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The Use of a Multipurpose Sampler for Headspace GC-MS Analysis of Volatile Organic Compounds in Human Urine and Plasma

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KEYWORDS

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ABSTRACT

A multi-purpose-sampler (Gerstel MPS), designed for liquid large volume, gaseous and headspace samples was used for the GC-MS analysis of organic volatiles in human urine and plasma. Headspace sampling with a volume, temperature and speed controlled gas tight syringe was combined with a temperature controlled cooled injection system for cold trapping, enrichment and focussing of analyte. Regular 2 ml GC-vials filled with 1 ml acidified urine or plasma were used as headspace sampling vials. A 100 vial autosampler tray was equipped with an additional temperature and heating time controlled preheating station for 5 vials. Profiles of organic volatiles were determined and 4-heptanone as a ketone of medical interest was quantified. Calibration curves and imprecision of the urine method for 4-heptanone concentrations in the range from 40 to 800 ng/ml showed a correlation coefficient of $r = 0,9980$ and a coefficient of variation (CV) between 3.0 and 3.4% respectively. In this pilot study including 92 patients with diabetes mellitus (Type I and II) and 51 controls the median for the diabetic group was 179 ng/l compared to 188 ng/l in the control group. Further studies have to show if there actually exists a relationship between 4-heptanone and diabetes mellitus - or if the origin of 4-heptanone is solely environmental. A possible source could be the widespread plasticizer Di-(2-ethyl hexyl)phthalate (DEHP), which in vivo could be hydrolyzed and oxidized to the corresponding β -keto-acid. Spontaneously and upon heating this acid would yield 4-heptanone. All major intermediates have now been found and identified in serum and urine. First results from human studies under current investigations show in fact that the plasticizer DEHP is the origin of elevated 4-heptanone concentrations in urine and plasma of patients receiving intravenously applied infusions.

INTRODUCTION

The concept of "metabolic profiling" has been widely applied in general to all different kinds of biological fluids such as urine, serum, cerebrospinal fluid, amniotic fluid, breast milk and to tissue homogenates [1-3]. Next to the organic acid fraction in urine and serum the profiles of organic volatiles have been intensively studied and linked to metabolic disorders [4-10]. The profile of organic volatiles in urine covers a diverse group of different polarity: alcohols, aldehydes, ketones, O- and N-heterocycles, sulfur containing compounds (isocyanates, sulfides) and hydrocarbons are found regularly and may be derived from nutrients, intermediates or environmental contaminants [11]. Pattern recognition of profiles [5] and especially the concentration of several ketones [6, 12], such as 4-heptanone [8, 13] were related to diabetes mellitus. In diabetic patients elevated levels of 4-heptanone in urine were found and tentatively related to more specific stages of the disease [8, 13]. A possible relationship also was found between endogenous volatile urinary metabolites with structures similar to

certain neurotoxins [14] and the development of the diabetic polyneuropathy [10, 15].

The sampling techniques used for the analysis of organic volatiles include static and dynamic headspace with condensation in a cryogenic trap [16, 17] or adsorption onto the hydrophobic porous polymer Tenax (poly 2,6-diphenyl-p-phenylene oxide) [5, 8, 18, 19], solvent extraction [4, 13] and the use of a transelevator [20-22]. Modifications have also been done concerning the instrumentation [23, 24]. Next to the GC-MS other selective detectors for complex sulfur, nitrogen, phosphorous or halogen can be very useful in headspace analysis [25]. Changes in the composition of the volatile sulfur containing compounds in the urine of diabetic persons can reliably be registered by use of a sulfur detector [25]. Mercaptanes such as methanthiole, ethanthiole, dimethyl sulfide and dimethyl disulfide can result from the enterobacterial degradation of methionine in the state of hepatic encephalopathy, but may also in some extent be due to sulfur compounds (methanthiole, dimethyl disulfide) found in coffee [26].

