Validated chromatographic methods for the simultaneous determination of a ternary mixture of sulfacetamide sodium and two of its official impurities; sulfanilamide and dapsone

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ABSTRACT

Sulfacetamide sodium is a widely prescribed sulfonamide drug due to its topical antibacterial action on eye and skin. Four impurities are stated in the British Pharmacopoeia among which are sulfanilamide and dapsone. This work presents two specific, accurate and precise chromatographic methods for the simultaneous determination of a mixture of sulfacetamide sodium, sulfanilamide and dapsone. The first method is an isocratic RP-HPLC where the separation of components was achieved on C18 column. A green mobile phase was used consisting of methanol:water (60:40, v/v). The flow rate was 1.0 mL/min and effluent was monitored at 273 nm. The second method is a TLC-spectrodensitometric one where good separation was achieved by using silica plates and a mobile phase consisting of chloroform: dichloromethane:acetic acid (6:2.5:1.5, by volume). Determination was done by densitometry in the absorbance mode at 273 nm. Both methods were validated in compliance with ICH guidelines. They were also successfully applied for the determination of sulfacetamide sodium and its impurities in Ocusol® ophthalmic solutions. The obtained results were statistically compared to the results obtained by applying the official methods of analysis of each component where no significant difference was found with respect to accuracy and precision.

KEYWORDS
dapsone, HPLC, sulfacetamide sodium, sulfanilamide, TLC- spectrodensitometry

1. INTRODUCTION

International Conference on Harmonization (ICH) and other national regulatory authorities focus on identification and quantification of possible impurities in drug substances [1]. Existence of impurities, even if in trivial quantities, may not only affect the efficacy of the drug but also the safety of pharmaceutical preparations [2]. Sulfacetamide sodium is chemically named as sodium acetyl((4-aminophenyl) sulfonyl)azanide [3]. It is principally used in topical preparations as antibacterial agent [4]. It is described for treatment of conjunctivitis, corneal ulcers and other superficial infections [5]. Sulfanilamide is chemically designated as 4-aminobenzenesulfonamide [3]. It is principally used in topical preparations as antibacterial agent [4]. It is described for treatment of conjunctivitis, corneal ulcers and other superficial infections [5]. Sulfanilamide is chemically designated as 4-aminobenzenesulfonamide [3]. It is reported to be impurity A for sulfacetamide sodium [3] as it is considered its major degradation product under the effect of light and temperature [6]. Dapsone is 4,4′-sulfonyldianiline and an official impurity for sulfacetamide sodium [3]. However, it is a medication used for the treatment of leprosy as it inhibits folic acid synthesis [3]. The structures and molecular weights of the studied components are illustrated in Fig. 1. Chromatographic techniques are considered the most suitable and commonly used analytical techniques for determination of drug substances in presence of impurities in pharmaceutical analysis [7–9]. Several chromatographic methods were reported for the simultaneous determination of sulfacetamide and sulfanilamide [10–14]. However, no
method was reported in the literature for the simultaneous determination of ternary mixture of sulfacetamide sodium, sulfanilamide and dapsone. Therefore, the aim of this work is to develop and validate sensitive, economic, accurate and precise chromatographic methods for the simultaneous determination of sulfacetamide sodium together with sulfanilamide and dapsone.

2. MATERIALS AND METHODS

2.1. Instruments

2.1.1. RP-HPLC method. HPLC system consists of an Agilent pump 1,100 series, equipped with a variable wavelength detector (model 1,100 series; Agilent, USA), and a 20 µL volume injection loop using X-bridge ODS column (5 µm, 250 mm × 4.6 mm i.d.), Waters, USA; a degasser (model DGU-12A). The samples were injected by using a 50-µL Hamilton analytical syringe, pH-meter (Jenway model 3,505, UK) was also used.

2.1.2. TLC-spectrodensitometric method. TLC system consists of Camag Linomat auto sampler (Muttenzl, Switzerland), Camag microsyringe (100 µL) and Camag TLC scanner 3S/N/30319 with winCATS software. A short wavelength UV lamp emitting at 254.0 nm was also used (Desaga, Wiesloch, Germany). TLC plates were pre-coated with silica gel F254 20 × 10 cm, 0.25 mm thickness (Merck, Darmstadt, Germany).

