Original Article

Validation of a Stability-Indicating Spectrometric Method for the Determination of Sulfacetamide Sodium in Pure Form and Ophthalmic Preparations

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Introduction: Sulfacetamide sodium is a widely used sulfonamide for ophthalmic infections. Objective: A number of analytical methods have been reported for the analysis of sulfacetamide but they lack the ability to determine both the active drug and its major degradation product, sulfanilamide, simultaneously in a sample. Materials and Methods: In the present study a simple, rapid and economical stability-indicating UV spectrometric method has been validated for the simultaneous assay of sulfacetamide sodium and sulfanilamide in pure form and in ophthalmic preparations. Results: The method has been found to be accurate (recovery 100.03 ±0.589%) and precise (RSD 0.587%) with detectable and quantifiable limits of 1.67×10−6 M (0.04 mg%) and 5.07×10−6 M (0.13 mg%), respectively for the assay of pure sulfacetamide sodium. The method is also found to be accurate and precise to small changes in wavelength, pH and buffer concentration as well as to forced degradation. The study further includes the validation of the method for the assay of pure sulfanilamide in solution, which has been found to be accurate, precise and robust. Conclusion: The results indicate that the proposed two-component spectrometric method is stability-indicating and can be used for the simultaneous assay of both sulfacetamide sodium and sulfanilamide in synthetic mixtures and degraded solutions.

Keywords: Degradation, ophthalmic preparations, sulfacetamide sodium, sulfanilamide, two-component ultraviolet spectrometric assay, validation

INTRODUCTION

Sulfacetamide (SC) sodium continues to be the most effective and widely prescribed sulfonamide for ophthalmic infections due to its bactericidal activity against susceptible microorganisms. Its effectiveness and low toxicity even at very high aqueous concentrations have proved to be nonirritating to the delicate eye tissues. Its penetration into ocular fluids and tissues in high concentration has been reported.[1-3] The sensitivity and toxic effects in therapeutic doses are usually mild and infrequent.[3,4] The official method for the assay of SC solution is based on sodium nitrite titration (diazoization) and determination of the end-point electrometrically, i.e., by amperometry.[5,6] However, this method does not take into account the presence of sulfanilamide (SN), a hydrolytic degradation product, which also undergoes diazotization, and a distinction between the two compounds in a degraded solution is not possible by this method. This is because of the presence of a primary aromatic amino group undergoing diazotization in both the compounds. One of the most widely used methods is that of Bratton and Marshall,[7] which consists of diazoitzing the sulfanamide with sodium nitrite in dilute acid and coupling the diazo compound with N-(1-naphthyl)-ethylenediamine to produce a pink color which is measured at 545 nm. This method is also not

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specific and a mixture of sulfonamides present in the solution would all diazotize producing a combined effect for the drugs present in the sample, thus giving false results. The methods for the assay of sulfonamides have been reviewed by Higuchi and Hanssen\cite{8} and Ahmad et al.\cite{9} The methods used for the assay of sulfonamides include chromatography,\cite{10-12} spectrometry,\cite{13-16} electrochemical techniques,\cite{5,6,17} thermal techniques,\cite{18} electrophoresis,\cite{19} chemiluminescence detection,\cite{20} supported liquid membrane extraction,\cite{21}

In view of the problems involved in the assay of SC in the presence of its degradation products, Ahmad and Ahmed\cite{13-15} reported a simple and rapid two-component spectrometric method for the simultaneous assay of SC and SN. However, this method was neither validated for the pure solutions nor applied to the assay of SC in ophthalmic dosage forms. In this study, validation of the assay procedure has been performed for the assay of SC in pure solutions and eye drops according to the guideline of International Council for Harmonization (ICH).\cite{22} The specificity of the method has also been investigated through thermal and photodegradation. Moreover, the same method has also been applied and validated for the assay of SN which has not been reported earlier.

