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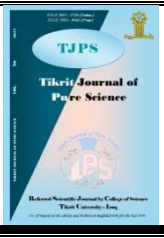

Ion-Pair Complex of Trimethoprim with Alizarin Yellow and its Determination and Extraction by LLE and DLLME

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ABSTRACT

For the analysis of trimethoprim medication in its pure form and pharmaceutical formulations, precise and accurate microextraction techniques with UV-Vis measurement have been developed and confirmed. In this study, LLE and DLLME procedures were utilized for the separation, pre-concentration, and evaluation of trimethoprim in pure form and pharmaceutical preparations by UV-Vis spectroscopy at 390 nm. The method was validated and applied to the evaluation of trimethoprim in pharmaceutical formulations. Several experimental factors containing the type of dispersive and extraction solvents and their volumes, pH, temperature, and centrifuging time were carried out. Under the ideal conditions, the procedures were linear in the range of 2–45 and 1-10 mg/L, with a coefficient of determination (R^2) of 0.9961 and 0.9992 for LLE and DLLME, respectively. The limit of detection (LOD) was 1.52 and 0.21 mg/L. Recovery of the target analyte in pharmaceutical formulations was 99.3%-104.9%.

معقد الازدواج الايوني لعقار الترايميثوبريم مع الاليزارين الاصفر و تقديره واستخلاصه باستخدام استخلاص سائل– سائل و الاستخلاص المايكروي سائل– سائل بالتشتت

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الملخص

تم استخدام طريقيتين مطورتين لتقدير عقار الترايميثوبريم في الشكل النقي و في المستحضرات الصيدلانية. في هذه الدراسة استخدم طريقة الاستخلاص سائل–سائل و طريقة الاستخلاص المايكروي بالتشتت لفصل و تقدير عقار الترميثوبريم.تم دراسة الظروف الفضلى العملية مثل نوع و حجم مذيب الاستخلاص و التشتت،و الاس الهيدروجيني،و درجة الحرارة ،و عدد وزمن الدورات اللازمة للاستخلاص. تحت الظروف المثالية تم الحصول على علاقة خطية بين التركيز و الامتصاصية على مدى تركيز (2–45) و (1–10) مكغم/ مل ومعامل ارتباط 0,9961 و 0,9992 لكلا الطريقتين على التوالي. بالاضافة الى ذلك تم الحصول على حد الكشف عند 1,52 و 0,21 مكغم/مل. اخيرا وجد ان معدل الاسترداد للعقار باستخدام الطريقتين يتراوح بين 99,3% و 104,9% . بعد LLE و DLLME ، تم إجراء اختبار قياس طيفي مباشر وقليل التكلفة ودقيق لترميثوبريم في التركيبات الصيدلانية والتحقق من إجراء التحليلات الروتينية.

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1. Introduction

Trimethoprim (TMP), is 2,4-diamine-5-(3,4,5-trimethoxybenzyl)pyrimidine, fig. 1. It has the formula [$C_{14}H_{18}N_4O_3$] and M.Wt 290.3 g.mol^{-1}. As a powerful bacteriostatic drug and well-known folic acid antagonist, trimethoprim is frequently used with sulfonamides to treat respiratory and digestive system infections [1-3]. It is frequently used to both prevent and cure several infections, including gastrointestinal, respiratory, and urinary infections [4-6] and has a half-life of about (8-11 hrs). Also its pharmaceutical formulations, it is in the form of syrup and tablets [7, 8]. Trimethoprim is an organic compound described by some of researchers [9, 10]. Various analytical methods were utilized to determine the TMP containing spectral techniques [11-15], liquid chromatography[16], HPLC [17], and the electrical method [18, 19]. The dispersive liquid-liquid microextraction (DLLME) has many advantages like rapid, low cost, and safety [20, 21]. The aim of this work was for the simultaneous spectrophotometric determination of TMP by ion-pair complex with alizarin yellow as a reagent combine with LLE and DLLME methods. For estimation and quality control in medication formulation, the established method must have certain properties, such as being easy, quick, and sensitive.

