Journal of Chromatography, 276 (1983) 11—18
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1702

GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF THE LOWER VOLATILE ALCOHOLS IN RAT BLOOD AND IN HUMAN STOOL SPECIMENS ON A FUSED SILICA CAPILLARY COLUMN

WILLIAM M. DOIZAKI* and MICHAEL D. LEVITT

Medical Research Laboratory, Veterans Administration Medical Center, Minneapolis, MN 55417 (U.S.A.)

(First received November 24th, 1982; revised manuscript received March 10th, 1983)

SUMMARY

A method is described for the simultaneous quantitation of the lower volatile alcohols in stool specimens and rat blood. The addition of potassium carbonate to the assay mixture markedly increased the sensitivity in the detection of these compounds. The method is shown to be simple and reproducible and is suitable for following the metabolism of ethanol in human stool specimens.

INTRODUCTION

We have been investigating the possibility that the metabolism of the colonic flora may influence the host's response to ethanol. Of particular interest has been the detection of 2-propen-1-ol (allyl alcohol)* during the metabolism of ethanol by human stool specimens [1]. Numerous gas chromatographic (GC) methods have been described for the measurement of alcohols in the blood and in other biological tissues. However, these methods were primarily adapted to the analysis of blood ethanol [2—5] and/or its metabolite, acetaldehyde [6—8], which precluded the determination of other volatile alcohols that may be present at much lower concentrations than ethanol. Volatile alcohols have been observed in the urine of diabetic patients [9, 10] and in some blood samples [11—13]. The quantitation of the aliphatic alcohols in serum and urine of these subjects was recently accomplished by GC—mass fragmentography [10]. The availability of such sophisticated instrumentation is not universal. Therefore, this paper describes a simple GC method for simul-

*The common names of some of the alcohols will be used in the text.

0378-4347/83/$03.00

taneous quantitation of the lower alcohols in stool specimens and rat blood in the presence of large quantities of ethanol.

EXPERIMENTAL

Reagents and chemicals

Standard solutions were prepared from dehydrated reagent-grade ethanol (U.S. Industrial Chemical Co., Tuscola, IL, U.S.A.), *n*-butanol (Mallinckrodt, St. Louis, MO, U.S.A.), *n*-propanol, *n*-pentanol, 2-propen-1-ol and 3-methyl-1-butanol (all from Fisher Scientific, Fair Lawn, NJ, U.S.A.). The internal standard was prepared from an aqueous stock solution containing 2000 nmol/ml of reagent grade 3-methyl-1-butanol (isoamyl alcohol)*.

INSTRUMENTATION

A Hewlett-Packard (HP) 5880A gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with a flame ionization detector, a column compensation unit, a cryogenic attachment, and a terminal was used. A fused silica capillary column (50 m × 0.2—0.21 mm I.D.) coated with Carbowax 20M (HP) was used. The chromatograph was operated isothermally at 20°C for 6 min, programmed at 5°C/min to 40°C followed by a 10°C/min increase to 90°C and maintained at this temperature for a further 5 min. The chromatograph oven was cooled with liquid carbon dioxide.

The instrument used for the mass spectral analysis was a HP 5992B GC—mass spectrometric (MS) unit with a 50-m fused silica capillary column as above. The separation was carried out isothermally at 20°C for 6 min, programmed at 10°C/min to 90°C and kept at this temperature for an additional 10 min. The oven was cooled with liquid carbon dioxide. The spectra were obtained at 23 eV.

Quantitation

Isoamyl alcohol was used as the internal standard since no endogenous isoamyl alcohol could be detected in the rat blood or in the human stool specimens and no isoamyl alcohol was detected during the anaerobic metabolism of ethanol by stool specimens. External standards for the volatile alcohol mixtures were prepared in distilled water in various concentrations from 250 to 2500 nmol/ml. The retention time of each of the alcohols was verified by injecting aliquots of each standard directly into the gas chromatograph. They were further verified by GC—MS by their abundance *m/e* peaks of 31, 31, 57, 56, 55, and 55 respectively for the alcohols as they were eluted from the GC column (see Fig. 2). The peak areas for each standard were automatically quantitated with the HP programmable GC terminal (5880A).