**Materials and Methods**

**Materials**

SC sodium and SN were purchased from Sigma-Aldrich Company Ltd. (Dorset, UK). The materials were stored in an air-tight container protected from light. The buffer system used in this study for the assay of SC was sodium acetate-acetic acid, 0.2 M (pH 4.0). All other solvents and reagents used were of analytical grade having the highest degree of purity obtained from Merck/BDH.

**Methods**

*Thin-layer chromatography*

One-way ascending thin-layer chromatography (TLC) technique was used for the identification of the compounds using precoated plates of silica gel G/ultraviolet (UV) 254 (Merck) and alumina H (Type E, Merck) with a layer thickness of 250 µm. The solvent systems employed included (1) chloroform-ethanol-heptane (1:1:1, v/v),\cite{23} (2) n-butanol-acetic acid-water (50:15:35, v/v),\cite{11} (3) n-butanol-acetic acid-water (100:20:48, v/v),\cite{24} and (4) ethanol-methanol (50:50, v/v).\cite{25} All experiments were performed at a temperature of 25°C ± 2°C with a tank saturation time of 1 h. The spots were sprayed with different reagents for the identification of products such as P-dimethylamino benzaldehyde spray, diazotization followed by alkaline beta-naphthol spray, diazotization followed by N-1-naphthyl-ethylene diamine spray, exposure to iodine vapors, and examination under UV light at 254 and 350 nm (Uvitec Lamp, UK).

**pH measurement**

The pH of the solutions was measured using a digital pH meter (Elmetron, model: CP501, sensitivity ±0.01 pH units, Poland), with a combination pH electrode and a temperature probe. The calibration of the instrument was carried out using commercially available buffer tablets of pH 4.00 and 7.00 (Merck). The electrode was immersed directly into the solution and kept for few seconds to equilibrate and the pH value was noted.

**Ultraviolet spectrometry**

The assay of SC sodium and SN was performed by UV spectrometry by recording their absorbencies at 271 and 258 nm, respectively. All absorbance measurements and spectral determinations were carried out on Shimadzu UV-visible recording spectrophotometer (model UV–1601) using quartz cells of 10 mm path length. The cells were employed always in the same orientation using appropriate control solutions in the reference beam. The baseline correction was made by the built-in baseline memory at the initializing period while auto-zero adjustment was made by one-touch operation. The wavelength scale was calibrated automatically by the instrument. The instrument was periodically checked for the absorbance scale using the following calibration standards:

Absorbance scale: 0.050 g/L of K$_2$Cr$_2$O$_7$ in 0.01 N H$_2$SO$_4$, absorbance at 257 nm = 0.725 and at 350 nm = 0.539 ± 0.005.\cite{8}

**Preparation of the stock and test solutions for validation studies**

The stock solutions of SC sodium and SN for validation studies were prepared in a concentration of $1.0 \times 10^{-4}$ M (2.54 mg%) in distilled water. The pH of the stock solution was found to be around 5.5. The test solutions were prepared in the range of $1.0–5.0 \times 10^{-5}$ M (0.25–1.27 mg%) from the stock by making appropriate dilutions with distilled water and adjusting the pH to 4.0 with acetate buffer (0.2 M). Each time, fresh solutions were prepared and protected from light, and their absorbance was recorded immediately. The solutions were found to be transparent in appearance.

**Validation of the analytical method**

The UV method for the assay of SC sodium was validated according to the guideline of ICH.\cite{22} The following validation parameters were studied.

**Linearity and range**

The linearity of the method was determined by plotting a calibration curve in the range of
1.0–5.0 × 10⁻⁵ M (0.25–1.27 mg%). The range was selected to maintain the absorbance values in the region of around 0.2–0.8 to obtain highest precision. The calibration curve was plotted as concentrations versus absorbance. The linearity was statistically calculated by regression analysis of five concentrations used in triplicate for SC sodium. The molar absorptivity and A (1%, 1 cm) values at the absorption maximum were also calculated from the curve.

