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Micro-Spectrophotometric Determination And Cloud Point Extraction Of Sulphamethoxazole In Pure Form And Pharmaceutical Preparation.

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ABSTRACT

Two methods were proposed for the determination of sulfamethaczole in pure matter and pharmaceuticals. Simultaneously, with ease, simplicity and speed, the first method was based on sodium sulfate (sodium sulfate) by sodium nitrite in the acid medium at 5 ° C followed by conjugation with beta naphthol in the base medium to form orange. And measured at 484 nm for the second method, the extraction was used at the cloud point and the surface material used was Triton X 114. Both methods obeyed the Per lambert law in a concentration range of 1-18 µg / mL. The sandal sensitivity of the two methods was 0.02358-0,02341 µg / ml and the detection limit was 0.118 and 0,24436 µg / mL. The quantitative estimate was 0.023799 and 0.38708 µg / ml. All the variables that affect the process of interaction have been studied to improve the reaction conditions. Among these variables are the best acid, base, concentration of reagent, reagent and reaction time. The two methods were applied successfully. The results showed that the overlapping materials did not affect the measurement process.

Keyword: Cloud Point Extraction, β –Naphthol, Triton X-114, Sulphamethoxazole.

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INTRODUCTION

Sulphamethoxazole(3-P-aminobenzenesulphonamido-5-methyl-isoxazole) has activity kind of the sulfonamides. Its has been essentially active in the cure of breathing and urinary-tract infections(1). Sulfamethoxazole is an antibacterial drug which has been used since the 1960s in the therapeutic of many systemic contagions in humans and other types (2). The key use has been in the therapeutic of serious urinary tract contagions. It has similarly been used contrary to gonorrhoea, meningitis and serious respiratory tract ·infections Pneumocystis carinii) and prophylactically against susceptible meningococci(3) . Despite its relatively unfavourable pattern of tissue distribution, it is the sulfonamide most commonly used around the world in combination with trimethoprim or pyrimethamine for the treatment of various systemic infections (4). Figure (1) display the structure of sulphamethoxazole (5).

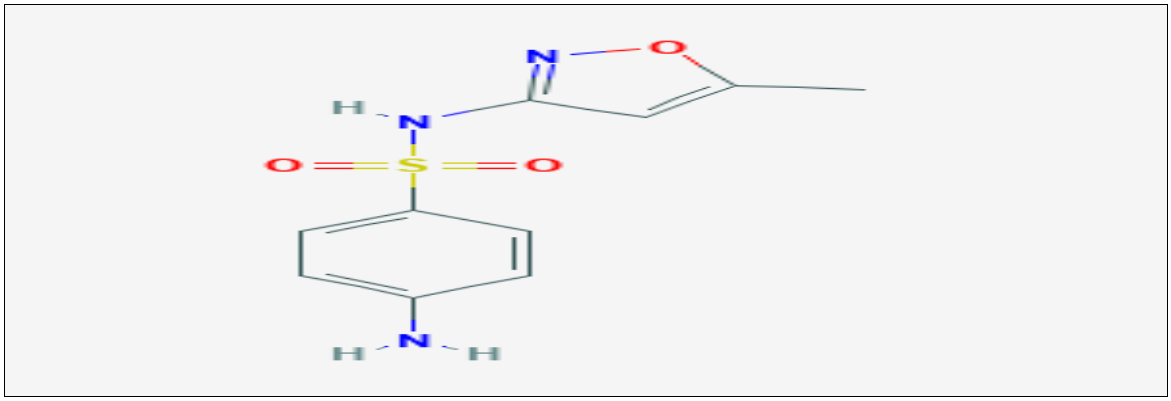

Figure 1: Structure of sulphamethoxazole

Instrumentation and Apparatus:

Instruments:

UV-Vis spectrophotometer: SHIMADZU, Double beam UV-Vis, model UV-1800 made in Japan. The range of wavelength (190-1100) nm, cell quartz with path 1cm.,Water Bath : A thermostat water bath, Memmert, made in Germany, Electric Balance: Sartorius (0.0000), made in Germany, Centrifuge: Triup International corp, TRIU 800 Centrifuge, made in Korea & PH meter: HANNA, PH meter, HI83141

General procedure for first methods Azo coupling:

The prepared Azo Coupling product are added in volumetric flask (10ml) in ice bath , 1ml of Sulphadimidine Sodium (SMX) (1000 µg ml^{-1}) ,1ml for hydrochloric acid , 1ml for sodium nitrate (1%) ,1ml for sulphamic acid (1%), 1ml for β- Naphthol(1000µg ml^{-1}) ,at last added 1ml for sodium hydroxide and complete the volume by distilled water .Then absorbance is measured by UV-VIS. And the maximum wave length show in figure:(1-1).

General procedure for second methods CPE:

A characteristic experiment of cloud point include the following steps: taking the volumetric flask (10ml) and added the optimum condition of azo coupling and added 1ml for surfactant (10%) and complete the volume by distilled water . The contain of volumetric flask transfer to centrifuge test tube then added the mixture in water bath 60 C^0 at 20 min and separated by centrifugation 4000 rpm at 20 min. Test tube taken in ice bath to increased viscosity micelles layer 1min. then become easily separated . The separated sediment s dissolved by 1ml of ethanol and measured the absorbance by UV-VIS. And the maximum wave length show in figure:(1-1).

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RESULT AND DISCUSSION

First methods: Spectrophotometric determination of sulphadimidine sodium (SDMS) by oxidation coupling reactions

Optimization Parameters for Reaction

All of the factors that affect to the absorbance of formation of azo dye product are optimized to improve the sensitivity and detection limit for the determination of the drugs .All optimization work under wavelength at 484 nm.

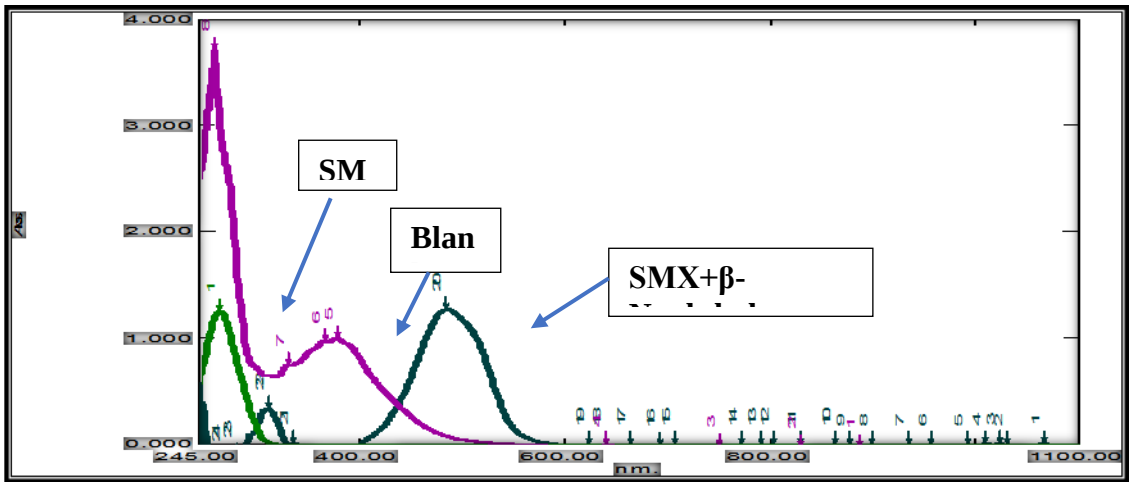

Figure 1-1: Absorbance spectra of the Resulting Dye.SMX

Effect of Acid Type

In this research, made chains experiment using (0.5 M) of different acid [HCl , H_2SO_4 , HNO_3 ,H_3PO_4 and CH_3COOH] that follow the same procedure that [1ml of drug SMX , 1 ml of each acid , 1ml of $NaNO_2$, 1ml of H_3NSO_3, 1ml β-Naphthol and 1ml of KOH] in volumetric flask 10 ml and complete the volume by distilled water to formation diazonium salt. After that measuring the absorbance at maximum wavelength for drug.