EXPERIMENTAL

Sample preparation. A total of 189 urine samples (spontaneous and 24h collecting period) were taken from 51 healthy controls and 92 diabetic patients. 2ml GC vials were filled with aliquots of 1 ml acidified (30µl conc. HCL) urine and analyzed in duplicates. Serum samples were collected from patients with diabetes, liver diseases and on hemodialysis.

Instrumentation. The applied system consists of a Multi Purpose Sampler (Gerstel GmbH, Mülheim an der Ruhr, Germany), operated in headspace-mode and equipped with a 1000 µl gas tight syringe, a HP-7673 tray for 2ml standard vials (Hewlett-Packard, Waldbronn, Germany) plus an additional pre-heating module for 5 vials with control of temperature and heating-time (Gerstel GmbH, Mülheim an der Ruhr, Germany), a temperature controlled cold injection system CIS-3, (Gerstel GmbH, Mülheim an der Ruhr, Germany) used as interface, cold trap and injection system for the subsequently following GC-MSD combination (HP 5890/5972, Hewlett-Packard, Waldbronn, Germany).

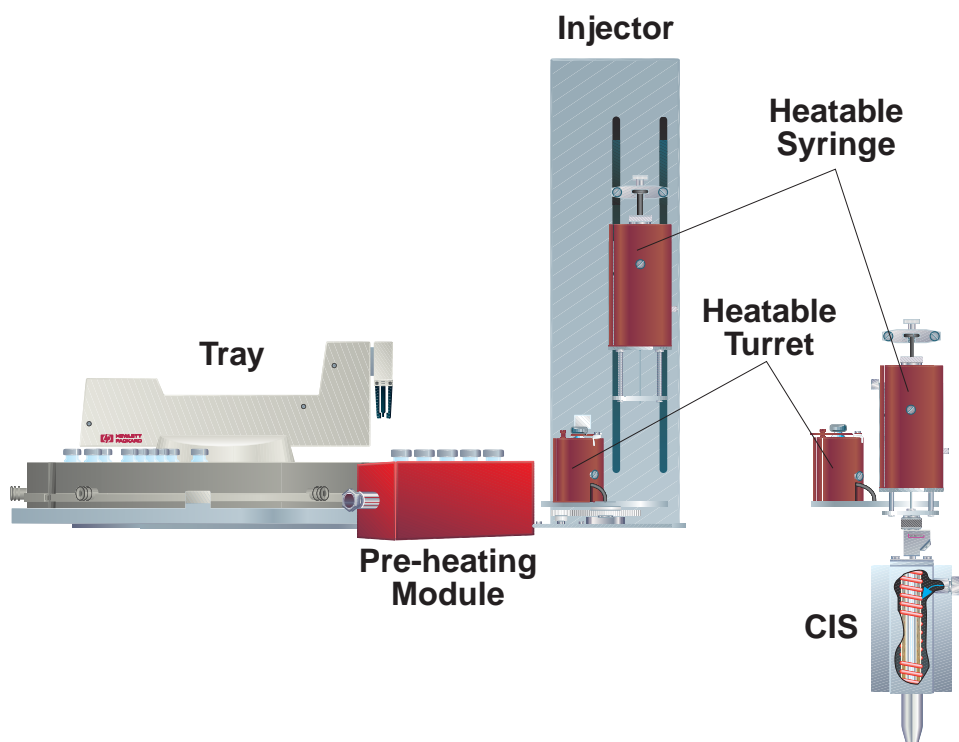


Figure 1. Gerstel Multi Purpose Sampler in standby (left) and injection mode (right).

Operation. Each sample is heated for the same period of time at the same temperature in the pre-heating module. Solvent flushing of the MPS with helium is done by injecting the special designed syringe into the CIS-3 for 8 min. The heated syringe can then be filled with a defined volume of helium and injected into the headspace vial. The depth of injection is controlled for both the position in the vial and in the injector. The sample is injected into the cooled CIS-3 for focussing and enrichment and after heating up to the desired temperature transferred to the capillary column in either split or splitless mode.