Accuracy
The accuracy of the proposed method was determined by adding known concentrations of the drug in the solutions followed by their analysis for recovery. Three different concentrations were selected from the studied range including low, medium and high, i.e. 1.0, 3.0, and 5.0 × 10⁻⁵ M (0.25, 0.76, and 1.27 mg%) and each was studied in triplicate for recovery. Mean recovery (%), standard deviation, and relative accuracy error were calculated from the recoveries obtained.

Precision
The precision of the method was determined at three different levels (i.e., 1.0, 3.0, and 5.0 × 10⁻⁵ M) by calculating the relative standard deviation (%RSD) of the mean recoveries.

Limit of detection and limit of quantitation
The limit of detection (LOD) and limit of quantitation (LOQ) of SC sodium by the studied method were calculated from the calibration curve using the following formulae:

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S}
\]

\[
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where σ is the standard deviation (SD) of the intercept and S is the slope of the calibration curve.

Robustness
The robustness of the method was investigated by making small changes in the parameters of the assay which includes as follows:

a. Wavelength (±2.0 nm)
b. pH of the solutions (±0.2 units)
c. Buffer concentration (±0.02 M).

Each parameter was studied thrice in the range used for the determination of SC sodium (i.e., 1.0–5.0 × 10⁻⁵ M). The mean of accuracy and precision of the method was determined for each parameter. Moreover, Student’s t-test was also applied to compare the significance of the difference between the means of unchanged and altered parameters.

Specificity
The pure solutions of SC (1.0 × 10⁻⁴ M, i.e., 2.54 mg%) were subjected to forced degradation by heating or exposing to UV light. The solutions were subjected separately for 10 h to 100°C in a thermostat water bath and for 1 h to UV light (TUV 30 Watts, Philips). The individual and total recoveries were determined by two-component spectrometric analysis after appropriate dilution (details in Section “Calculation of concentrations of SC and its degradation product, SN, by two-component spectrometric method [additive absorbencies]”). The specificity of the method was further investigated by adding known amounts of the major degradation product of SC sodium, i.e., SN, to its solutions and calculating the recoveries of each compound by the proposed method. The accuracy, relative accuracy error, and precision were determined.

Calculation of concentrations of sulfacetamide and its degradation product, sulfanilamide, by two-component spectrometric method (additive absorbencies)
In a two-component spectrometric assay, absorbance measurements are made at two suitably selected wavelengths and the concentrations are determined by solving two simultaneous equations. This may be done directly or using matrix method:

\[
A_1 = K_1 C_1 + K_2 C_2
\]

\[
A_2 = K_1 C_1 + K_2 C_2
\]

Where \(A_1\) is the absorbance at wavelength \(\lambda_1\), \(A_2\) is the absorbance at wavelength \(\lambda_2\), \(K_1\) is absorptivity-cell path product for component 1 at wavelength \(\lambda_1\), \(K_2\) is absorptivity-cell path product for component 1 at wavelength \(\lambda_2\), \(K_1\) is absorptivity-cell path product for component 2 at wavelength \(\lambda_1\), \(K_2\) is absorptivity-cell path product for component 2 at wavelength \(\lambda_2\), \(C_1\) is concentration of component 1, and \(C_2\) is concentration of component 2. The solution of equation (1a) and (1b) for \(C_1\) and \(C_2\) is made as:

\[
C_1 = (K_2 A_1 - K_1 A_2)/(K_1 K_2 - K_1 K_2)
\]

\[
C_2 = (K_1 A_1 - K_2 A_2)/(K_1 K_2 - K_1 K_2)
\]

The solution of these simultaneous equations may be achieved using suitable software such as “multicomponent analysis by full-spectrum quantitation” or Microsoft Excel.

Application of the method to liquid ophthalmic preparations
The current method was also applied for the assay of SC sodium in different ophthalmic solutions and suspensions to observe any interference from the excipients and other active ingredients of different formulations. The details of the ophthalmic preparations of SC sodium procured from the local pharmacy are given in Table 1.