2.2. Materials

2.2.1. Pure standards. Sulfacetamide sodium monohydrate was kindly offered by the Egyptian International Pharmaceutical Industries Company (EIPICO), Egypt and its purity was checked and found to be 99.96% ± 0.722 according to the British Pharmacopoeia method (BP) (titrimetric method) [3]. Sulfanilamide was obtained from El-Nasr Pharmaceutical Chemicals Co., Egypt. Its purity was checked and found to be 99.86% ± 1.548 according to the United States Pharmacopeia (USP) method (titrimetric method) [15]. Dapsone was kindly supplied by The Nile for Pharmaceuticals & Chemical Industries, Egypt. Its purity was found to be 99.87% ± 1.046 according to USP method (HPLC method) [15].

2.2.2. Pharmaceutical formulations. Ocusol® 10% eye drops, batch No.5518007, labeled to contain 100 mg of sulfacetamide sodium per one mL. Ocusol® 20% eye drops, batch No.6519001, labeled to contain 200 mg of sulfacetamide sodium per one mL. Both are manufactured by Alexandria Co. for Pharmaceuticals & Chemical Industries, Egypt and were purchased from local pharmacies.

2.2.3. Chemicals and solvents. All chemicals used throughout this work were of analytical grade, and the solvents were of HPLC grade. They are as follows: methanol, chloroform, and dichloromethane (Sigma-Aldrich, Steinheim, Germany). Glacial acetic acid (El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt), double-distilled deionized water (Otsuka, Cairo, Egypt), Orthophosphoric acid (Adwic, Cairo, Egypt).

2.3. Standard solutions

2.3.1. RP-HPLC method

2.3.1.1. Stock standard solutions. Stock standard solutions of sulfacetamide sodium, sulfanilamide and dapsone (1.00 mg mL⁻¹) were prepared by accurately weighing and transferring 100.00 mg of each pure drug into three separate 100-mL volumetric flasks in methanol. The volume was completed in methanol and the solution was then completed with volume with the same solvent.

2.3.1.2. Working standard solutions. Working standard solutions of sulfacetamide sodium (50.00 µg mL⁻¹), sulfanilamide and dapsone (25.00 µg mL⁻¹) were prepared by transferring 5.0 mL of sulfacetamide sodium and 2.5 mL of sulfanilamide and dapsone stock standard solutions into three separate 100-mL volumetric flasks. The volume was then completed with methanol.

2.3.2. TLC-spectrodensitometric method. Standard solutions of sulfacetamide sodium (1.00 mg mL⁻¹), sulfanilamide and dapsone (0.10 mg mL⁻¹) were prepared in three separate 100-mL volumetric flasks by accurately weighing and dissolving 100.00 mg of sulfacetamide sodium, 10.00 mg of sulfanilamide and dapsone in methanol. The volume was then completed with the same solvent.
2.4. Procedures

2.4.1. Construction of calibration graphs

2.4.1.1. RP-HPLC method. Aliquots of sulfacetamide sodium, sulfanilamide and dapsone equivalent to 0.10–300.0 μg, 5.00–80.00 μg and 10.00–160.00 μg, respectively, were accurately transferred from their corresponding working standard solutions, into three separate series of 10-mL volumetric flasks. The volumes were completed to the mark with methanol. A 1 μL aliquot of the solution claimed to contain 1 μg band⁻¹ of sulfacetamide sodium was applied onto the TLC plate by the Camag micro syringe. The general procedure previously described was followed. The concentration of sulfacetamide sodium was calculated using the corresponding regression equation.