Quantification of 4-heptanone in urine. Acidified pooled urine samples spiked with 4-heptanone were used for the calibration curves in a concentration range from 40 to 800 ng/ml. The ion m/z 71.15 was used for quantification and the ions m/z 43.1 and m/z 114.15 were used as qualifier ions for the identification. Intra assay imprecision of the method was determined for different urine samples in the observed concentration range by measuring 10 aliquots from each sample in a row.

Analysis conditions.

Column: 60 m DB-5 (J&W), $d_i=0.25$ mm, $d_f=0.25$
Pneumatics: Carriergas He, $p_i=100$ kPa, split x:30,
1 min splitless
Temperatures: HSS pre-heating module: 70°C (10 min)
HSS turret: 70°C
HSS syringe: 70°C
CIS: -150°C; with 12°C/s to 300°C (3 min)
Oven: 60°C; with 5°C/min to 100°C;
with 25°C/min to 240°C
MSD: 280°C
Detector: MSD, scan 10-260 amu

RESULTS AND DISCUSSION

Identification. **Figure 2** shows the chromatogram from an acidified urine sample of a healthy person. Without acidifying the number of peaks is significant smaller, but on the other hand some peaks become more prominent as is the case for allylisothiocyanate. The identified volatiles are listed in **Table I**.

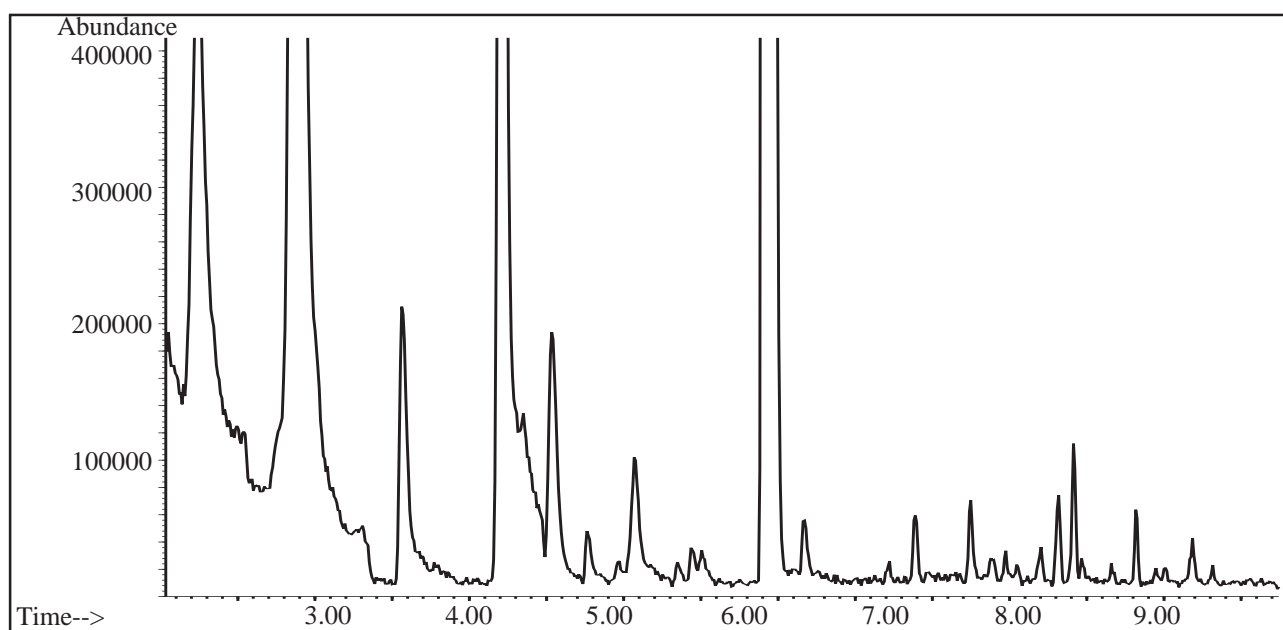


Figure 2. Chromatogram of acidified urine sample from healthy person.

