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ANALYSIS OF TRACE VOLATILE METABOLITES IN SERUM AND PLASMA

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SUMMARY

A method is described for analyzing volatile constituents in 5- to 10-ml samples of human serum and plasma. A headspace sampling technique was employed by which volatile sample constituents are carried in a stream of ultra-pure nitrogen and adsorbed onto a porous polymer with subsequent heat desorption. The concentrated volatiles were separated on highly efficient capillary columns. Mass spectrometric identifications were made with 55 ml of serum. Characteristic components in normal serum are: ethanol, 4-methyl-2-pentanone, hexanal, 1-butanol, 2-hexanol, 2-heptanone, and benzaldehyde.

INTRODUCTION

Profiles of volatile metabolites in human urine have been extensively studied using both extraction and adsorption techniques for concentration of the volatile organics, followed by gas chromatographic (GC) separation and mass spectrometric (MS) identification of the components[1–3]. The adsorption procedure has been employed also to examine pathological conditions such as diabetes mellitus[4]. With this technique, determinations of urinary components are generally performed with 100-ml aliquots of 24-h urines. If the metabolic studies require controls in shorter intervals, *e.g.* for correlation of the volatile components to blood sugar levels in diabetes mellitus, whole blood, plasma or serum should be used for the analysis.

Studies of volatiles in these biological fluids so far have been mainly concerned with some lower alcohols, especially ethanol, aldehydes, ketones, low-boiling hydrocarbons, and chlorinated hydrocarbons in toxicological examinations. Blood specimens with abnormal and toxicologically significant levels of these components can be examined gas chromatographically using headspace sampling[5], solvent extraction[6], or direct injection of blood[7,8].

This paper describes the application and evaluation of the technique previously used for urinary profiles to normal trace levels of volatile components in human serum and plasma.

EXPERIMENTAL

Sample preparation

The detailed sampling procedure has been previously described for urine[2] and was adapted for this work without change. Serum and plasma volatiles were adsorbed on Tenax GC (Applied Science Labs., State College, Pa., U.S.A.) using 5–10 ml of sample heated in a water-bath of 95° and stirred with a magnetic stirrer. Nitrogen of "Zero" grade (Iweco, Houston, Texas, U.S.A.) was passed over the sample for 1 h at a flow-rate of 15 ml/min.

For MS studies larger volumes of serum were necessary (50–70 ml) to insure sufficient concentration of the substances for MS identifications. In general, three adsorbent traps were attached in parallel on top of the sampling system.

Gas chromatography

A Shimadzu Model GC-5 AP_5 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a sulfur-specific flame photometric detector was used in this work. The capillary column material was nickel (Handy and Harman tube, Norristown, Pa., U.S.A.), 100 m × 0.5 mm I.D., coated with Emulphor ON 870. (American Instrument Co., Silver Spring, Md., U.S.A.). The column generated 125,000 theoretical plates for a tetraline standard. Its preparation and properties will be described elsewhere[9]. The trap was 3 m × 0.5 mm I.D., and had the same coating. Desorption of the volatiles from the adsorbent was effected in a specially designed injector block[2] at 300° at a carrier gas flow-rate of 20 ml/min using dry ice as the coolant of the pre-column. After a trapping period of 15 min the pre-column was connected manually to the analytical column and the GC separation commenced at 80°. After 15 min the column temperature was programmed to 180° at 2°/min.

Mass spectrometry

GC–MS analyses were made on a Model CH 5 mass spectrometer connected to a Spectrosystem 100 computer and coupled to a Model 2701 gas chromatograph (all from Varian-Mat, Bremen, G.F.R.). A two-stage separator of the Biemann-Watson design was employed and operated at a flow-rate of 6 ml helium/min through the GC column. Additional helium (make-up gas) was introduced at the end of the column. The amount of helium added increased the pressure in the ion source to 6×10^{-6} Torr, which gave the optimal response. Spectra were recorded at 70 eV at an exponential scan mode, 10–300 mass units in 4 sec. Temperatures were 250° for the ion source and 220° for separator and transfer lines. Sampling, trapping, and GC conditions were the same as on the Shimadzu instrument except that a 200 m × 0.50 mm I.D., nickel column was used and the carrier gas was flow controlled.

RESULTS AND DISCUSSION

The sampling procedure was tested at several temperatures, especially at 25° and 95°. Because of the considerably higher yield of volatiles and because the chro-

matograms did not reveal any obvious artifacts, the higher temperature was chosen. At 95° a sampling time of 1 h and a sample size of 5–10 ml of serum or plasma are sufficient for a GC profile, thus making the method a potential screening procedure. The method gives reproducible results and allows detection of trace amounts of constituents. For MS identifications, higher concentrations of volatiles are required. The total sample size, however, can also be increased by desorbing two or three adsorbent traps, collected in a parallel manner, into one pre-column trap.

