	3.244	0.676	1042.817
	Meta	6.957	2.595	1997.819
35:65	Sulfa	2.367	0.223	1143.406
	Tri	3.251	0.680	1304.815
	Meta	7.133	2.686	2595.903
40:60	Sulfa	2.373	0.226	1105.906
	Tri	3.310	0.710	1299.202
	Meta	7.375	2.811	2863.896
45:55	Sulfa	2.369	0.224	509.0395
	Tri	3.407	0.761	1052.848
	Meta	7.631	2.944	4217.812

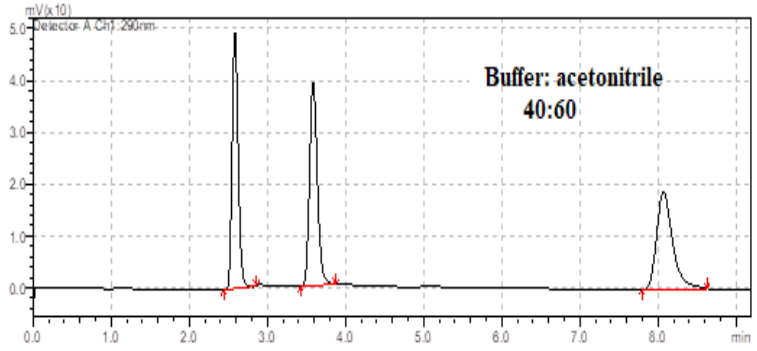

Figure 2: Chromatogram for three pharmaceutical compounds at (60:40)

The flow rate.

Various flow rate for the mobile phase (0.8, 0.9, 1, 1.1, 1.2, 1.3, and 1.6 ml/mint) was studied for the best the flow rate to achieve the best separation thus leads to a short analysis time. The results in fig 3 show that the optimal flow rate is 1.2 mL/mint.

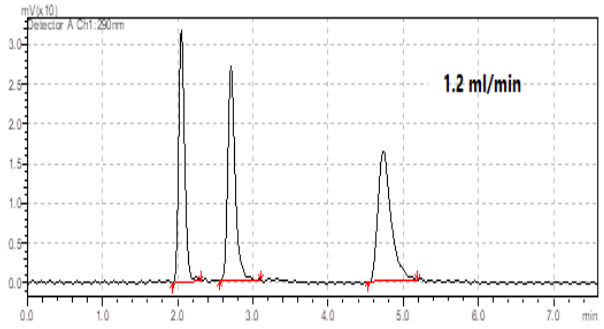

Figure (3): Chromatograms for different flow rate mobile phase (40:60 Buffer: Acetonitrile) 1.2 ml/ min

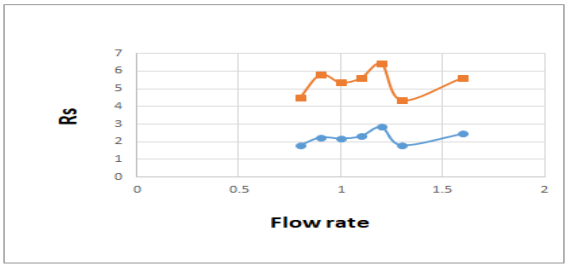

Figure 4. Efect flow rate on the Rs.

The effect of temp on the resolution

The temperature of the column oven at different temperature (40, 45, 50, 55 and 60 °C) was studied using a heat oven. Increasing the column temperature in RP-chromatography was observed to decrease in separate bands retention times and column efficiency increased by reducing the viscosity of the mobile phase [16]. The optimum temperature was chosen to be 45 °C.

Table (3): Results of tR, k, α, N and Rs of three pharmaceutical compounds at different temperatures.