3. RESULTS AND DISCUSSION

Nowadays, the mainstream of drug-related impurity determinations is performed by HPLC as it offers the desired level of sensitivity with high degree of automation [16, 17]. Selection from wide variety of stationary phases, mobile phases and operation modes makes HPLC applicable to all drug classes [18]. Consequently, a RP-HPLC method is described for the simultaneous determination of sulfacetamide sodium with its BP stated related impurities; sulfanilamide and dapsone without prior separation. Furthermore, the development of an HPLC method which uses eco-friendly solvents would be favored to decline the harmful effects of used solvents on both human and environment. Despite all the mentioned advantages of HPLC methods, economic issues and the need for high operated instruments makes sometimes its implementation difficult. So, TLC would be found as a powerful tool for the screening and isolation of impurities owing to its simplicity and low cost [19]. Therefore, another TLC-spectrodensitometric method was developed for the quantitative determination of the studied ternary mixture.

3.1. Methods development

3.1.1. RP-HPLC method. Different green mobile phases were tried to approach the goal of green chemistry such as; ethanol:water (80:20, v/v), ethanol:water (60:40, v/v), methanol:water (70:30, v/v) and methanol:water (80:20, v/v). The best resolution with sharp and symmetric peaks was accomplished upon using mobile phase consisted of methanol:water (80:20, v/v), (pH 5.0 adjusted by orthophosphoric acid). The suggested mobile phase composition proved to be one of the most green solvents [20]. Different flow rates and detection wavelengths were tested; satisfactory resolution was obtained with isocratic mode at flow rate 1.0 mL min⁻¹ and UV detection at 273.0 nm. The retention time obtained was 4.3, 3.1 and 6.0 min for sulfacetamide sodium, sulfanilamide and dapsone, respectively, under the described conditions, Fig. 2.

3.1.2. TLC-spectrodensitometric method. Studying the optimum parameters for maximum separation was carried out by trying different two and three components developing systems with different ratios such as; chloroform:methanol (7:3, v/v), chloroform:methanol (9:1, v/v) and chloroform: dichloromethane (7:3, v/v), but highly tailed peaks were observed. Other systems were tried such as; chloroform:dichloromethane:acetic acid and chloroform:methanol:acetic acid, in different ratios, where incomplete resolution of the
drugs was obtained with tailed peaks. Eventually, chloroform:dichloromethane:acetic acid (6:2.5:1.5, by volume) was the developing system of choice which permits the determination of the studied mixture with minimum tailing and maximum separation. The corresponding retardation factors ($R_f$) values were found to be 0.60, 0.41 and 0.75 for sulfacetamide sodium, sulfanilamide and dapsone, respectively, Fig. 3.

UV detection at 273.0 nm and Slit dimensions (3.00 × 0.45 mm) offer best results; regarding sensitivity, peak symmetry and peak sharpness.

3.2. Methods validation

Calculation of system suitability parameters is necessary to verify the performance of the proposed RP-HPLC and TLC-spectrodensitometric systems [21, 22]. Good results were obtained as shown in Tables 1 and 2. ICH guidelines of the proposed chromatographic methods were followed by measuring linearity, range, LOD, LOQ, accuracy, precision, specificity and robustness [23]. Results are shown in Tables 3 and 4.

3.2.1. Linearity

3.2.1.1. RP-HPLC method. A linear relationship was obtained between the integrated peak areas and the corresponding concentrations of sulfacetamide sodium, sulfanilamide and dapsone in the ranges of 1.00–30.00 µg mL$^{-1}$, 0.10–8.00 µg mL$^{-1}$ and 0.50–16.00 µg mL$^{-1}$, respectively. The regression equations were computed and found to be:

- For sulfacetamide sodium:
  $$A = 82.269C + 35.237 \quad r = 0.9998$$

- For sulfanilamide:
  $$A = 70.583C + 7.7446 \quad r = 1.0000$$

- For dapsone:
  $$A = 70.997C + 7.0395 \quad r = 0.9999$$

Where, $A$ is the integrated peak area, $C$ is the concentration in µg mL$^{-1}$ and $r$ is the correlation coefficient.