Volatiles	RT (min)	Volatiles	RT (min)
Acetone	2.23	Allylisothiocyanate	6.10
2-Butanone	2.73	Chloro cyclohexane	6.13
Chloroform	2.87	2-Heptanone	6.16
Benzene	3.29	2,4-Dimethyl thiophene	6.36
Cyclohexene	3.45	Methyl-2-propenyl disulfide	6.56
2-Pentanone	3.56	Methyl propyl disulfide	6.71
2,5-Dimethylfuran	3.78	3-Methyl-2-heptanone	6.87
Phenol	3.98	Dimethyl trisulfide	7.23
3-Methyl-1-butanol	4.11	Phellandrene	7.66
Dimethyl disulfide	4.20	1,4-Dichloro benzene	7.69
Toluene	4.52	α -Terpinene	7.80
3-Hexanone	4.75	1-Methyl-2-(1-methyl ethyl)	7.90
2-Ethyl-5-methylfuran	4.94	benzene	
Tetrachloro ethylene	5.06	γ -Terpinene	8.31
5-Methyl-3-hexanone	5.43	4-Methyl phenol	8.50
3-Heptanone	5.45	α -Terpinolene	8.64
4-Heptanone	5.90	3-Methyl hexane-2-one	8.86
Cyclohexanol	6.07		

RT: retention time

Table I. Identified volatiles in human urine.

The analysis of ketones is of clinical relevance in metabolic disorders, especially in the case of diabetes mellitus. Next to methylketones, found at elevated levels in ketoacidosis 4-heptanone proved to be an interesting metabolite [8,13]. **Figure 3** shows an extracted ion chromatogram (m/z 43) of an urine specimen from a healthy control person, which can be chosen to monitor the following ketones: 2-propanone (1), 2-butanone (2), 2-pentanone (3) and 4-heptanone (4), which is often accompanied by a smaller peak of 2-heptanone (5).