For recovery studies, rat blood was diluted 1:1 with cold physiological saline, then with an equal volume of isoamyl alcohol as internal standard (2000 nmol/ml). Stool samples were prepared by thoroughly mixing 1 g of the stool specimen (wet weight) with 2 ml of 0.1 *M* phosphate buffer in saline (pH 7.4),

*The common names of some of the alcohols will be used in the text.

followed by equal portions of the isoamyl alcohol internal standard as above.

For GC analyses, a 0.2-ml aliquot of the above sample was added to a 9-ml septum bottle containing 200 mg of anhydrous potassium carbonate (previously dried overnight at 100°C), the bottle was immediately sealed with a rubber stopper, heated in a water bath with constant stirring for 20 min at 70 ± 2°C. A 0.2-ml aliquot of gas was withdrawn through the stopper with a 1.0-ml gas-tight A-2 Pressure-Lok syringe (Precision Sampling, Baton Rouge, LA, U.S.A.) and injected directly into the gas chromatograph.

Incubation of the stool specimens with ethanol was carried out anaerobically in a 50-ml rubber stoppered Erlenmeyer flask. The stool sample in 0.1 *M* phosphate buffer in saline (pH 7.4) was deaerated in the presence of carbon dioxide and then incubated with 0.1% (v/v) ethanol. Aliquots of the reaction mixture were taken at various time intervals and assayed as above on the gas chromatograph.

RESULTS AND DISCUSSION

Very low concentrations of alcohols in biological fluids may be difficult to quantitate by GC because of the incomplete four-variable recovery of these compounds from the gas phase [13]. The vapor pressures of the alcohols can, however, be substantially increased by the addition of different salts to the alcoholic solutions [3, 14]. Anhydrous potassium carbonate was selected as the salt of choice in this present method because of its greater ability to increase the vapor pressure of these alcohols [3]. As indicated in Fig, 1. the release of the various alcohols into the vapor phase was 2—10 times greater in the presence of the salt than in its absence. The strong affinity of water for each alcohol apparently limits the release of these alcohols into the vapor phase [15].

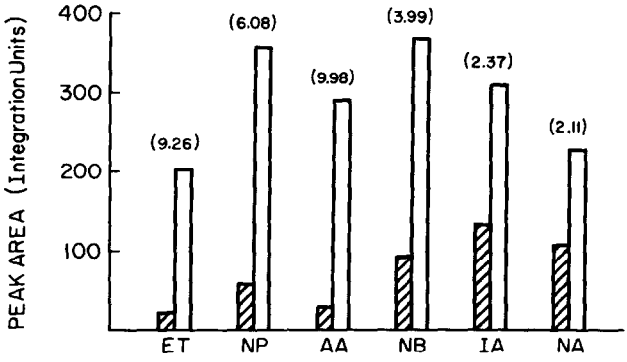

Fig. 1. Recovery of alcohols from aqueous solutions in the presence (▫) and absence (▪) of potassium carbonate. Concentration of each alcohol at 1000 nmol/ml. ET = ethanol; NP = *n*-propanol; AA = 2-propen-1-ol (allyl alcohol); NA = *n*-pentanol (*n*-amyl alcohol). Values in parentheses denote the recovery enhancement factor in the presence of potassium carbonate.

