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Note

Rapid and simple gas—liquid chromatographic method for the determination of 5,5-dimethyl-2,4-oxazolidynedione

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5,5-Dimethyl-2,4-oxazolidynedione (DMO) is a weak non-metabolizable organic acid with a pK_a of 6.13 at 37°C and should act as a modifier of intracellular pH [1—3]. DMO distributes between intracellular and extracellular space: its acid form permeates into cells, while its anionic form does not. Changes of DMO concentration in serum are thus dependent on intracellular pH changes only, so DMO should be a sufficient marker of intracellular pH and acid—base balance changes [4, 5].

Previously described methods for the determination of DMO were based either on spectrophotometric measurement, when large doses of DMO were administered [1, 2], or on radioisotope techniques [6—9]. Although these isotopic methods for determining DMO are very sensitive, they are not practical for most laboratories because of the high costs involved.

A new, alternative and rapid method for the determination of DMO in serum samples, with high sensitivity and low cost, is described below.

EXPERIMENTAL

Reagents

Reagent-grade dichloromethane, methanol, chloroform and sulphuric acid were obtained from Merck (Darmstadt, F.R.G.). DMO was from Sigma (St. Louis, MO, U.S.A.). The internal standard 3-ethyl-3-methyl-2,5-pyrrolidinedione (ethosuximide; ETS) was from Applied Science (State College, PA, U.S.A.). The internal standard stock solution (1 mg/ml) was prepared by dis-

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solving ETS in methanol. The stock solution of DMO (1 mg/ml) was prepared by dissolving DMO in methanol. The extraction solvent was dichloromethane.

Calibration procedure

Using the DMO standard solution, separate series of blank control serum samples (500 μl) were added with concentrations ranging from 5 to 200 μg/ml and with the internal standard at a fixed concentration of 25 μg/ml. These calibration samples were taken through the extraction procedure described below.

Apparatus and operating conditions

A Pye-Unicam 304 gas chromatograph equipped with a flame-ionization detector, a PU 4810 computing integrator and a PM 8251 recorder was used. The glass column was 1.8 m × 2 mm I.D. packed with 20% ethylene glycol adipate (EGA) on 100—120 mesh Chromosorb W HP. The EGA was obtained from Anlabs (North Haven, CT, U.S.A.) and the Chromosorb W HP was from Applied Science. The flow-rate of hydrogen was 30 ml/min and the flow-rate of air was 300 ml/min. The flow-rate of argon (the carrier gas) was 35 ml/min. The injector temperature was 250°C, the oven temperature was 190°C and the detector temperature was 250°C.

Procedure

Into a 10-ml stoppered glass centrifuge tube, 500 μl of serum, 25 μl of the internal standard (stock solution), 50 μl of sulphuric acid and 5 ml of dichloromethane were added. The contents were stirred with a Vortex-type mixer for 5 min and centrifuged for 5 min at 3500 *g*. After centrifugation, a glass centrifuge tube was kept at −30°C for 15 min. Then, the organic phase was transferred into a glass conical centrifuge tube and evaporated to dryness at 40°C under a stream of nitrogen. A 50-μl aliquot of chloroform was pipetted onto the dry mass, dissolved using the Vortex-type mixer for 30 s, and centrifuged for 2 min at 2000 *g*. A 1-μl aliquot of the supernatant was injected into the chromatograph.

Calculations

Ultimate sample concentrations were calculated by determining the peak-area ratio of DMO, with respect to the internal standard, and comparing this ratio with the standard curve obtained after analysis of the calibration samples.

RESULTS AND DISCUSSION

The recovery rate, calculated on the basis of the amount of DMO measured after extraction compared with standard methanol solutions, was 96 ± 0.3%. Fig. 1 shows that no interfering peaks were obtained at the retention times of DMO or its internal standard with the described method and chromatographic conditions. All the compounds eluted as completely separate symmetrical peaks. The retention times for DMO and its internal standard were 7.5 and 9.0 min, respectively. A linear relationship (r = 0.9989) was found when the ratios of the peak area of DMO to the peak area of its internal standard (50

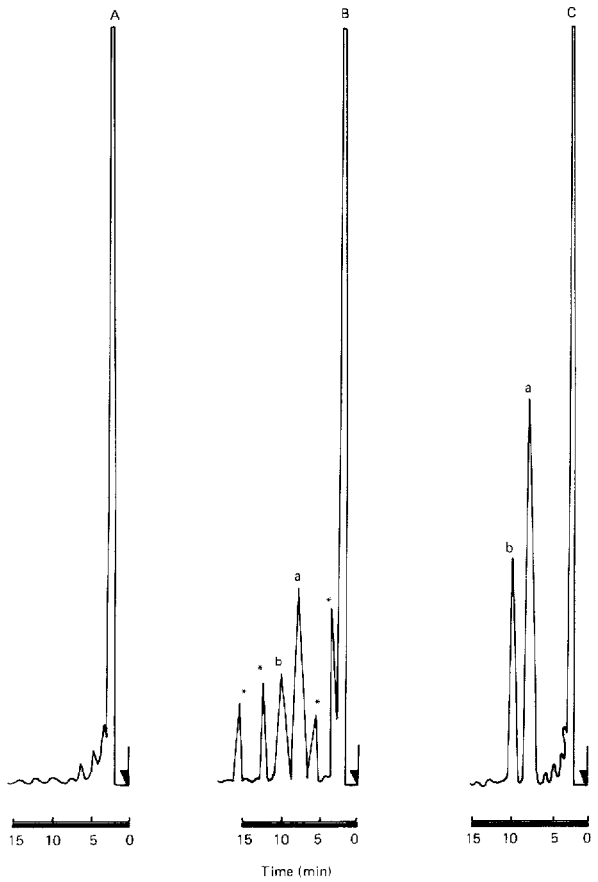

Fig. 1. Chromatographic determination of DMO in serum. (A) Control serum extract; (B) rabbit serum after DMO administration; (C) spiked serum (50 μg/ml internal standard and 75 μg/ml DMO). Peaks: a = internal standard; b = DMO; * = unknown.

TABLE I

ACCURACY AND PRECISION OF THE DESCRIBED METHOD

DMO serum concentration (μg/ml)		Coefficient of variation (%)	Accuracy (%)
Theoretical	Observed (mean ± S.D., n = 21)		
5	5.9 ± 0.5	9.0	118.0
10	9.8 ± 0.3	3.9	98.6
15	14.8 ± 0.3	3.8	98.7
20	19.5 ± 1.0	4.0	98.0
50	51.3 ± 2.2	2.2	102.6
100	100.5 ± 2.7	2.7	100.7
150	150.5 ± 2.6	3.0	101.8
200	197.0 ± 4.6	1.9	98.8

μg/ml) were plotted on the y-axis against various concentrations of DMO (μg/ml) on the x-axis. Equations by the least-squares method were $y = 0.00925x - 0.0237$ for the DMO calibration samples. The detection limit was

0.2 μg/ml at a signal-to-noise ratio of 10:1. The accuracy and precision of the procedure were ascertained by adding different amounts of DMO to drug-free serum and analysing 21 samples of each concentration with the method described. The results are summarized in Table I.

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