These preparations are available in 10% and 20% concentration range containing different excipients.
including stabilizers. Two of the preparations were solutions of SC sodium only while the other three were suspensions that contained prednisolone acetate (0.2%) and phenylephrine HCl (0.12%) as other active drugs [Table 1]. All the samples were diluted appropriately (10,000 for 10% and 20,000 for 20%) before analysis to achieve the required concentration for the assay. In addition, the suspensions were filtered to remove the insoluble particles of other active drugs.

Validation of the proposed method for sulfanilamide
The current method was also validated for the analysis of SN. Similar validation protocols as used for SC sodium were investigated.

RESULTS AND DISCUSSION
Identification of degradation products of sulfacetamide
The solutions of SC sodium, pure and those used for forced degradation by heat or light were subjected to TLC for the detection and identification of the degradation products using solvent systems 1 to 4. No degradation products have been identified in freshly prepared solutions of SC sodium, whereas SN has been found to be the major degradation product in the solutions exposed to heat or UV light and in ophthalmic preparations. Both SC sodium and SN have been identified by comparison of their Rf values and shapes of the spots under UV light with those of the reference standards.

Assay of sulfacetamide sodium and degradation product
SC and its degradation product, SN, at pH 7.0 exhibit absorption maxima at 260 and 259 nm, respectively. However, at pH 4.0 (acetate buffer), the absorption maximum of SC (271 nm) is quite distinct from that of SN (258 nm) [Figure 1]. This is due to difference in the ionization behavior of SC (pK\textsubscript{a} 5.40) and SN (pK\textsubscript{a} 10.45). Therefore, it is possible to conduct a two-component spectrometric assay of SC and SN by absorbance measurements at 271 and 258 nm. This method has been applied for the assay of SC sodium in thermal and photodegraded solutions containing SN as the only degradation product as well as in synthetic mixtures containing both compounds.

Table 1: Ophthalmic preparations of sulfacetamide sodium used in the present study

| Sample number | Formulation type | Other active ingredient | pH |
|---------------|------------------|------------------------|-----|
| 1             | Suspension\(^a\) | Prednisolone acetate (0.2%), phenylephrine HCl (0.12%) | 7.4 |
| 2             | Suspension\(^a\) | Prednisolone acetate (0.2%), phenylephrine HCl (0.12%) | 7.5 |
| 3             | Solution\(^a\)  | Prednisolone acetate (0.25%), phenylephrine HCl (0.12%) | 7.6 |
| 4             | Solution\(^a\)  | -                      | 8.0 |
| 5             | Solution\(^a\)  | -                      | 8.2 |

\(^a\)Concentration 10% per 5 mL, \(^b\)Concentration 20% per 10 mL, \(^c\)pH of the eye drops measured in the laboratory

Validation of the assay method
Linearity
The absorption maximum of the SC sodium has been found to be at 271 nm that shows a linear relationship between absorbance and concentration of the drug over the range of 1.0–5.0 × 10\textsuperscript{-5} M (i.e. 0.25–1.27 mg%). The data show very low scatter of the points around the calibration curve with a regression value (R\textsuperscript{2}) of 0.99986 [Figure 2a]. The intercept is also not significantly different from zero indicating acceptable peak purity. The statistical evaluation of the data is presented in Table 2, and the overlay spectra of the drug in the studied concentration range are presented in Figure 2b.

Accuracy
Accuracy of the method has been determined by adding known amounts of the drug in a solution within the linearity range. The results obtained for three different concentrations in triplicate show relative accuracy errors in the range of −0.92 to +1.15 and overall mean recovery of 100.03% with an SD of ±0.5888 [Table 3]. The small errors in the results indicate that the method is highly accurate and could be applied to the analysis of SC sodium.

