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Determination of Sulfacetamide Sodium in Pure and Their Pharmaceutical Formulations by Using Cloud Point Extraction Method

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Abstract.

In this study, simple, low cost, precise and speed spectrophotometric methods development for evaluation of sulfacetamide sodium are described. The primary approach contains conversion of sulfacetamide sodium to diazonium salt followed by a reaction with p-cresol as a reagent in the alkaline media. The colored product has an orange colour with absorbance at λmax 450 nm. At the concentration range of (5.0-100 µg.mL^{-1}), the Beeŕ s Low is obeyed with correlation coefficient (R^2= 0.9996), limit of detection as 0.2142 µg.mL^{-1}, limit of quantification as 0.707 µg.mL^{-1} and molar absorptivity as 1488.249 L.mol^{-1}.cm^{-1}. The other approach, cloud point extraction was utilized to an estimation of a trace amount of the colored product in the previous procedure followed by a measuring process with a UV-Vis spectrophotometer. The linearity of the calibration graph was above the range of (1.0-60 µg.mL^{-1}), the correlation coefficient (R^2= 0.9991) and molar absorptivity was 7417.622 L.mol^{-1}.cm^{-1}. The detection limit(LOD) and quantification limit(LOQ) were based to be 0.070 and 0.231 µg.mL^{-1} , respectively. This approach was successfully employed for sulfacetamide sodium detection within the pure and pharmaceutical formulation.

Key words: Cloud point extraction, Determination, Ecological – friendly, Spectrophotometry, Sulfacetamide sodium.

Introduction:

Sulfacetamide sodium (SAC) is sodium acety l [(4-aminophenyl) sulfonyl] azanide (Fig.1) (1). It is utilized as an antibacterial agent for the treatment of conjunctivitis and ophthalmic infections. It has high activity for topical use and so it is utilized also for the treatment of acne. It is an individual corticoids group. It is utilized to decrease swelling, redness and allergy, which has an effect upon the eyes. It is additionally utilized for the treatment of a wide range of outer eye inflammations related to some infections (2). Various analytical procedures have been applied for the evaluation of SAC in its biological fluids, pharmaceutical formulations and water samples. They contain UV-visible spectrophotometry (3-6), capillary electrophoresis (7,8), voltammetry (9), liquid chromatography (10-12). spectrofluorimetric (13,14), TLC (15), enthalpimmetric (13) and HPLC with fluorescence detection(15). The cloud point extraction has a great importance due to safety , speed and low cost , consequently it has applied as one of the evaluation and pre-concentration techniques in analytical chemistry(16-20). In this work, the proposed technique is based totally on the azo coupling reaction of SAC with para-cresol to form an orange solution, then on the estimation and pre-concentration the usage cloud point extraction (CPE) which suggests an absorbance at 450 nm. The aim of the current study is to estimate and to find the optimal conditions for estimating the SAC medication in two methods: first through the azo coupling reaction with para-cresol at the maximum wavelength of 450 nm and the second method is the extraction by cloud point using Triton-X-100 as a surfactant, and then comparing the two methods.

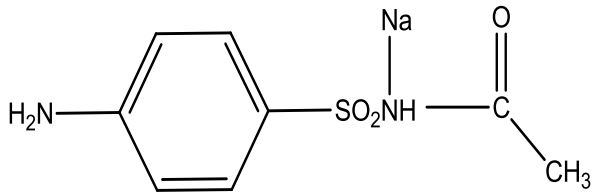


Figure 1. Sulfacetamide sodium Structure.

Materials and Methods:

UV-V is spectrophotometer (160) single beam which was employed for all spectral and absorption intensity measurements with utilized 1 cm quartz cells. The reagents and chemicals substances had been of analytical grade. However, SAC was purchased from Samarra Drug Company (SDI) and p-cresol from Merck Company. A stock P- cresol solution (1000 µg/ mL) was prepared by dissolving (0.1 g) of P-cresol in distilled water and diluted in the volumetric flask (100 mL) to the mark. Stock solution of SAC (1000 µg/ mL) was prepared by dissolving (0.1 g) in distilled water and diluted in a volumetric flask (100 mL) to the mark. Then the other materials were prepared in the following percentages (25%) sodium hydroxide, (10 %) of TritonX-100, (4%) of urea, (1%) of $NaNO_2$ and hydrochloric acid (50 %) by dissolving (25g, 10g, 4 g, 1g and 50ml) respectively in distilled water and diluted in a volumetric flask (100 mL)

A general method of diazotization.

