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# Spectrophotometric Determination of Sulfacetamide in Pure Form and Pharmaceutical Formulations with Metol and potassium hexacyanoferrate (III)

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## الخلاصة

طريقة بسيطة، عالية الحساسية وذات دقة عالية لتقدير سلفااسيدامايد بشكل نقي او في المستحضرات الصيدلانية من خلال تكوين معقد.

تضمنت الطريقة تفاعل سلفااسيدامايد مع مزيج الميتول وبوتاسيوم سداسي سيانيد الحديديك وتكوين معقد ملون لتقدير سلفااسيدامايد طيفيا عند اقصى طول موجي 540 نانوميتر.

تم تحديد الظروف الفضلى للتفاعل للحصول على اعلى حساسية واطول استقرارية. عند الظروف الفضلى للامتصاصية لمعقد الملون وجد ازدياد بالخطية مع ازدياد تركيز سلفااسيدامايد والموثق من خلال قيمة معامل الارتباط. كان مدى التركيز المستخدم هو 0.1–100 مايكروغرام مل$^{-1}$ وبحدود كشف 0.01842 مايكروغرام مل$^{-1}$ وبانحراف قياسي نسبي0.74%.

طبقت الطريقة المقترحة بدقة وضبط عالي وبنجاح لتقدير نسبة سلفااسيدامايد بشكل نقي او في المستحضرات الصيدلانية وتم مقارنة النتائج الاحصائية باستخدام اختباري وقد وجد ان ان قيمهما اقل من قيمهما الواردة بالطريقة المستخدمة في تقديره بالدستور البريطاني.

## Abstract:

A simple, sensitive and accurate spectrophotometric method of determination of Sulfacetamide (SAC) in pure form and pharmaceutical formulation.

The method is based on the formation of (SAC) complex. The reaction between of the Sulfacetamide with the mixture of metol and potassium hexacyanoferrate (III) was evaluated for the spectrophotometric determination of the Sulfacetamide. The maximum absorbance of the colored complex occurred at λ=540nm. Reaction conditions have been optimized to obtain (SAC) complex of high sensitivity and longer stability. Under optimum conditions the absorbance of the (SAC) complex where found to increase linearly with increase in concentration of the Sulfacetamide, which corroborated with correlation coefficient value. The concentration ranges are 0.1-100 µg ml$^{-1}$ with detection limit 0.01842µg ml$^{-1}$ and

relative standard deviation 0.74 % and relative error of prediction for drug were lower.

The proposed method was successfully applied to determine of the selected Sulfacetamide in pure form and pharmaceutical formulations with good precision and accuracy compared to standard method as revealed by t- and F- values and the results obtained agree well with the labeled contents.

**Key word:** Sulfacetamide, spectrophotometric, oxidation–reduction reaction.

## Introduction:

Sulfacetamide, *N*-[(4-aminophenyl)sulfonyl]acetamide monosodium salt monohydrate($C_8H_9N_2NaO_3S,H_2O$),) (Fig.1). Sulfonamides are widely used antibiotics in today's human and veterinary medicine practice. Sulfacetamide (SAC) are N-substituted derivatives of sulfanilamide and compete with p-aminobenzoic acid in enzymatic synthesis of dihydrofolic acid. This leads to a decreased synthesis of nucleic acids [1]. SAC have been widely used for both prevention and treatment of diseases and as feed additives to promote growth in animal feeding operations and concentrated animal feeding operations [2].

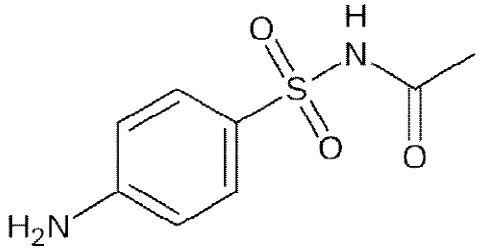



**Fig-1**

There are various analytical procedures for the assay of SAS, Micellar Electrokinetic Capillary Chromatography[3,4], TLC[5], column chromatographic[6], spectrophotometric[7,8], High-performance liquid chromatography (HPLC)[9], HPTLC [10], voltammetry [11], Spectrofluorimetric [12], Enthalpimetric [13], Liquid Chromatography and Spectrophotometric Detection [14], HPLC with Fluorescence Detection [15].

## Materials and Methods:

### Apparatus:

Spectral and absorbance measurements were made on UV 1650 Shimadzu spectrophotometer by using 1 cm quartz cell.

### Materials and Reagents:

All reagents used were analytical grade and water was always double distilled.

**Pure samples:**

Sulfacetamide pure grade was provided by SID- Samara factory.

