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Note

# Thin-layer chromatographic determination of mafenide [(*p*-aminomethyl) benzenesulphonamide] in human serum

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Mafenide (Sulfamylon) is often used to combat infection in patients with burn wounds [1, 2], but it has been found [1—3] that an undesirable side-effect is the inhibition of carbonic anhydrase. As this enzyme is important with regard to the buffering system by which the kidney rids the body of excess of hydrogen ions, its inhibition would have the effect of reducing the kidney's efficiency as a protective organ against metabolic acidosis. High blood levels of mafenide may lead to acidosis and therefore the ability to monitor the blood levels of this drug may be of benefit to the patient.

This paper describes a simple, accurate method for the determination of mafenide in human serum. The method consists in partially deproteinating a small volume of serum, spotting the supernatant on a thin-layer chromatographic (TLC) plate together with serum standards, developing the plate and subjecting the mafenide to reaction in situ with fluorescamine to induce fluorescence. The intensities of the fluorescence of the various spots are then measured and peak heights of the standards are used to calculate the concentration of mafenide in unknown samples.

## EXPERIMENTAL

### *Reagents*

All reagents and solvents were of guaranteed reagent grade (E. Merck, Darmstadt, G.F.R.). Mafenide (Sulfamylon) was supplied by the Winthrop Laboratories Division of Sterling Drugs (Mobeni, Durban, South Africa). Fluorescamine was obtained from Hoffman-La Roche Diagnostica (Basle, Switzerland).

The spray reagent was 15 mg of fluorescamine dissolved in 200 ml of

acetone. The thin-layer developing solvent was an ethyl acetate—methanol—ammonia (75 : 20 : 5) solution.

*Apparatus*

A Perkin-Elmer MPF 3 spectrofluorimeter equipped with a thin-layer scanning attachment was used to measure the fluorescence of the spots on the thin-layer plates using the following operating conditions: light source, xenon lamp; excitation wavelength, 390 nm; excitation slit width, 10 nm; emission wavelength, 490 nm; scan speed, "high"; paper speed, 2.5 cm/min. The emission slit width, amplifier sensitivity and sample adjustment were set to obtain about 80% of full-scale deflection on the recorder when the strongest spot in the chromatogram was being scanned.

Other equipment included silica gel 60 TLC plates (Merck) and 5-µl disposable glass micropipettes (Clay-Adams, Division of Becton, Dickinson & Co., Parsippany, N.Y., U.S.A.).

*Stock solutions*

Stock solutions were made up in absolute methanol and stored at −20°. Stock solutions containing 1, 2, 4, 8, 10, 15, 20 and 40 µg mafenide per 100 µl of methanol were prepared as follows. The mafenide was weighed on a Mettler ME 22 electronic microbalance and dissolved in absolute methanol to yield a solution containing 10 mg of mafenide per 10 ml of methanol. By further appropriate dilutions, the above stock solutions were prepared. These stock solutions can be stored at −20° for at least a month without deterioration.

*Standard solutions*

Eight standard solutions were prepared by evaporating under nitrogen 100 µl of each of the above stock solutions in screw-capped bottles and then adding 1 ml of fresh, drug-free human serum. The containers were tightly closed and allowed to stand at room temperature for 1 h, with agitation at regular intervals to ensure complete dissolution of the mafenide. These standard solutions were freshly prepared on each occasion when a series of analyses were to be performed.

*Preparation of serum*

A 50-µl volume of serum (standard or unknown) was measured accurately into a small, stoppered, conical centrifuge tube, then 150 µl of methanol were added in order to precipitate the proteins. The contents of the tubes were mixed thoroughly by means of a Whirlimixer and centrifuged for 2 min to produce a clear supernatant liquid.

*Spotting the plates*

A 10-µl volume of the clear supernatant liquid was applied to the thin-layer plate in two equal portions of 5 µl. The liquid was applied in one smooth application and it was allowed to run on to the plate by the natural capillary action of the plate and gravity alone. The spot was dried with a hair-drier between applications. In this fashion, standard sera and unknown sera,

alternating in duplicate, were applied to a 10 × 20 cm plate. This procedure allowed four determinations on unknown sera to be carried out in duplicate.

*Spraying the plate*

The solvent was allowed to migrate to a height of 5 cm above the point of application, whereupon the plate was dried in an oven. After cooling, the plate was sprayed with the fluorescamine solution. The spraying apparatus must deliver a very fine mist and for optimal results great care must be taken to spray the plate evenly.

## RESULTS AND DISCUSSION

The fluorescence peak heights measured for the serum standards were used to plot a standard graph of peak height versus mafenide concentration. Mafenide concentrations of unknown sera could be obtained by interpolation from this graph.

When determining the concentration of mafenide from unknown sera, standards between 1.0 and 8.0 μg/ml were applied to the plate. Whenever a sample with a higher concentration than 8.0 μg/ml was found, the determination was repeated with serum standards between 10 and 40 μg/ml. In these instances it was sufficient to apply only 5 μl of the supernatant.

*Reproducibility and accuracy*

The accuracy and reproducibility of the method were determined by preparing sera containing known amounts of mafenide and having these sera analyzed by a technician to whom the actual concentrations had not been revealed. The results were very satisfactory and are presented in Table I.

TABLE I

REPRODUCIBILITY AND ACCURACY OF THE DETERMINATION OF MAFENIDE IN SERUM

| Mafenide added (μg/ml) | Mafenide recovered (μg/ml) | Mean ± S.D. (μg/ml) |
|---|---|---|
| 1.6 | 1.45 | 1.49 ± 0.03 |
| | 1.52 | |
| | 1.48 | |
| | 1.50 | |
| 8.5 | 8.0 | 8.39 ± 0.54 |
| | 7.85 | |
| | 8.90 | |
| | 8.80 | |

*Specificity*

A variety of sulpha compounds (sulphisoxazole, sulphasomidine, sulpha-

dimethoxine, sulphafurazole, sulphamoxole, sulphatolamide and sulphanilamide) were tested for possible interferences, but none was found.

*Limit of detection*

The lower limit of detection that was possible without losing accuracy was 1 μg/ml. The response of the fluorimeter to the fluorescence was found to be linear up to 600 ng of mafenide.

REFERENCES

1 J.M. Shuck and J.A. Moncrief, *Amer. J. Surg.*, 118 (1969) 864.
2 Editorial Staff, *Drugs*, 1 (1971) 435.
3 H.N. Harrison, H.W. Bales and F. Jacoby, *J. Trauma*, 12 (1972) 994.