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SPECIFIC DETECTION OF VOLATILE METABOLITES IN URINES OF NORMAL SUBJECTS AND PATIENTS WITH *DIABETES MELLITUS* USING COMPUTERIZED MASS FRAGMENTOGRAPHY

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SUMMARY

Volatile urinary components are analyzed by a combination of gas chromatography, mass spectrometry and a data-acquisition system. Mass fragmentograms using the mass spectrometric data on magnetic tape are recorded for the primary aliphatic alcohols ethanol, *n*-propanol, isobutanol, *n*-butanol and isopentanol and the ketones 4-heptanone and cyclohexanone. The mass fragmentograms are used as selective profiles to facilitate recognition of abnormalities in the urinary components in cases of *diabetes mellitus*.

INTRODUCTION

It has been shown that the profile of low-molecular-weight and gas chromatographically volatile metabolites in human urine is characterized by a complex variety of constituents[1–5] with levels estimated at between 10 ng and 200 μg in normal 24-h urine[6]. Chemically, these substances are mainly ketones, alcohols, aldehydes, sulphides, isothiocyanates, pyrroles and furan derivatives with molecular weights ranging from 32 (methanol) to approximately 160. Volatile urinary compounds are subject to physiological variations, which, for some components (such as allyl isothiocyanate, carvone and piperitone) are very pronounced; these substances may be completely absent from normal urine.

For more detailed studies of the profile of normal urine, it is desirable to use selective detection in order to reduce the complexity of the profile. Specific detection is even more useful for characterizing urinary-profile abnormalities due to metabolic disorders. Such abnormalities have been described for urine from patients with *diabetes mellitus*[6] and were attributed to the aliphatic alcohols ethanol, *n*-propanol, isobutanol, *n*-butanol and isopentanol and the ketones 4-heptanone and cyclohexanone, which were found to correlate with diabetes. A flame ionization detector and an alkali flame detector can be used as selective detectors for compounds containing the hetero-atoms sulphur and nitrogen, respectively; for the oxygenated substances of interest here, such a detector is not available.

In this paper, a study on low-molecular-weight alcohols and ketones is described, in which computerized mass fragmentography (MF) is used. Selective profiles

of a group of metabolites, or single-compound detection, facilitates recognition of pathological changes in the pattern of volatile urinary components.

EXPERIMENTAL

Concentration of urinary components

The volatile constituents from 5% of a 24-h urine collection were adsorbed on Tenax GC, a porous polymer of 2,6-diphenyl-*p*-phenylene oxide (35–60 mesh; Applied Science Labs., State College, Pa., U.S.A.); experimental details have been reported[6].

Analysis of the volatiles

The analyses were performed on a combination of a model 2700 gas chromatograph, a CH5 spectrometer and a Spectrosystem 100 MS computer with two magnetic-tape units (Varian-MAT, Bremen, G.F.R.). The gas chromatograph and mass spectrometer were directly interfaced by a platinum capillary 30 cm long. After desorption and recondensation of the volatile compounds[6], the samples were separated by gas chromatography (GC) on a stainless-steel column (100 m × 0.5 mm I.D.) coated with Emulphor ON-870 (Supelco, Bellefonte, Pa., U.S.A.). Helium was used as carrier gas at a flow-rate of 5 ml/min, and the column temperature was maintained at 60° for 16 min, then programmed to 175° at 2°/min and kept at this value. The total ion current from a second ion source (the total pressure monitoring source), operated at an ionisation potential of 20 eV, was used as the signal for the GC monitoring. Mass spectra were recorded exponentially over the *m/e* range 15 to 200 at a scan rate of 2.5 sec/decade; automatic repetitive scanning was used, with a programmed delay of 4 sec after each scan. Experimental conditions for mass spectrometric (MS) recording were as follows: ionisation potential, 70 eV; emission current, 100 μA; accelerating voltage, 3 kV; multiplier voltage, 2 kV; ion-source temperature, 220°; interface temperature, 220°; resolution, 750; and operating pressure 50 μtorr.

During acquisition, data were recorded as position spectra. After calibration of the computer with perfluorokerosene, the data were converted into mass spectra to be stored on magnetic tape; mass fragmentograms from the MS data were recorded on a Complot plotter (Houston Instrument, Bellaire, Texas, U.S.A.).