Table 1-1: Data of Absorbance of Effect acids

Acids	HCl	HNO_3	H_2SO_4	$H3PO_4$	CH_3COOH
Absorbance	**0.290**	0.145	0.098	0.140	**0.222**

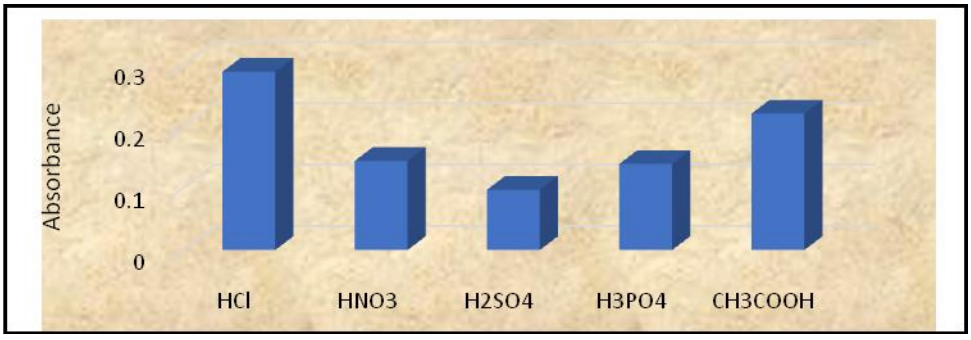

Figure 1-2: absorbanse of different acid

Effect of Optimum Volume of 0.5M Hydrochloric Acid

The same addition for SMX is [1ml drug , with varying volumes of 0.5 HCl from (0.1-1) ml , 1 ml $NaNO_2$, 1ml H_3NSO_3, 1ml β-Naphthol and 1ml of KOH in 10 ml volumetric flask and complete the volume by distill

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water .Then measured the absorbance and the optimum volume for higher absorbance that fixed for sequence experiment 0.5 ml show in table (1-2) and figure (1-3).

Table 1-2: Data of the absorbance of different volume to Hydrochloric acid with SMX

Volume of (HCl) 0.5 M	Abs.	Volume of (HCl) 0.5 M	Abs.
0.1	0.039	0.6	0.253
0.2	0.097	0.7	0.315
0.3	0.106	0.8	0.279
0.4	0.189	0.9	0.228
0.5	0.201	1	0.170

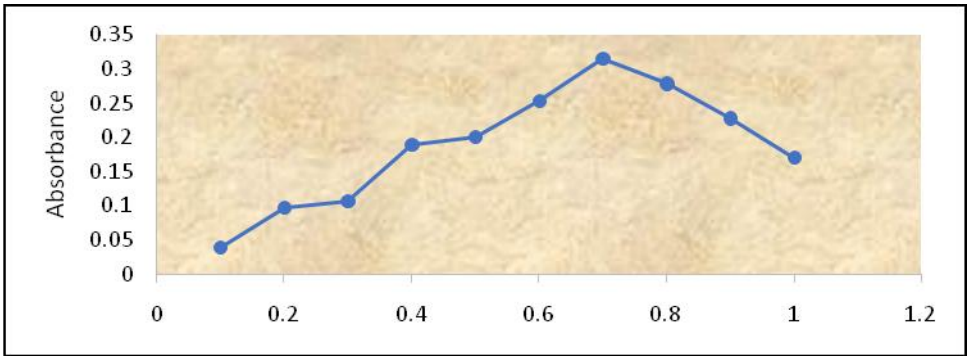

Figure 1-3: The Volume of 0.5 M (HCl) ml with SMX

It is noticeable that absorbance growth with rise the volume of acid, all of a sudden the absorbance drop because the primary amine becomes inactive (6).

Effect of Base Type

In this experiment using different basic [NaOH, KOH, K_2CO_3, Na_2CO_3, NH_4OH, $NaHCO_3$] and that follow the addition SMZ [1ml SMX, **0.7**ml HCl, 1ml $NaNO_2$, 1ml H_3NSO_3, 1ml β-Naphthol and 1ml of each base in volumetric flask10 ml and complete the mark by distill water. .The absorbance is measured the absorbance results are shown in table (1-3) and figure (1-4).

Table 1-3: Data of the absorbance of different base with SMX

Volume of 0.5M acids	NaOH	KOH	NH_4OH	Na_2CO_3	K_2CO_3	$NaHCO_3$
Absorbance	0.119	0.130	0.022	0.373	0.059	0.004

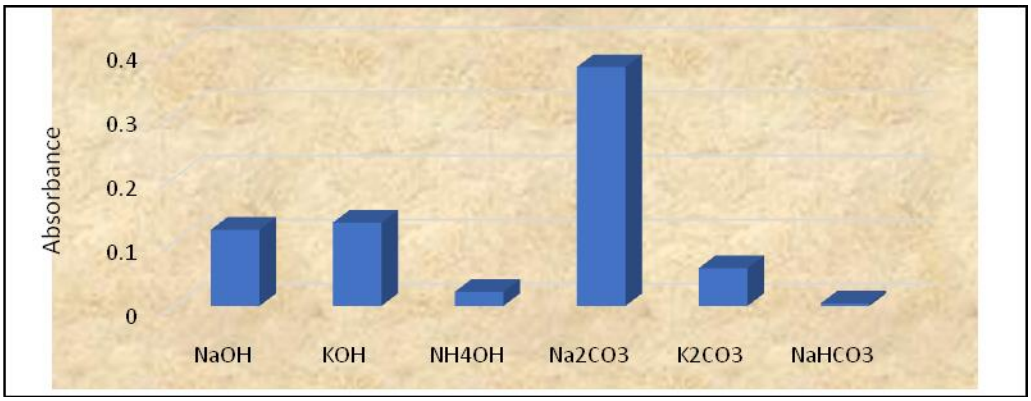

Figure 1-4: the absorbance different base with SMX

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It is clear the Sodium hydroxide give the higher absorbance , this base it is fixed in subsequent(7) show in table (1-3)& figure (1-4).

Effect of Optimum Volume of 0.5M [Na_2CO_3]

The same addition for SMX [1ml SMX , **0.7** ml HCl, 1 ml $NaNO_2$, 1ml H_3NSO_3, 1ml β-Naphthol and varying volume of o.5 M Na_2CO_3 (0.1-1) ml] in volumetric flask10 ml and complete the volume by distill water .Then measured the absorbance and the optimum volume for higher absorbance that fixed for sequence experiment. The absorbance result show in table (1-4) and figure (1-5).

