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Short Communication

A normal-phase high-performance liquid chromatographic assay for aziridine residue in trientine dihydrochloride

E. M. MAY[1]*, D. C. HUNT[1] and J. SLOGGEM[2]

[1] *Laboratory of the Government Chemist, Cornwall House, Waterloo Road, London SE1 8XY, UK*
[2] *Scientific and Technical Branch, DHSS, 14 Russell Square, London WC1 5EP, UK*

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Introduction

In order to issue a product licence for the new drug trientine dihydrochloride, which is used in the treatment of Wilson's disease, the Department of Health and Social Security as the Licensing Authority in the UK needed to be satisfied that the potent carcinogen aziridine was not present. This required the development of a method capable of detecting the compound at very low levels.

The starting material for the drug trientine dihydrochloride is the commercially available trientine base (triethylenetetramine, TETA), which is made by reacting ammonia with 1,2-dichloroethane at high temperature. The product is a mixture of polyamines in various isomeric forms and varying chain lengths. This mixture can contain aziridine but by subjecting it to fractional distillation aziridine is separated from the higher boiling fractions. The trientine fraction contains four isomeric tetramines and smaller amounts of other amines [1]. Since aziridine is stable in bases in the absence of water, it is conceivable that, if distilled over the trientine fraction, trace amounts could remain. Accordingly, it was necessary to analyse commercial trientine base, the purified intermediate and trientine dihydrochloride to determine any trace levels of aziridine. Headspace analysis was not considered because it could be argued that aziridine was present as an involatile salt. A method utilising the rapid reaction between aziridine and Folin's reagent (sodium salt of 1,2-naphthoquinone-4-sulphonic acid) to form the 4-aziridinyl-1,2-naphthoquinone complex was evaluated. This complex is readily soluble in chloroform, giving a yellow solution which absorbs light at 410 nm [2].

*To whom correspondence should be addressed.

Preliminary work showed that trientine and other amines also formed complexes with Folin's reagent which give similar UV spectra (Fig. 1). Thus it was not possible to carry out a simple spectrophotometric assay.

Thin-layer chromatography of the chloroform extracts of the complexes of trientine base, its dihydrochloride and aziridine showed that these complexes could be separated by normal-phase chromatography. As the aziridine complex was separated from the TETA complexes (Fig. 2), the system was then transferred to HPLC using conditions similar to those described by Evans and Mayfield [3]. It was not possible to resolve peaks of both complexes isocratically. Since resolution of the trientine complexes was not important, it was decided to optimize the system for the resolution of the aziridine complexes.

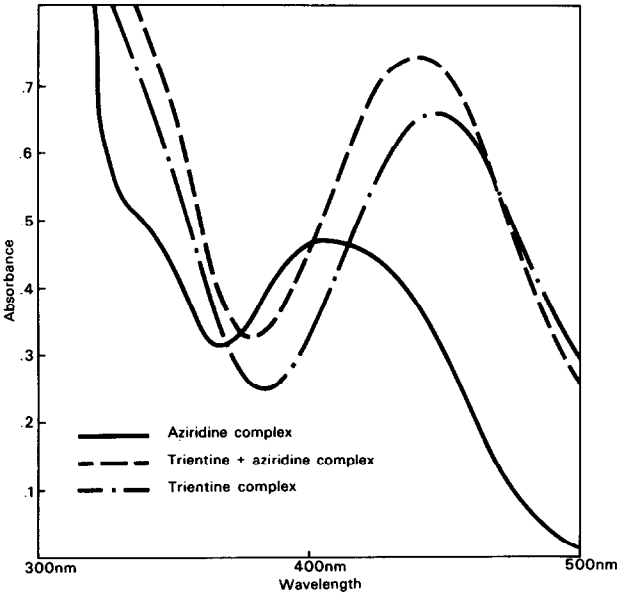

Figure 1
Ultraviolet spectra of trientine, aziridine and trientine/aziridine mixture at concentrations of 0.125% trientine and 0.0005% aziridine in chloroform.

