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THE IDENTIFICATION OF VOLATILE COMPOUNDS IN HUMAN URINE*

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SUMMARY

Since we are using the gas chromatography of volatile urine constituents in a procedure to diagnose diseases, we wished to identify the components of our sample. Forty-two compounds were identified by gas chromatography–mass spectroscopy, using a modified head-space collection technique. A co-injection procedure was used to place these components on a chromatogram from our disease diagnosis instrument.

INTRODUCTION

As part of our work on the development of a mass screening procedure for the diagnosis of diseases based on the gas chromatography of volatile urine constituents[1–3], we wished to identify the components of our analysis sample. We chose the technique of coupled gas chromatography–mass spectroscopy (GC–MS) as being the most suitable for our purposes. Other investigators (see for example refs. 4–7) have used the GC–MS technique to study urine composition. Zlatkis and Liebich[7] have also examined some volatile urine constituents. They obtained their sample by an extraction–distillation procedure. We have used a modified head-space procedure whereby the organic components were separated from the water-saturated sweep gas by adsorption on to a Chromosorb-101 pre-column[8]. Backflushing the heated pre-column yielded a sample suitable for injection into the GC–MS system.

As the chromatographic system used for the GC–MS runs was different from that used for the disease diagnosis studies[3], a co-injection procedure with known compounds was used to identify the peaks in the urine chromatogram obtained on the latter system.

EXPERIMENTAL

Apparatus

A modified Beckman Thermotrac oven, equipped with dual thermistor detectors, was interfaced with an EAI Quad 300 quadrupole mass spectrometer through

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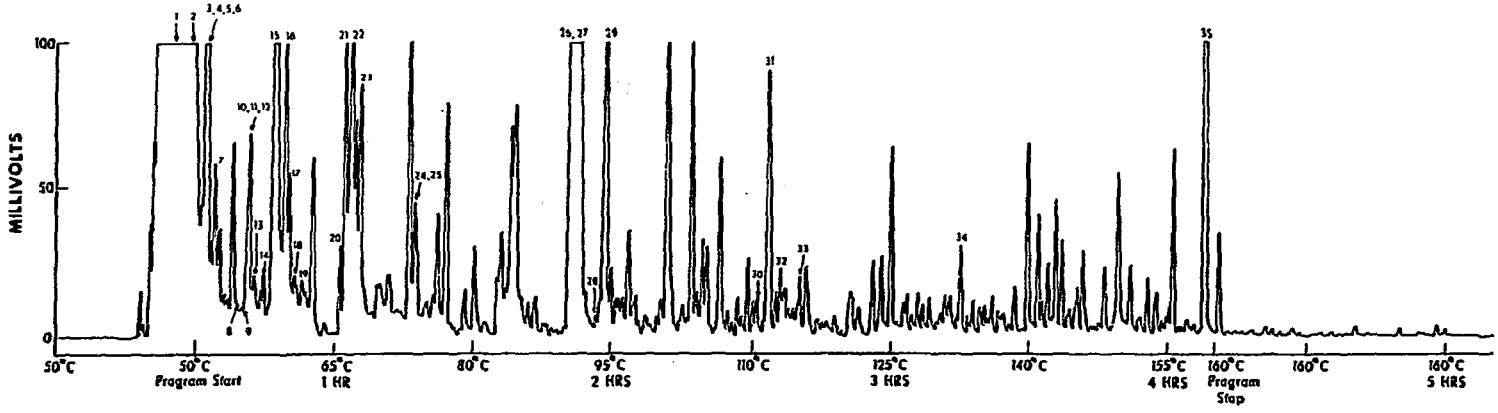

Fig. 1. Chromatogram of the standard urine sample. 1=2-Propanone; 2=2-methylpropanal; 3=butanal; 4=2,3-butanedione; 5=2-butanone; 6=hexane; 7=2-methylfuran; 8=propylene sulfide; 9=3-methylbutanal; 10=3-methyl-2-butanone*; 11=chloroform; 12=2-methylbutanal; 13=benzene; 14=thiophene; 15=2-pentanone*; 16=3-pentanone; 17=2,3-pentanedione; 18=2-ethylfuran; 19=2,5-dimethylfuran; 20=4-methyl-2-pentanone*; 21=pent-3-en-2-one*; 22=2,3-dithiabutane*; 23=3-methyl-2-pentanone*; 24=2-hexanone*; 25=toluene; 26=4-heptanone*; 27=pyrrole*; 28=3-heptanone*; 29=2-heptanone*; 30=4-octanone; 31=benzaldehyde*; 32=3-octanone*; 33=2-pentylfuran; 34=2-nonanone; 35=carvone*.