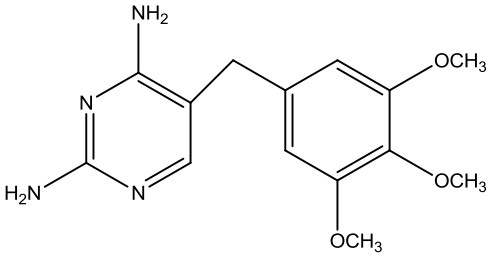

Fig. 1: Trimethoprim structure

2. Experimental

2.1. Apparatus

UV-VIS spectrophotometer (cintra 5) with 1.0- cm quartz cells and pH meter (DW-9421 from the Philips instrument) were utilized. To separate the organic phase from the aqueous phase, a hermle Z-300 centrifuge (Germany, Wehingen) was utilized.

2.2. Materials and methods

Trimethoprim (TMP, purity 98.9%) was obtained from the state company for drug industries and medical appliances, Samara-Iraq(SDI). Alizarin yellow(ALZ) was obtained from (Sigma-Aldrich). A stock solution of (TMP) 0.05 gm was weighed and dissolved in 100 mL of 0.1N HCl solution to prepare 500 mg/L. In the volumetric flask, 0.05 g of ALZ was dissolved in 100 mL of distilled water to produce a stock solution (500 mg/L) (100mL). Buffer's solution was prepared according to reference [22]. The working standard solutions were prepared daily by diluting the stock solution with the same solvent.

2.3. Assay procedure for dosage forms

Ten tablets of TMP, 80 mg (Supreme, India & Co-trimoxazole, Iraq) were weighed and powdered. The amount weighed was dissolved in 100 mL of (0.1 N)HCl solution in a volumetric flask. The content was mixed well and filtered through filter paper to remove the insoluble compounds.

2.4. General procedure of LLE.

A 2.0-45 mg. L^{-1} of TMP standard solutions were added to volumetric flasks stoppered tube including 2 mL ALZ solution (100 mg/ L). The volume was completed to 5 mL with 0.1 N HCl solution. The mixture was shaked for 7 min, heated for 10 min at 30 ^{0}C and then $CHCl_3$ 5 mL was added to the mixture and centrifuged at 4000 rpm for 5 min. A syringe was used to separate the organic phase containing the ion-pair complex, and the absorbance at 390 nm was measured against a blank.

2.5. General procedure of DLLME.

A 1.0 – 10.0 mg/L of TMP standard solutions were added to volumetric flasks stoppered tube containing 2 mL ALZ solution (20 mg/L) and 2 mL of the HCl solution (0.1 N) . The volume was completed to 10 mL with distilled water. The mixture was shaked for 7 min, then heated for 10 min at 30 ^{0}C. A cloudy solution was obtained by rapidly injecting 300 µL chloroform as an extraction solvent and 1000 µL ethanol as a dispersive solvent into the mixture utilizing a microsyringe. For 5 min, the mixture was centrifuged at 4000 rpm. The yellow ion-pair complex was obtained using a microsyringe, and the absorbance at 390 nm was measured.

3. Results and discussion

Trimethoprim (TMP) forms a yellow ion-pair complex, in an acidic media, with alizarin yellow. The spectrum of the quantitatively extracted complex TMP-ALZ into $CHCl_3$ exhibited maximum absorbance at 390 nm as shown in figure.2. The procedure was depended on the ion transfer complex formed by ALZ, and TMP that has basic nitrogen as an electron donor to produce ion-pair salts that are colored and extracted from aqueous solutions into the organic phase [23].

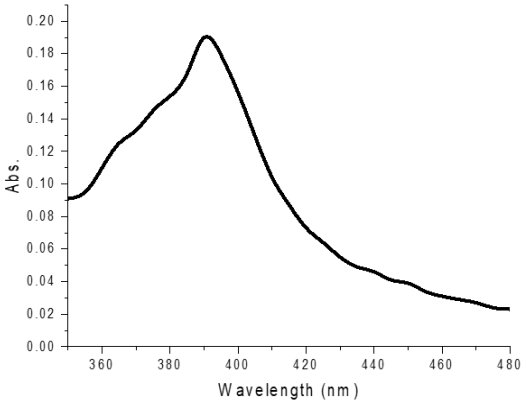

Fig.2: Spectrum of the resulting ion-pair complex (TMP-ALZ)

3.1. Optimization of liquid-liquid extraction(LLE).

The optimum conditions required for the quick and efficient production of the MTP ion-pair complex

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were accomplished. The influence of pH on the TMP-ALZ complex was studied by extracting the colored complexes in the presence of phosphate buffer and 0.1N of hydrochloric acid. At 0.1N of HCl solution, the highest color intensity and best absorbance value were recorded, the table. 1. Various solvents such as chloroform, dichloromethane and tetra chloromethane were investigated. Chloroform was found to be the most effective solvent for its quantitative and selective extraction, table. 2.