3.2.1.2. TLC-spectrodensitometric method. For sulfacetamide sodium, a polynomial relationship was found to exist between the integrated peak area and the corresponding concentrations in the range of 0.10–4.00 µg band$^{-1}$. The second order polynomial regression was conducted to improve the sensitivity level, concentration range and the regression coefficient of the method. For sulfanilamide and dapsone, a linear relationship was established between the integrated peak areas and the corresponding concentrations in the range of 0.03–0.60 µg band$^{-1}$ for both components. The regression equations were computed and found to be:

- For sulfacetamide sodium:
  $$A = -1022.1C^2 + 9796.1C + 2912.8 \quad r = 0.9999$$
Table 1. System suitability parameters for the proposed HPLC method for the simultaneous determination of sulfacetamide sodium, sulfanilamide, and dapsone

| Parameter                                      | Sulfanilamide | Sulfacetamide sodium | Dapsone | Reference values [21] |
|------------------------------------------------|---------------|----------------------|---------|-----------------------|
| Retention time (tR)                           | 3.1           | 4.3                  | 6.0     |                       |
| Resolution factor (Rf)a                       | 0.24          | 0.32                 | 0.46    |                       |
| Tailing factor (T)                            | 1.91          | 1.71                 |         |                       |
| Capacity factor (K')                           | 1.43          | 2.13                 | 0.34    | 1 < K' < 5            |
| Selectivity factor (α)                        |               |                      |         | α > 1                 |
| Number of theoretical plates (N)d             | 2,843         | 4,442                | 6,400   | High N indicates better separation |
| Height equivalent to theoretical plate (HETP) (mm)d | 0.0028    | 0.0018               | 0.0013  | Low HETP indicates better separation |

Table 2. System suitability parameters of the proposed TLC-spectrodensitometric method for the simultaneous determination of sulfacetamide sodium, sulfanilamide, and dapsone

| Parameters                                      | Sulfanilamide | Sulfacetamide sodium | Dapsone | Reference values [22] |
|------------------------------------------------|---------------|----------------------|---------|-----------------------|
| Retardation factor (Rf)                        | 0.24          | 0.32                 | 0.46    |                       |
| Resolution (Rf)b                               | 1.91          | 1.71                 |         |                       |
| Capacity factor (K')                            | 1.43          | 2.13                 | 0.34    | 1 < K' < 5            |
| Selectivity factor (α)                         |               |                      |         | α > 1                 |
| Number of theoretical plates (N)c              | 2,843         | 4,442                | 6,400   | High N indicates better separation |
| Height equivalent to theoretical plate (HETP) (mm)d | 0.0028    | 0.0018               | 0.0013  | Low HETP indicates better separation |

A = 14636C + 588.84   r = 0.9999  for sulfanilamide
A = 20389C + 1050.4 r = 0.9999  for dapsone

Where A is integrated peak area, C is the concentrations in µg band\(^{-1}\) and r is the correlation coefficient.

3.2.2. Detection and quantification limits. The BP monograph of sulfacetamide sodium specifies a range between 99 and 101% for sulfacetamide contents and a limit for sulfanilamide and total related substances not exceeding 0.2% and 0.5% of the declared content of sulfacetamide sodium, respectively [3]. The LOD and LOQ values of both impurities were calculated by using the following calculations: LOD = (SD of regression residuals/slope) × 3.3; LOQ = (SD of regression residuals/slope) × 10. The low LOD and LOQ values demonstrate the high sensitivity of the proposed methods.

3.2.3. Accuracy. The accuracy of the results was checked by applying the proposed RP-HPLC method for determination of different samples of sulfacetamide sodium (2.00, 6.00, 10.00, 16.00, 25.00 µg mL\(^{-1}\)), sulfanilamide (0.80, 3.00, 5.00, 7.00, 8.00 µg mL\(^{-1}\)) and dapsone (3.00, 5.00, 6.00, 8.00, 10.00 µg mL\(^{-1}\)). Other five different concentrations of sulfacetamide sodium (0.20, 0.60, 1.50, 2.50, 4.00 µg band\(^{-1}\)), sulfanilamide and dapsone (0.05, 0.15, 0.25, 0.35, 0.45 µg band\(^{-1}\)) were analyzed to study the accuracy of TLC-spectrodensitometric method. The calculated percentage recoveries indicate good accuracy of both chromatographic methods.

