THE PHOTOMETRIC DETERMINATION OF 2-AMINO-5-NITROTHIAZOLE AND CERTAIN ACYL DERIVATIVES

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The use of 2-amino-5-nitrothiazole in veterinary medicine makes it desirable to have available a method for its determination in aqueous solution and in blood. Although diazotisation of aminothiazoles in hydrochloric acid yields chlorine substituted products,[1] in sulphuric acid the normal diazo-salt is formed. Optimum conditions were, therefore, established for diazotisation of aminonitrothiazole in sulphuric acid followed by coupling with *N*-naphthylethylenediamine and a method for the determination of aminonitrothiazole in aqueous solution was evolved. This method was applicable to the phthalyl and succinyl derivatives after hydrolysis and to blood after a suitable deproteinisation procedure.

Proposed Method

Reagents

(1) Sulphuric acid 20N (55 per cent. v/v).
(2) Sodium nitrite solution 2 per cent.
(3) Sulphamic acid solution 2·5 per cent.
(4) *N*-naphthylethylenediamine hydrochloride solution 0·4 per cent.
(5) Trichloracetic acid 10 per cent.

Procedures

(*a*) *For simple solutions of aminonitrothiazole.* Into a 25-ml. flask, pipette 1 ml. of a solution containing up to 0·5 mg. of aminonitrothiazole in dilute sulphuric acid, add 5 ml. of 20N sulphuric acid and cool in ice-water for 2 minutes. Add 1 ml. of 2 per cent. sodium nitrite, mix and leave for 4 minutes; add 1 ml. of 2·5 per cent. sulphamic acid, mix and leave for 4 minutes. Add 1 ml. of 0·4 per cent. *N*-naphthylethylenediamine hydrochloride solution, mix and leave for 2 minutes. Remove the flask from the ice bath, dilute to 25 ml. with ethanol (95 per cent.) and within 5 minutes of dilution measure the extinction of 1 cm. at 580 mμ. or using a suitable filter. Read off the amount of aminonitrothiazole from a standard curve prepared using pure aminonitrothiazole. Calibration data are given in Table I.

(*b*) *For succinylaminonitrothiazole.* Pipette 1 ml. of solution containing up to 0·8 mg. of succinylaminonitrothiazole into a 25-ml. flask, add 5 ml. of 20N sulphuric acid and heat in a boiling water bath for 40 minutes. Cool in ice-water for 5 minutes and proceed as in (*a*) commencing with the words "Add 1 ml. of 2 per cent. sodium nitrite solution." Read off the amount of succinylaminonitrothiazole from a standard curve prepared by this method, using pure succinylaminonitrothiazole.

(*c*) *For phthalylaminonitrothiazole.* Proceed as for succinylamino-nitrothiazole, but heat for 90 minutes at 100° C. instead of for 40 minutes.

(*d*) *For aminonitrothiazole and acyl derivatives in blood.* To 1 ml. of blood in a small centrifuge tube add 2 ml. of water and mix, add 1 ml. of 10 per cent. trichloracetic acid, mix well and centrifuge for 2 minutes. If aminonitrothiazole is present, pipette 1 ml. of the supernatant liquid into a 25 ml. flask, add 5 ml. of 20N sulphuric acid and continue as in (*a*), commencing with the words "cool in ice/water for 2 minutes." If succinyl- or phthalyl-aminonitrothiazole is present, treat 1 ml. of solution as in (*b*) and (*c*) respectively.

Notes on the Method

Procedure (*a*). Variation in acidity affects the tint but not the intensity of the final colour. The purpose of the ethanol is to prevent precipitation of the pigment and minimise fading which, in the final method, is at the rate of about 1 per cent. in 3 minutes. The reproducibility of the method is illustrated by the following values obtained in determinations carried out in pairs at different times; 0·810, 0·810; 0·800, 0·810; 0·813, 0·800; 0·815, 0·815; 0·810, 0·805.

TABLE I

CALIBRATION DATA FOR THE DETERMINATION OF AMINONITROTHIAZOLE IN AQUEOUS SOLUTION

Aminonitrothiazole mg.	Extinction of 1 cm. at 580 mμ	Slope
0·05	0·165	3·3
0·10	0·335	3·4
0·15	0·505	3·4
0·20	0·65	3·3
0·25	0·77	3·1
0·30	0·88	3·0
0·35	1·01	2·9
0·40	1·145	2·9
0·45	1·26	2·8

Procedures (*b*) *and* (*c*). Under the conditions used for the hydrolysis of phthalyl- and succinyl-aminonitrothiazole, hydrolysis to aminonitrothiazole is complete and no decomposition of the latter occurs.

Procedure (*d*). When determinations were made on blood to which known amounts of aminonitrothiazole had been added, recovery was 70 per cent. owing to the adsorption which commonly occurs in deproteinisation. Allowance may be made for this either by applying a correction or by using a calibration curve based on data obtained by applying the method to aminonitrothiazole in the presence of blood.

SUMMARY

1. A photometric method, based on diazotisation and coupling, for the determination of aminonitrothiazole and its phthalyl- and succinyl-derivatives is proposed.

2. The method is applicable to blood after the deproteinisation procedure described.

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REFERENCE

1. Morgan and Morrow, *J. chem. Soc.*, 1915, **107**, 1291.