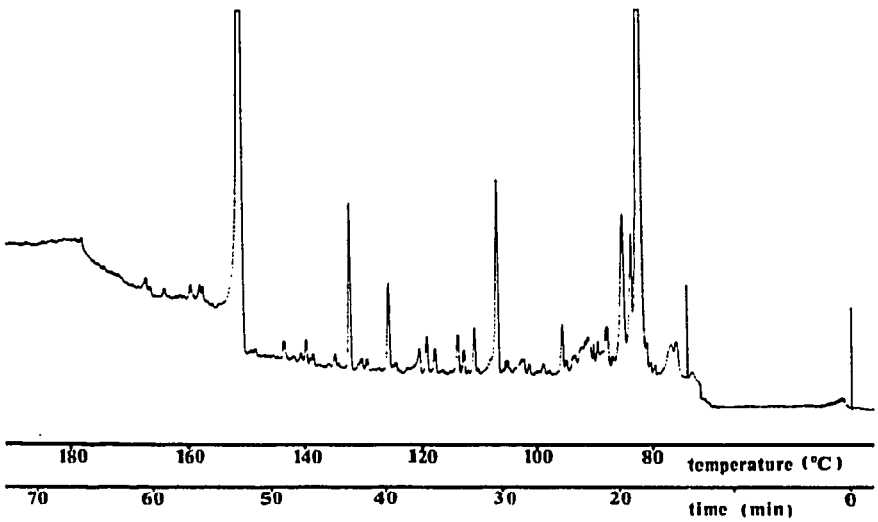

Fig. 1. Volatile components in 5 ml of plasma (conditions in text).

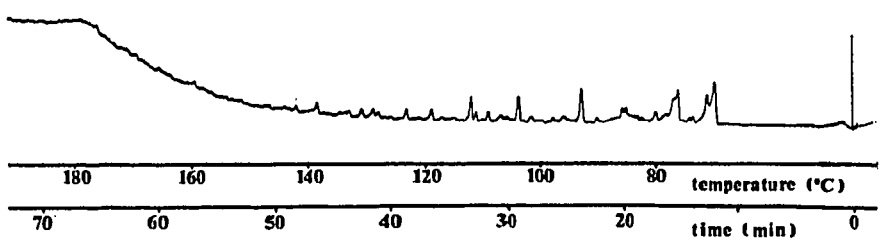

Fig. 2. Blank for the entire procedure. Conditions and recorder sensitivity, as in Figs. 1 and 3.

Fig. 1 shows the chromatogram of a 5-ml plasma sample and Fig. 3 represents a 55-ml pooled serum sample which was also used for GC–MS analysis. In regular intervals blanks were run to control the total sampling, trapping, and GC procedure (Fig. 2) for contaminants. This was particularly important since the amounts encountered with these samples were quite small (several orders of magnitude lower than in urine).

Possible sources for contaminations are the glassware, the gas which is passed over the sample and through the adsorbent, substances remaining on the adsorbent

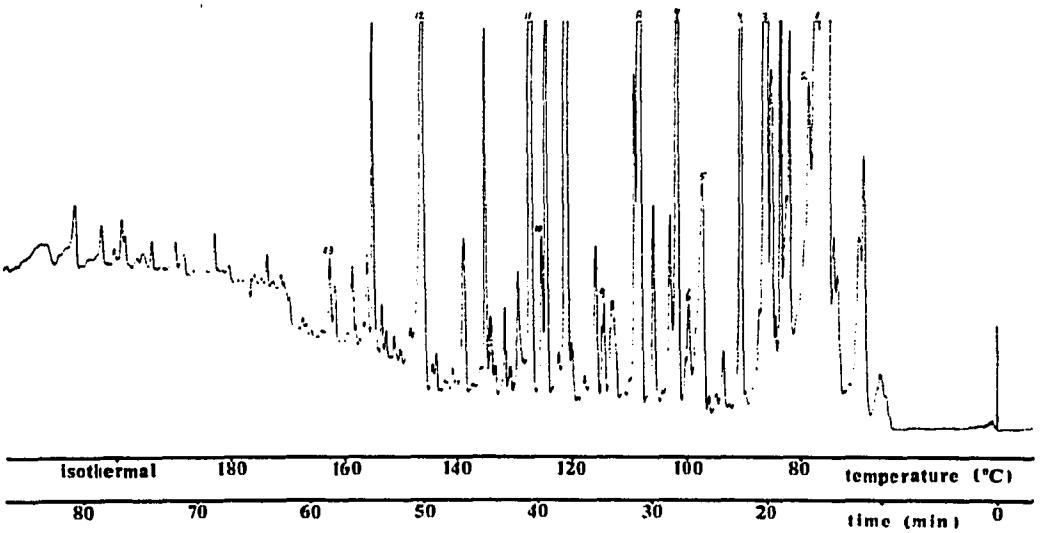

Fig. 3. Volatile components in 55 ml of pooled serum from normal individuals (conditions in text).

when not properly conditioned, and residues in the GC system. It should be emphasized that during blood collection and preparation of the serum or plasma special precautions have to be followed. Clean glass tubes preheated in an oven at 200° to evaporate any volatile contaminants should be used and possible contaminations from the disinfectant during venipuncture should be controlled.

Table I shows a list of some components identified in serum. Peak numbers refer to Fig. 3. The substances were identified by using reference mass spectra of authentic compounds and spectra from the literature. As expected, several components known from the urinary volatiles can be detected in serum as well. The GC profile of the volatile organics in serum or plasma of normal individuals is not as complex as the urinary pattern. Sulfur compounds are present in detectable quantities and further studies will be made in this area.

Changes in metabolic pathways due to pathological conditions can lead to abnormal profiles of volatile metabolites in serum and plasma. This may give analytical data for the early detection of disease.

TABLE I

IDENTIFIED COMPONENTS IN HUMAN SERUM

Peak No.	*Compound*	*Peak No.*	*Compound*
1	Ethanol	8	2-Hexanol
2	2-Pentanone	9	2-Heptanone
3	4-Methyl-2-pentanone	10	Cyclohexanone
4	Toluene	11	Limonene
5	1-Hexanol	12	2-*n*-Butoxyethanol (tentative)
6	3-Penten-2-one	13	Benzaldehyde
7	1-Butanol		

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