RESULTS AND DISCUSSION

From the MS data on the magnetic tape, computer reproductions of the complete chromatogram, as well as selective chromatograms of groups of compounds or single compounds, can be obtained. These computer-produced fragmentograms, using one or several characteristic ions of the MS pattern of a substance or a group of substances in conjunction with the gas chromatographic retention time, have a good degree of specificity.

In Fig. 1, the computer reproduction of the early portion of the profile of the volatile compounds in urine from a patient with *diabetes mellitus* is shown. The abscissa indicates the spectrum numbers within the repetitive scan and the ordinate the sum of the intensities of all ions. Examples of selective detection by MF using the MS data from the same analysis as in Fig. 1 are shown in Figs. 2–6. The sulphur compounds dimethyl disulphide and a component tentatively identified as dimethyl

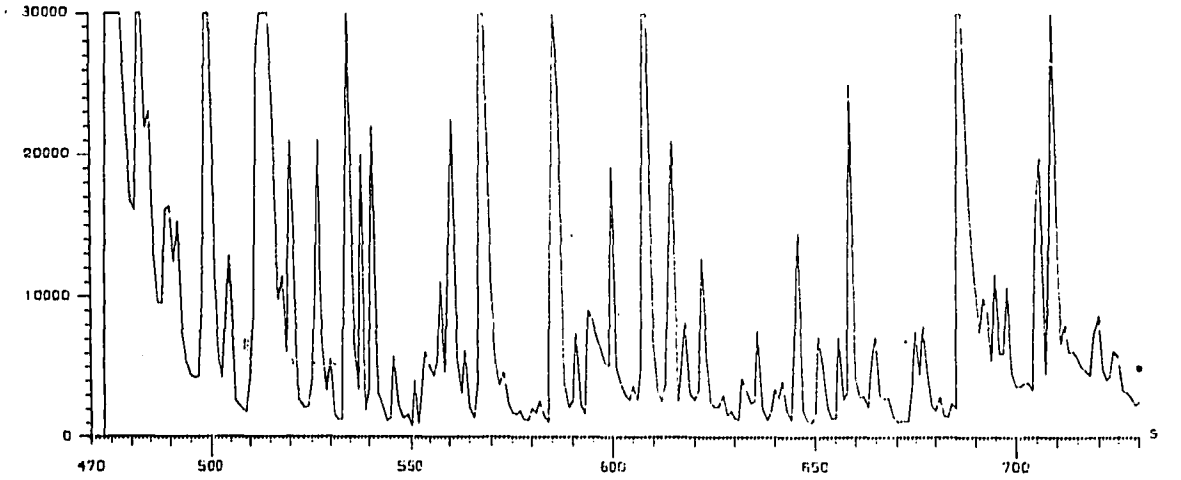

Fig. 1. Computer reproduction of gas chromatogram of volatile compounds in urine from a diabetic patient.

trisulphide, forming the ion S_2^+ (*m/e* 64) are shown in Fig. 2. The ketones 4-heptanone and cyclohexanone can be represented using the molecular ions *m/e* 114 and *m/e* 98, respectively (see Figs. 3 and 4); the additional peaks represent isomeric ketones. For detection of the entire group of primary aliphatic alcohols in diabetic urine, the fragment *m/e* 31 (corresponding to the ion $H_2C{=}{}^+OH$) is suitable, as shown in Fig. 5. Other urinary components, such as aldehydes and ketones, also produce the fragment *m/e* 31, but with very low relative intensity. Interference with the detection of primary alcohols occurs only when these components are present in very high concentrations.