Precision
Precision is the closeness of agreement between a series of measurements obtained from multiple samples of the
studied drug under prescribed conditions. The overall mean of RSD for SC sodium has been found to be below 1% [Table 3], indicating very low degree of scatter thus proving high precision of the method.

Limit of detection and limit of quantification
LOD is the minimum amount of an analyte that can be detected but may not be quantified under the used analytical conditions. The lowest amount of the analyte that is quantifiable under the applied analytical conditions with acceptable accuracy and precision is termed as LOQ. In this study, the LOD and LOQ of SC sodium are found to be $1.67 \times 10^{-6}$ M (0.04 mg%) and $5.07 \times 10^{-6}$ M (0.13 mg%), respectively.

Robustness
It is the capability of the method to remain unaffected by deliberate but small changes in the parameters of the analytical method. The reliability of the method has been tested by changing assay wavelength, pH of the medium, and buffer concentration of the solutions. The RSD in all cases has been found to be within 2% with an accuracy around 99.7%–100.1% [Table 4], indicating that the method is sufficiently robust and can be used for the assay of the drug effectively even with minor changes taking place during analysis. In addition, Student’s $t$-test has also been applied to compare the % recovery at different levels [Table 4], which showed nonsignificant differences between the results ($t_{\text{tabulated}} < t_{\text{calculated}}$), thus further confirming the reliability and robustness of the method during normal use.

Specificity
It is of utmost importance to assess unequivocally an analyte in the presence of components that are expected to co-elute with the peak of the analyte and interfere with its quantification. Table 3 shows the results of the specificity studies indicating that the method is specific for SC sodium as the amount found was found to be near theoretical amount.

Table 2: Analytical parameters for the determination of sulfacetamide sodium

| Parameter          | Value     |
|--------------------|-----------|
| $\lambda_{\text{max}}$ | 271 nm    |
| Concentration range| 1.0-5.0×10^{-3} M (0.25-1.27 mg%) |
| Correlation coefficient (R) | 0.99993 |
| Molar absorptivity (ε) | $1.69 \times 10^4$/M/cm     |
| A (1%, 1 cm) | 665 |
| Slope              | 16,900    |
| Intercept          | 0.0300    |
| SE of slope        | 0.0037    |
| SE of intercept    | 0.0038    |
| SD of intercept    | 0.0086    |

SE: Standard error, SD: Standard deviation

Table 3: Accuracy and precision of sulfacetamide sodium by the proposed method

| Amount added (M × 10^{-5}) | Amount found (M × 10^{-5}) | Recovery (%) | Mean recovery (%) ±SD | Relative accuracy error (%) | Precision (% RSD) |
|----------------------------|----------------------------|--------------|------------------------|----------------------------|--------------------|
| 1.00                       | 1.01                       | 100.59       | 100.81±1.0619          | +0.99                      | 1.0533             |
| 1.00                       | 0.99                       | 99.88        |                        | -0.92                      |                    |
| 1.00                       | 1.02                       | 101.97       |                        | +1.15                      |                    |
| 3.00                       | 2.98                       | 99.41        | 99.68±0.5211           | -0.27                      | 0.5228             |
| 3.00                       | 1.98                       | 100.28       | 99.61±0.1835           | +0.60                      |                    |
| 3.00                       | 1.97                       | 99.34        |                        | -0.34                      |                    |
| 5.00                       | 4.99                       | 99.76        | 99.61±0.1835           | +0.15                      | 0.1842             |
| 5.00                       | 4.97                       | 99.41        |                        | -0.20                      |                    |
| 5.00                       | 4.98                       | 99.66        |                        | +0.05                      |                    |
| Mean                      |                            | 100.03±0.5888 |                        | -                          | 0.5868             |

Recovery (%)=(amount found/amount added) × 100, where amount found was calculated from (mean absorbance of three determinations-intercept)/slope (Ahmed et al. 2013), Relative accuracy error (%)=(recovery-mean recovery)/(mean recovery) × 100. SD: Standard deviation, RSD: Relative standard deviation

