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Note

Quantitative determination of sulfanilamide in sodium sulfacetamide raw material and ophthalmic solutions by high-performance liquid chromatography

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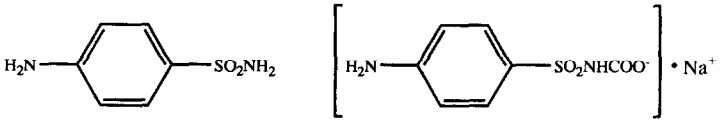
Sodium sulfacetamide (SUL) is a sulfonamide anti-infective used in ophthalmic solutions for the treatment of conjunctivitis, corneal ulcer and other superficial ocular infections[1]. Both the British Pharmacopoeia[2] and the Deutsches Arzneibuch[3] require the determination of sulfanilamide (SFN) in SUL raw material. (The structures of SFN and SUL are shown in Fig. 1.) The current compendial method shared by both is a semi-quantitative thin-layer chromatographic (TLC) analysis. A method that is more accurate and reproducible and which can be automated is desirable.

There are a number of procedures for the analysis of sufonamides in the literature, including colorimetric[4–5], TLC[6–7], gas chromatography (GC)[9–10], electroanalytical[11], and high-performance liquid chromatography (HPLC)[10,12–22]. Only a few references addressed analysis of SFN and SUL[12,18,20]. The methods described by Penner[12] and by Elrod *et al.*[20] separate SFN from SUL but do not permit quantitation of SFN due to its very low capacity factor (k'). Jandera *et al.*[18] carried out extensive optimization studies on mobile phase composition which led to our decision to use methanol–water (1:9, v/v). However, the columns they used are not readily available. The HPLC method reported herein permits quantitative determination of SFN and SUL.

EXPERIMENTAL

Apparatus

The HPLC system consisted of the following: a 6000A pump (Waters Assoc., Milford, MA, U.S.A.); a Model 440 UV detector (Waters Assoc.); a μBondapak C_{18}



SFN SUL

Fig. 1. Chemical structures of sulfanilamide (SFN) and sodium sulfacetamide (SUL).

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column (10 μm particle size), 30 cm × 3.9 mm I.D. with USP Packing Type L1 (Waters Assoc.); a 710B WISP autoinjector (Waters Assoc.); a 3357 data collection system integrator (Hewlett-Packard, Fullerton, CA, U.S.A.); and an OmniScribe recorder (Houston Instruments, Austin, TX, U.S.A.). UV spectra analysis was performed on a HP1040M photodiode array detector (Hewlett-Packard).

Materials and reagents

Methanol (HPLC grade) was obtained from Baxter (McGraw Park, IL, U.S.A.). Acetic acid, glacial (ACS reagent grade) was obtained from Mallinckrodt (Paris, KY, U.S.A.). Water was distilled twice from an all glass apparatus then deionized and filtered through activated carbon through a Milli-Q system (Millipore, Waters Assoc.).

Standards and samples

The stock standard solution was prepared by weighing 100 ± 10 mg SFN (Aldrich, Milwaukee, WI, U.S.A.) into a 50-ml volumetric flask which was brought to volume with methanol–water (20:80, v/v) and mixed well. Then 5.0 ml of this solution was pipetted into a 100-ml volumetric flask and brought to volume with methanol–water (20:80, v/v). The working standard was prepared by pipetting 3.0 ml of 0.1 mg/ml SFN stock standard solution into a 100-ml volumetric flask and bringing to volume with methanol–water (20:80, v/v).

SUL raw material samples were prepared by weighing 250 ± 10 mg into a 25-ml volumetric flask and bringing to volume with methanol–water (20:80, v/v). Ophthalmic solutions containing 10% (w/v) SUL were prepared by pipetting 1.0 ml into a 100-ml volumetric flask. In either case, 3.0 ml of the resulting solution was pipetted into a 100-ml volumetric flask and brought to volume with methanol–water (20:80, v/v).