Table 1-4: Data of the absorbance of different base with SMX

Volume of 0.5M bases	Absorbance at λmax =484 for SMX+Na_2CO3	Volume of 0.5M bases	Absorbance at λmax =484 for SMX+Na_2CO3
0.1	-0.010	0.6	0.271
0.2	0.037	0.7	0.312
0.3	0.091	0.8	0.396
0.4	0.146	0.9	0.367
0.5	0.209	1	0.295

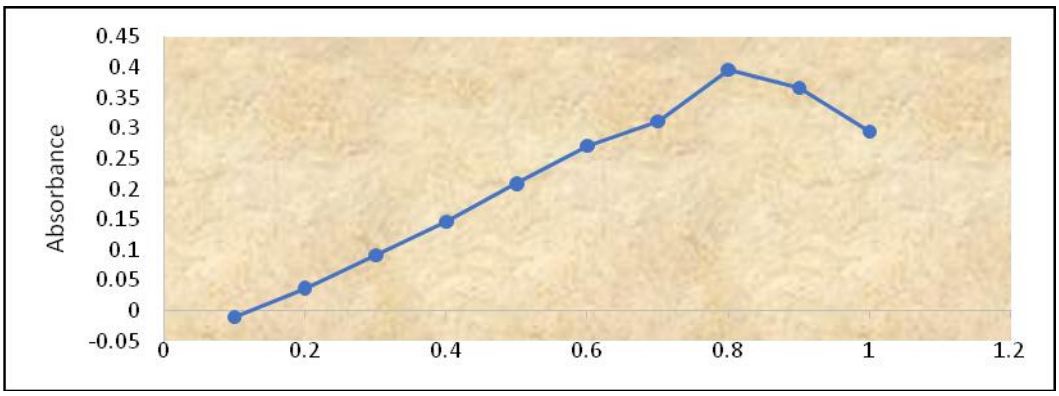

Figure 1-5: Deferent Volume of Na $_2CO3$ with SMX

It is evident that absorbance increase with increase the volume of Na $_2CO3$, but suddenly decrease the absorbance because the decomposition happen when increase basicity and formation diazotate ions may coupling and agreement with previous studies (8) . The optimum value of **0.8** ml for Na_2CO_3 with SMX.

Effect of Optimum Volume of 1% Sodium Nitrite

The same additions are for SMZ[1ml for SMZ,**0.7** ml HCl, with varying volume of 1% $NaNO_2$ from (0.1-1) ml, 1ml H_3NSO_3, 1ml β-Naphthol and **0.8** ml Na_2CO_3] in volumetric flask 10 ml and complete the mark by distill water .Then the higher absorbance of optimum volume are fixed for sequence experiment show in table (1-5) and figure (1-6).

Table 1-5: Data of Absorbance to Optimum Volume of 1% NaNO2

Volume of 1% Sodium Nitrite	Absorbance at λmax =484 for SMX	Volume of 1% Sodium Nitrite	Absorbance at λmax =484 for SMX
0.1	0.194	0.6	0.376
0.2	0.249	0.7	0.288
0.3	0.361	0.8	0.239
0.4	0.421	0.9	0.195
0.5	0.395	1	0.152

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Scheming of the absorbance values against the volume of Volume 1% NaNO2 displayed in figure (1-6).

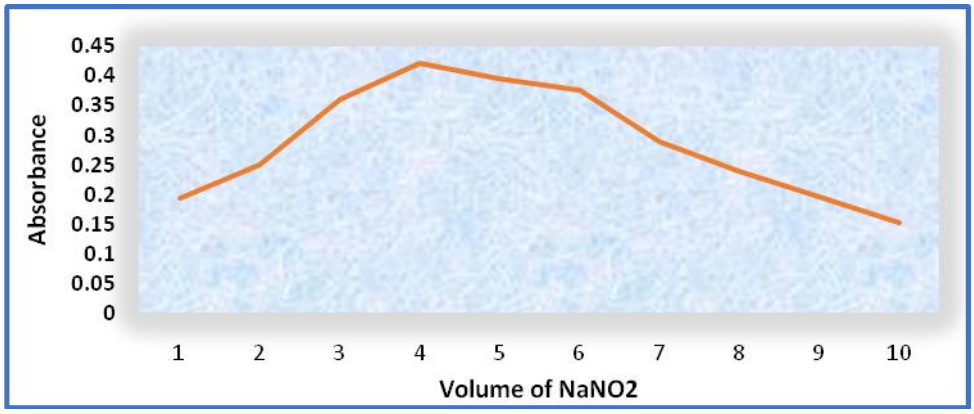

Figure 1-6: Data of Absorbance the volume of Volume 1% $NaNO_2$.

It is clear the absorbance increase with increase the volume of $NaNO_2$,but the signals decrease because the nitrate toxic may because a high rate of pollutants affecting on diazonium salt (9) .

Effect of Optimum Volume of 1% Sulphamic Acid

The additions for experimental are [1ml for SMZ,**0.7** ml HCl, **0.4** ml 1% $NaNO_2$ with varying volume of 1% H_3NSO_3 from (0.1-1) ml, 1ml β-Naphthol and **0.8** ml Na_2CO_3] in volumetric flask 10 ml and complete the volume by distill water. Then the higher absorbanceof optimum volumeare fixed for sequence experiment . Table (1-6) show the data of the absorbance

Table 1-6: Data of the Absorbance od Optimum Volume of 1% Sulphamic Acid

Volume of 1% Sulphamic Acid	Absorbance at λmax =484 for SMX	Volume of 1% Sulphamic Acid	Absorbance at λmax =484 for SMX
0.1	0.178	0.6	0.417
0.2	0.203	0.7	0.376
0.3	0.291	0.8	0.255
0.4	0.367	0.9	0.201
0.5	0.432	1	0.139

Scheming of the absorbance values against the volume of Volume of 1% Sulphamic Acid displayed in figure (3-9) .

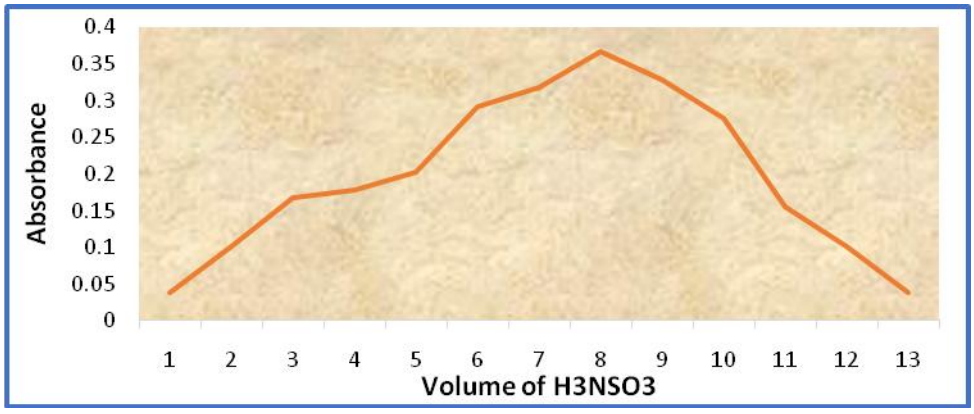

Figure 1-7: Data of Absorbance Volume of 1% Sulphamic Acid

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In this graph is clear the absorbance increase with increase the volume of Sulphamic acid , but the signals decrease suddenly because this volume remove nitrite and escape of nitrogen gas(10). The optimum volume of Sulphamic acid is 0.5 ml.

Effect of Optimum Volume of (100 µg ml^{-1}) Reagent

The same additions are [1ml for SMZ, **0.7** ml HCl, **0.4** ml 1% $NaNO_2$,**0.5**ml H_3NSO_3, with varying volume of (100 µg mL^{-1}) β-Naphthol from (0.1-1) ml and **0.8** ml Na_2CO_3in volumetric flask 10 ml and complete the volume by distill water. Then the higher absorbance of optimum volume at maximum wavelength's are fixed for sequence experiment show in table (1-7).

Table 1-7: Data of Absorbance of Optimum Volume of (100 µg ml^{-1}) Reagent

Volume of (100 µg ml^{-1}) Reagent	Absorbance at λmax =484 for SMX+ 2-NAP	Volume of (100 µg ml^{-1}) Reagent	Absorbance at λmax =484 for SMX+ 2-NAP
0.1	0.178	0.6	0.6
0.2	0.209	0.7	0.7
0.3	0.298	0.8	0.8
0.4	0.319	0.9	0.9
0.5	0.388	1	1

Scheming of the absorbance values against the volume of Volume of Reagent displayed in figure (1-8).

