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QUANTIFICATION OF ENDOGENOUS ALIPHATIC ALCOHOLS IN SERUM AND URINE

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SUMMARY

The endogenous aliphatic alcohols ethanol, *n*-propanol, *n*-butanol, isobutanol and isopentanol in serum and urine were measured by gas chromatography–mass fragmentography. Whereas for the higher-molecular-weight alcohols extraction with dichloromethane is used, ethanol is determined by direct injection of serum and urine.

When zero is assigned to all values below the detection limits of the procedures (0.1 mg/l for ethanol and 2 μg/l for each of the higher-molecular-weight alcohols), the following normal ranges are found: ethanol, 0–39 mg/l in serum and 0–46 mg per 24 h in urine; *n*-propanol, 0–48 μg/l in serum and 0–300 μg per 24 h in urine; *n*-butanol, 0–20 μg/l in serum and 0–18 μg per 24 h in urine. The isobutanol and isopentanol levels in the serum and urine of normal subjects are below the detection limit. For diabetic patients, on average increased levels are found for ethanol, *n*-propanol and *n*-butanol in serum and urine.

INTRODUCTION

Primary aliphatic alcohols of endogenous origin have been detected in blood serum and urine within the gas chromatographic (GC) profile of low-molecular-weight and volatile components in these fluids[1–5]. The occurrence of endogenous ethanol in blood has long been known. Bücher and Redetzki[6] determined ethanol quantitatively in the serum of normal individuals and diabetics using the alcohol dehydrogenase method. Higher-molecular-weight endogenous alcohols have not been studied. In the presence of ethanol they cannot be quantified separately by enzymatic methods because of their cross-reactions with ethanol.

Several GC procedures have been developed for the quantitative determination of ethanol involving distillation, extraction, direct injection or headspace techniques. Of these, direct injection[7,8] and headspace analysis[9,10] are the favoured methods. In general, the GC methods are suitable for quantifying other volatile substances such as acetaldehyde, *n*-propanol and isopropanol in addition to ethanol[11,12]. However, all of the existing GC methods have been applied only to the quantification of ethanol after consumption of alcoholic beverages or to some higher-molecular-weight alcohols from exogenous sources in conjunction with intoxication.

Interferences from other constituents in serum and urine and the low concen-

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trations of the alcohols, especially of the higher-molecular-weight ones, are the reasons why GC methods cannot be applied satisfactorily to the quantification of endogenous alcohols. In this paper two methods are described for the quantitative determination of endogenous ethanol and endogenous higher-molecular-weight alcohols, taking advantage of the high specificity and sensitivity of mass fragmentography (MF).

EXPERIMENTAL

Serum and urine samples

Serum and urine samples were collected from normal individuals, from hospital patients without obvious metabolic defects and from diabetic patients who had abstained from drinking alcoholic beverages for 3 days before sample collection. Serum was obtained from venous blood by centrifugation for 10 min at 1600 *g*. Urine was collected for 24 h.

Apparatus

The GC–MF determinations were performed on a combination of a Model 2700 gas chromatograph and a CH 5 mass spectrometer (Varian-MAT, Bremen, G.F.R.) interfaced with a 30 cm × 0.1 mm I.D. platinum capillary.

Determination of ethanol in serum and urine

Ethanol was determined by direct injection of a serum or urine sample.

To 0.5 ml of serum or urine 2 μl of internal standard (50 μl of diethyl ether in 100 ml of distilled water) were added. The mixture was thoroughly shaken and 1 μl was analysed by GC–MF under the conditions described in Table I.

TABLE I

GAS CHROMATOGRAPHIC–MASS FRAGMENTOGRAPHIC CONDITIONS

Parameter	*Value*	*Parameter*	*Value*
Column	100 m × 0.5 mm I.D. stainless steel, coated with Emulphor ON-870	Electron energy of ion source	70 eV
		Accelerating voltage	3 kV
		Ion source temperature	220°C
Column temperature:		Interface temperature	220°C
For ethanol	65°C	Resolution	400
For higher-molecular-weight alcohols	70°C	Emission current	300 μA
		Multiplier voltage	3 kV
Injector block temperature	150°C	Specific ion	*m/e* 31
Carrier gas	Helium at 4 ml/min		

The water from the directly injected serum or urine sample was by-passed between the outlet of the GC column and the interface, *i.e.*, after its separation from the ethanol to be measured, by using a system of a T-connection and two valves (Kontron-Technik, Eching, G.F.R.), located inside but operated from outside the oven. All connections were 1/16 in. Two normal valves and a T-connection were used instead of a three-way valve because they had a low dead volume and because they

were found to perform well with respect to thermal stability and tightness. After the elution of the ethanol, the effluent of the column was by-passed by turning the valves. The gas flow into the ion source was shut off for 9 min, during which time the elution of the water was completed. Then the valves were turned back into the flow-through position, and the system was ready for the next analysis.