The excellent technique was to develop synthesis azo coupling by putting (1mL) of SAC 1000 µg/mL in the volumetric flask (10 mL) immersed in an ice bath 0-5 ^{0}C, adding (1mL) of hydrochloric acid (50 %) and gradually adding (1mL) of sodium nitrite(1%) and then waiting for 20 min, as well as, adding (1mL) of urea solution (4%) then stir the mixture to remove the excess of nitrite (21)followed by adding (1 mL) of P-cresol 1000 µg/mL. Finally adding (1mL) of sodium hydroxide (25%) and diluting this mixture to (10 mL) by D.W. The azo dye solution has an orange color that has an absorbance at λ_{max} 450 nm.

Cloud point extraction procedure of sulfacetamide sodium.

Various concentrations ranging from (1.0-60 µg.mL^{-1}) of azo dye of formed SAC were put in 10 mL centrifuge tubes , then (1.8 mL) of Triton X - 100(10% v/v) was added and completed using D.W to the mark. Solutions were put in the water bath for 30 min at 80 ^{0}C .The obtained solutions were centrifuged for 1 min at 2000 rpm and the solutions were cooled in an ice bath for 20 min. The surfactant rich-phase was removed and (1 mL) of ethanol was added to dissolve the micellar phase and transferred into quartz cell to measure its absorption intensity at 450 nm.

Method of pharmaceutical formulations.

Sulfacetamide sodium drops provided from cooper(Union) (each ml contains: 100mg Sulfacetamide sodium monohydrate) 50µL was taken in 50 ml volumetric flask and complete to the mark of Distilled water to prepare 1000 µg/mL
Ocusul (Egypt) 10% (10 ml contains: 1mg Sulfacetamide sodium) 500 µL was taken to prepare the solution of sulfacetamide sodium 1000 µg/mL in the 50 ml volumetric flask and complete to the mark of D.W.

Results and Discussion:

The fundamental study shows the diazotization reaction of SAC with HNO_2 and coupling with p-cresol as a reagentto producing an orange color mixture at λ_{max} (450 nm) in the present NaOH solution. The absorption spectra of orange dye against the blank is appeared in Fig.2.

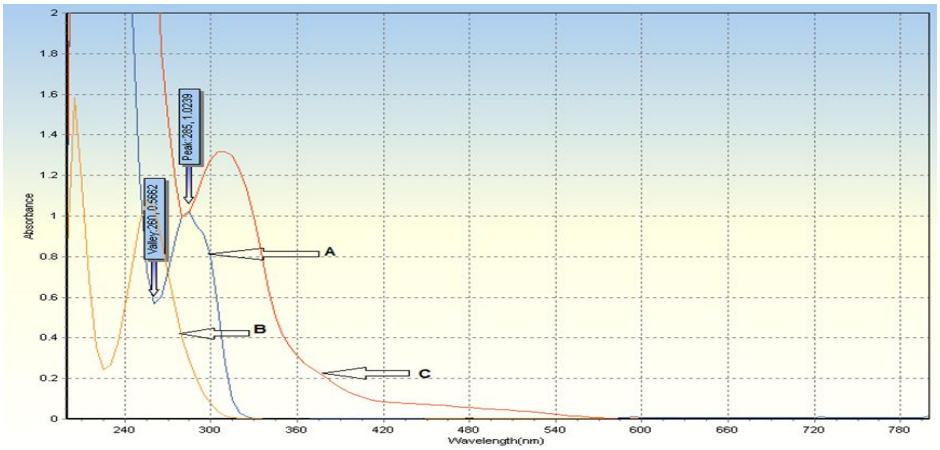


Figure 2. Uv-Visible spectrum of sulfacetamide sodium

A: Reagent (P-Cresol 1000ppm). ,B: Drug (sulfacetamide sodium 1000 pppm), C: New Compound (Sulfacetamide sodiume &P-cresol), againt ablank Prapered under the same conditions without drug.