**Standard stock solution** (preparation 100 ppm)**:**

Stock solutions of Sulfacetamide were prepared by dissolved (0.01g) of standard Sulfacetamide in 5ml ethanol, then transferred into 100 ml volumetric flasks and diluted to the mark with bidistilled water.

**Market samples:**

Sulfacetamide tablets, labeled to contain (20%). They were obtained from commercial sources in the local market.

**Reagents:**

Metol solution (13 and 20mM) were freshly prepared by dissolved (0.225g) and (0.68889g) of metol respectively, and diluting to 100ml with bidistilled water in volumetric flasks. (20mM) potassium hexacyanoferrate (III) were prepared by dissolved (0.332 g) $K_3[Fe(CN)_6]$ and diluting to 100 ml with bidistilled water in volumetric flask.

**Recommended analytical procedure:**

**Method:**

Different aliquots of Sulfacetamide standard stock (100 µg $ml^{-1}$) were transferred into a series of 10 ml volumetric flasks, equivalent to (0.1-10) mg $ml^{-1}$ to each these were added 1ml of buffer (pH= 5) and metol (1ml) and potassium hexacyanoferrate (III) (1ml) were diluted to the mark with bidistilled water. The absorbance was measured at λ=540nm against a reagent blank prepared similarly. A calibration graphs were drawn by plotting the absorbance against the drug concentration.

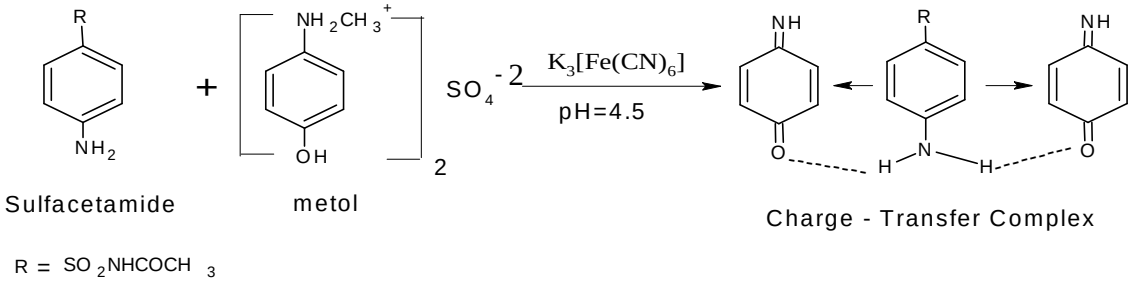
**Analytical of pharmaceutical formulation:**

Ten tablets were accurately weighted and finally powdered. An amount of the powder equivalent (0.01mg) Sulfacetamide was dissolved in 5ml of ethanol and transferred to 100 ml calibrated flask. The contents of the flask were shaken and then make up to the mark with bidistilled water to obtain (100 µg $ml^{-1)}$ of SAC.

## Results and Discussion:

**Absorption spectra:**

Throughout the preliminary investigation on the reaction[15], between drugs (SAC) with metol in the presence of potassium hexa cyanoferrate (III), colored (red purple) products obtained (Scheme-1) with a maximum absorption at λ=540nm (Fig. 2). The absorbance of the colored products measured against reagent blank which has minimum absorbance at the same wavelength from the results obtained, appeared that it is possible to determine a microgram of this drug.




**Reaction scheme**

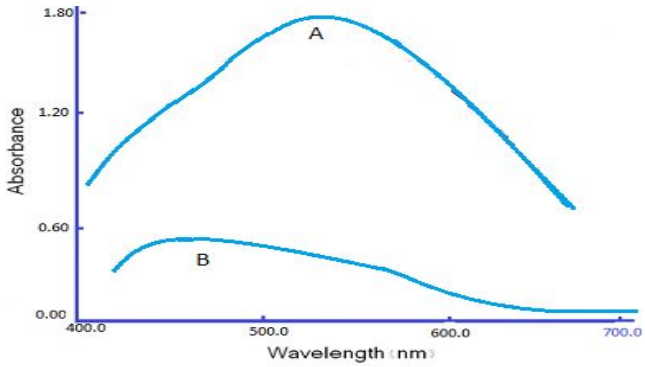



**Fig-2: Absorption spectra of A= drug Sulfacetamide complex,**
B=metol/ potassium hexacyanoferrate (III) reagent

**Optimization of Experimental Conditions:**

The effect of various variables on the color development was tested to establish the optimum conditions for the determination of Sulfacetamide by using metol and potassium hexacyanoferrate (III).

**Effect of pH:**

The optimum pH for complete color development is 5 (Fig 3). The buffer solution is added to give the required pH.