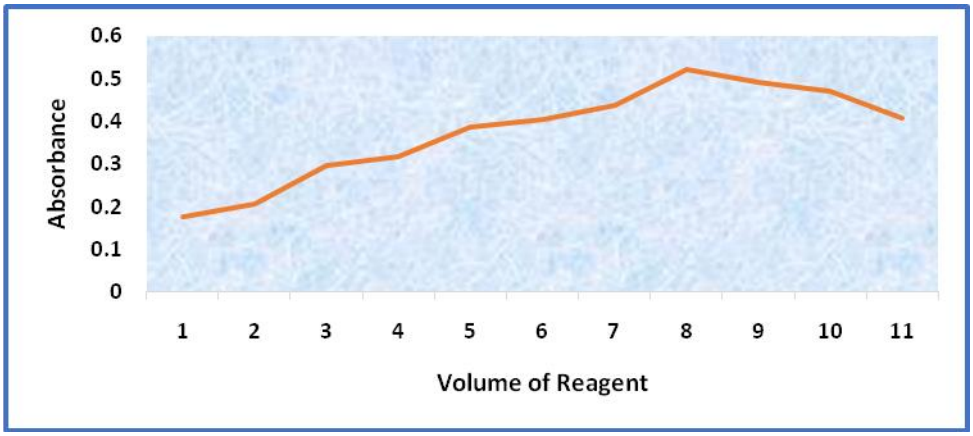

Figure 3-8: Data of Absorbance Optimum Volume of (100 µg ml^{-1}) Reagent

The absorbance increase when increase the volume of reagent but ,suddenly decrease because this is required volume to coupling with drug (11). The optimum volume of reagent [**0.8** ml β-Naphthol with SMX].

Effect of Reaction Time on Stability Color Product

The optimum volumes of parameters are complete [1ml for SMZ, **0.7** ml HCl, **0.4** ml 1% $NaNO_2$,**0.5**ml H_3NSO_3, **0.8** ml β-Naphthol and **0.8** ml Na_2CO_3]. The time on stability color of product one of the important factors to cloud point and diazotization , as well as , we needed to study time (0-60) min. ,then absorbance is measured and fixed the higher absorbance at maximum wavelength show in table (1-8) .

Table 1-8: Data of Absorbance of Reaction Time on Stability Color Product.

Volume of (100 µg ml^{-1}) Reagent	Absorbance at λmax =473 for SDMS	Absorbance at λmax =484 for SMX	Absorbance at λmax =400 for CFX
0	0.297	35	0.602
5	0.308	40	0.613
10	0.359	45	0.618

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15	0.406	50	0.603
20	0.467	55	0.596
25	0.511	60	0.587
30	0.533	65	0.537

Scheming of the absorbance values against the time of reaction (0- 65) min is displayed in figure (1-9).

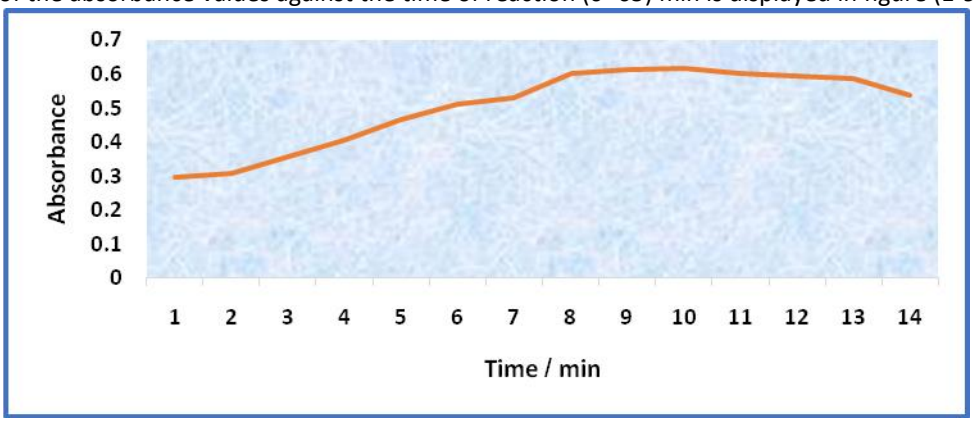

Figure 1-9: Effect of Reaction Time on Stability Color Product

This clear the time of product remain stable for SMX is 45 min display in table (1-8) and figure (1-9).

Effect of Order Addition

It has been taken for the sequence of addition with optimum volume but different addition.

Table 1-9: Effect of Order Addition

No	Addition	Absorbance at λmax =484 for SMX
1	**R+H+N+S+D+B**	0.213
2	**D+H+N+S+R+B**	0.314
3	**D+H+N+B+R+S**	0.643
4	**D+B+R+N+H+S**	0.044
5	**R+B+D+H+N+S**	0.288
6	**R+H+N+B+D+S**	0.100

D= Drug (SMX) , H= acid (HCl) , N= $NaNO_2$,S=H_3NSO_3 , B = Base (Na_2CO_3) , R= Reagent (β- Naphthol).
Scheming of the absorbance values against Effect of Order Addition is displayed in figure (1-10).

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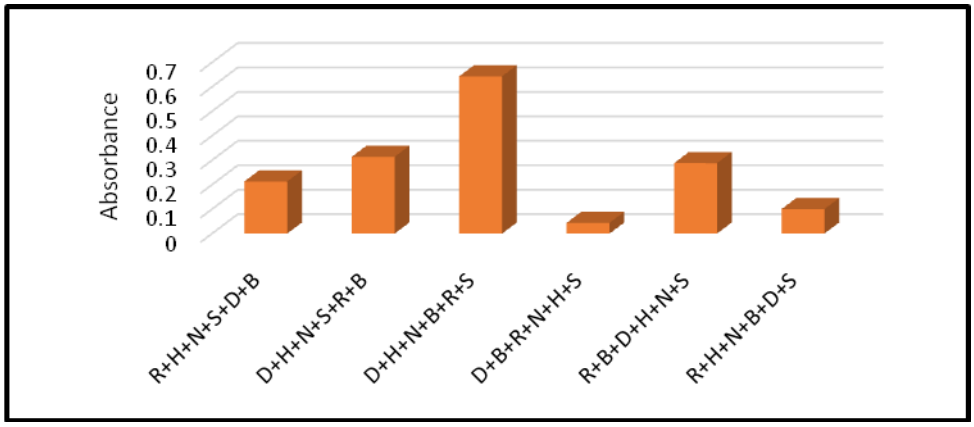

Figure 1-10: Effect of Order Addition

At the maximum wavelength the absorbance is measured, and fixed the higher absorbance for the best order addition display in table (1-9) and figure (1-10).

Effect of Solvents

All additions of diazotization and coupling reaction are added with optimum condition . Then followed diluted by different polar solvent [water , ethanol , methanol ,1- propanol, acetonitrile & acetone] in volumetric flask 10 ml ,at maximum wavelength for each drug the absorbance are measured and recorded for the best solvent . The effect of absorbance show in table (1-10).

Table 1-10: Data of Absorbance to Solvents

Solvent	Water	Ethanol	Methanol	Acetonitrile	1-Propanol	Acetone
Absorbance	0.692	0.414	0.435	0.365	0.201	0.114

Scheming of the absorbance values against Effect of Solvents is displayed in figure (1-11).