The calculation of the ethanol concentration was based on the ratio of the peak heights of ethanol and internal standard and on a calibration graph obtained from four aqueous standard solutions with ethanol concentrations between 0.16 and 79 mg/l.

Determination of higher-molecular-weight alcohols in serum and urine

The higher-molecular-weight alcohols *n*-propanol, *n*-butanol, isobutanol and isopentanol were determined after extraction from serum or urine.

To 1 ml of serum or urine 4 μl of internal standard (25 μl of 2-buten-1-ol in 100 ml of distilled water) were added. The mixture was extracted with 1 ml of dichloromethane by shaking for 1 min. After aspiration of the supernatant aqueous phase, the organic phase was concentrated to a volume of approximately 50 μl under a stream of nitrogen. A 1-μl volume of the concentrated extract was analysed by GC–MF under the conditions described in Table I.

The calculation of the concentrations of each alcohol was based on the ratios of the peak heights of the alcohols and the internal standard and on calibration graphs for each alcohol obtained from four aqueous standard solutions with alcohol concentrations between 10 and 320 μg/l.

RESULTS AND DISCUSSION

Specificity of the methods

The quantitative determination of ethanol, *n*-propanol, *n*-butanol, isobutanol and isopentanol by GC–MF uses the ion of *m/e* 31, corresponding to the fragment $H_2C{=}\overset{+}{O}H$, as the specific ion. The ion is characteristic of primary aliphatic alcohols and gives the procedure satisfactory specificity. The only endogenous substance with certain interference in the determination of ethanol is isopropanol. Under the experimental conditions described it was not separated from ethanol. However, in urine isopropanol was present in only trace amounts, and in serum its concentration was estimated to be approximately one tenth of that of ethanol. Further, because of the low intensity of the *m/e* 31 fragment in isopropanol, its detection sensitivity was only 5% of that of ethanol. For the higher-molecular-weight alcohols no interfering substances were observed.

Sensitivity and precision of the method

The detection limits of the procedures were 0.1 mg/l for ethanol and 2 μg/l for each of the higher-molecular-weight alcohols. The coefficients of variation were 5% for ethanol (at a concentration of 1.7 mg/l) and 10% for the other alcohols (at a concentration of 15 μg/l).

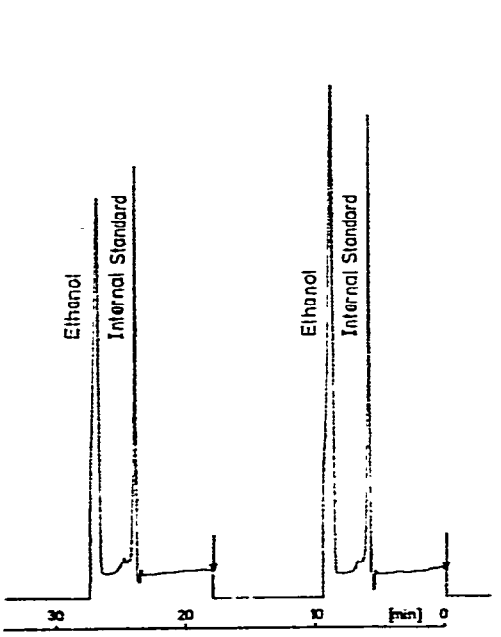
Practicability of the methods

Both methods are characterized by their simplicity, which is a particularly

significant factor in the quantification of ethanol. The method of direct injection of 1 μl of serum or urine after the addition of the internal standard, avoids any sample work-up procedure. The basic requirement for the method is the valve system for by-passing the water, which otherwise would cause irreproducible decreases in sensitivity.

For the higher-molecular-weight alcohols the extraction method gave better results than the direct injection procedure, because by-passing the water interferes with the elution of the alcohols, especially of *n*-propanol, and because owing to their low concentrations these alcohols require a concentration step.

For the ethanol determination the time between two injections was approximately 18 min, half of which was necessary for the elution of the water (Fig. 1). For the determination of all four higher-molecular-weight alcohols the corresponding time was only 12 min (Fig. 2) because overlapping injections were possible.



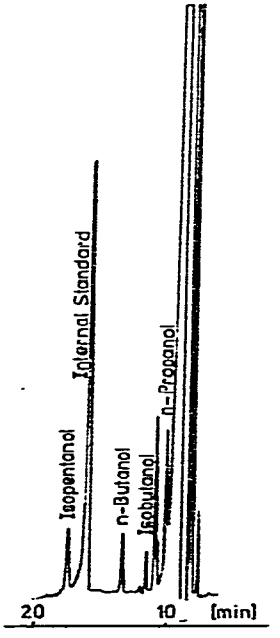


Fig. 1. Mass fragmentograms of subsequent measurements of ethanol in two urine samples, *m/e* 31. The decreases in the baseline after the ethanol peaks are caused by the by-passing of the water.

