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Short communication

A reverse phase ion-pairing HPLC method for the stability monitoring of sulphacetamide ophthalmic preparations

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1. Introduction

Sulphacetamide sodium solutions in different concentrations are among the most commonly prescribed drugs for repeated topical application in the local management of ophthalmic infections susceptible to sulphonamide therapy. Sulfacetamide ophthalmic solutions are sterilized by autoclaving, where 1% hydrolysis is known to take place [1,2]. A practical problem of colour development due to oxidative decomposition of suphacetamide (I) and its hydrolysis product sulphanilamide (II) in such a formulation is well documented [3,4]. Scheme 1 represents the hydrolysis of sulphacetamide (I) to yield sulphanilamide (II) in the absence of oxygen [5].

Ophthamic solutions containing sulphacetamide sodium (10, 20 and 30%) may undergo hydrolysis during sterilization or storage under adverse conditions. The BP1993 monograph of sulphacetamide sodium eye drops [6] specifies a range between 95 and 105% for sulphacetamide contents and a limit for the related substances not exceeding 5% of the declared content of sulphacetamide. On the other hand, the USP23 monograph [7] specified no limit for the hydrolytic product (II).

The BP monograph of sulphacetamide eye drops specified a titrimetric method for the determination of (I) and a TLC method for (II). Several reports are available to determine the 'intact' sulphacetamide which may not reflect the exact stability of the product. The determination of sulphacetamide degradation by using spectroscopic methods and TLC have been reported earlier [8–12]. The analysis of sulphacetamide in mixtures and in triplesulpha cream by HPLC was also given [13,14]. Recently, Garcia-Alvarez-Coque et al. [15] developed a high performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azo dye precolumn derivatization. These described meth-

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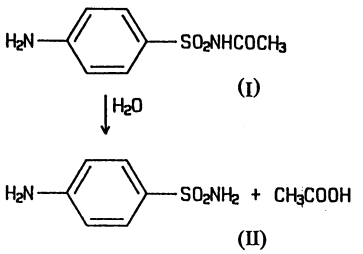

Scheme 1. Formation of sulphanilamide (II) by the hydrolysis of sulphacetamide (I).

ods are time consuming and unsuitable for the simultaneous determination of sulphacetamide and its hydrolytic product in different formulations. Hence, for post-marketing stability monitoring of sulphacetamide ophthalmic solutions a rapid, specific and stability indicating assay method would be an advantage.

In this communication we wish to report a reverse phase ion-pairing HPLC procedure capable of determining both 'intact' sulphacetamide (I) and its hydrolytic product (II) in one run, within 8 min. The validated procedure was applied to monitor the stability of commercial batches of sulphacetamide sodium ophthalmic solutions randomly obtained from the local market.

2. Experimental

2.1. Materials

Sulphacetamide and sulphanilamide USP reference standards were used as received. Methanol HPLC grade (Hiper Solv, BDH, Poole, UK), acetic acid (96%) analytical grade (E. Merck, Darmstadt, Germany), hexane-1-sulphonic acid sodium salt HPLC grade (E. Merck, Darmstadt, Germany) were used. Water was bidistilled in an all-glass still for all analytical purposes. Five commercial batches of sulphacetamide eye drops and one batch of sulphacetamide ointment with different manufacturing dates were obtained from local drug stores in Riyadh (Saudi Arabia). All the drug batches were found stored in open shelves in air conditioned pharmacies. The specifications of the batches examined are given in Table 1.

2.2. Chromatography equipment

The HPLC system used comprised of: a Waters 600E System Controller, Waters 715 Ultra WISP Sample Processor, Waters 991 Photodiode array detector and PDA integrator. A reverse phase Nucleosil C-18, 125-5, 5μ (Macherey-Nagel) column was used.