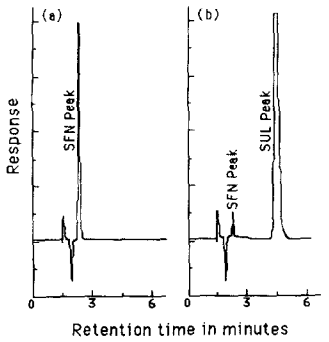
Reversed-phase HPLC analysis

A mobile phase of methanol–glacial acetic acid–water (10:1:89) was used. The system had the following parameters: flow-rate of 1.5 ml/min; injection volume, 90 μl; detection at 254 nm, 0.2 a.u.f.s.; analysis time, 7 min; chart speed, 0.25 cm/min. HPLC was performed at room temperature. Calculations for the samples were based on peak area measurements.

RESULTS AND DISCUSSION

Typical chromatograms of SFN standard and of SUL raw material sample are shown in Fig. 2. Samples of an ophthalmic solution placebo containing polyvinyl alcohol, benzalkonium chloride, sodium edetate and excipients but without SUL showed no interferences at the locations of either the SFN or SUL peaks (Fig. 3).

Linearity was checked from 0.000149 to 0.00596 mg/ml (equivalent to 0.05% to 2.0% degradation of the sodium sulfacetamide raw material after dilution for analysis). The correlation coefficient was 0.999 for both peak area and peak height data. For both peak area and peak height data, there were no significant differences (α = 0.05) between the y-intercept and the origin (Figs. 4 and 5). Therefore, a single point standard was used.



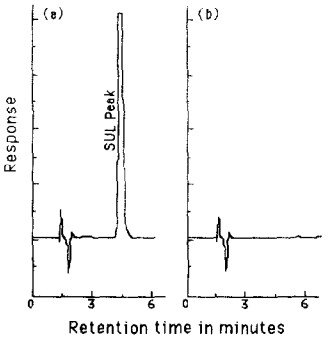


Fig. 2. Chromatograms of SFN standard (a) and SUL raw material (b).

Fig. 3. Chromatograms of ophthalmic solution containing 10% SUL (a) and containing no SUL (b).

Recovery studies to show method accuracy were completed at levels equivalent to 1.014% and 0.334% degradation of SUL. Results are summarized in Table I.

The limit of SFN detection is less than $1.8 \cdot 10^{-6}$ mg/ml. An average of three peak height measurements at this concentration gave a signal-to-noise ratio greater than 10:1 with a relative standard deviation (R.S.D.) of $\pm 3.1\%$. This demonstrates