Figure 2: (a) The calibration curve and (b) overlay spectra of sulfacetamide sodium at pH 4.0
to be present within the sample. All the above-studied parameters have indicated that the current method is highly accurate and precise for the analysis of SC sodium in pure solutions. However, in the presence of its major degradation product, SN, which absorbs nearly in the same region at 258 nm, the two-component spectrometric method can be used. A general scheme for the calculation of concentration in a two-component spectrometric assay has already been presented in Materials and Methods (Section “Calculation of concentrations of SC and its degradation product, SN, by two-component spectrometric method [additive absorbencies]”). The values of absorptivity constants used in the assay have been determined at pH 4.0 and are as follows:

a. SC sodium at 271 nm (εK) = 665 and at 258 nm (εK) = 510
b. SN at 271 nm (εK) = 640 and at 258 nm (εK) = 830.

The two-component analysis showed a recovery in the range of ~98%–101% for the thermally degraded and UV exposed solutions with an average molar balance of around 4.98 × 10^4 and 4.97 × 10^4 M, respectively [Table 5]. This is in close agreement with the actual composition of the mixture (5.00 × 10^4 M). These results indicate that an exposure of 10 h to heat or 1 h to UV light caused degradation of SC solutions (pH ~5.5) to about 15 and 29%, respectively [Table 5].

No degradation product other than SN was identified by TLC which is also supported by the molar balance of two-component recovery studies [Table 5]. In such type of studies, degradation up to 15%–20% is generally considered as acceptable as higher degradation may produce different degradation products which are generally not encountered during routine stability studies or shelf-life of the product[27,28]. The forced degradation does not produce any measurable thermal or photochemical change to affect the accuracy of the assay method. However, on prolonged light exposure (at least for 200 h at 70°C–90°C), SN is known to be further photo-oxidized to azobenzene-4,4-disulfoxamide which exhibits absorption maxima at 320 nm. Therefore, the current method should be applied to the samples stored in the dark or not exposed to light for prolonged period or undergone thermal degradation without any interference from the photoproducts of SN.

In addition to the forced degradation study, known amounts of SN have been added to the solutions of SC sodium and the quantities of both compounds have been determined in synthetic mixtures. The results of the assay of SC sodium and SN by the two-component analysis are reported in Table 6. The results indicated the achievement of a good molar balance of SC and SN showing that the method is capable of determining the two compounds in a mixture within an average accuracy of around ±1% and precision of <2% [Table 6].

### Table 4: Robustness parameters of the proposed method

| Parameters          | Accuracy (%) ±SD | Precision (% RSD) | Student t-test |
|---------------------|------------------|-------------------|---------------|
| Wavelength (271±2 nm) | 269              | 100.05±0.5934  | 0.5931        |
|                     | 273              | 100.02±0.5240  | 0.5239        |
| pH (4.0±0.2 units)   | 3.8              | 99.94±0.7329  | 0.7333        |
|                     | 4.2              | 99.90±0.9729  | 0.9739        |
| Buffer (0.2±0.02 M)  | 0.18             | 100.14±1.3763 | 1.3744        |
|                     | 0.22             | 99.73±1.6841  | 1.6887        |

The concentration range is similar in each case to that of Table 2.

The values of accuracy are the mean of percentage recovery where n=3 in each case. The comparison has been made between the means of original and altered parameters.

| Time (h)  | SC (M × 10^6) | SN (M × 10^6) | Total (M × 10^6) | Recovery (%) |
|-----------|---------------|---------------|------------------|--------------|
| Solutions exposed to heat  | 0  | 5.00 | 0.00 | 5.00 | 100.00 |
|            | 2  | 4.94 | 0.05 | 4.99 | 99.80 |
|            | 4  | 4.78 | 0.20 | 4.98 | 99.60 |
|            | 6  | 4.59 | 0.37 | 4.96 | 99.20 |
|            | 10 | 4.27 | 0.68 | 4.95 | 99.90 |