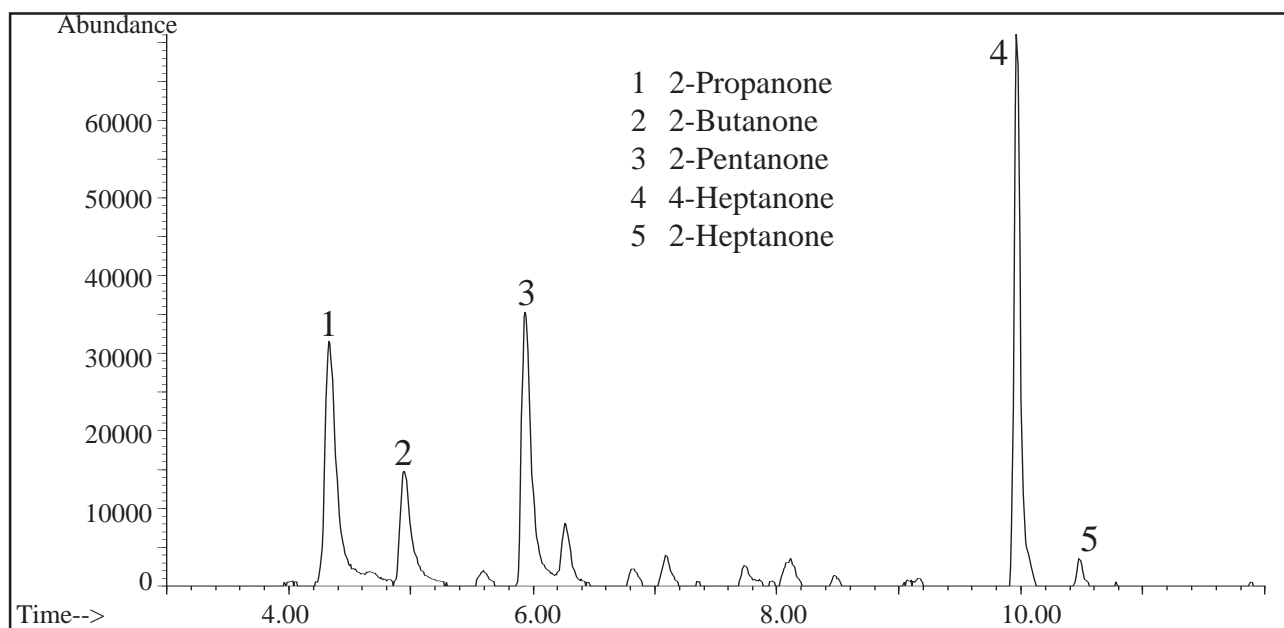


Figure 3. Extracted ion chromatogram (m/z 43), urine sample from healthy person.

Quantification. The calibration curve for 4-heptanone in urine was linear in the range from 40 to 800 ng/ml with a correlation coefficient of $r = 0,9980$. The determination of intra assay imprecision showed a coefficient of variation (CV) between 3.0 and 3.4% for the total concentration range.

Clinical study. The median concentration of 4-heptanone in urine from the healthy control group was 188 ng/ml, ranging from 27 to 1044 ng/ml compared to 179 ng/ml, ranging from 17 to 978 ng/ml in the diabetic patient group (**Figure 4**). The diabetic patient group was rather inhomogenous regarding duration, metabolic control and type of diabetes and there was no significant correlation to any of these parameters.