Table 1: Effect of pH value on formation of complex TMP-ALZ

pH effect	Abs.
0.1 N HCl	0.186
1	0.170
2	0.152
3	0.132
4	0.123
5	0.120
6	0.115
7	0.008
8	0.008

Table 2: Effect of solvent type on the extraction of complex TMP-ALZ

Type of solvent	Abs.
$CHCl_3$	0.185
CH_2Cl_2	0.109
CCl_4	--

The optimum reaction time and the temperature was studied by following the color development. A water bath was employed to study the effect of temperature, ranging from (25-50^{0}C), and it was found that 30 ^{0}C had the best absorption value at 390 nm, figure.3. The incubation time (5-30) minutes were investigated and found that 10 minutes gave the greatest absorbance at 390 nm, figure.4.

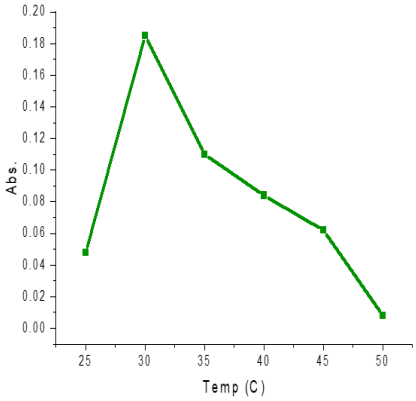

Fig.3: Effect of temperature on the formation of TMP-complex

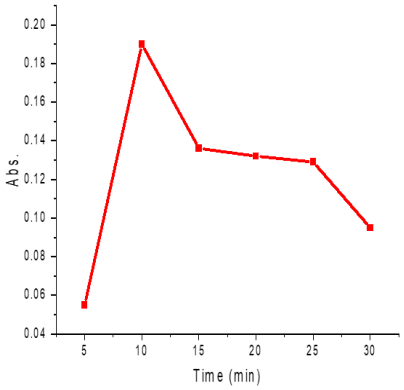

Fig.4: Effect of incubation time on the formation of TMP-complex

The influence of the ALZ volume was investigated by measuring the absorbance of solutions including a fixed concentration of TMP drug and different volumes of the respective reagent. A 2.0 mL reagent ALZ solution (100 mg/L) was used to produce the complex's highest color. The effect of the reagent's volume on the absorbance is shown in Figure 5. The absorbance of the ion-pair complex was unaffected by adding more ALZ reagent. The influence of number and time rotation on the extraction of the yellow ion-pair complex in the centrifuge is crucial. 4000 rpm at 5 min gives the best absorbance as shown in the table.5 and 6. The influence of excipients such as glucose, starch, and others was investigated and it was found that they have no important effect on the estimation and extraction of the TMP drug, as shown in the table. 7.

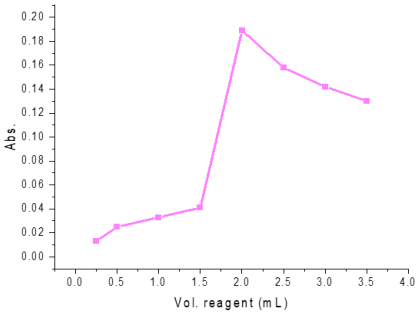

Fig.5: Effect of reagent ALZ volume on the extraction of TMP-complex

Table5: Effect of rotation number on theextraction of TMP-complex

Rotation No. (rpm)	Abs.
1000	0.116
2000	0.127
3000	0.145
4000	0.191

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Table.6: Effect of the rotation time on the extraction of TMP-complex

Rotation time (min)	Abs.
1	0.090
2	0.102
3	0.107
4	0.126
5	0.192

Table.7: Effect of excipients on the extraction of TMP-complex

Rec. %	Comp.
-	Drug without excipients
91.8	Galactose
87.5	Maltose
93.3	Sucrose
97.8	Glucose
96.4	Talic acid
97.6	Ribose
93.1	Starch

The formation of the yellow ion-pair complex TMP-ALZ followed a ratio of 1:1, according to the results of the reaction stoichiometry between the TMP drug(3.2×10^{-4}M) and alizarin yellow(3.2×10^{-4}M) , Figures 6 and 7.