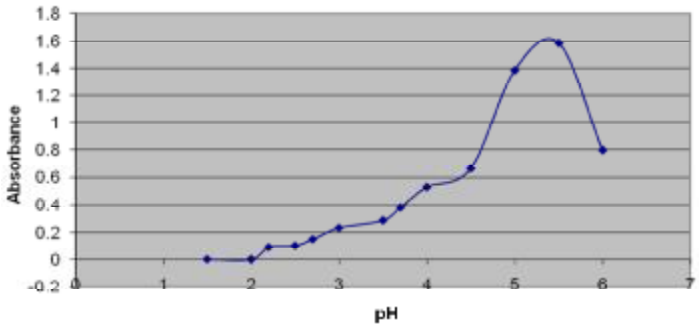



**Fig-3: Optimum pH for complete color development**

**Effect of Concentration of potassium hexacyanoferrate (III):**

The effect of the different volumes of (20mM) of $k_3[Fe(CN)_6]$ solution was examined on the maximum absorbance of the colored product in the presence of (1ml) metol (20mM). Fig.4 shows that 1ml of the solution of potassium hexacyanoferrate (III) was enough to obtain the maximum absorbance.

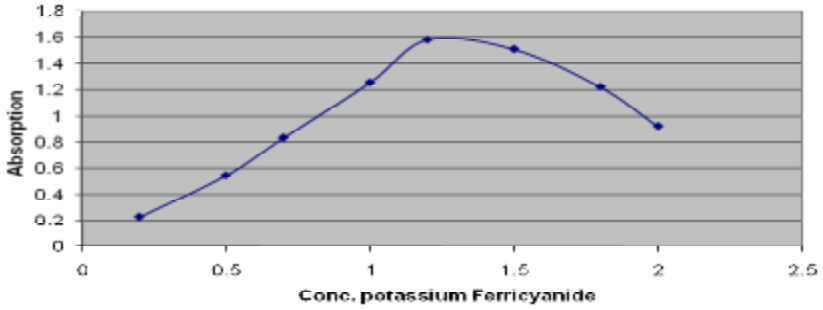



**Fig-4: Effect of Concentration of (20mM) potassium hexacyanoferrate (III) on the absorbance intensity of Sulfacetamide drug at 540nm.**

**Effect of volume of metol reagent:**

Metol was found to be a useful for charge transfers reaction, because it was produced a stable charge transfer complexes rapidly with drugs in presence of potassium hexacyanoferrate (III). More over this reagent is easily obtainable and is soluble in water. The effects of the different volumes of (20mM) metol solution were examined on the maximum formation of the colored product. Fig. 5 shows that (1ml) of the solution was enough to obtain the maximum absorbance, and it was used in the subsequent experiments.

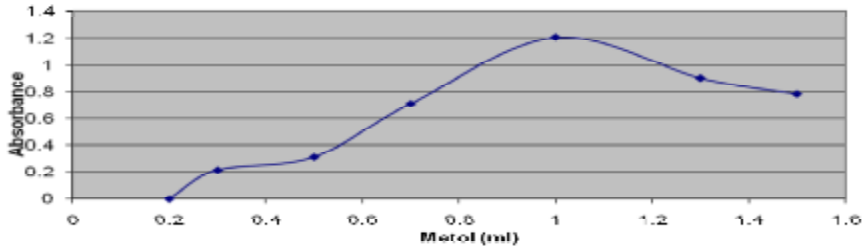



**Fig-5: Effect of volume of metol reagent (20 mM) on the absorbance intensity of Sulfacetamide drug using (1ml) of potassium hexacyanoferrate at 540nm.**

**Effect of addition orders:**

Seven orders of addition were examined as shown below:

| NO. | Addition order | Absorbance (nm) |
|---|---|---|
| 1 | B+D+M+F | 0.230 |
| 2 | M+D+B+F | 0.263 |
| 3 | D+B+M+F | 0.369 |
| 4 | F+M+D+B | 0.535 |
| 5 | D+B+F+M | 0.444 |
| 6 | F+D+B+M | 1.522 |
| 7 | F+M+B+D | 0.689 |

**D= Drug, R= Reagent (metol), O= Oxidant $k_3[Fe(CN)_6]$ , B=buffer**

**Effect of temperature:**

The effect of temperature on the color intensity of the product was studied Fig.6 in practice a maximum absorbance was obtained when the color was developed at room temperature ($25^0$C), but when the color was developed in an ice bath ($5^0$C) or in a water bath($45^0$C) a loss in color intensity and stability were observed. It is therefore recommended that the color reaction should be carried out at room temperature ($25^0$C) after 20min.