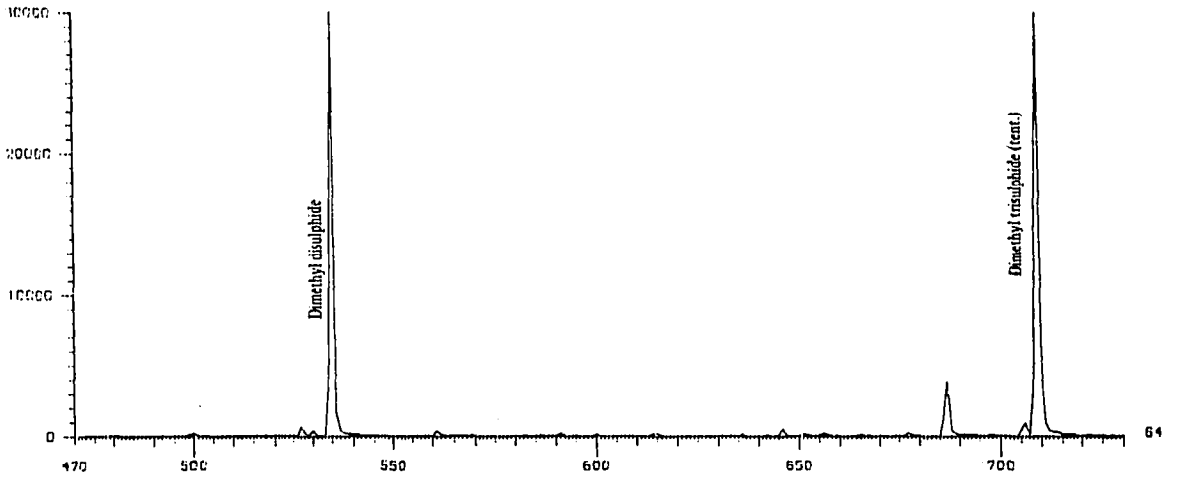

Fig. 2. Mass fragmentogram with *m/e* 64 (diabetic urine).

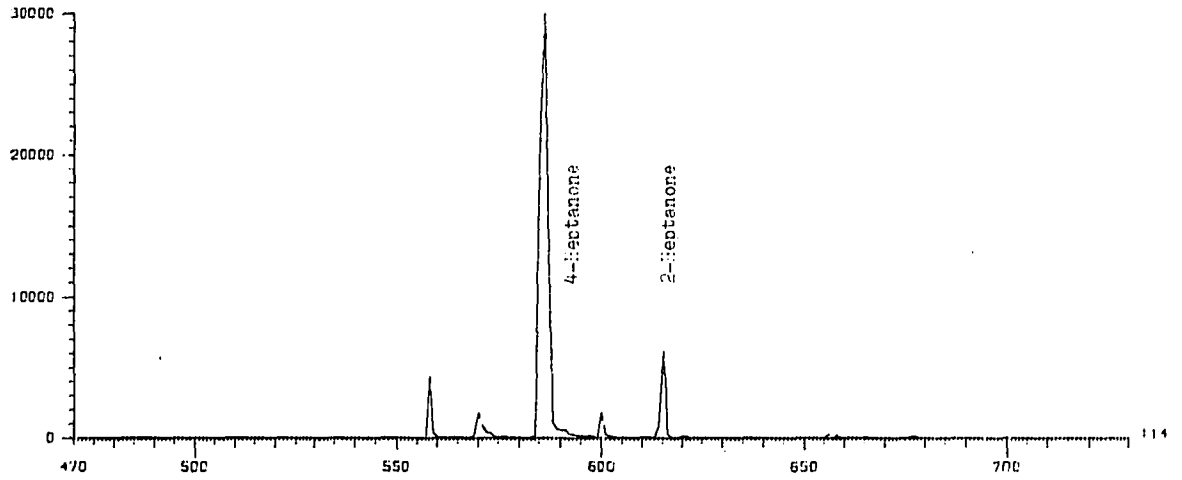

Fig. 3. Mass fragmentogram of C_7 ketones, *m/e* 114 (diabetic urine).