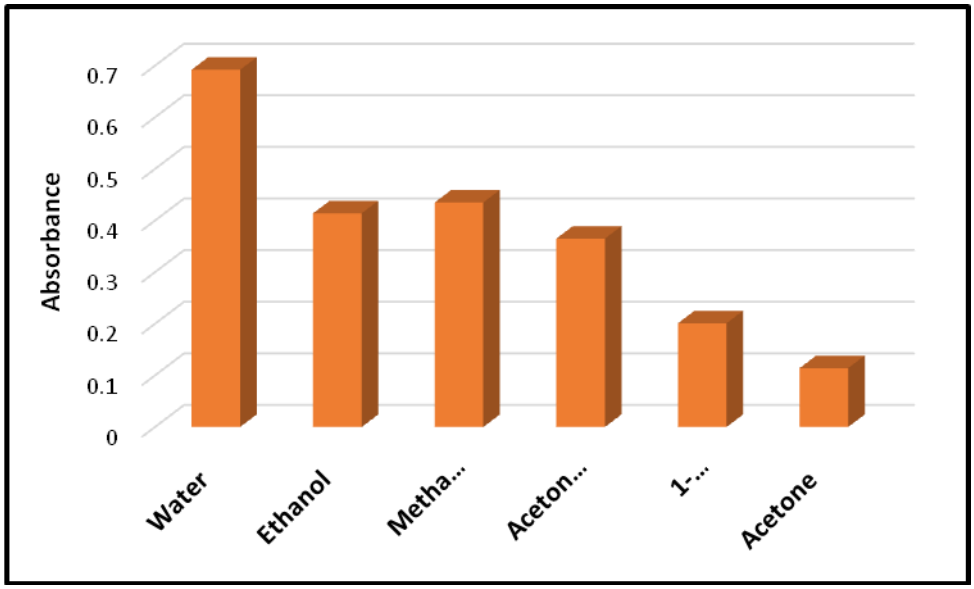

Figure 1-11: Effect of Order Addition

In this study show the best of solvent is water to SMX . The water & ethanol is sensitive ,cheap , economically and nontoxic(12) show in table (1-10) and figure (1-11).

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Effect Temperature in the Formation of Color Product and Stabilization

The conclusion of different temperature on color product has been studied from (5-60)C^0 . And the rest of adding are optimal settings then dilution with distill water in volumetric flask 10 ml . Then absorbance are measured at the maximum wavelength.

Table 1-11: Data of Absorbance to Temperature in the Formation of Color Product and Stabilization

Time	5	15	20	30	40	50	60
Absorbance	0.689	0.693	0.675	0.653	0.604	0.591	0.582

Scheming of the absorbance values against Effect of Solvents is displayed in figure (1-12).

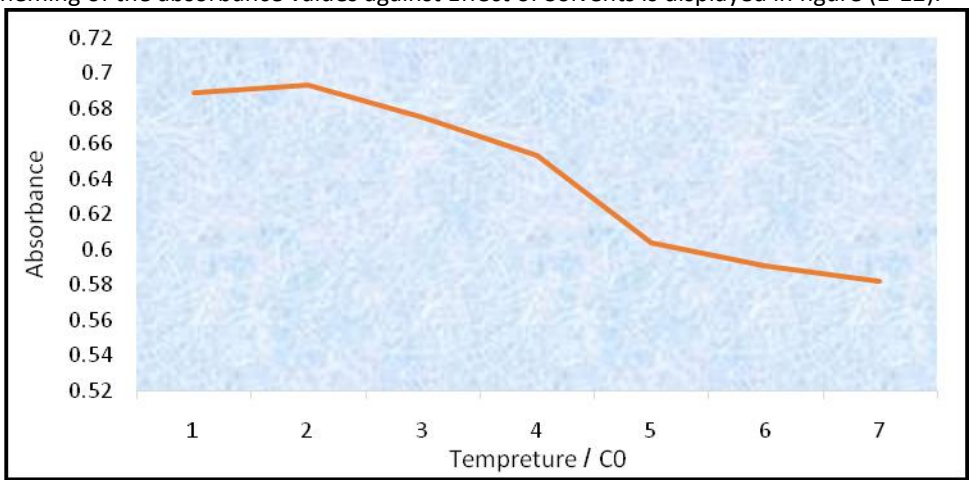

Figure 1-12: Effect Temperature in the Formation of Color Product and Stabilization.

It's clear that at temperature (15C^0) is the greatest absorbency for SMX , on the other hand when temperature rises the absorbency starts lessening suggestion dissociation of product and can be notice from strength of color. The results are in arrangement with literatures (13) ,and this temperature is stable in later experiment.

Stoichiometric Determination of Product

Continuous Variation Method (14)

A series of different volumes of reagent and drug are prepared (0.1-0.9) ml ,with concentration ($4x10^{-4}$ M) in volumetric flask 10 ml .The additions are optimal condition and complete the volume by distilled water (10). Then absorbance are measured by UV-VIS at λmax =484 nm . the stoichiometric ratio between reagent[R] and drug[D] result 1:1 show in table (1-12) and figure (1-13).

Table 1-12: Data of absorbance for Continuous Variation Method Result for SMX

Volume of Drug/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Volume of Reagent/ ml	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
Abs.	**0.039**	**0.090**	**0.132**	**0.170**	**0.203**	**0.185**	**0.155**	**0.114**	**0.070**

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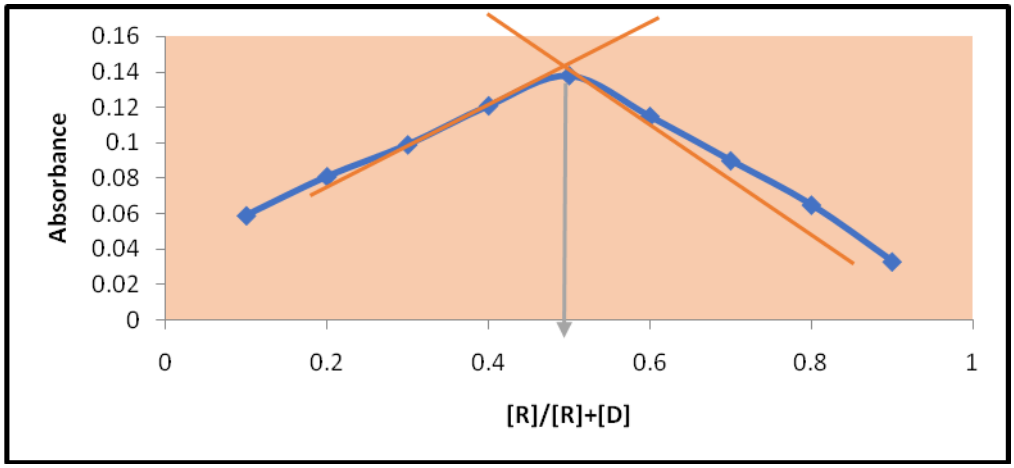

Figure 1-13: Continuous Variation method of SMX

Mole Ratio Method

In this method the volume of drug is fixed at I ml with concentration ($4x10^{-4}$M) and the volume of reagent is change (0.5-4.5 ml) . The optimum of addition is complete by distill water in volumetric flask 10 ml and the absorbance is measured by UV-VIS at λmax =484 nm. The stoichiometric ratio between reagent[R] and drug[D] result 1:1 show in table(1-13) and figure (1-14).

Table 1-13: Data of Absorbance Mole Ratio Method.