Temp ºC	pharmaceutical	t_R	k	α	N	Rs
40	Sulfa	2.212	0.143152	3.541516	639.0784	1.828571
	Tri	2.916	0.506977		771.2522	
	Meta	5.123	1.647545	3.249745	1679.688	4.797826
45	Sulfa	2.204	0.139018	3.486989	1078.09	2.158065
	Tri	2.873	0.484755		1066.143	
	Meta	4.983	1.575194	3.249467	2052.09	5.341772
50	Sulfa	2.172	0.122481	3.734177	1035.409	2.16
	Tri	2.820	0.457364		1168.397	
	Meta	4.871	1.517313	3.317514	1960.879	5.327273
55	Sulfa	2.136	0.103876	4.149254	931.1216	1.978125
	Tri	2.769	0.431008		946.5878	
	Meta	4.757	1.458398	3.383693	1196.908	4.369231
60	Sulfa	2.108	0.089406	4.491329	652.8799	1.830303
	Tri	2.712	0.40155		1080.616	
	Meta	4.632	1.393798	3.471042	1773.176	4.987013

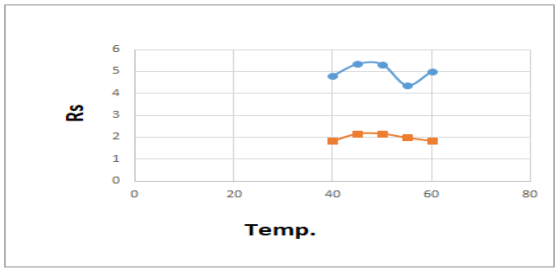

Figure 5. The effect of temperature on Rs.

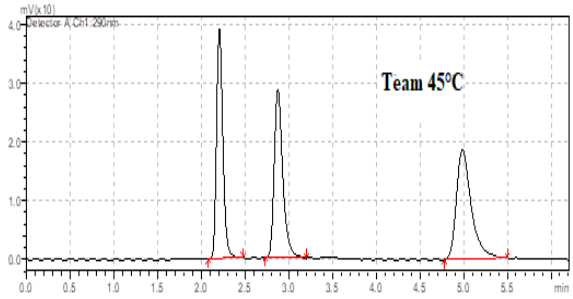

Figure 6: Chromatograms of three pharmaceutical compound at 45 ºC.

Calibration Curve

Linearity was tested under optimum conditions, the conc range of 0.1-100 µgml^{-1} for the Sulfa, Tri, and Meta mixture. The calibration curve linearity was calculated using the linear regression method, calibration plots for three pharmaceutical compounds were constructed by plotting the peak area versus concentrations as It is shown in Figure (7). Regression Equation, Linear Correlation, (LOD), (LOQ) Slope, Intercept and r^2 are given in the table (4).