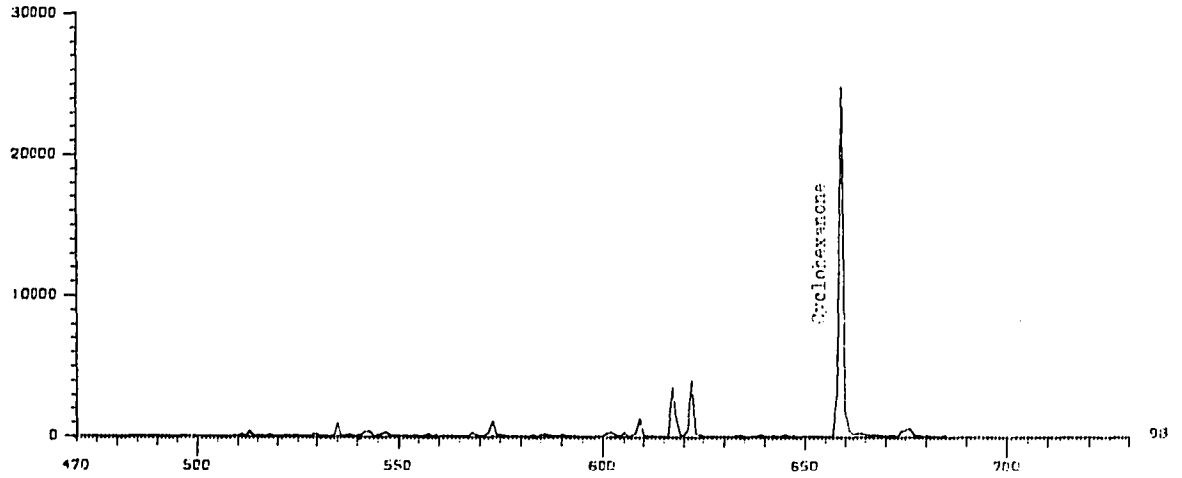

Fig. 4. Mass fragmentogram of cyclohexanone, *m/e* 98 (diabetic urine).

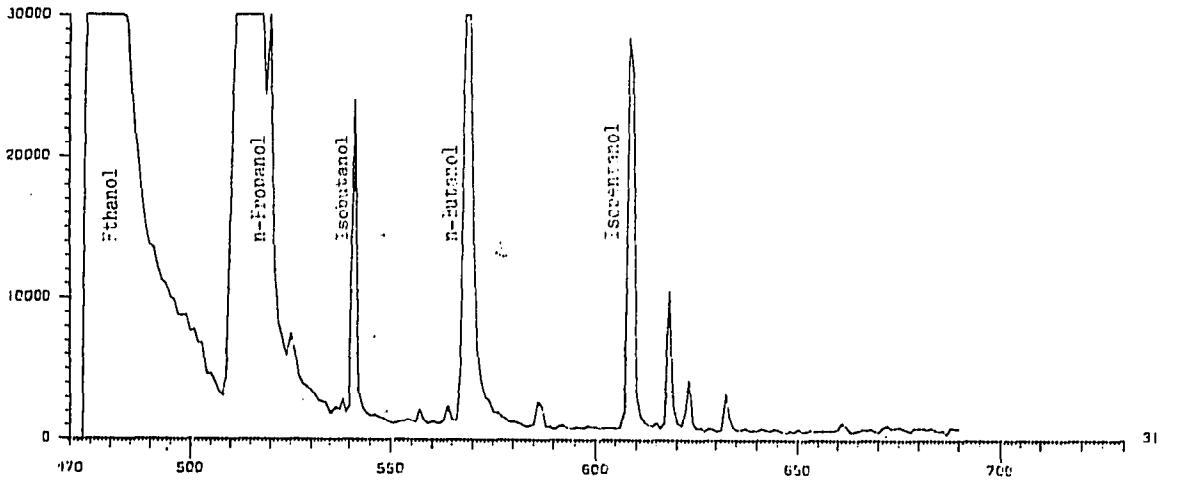

Fig. 5. Mass fragmentogram of primary alcohols, *m/e* 31 (diabetic urine).

