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Quantitative gas chromatographic determination of 5,5-dimethyl-2,4-oxazolidinedione

5,5-Dimethyl-2,4-oxazolidinedione (DMO) is a weak acid whose acid hydrogen linked with nitrogen can be titrated with soda in the presence of phenolphthalein as described by STOUGHTON[1]. This simple method for DMO evaluation has been adopted in control laboratories where relatively concentrated solutions (*e.g.*, 2.5 %) are analysed. Unfortunately, when the quantitative estimation is required in biological fluids such as urine, plasma, and tissue homogenates, this method presents insuperable difficulties. These are mainly due to the small amount of substance present and the great variety of other matter which interferes with the titration in such fluids.

BUTLER AND WADDEL have developed a selective extraction method for DMO from biological liquids using a borate buffer, and a quantitative determination of DMO based on characteristic UV absorption[2-4]. We have already proved that this method is valid[5-8].

Apart from BUTLER AND WADDEL's method and the radioisotopic evaluation of [2-14C]DMO[9, 10], no other procedures for the determination of DMO in biological fluids have been described in the literature.

The purpose of this study was to investigate the gas-chromatographic behaviour of DMO and see whether it might be applied to the determination of this product. It is a well-known fact that the most reliable method at present available for calculating body and muscle intracellular pH is based on DMO distribution in the body, and the quantitative determination of this material is thus a prerequisite[2, 6, 10].

Equipment and working conditions

A Carlo Erba Fractovap GV gas chromatograph equipped with a flame ionization detector and stainless steel columns, 2 m × 2 mm I.D., was used. The columns were filled with 10 % butanediol succinate on Gas-Chrom Q (90–100 mesh), 10 % Carbowax 20 M on Chromosorb W (60–80 mesh), and 2 % SE-30 on Chromosorb W (60–80 mesh).

The working conditions for the 10 % butanediol succinate on Gas-Chrom Q column are as follows: column temperature 213°; detector temperature 130°; injector temperature 263°; nitrogen flow 20 ml/min.

Synthesis of 5,5-dimethyl-2,4-oxazolidinedione

The DMO was prepared according to STOUGHTON's method[1] starting from the methyl ester of α-hydroxy-isobutyric acid and anhydrous urea in the presence of sodium and anhydrous ethyl alcohol.

The final product, when crystallized three times from benzene and dried in a vacuum at 40°, proved to be pure from a gas chromatographic standpoint. The corrected m.p. was 76–77°. Elementary analysis for nitrogen gave $N_2 = 10.87$ % (theoretical $N_2 = 10.85$ %).

Solutions in distilled water were injected into the gas chromatograph.

Synthesis of 5-methyl-2,4-oxazolidinedione (MO)

MO was synthesized by the same method as DMO starting from the methyl

ester of lactic acid. The final product when distilled in a vacuum at 142° at 1 mm Hg also proved to be pure from a gas chromatographic angle. The m.p. was 49°. Elementary analysis for nitrogen gave N_2 = 12.03 % (theoretical N_2 = 12.17 %).

Solutions in distilled water were injected into the gas chromatograph.

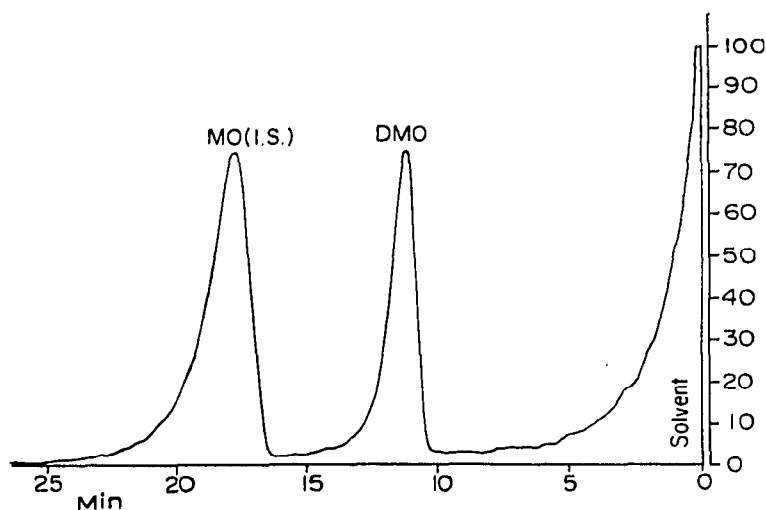Fig. 1. Gas chromatogram showing a separation between DMO and MO (internal standard). Analytical conditions: column, 2 m × 2 mm I.D., filled with 10 % butanediol succinate on Gas-Chrom Q (90–100 mesh); column temperature = 213°; evaporator temperature = 263°; detector temperature = 130°; nitrogen flow = 20 ml/min; flame ionization detector.