Volume of Reagent/ ml	0.5	1	1.5	2	2.5	3	3.5	4	4.5
Abs.	0.151	0.333	0.399	0.401	0.410	0.415	0.425	0.427	0.433

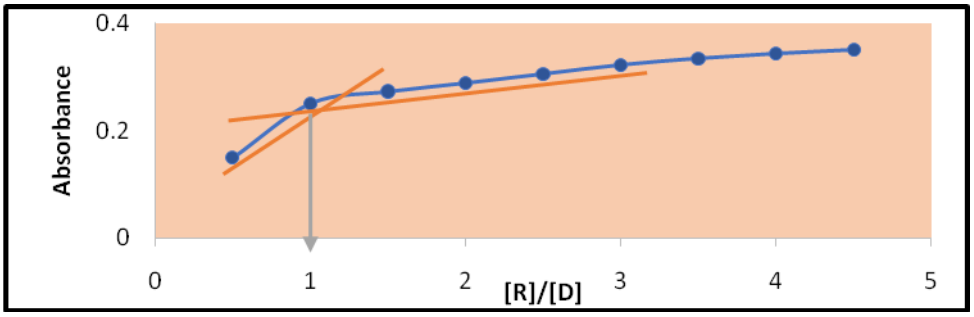

Figure 1-14: Mole Ratio Methodof SMX

The structure of complex between SMX and β-Naphthol shown in figure (1-14)

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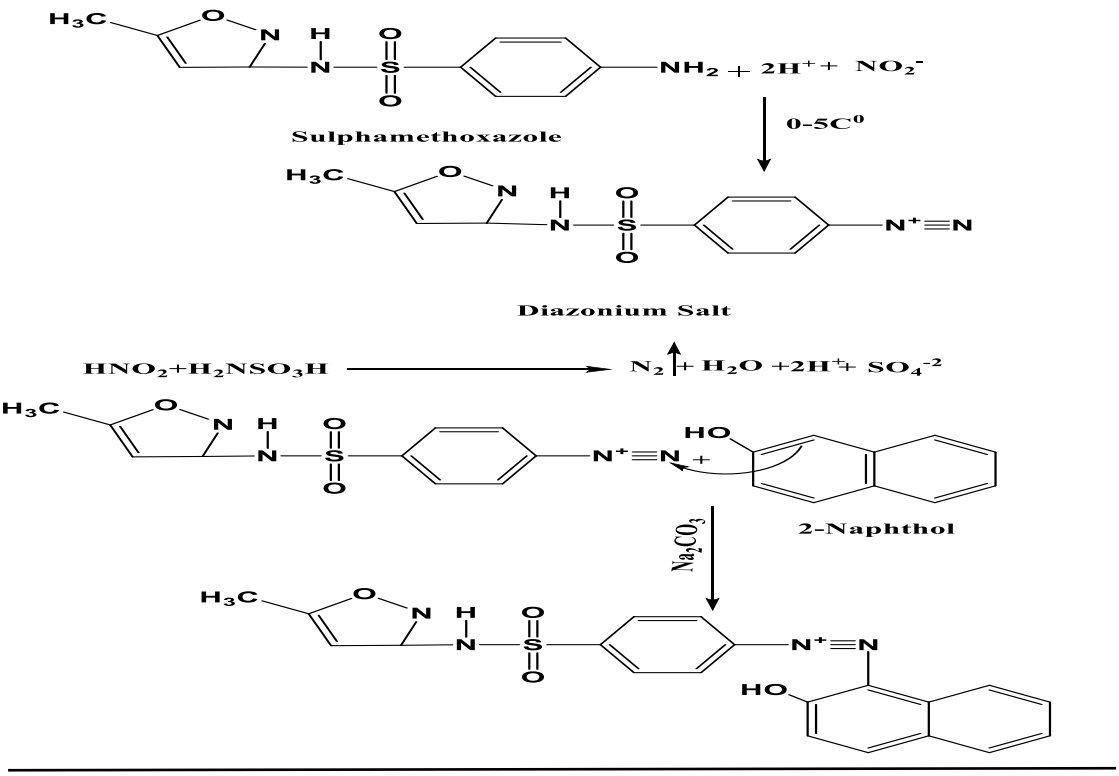

Figure 1-14: Structure of SMX and β-Naphthol

Calibration Curve for complex of SMX - β-Naphthol

In this study the solution are prepared in volumetric flask 10 ml continue of different volume of SMX (1-12) µgmL^{-1} by taken [varying volume of SMX(0.1-1.8 ml) with concentration (1-18 µg ml^{-1}) , **0.7** ml HCl, **0.4** ml 1% $NaNO_2$, **0.8** ml Na_2CO_3, **0.8** ml β-Naphthol and **0.5**ml H_3NSO_3] . The complete the volume by distill water and measured the absorbance by UV-VIS at maximum wave length against a blank solution prepared same condition without drug . Linear calibration graph is established by blotting absorbance against concentration of SMX ,it found (1-18) µgml^{-1} obeys the Bear Law . The molar absorption coefficient of product equals (10.738 x10^{3}L.mol^{-1}.cm^{-1}) and sandal's sensitivity (0.02358 µgmL^{-2}). The result show in figure (1-15).

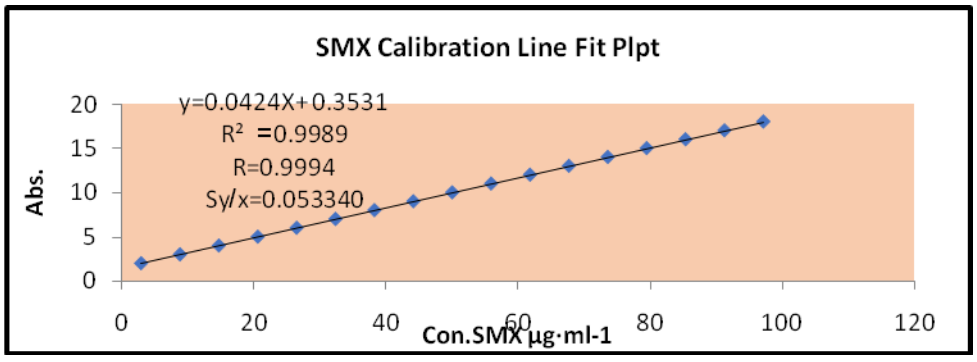

Figure 1-15: Calibration Graph of SMX.

Effect of interference

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In this study effect of interference expected present with SMX by added 1ml (1000 ppm), and the rest of optimum addition in volumetric flask 10 ml and complete by distill water. Then measured the absorbance by UV-VIS.

Table 1-14: Data of Absorbance of interference

NO.	100ppm interference	Abs.	Recovery %	E_{rel}%
1	Lactose	0.735	90.28	-9.72
2	Starch	0.736	90.25	-9.75
3	Arabic Gum	0.741	91.674	-8.326
4	Glucose	0.752	94.27	-5.73
5	Talc	0.756	95.08	-4.92
6	$Ca_3(PO_4)_2$	0.772	98.93	-1.07
7	$CaCl_3$	0.762	96.63	-3.37
8	$FeCl_3$	0.765	97.21	-2.78
9	$CoCl_2$	0.738	90.79	-9.21
10	$CaCl_2$	0.745	92.46	-7.54
11	$NiCl_2$	0.749	93.46	-6.53
12	Tri methyprine	0.773	99.03	-0.96

This result show in table (1-14) there is no interaction between interference and SMX (15)

The Stability Constant of Color Product

The stability constant K Show in the table (1-15)

Table 1-15: Data of The Stability Constant of Color Product of SMX.

Volume of $4x10^{-4}$M of SDMS / ml	Final con. SDMS /M	As*	Am*	α	K(L..Mol^{-1})	Mean of K (L.Mol^{-1})
0.3	1.2×10^{-3}	0.471	0.473	0.0422	3.574×10^{3}	3.307×10^{6}
0.5	2×10^{-3}	0.574	0.579	0.0863	3.566×10^{3}	
0.7	2.8×10^{-3}	0.641	0.645	6.2015×10^{-3}	2.1355×10^{4}	

It is clear the stability constant is high ,so the dye formed is very stable.
Am= the high absorbance , As = the few absorbance.

Accuracy and Precision Test

The table (1-16) show the accuracy and precision of SMX ,which study at different concentration (12,9.6,3) . It is clear this result has a good accuracy and precision.

Table 1-16: Accuracy and Precision Test of SMX

Amount of SMX /µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
12	12.0966	100.805	100.6360	0.805	0.636	0.6825
9	9.1108	101.2313		1.2313		0.3471
6	6.1863	103.105		3.105		0.9178
3	2.9221	97.4033		-2.5966		3.4718

*= Average for five determination

Application

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The proposed method applied on (Syrup Bactrim, the manufacture company is [Roche FarmaceuticaQuimica , Amadora, Portugal]) .That contain (200mg) in 5 ml.The result is good and summarized in table (1-17).

