Journal of Chromatography, 133 (1977) 263–266
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CHROM. 9741

RAPID METHOD FOR THE SIMULTANEOUS DETERMINATION OF 1,4-DIOXAN AND ITS MAJOR METABOLITE, β-HYDROXYETHOXYACETIC ACID, CONCENTRATIONS IN PLASMA AND URINE

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(Received September 13th, 1976)

SUMMARY

1,4-Dioxan and its principle metabolite, β-hydroxyethoxyacetic acid (HEAA), are determined by gas chromatography–mass spectrometry (GC–MS) on a 3% OV-17 column using selected ion monitoring, following the methylation of HEAA directly in plasma or urine without extraction. The recoveries of dioxan from plasma and urine are 98 and 94%, respectively, and the recoveries of HEAA from plasma and urine are 86 and 94%, respectively. The detection limits of 1,4-dioxan in plasma and urine are 0.07 ppm, and the detection limits of HEAA in plasma and urine are 0.5 and 0.1 ppm, respectively. Separate simultaneous measurements of 1,4-dioxan and HEAA methyl ester concentrations in urine and plasma are obtained after the methylation via GC–MS without additional preparation of the samples.

INTRODUCTION

A recent pharmacokinetic study by Young and Gehring[1] has demonstrated that the fate of 1,4-dioxan in rats is markedly dose-dependent due to an apparent saturation of the metabolic pathway for detoxification of 1,4-dioxan. Braun and Young[2] showed that, contrary to the data presently available in the literature, the major metabolite of 1,4-dioxan in rats is β-hydroxyethoxyacetic acid (HEAA). Mather and Assimos[3] and other workers have described gas chromatographic methods for the quantitation of 1,4-dioxan. However, these methods lack specificity and sensitivity. The literature contains no references to the determination of HEAA. To elucidate further the apparent dose-dependent kinetics of 1,4-dioxan, a selective and sensitive method for the determination of 1,4-dioxan and HEAA concentrations in plasma and urine was required.

EXPERIMENTAL

Reagents

1,4-Dioxan was obtained from Burdick & Jackson (Muskegon, Mich., U.S.A.).

HEAA was supplied by Dow Chemical (Midland, Mich., U.S.A.). Both 1,4-dioxan and HEAA were used without additional purification. All inorganic and organic chemicals used were ACS reagent grade.

Gas chromatography–mass spectrometry

Gas chromatographic–mass spectrometric (GC–MS) determinations were performed on a Finnigan Model 3000D GC–MS instrument equipped with a Finnigan 6000 MS Data System. Sequential GC columns (0.5 ft. × 2 mm I.D. and 5.5 ft. × 2 mm I.D.) packed with 3% OV-17 on 80–100 mesh Supelcoport were used for separation. The injector, separator, and ion source were maintained at 250°, 255° and 70°, respectively. The column oven was programmed from 80°–150° at a rate of 20°/min. The helium carrier gas was maintained at 18 p.s.i. at the head of the column. The separator and ion source pressures were 5×10^{-1} and 5×10^{-5} torr, respectively. The mode of ionization was either electron impact (EI) or chemical ionization (CI) with methane as the reagent gas. The data were obtained by selected ion monitoring of *m/e* 88 (M^+ of dioxan) and *m/e* 116 ($[M-18]^+$ of the methyl ester of HEAA) during EI, and of *m/e* 89 ($[M+1]^+$ of dioxan), *m/e* 135 ($[M+1]^+$ of the methyl ester of HEAA), and *m/e* 116 ($[M-18]^+$ of the methyl ester of HEAA).

Simultaneous determination of 1,4-dioxan and HEAA

The HEAA in all standards and samples was converted to methyl hydroxyethoxy acetate (MHEA) prior to analysis. The ester was formed by heating 0.5 ml of sample with 100 μl of concentrated hydrochloric acid and 1.0 ml of methanol for 5 min at 60°. While still warm, 2.0 ml of acetic anhydride was slowly added to destroy the acid–ester equilibrium by removing the water. The sample was diluted exactly to 5 ml with methanol.

Samples and standards were injected (1 μl) into the GC–MS instrument under the conditions described above. The selected ions monitored for 1,4-dioxan (*m/e* 88 during EI and *m/e* 89 during CI) and MHEA (*m/e* 116 during EI, and *m/e* 135 and 116 during CI) were electronically integrated to obtain the area. Calibration curves of 1,4-dioxan and HEAA were constructed from standards in plasma and urine. From these curves the concentrations of 1,4-dioxan and HEAA in the samples were calculated.