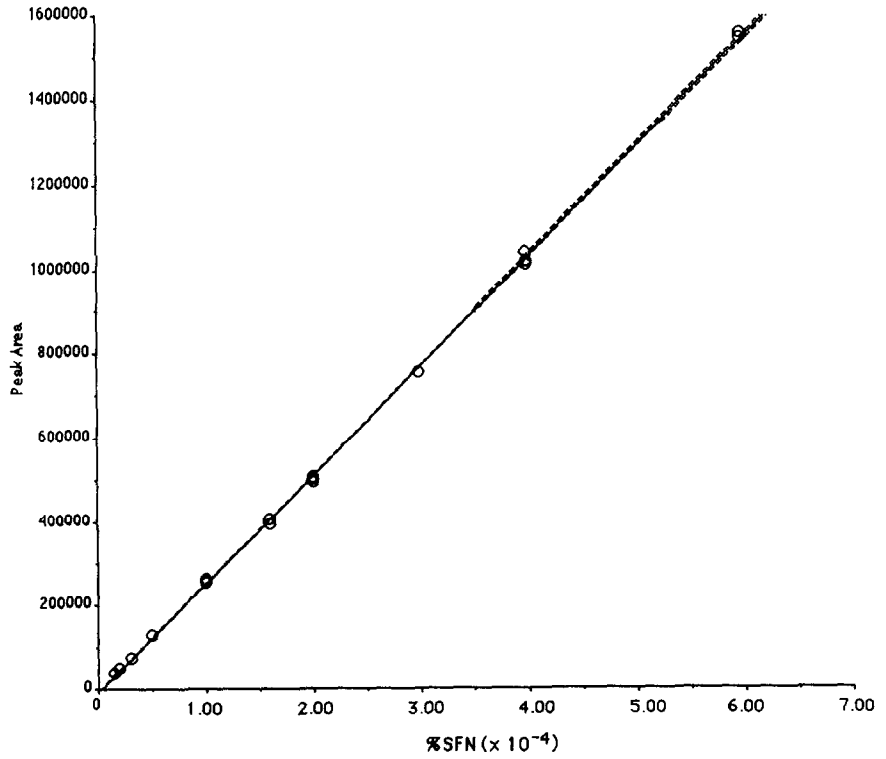


Fig. 4. Peak area linearity. —— = Calibration curve: $y = 2.59 \cdot 10^9 x - 3597$. - - - = Two-tailed 95% confidence intervals.

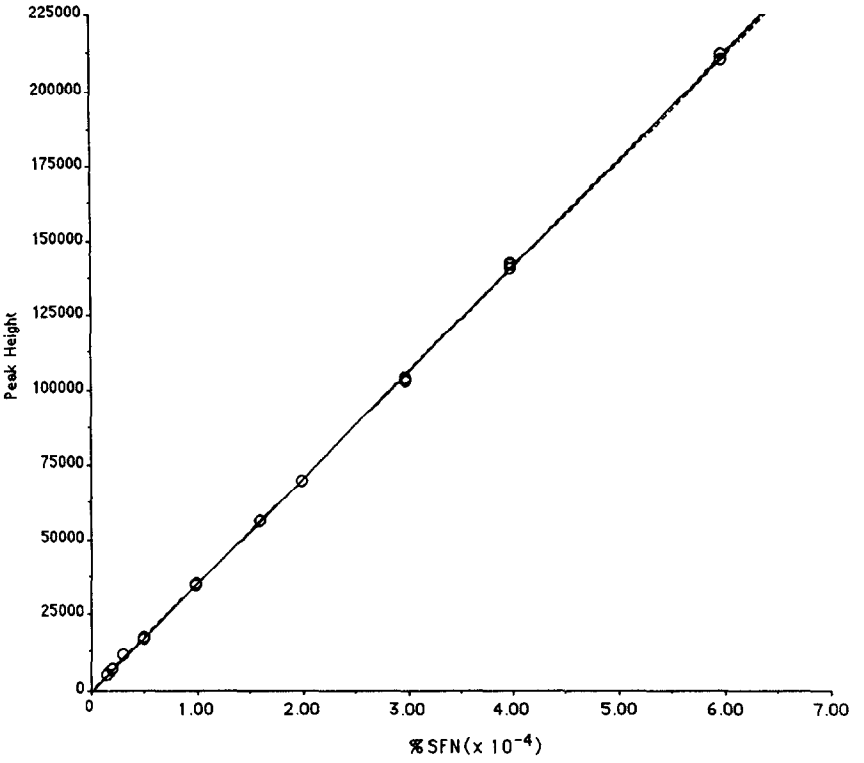


Fig. 5. Peak height linearity. —— = Calibration curve: $y = 3.53 \cdot 10^8 x - 68.4$. - - - = Two-tailed 95% confidence intervals.

excellent detectability at a level representing only 0.006% degradation of SUL raw material.

Six replicates of the 1.0% (w/v) spike used in the accuracy studies were obtained to determine single-day precision. The percent (w/w) SFN was calculated. On a second day, six replicates of the same sample were obtained to provide information concerning day-to-day precision values. Person-to-person precision experiments were run on the same sample to provide information about precision values between different analysts as well as providing feedback with respect to clarity of method write-up. Peak area data are summarized in Table II.