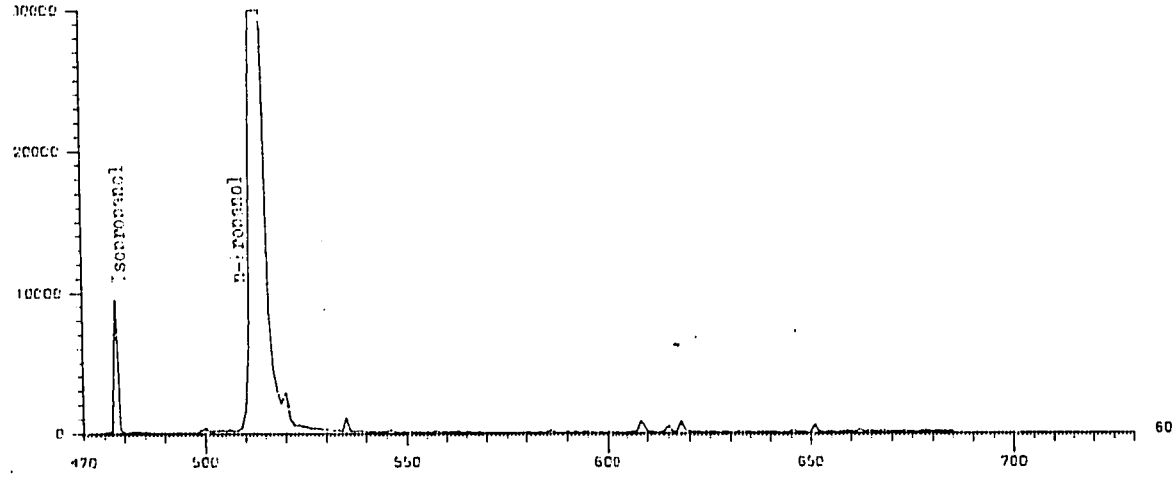

Fig. 6. Mass fragmentogram of isopropanol and *n*-propanol, *m/e* 60 (diabetic urine).

Even better selectivity in the detection of alcohols can be achieved by using additional characteristic ions; Fig. 6 demonstrates the detection of *n*-propanol and isopropanol (molecular ion *m/e* 60).

Instead of using the single-ion plots, mass fragmentograms with several characteristic ions can be used leading to higher specificity. Cyclohexanone in a second sample of diabetic urine is represented in Fig. 7, using the molecular ion *m/e* 98 and the fragments *m/e* 83 ($M-CH_3$) and 80 ($M-H_2O$) as characteristic masses in the fragmentation pattern of cyclohexanone.

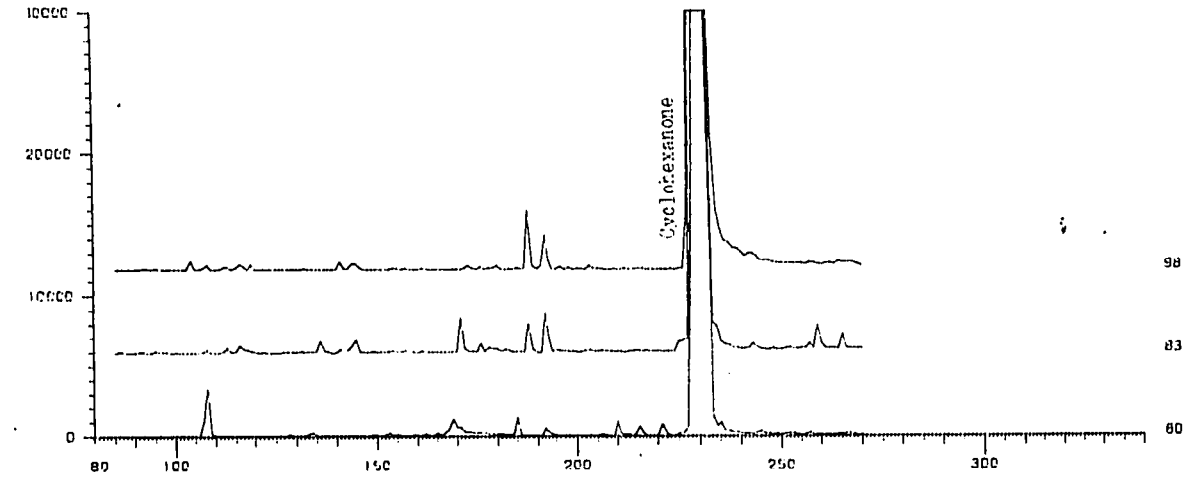

Fig. 7. Mass fragmentogram of cyclohexanone, multiple ions *m/e* 98, 83 and 80 (diabetic urine).