Investigation of optimization reaction of diazonium salt.

Different parameter influenced the absorption intensity of colored azo product for example, kind and volume of acid, sodium nitrite volume, reagent volume and sodium hydroxide volume. The influence of various acids (HCl, $H2SO_4$, HNO_3 and CH3COOH)(50%) was investigated for the synthesis of diazonium salt and the results were observed as in Table 1. The better volume of 50% hydrochloric acid was (0.2 mL) as shown in Fig. 3.

Table 1. Effect of acid type

Type of acid	HCl	H_2SO_4	HNO_3	CH_3COOH
Abs.λ_{max} 450 nm	0.2041	-	0.1527	0.1865

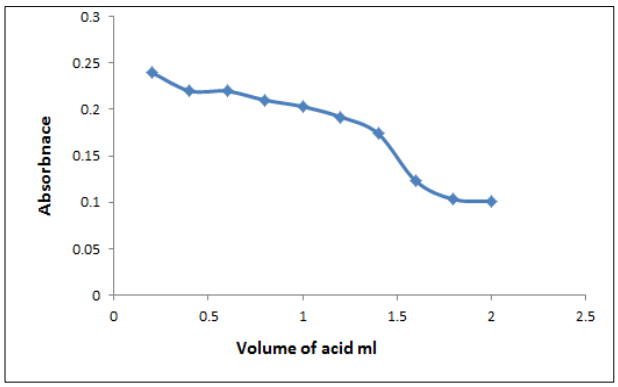


Figure 3. Effect volume of acid

The influence of the amount of sodium nitrite was investigated by changing the volumes of $NaNO_2$ solution utilized from (0.2-2.0 mL) in the diazotization procedure and founded that 1.0 mL gave the batter absorbance as shown in Fig. 4.

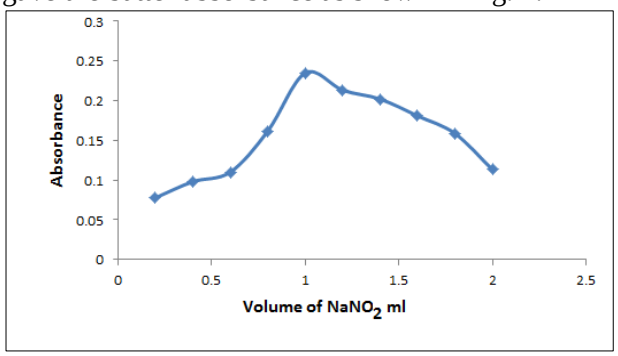


Figure 4. Effect of 1% sodium nitrite

To evacuate the excess of HNO_2, a series of different volume of (4%) urea from (0.2-2.0 mL) was utilized; the results showed 1 mL is sufficient to evacuate the excess of acid as in Fig. 5.

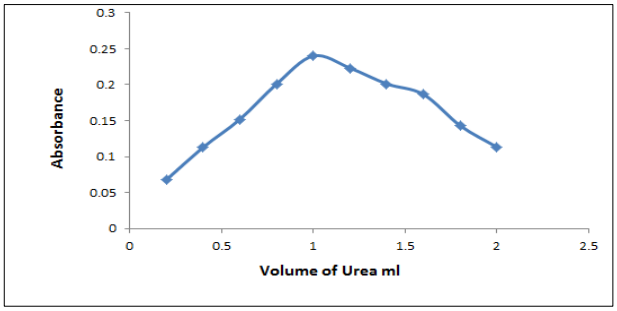


Figure 5. Effect volume of urea (4%)

The influence of various bases on the reaction of synthesis of the azo compound, a (25%) of KOH, NaOH and NH_4OH was examined. The results show the better base was sodium hydroxide as showed in Table 2. Various volumes of (25% NaOH) from 0.2 to 2.0 mL were investigated, the greatest absorbance was observed by the adding (0.2 mL) of sodium hydroxide as shown in Fig. 6