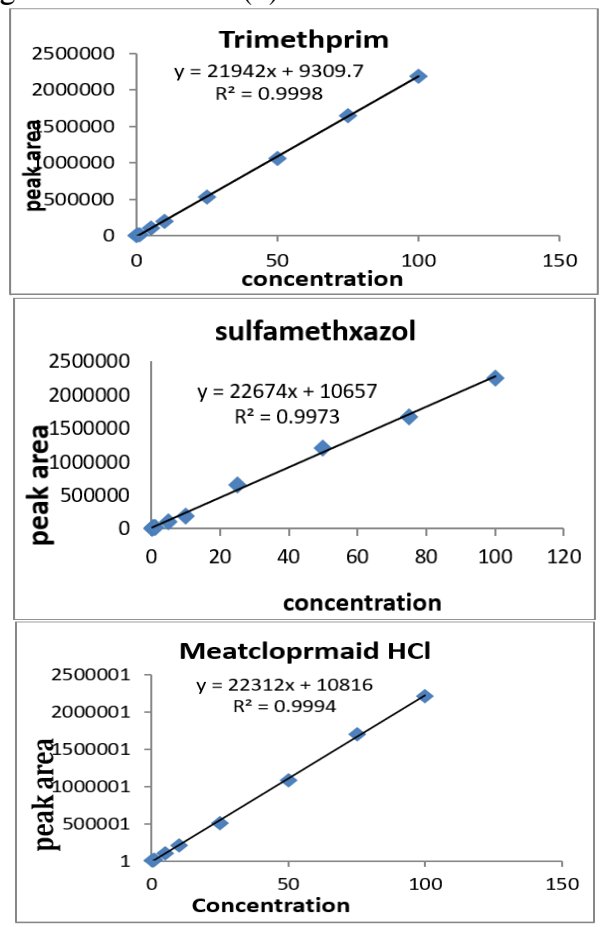

Figure 7. Calibration curve, conc (µg/ml) vs peak area for Sulfa, Tri, Meta

Table 4. Calibration curve results

pharmaceutical compounds	Linearity range(μg/ml)	Calibration graph	r^2	slop	Intercept (μv)	LOD (μg/ml)	LOQ (μg/ml)
Sulfa	0.1-100	Peak area	0.9973	22672	10657	$6.54Ч10^{-3}$	0.021
Tri	0.1-100	Peak area	0.9998	21942	9309.7	$6.65Ч10^{-3}$	0.022
Meta	0.1-100	Peak area	0.9994	22312	10816	$2.8Ч10^{-3}$	$9.33Ч10^{-3}$

Precision and accuracy

precision and accuracy were tested by injecting three different concentration levels 12, 15, and 18 μg mL^{-1} of sulfa-tri and meta. Three repeated injections were made for each pharmaceutical compound within the linear calibration range.

Table (5): Precision and accuracy of the method

pharmaceutical compounds	Injected (μg.mL^{-1})	Sample Conc. calculated from peak area		
		Mean*	RSD %	Er %
Sulfa	12	11.91	2.0	-0.76
	15	14.87	2.52	-0.87
	18	18.52	0.81	2.80
Tri	12	11.83	1.53	-9.2
	15	14.97	0.58	-0.20
	18	18.19	0.31	1.04
Meta	12	11.87	0.47	-6.95
	15	15.06	0.64	0.39
	18	18.15	1.1	0.83
Mean*of three time.				

Application of the method

The contents of Sulfa, Tri and Meta were determined in commercial pharmaceutical formulations. The results are shown in Table 6. The prepared commercial formulations chromatograms are shown in Figure 6. The recoveries ranged between 92.8 - 109.8% and RSD ranged between 0.11-1.22%. The results showed correlation and agreement between the declared and determined values of the studied pharmaceutical compounds for all analyzed samples, which indicates that the method that was used is more specific, efficient and highly selective.

Table 6. Application of the method for measurement of the pharmaceutical compounds at two concentrations in different commercial pharmaceutical formulation.

Commercial Name Company	pharmaceutical compounds	Conc. taken (μgmL^{-1})	Conc. calculated from peak area		
			Conc.* found (μg.mL^{-1})	RSD %	Recovery
Septrin tablet	Sulfa	50	50.37	0.24	100.74
		25	25.14	0.19	100.56
	Tri	10	9.88	0.58	99.8
		5	5.21	0.46	104.2
Septrin suspension	Sulfa	50	50.41	0.92	100.82
		25	25.68	0.72	102.72
	Tri	10	10.09	0.41	100.9
		20	19.02	0.15	95.1
Kindi tablet	Sulfa	50	49.85	1.22	99.7
		25	24.88	0.36	99.52
	Tri	10	9.85	0.63	98.5
		5	4.67	0.78	92.8
Kindi suspension	Sulfa	50	48.65	0.25	97.3
		25	24.19	0.41	96.76
	Tri	10	10.92	0.69	109.2
		20	20.18	0.14	100.9
Kindi syrup	Meta	10	10.84	0.11	108.4
		5	4.79	0.64	95.8
Kindi tablet	Meta	10	9.74	0.39	97.4
		5	4.89	0.22	97.8
Julphar tablet	Meta	10	10.66	0.14	106.6
		5	5.17	0.24	103.4
Neosil syrup	Meta	10	9.84	0.67	98.4
		5	5.49	0.37	109.8
*Average of three time					