Fig. 2. Mass fragmentogram of a measurement of *n*-propanol, *n*-butanol, isobutanol and isopentanol in a diabetic urine sample, *m/e* 31.

The primary alcohol 2-buten-1-ol is a suitable internal standard for the quantification of the higher-molecular-weight alcohols. It is well separated from the four alcohols to be determined and, owing to its chemical structure, its behaviour during the work-up procedure is similar to that of the alcohols. Less similar is the structure of diethyl ether in the ethanol quantification. However, as no work-up procedure is involved, diethyl ether is acceptable as an internal standard.

TABLE II

ETHANOL IN SERUM AND URINE

Sample	*Group*	*Number of samples (n)*	*Range (mg/l)*	*Mean value (mg/l)*
Serum	Control subjects	42	0– 39	6.6
	Diabetic patients	168	0–159	10.0
			Range (mg per 24 h)	*Mean value (mg per 24 h)*
Urine	Control subjects	57	0– 46	7.2
	Diabetic patients	247	0–484	19.8

Ethanol in serum and urine

In the control group consisting of healthy individuals and hospital patients without obvious metabolic defects, the ethanol concentrations in 42 serum samples ranged from 0 to 39 mg/l and in 57 urine samples from 0 to 46 mg per 24 h. Values below the detection limit of the described quantification procedure, *i.e.*, below 0.1 mg/l, are reported as zero. By GC profile analysis[2,3,5], which uses much larger sample volumes, it has been shown that the ethanol concentration in serum and urine is always different from zero.

In the group of diabetic patients, the range of ethanol concentrations was much greater than that in the control group, in some instances by a factor of up to 10.

The results are summarized in Table II. They show that in the group of the diabetics, extreme concentrations of ethanol occurred, and that with regard to the mean values, the concentration of ethanol in serum and the excretion of ethanol in urine were increased compared with the control group. On the other hand, normal levels and levels below the detection limit were also observed in some diabetics. Fig. 3

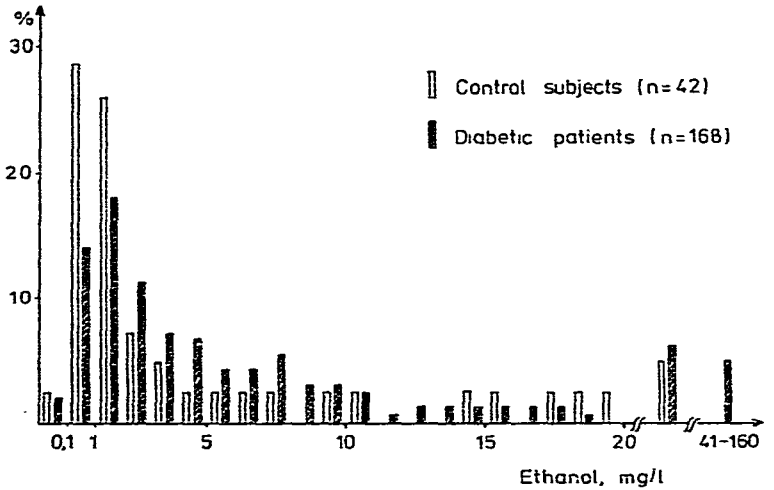


Fig. 3. Distribution curve of ethanol in sera of control subjects and diabetic patients.

shows considerable overlap of the distribution curves of the ethanol concentrations in the serum of the control subjects and the diabetic patients. A similar behaviour was observed for ethanol in urine. The highest ethanol levels observed in serum were *ca.* 160 mg/l, corresponding to 0.16 per 1000.

High-molecular-weight alcohols in serum and urine

In the control group the *n*-propanol concentrations were in the ranges 0–48 μl/l and 0–300 μg per 24 h, respectively, and the *n*-butanol concentrations were in the ranges 0–20 μg/l and 0–18 μg per 24 h, respectively. The isobutanol level was zero, *i.e.*, below the detection limit of the procedure, in all serum samples ($n = 44$) and urine samples ($n = 64$) of the control group. In the same samples the isopentanol level was found to be above the detection limit in only a few instances.

In the group of diabetic patients in many instances elevated levels of *n*-propanol (Table III) and *n*-butanol (Table IV) were found in serum and urine. Also, the mean values were increased in comparison with the control group. As with ethanol, the biological range of *n*-propanol and *n*-butanol concentrations in diabetics was very wide (Figs. 4 and 5).