Experimental

Apparatus

The modular apparatus comprised: Waters 6000A pump; a dual channel UV detector (Beckman model 165) adjusted to 260 and 410 nm at 0.05 a.u.f.s.; a column 250 × 4.6 mm (Lichrosorb 5-μm silica 60) and Rheodyne 7125 injection valve with 200 μl loop. The mobile phase used was dichloromethane–2-propanol (98.5:1.5 v/v). The flow rate was 2.0 ml min^{-1} at 2000 psi.

Peak areas were evaluated using a Shimadzu integrator at 410 nm, this wavelength being more selective for the aziridine complex. Monitoring at 260 nm was employed to confirm the absence of any co-eluting interfering decomposition products from Folin's reagent.

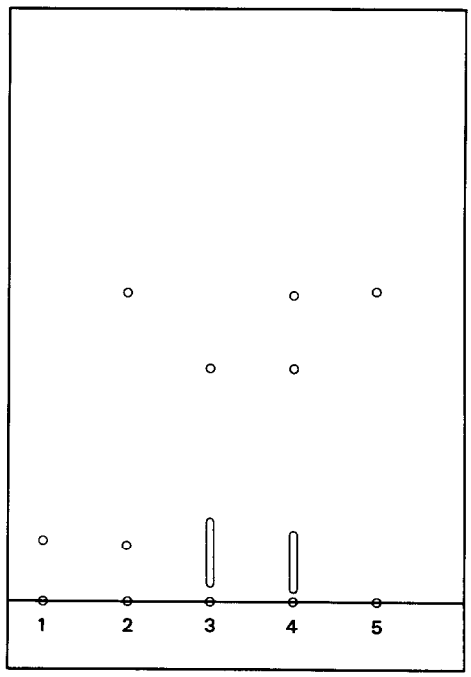

Figure 2
Thin-layer chromatograms of chloroform extracts used in the UV scans. 1, Trientine dihydrochloride; 2, trientine dihydrochloride + aziridine; 3, trientine base; 4, trientine base + aziridine; 5, aziridine. Loading equivalent to 125 μg of trientine and 0.1 μg of aziridine. Plate Si F_{254}, mobile phase chloroform–methanol 90:10.

Reagents

The reagents and materials used were: 1,2-naphthoquinone sulphonic sodium salt specially purified (BDH); sodium phosphate buffer 0.05 M Na_3PO_4 (pH 11.7); chloroform (AnalaR grade) dried by passing through a column of silica (Merck 230 μm); dichloromethane (HPLC grade), dried by passing through a column of silica (Merck, 230 μm). Trientine base was obtained from Dow Chemical Co. The dihydrochloride was obtained from K & K Greef. Aziridine (99%) obtained from Serva was stored over sodium hydroxide.

Evaporation under vacuum was carried out using a Büchi Rotavapor RE.

It should be noted that aziridine is a potent carcinogen and must be handled with care.

Preparation of the complex

1 ml of standard aziridine (1 μl/100 ml water), 50 ml water, 1 ml phosphate buffer (pH 11.9) and 25 mg of Folin's reagent were shaken until the Folin's reagent was dissolved and the mixture was then allowed to stand for 1 min. Dry chloroform (20 ml) was added, shaken well and allowed to stand for 1 min. The chloroform extract was filtered through a silicone-treated filter paper and 200 μl injected onto the HPLC system.

A typical chromatogram showed two peaks (Fig. 3), the first arising from the Folin's reagent. This reagent slowly decomposes in alkaline solutions, so a blank determination was carried out daily to confirm the absence of any interfering decomposition products. The aziridine standard should be freshly prepared daily, and all reactions must be carried out in an effective fume cupboard, in subdued light and with appropriate precautions in handling aziridine.