2.3. Preparation of mobile phase

The mobile phase consisted of water:methanol:acetic acid:hexane-1-sulphonic acid sodium salt in the following ratio 890:100:10:2 (v/v/v/w). It was filtered through a membrane filter (0.45 μm porosity, Millipore, MA, USA) and degassing was carried out by on-line helium purging.

2.4. Chromatographic conditions

The detection wave length 254 nm was found

Table 1
Details of the commercial batches of sulphacetamide ophthalmic preparations collected from Riyadh, Saudi Arabia for the present study

No.	Brand name	Manufacturer	Batch No.	Mfg.[a] date	Exp.[b] date
1.	Sulphacetamide 20% Eye Drops	Laboratories Cusi SA, Spain	K 2	3/96	3/98
2.	Sulphacetamide 20% Eye Drops	Laboratories Cusi SA, Spain	K 3	3/96	3/98
3.	Sulphacetamide 20% Eye Drops	Laboratories Cusi SA, Spain	K 4	4/96	4/98
4.	Sulphacetamide 20% Eye Drops	Laboratories Cusi SA, Spain	K 9	5/96	5/98
5.	Sulphacetamide 20% Eye Drops	Laboratories Cusi SA, Spain	K 13	9/96	9/98
6.	Sulphacetamide 10% Eye Ointment	Laboratories Cusi SA, Spain	96 C 19	3/96	3/98

[a] Mfg., Manufacturing.
[b] Exp., Expiration.

suitable for the determination of (I) and (II) using photodiode array detector. The sensitivity was set at 0.4 AUFS and chart speed was 5 cm min^{-1}. The column was maintained at ambient temperature. The autosampler was programmed to inject 20 μl of both standards and samples. The mobile phase was pumped isocratically with a flow rate of 1.3 ml min^{-1}.

2.5. Preparation of standard solutions

2.5.1. Sulphacetamide standard stock solution

A 50 mg quantity of suphacetamide sodium, accurately weighed, was dissolved in 50 ml of methanol. From this stock solution 5 ml were taken and diluted to 50 ml with mobile phase to give a working standard solution 100 μg ml^{-1}.

2.5.2. Sulphanilamide stock standard solution

A 50 mg quantity of sulphanilamide, accurately weighed, was dissolved in 50 ml of methanol. One milliliter of this solution was diluted to 100 ml with the mobile phase to give the working standard solution of 10 μg ml^{-1}.

2.5.3. Standard solutions for testing linearity

Various concentrations of sulphacetamide sodium were prepared by diluting the stock standard solution with the mobile phase covering the range 1–50 and 50–500 μg ml^{-1}. On the other hand, for the sulphanilamide various concentrations were prepared by diluting the stock standard solution with the mobile phase covering the range 0.1–5 and 5–50 μg ml^{-1}.

2.5.4. Synthetic mixtures for testing recovery

Synthetic mixtures containing (I) and its hydrolytic product (II) were prepared corresponding to varying levels of hydrolysis (1–10%).

2.6. Preparation of the sample solution

A 0.5 ml volume of the ophthalmic solution was diluted to 100 ml with the mobile phase. This solution was further diluted to obtain 100 μg ml^{-1} concentration.

2.7. Preparation of the samples of eye ointments

The extraction method described by BP 1993 was followed [6]. A quantity of the ointment equivalent to 0.25 g of sulphacetamide sodium was taken in a separating funnel containing 20 ml of petroleum spirit (40–60°C), 30 ml of ether and 10 ml of 2 M HCl. After thorough shaking, the acid layer was retained and the organic layer was twice extracted with 30 ml quantities of 2 M HCl. All the acidic layer was transferred to a 100 ml volumetric flask and the volume made up to 100 ml with 2 M HCl. After filtration, this solution was further diluted with the mobile phase to obtain 100 μg ml^{-1} concentration.

2.8. Assay procedure

Equal volumes (20 μl) of the standard and sample solutions were injected. The concentration of each component injected was always within the linearity range.

