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Ion-pair chromatographic method for the analysis of mafenide acetate

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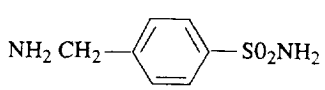
Abstract

Mafenide acetate (Sulfamylon) is an antibacterial agent used to prevent and treat burn wound sepsis. No LC method is currently available for the analysis of this drug. A simple LC method was developed and validated for the analysis of sulfamylon. The chromatographic separation was achieved on a reversed-phase, C_{18} column with UV detection at 270 nm. This isocratic system was operated at ambient temperature and required a 6-min run-time. The mobile phase consisted of methanol–phosphate buffer pH 5.0 (65:35, v/v) and 2 m*M* 1-heptanesulfonic acid as the ion-pairing agent. The flow-rate was maintained at 1.0 ml/min. Standard curves were linear over the concentration range of 2 to 100 mg l^{-1}. Within-day and day-to-day relative standard deviations were less than 2.4% and 4.4%, respectively. This method was used to quantify mafenide acetate in pharmaceutical formulation.

1. Introduction

Mafenide is the *p*-aminomethyl derivative of benzenesulfonamide (Fig. 1). This is a broad spectrum antibacterial agent used in the treatment of burn wounds [1]. It is available as a water-miscible cream containing 11.2% (w/w) mafenide acetate and its antibacterial activity is not antagonized by *p*-amino benzoic acid (PABA). The drug itself or in cream formulation is analyzed by a spectrophotometric method [2]. A thin-layer chromatographic method has also been reported for the analysis of mafenide in biological fluids [3]. No liquid chromatographic method is currently available for the analysis of the drug. Sulfonamides have been analyzed by various chromatographic techniques such as column [4–7], thin-layer [8–11] and paper chromatography [12]. Various exhaustive and updated reviews of LC methods for the determination of sulfonamides in tissues, milk and food products have also been reported [13–15]. Ion-pair thin-layer chromatography [16–21] and reversed-phase ion-pairing LC methods have also been utilized for the analysis of various sulfonamides [22,23]. All of these ion-pairing methods used basic pH media, cationic counterion (tetrabutylammonium ion) and various expensive organic solvents. The mechanism of ion-pair ex-



Mafenide

Fig. 1. Structure of mafenide.

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traction and the separation of ionic compounds by reversed-phase liquid chromatography have also been documented [24,25]. Objective of this investigation was to develop a simple, sensitive and specific LC method for the determination of mafenide acetate in pharmaceutical formulation without any complex sample extraction procedure.

2. Experimental

2.1. Materials

Mafenide acetate (Professional Compounding Centers of America, Houston, TX, USA), mafenide acetate reference standard (United States Pharmacopeial Convention, Rockville, MD, USA), mafenide acetate cream (Dow B. Hicam, Sugar Land, TX, USA), lidocaine hydrochloride (Sigma, St. Louis, MO, USA), water (HPLC grade), methanol, monobasic potassium phosphate, dibasic sodium phosphate (Fisher Chemical, Fairlawn, NJ, USA) and 1-heptanesulfonic acid monohydrate as the sodium salt (Eastman Kodak, Rochester, NY, USA) were used as received.

2.2. Chromatography

The HPLC system consisted of a pump (Model LC-600) programmed by a system controller (Model SCL-6B), an auto-injector (Model SIL-9A), an UV–Vis spectrophotometric detector (Model SPD-6AV) and a recorder (Model CR-501), all from Shimadzu (Tokyo, Japan). The separation was carried out on a 250×4.6 mm I.D. C_{18} Spherisorb column (Phase Separations, Norwalk, CT, USA). The mobile phase was methanol–phosphate buffer (pH 5) (65:35, v/v) containing 2 m*M* 1-heptanesulphonic acid. The apparent pH of the mobile phase after addition of the counterion was 4.2. The flow-rate was maintained at 1.0 ml min^{-1}. The column effluent was monitored at 270 nm.