Table (1-17): Data of Accuracy and Precision Test

Amount of SMX /µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
12	11.9173	99.3108	99.2269	-0.6891	-0.772	0.4110
9	8.6674	96.304		-3.695		2.0503
6	6.0447	100.745		0.745		0.3490
3	3.0164	100.548		0.5486		0.9547

Second Method: Spectrophotometric determination of Sulphamethoxazole(SMX) with using Cloud Point Extraction Technique

Effect Type of Surfactant with SMX

The surfactant plays an important role in cloud point extraction process .The basic practical depended of micells for extraction .The solution contains [1ml for SMZ, **0.7** ml HCl, **0.4** ml 1% $NaNO_2$, **0.8** ml Na_2CO_3 , **0.8** ml β-Naphtholand **0.5**ml H_3NSO_3]and added 1ml of each surfactant in volumetric flask 10 ml and complete the volume by distilled water.at $60C^O$ for 20 min. then separated by centrifugated at 4000rpm for 15 min. ,that separated and dissolved in 1 ml ethanol and measured by UV-VIS at λmax=484nm and show the result in table(2- 1)

Table 2-1: Data of Absorbance to Type of Surfactant with SMZ.

Addition	Tween 20	Triton X-100	Tween 80	SDS	Triton X-114	CTAP
Absorbance	0.131	0.118	0.102	0.145	0.360	0.226

Scheming the absorbance values of the cloud point against the type of surfactant is displayed in Figure (2-1).

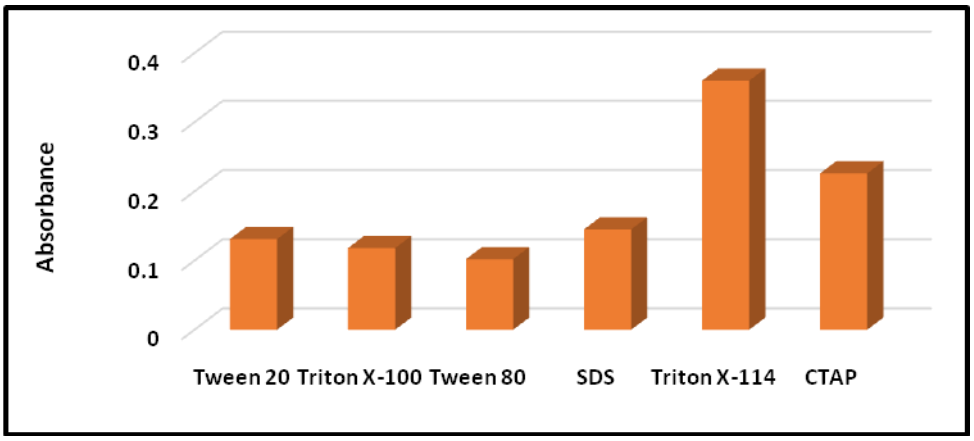

Figure 2-1: Type of Surfactant

It is clear in this result the surfactant Triton X-114 increase the Absorbance and efficiently of cloud point extraction (16) show in figure (2-1).

Effect of Triton X-114 Volume

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Sum of 10 ml solution is primed SMZ[1ml for SMZ, **0.7** ml HCl, **0.4** ml 1% $NaNO_2$, **0.8** ml Na_2CO_3 , **0.8** ml β-Naphthol and **0.5**ml H_3NSO_3] in volumetric flask 10 ml and custom changing volumes of 10% (v/v) Triton X-114 (0.2-2) ml for all drug, then whole the volume by distill water, are heated at 60 C^0 for 20 min to practice cloud point then centrifugation at 4000 rpm for 20 min . The surfactant – opulent phase softened by 1ml ethanol then at maximum wavelength at λmax = 484 nm the absorbance are measured and the best is recorded . This results displayed in table (2-2).

Table 2-2: Data of Absorbance to Triton X-114 Volume with SMZ

Volumeof Triton X-114	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2
Abs.	0.212	0.249	0.254	0.260	0.361	0.376	0.385	0.407	0.391	0.390

Plotting the absorbance values of the cloud point against the volume of Triton X-114 is displayed in Figure (2-1).

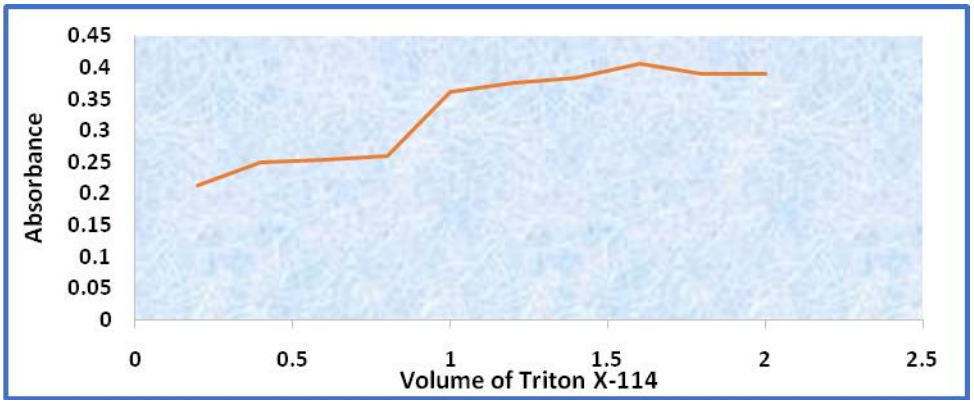

Figure 2-2: Volume of Triton X-114 with SMX Drug.

Most researches approve that the quantity of a nonionic surfactant-type TX-114 as an take out medium effects an central role for exploiting the extraction adeptness by lessening the phase volume ratio (V_s/V_a), therefore improving the pre-concentration factor of the CPE procedure **(17)**.

Effect of Equilibrium Temperature

In series of 10 ml contain [1ml for SMZ, **0.7** ml HCl, **0.4** ml 1% $NaNO_2$, **0.8** ml Na_2CO_3 , **0.8** ml β-Naphthol, **0.5**ml H_3NSO_3 and **1.6** ml 10%(v/v) Triton X-114]in volumetric flask 10 ml ,then complete the mark by Distilled water. The varied temperature (35-70̊) for 20 min. to formed cloud point and separated by centrifugation at 4000rpm for 20 min, 1ml ethanol will be added and measured by UV-VIS at λmax=484nm and recorded

Table 2-3: Data of Absorbance of Varied Temperature

Temperature	35	40	45	50	55	60	65
Absorbance	0.368	0.370	0.396	0.401	0.408	0.402	0.399

Plotting the absorbance values of the cloud point against the temperature is displayed in Figure (2-3).

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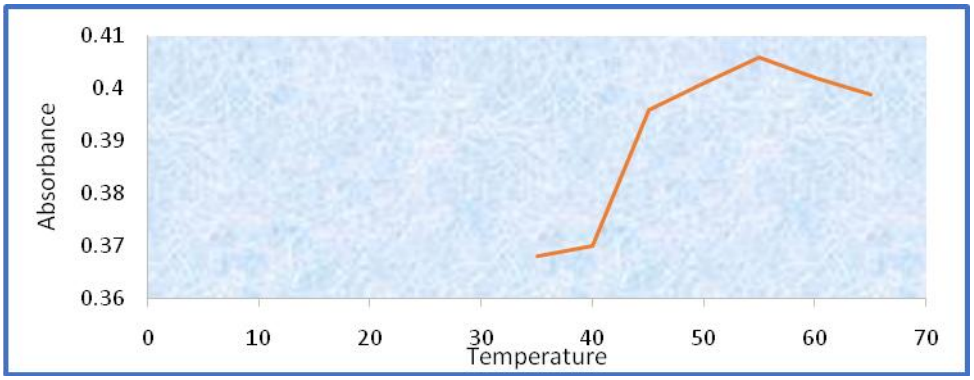

Figure 2-3: Absorbance Versus Temperature for SMX drug.