2.9. Calculations

These results were calculated using the peak area ratio between the sample and standard.

3. Results and discussion

A typical chromatogram obtained by using the described conditions is shown in Fig. 1. For (II), the retention time observed was 4.3 min, relative retention time (RRT) relative to the retention time of (I) was 0.59 and the calculated capacity factor (K') was 3.3. The retention time of (I) was found to be 7.3 min and K' was 6.3.

3.1. Precision

Replicate analyses of three concentrations (5, 50 and 300 μg ml^{-1}) of the standard solution of (I) showed good reproducibility. The relative standard deviation (RSD%) values for the three concentrations were 1.8%, 1.5% and 0.8% ($n = 6$), respectively. Similarly for the hydrolytic product (II) also three concentrations (0.5, 1 and 10 μg

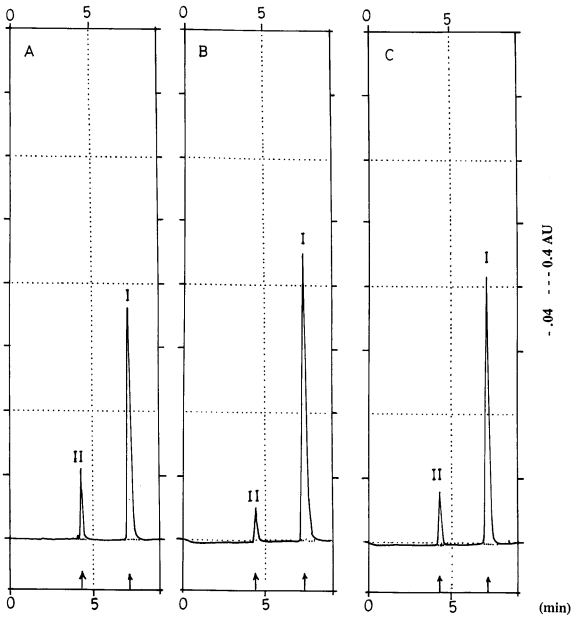

Fig. 1. Chromatograms showing the separation of sulphacetamide (I) and sulphanilamide (II) in: (A) synthetic mixture of (I) and (II); (B) sulphacetamide 20% eye drops aged 6 months; (C) Sulphacetamide 20% eye drops aged 12 months using water:methanol:acetic acid:hexane-1-sulphonic acid sodium salt (890:100:10:2; v/v/v/w) as mobile phase. UV detection 254 nm, flow rate = 1.3 ml min^{-1}.

ml^{-1}) were used where RSD% was found to be 1.9, 1.2 and 0.5% ($n = 6$), respectively.

3.2. Linearity

For sulphacetamide sodium (I), the linearity was tested over a concentration range of 1–50 and 50–500 μg ml^{-1}. The values of the correlation coefficient 'r' were 0.99998 and 0.9998, the slope 0.06030 ± 0.009 and 0.03562 ± 0.001 (SE) and the intercept 0.00056 ± 0.0042 and 0.002619 ± 0.0028, respectively. Upon linear regression analysis, the equations obtained were: $Y = 0.06030X + 0.00056$ and $Y = 0.0356234X + 0.002619$.

For the degradation product (II) the linearity tested over a range of 0.1–5 and 5–50 μg ml^{-1} which gave correlation coefficients 'r' = 0.9985 and 0.9999, with the slopes 0.01067 ± 0.0041 and 0.05487 ± 0.006, and the intercepts 0.00143003 ± 0.0007 and 0.00002819 ± 0.0003, respectively. The linear regression analysis equations obtained were: $Y = 0.01067X + 0.00143003$ and $Y = 0.05487X \pm 0.00002819$.

Intra-day and inter-day variations were found to be non-significant (RSD < 2).