RESULTS AND DISCUSSION

Selected ion monitoring provides a selective and sensitive method for quantitating 1,4-dioxan and HEAA (Table I). The methylation of HEAA by the procedure described above is rapid, reliable and quantitative yielding complete conversion of HEAA to MHEA without any degradation of 1,4-dioxan. Urine and plasma samples to which were added 1,4-dioxan and HEAA (0.1–100 μg/ml) were analyzed by the described method (Table II). Mean recoveries of 1,4-dioxan from urine and plasma were 94.4 and 97.8%, respectively, and the mean recoveries of HEAA from urine and plasma were 93.8 and 85.8%, respectively. Considering the minimal amount of sample preparation these recoveries are satisfactory. At a signal-to-noise ratio (*S/N*) of 2, the procedure is capable of quantitating 0.07 μg/g of 1,4-dioxan in 0.5 ml of urine and plasma, 0.1 and 0.5 μg/g of HEAA in 0.5 ml of urine and plasma, respectively. The

TABLE I

RESPONSES OBTAINED FROM 1,4-DIOXAN AND β-HEAA STANDARDS IN METHANOL

Peak areas are expressed in arbitrary units. Peak areas are means of five determinations ± standard deviation. C.V. = coefficient of variation.

Amount injected (μg/μl)	*Peak area (mV)*		*Response × 10^{-4} (mV/μg)*
1,4-Dioxan			
1.0	41,000 ± 4,100		4.1 ± 0.41
0.1	3,900 ± 400		3.9 ± 0.40
0.01	390 ± 35		3.90 ± 0.35
0.001	38 ± 3.9		3.8 ± 0.39
0.0001	3.0 ± 0.042		3.0 ± 0.42
		Mean	3.7 ± 0.39 (10% C.V.)
β-HEAA			
1.0	11,000 ± 970		1.1 ± 0.097
0.1	970 ± 98.0		0.97 ± 0.098
0.01	91 ± 8.8		0.91 ± 0 088
0.001	9.4 ± 0.89		0.94 ± 0.089
0.0001	1.3 ± 0.21		1.3 ± 0.21
		Mean	1.0 ± 0.12 (11% C.V.)

S/N appears to be the only factor limiting the sensitivity. The sensitivity is sufficient to measure dioxan and HEAA in rats and men exposed to dioxan[1,4].

Critical evaluation of procedure

Small day-to-day variations in sensitivity were observed; however, the stability of the instrument was sufficient to allow the use of external standards. By injecting an external standard after each sixth sample, the slow changes in relative sensitivity posed no problem in the calculation of 1,4-dioxan and HEAA concentrations.

Although the ratios of the reagents were not critical, optimal methylation of the HEAA was achieved using 100 μl of concentrated hydrochloric acid, 1.0 ml of

TABLE II

RECOVERY OF 1,4-DIOXAN AND β-HEAA FROM URINE AND PLASMA

Amounts recovered are means of quadruplet determinations ± standard deviation. N.D. = not detected.

Amount added to 0.5 ml urine or plasma (μg)	*Amount recovered from urine (μg)*	*Amount recovered from plasma (μg)*
1,4-Dioxan		
0.05	0.051 ± 0.013	0.055 ± 0.011
0.5	0.49 ± 0.049	0.52 ± 0.066
5.0	4.4 ± 0.37	4.7 ± 0.24
50.0	45 ± 5.0	44 ± 3.2
500.0	460 ± 14	450 ± 18
β-HEAA		
0.05	0.043 ± 0.0066	N.D.
0.5	0.49 ± 0.051	0.43 ± 0.041
5.0	4.6 ± 0.25	4.2 ± 0.48
50.0	46 ± 4.4	41 ± 3.3
500.0	490 ± 37	440 ± 29

methanol and 0.5 ml of sample. However, the incubation time and temperature were more critical. Temperatures below 50° and above 75° gave consistently low recoveries of MHEA as did heating times less than 5 and more than 20 min. The recoveries of MHEA within these temperature and time limits were constant. Also, the recoveries of 1,4-dioxan were not affected by these conditions.

It was observed that in the presence of water HEAA and MHEA were in equilibrium. By adding excess acetic anhydride, water was removed and HEAA was quantitatively converted to MHEA. The exact quantity of acetic anhydride added is not critical as long as it is in excess. Extreme caution should be observed during the addition of the acetic anhydride since the reaction is exothermic.

Direct injection of plasma and urine caused peak broadening after approx. 30 injections necessitating repacking and reconditioning the column. This time-consuming problem was eliminated by the addition of the 6-in. pre-column which can be easily replaced when peak broadening occurs.

The retention times (t_R) for 1,4-dioxan and MHEA on the column were 40 ± 2 and 258 ± 15 sec. The variability in the t_R value of MHEA was apparently due to the difficulty in reproducing the 20°/min heating rate accurately. Regardless of the t_R variability, with the high degree of specificity of selected ion monitoring, no problems were encountered in the recognition of the MHEA peak.

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