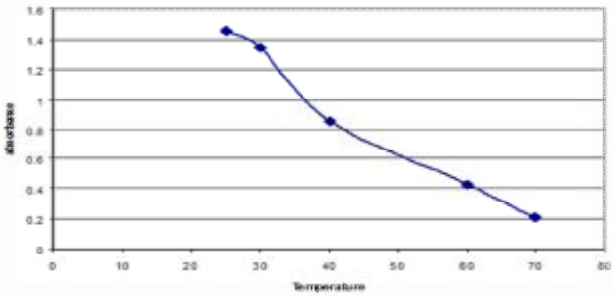


**Fig-6: Effect of Temperature**

**Effect of time on stability:**

The color intensity reached a maximum after drug solution had been reacted immediately with metol and potassium hexacyanoferrate (III) in aqueous medium and became stable after (10min), remained stable for at least (Fig. 7).

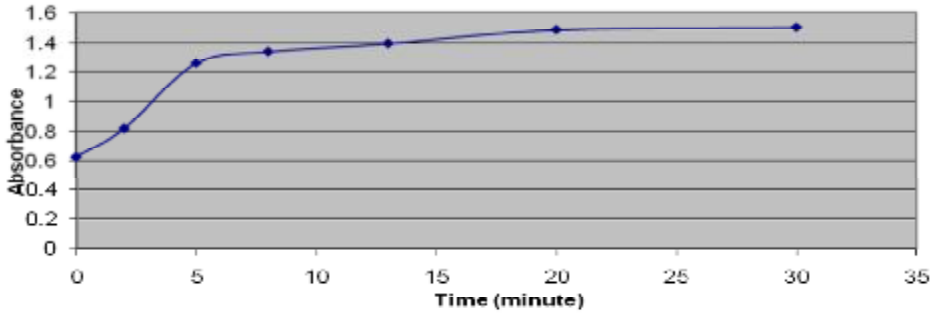



**Fig-7: Effect of time (min) on the absorbance of the formed complexes of Sulfacetamide drug.**

## Calibration Graph:

After the optimizing reaction conditions describe above, calibration curve (Fig.8) for SAC was constructed by plotting absorbance of SAC complex and the concentration of the SAC drug. The calibration curve was linear. The analytical values of statistical treatments for the calibration curve are summarized in Table-1.

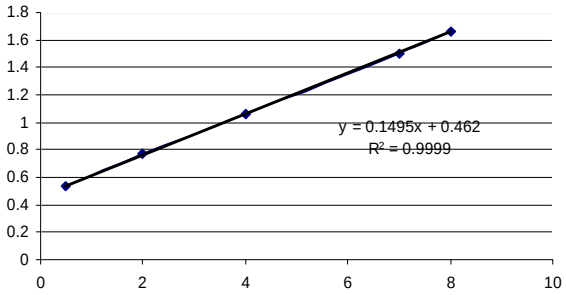



**Fig-8: Calibration curve of SAC**

| **Parameter** | **Value** |
|---|---|
| Correlation coefficient ® | 0.9998 |
| Linearity percentage ($r^2$ %) | 99.98 |
| Test for a significant correlation (t) | 154.906 |
| Regression equation | Y=1.4953+0.463 |
| Slope (ml $\mu g^{-1}$) | 1.4953 |
| Intercept | 0.462 |
| Standard deviation of the residuals,$S_{y/x}$ | 0.00614152329 |
| Standard deviation of the slope, $S_b$ | 0.0096111927685 |
| Standard deviation of intercept Sa | 0.007770778809 |
| Linearity range (µg $ml^{-1}$) | 2-90 |
| Molar absorptivity, ε (l $mol^{-1}$ $cm^{-2)}$ | $3.8X10^{-5}$ |
| Sandell's sensitivity, S(µg $cm^{-2}$) | $6.6875X10^{-4}$ |
| Limit of detection LOD (µg $ml^{-1}$) | 0.01842 |
| Limit of quantification LOQ (µg $ml^{-1}$) | 0.06142 |

**Table-1: Performance data of the proposed method**

## Accuracy and precision:

The accuracy and precision of the determination of SAC were studied depending upon the value percentage of the relative error (E%), recovery (REC%) and relative standard deviation (RSD%) respectively. For five replicates of each concentration of SAC (0.3- 0.75) µg.ml$^{-1}$. The results in table-2 show a good accuracy and precision.