3.3. Limit of quantification (LOQ)

The method has the following limit of quantification for different components:

Sulphacetamide (I)

$= 2.5$ μg ml^{-1} ($n = 6$, RSD% $\pm$ 2.21)

Sulphanilalmide (II)

$= 0.5$ μg ml^{-1} ($n = 6$, RSD% $\pm$ 1.72).

3.4. Limit of detection (LOD)

The limit of detection based on signal-to-noise ratio for (I) and (II) was found to be:

Sulphacetamide (I) = 1.25 μg ml^{-1}

Sulphanilalmide (II) = 0.25 μg ml^{-1}

3.5. Accuracy

The accuracy of the proposed method was tested by using five synthetic mixtures (containing varying levels of the hydrolytic product) and the results are presented in Table 2. The results obtained indicated a good percentage of recovery for (I) which ranged between 99.6 and 101.0 (RSD%: 0.7–1.1) and for (II) ranging between 98.9 and 101.2 (RSD%: 0.8–1.2).

3.6. Selectivity

Identical chromatograms were obtained for a synthetic mixture of sulphacetamide (I), hydrolytic product (II) and batches of the ophthalmic solutions which suffered varied degrees of hydrolysis upon storage (Fig. 1).

4. Conclusion

Sulphacetamide (I) and its degradation product

Table 2
Results of analysis of synthetic mixtures containing sulphacetamide (I) and its hydrolytic product (II) by the suggested HPLC procedure

Component	Mixture number-Composition (mg%)				
	1	2	3	4	5
Sulphacetamide (I)					
—Added (mg%)	20.0	20.0	20.0	20.0	20.0
—Recovery (%) ± RSD*	99.8 ± 1.1	99.8 ± 1.1	101.0 ± 0.8	100.4 ± 0.7	99.9 ± 0.9
Sulphanilamide (II)					
—Added (mg%)	0.2	0.5	1.0	1.5	2.0
—Recovery (%) ± RSD*	98.9 ± 1.2	99.5 ± 1.01	101.2 ± 0.90	100.7 ± 1.00	100.2 ± 0.80

* Mean of six replicates.

Table 3
Results of analysis of commercial batches of Sulphacetamide ophthalmic preparations collected from Riyadh, Saudi Arabia

Name of the Product (batch No.)	Age (months)	pH	Sulphacetamide (I) assy (± RSD)	Hydrolytic product (II) % (± RSD)
Sulphacetamide 20% Eye Drops (K2)	12	7.3	96.9 (± 0.80)	2.85 (± 0.85)
Sulphacetamide 20% Eye Drops (K3)	12	7.4	95.6 (± 0.45)	3.15 (± 1.45)
Sulphacetamide 20% Eye Drops (K4)	11	7.2	96.0 (± 0.65)	1.85 (± 1.21)
Sulphacetamide 20% Eye Drops (K9)	10	7.4	96.5 (± 0.64)	1.94 (± 0.86)
Sulphacetamide 20% Eye Drops (K13)	6	7.5	98.5 (± 0.75)	1.75 (± 0.45)
Isoptocetamide Eye Ointment 10% (96C19)	12	–	95.9 (± 1.51)	1.46 (± 0.94)

(II) which had previously been assayed by separate methods can now be assayed simultaneously using a single HPLC method. The developed method was found to be precise, reproducible, linear, accurate and rugged. The analysis of the samples obtained from local markets revealed that both ophthalmic solutions and ointment suffered varied degrees of hydrolysis (Table 3). However, ophthalmic solutions contained relatively high levels of sulphanilamide as compared with ointment. In earlier reports it has been described that suphanilamide has limited solubility (1 in 170) in water [16,17]. The concentrations of hydrolytic product (II) found in the present post-marketing stability studies of Suphacetamide ophthalmic solution revealed that storage under the prevailing conditions for longer durations of time may lead to microcrystal formation which might be injurious to eyes.

It is concluded that the proposed method has been found to be highly sensitive, time saving and appropriate for use in quality control and post-marketing stability testing of such products.

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