3.2.4. Precision. To study the precision of the RP-HPLC method, three different concentrations of pure samples of sulfacetamide sodium (4.00, 12.00, 24.00 µg mL\(^{-1}\)), sulfanilamide (2.00, 4.00, 8.00 µg mL\(^{-1}\)) and dapsone (4.00, 8.00, 10.00 µg mL\(^{-1}\)) were analyzed in triplicate on a single day (intraday precision) and on three consecutive days (interday precision). For TLC-spectrodensitometric method, three different concentrations of pure samples of sulfacetamide sodium (0.50, 2.00, 3.00 µg band\(^{-1}\)), sulfanilamide and dapsone (0.20, 0.40, 0.60 µg band\(^{-1}\)) were also analyzed in triplicate on a single day (intraday precision) and on three consecutive days (interday precision). The percentage
relative standard deviations (RSD %) for the mentioned drugs were calculated. Low RSD % shows that the developed methods are precise enough.

### 3.2.5. Specificity

The specificity was accomplished by the analysis of several laboratory prepared mixtures containing the studied components in different ratios within the linearity range using the previously mentioned procedure under each method. The specificity of the proposed methods was checked by the calculated percentage recoveries and small SD value.

### 3.2.6. Robustness

Robustness of the developed RP-HPLC method was assessed through deliberately changing

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**Table 3.** Regression and validation parameters of the proposed HPLC method for the determination of pure sulfacetamide sodium, sulfanilamide and dapsone

| Parameter                  | Sulfacetamide sodium | Sulfanilamide | Dapsone   |
|----------------------------|----------------------|---------------|-----------|
| **Linearity**              |                      |               |           |
| Linearity range (μg mL⁻¹)  | 1.00–30.00           | 0.10–8.00     | 0.50–16.00|
| Slope                      | 82.869               | 70.583        | 70.997    |
| Intercept                  | 35.237               | 7.745         | 7.0395    |
| Correlation coefficient (r)| 0.9998               | 1.0000        | 0.9999    |
| **Accuracy (mean ± SD)**   | 100.63 ± 0.643       | 100.90 ± 1.708| 99.37 ± 1.339|
| **Precision (RSD%)**       |                      |               |           |
| Repeatabilitya             | 0.421                | 0.912         | 0.425     |
| Intermediate precisionb    | 0.623                | 1.992         | 0.826     |
| **Specificity (mean ± SD)**| 99.76 ± 1.299        | 99.67 ± 1.816 | 99.68 ± 0.932|
| Robustness (RSD%)          | 1.570                | 1.123         | 1.940     |
| LOD (μg mL⁻¹)c             | —                    | 0.028         | 0.153     |
| LOQ (μg mL⁻¹)c             | —                    | 0.084         | 0.469     |

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**Table 4.** Regression and validation parameters of TLC-spectrodensitometric method for the simultaneous determination of sulfacetamide sodium, sulfanilamide and dapsone samples

| Parameter                  | TLC-spectrodensitometric method |
|----------------------------|---------------------------------|
| **Linearity**              | Sulfacetamide sodium            |
| Range (μg band⁻¹)          | 0.10–4.00                       |
| Slope                      | 14,636                          |
| Intercept                  | 2912.8                          |
| Correlation coefficient (r)| 0.9999                          |
| **Accuracy (mean ± SD)**   | 99.03 ± 1.776                   |
| **Precision (RSD%)**       |                                  |
| Repeatabilityb             | 1.702                           |
| Intermediate precisionc    | 1.913                           |
| **Specificity (mean ± SD)**| 100.29 ± 1.828                  |
| Robustness (RSD%)          | 1.791                           |
| LOD (μg band⁻¹)d           | —                               |
| LOQ (μg band⁻¹)d           | —                               |