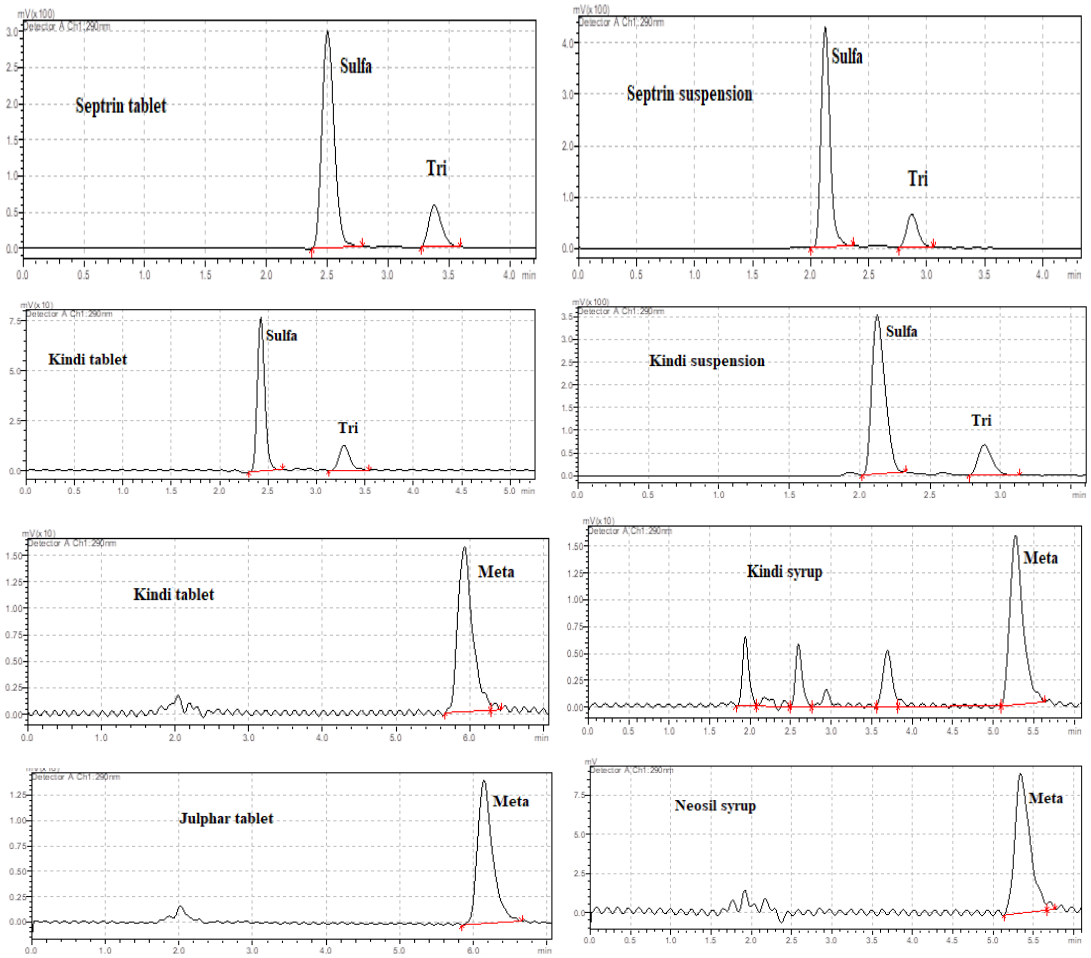

Figure 8. chromatograms of three pharmaceutical compounds in their commercial pharmaceutical formulation.

Conclusion

A very sensitive, highly accurate, sample and relatively fast analytical method for the determination of sulfamethoxazole, trimethoprim and metoclopramide hydrochloride. This validated method can be used in an identification of these active ingredient pharmaceutical in commercial pharmaceuticals formulation. Organic rates, flow rate and temperature were effective factors to achieve high column efficiency, resolution and purification separate bands. The results showed a good correlation and agreement between the declared and determine value of the studied active ingredient pharmaceutical in general analyzed commercial pharmaceuticals formulation. This developed method used to separate the studied compounds innovations compared to previous studies because none of these studies can identify all of them the three active ingredient pharmaceutical with a short time.

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