

# Colorimetric Estimation of Sulphacetamide Sodium in Bulk and in Formulations

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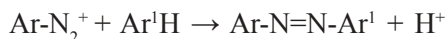
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**A new simple and sensitive colorimetric method has been developed for estimation of sulphacetamide sodium in bulk and in pharmaceutical formulations. The method is based on the formation of a yellow colored azo dye by diazotization of sulphacetamide sodium, followed by a diazo-coupling reaction between the resulting product and acetyl acetone. The azo dye exhibited maximum absorbance at 420 nm with apparent molar absorptivity  $7.8983 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ . Beer's law was found to be obeyed in the concentration range of 2-20  $\mu\text{g/ml}$ . Results of the analysis were validated statistically and by recovery studies.**

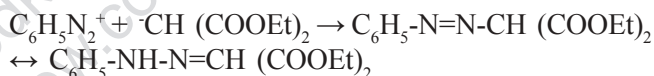
Sulphacetamide sodium is the monohydrate of the sodium salt of *N*<sup>1</sup>-acetylsulfanilamide. Sulphacetamide sodium is a short acting sulfonamide which is used topically for ocular infections<sup>1</sup>. The official method of IP, BP and USP recommends nitrite titration for estimation of sulphacetamide sodium, where the end point is determined by potentiometry<sup>2-4</sup>. The titrimetric method has its own limitations is that, it is very difficult to measure small quantities of drug samples and it is also less sensitive. A number of spectrophotometric methods are reported for estimation of sulphacetamide sodium, but these methods require expensive reagents like dopamine, 3-aminophenol, cerium(IV), iminodibenzyl and primaquine phosphate<sup>5-9</sup> and are not so sensitive and do not have wide applicability, which limit their use. The present work describes a simple, rapid, reliable and sensitive spectrophotometric method for estimation of sulphacetamide sodium in bulk and in pharmaceutical formulations.

A Systronic model 119 UV/Vis spectrophotometer with 1 cm matched quartz cell was used for all the spectral measurements. Sulphacetamide sodium was obtained as a gift sample from RDPL Jaipur which was manufactured by Ishita Drug and Ind. Ltd., Ahmedabad (Batch No. 1111/04). All reagents were of analytical grade and distilled water was used for dilutions. The ophthalmic formulations used were: Albucid (10%) manufactured by Allergan India Pvt. Ltd., MP, Locula (10%) manufactured by East India Ph. Works Ltd., Kolkata; Andremide (20%) manufactured by Intas Ph. Ltd., Ahmedabad; Sulfacid+ (20%) manufactured by Jawa Pharmaceuticals Pvt. Ltd., Gurgaon.

When a primary aromatic amine is treated with nitrous acid in an ice-cooled solution, the product is a diazonium salt<sup>10</sup>.  $\text{Ar-NH}_2 + \text{HNO}_2 + \text{HCl} \rightarrow \text{Ar-N}_2^+ \text{Cl}^- + \text{H}_2\text{O}$ . Under proper conditions diazonium salts react with certain aromatic compounds to yield products of general formula  $\text{Ar-N=N-Ar}^1$ , called as azo compounds and the reaction is called as coupling reaction. In this reaction, the nitrogen is retained in the product.



Aromatic amines undergo coupling in weak acid solution but not in strong acid solution. Coupling also occurs in sodium acetate buffered solution with compounds containing an active methylene; the product is usually the phenylhydrazone, formed by the tautomerisation of the azo compound to the more thermodynamically stable hydrazone. This coupling reaction is often used to detect the presence of enols and is called as Japp-Klingermann reaction<sup>10-11</sup>.



The new developed method involves the diazo-coupling reaction of diazonium salt of sulphacetamide sodium with acetyl acetone in an alkaline medium. Sulphacetamide sodium was treated with cooled nitrite solution in acidic medium, resulting in diazotization to give the diazonium chloride. This diazonium chloride was coupled with the active methylene group of acetyl acetone to form an azo dye in an alkaline medium. The formed yellow color azo dye is the basis of quantitative estimation of sulphacetamide sodium.

Sulphacetamide sodium stock solution (1 mg/ml) was prepared in distilled water. Solutions of lower concentrations were prepared by diluting the standard stock solution. Aliquots of standard solution of sulphacetamide sodium were suitably diluted with distilled water to get the concentrations in the range of 2-20  $\mu\text{g/ml}$ . Ten ml of each dilution was transferred into a series of 25 ml volumetric flask and 1ml of 1 M hydrochloric acid was added to each of them. The dilutions were maintained below 5<sup>0</sup> and 2 ml of ice-cooled 0.1% sodium nitrite solution was added drop wise with stirring. After 3 min, 1 ml of 3% sulfamic acid solution was added to each flask and set aside for 5 min. Then 4 ml of 5% acetyl acetone and 4 ml of 4 M sodium hydroxide solution were added consecutively to each flask and mixed well. After 10 min, the azo dye solution was scanned by UV/Vis spectrophotometer and the wavelength of maximum

absorbance ( $\lambda_{\max}$ ) of Gaussian spectra was found to be 420 nm. Absorbances of all above dilutions were measured against reagent at 420 nm. Calibration curve was prepared by plotting absorbance versus concentration ( $\mu\text{g/ml}$ ).