Results and discussion

All three columns used gave satisfactory peaks both with DMO and MO. The best separation of both peaks was obtained with the column containing 10 % butanediol succinate on Gas-Chrom Q (90–100 mesh).

When this column was used under the conditions described above the DMO retention time, relative to that of MO, was 0.64 (Fig. 1). Although the molecular weight of MO is less than that of DMO, it is retained in the column longer probably because the MO molecule has a higher polarity than the DMO molecule.

Results obtained by analysing a number of solutions with various MO/DMO weight ratios are reported in Table I. Fig. 2 shows that there is a linear correlation between area ratios (A_R) and weight ratios (W_R). The best straight line was obtained by the least squares method using an Olivetti P 101 computer. The standard deviation of the mean weight factor of response $(f = 2.59 \pm 0.088)$ shows that the analytical method adopted is highly reproducible. These data indicate that MO may be used as an internal standard for the quantitative determination of DMO. The smallest amount of DMO which could be determined quantitatively was 1 μg.

In conclusion, the gas chromatographic evaluation of DMO using MO as an internal standard appears to be highly sensitive. In addition, it is superior to WADDEL AND BUTLER's UV-spectrophotometric method as regards selectivity.

In view of these properties we believe that the gas chromatographic method may be used instead of the costly radioisotopic method even when bioptical samples are available.

The method described here has already been applied[11] to the measurement of

TABLE I

AREA RATIOS (A_R) BETWEEN MO AND DMO AND RESPONSE WEIGHT FACTORS $(\bar{f})$ OBTAINED BY GAS CHROMATOGRAPHIC ANALYSIS OF 6 SOLUTIONS CONTAINING DIFFERENT MO/DMO WEIGHT RATIOS (W_R)

Analytical details are given in Fig. 1.

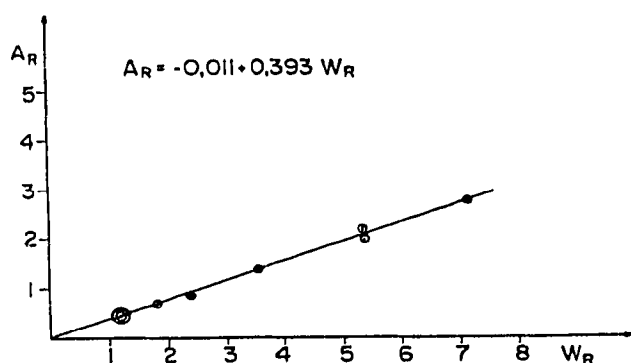| Solution	$W_R = MO/DMO$	$A_R = MO/DMO$	$\bar{f}$
I	7.16	2.79	2.68
2a	5.37	2.22	2.42
2b	5.37	2.01	2.67
3	3.58	1.40	2.56
4	2.39	0.89	2.68
5	1.79	0.70	2.54
6a	1.19	0.46	2.60
6b	1.19	0.46	2.60

$\bar{f} \pm$ d.v. $= 2.59 \pm 0.088$.

Fig. 2. Linear correlation between area ratios (A_R) and weight ratios (W_R) of the solutions reported in Table I. The equation of the best straight line was obtained by the least squares method.

the intracellular pH of human muscle in normal and pathological subjects; further experiments will be reported soon.

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