2.3. Solutions

Phosphate buffer (pH 5.0)

Monobasic potassium phosphate (9.073 g l^{-1}) (solution A) and disodium phosphate (9.48 g l^{-1}) (solution B) were prepared in water (HPLC grade). Solution A (992 ml) was mixed with solution B (8 ml) to make the phosphate buffer.

Mobile phase

Methanol (650 ml) was mixed with 350 ml of phosphate buffer. 1-Heptanesulfonic acid monohydrate as a sodium salt (0.44 g) was then added to this solution and stirred with a magnetic stirrer. The solution was filtered through a prefilter and a 0.4-μm polycarbonate filter (Nucleopore, Pleasanton, CA, USA).

Standard solutions

Mafenide acetate standard solutions (2.0 to 100.0 mg l^{-1}) were prepared in mobile phase. The stock standard solution was prepared by dissolving 100.6 mg of mafenide acetate in 100 ml mobile phase in a volumetric flask. Various standard solutions were then prepared from this stock solution after adequate dilution with the mobile phase.

Internal standard solution

Lidocaine hydrochloride solution (616 mg l^{-1}) was prepared by dissolving 61.6 mg of lidocaine hydrochloride in 100 ml of methanol in a volumetric flask.

2.4. Sample preparation for LC

The internal standard solution (60 μl) was added to a borosilicate culture tube and evaporated to dryness at 40°C in an oven. The standard solution or sample to be analyzed (200 μl) was spiked to the test tube and vortexed for 15 s. An aliquot (20 μl) was analyzed by LC.

2.5. Calculation

The ratios of the peak height of mafenide acetate to that of the internal standard were

calculated. The unknown mafenide acetate concentration was determined from the regression equation relating the peak-height ratio (PHR) of the standards to their nominal concentrations.

2.6. Analysis of mafenide acetate in cream formulation

Mafenide acetate cream contains 11.2% (w/w) of mafenide acetate corresponding to 8.5% (w/w) of the free base along with methyl paraben, propyl paraben, sodium bisulfite and disodium EDTA as the inactive ingredients. A weighed amount of the cream (25–30 mg) was placed into 50-ml volumetric flasks and 25 ml of mobile phase was added and kept at 40°C under tightly closed condition for 30 min in a controlled temperature oven. The mixture was shaken thoroughly and the volume was adjusted to 50 ml with the mobile phase. Mafenide concentration in the solution was determined after filtration through a 0.45-μm Nylon filter.

3. Results and discussion

Conventionally the use of ion-pair partition chromatography of sulfonamides involves the interaction of the acidic-NH linkage adjacent to the sulfonyl group and a cationic counterion under basic condition. Tetrabutylammonium ion (TBA) is generally used as the counterion to form this ion-pair with the sulfonamides [22,23]. Because of the low solubility of these ion-pairs in polar medium, various non-polar solvents containing varied proportions of butanol are used as the mobile phase. However, in addition to the sulfonyl-NH group, the mafenide molecule also contains an amino group attached to the methylene group. At an acidic pH, the basic–NH group will be protonated and forms an ion-pair with the available anionic counterion, e.g. 1-heptanesulfonic acid. These ion-pairs will have a better solubility in the polar mobile phase (methanol and aqueous buffer solutions) as compared to the ion-pairs formed with the TBA and thereby simple, inexpensive polar mobile phase can be utilized for their separation. With this hypothesis in mind, we investigated the effect of different anionic counterion concentrations in the mobile phase on the peak shape and separation of mafenide acetate. Presence of 2 m*M* 1-heptanesulfonic acid showed the best results and the chromatograms of mafenide acetate and the internal standard in mobile phase did not show any interfering peaks. The repeatability of the retention time of mafenide acetate and lidocaine hydrochloride was determined from 30 consecutive injections during an analysis of a series of mafenide acetate samples. The relative standard deviations (R.S.D., %) were found to be 0.25 and 0.36% for mafenide acetate and lidocaine hydrochloride, respectively.