Table 2. Effect of bases type

Type of bases	NaOH	KOH	NH_4OH
Abs λ_{max} 450 nm	0.2499	0.0906	0.1345

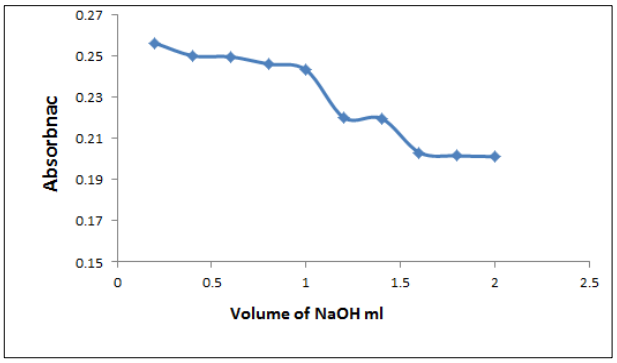


Figure 6. Effect of NaOH volume

Figure 7 shows that 1.8 mL of P-cresol reagent gave high absorbance at λ_{max}(450 nm). It is worth noting that the best addition sequence for the reactants we obtained and the greatest absorbance value was formed with a high sensitivity recorded in Table 3.

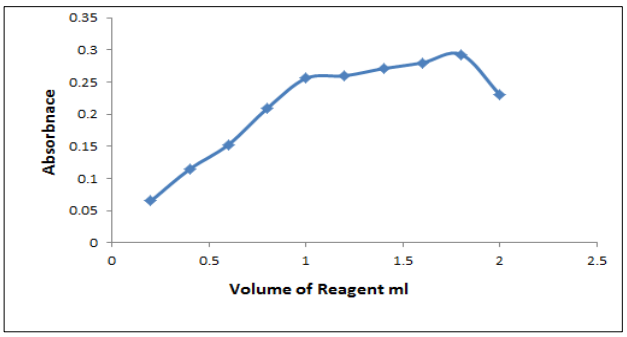
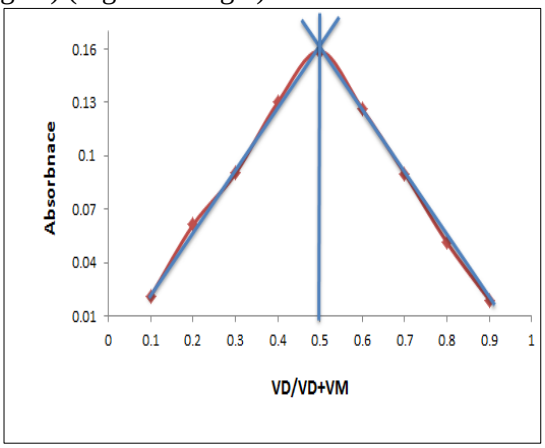


Figure 7. Effect of reagent volume

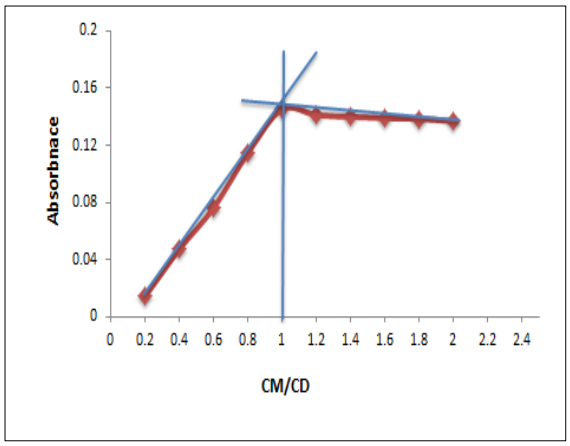
Table 3. Effect of addition sequence on absorbance of azo dye

No.	Order Additions	Abs.
1	Salt+ Reagent+Base	0.2927
2	Salt+Base+Reagent	0.1031
3	Salt+(Reagent+Base)	0.3295

The continuous variation and mole ratio methods were achieved to assess the stoichiometry of SAC : p-cresol ratio. The results indicated that the ratio of **SAC** – p-cresol is equal to 1:1 (drug: reagent) (Fig.8 and Fig.9).