* Also identified by Zlatkis and Liebich[7].

a membrane-type (Lewellyn-type) molecular separator, but with a film of methylsilicone rubber (0.001 in. nominal thickness) in place of the silicone-painted silver membrane described by Black *et al.*[9]. A 1000 ft. (305 m) long, 0.03 in. (0.076 cm) I.D. stainless-steel open tubular column[10] coated with methylsilicone oil SF 96(50) mixed with 5% of Igepal CO-880[11] was used. The carrier gas was helium at a pressure of 25 p.s.i. The oven temperature was programmed as follows: 25–30° (20 min), 30–172° (122 min), 172° (isothermal, 20 min). The mass spectra were recorded on 1 sec (0–120 *m/e*) or 1.5 sec (0–240 *m/e*) scans using a Datagraph light-deflecting galvanometer.

The GC system for disease diagnosis has been described elsewhere[3].

GC–MS procedure

The urine used in the analysis was collected during standard working hours over a 3-day period from ten male chemists working at the U.S.D.A. laboratory. The vapor was collected by bubbling helium at a flow-rate of 36–41 ml/min through a magnetically stirred mixture of 800 ml of urine and 80 ml of phosphate buffer (prepared by dissolving 200 g of K_2HPO_4 and 154 g of KH_2PO_4 in 1000 ml of water) heated to 85° (25 min) and maintained at 85±2° for 1 h. The helium sweep gas was then passed through a water-cooled condenser into a 0.25 in. (0.63 cm) U-tube filled to a length of 12 cm with Chromosorb-101 (50–60 mesh). This was repeated for a further ten 800-ml samples and one 1100-ml sample (110 ml of buffer, 2 h sweep) using the same Chromosorb trap. The trap was then swept with helium for 40 min.

The volatiles were transferred to a stainless-steel helical trap cooled with liquid nitrogen by backflushing the Chromosorb trap (heated in a 150–154° oil-bath) with nitrogen (20 p.s.i.) for 90 min. The sample was flashed from the helical trap on to the chromatographic column with a heat gun.

Preliminary identifications of the compounds were made using Cornu and Massot's compilation of mass spectra[12,13]. Final identities were confirmed, in most cases, by comparisons of both relative retention times and mass spectra with those of authentic compounds obtained on the same GC–MS system.

Procedure for the correlation of GC–MS and disease-diagnosis chromatographic systems

The compounds to be studied were arranged in the order of expected retention times. These were divided into five groups, every fifth compound being put into the same group. A sample for each group was prepared containing *ca.* 0.1 g of each compound in the group. Five mixtures of these five samples were prepared in the following form: the first contained *ca.* 0.01 g of the first sample, *ca.* 0.03 g of the second, *ca.* 0.06 g of the third, *ca.* 0.12 g of the fourth and *ca.* 0.24 g of the fifth; the second mixture contained *ca.* 0.03 g of the first sample, *ca.* 0.06 g of the second, *ca.* 0.12 g of the third, *ca.* 0.24 g of the fourth and *ca.* 0.01 g of the fifth; this rotation was continued for the other three mixtures. Solutions were prepared, each containing 1.5 μl of a mixture in 250 ml of water. On the disease diagnosis system, 5-ml aliquots of each of these were run. Comparisons of the five chromatograms as to the relative order and the relative sizes of the peaks made possible the assignment of the elution sequence of the compounds of interest on this system. For the co-injection experiment, 5 ml of the aqueous solution of the first mixture were added to a standard urine

sample, and this solution was chromatographed in the usual way[3]. From the comparison of this chromatogram with that of the unadulterated standard urine sample, the identities of several peaks of the standard chromatogram could be ascertained.

RESULTS

Fig. 1 displays the chromatogram of the standard urine sample. Also identified in the GC–MS work, but not included in the co-injection experiment, were acetaldehyde, trimethylamine*, 2-pentenal*, 3-hexanone**, 2-propylfuran, 4-methylpent-3-en-2-one** and 2-butylfuran. Of the compounds noted here, 22 have not been identified in urine before.

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* Identification by mass spectral comparisons only.
** Also identified by Zlatkis and Liebich[7].