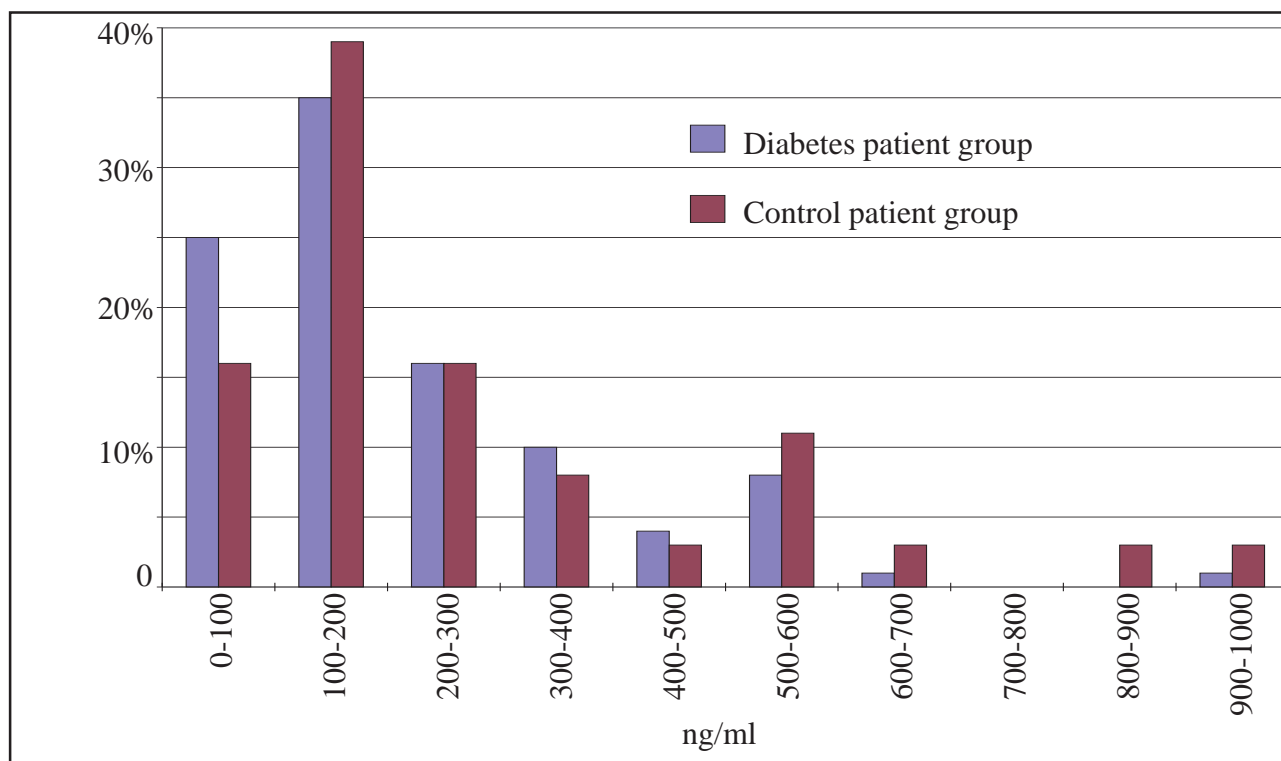


Figure 4. Distribution of urinary 4-heptanone concentrations.

In the group of healthy controls 4 persons had very high concentrations of 4-heptanone in urine: 1390 ng/ml, 1650 ng/ml, 1780 ng/ml and 3720 ng/ml. As there is no difference in the median concentration of 4-heptanone between the diabetic and the control group and considering the highly elevated levels in a few controls the source of 4-heptanone might be solely environmental. A possible source could be the widespread plasticizer 1,2-benzenedicarboxylic acid-bis-(2-ethylhexyl)-ester (DEHP), which in vivo could be hydrolyzed and then oxidized to the corresponding β -keto-acid excreted in urine. Spontaneously and upon heating this acid would yield 4-heptanone and 2-heptanone. First results from studies under current investigations show the plasticizer DEHP to be the origin of 4-heptanone as well as 2-heptanone in human urine and plasma. As DEHP is a common plasticizer used in medical tubings the elevated level in patients of intensive care units and on hemodialysis can easily be explained. In a pilot study a healthy control person was infused 500 ml NaCl in a regular infusion set, which was shown to contain DEHP as plasticizer. Specimens of 24 hours urine collected before and after the infusion were analyzed for the content of 4-heptanone:

4-heptanone ($\mu\text{g}/24\text{h}$)

before infusion	236
after infusion	685

Serum samples. Serum samples were analyzed for the presence of alcohols and ketones, especially for 4-heptanone and 2-ethyl hexanol. Samples from patients undergoing hemodialysis and patients with liver diseases showed increased levels of both, further indicating DEHP to be its possible source. **Figure 5** shows the SIM-chromatogram of a serum sample from a critical ill patient in the intensive care unit of our hospital. **Figures 6** and **7** show the mass spectra of 4-heptanone (**6**) and 2-ethyl hexanol (**7**).