TABLE I

RECOVERY OF SFN FROM SPIKED SAMPLES

	1.014% (w/w)		*0.334% (w/w)*	
	Peak area	*Peak height*	*Peak area*	*Peak height*
Mean	98.7	100.4	101.7	103.4
S.D.	0.4	0.8	0.6	0.4
R.S.D. (%)	0.4	0.7	0.6	0.4
n	6	6	3	3

TABLE II

SUMMARY OF DAY-TO-DAY AND OPERATOR-TO-OPERATOR PRECISION

	Operator A		*Operator B*
	Day 1	*Day 2*	
Mean	1.001	1.013	1.019
S.D.	0.004	0.009	0.022
R.S.D. (%)	0.4	0.9	2.2
n	6	6	6

The following parameters for system suitability are suggested, based on measurements made of "suitable" and "unsuitable" chromatograms obtained in our lab (Fig. 6): no less than 4400 plates/m for SFN; k' values of 0.2 to 1.0 for SFN and $\geqslant 2$ for SUL; tailing factor $\leqslant 1.5$ for SFN; and a resolution of $\geqslant 2$ between SFN and SUL peaks[23]. It is necessary to examine chromatograms to assure a suitable system for quantitative analysis. There is a large negative signal that elutes just before the SFN peak. There must be some observable baseline between this negative signal and the forward edge of the SFN peak to assure accurate and reproducible quantitation (Fig. 6). The "suitable" separation was run on a relatively new column; the "unsuitable" separation on a very old column that had been used for other analyses for several months. All other analytical conditions were identical for both separations.

Samples of a 10% SUL ophthalmic solution were adjusted to pH 2 with 1 *M* hydrochloric acid or left untreated. All samples, treated and untreated, were stored at a temperature of 45°C for nineteen days. All samples showed loss of SUL and increase of SFN, indicating degradation of SUL to SFN via hydrolysis. The absence of other detectable peaks, and the fact that the mass balance of SUL and SFN accounted for 99.9% of the SUL originally present in undegraded samples, indicates that no significant hydrolysis of SFN to sulfanilic acid occurs. See Fig. 7 for chromatograms of these samples.

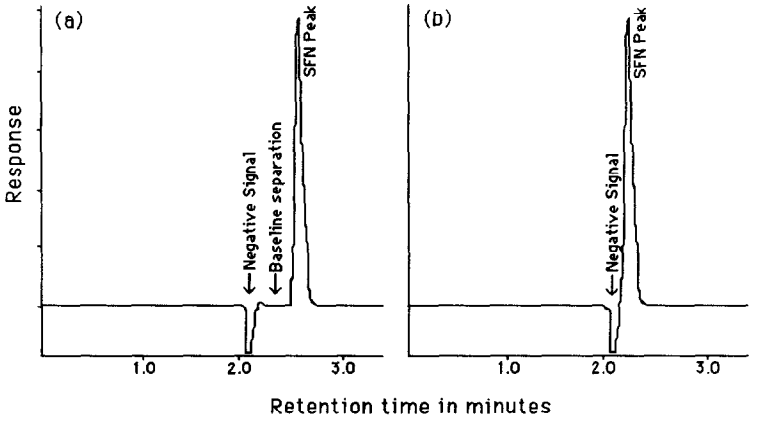


Fig. 6. Suitable (a) and unsuitable (b) retention of SFN.