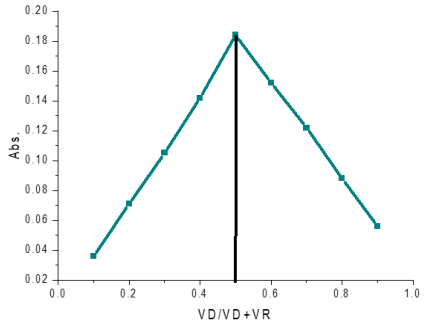

Fig.6: Continuous variation method of TMP-ALZ

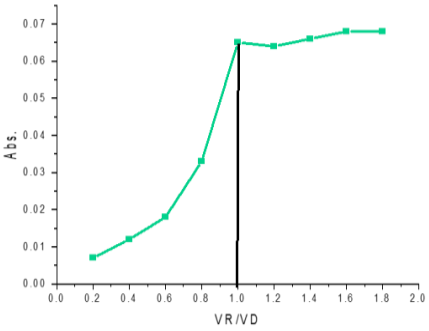

Fig.7: Mole ratio method of TMP-ALZ

3.1.1. Calibration Curve

An analytical method is shown to be linear if it can produce test findings that are proportionate to the concentration of the drug in the sample. Over the concentration range of 2.0 to 45.0 µg/mL, the TMP calibration curve was linear, figure. 8.

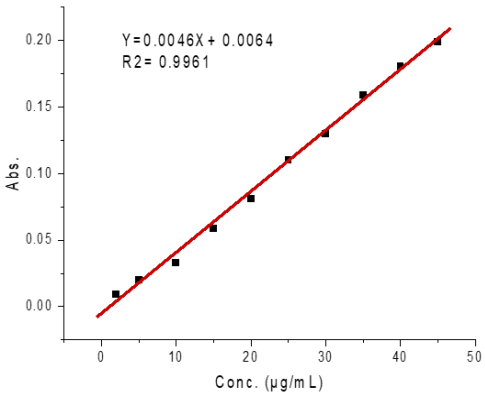

Fig. 8: Calibration curve of TMP-complex using LLE at 390 nm

3.2. Optimization of dispersive liquid-liquid microextraction(DLLME)

DLLME microextraction method was developed for the detection and preconcentration of trimethoprim. A trimethoprim standard solution in its pure form and pharmaceutical formulations were used to achieve various experimental parameters to find the ideal conditions for DLLME. Some extraction solvents such as chloroform, dichloromethane, and tetra chloromethane were utilized for DLLME. Then optimizing the extraction solvent and volume. Referring to results shown in the table. 8, and 9, a 300 µL of $CHCl_3$ was chosen for DLLME.

Table 8: Effect of extraction solvent

Type of solvent	Abs.
$CHCl_3$	0.250
CH_2Cl_2	0.112
CCl_4	--

Table 9: Effect of extraction solvent volume

volume of extraction solvent µmL	Abs.
200	0.120
300	0.254
400	0.089
500	0.054

A good disperser solvent for DLLME must be miscible with both the organic and aqueous phases and produce a cloudy state that increases the contact area between the two phases. Dispersive solvents such as methanol, ethanol, and acetone, were investigated. Since the best absorbance signal was obtained when using ethanol, table.10 and it was chosen as a dispersive solvent for the following study. Then, a dispersive solvent volume range of 500 to 1500 µL was examined. The obtained results showed a higher response for 1000 µL ethanol, figure.9.