The optimum conditions were established by OVAT method (Varying one parameter and keeping others constant and measuring absorbance at  $\lambda_{\max}$  until constant value of absorbance is obtained).

Validation of the developed method was done as per ICH guidelines<sup>12</sup>. Accuracy of the developed method was determined by recovery studies and it was performed by adding definite amounts of the drug to the preanalyzed formulation and reanalyzing the mixture by the developed method. Precision of the developed method was determined by repeatability, interday and intraday precision with six replicates. Limit of detection and limit of quantification were determined by using standard deviation of absorbance of blank.

One ml of ophthalmic formulation of sulphacetamide sodium was diluted with distilled water up to 100 ml. Appropriate aliquots of the drug solution were taken and the standard procedure was followed for analysis of drug content. The absorbance was measured at 420

nm and the amount of drug was determined by linear regression equation (Table 1).

Sulphacetamide sodium contains primary aromatic amino group, that undergoes diazotization reaction and the formed diazonium salt can be further coupled with acetyl acetone in alkaline medium. The formation azo dye was the basis of quantitative estimation of sulphacetamide sodium. As diazotization requires acidic medium, 1-2 ml of 1 M hydrochloric acid was added. Two ml of sodium nitrite (0.1%) was sufficient to diazotize sulphacetamide sodium and excess of nitrite during diazotization was removed by addition of 1 ml of sulfamic acid solution (3%). Higher reagent concentration of coupling agent, acetyl acetone (5%) gives high intensity color which fades quickly with time and 4 ml of coupling agent was found to be optimum. Alkaline medium is necessary for diazo coupling reaction and 2-5 ml of 4 M sodium hydroxide was used for the same. Stability study of the azo dye was carried out by measuring the absorbance values at time intervals of 10 min for 3 h and it was found to be stable for 2 h.

The wavelength of maximum absorbance ( $\lambda_{\max}$ ) of azo dye was 420 nm and it was selected for measurement of absorbance. The Beer's law was obeyed in the concentration range 2-20  $\mu\text{g/ml}$  of sulphacetamide sodium. The linear regression equation was found  $Y = 0.0461c - 0.0564$ , with correlation coefficient  $r^2 = 0.9956$  (where  $Y$  = absorbance and  $c$  = concentration in  $\mu\text{g/ml}$ ). The molar absorptivity and Sandell's sensitivity for the developed method were found as  $7.8983 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$  and  $0.02990 \mu\text{g/cm}^2$  per 0.001 absorbance unit from 6 replicates. Results of the analysis were statistically validated for the developed method (Table 1). By using the developed method, sulphacetamide sodium was estimated in the range of 98.71-99.62% with 0.17-0.35 standard deviation in ophthalmic formulations. Recovery studies were performed and the % recovery was obtained in the range of 98.96-99.79% with 0.08-0.31 standard deviation (Table 2).

**TABLE 1: OPTICAL CHARACTERISTICS AND OTHER PARAMETERS**

| Parameters  | Value                   |
|---|-------------------------|
| $\lambda_{\max}$ (nm)   | 420                     |
| Beer's law limit ( $\mu\text{g/ml}$ )                                 | 2-20                    |
| Molar absorptivity ( $\text{l mol}^{-1} \text{ cm}^{-1}$ )            | $7.8983 \times 10^3$    |
| Sandell's sensitivity ( $\mu\text{g/cm}^2$ per 0.001 absorbance unit) | 0.02990                 |
| Correlation coefficient ( $r^2$ )                                     | 0.9956                  |
| Regression equation ( $Y = a+bc$ )                                    | $Y = 0.0461 c - 0.0564$ |
| Intercept (a)   | -0.0564                 |
| Slope (b)   | 0.0461                  |
| Repeatability % RSD   | 0.0028                  |
| Interday precision % RSD  | 0.0692                  |
| Intraday precision % RSD  | 0.0616                  |
| Limit of quantification ( $\mu\text{g/ml}$ )                          | 0.2535                  |
| Limit of detection ( $\mu\text{g/ml}$ )                               | 0.0836                  |

SD= standard deviation, % RSD= % relative standard deviation,  $r^2$  = correlation coefficient, a= intercept and b = slope

**TABLE 2: RESULTS OF ANALYSIS OF SULPHACETAMIDE SODIUM IN OPHTHALMIC FORMULATIONS**

| Formulation     | % Amount found* $\pm$ SD | SEM.  | % Recovery* $\pm$ SD |
|-----------------|--------------------------|-------|----------------------|
| Albucid (10%)   | 99.62 $\pm$ 0.17         | 0.069 | 99.79 $\pm$ 0.08     |
| Locula (10%)    | 99.35 $\pm$ 0.21         | 0.085 | 99.51 $\pm$ 0.10     |
| Andremide (20%) | 99.29 $\pm$ 0.27         | 0.110 | 99.37 $\pm$ 0.21     |
| Sulfacid+ (20%) | 98.71 $\pm$ 0.35         | 0.142 | 98.96 $\pm$ 0.31     |

\* = Mean of 6 replicate readings, SD= standard deviation, SEM= standard error of mean

Hence the method developed in the present investigation is simple, reliable, sensitive, accurate and precise and can be successfully applied for routine estimation of sulphacetamide sodium in bulk drug and in pharmaceutical formulations. This method can also extend to estimation of other medicinal compounds, which have primary aromatic amino group.

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