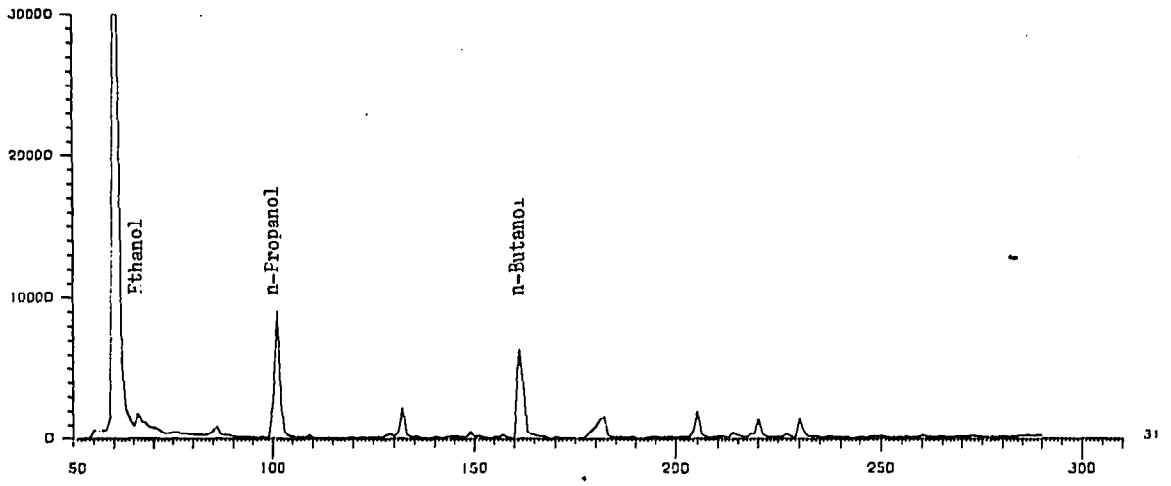

Fig. 8. Mass fragmentogram of primary alcohols, *m/e* 31 (normal urine).

In contrast to the diabetic urines, Figs. 8 and 9 show examples of mass fragmentograms of the volatile compounds in normal urine using the fragments *m/e* 31, 98, 60 and 114. It is obvious that the concentration of the alcohols is low in the normal urine, while cyclohexanone is either absent or can be detected only in trace amounts. The concentration of 4-heptanone is similar in both the normal and the diabetic urines. Selective plotting for ethanol, *n*-propanol, isobutanol, *n*-butanol, isopentanol and cyclohexanone can be used to recognize abnormalities indicative of metabolic disorders related to *diabetes mellitus*. The concentrations of these components are enhanced in diabetic urine.

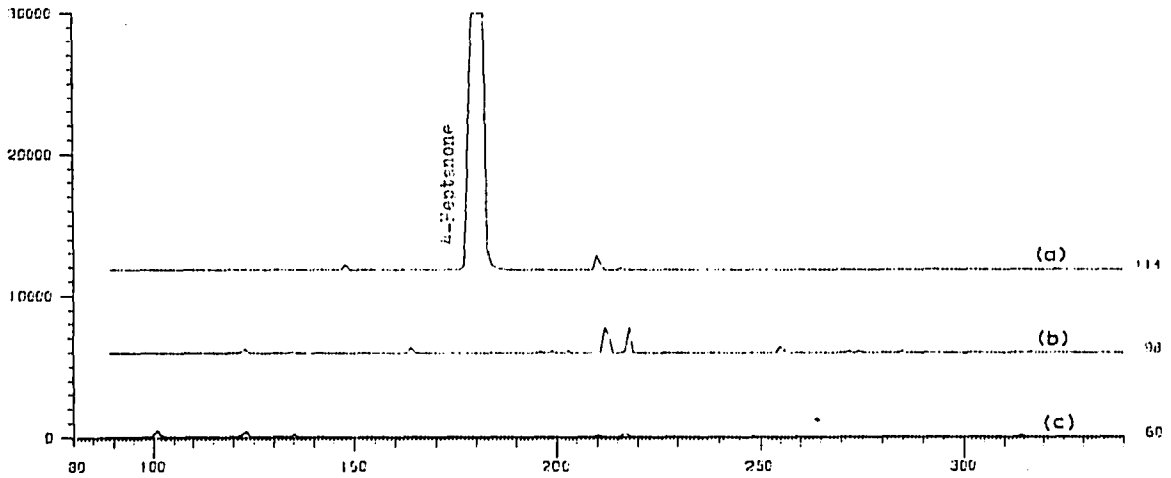

Fig. 9. Mass fragmentogram of normal urine. (a) 4-heptanone, *m/e* 114; (b) *m/e* 98; (c) *m/e* 60.

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