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Table 10: Effect of dispersive solvent

Type of dispersive solvent	Abs.
Ethanol	0.252
Methanol	0.029
Acetone	0.052

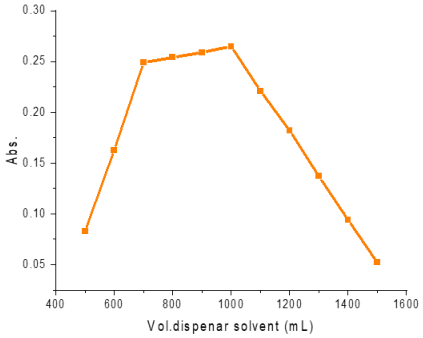

Fig. 9: Effect of ethanol volume as dispersive solvent

The effect of reagent volume (0.25-3.0)mL was investigated. The results (figure 10) showed that adding a volume of reagent first enhanced the analytical signal before adding more reagent caused it to diminish. Because of this, 2.0 mL was chosen as the ideal reagent volume for the DLLME method. The effects of centrifugation rate and time were observed in this study at speed between 1000 and 4000 rpm and times between 1.0 and 5.0 min, respectively. A faster transfer of the analyte from the aqueous phase into the extraction phase was observed in DLLME because of the large surface area between the extraction solvent and the aqueous phase. According to the got results, 4000 rpm, and 5 min were chosen as centrifuge rate and time, respectively. Numerous saccharides were investigated as potential interfering agents to determine the presence of trimethoprim (TMP) in pharmaceutical formulations. The results are reported in Table 11.

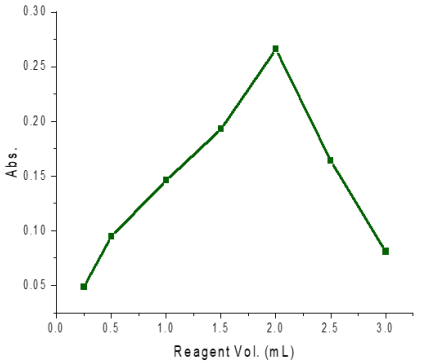

Fig. 10: Effect of reagent volume

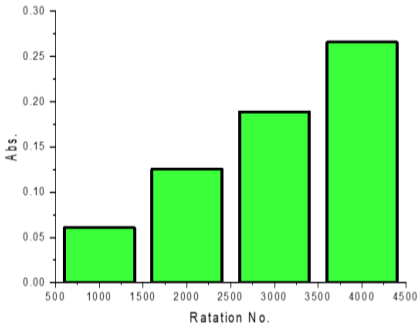

Fig. 11: Effect of rotation number

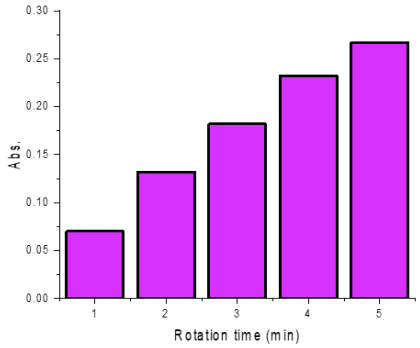

Fig. 12: Effect of the rotation time

Table 11: Effect of excipients on the extraction of TMP

Rec. %	Comp.
-	Drug without excipients
89.1	Galactose
97.1	Maltose
95.5	Sucrose
89.7	Glucose
96.7	Talic acid
95.5	Ribose
96.3	Starch

3.2.1. Linearity and range

Beer's law range, regression equation, molar absorptivity, Sandell's sensitivity, and correlation coefficient for the two the suggested approach is listed in (table. 12). For the DLLME method, a linear relationship between the absorbance at maximum and MTP drug concentration in the range (1.0- 10.0) mg/L was established in the final measured volume of 10mL. (figure.13). A strong association may be seen in the Beer's law plots at max after regression analysis. The resulting ion-pair complex's high molar absorptivities show how sensitive the technique is.

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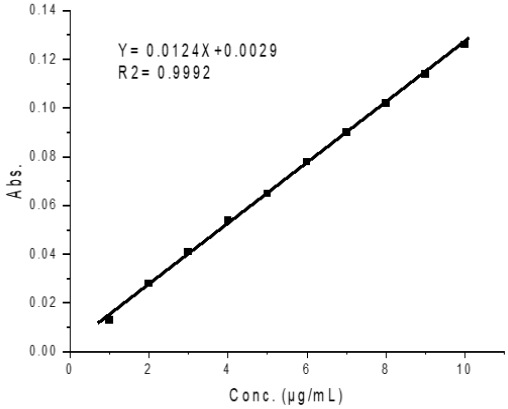

Fig. 13: Calibration curve of TMP using DLLME

3.3. Validation of the method

The two approaches' applicability for analyzing TMP in both pure and pharmaceutical forms was investigated. The results obtained for pure drugs are given in the table. 13. By examining three replicates of the medicine, the procedures' accuracy and precision were confirmed. The best precision and reproducibility of the procedures are indicated by the low relative standard deviations (RSD %) values. Table 13, provides the findings of the dose form analysis. The RSD% values were low, and the results were reproducible. The average percent recoveries (Rec. %) were quantitative (99.3%–104.9%), which indicates that the approaches were accurate.