The retention times of the alcohols were sufficiently separated to allow good baseline resolution (see Fig. 2). Reproducibility of the method was measured from 22 separate determinations of the external standard alcohol mixture (con-

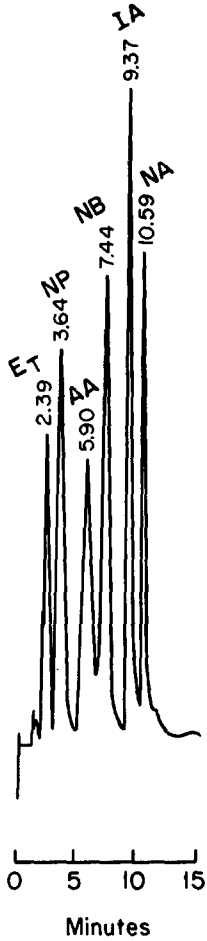

Fig. 2. Gas chromatogram of a standard alcohol mixture. Concentration of each alcohol at 1000 nmol/ml in aqueous solution. Abbreviations as in Fig. 1. Numbers at each peak denote the retention times of the individual alcohols.

taining 1000 nmol/ml of each) over a period of 3—4 weeks. The standard error of mean ranged from 1.3 to 2.8% for these alcohols.

Standard curves (Fig. 3), prepared using 250—2500 nmol/ml of the alcohols in aqueous solutions as a mixture, yielded straight lines through the origin when peak area units were plotted against the concentrations of the alcohols. For the detection of ethanol, this represents a 5- to 10-fold increase in sensitivity as compared to those previously reported by a GC method [7, 8].

As seen in Table I, the recovery of known amounts of ethanol added to rat blood was 104.8 ± 3.0% (mean ± S.E.M.). Trace amounts of ethanol only were detected in the normal rat blood. Consequently, no corrections for endogenous ethanol content were made in the recovery studies (Table I). In absence of potassium carbonate, however, the recovery of ethanol from the rat blood was less than 30%. Concentrations below 1000 nmol/ml of ethanol were barely detectable and could not be quantitated.

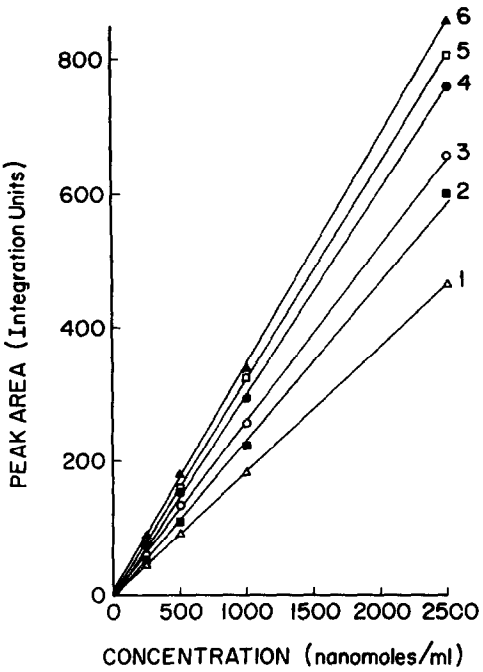

Fig. 3. Plot of peak area vs. concentration of the lower volatile alcohols in aqueous solutions. 1 = Ethanol; 2 = *n*-pentanol (*n*-amyl alcohol); 3 = 2-propen-1-ol (allyl alcohol); 4 = 3-methyl-1-butanol (isoamyl alcohol); 5 = *n*-propanol; 6 = *n*-butanol.

TABLE I

RECOVERY OF ETHANOL ADDED TO RAT BLOOD

Isoamyl alcohol was used as the internal standard.

Amount added (nmol)	Amount recovered			
	With potassium carbonate		Without potassium carbonate	
	nmol ± S.E.M.	% ± S.E.M.	nmol ± S.E.M.	% ± S.E.M.
543	591 ± 16	108.6 ± 2.9	trace	0
1085	1094 ± 35	100.8 ± 3.2	287 ± 24	26.3 ± 2.1
10,850	11,144 ± 277	102.6 ± 2.5	2954 ± 159	27.2 ± 1.4
21,700	23,221 ± 706	107.1 ± 3.2	6615 ± 242	30.4 ± 1.4