Figurer 8. Mole-ratio method of SAC –Reagent



Figurer 9. Continuous variation method of SAC -Reagent

Calibration Curve

Under the optimized conditions established by a spectrophotometric determination for the estimation of SAC, linear calibration curve was established by plotting concentration versus absorbance of SAC (5.0-100 µg. mL^{-1})(Fig.10).

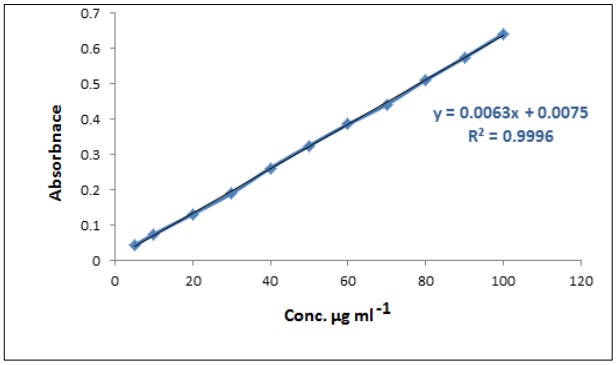


Figure 10. Standard calibration curve of sulfacetamide sodium

Investigation of optimization of cloud point extraction for sulfacetamide sodium.

A series of different volumes of (10%) TX-100 from 0.2 to 2.0 mL to enhance cloud point extraction was examined. The results showed 1.8 mL gave better absorbance as shown in Fig. 11.

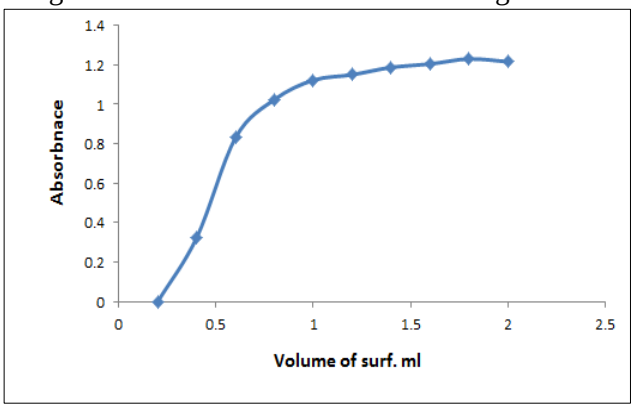


Figure 11. Effect volume of (10% v/v) Triton X-100

The two conditions equilibrium temperature and time incubation were considered as the necessary steps to complete the cloud point extraction in order to enhance effective extraction and pre-concentration of SAC drug. The temperature was varying from 60-90 ^{0}C and the incubation time ranged between 15-30 min. It was indicated that an equilibration temperature of 80 ^{0}C and time of 30 min were chosen in the subsequent steps, and centrifuged by 1 min in 2000 rpm and then, cooling in the 15 min lead to the high recovery of SAC in brief time. After completing the extraction technique (CPE), the aqueous solution was removed by decantation and EtOH was added to the surfactant-rich phase to lower the viscosity of the surfactant-rich phase and ease its transfer into a spectrophotometric cell. 1 mL of ethanol was chosen in the subsequent work.

Analytical data

Under the optimized parameters established by cloud point extraction (CPE) technique for the

evaluation of SAC, a linear calibration curve was established by a plotting concentration of SAC (1.0-60 µg/mL) versus absorbance as shown in Fig 12. Analytical parameter of with and without cloud point are tabulated in Table 4. Where it was found that the method of extracting at the cloud point is an excellent novelty in extracting trace quantities of the SAC drug and it has high enrichment and pre-concentration factors as shown in the Table4.