Table 12: Analytical and statistical parameters of LLE and DLLME

Parameter	***LLE***	***DLLME***
λ max(nm)	390	
color	yellow	
linearity range mg/L	2.0-45	1.0-10.0
Molar absorptivity (L.mol^{-1} cm^{-1}), Ɛ	1.33×10^{3}	3.6×10^{3}
Sandell's sensitivity µg/cm^2	0.21	0.08
Correlation coefficient (R^2)	0.9961	0.9992
Regression equation	Y= 0.0046X- 0.0064	Y= 0.0124X- 0.0029
Slope(b)	0.0046	0.0124
Intercept(a)	0.0064	0.0029
Limit of detection mg /L (LOD)	1.52	0.21
Limit of quantification mg/L(LOQ)	4.56	0.63
C.L. for the slope at 95%	$0.0046\pm1.023\times10^{-4}$	$0.0124\pm1.23\times10^{-4}$
C.L. for Intercept at 95%	0.0.0064± 0.00273	0.0029± 7.67×$^{-4}$
*C.L. for the X1 mg/L at 95%	$9.36 \pm 3.4\times10^{-3}$	$3.12 \pm 3.7\times10^{-3}$
*C.L. for the X2 mg/L at 95%	$14.39 \pm 2.2\times10^{-3}$	$5.00 \pm 4.4\times10^{-3}$
*C.L. for the X3 mg/L at 95%	$19.79 \pm 3.7\times10^{-3}$	$7.21\pm 4.5\times10^{-3}$

*LLE (X1=10, X2=15, X3=20), DLLME (X1=3, X2=5, X3=7)

Table 13: Application of the suggested methods (LLE & DLLME) for the evaluation of TMP

drug	**Liquid-liquid extraction**					
	Conc. of drug mg/L		**Relative Error%**	**Rec. %**	**Average Rec.%**	**RSD% (n=3)**
	Taken	Found				
Supreme	10	10.1	0.67	100.8	101.03	5.7
	15	15.1	1.02	101		2.97
	20	20.29	1.48	101.3		2.1
Co-trimoxazole	10	9.72	-2.8	97.2	98.7	1.2
	15	14.94	-0.35	99.6		0.89
	20	19.86	-0.15	99.3		1.1
Dispersive liquid-liquid microextraction						
Supreme	3	3.15	4.9	104.9	102.4	4.3
	5	5.00	0.0	100.0		2.7
	7	7.18	2.5	102.5		2.2
Co-trimoxazole	3	3.03	1.2	101.1	101.1	2.1
	5	5.00	0	100.0		2.7
	7	7.09	1.2	102.2		3.1

Table 14: Comparison of the linearity, and LOD with previous studies

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Method	Linearity mg/L	LOD mg/L	Ref.
HPLC	2-60	0.0098	[4]
Spectrophotometric method	7.5-60	0.0269	[11]
Micellar electrokinetic capillary chromatography	0.5-200	1.3	[24]
RP-HPLC	0.16-0.24	3	[25]
Liquid chromatography	25-400	0.5	[26]
Ion-pair extraction method	1.25-10.71	0.154	[14]
Extractive spectrophotometric method	4-24	-	[27]
HPLC	0.5-40	0.18	[28]
LLE method	2-45	1.52	Present work
DLLME method	1-10	0.21	Present work

4. Conclusion

For the examination of trimethoprim in pure and pharmaceutical formulations, a quick, low-cost LLE extraction technique with UV-VIS measurement was developed and verified. The results were compared to the DLLME approach. The proposed method is comparable to other reported techniques, table. 14. Additionally, the UV-Vis detection system, which was simple to use, is a significant benefit of this study. To the best of our knowledge, only a few techniques exist for the evaluation of trimethoprim, and this method provides a simple method for determining out how much trimethoprim is present in pharmaceutical formulations. According to the findings, the suggested approach showed good recoveries. As a result, this method can be used in routine examination to uantitatively analyze trimethoprim in pure materials.

5. References

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