The possibility that the presence of other alcohols in the mixture would affect the release of any of the alcohols at various concentrations from stool specimens was also tested. The endogenous content of the volatile alcohols in stool specimens from 21 normal human subjects were as follows: ethanol, 21—549 nmol/g (wet weight) with a mean of 134 ± 31 (S.E.M.); *n*-propanol, 0—330 nmol/g with a mean of 37 ± 15; *n*-butanol, 0—86 nmol/g with a mean of 16 ± 4; and *n*-amyl alcohol, 0—13 nmol/g with a mean of 1.3 ± 0.6. Trace amounts of isobutanol and allyl alcohol were observed in few samples. How-

TABLE II

RECOVERY OF THE LOWER VOLATILE ALCOHOLS ADDED TO HUMAN STOOL SPECIMENS

Isoamyl alcohol was used as the internal standard. Alcohols were added to the stool specimens as a single mixture.

Amount added (nmol)	Amount recovered			
	Ethanol		*n*-Propanol	
	nmol ± S.E.M.	% ± S.E.M.	nmol ± S.E.M.	% ± S.E.M.
250	259 ± 4.6	103.8 ± 1.8	269 ± 4.8	107.9 ± 1.9
500	500 ± 11	100.0 ± 2.2	472 ± 8	94.6 ± 1.5
1000	952 ± 18	95.2 ± 1.8	996 ± 22	99.6 ± 2.2
2000	1948 ± 32	97.3 ± 1.5	1955 ± 32	97.6 ± 1.6

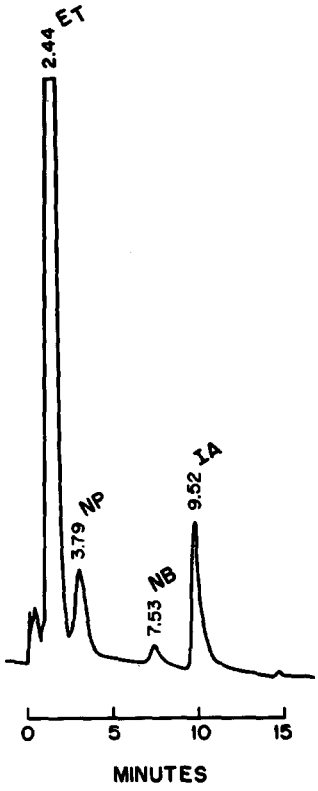

Fig. 4. Gas chromatogram of a typical run during the anaerobic metabolism of ethanol by a normal human stool specimen. Abbreviations as in Fig. 1.

ever, only trace amounts of ethanol were detected in the specimens used in the recovery studies (Table II). Therefore, corrections for the endogenous content of these alcohols in the stool specimens are not included in Table II. As shown

2-Propen-1-ol (allyl alcohol)		*n*-Butanol		*n*-Pentanol (*n*-amyl alcohol)	
nmol ± S.E.M.	% ± S.E.M.	nmol ± S.E.M.	% ± S.E.M.	nmol ± S.E.M.	% ± S.E.M.
240 ± 5.3	96.1 ± 2.1	254 ± 4.7	101.5 ± 1.9	258 ± 3.6	103.1 ± 1.4
487 ± 9	96.2 ± 2.1	491 ± 7	98.3 ± 1.5	498 ± 6	99.2 ± 1.3
1039 ± 24	103.9 ± 2.4	1101 ± 16	110.1 ± 1.9	998 ± 15	98.8 ± 1.5
1922 ± 39	96.1 ± 2.0	1856 ± 24	92.9 ± 1.2	1893 ± 39	94.7 ± 2.0

in Table II, the presence of these alcohols as a mixture in human stool specimens did not interfere with the recovery of each of the individual alcohols. The apparent recovery for these volatile alcohols ranged from 98.1 to 100.7% with standard error of mean of 1.6—2.2%. Although not shown, similar recoveries were observed when varying amounts of the alcoholic mixture were added to rat blood. Excessive amounts of ethanol did not interfere with the quantitation of other alcohols. A typical chromatogram obtained during an anaerobic metabolism of ethanol by a stool specimen shows that *n*-propanol is adequately separated form a large excess of ethanol to allow quantitation (Fig. 4). In this instance, the amount of ethanol (approximately 40 μmol/ml) was 32 times as much as *n*-propanol.