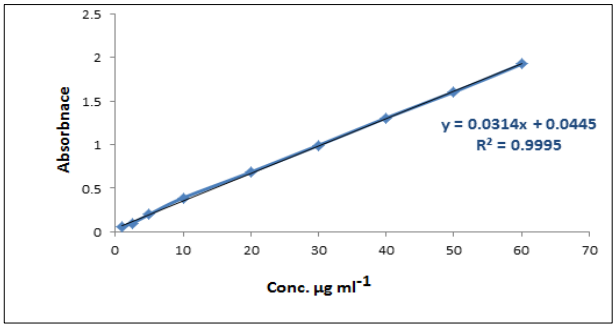


Figure 12. Calibration curve of CPE of sulfacetamide sodium

Table 4. Analytical parameter of cloud point extraction method

Parameters	*Before CPE*	*After CPE*
λ_{max} nm	450	450
Color	Orange	Orange
Regression equation	Y=0.0063X- 0.0075	Y=0.0314X- 0.0445
Linearty range(µg/mL^{-1})	5-100	1-60
Correlation Cofficient (R^2)	0.9996	0.9995
Sandell's sensivity (µg . cm^{-2})	0.1587	0.03
Slope (b)	0.0063	0.0314
Intercept(a)	0.0075	0.0445
Limit of detection(µg/mL^{-1})	0.2142	0.070
Limit of quantification(µg/mL^{-1})	0.707	0.231
C.L.for the slope(b±ts$_b$) at 95%	0.0063 ± 8.5 x10^{-5}	0.0314 ± 6.38x10^{-4}
C.L.for the intercept(a±ts$_a$) at 95%	0.0075 ± 5.2x10^{-3}	0.0445 ± 2x10^{-2}
Standard error for regression line ($S_{y/x}$)	0.004	0.0107
C.L for Conc.20 µg ml^{-1}at 95%	20.09±2.05	20.199 ± 2.882
C.L for Conc.40µg ml^{-1}at 95%	39.58±1.98	39.471 ± 2.114
C.L for Conc.60 µg ml^{-1}at 95%	60.34±2.20	58.986 ± 1.905
Enrich Factor(EF)		498.4
Pre-concentration Factor(PF)		15.384

Accuracy and Precision.

The accuracy was assessed by estimating the percentage, relative error and recovery, while the precision evaluated by the relative standard deviation (RSD%) as recorded in Table 5 for pure material and Table 6 for the application of the proposed CPE on pharmaceutical formulation of SAC.

Table 5. accuracy and precision of suggested procedure for evaluation of sulfacetamide sodium.

Drug	Conc. of drug µg.mL^{-1}		Relative Error%	Recov %	Average Recov%	RSD% (n=3)
	Taken	Found				
	Before cloud point extraction					
	20	20.09	0.45	100.45	99.98	4.106
Sulfacetamide.Na	40	39.58	-1.05	98.95		2.021
	60	60.34	0.566	100.56		1.470
	After Cloud point extraction					
Sulfacetamide.Na	20	20.199	0.995	100.99	99.32	4.757
	40	39.471	-1.32	98.67		2.156
	60	58.986	-1.69	98.31		1.298

Table 6. Application of the proposed CPE for the evaluation of sulfacetamide sodium

drug	Before cloud point extraction					
	Conc. of drug µg/mL^{-1}		Relative	Rec%.	Average	RSD%
	Taken	Found	Error%	%	Rec%	(n=5)
(sulfacetamide sodium/cooper)	20	19.65	-1.75	98.25	99.37	0.452
	40	40.05	0.125	100.125		0.212
	60	59.86	-0.233	99.76		0.053
Ocusul (sulfacetamide sodium)	20	19.58	-2.1	97.9	99.11	0.20
	40	40.18	0.45	100.45		0.28
	60	59.93	-0.11	99.88		0.100
	After cloud point extraction					
(sulfacetamide sodium/cooper)	20	20.32	1.6	101.6	100.62	0.123
	40	40.19	0.475	100.47		0.062
	60	59.88	-0.2	99.8		0.025
Ocusul (sulfacetamide sodium)	20	20.35	1.75	101.75	100.685	0.049
	40	40.21	0.525	100.525		0.037
	60	59.87	-0.21	99.78		0.028

Figure 13 explains the suggested mechanism of diazotization reaction of azo dye of SAC with p- cresol

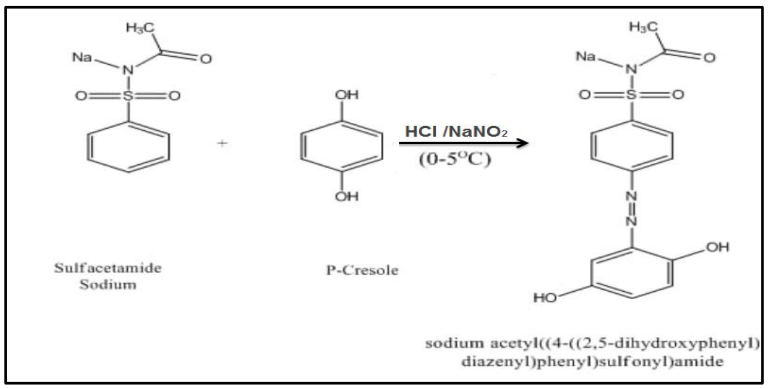


Figure 13. The proposed mechanism of diazotization reaction.