Solutions exposed to UV light

| Time (h)  | SC (M × 10^6) | SN (M × 10^6) | Total (M × 10^6) | Recovery (%) |
|-----------|---------------|---------------|------------------|--------------|
|            | 0  | 5.00 | 0.00 | 5.00 | 100.00 |
|            | 10 | 4.72 | 0.24 | 4.96 | 99.20 |
|            | 20 | 4.39 | 0.65 | 5.04 | 100.80 |
|            | 30 | 4.15 | 0.77 | 4.92 | 98.40 |
|            | 60 | 3.55 | 1.36 | 4.91 | 98.20 |

The values in M/L have been reported to indicate the molar loss of SC sodium on the basis of mole to mole conversion to SN. UV: Ultraviolet, SN: Sulfanilamide, SC: Sulfacetamide

### Application of the method to ophthalmic liquid preparations

The current assay method has also been applied to five different ophthalmic preparations, containing two solutions and three suspensions, of commercially available SC sodium [Table 1]. The spectrum of all formulations is similar to the pure drug in the studied concentration range apparently showing no interference of any compound or excipient after appropriate dilutions [Figure 3]. The filtration and rinsing followed by the dilution of the suspensions have been found adequate to remove prednisolone acetate (0.2%) and phenylephrine HCl (0.12%), and thus, no spectral interference has
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been observed in the assay at the dilutions required to determine SC sodium (10% or 20%). The recovery of all the preparations has been found in the range of 93%–99% [Table 7] for different batches of the studied brands indicating that the current method is effective in the determination of SC in eye drops containing various excipients and other active drugs. Moreover, the commercial samples have also been spiked up with known amount of SC sodium that has also been accurately quantified [Table 7], indicating that the method is applicable to both pure solutions and eye drops.

Validation of the assay method for the analysis of sulfanilamide SN is the major degradation product of SC.\[^{[9,13-15,29-31]}\] However, its antibacterial activity is comparatively weaker than the parent compound, thus limiting its clinical use.\[^3\] It has been observed that the current method of analysis can accurately and precisely determine both SC and SN in a mixture using the two-component analysis. Therefore, the current method has also been validated for the assay of SN. The assay method has shown good linearity for SN over the concentration range of 1.0–5.0 × 10^{-5} M (0.17–0.86 mg%) with a regression value ($R^2$) of 0.99936 [Figure 4a]. The absorption maximum of SN at pH 4.0 is 258 nm which is constant over the studied concentration range [Figure 4b]. The statistical results are

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### Table 6: Determination of accuracy and precision of sulfacetamide sodium and sulfanilamide in synthetic mixtures by two-component spectrometric assay

| Amount added (M × 10^{-6}) | Amount found (M × 10^{-6}) | Total (M × 10^{-6}) | Recovery (%) | Mean recovery (%) ±SD | Relative accuracy error (%) | Precision (% RSD) |
|---------------------------|---------------------------|---------------------|--------------|------------------------|----------------------------|-------------------|
| SC SC SN SC SN SC SN SC SN | 5.00 0.00 5.01 - 5.01 100.20 - | 98.96±1.56 100.30±1.40 | +2.06 -1.30 | 1.39 | -0.30 |
| 2.00 3.00 1.95 3.06 5.01 97.50 102.00 | 3.00 2.00 2.95 2.03 4.98 98.33 101.50 | 3.00 2.00 2.95 2.03 4.98 98.33 101.50 | -1.22 -1.30 | 1.48 +1.69 | 1.25 -1.30 |
| 1.00 4.00 1.01 3.96 4.97 101.00 99.00 | 1.00 4.00 1.01 3.96 4.97 101.00 99.00 | 1.00 4.00 1.01 3.96 4.97 101.00 99.00 | -1.22 -1.30 | 1.48 +1.69 | 1.25 -1.30 |
| 0.00 5.00 - 5.00 5.00 - 100.00 | 0.00 5.00 - 5.00 5.00 - 100.00 | 0.00 5.00 - 5.00 5.00 - 100.00 | -1.22 -1.30 | 1.48 +1.69 | 1.25 -1.30 |