3.1. Validation of the mafenide acetate assay

Linearity

The standard curves were linear over the concentration range 2.0–100 mg l^{-1}. The equation of the standard curve relating the peak-height ratio (P) to the mafenide acetate concentration (C in mg l^{-1}) in this range was:

$$P = 0.026C + 0.0012,\ r^2 > 0.999.$$

Precision

Within-day precision was determined by analysis of four different standard curves on the same day. Day-to-day precision was determined by the analysis of the same solutions on seven different days over a period of 45 days. During this period, the solutions were stored at room temperature (25°C) and solutions for the standard curves were prepared fresh each day from the stock solution. The variability in the peak-height ratio at each concentration was used to determine the precision of the assay procedure and presented in Table 1. Within-day and day-to-day R.S.D. values ranged from 0.3 to 2.4% and 0.3 to 4.4%, respectively.

Accuracy

Three quality control samples and the standard solutions were stored at room temperature

Table 1
Within-day and day-to-day analytical precision

Within-day[a]			Day-to-day[b]	
Concentration ($mg\ l^{-1}$)	Mean peak-height ratio[c]	R.S.D. (%)	Mean peak-height ratio[d]	R.S.D. (%)
0.00	0.00	–	0.00	–
2.0	0.06	2.4	0.06	1.8
6.0	0.15	0.9	0.16	4.4
10.1	0.26	1.1	0.26	1.5
20.1	0.50	0.9	0.51	3.6
50.3	1.33	1.0	1.32	1.2
70.4	1.82	0.3	1.83	0.6
100.6	2.59	0.3	2.59	0.3
Slope	0.026 ± 0.0005	1.8	0.026 ± 0.0004	1.5

[a] Analyzed on the same day.
[b] Analyzed on seven different days within a period of 45 days.
[c] Mean; $n = 4$.
[d] Mean; $n = 7$.

(25°C) over a period of 45 days. The quality control samples were prepared from the USP reference standard. These samples were analyzed several times during this period and the accuracy of the assay was determined by comparing the measured concentration to its nominal value (Table 2). The R.S.D. ranged from 1.0 to 2.2%.

Sensitivity

The sensitivity criteria were determined from seven different standard curves using the lowest limit of reliable assay measurement criteria as described by Oppenheimer et al. [26]. The critical level is the assay response above which an observed response is reliably recognized as detectable. This was $0.25 \pm 0.11\ mg\ l^{-1}$ (mean ± S.D.). The detection level is the actual net response which may a priori be expected to lead to detection. This was $0.49 \pm 0.22\ mg\ l^{-1}$ (mean ± S.D.). The determination level, the concentration at which the measurement precision will be satisfactory for quantitative determination, was $1.15 \pm 0.49\ mg\ l^{-1}$ (mean ± S.D.) for a level of precision of 10% R.S.D.

Table 2
Accuracy in the analysis of mafenide acetate in quality control samples

Actual concentration ($mg\ l^{-1}$)	Measured concentration[a] ($mg\ l^{-1}$)	Accuracy[b]
4.0	4.0 ± 0.15	99.9 ± 1.8
30.2	30.8 ± 1.3	101.9 ± 2.2
80.5	81.7 ± 1.7	101.5 ± 1.0

[a] Mean ± S.D.; $n = 7$.
[b] Accuracy = (measured concn/actual concn) · 100.

3.2. Applications of the LC method

This LC method was used to measure mafenide acetate content in pharmaceutical formulation and also to evaluate its stability in stock standard solutions.