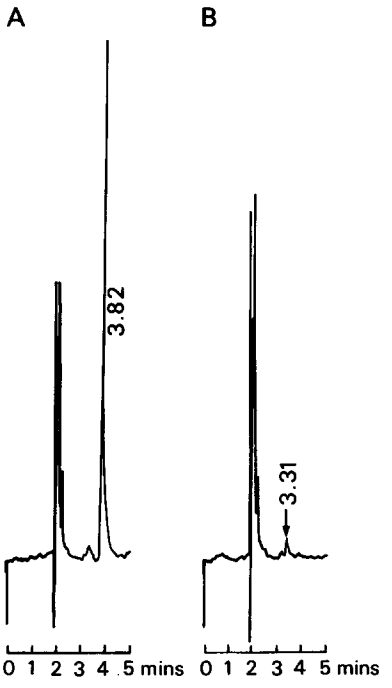

Figure 3
HPLC chromatograms. (A) Aziridine standard complexed with Folin's reagent; (B) Folin's reagent alone. Conditions as in text.

Calibration

Aziridine standards (0.05 μl/100 ml to 1 μl/100 ml; $n = 6$) were subjected to the proposed procedure. A calibration graph of peak area against concentration was found to be linear and passed through the origin, the regression being $y = 0.54x$. The relative standard deviation of six injections was 1.5% at 1 μl/100 ml.

Determination of aziridine in the presence of trientine

In order to ascertain whether aziridine could be detected in the presence of large amounts of trientine compounds, experiments were carried out in which aziridine was added at the ppm level to strong solutions (i.e. 10%) of trientine. The two complexes should then have been extractable with chloroform. However, problems arose due to the incompatibility of trientine and Folin's reagent in strong solutions. Naphthoquinones are unstable in strong alkali and form a humus-like precipitate above pH 11.0. Both trientine base and the intermediate have a pH above 11 in strong solution, and sufficient Folin's reagent must be added to complex all the amino groups of trientine, in addition to the aziridine. 10 g of trientine plus 10 μg of aziridine require 100 g of Folin's reagent, but the addition of this large quantity gave a thick dark brown precipitate which prevented satisfactory extraction. These difficulties limited this method to a sensitivity of 12 ppm of aziridine, which was considered unsatisfactory. Other methods involving the separation of aziridine from the trientine base before complexing were therefore considered.

Purging the aziridine from aqueous solutions of trientine into Folin's reagent using nitrogen was unsuccessful. The aziridine remained intact in the original solution, even after heating at 70°C for 30 min. This led to the conclusion that aziridine forms a constant boiling mixture and can only be removed by distilling to dryness under reduced pressure.