TABLE III

n-PROPANOL IN SERUM AND URINE

Sample	*Group*	*Number of samples (n)*	*Range (μg/l)*	*Mean value (μg/l)*
Serum	Control subjects	43	0– 48	8
	Diabetic patients	173	0–226	17
			Range (μg per 24 h)	*Mean value (μg per 24 h)*
Urine	Control subjects	63	0–300	24
	Diabetic patients	245	0–960	47

TABLE IV

n-BUTANOL IN SERUM AND URINE

Sample	*Group*	*Number of samples (n)*	*Range (μg/l)*	*Mean value (μg/l)*
Serum	Control subjects	44	0– 20	6
	Diabetic patients	167	0–180	8
			Range (μg per 24 h)	*Mean value (μg per 24 h)*
Urine	Control subjects	64	0– 18	0
	Diabetic patients	246	0–320	10

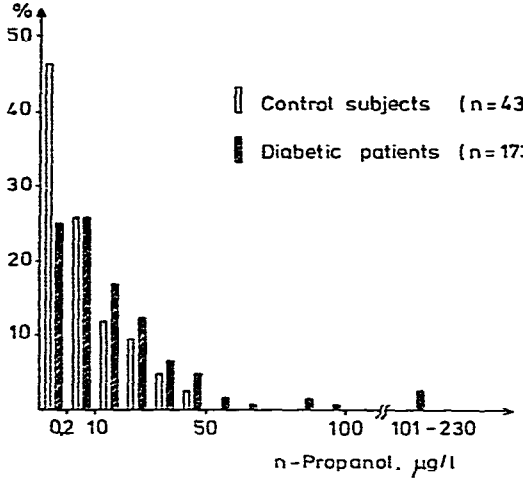


Fig. 4. Distribution curve of *n*-propanol in sera of control subjects and diabetic patients.

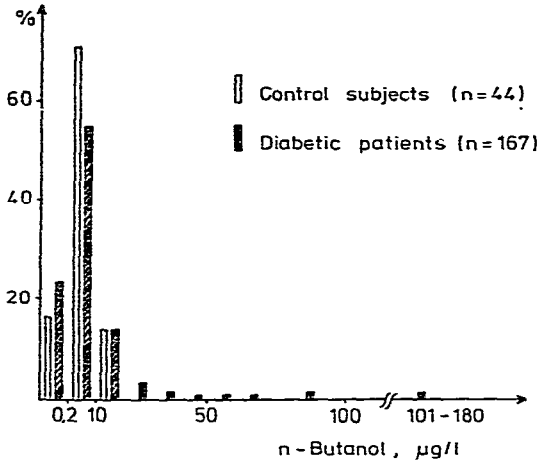


Fig. 5. Distribution curves of *n*-butanol in sera of control subjects and diabetic patients.

The methyl-branched alcohols isobutanol and isopentanol showed elevated levels less frequently. These alcohols were studied mainly in urine. Proceeding on the observation that in the control group the excretion of isobutanol and isopentanol was below the detection limit, in a group of 138 diabetics 45 patients (32%) showed a measurable excretion of isobutanol and 38 patients (28%) a measurable excretion of isopentanol. When, however, of the total group of diabetics only the group of patients with proved diabetic complications ($n = 37$) was considered, it was found that in 28 patients (76%) the excretion of isobutanol and in 30 patients (81%) the excretion of isopentanol were increased. When the levels of methyl-branched alcohols were high, normally also the excretion of ethanol, *n*-propanol and *n*-butanol was increased. The presence of isobutanol and isopentanol can be considered to be an indication of the existence of diabetic complications.

REFERENCES

1 A. Zlatkis, W. Bertsch, H. A. Lichtenstein, A. Tishbee, F. Shunbo, H. M. Liebich, A. M. Coscia and N. Fleischer, *Anal. Chem.*, 45 (1973) 763.
2 H. M. Liebich, O. Al-Babbili, A. Zlatkis and K. Kim, *Clin. Chem.*, 21 (1975) 1294.
3 H. M. Liebich and O. Al-Babbili, *J. Chromatogr.*, 112 (1975) 539.
4 H. M. Liebich, *J. Chromatogr.*, 112 (1975) 551.
5 H. M. Liebich and J. Wöll, *J. Chromatogr.*, 142 (1977) 505.
6 T. Bücher and H. Redetzki, *Klin. Wochenschr.*, 29 (1951) 615.
7 N. C. Jain, *Clin. Chem.*, 17 (1971) 82.
8 W. H. Swallow and P. R. Hentschel, *J. Chromatogr.*, 130 (1977) 403.
9 P. K. Gessner, *Anal. Biochem.*, 38 (1970) 499.
10 M. A. Korsten, S. Matsuzaki, L. Feinman and C. S. Lieber, *N. Eng. J. Med.*, 292 (1975) 386.
11 S. Szczepaniak, *Mikrochim. Acta*, I (1978) 513.
12 J. F. Brien and C. W. Loomis, *Clin. Chim. Acta*, 87 (1978) 175.