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### Notes

- a Intra-day (n = 3), average of three concentrations of sulfacetamide sodium (4.00, 12.00 and 24.00 μg mL⁻¹), sulfanilamide (2.00, 4.00, 8.00 μg mL⁻¹) and dapsone (4.00, 8.00 and 12.00 μg mL⁻¹), repeated three times within the same day.
- b Inter-day (n = 3), average of three concentrations of sulfacetamide sodium (0.50, 2.00 and 3.00 μg mL⁻¹), sulfanilamide (2.00, 4.00, 8.00 μg mL⁻¹) and dapsone (0.20, 0.40 and 0.60 μg mL⁻¹), repeated three times in three consecutive days.
- c Calculated from equation [LOD = 3.3 (SD/S), LOQ = 10 (SD/S); where SD is the standard deviation of regression residuals and S is the slope of the calibration curves.

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**Parameters:**

- **A** = ax² + bx + c Where, A is the peak area, x is the concentration in (μg/band), a and b are coefficients 1 and 2, respectively and c is the intercept.
- a Slope a and slope b are the coefficients of X² and X, respectively. Following a polynomial regression.
Table 5. Quantitative determination of sulfacetamide sodium in pharmaceutical formulations by the proposed chromatographic methods

| Pharmaceutical formulation | RP-HPLC method | TLC-spectrodensitometric method |
|-----------------------------|----------------|-------------------------------|
|                             | Claimed (µg mL⁻¹) | Founda % ± SD | Claimed (µg band⁻¹) | Foundb % ± SD |
| Ocusol® 10% eye drops       | 10             | 99.45 ± 1.598               | 1                 | 100.10 ± 1.005 |
| Claimed to contain 100 mg of |                |                              |                   |                |
| sulfacetamide sodium per one mL |              |                              |                   |                |
| Batch No. 5518007          |                |                              |                   |                |
| Ocusol® 20% eye drops      | 10             | 99.73 ± 1.070               | 1                 | 101.77 ± 0.623 |
| Claimed to contain 200 mg of |                |                              |                   |                |
| sulfacetamide sodium per one mL |              |                              |                   |                |
| Batch No. 6519001          |                |                              |                   |                |

* Average of five experiments.

Table 6. Statistical analysis of the results obtained by the proposed HPLC method and the official methods for the determination of sulfacetamide sodium in its eye drops, sulfanilamide and dapsone in their pure forms

| Item                              | HPLC                        | Official methods [15]                        |
|-----------------------------------|------------------------------|---------------------------------------------|
|                                  | Sulfacetamide sodium         | Sulfacetamide sodium                        |
|                                  | (Ocusol® 10%) (ocusol® 20%) | (Ocusol® 10%) (ocusol® 20%)                 |
|                                  | Sulfanilamide                | Sulfanilamide                               |
|                                  | Dapsone                      | Dapsone                                     |
| Mean                             | 99.40                        | 101.04                                      |
| SD                               | 1.598                        | 0.772                                       |
| Variance                         | 2.554                        | 0.596                                       |
| n                                | 5                            | 5                                           |
| Student's t-test (2.306) d       | 2.066                        | 5.5                                          |
| F value (6.39) d                 | 4.285                        | 5.801                                        |

* HPLC method using C18 column with mobile phase containing a mixture of water:methanol:glacial acetic acid (89:10:1, by volume) with flow rate 1.5 mL min⁻¹ and UV detection wavelength at 254.0 nm.
* Potentiometric titration method using 0.1M sodium nitrite as a titrant and detection of end point by starch iodide paste as an external indicator.
* HPLC method using silica column with mobile phase containing a mixture of isopropylacetoniitrile:ethyl acetate:hexane (10:10:70, by volume), flow rate 1.0 mL min⁻¹ and UV detection wavelength at 254.0 nm.
* Figures in parentheses are the corresponding tabulated values for t and F at P = 0.05.