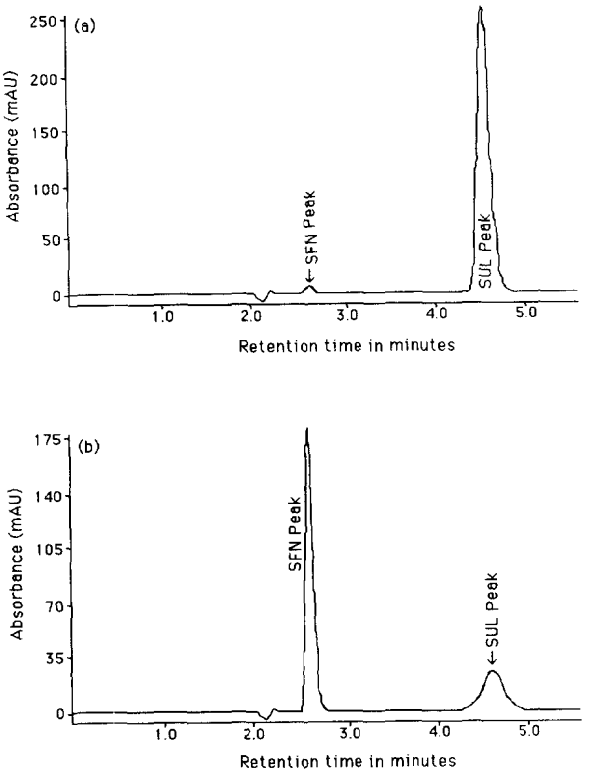


Fig. 7. Chromatograms of thermally stressed samples: untreated (a) and pH 2 (b).

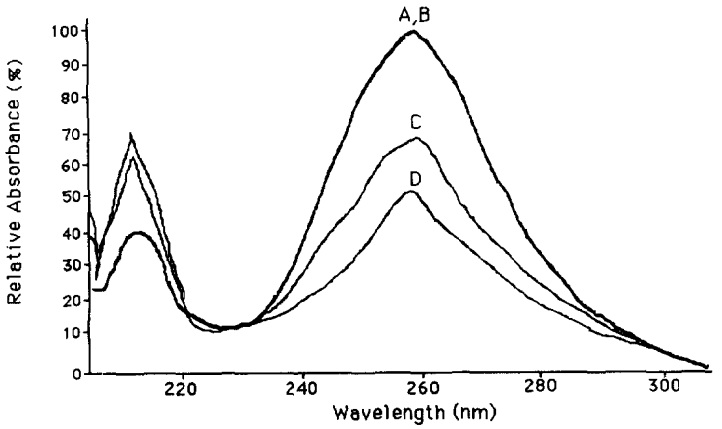


Fig. 8. UV spectra of the SFN peak in a thermally stressed, pH 2 sample. (A) Standard UV scan; (B) peak apex UV scan; (C) peak front UV scan; (D) peak tail UV scan.

TABLE III

SUMMARY OF SFN DATA FROM SIX RAW MATERIALS

Raw material	*Date of manufacturing*	*Mean SFN concentration, % (w/w) of SUL*	*R.S.D. (%)*
13690	May 1985	0.0882	6.7
15233	Aug 1985	0.0459	7.8
15562	Dec 1987	0.0881	3.1
15714	Jun 1988	0.0532	3.0
16215	Jan 1989	0.0637	3.3
IPL No. 15588-208	Feb 1988	0.0838	0.7

Each stressed sample was analyzed by the proposed analysis procedure, using a diode array detector to obtain UV spectra of all eluting peaks. Overlays of UV spectra from the front, apex and back of the eluting SFN peak in each sample demonstrated the homogeneity of this peak throughout its elution. Fig. 8 shows UV spectra from the SFN peak in the pH 2-adjusted sample compared with a SFN standard. These data demonstrate that the method is specific and stability indicating for the determination of SFN in the presence of SUL.

Six SUL raw materials supplied by Napp Chemicals were analyzed for SFN. Mean SFN concentrations for all raw materials were $\leqslant 0.09\%$ (w/w). The results are summarized in Table III as the mean of three replicates.

CONCLUSION

A simple, accurate, sensitive and precise HPLC method was developed to determine SFN in SUL raw materials and ophthalmic solutions. With this method, the SFN resulting from the degradation of SUL can be monitored accurately at concentrations representing 0.05 to 2.0% degradation of SUL.

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