The result recorded the best Temperature are 55 C^0 show in table (2-3) and figure (2-3).

Effect of Incubation Time

The solution prepared [1ml for SMZ, **0.7** ml HCl, **0.4** ml 1% $NaNO_2$, **0.8** ml Na_2CO_3 , **0.8** ml β-Naphthol, **0.5**ml H_3NSO_3 and **1.6** ml 10%(v/v) Triton X-114]in volumetric flask 10 ml and complete the volume by distilled water, at temperature 55c^o and the incubation time for(5-35min) to form cloud point and separated by centrifugation at 4000rpm for 20 min, 1ml ethanol will be added and measured by UV-VIS at λmax=484 nm.

Table 2-4: Data of absorbance of Incubation Time.

Time /min	5	10	15	20	25	30	35
Absorbance	0.271	0.273	0.278	0.283	0.281	0.279	0.278

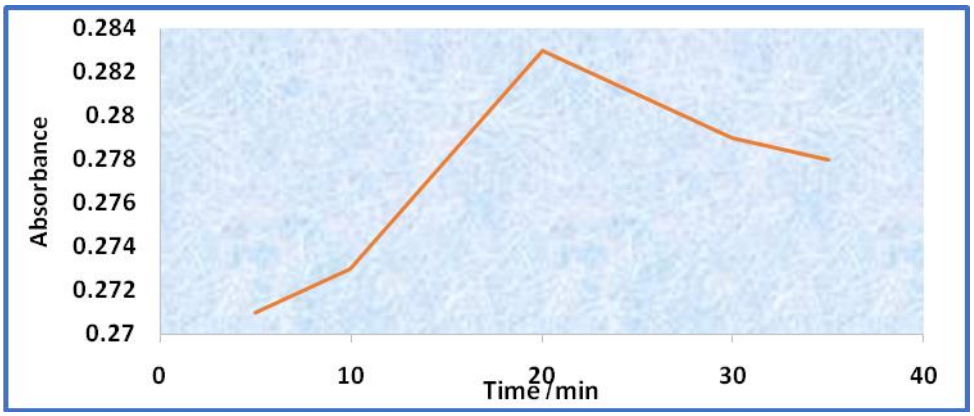

Figure 2-4: Absorbance of different incubation time

The best of incubation time is 20 min (18) . show in the table (2-4) and figure (2-4)

Preparation of Calibration Curve in CPE

The solution prepared increasing concentration (1-18µg mL^{-1}) by taking [changing volume of SMX (0.1-1.8ml) with concentration (1-18 µg ml^{-1}), **0.7** ml HCl, **0.4** ml 1% $NaNO_2$, **0.8** ml Na_2CO_3 , **0.8** ml β-Naphthol, **0.5**ml H_3NSO_3 and **1.6** ml 10%(v/v) Triton X-114] in volumetric flask 10 ml and complete the volume by distilled water, at temperature 55c^o and the incubation time for(20min) to form cloud point and separated by centrifugation at 4000rpm for 20 min, 1ml ethanol will be added and measured by UV-VIS at λmax=484nm and show the result in figure (2-5).

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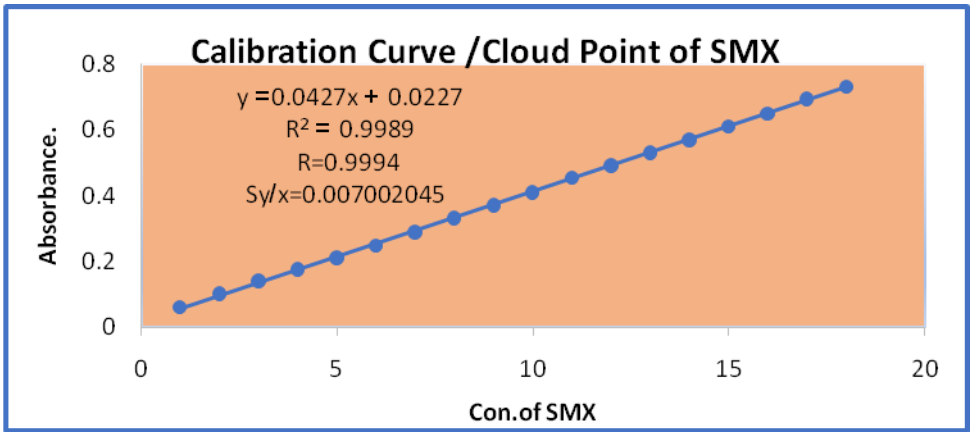

Figure 2-5: Calibration Curve AZO + Cloud Point of SMX

Accuracy and Precision Test

The table 21 show the accuracy and precision for SMX with CPE, which study at different concentration (12,9.6,3) . It is clear this result has a good accuracy and precision show in table (2-5).

Table 2-5: Data of Accuracy and Precision Test

Amount of SMX /µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
12	12.0179	100.149	102.3839	0.1491	2.3839	0.4157
9	9.0795	100.8833		0.8833		1.1541
6	6.0436	100.7266		0.7266		3.4091
3	3.2333	107.7766		7.7766		2.2698

Application

The proposed method applied on (Bactrim, the manufacture company is [Roche FarmaceuticaQuimica , Amadora , Portugal] syrup (Bactrim) that contains (200mg) in 5 ml .The result is good and summarized in table (2-6).

Table 2-6: Data of Accuracy and Precision Test

Amount of SMX /µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
12	12.4845	104.0375	107.3796	4.0375	7.3803	0.2343
9	9.1513	101.6811		1.6811		0.3192
6	6.0589	100.98		0.9826		2.7587
3	3.6846	122.82		22.82		3.2848

Comparison of the AZO-Coupling with Cloud Point Extraction Methods for SMX

The final result of the AZO-Coupling with Cloud Point Extraction for SDMS Methods show in table (2-7)

Parameter	AZO-Coupling Method	CPE Method
Color of Product	Orange	Red
λ max	484 nm	484
Regression equation	y=0.0424X+ 0.3531	y =0.0427x + 0.0227

Standard deviation of regression	0.007782	0.007002045
Correlation coefficient (r)	0.9994	0.9994
C.L for slop ($b \pm tS_b$) at 99%	0.0424 ± 0.00103	0.0427±0.000929
C.L for Intercept ($b \pm tS_a$) at 99%	0.3531 ± 0.01118	0.0227±0.01006
Concentration range (µg ml^{-1})	1-18 µg ml^{-1}	1-18
Limit of Detection (µg ml^{-1})	0.1189	0.24436
Limit of Quantitative (µg ml^{-1})	0.023799	0.38708
Sandell's Sensitivity (µg ml^{-1})	0.02358	0.02341
Molar absorbance ($L.mol^{-1}.cm^{-1}$)	$10.738X10^3$	10.814×10^3
Composition of product	1:1	1:1
Recovery %	96.304 – 100.745	100.149-107.776
RSD%	0.3471– 3.4718	0.4157-3.4091
C.L for con.12(µg ml^{-1})	12.0966±0.1699	12.0179±0.1028
C.L for con.9(µg ml^{-1})	9.1108±0.06512	9.0795±0.2157
C.L for con.6(µg ml^{-1})	6.1863±0.1169	6.0436±0.4242
C.L for con.3(µg ml^{-1})	2.9221±0.2088	3.233±0.1511
Enrichment Factor		100.7075

CONCLUSION

Cloud point extraction is demeans, cool, safe and useful pre-concentration technique to determine Sulphamethoxazole by UV/VIS. In planned method is a kindliness, selectivity and gave a good RSD and low limit of detection.

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