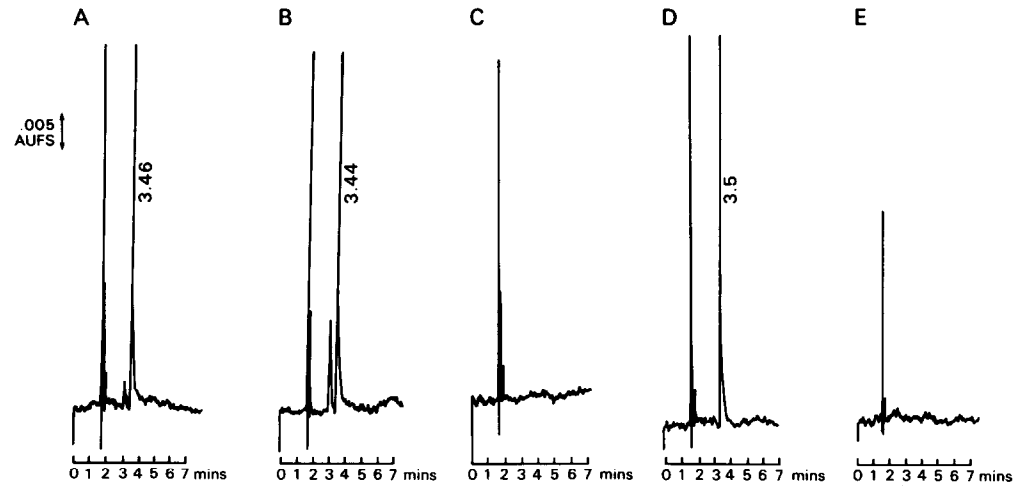

Figure 4
HPLC chromatograms. (A) 1 ml aziridine standard complexed after passing through rotary evaporator, 80% recovery when compared with B. (B) 1 ml aziridine standard complexed, not passed through rotary evaporator. (C) 10 g trientine dihydrochloride complexed after passing through rotary evaporator. (D) 10 g trientine dihydrochloride plus 1 ml (10 μg) aziridine standard complexed. (E) Blank of Folin's reagent, through rotary evaporator. Conditions as in the text. The 200 μl injection is 0.1 μg and represents 1 ppm in the sample.

Using a rotary evaporator it was found possible to recover 100% of 10 μg aziridine added to 10 g of trientine. This method has the advantage of leaving behind the main bulk of the trientine, since only minor volatile components from the trientine base pass over with aziridine.

The final experimental procedure developed was as described below:

(1) 1.0 ml of standard aziridine solution, 50 ml water, 1 ml phosphate buffer (pH 11.9) and 25 mg of Folin's reagent were treated as previously described.
(2) 1.0 ml of standard aziridine plus 50 ml water was heated at 70°C on a rotary evaporator to dryness, the distillate collected in a cooled flask and then complexed and prepared as in (1) above. 80–100% recovery was achieved when compared with (1) which showed that no significant losses are sustained during the rotary evaporation.
(3) 10 g of trientine base, intermediate or dihydrochloride, plus 1.0 ml of aziridine standard and 50 ml of water were heated on a rotary evaporator at 70°C until no more distillate was produced. The distillate was complexed using 500 mg of Folin's reagent. 80–100% recovery should be achieved when compared with (1) and (2) before proceeding to (4) to prove that aziridine can be recovered from the sample under test.
(4) As step (3) but no aziridine was added.

Sensitivity and detection limit

Using the method as described, samples of trientine base, the intermediate and of the dihydrochloride spiked with aziridine at the 1 ppm level gave in each case a peak height 75% full scale deflection for aziridine by HPLC. The detector response is equivalent to 1 ppm in the sample. The detection limit is 0.05 ppm.

Results

Seven samples of trientine were examined for the presence of aziridine by the described method. No aziridine was detected in any of the samples.

Discussion

Although aziridine will be formed during the reaction between ammonia and 1,2-dichloroethane, subsequent fractional distillation of the trientine portion should remove any aziridine, as the boiling point of aziridine is 56°C compared with 266°C for trientine.

Aziridine is very reactive, particularly when protonated (pKa = 7.98): for example, in aqueous solutions it forms ethanolamine. Aziridine is miscible with water and virtually all organic solvents. It is volatile, with a vapour pressure of 160 mm at 20°C. During the production process for trientine dihydrochloride, there are a number of stages at which any aziridine, if present, would be removed due to its solubility in solvents, its reactivity and its volatility.

In conclusion, an analytical method has been developed which can detect aziridine below 0.1 ppm level in basic related drugs. The method should be adaptable for basic drugs in general. Samples of trientine base, its intermediate and the final product, the new drug trientine dihydrochloride, have been examined and no aziridine detected.

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References

[1] L. Bergstedt and G. Widmark, *Acta chem. scand.* **24**, 2713–2723 (1970).
[2] D. H. Rosenblatt, P. Hlinka and J. Epstein, *Anal. Chem.* **27**, 1290–11293 (1955).
[3] D. J. Evans, R. J. Mayfield and I. M. Russell, *J. Chromatogr.* **115**, 391–395 (1975).

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