Table 7. Statistical analysis of the results obtained by the proposed TLC-spectrodensitometric method and the official methods for the determination of sulfacetamide sodium in its eye drops, sulfanilamide and dapsone in their pure forms

| Item                              | TLC- spectrodensitometric method | Official methods [15]                        |
|-----------------------------------|----------------------------------|---------------------------------------------|
|                                  | Sulfacetamide sodium              | Sulfacetamide sodium                        |
|                                  | (Ocusol® 10%) (Ocusol® 20%)      | (Ocusol® 10%) (Ocusol® 20%)                 |
|                                  | Sulfanilamide                     | Sulfanilamide                               |
|                                  | Dapsone                           | Dapsone                                     |
| Mean                             | 100.20                           | 101.04                                      |
| SD                               | 1.005                            | 0.772                                       |
| Variance                         | 1.010                            | 0.596                                       |
| n                                | 5                                | 5                                           |
| Student's t-test (2.306) d       | 1.482                            | 5.5                                          |
| F value (6.39) d                 | 1.695                            | 4.271                                        |

* HPLC method using C18 column with mobile phase containing a mixture of water:methanol:glacial acetic acid (89:10:1, by volume) with flow rate 1.5 mL min⁻¹ and UV detection wavelength at 254.0 nm.
* Potentiometric titration method using 0.1M sodium nitrite as a titrant and detection of end point by starch iodide paste as an external indicator.
* HPLC method using silica column with mobile phase containing a mixture of isopropylacetoniitrile:ethyl acetate:hexane (10:10:70, by volume), flow rate 1.0 mL min⁻¹ and UV detection wavelength at 254.0 nm.
* Figures in parentheses are the corresponding tabulated values for t and F at P = 0.05.
experimental conditions. Three different concentrations of each drug were analyzed under a variety of conditions, such as small changes in mobile phase ratio ±2%, pH ± 0.2, flow rate± 0.1 and the detection wavelength ±1.0 nm. For TLC-spectro densitometric method, the changed experimental conditions were small modifications in proportions of the developing system ±1% and detection wavelength ±1.0 nm. For both methods, the RSD % for the studied compounds were calculated and found to be less than 2% which confirms the robustness of the chromatographic methods.

3.3. Application to pharmaceutical formulations
The developed chromatographic methods were successfully applied for the determination of sulfacetamide sodium in both Ocusol® 10% and 20% eye drops. Results presented in Table 5 proved the applicability of the suggested methods for the determination of sulfacetamide sodium in its ophthalmic solutions without any interference of the excipients or impurities that may be found in the pharmaceutical formulations.

3.4. Statistical analysis
The results obtained from the analysis of sulfacetamide sodium by the suggested chromatographic methods were statistically compared to those obtained by applying the USP official method for the determination of sulfacetamide sodium in its ophthalmic solutions [15]. Sulfanilamide and dapsone were analyzed in their pure forms by the established chromatographic methods and the USP official ones [15], where the results acquired by the mentioned techniques were statistically compared [24]. The calculated t and F-values were less than the corresponding theoretical ones indicating that there is no significant difference between the proposed methods and the official ones regarding accuracy and precision, Tables 6 and 7.

4. CONCLUSION
Ultimately, the presented RP-HPLC and TLC-spectro densitometric methods provide a quantitative approach for the simultaneous determination of sulfacetamide sodium and its official impurities; sulfanilamide and dapsone. The presented eco-friendly RP-HPLC method shows high sensitivity, accuracy and precision. From the economic point of view, the shorter run time (~6 min) without affecting the resolution of separation leads to low mobile phase consumption and makes it a feasible method for the routine analysis of the drugs. The suggested TLC-spectro densitometric method has the merits of running several samples simultaneously, using simple composition of mobile phase, and offering high sensitivity and specificity with relatively low cost. The proposed chromatographic methods have been validated in accordance with ICH guidelines. Both chromatographic methods can be applied in routine and quality control analysis of sulfacetamide sodium in its pure powdered forms and in its commercially available ophthalmic formulations without the interference of commonly encountered dosage form additives. In addition, the successful application of the suggested techniques to detect trace amounts of sulfanilamide and dapsone reveals the usefulness of these methods for impurity profiling.

Conflict of interest: The authors have declared no conflict of interest.

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