Cream formulation

Mafenide acetate is only available as a cream formulation. The analysis of the drug in this formulation is generally carried out by a spectrophotometric method [2]. According to USP, it should contain 90–110 percent of mafenide acetate in terms of the labeled amount of mafenide. We were interested to determine the mafenide

acetate content in the cream formulations using this LC method. Sample chromatograms of the cream formulation and cream spiked with the internal standard are shown in Fig. 2. In Fig. 2a the peak with a retention time of 2.6 min corresponds to the drug and peaks with retention time of 3.7 and 5.9 min correspond to the inactive ingredients in the cream. From the chromatograms, it was evident that slight interference exists with one of the inactive ingredients of the cream and the internal standard peak (retention time 5.3 min; Fig. 2b). The accuracy of this method in determining the mafenide acetate in the cream formulation was then determined by using peak-height ratio (PHR), absolute peak area (PA) and absolute peak heights (PH). The drug content in the cream was determined and reported as the mean percentage of the nominal concentration. The mean percentage of the nominal concentration determined by the three different measurements were 98.4 ± 3.6, 98.3 ± 3.5 and 97.6 ± 3.8 (mean ± S.D., n = 8) for PHR, PA and PH respectively. This study also indicated that there is no significant differences among the methods as determined by one way ANOVA. The potency of all the cream products analyzed also falls within the range set by the USP. The analysis of mafenide acetate in the commercial product required a sample filtration step through a 0.45-μm Nylon syringe filter prior to injection onto the LC system. The loss of drug, if any, during this process was then evaluated. Standard solutions were injected onto the LC system prior to and after filtration through 0.45-μm Nylon filters. The absolute peak heights of the standard solutions were compared. The results indicate that the filtration step does not have any influence on the absolute peak height of the drug.

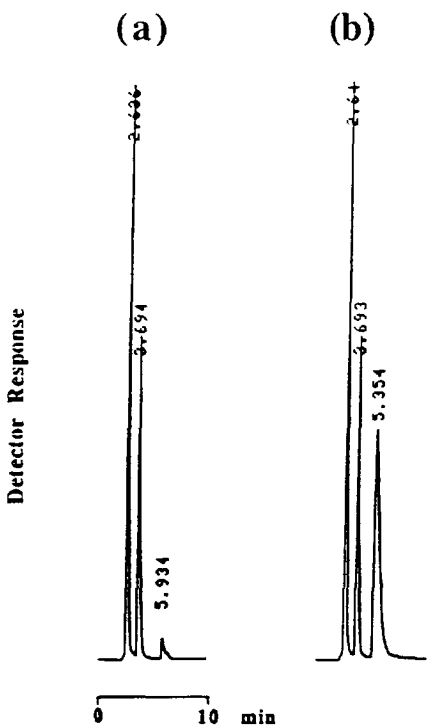

Fig. 2. Representative chromatograms obtained following injection of (a) sample from mafenide acetate cream (with a retention time of 2.6 min for the drug), and (b) sample from mafenide acetate cream spiked with the internal standard (lidocaine-HCl 185 mg l^{-1}).

Stability of mafenide in solution

During the preliminary method development work, the standard and the internal standard solutions were kept at refrigerated temperature (≈4°C). After four days of storage, the low soluble component (disodium phosphate) of the phosphate buffer in the mobile phase was precipitated out from the solution and this was confirmed by IR analysis. Therefore, all the solutions were kept at room temperature (≈25°C) over a period of 45 days. The precipitation problem was avoided by storing these solutions at room temperature. No degradation product was detected in the chromatograms during this period. The slopes of the standard curves were consistent over this period indicating that the compound is quite stable in the mobile phase over a period of at least 45 days at room temperature.

4. Conclusions

A simple, sensitive, and reproducible method was developed for the analysis of mafenide acetate alone, and in pharmaceutical formulation. Ion-pairing was achieved by an anionic

counterion and mafenide acetate, in acidic solution. The method did not require any complex extraction procedure prior to the LC analysis and used a less expensive mobile phase system. The stability studies indicated that the drug was stable in the mobile phase at 25°C over a period of at least 45 days.

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