Table 7 showing the comparison of our proposed method for estimating the SAC drug by the cloud point extracting and between several spectral methods with the literature

Table 7. Compassion the values of the CPE method with various methods reported in literature to sulfacetamide sodium determination

Type of method	Reagent	Colour	λ_{max} nm	LOD µg.mL^{-1}	Linear range µg.mL^{-1}	RSD(%)	Ref.
Spectrophotometric	8-hydroxyquinoline	Red	500	0.11	0.1–7.0	0.1	(22)
Spectrophotometric	8-Hydroxy-7-iodoquinoline-5-sulfonic Acid	yallow	490	-	2-28	1.9178	(23)
Derivative UV Spectrophotometry	-	-	258	0.55	0.55-25.4	1.25	(24)
Spectrophotometric	2,6-dihydroxytoluene	Yallow	435	0.036	0.25-12.5	1.25	(25)
CPE method	P-cresol	Orange	450	0.070	1-60	1.298	Present work

Conclusion:

A simple, speed and spectrophotometric technique has been developed for the estimation of trace amount of sulfacetamide sodium with p-cresol. The first technique containing conversation sulfacetamide sodium to azo dye was measured spectrophotometrically. The second technique included determination and pre-concentration of sulfacetamide sodium using cloud point extraction. It was found that our proposed method is highly efficient and highly recoverable and was applied to some pharmaceutical preparations in the local market. Through a comparison with other methods in the literature, it has been found that it is the best

method for easing the application and it is considered environmentally friendly because it does not use organic materials that are harmful to the environment and also has a high linear range.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Anbar.

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تقدير دواء صوديوم سلفاسيتاميد في المادة النقية والمستحضرات الصيدلانية باستخدام الاستخلاص بنقطة الغيمة

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الخلاصة:

في هذه الدراسة تم وصف تطوير طرائق قياس طيفية بسيطة ومنخفضة التكلفة ودقيقة وسريعة لتقدير سلفاسيتاميد الصوديوم.الطريقة الاولى والتي تتضمن تحويل سلفاسيتاميد الصوديوم الى ملح الدايازونيوم ثم التفاعل مع بارا-كريسول ككاشف في الوسط القلوي. المركب الناتج ملون ذو لون برتقالي يمتص عند اعلى قمة امتصاص 450 نانوميتر . عند مدى (5-100ميكروغرام)،حيث يطبق قانون بير لامبرت بمعامل ارتباط (0.9996) وحد الكشف هو 0.2142ميكروغرام مل، حد القياس الكمي هو 0.707ميكروغرام مل والامتصاصية المولية 1488.249 لتر/مول.سم. الطريقة الاخرى، تم استخدام الاستخلاص بنقطة الغيمة لتقدير كمية ضئيلة من المركب الملون في التفاعل السابق متبوعا بالقياس باستخدام مقياس الطيف الضوئي للاشعة فوق البنفسجية. كان خط الرسم البياني لمنحني المعايرة والذي يبدأ من (10-60 ميكروغرام)، وكان معامل الارتباط (0.9991) ومعامل الامتصاص المولي 7417.622 لتر/مول.سم وتم تحديد حد الكشف والحد الكمي ليكونا 0.070 و 0.231 ميكروغرام على التوالي. تم استخدام هذه الطريقة (الاستخلاص بنفقطة الغيمة) بنجاح للكشف عن السلفاسيتاميد الصوديوم داخل المركبات الصيدلانية.

الكلمات المفتاحية: الاستخلاص بنقطة الغيمة، التقدير، صديقة للبيئة، القياس الطيفي الضوئي، سلفاسيتاميد الصوديوم.