| No. | Conc µg/ml | | E%* | REC%* | RSD%* |
|---|---|---|---|---|---|
| | present | Found | | | |
| 1 | 0.3 | 0.302 | 0.667 | 100.667 | 0.748 |
| 2 | 0.5 | 0.496 | - 0.8 | 99.2 | 0.266 |
| 3 | 0.75 | 0.752 | 0.267 | 100.267 | 0.396 |

**Table-2: Accuracy and precision of the proposed method.**
***Average of five determinations.**

## Stoichiometry of the formed product:

The stoichiometry of the formed product was investigated by mole ratio. Continuous variation (job's method), and slope ratio methods. In the mole ratio method increased volumes of (20 mM) metol were added to a (1ml) of (20mM) SAC in a series of (10ml) volume flasks, followed by 1ml of 20mM potassium hexacyanoferrate (III), the volumes were made up to the mark with distill water, allowed to stand to 15min. and the absorbenc were measured at 540nm versus the reagent blanks. The results were plotted as shown in (Fig.9-10) which indicated the existence of 1:1 metol: SAC

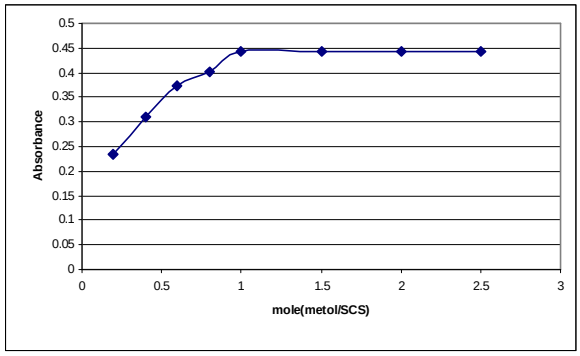



**Fig-9: Mole ratio plots**

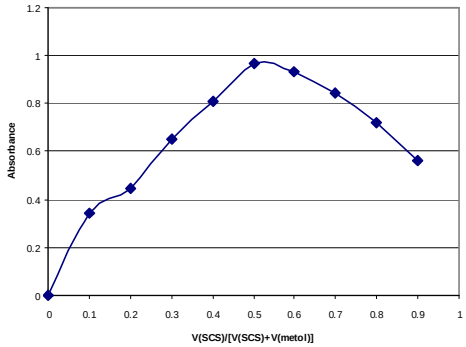



**Fig-10: Continuous variation plots**

The job method was applied by placing 1 to 9ml of 20mM SAC solutions in to a series of 25ml volume flasks; this was followed by placing 1 to 9ml of 20mM reagent metol and 7ml of potassium hexacyanoferrate (III). The solutions were diluted to the mark with distilled water, allowed to stand for 15 min, versus reagent blank. The results were plotted as shown in figure which indicated the existence of 1:1 (metol: SAC)

## Pharmaceutical application:

## Evaluation of the proposed method:

For evaluation, the competence and the success of the proposed method the result obtained were compared with those obtained by British pharmacopeia. The results obtained by the different method stable were statistically compared using the student t- test and variance ration F-test at 95% confidence level in all cases, the calculated t- test and F-values Table-3 did not exceed the theoretical values which indicated that there is no significance difference between either methods in accuracy and precision in determination of SAC in pharmaceutical preparation.

| No. | Proposed method | Standard method | | Value | | | |
|---|---|---|---|---|---|---|---|
| Pharmaceutical preparation | Rec%* Xi | $(X_i - \overline{X})^2$ | Rec% $(Xi)_2$* | $(X_i - \overline{X})^2$ | S** | t (theo.) | F (theo.) |
| SCS pure | 100 | 0.154 | 100 | 0.123 | 0.345 | 0.149 (2.776) | 1.340 |
| Samacetamide Eye Drops (10%) | 99.55 | 0.0028 | 99.28 | 0.006 | | | |
| Samacetamide Eye Drops (20%) | 99.27 | 0.116 | 99.373 | 0.076 | | | |
| | X1=99.61 | $\sum$=0.273 | X2=99.650 | $\sum$=0.204 | $n_1+n_2-2=4$ | $n_1-1=2$ $n_2-1=2$ | |

**Table-3: Comparison of the proposed method with standard method using t- and F- statistical tests.**
*** Average of five determinations, S ** Pooled standard deviation**

## Conclusion:

Despite of the great number of methods described in the literature for analysis of Sulfacetamide, the proposed method for the determination of Sulfacetamide in pharmaceutical samples have the advantage to be simple, sensitive, accurate and inexpensive. The method represented good accuracy and precision so that the respective relative standard deviation and relative error of prediction for drug were lower. The proposed method was applied successfully to analysis of drugs in tablets and thus is very appropriate for routine quality control analysis of drug.

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