*Same as described in Table 3. SD: Standard deviation, RSD: Relative standard deviation, SC: Sulfacetamide, SN: Sulfanilamide

### Table 7: Assay of sulfacetamide sodium in different ophthalmic preparations

| Sample (%) | Recovery (%) ±SD |
|------------|------------------|
| 1 (10) | 94.97±1.05 |
| 2 (10) | 98.63±1.96 |
| 3 (10) | 93.63±1.52 |
| 4 (10) | 96.43±1.55 |
| 5 (20) | 96.80±1.21 |

*Same as described in Table 3. SD: Standard deviation, RSD: Relative standard deviation, SC: Sulfacetamide, SN: Sulfanilamide

### Table 8: Analytical parameters for the determination of sulfanilamide

| Parameter | Value |
|-----------|-------|
| $\lambda_{\text{max}}$ | 258 nm |
| Concentration range | 1.0-5.0×10^{-5} M (0.17-0.86 mg%) |
| Correlation coefficient ($R$) | 0.99968 |
| Molar absorptivity ($\epsilon$) | 1.38×10^{4}/M/cm |
| A (1%, 1 cm) | 830 |
| Slope | 13,830 |
| Intercept | 0.0209 |
| SE of slope | 0.0064 |
| SE of intercept | 0.0067 |
| SD of intercept | 0.0149 |
| LOD | 3.57×10^{-6} M (0.06 mg%) |
| LOQ | 1.08×10^{-5} M (0.19 mg%) |

LOD: Limit of detection, LOQ: Limit of quantitation, SE: Standard error, SD: Standard deviation

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Figure 3: Ultraviolet spectra of the eye drop preparations (sample 1–5) of sulfacetamide sodium at pH 4

Validation of the assay method for the analysis of sulfanilamide SN is the major degradation product of SC.\[^{[9,13-15,29-31]}\] However, its antibacterial activity is comparatively weaker than the parent compound, thus limiting its clinical use.\[^3\] It has been observed that the current method of analysis can accurately and precisely determine both SC and SN in a mixture using the two-component analysis. Therefore, the current method has also been validated for the assay of SN. The assay method has shown good linearity for SN over the concentration range of 1.0–5.0 × 10^{-5} M (0.17–0.86 mg%) with a regression value ($R^2$) of 0.99936 [Figure 4a]. The absorption maximum of SN at pH 4.0 is 258 nm which is constant over the studied concentration range [Figure 4b]. The statistical results are
reported in Table 8. The minimum amount of SN, that is, detectable (LOD) and quantifiable (LOQ), is found to be $3.57 \times 10^{-6}$ M (0.06 mg%) and $1.08 \times 10^{-5}$ M (0.19 mg%), respectively [Table 8]. The recovery studies show that the relative accuracy error is in the range from −1.68 to +0.63, with an overall mean recovery of 100.00% and SD of ±0.9864, whereas the mean RSD is found to be 0.9862%. This indicates that the present method is highly accurate and precise for the assay of SN [Table 9]. The deliberate small changes in wavelength, pH, and buffer concentration do not affect the accuracy (99.8%–100.1%) and precision (RSD <2%), thus indicating the high robustness of the UV method with statistically nonsignificant difference between the results [Table 10].

**Conclusion**

The present study has reported the validation of a stability-indicating simple, economical, accurate, and precise UV spectrometric method for the assay of SC sodium and SN. Since SN is known to photo-oxidized to various products such as azo and azoxy compounds, it would be of value to perform three- and four-component analysis to further enhance the utility of the proposed method and to investigate the kinetics of their formation. In addition, other dosage forms such as ophthalmic ointments of SC sodium can also be studied by the application of this assay method.

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**Conflicts of interest**

There are no conflicts of interest.
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