During the anaerobic metabolism of ethanol by a normal human stool specimen, *n*-propanol, *n*-butanol and *n*-amyl alcohol were formed (Fig. 5). Although not shown here, allyl alcohol was also noted in lesser amounts in some specimens. Trace amounts of isobutanol were detected in few samples.

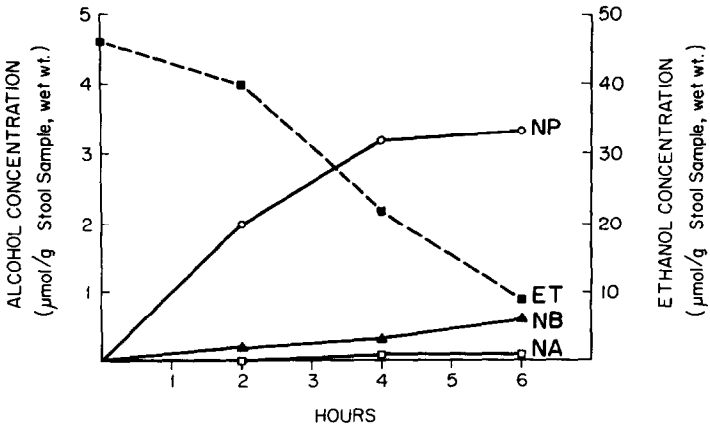

Fig. 5. Formation of various alcohols during the anaerobic metabolism of ethanol by a normal human stool specimen. Abbreviations as in Fig. 1.

Isobutanol elutes prior to allyl alcohol and can interfere with its determination. However, the amount of isobutanol found in these samples was not significant to hinder the quantitation of allyl alcohol. The identity of the formation of these alcohols was further confirmed by GC—MS. Implications of these findings are discussed elsewhere [1].

The advantages of the method reported here are its simplicity, reproducibility and accuracy. Utilizing this method, simultaneous quantitation of the lower volatile alcohols is now attainable in the presence of a large excess of ethanol.

REFERENCES

1 M.D. Levitt, W. Doizaki and A.S. Levine, Hepatology, 2 (1982) 598.
2 P.K. Gessner, Anal. Biochem., 38 (1970) 499.
3 G. Machata, Clin. Chem. Newsletter, 4 (1972) 29.
4 R.P. Sturtevant, Microchem. J., 22 (1977) 574.
5 B.R. Manno and J.E. Manno, J. Anal. Toxicol., 2 (1978) 257.
6 C.J.P. Eriksson, H.W. Sippel and O.A. Forsander, Anal. Biochem., 80 (1977) 116.
7 C.L. Mendenhall, J. MacGee and E.S. Green, J. Chromatogr., 190 (1980) 197.
8 J.F. Brien and D.J. Hoover, J. Pharmacol. Methods, 4 (1980) 51.
9 H.M. Liebich and O. Al-Babbili, J. Chromatogr., 112 (1975) 539.
10 H.M. Liebich, H.J. Buelow and R. Kallmayer, J. Chromatogr., 239 (1982) 343.
11 M. Novotny, M.L. McConnell, M.L. Lee and R. Farlow, Clin. Chem., 20 (1974) 1105.
12 B. Dowty, D. Carlisle, J.L. Laseter and J. Storer, Science, 187 (1975) 75.
13 K.Y. Lee, D. Nurok and A. Zlatkis, J. Chromatogr., 158 (1978) 377.
14 S.L. Friant and I.H. Suffet, Anal. Chem., 51 (1979) 2167.
15 R. Bassette, S. Ozerus and C.H. Whitnah, Anal. Chem., 34 (1962) 1540.