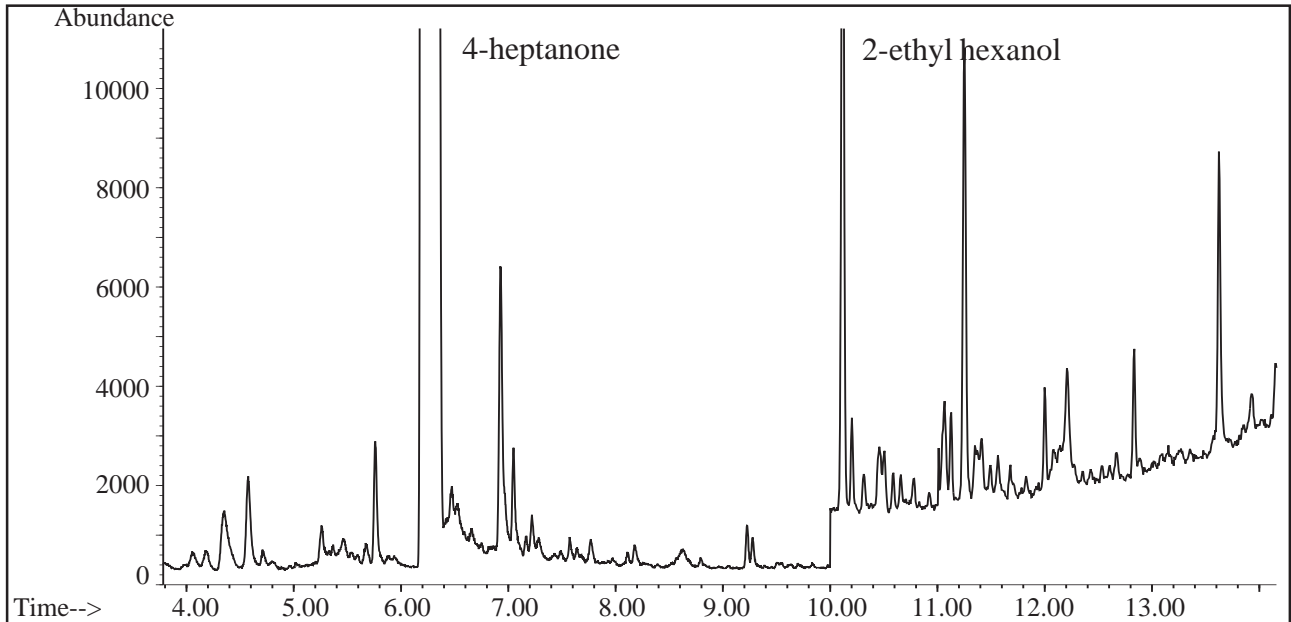


Figure 5. Selected ion monitoring chromatogram of serum sample from critical ill patient.

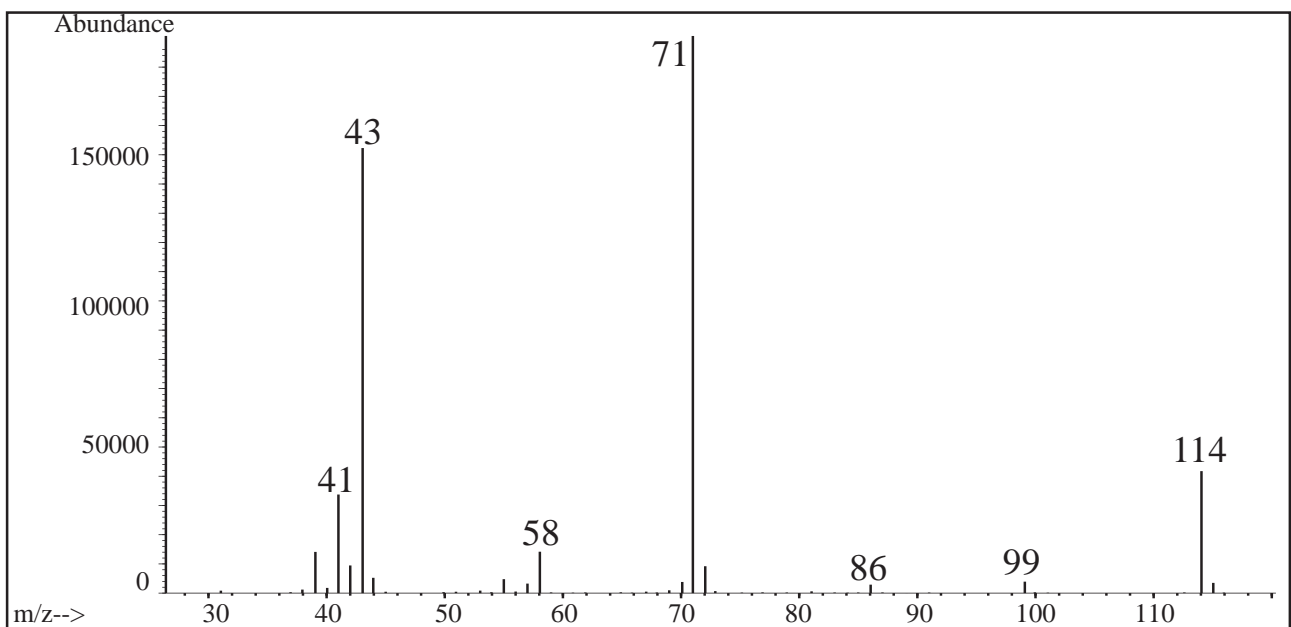


Figure 6. Mass spectrum of 4-heptanone (RT 6.3 min).

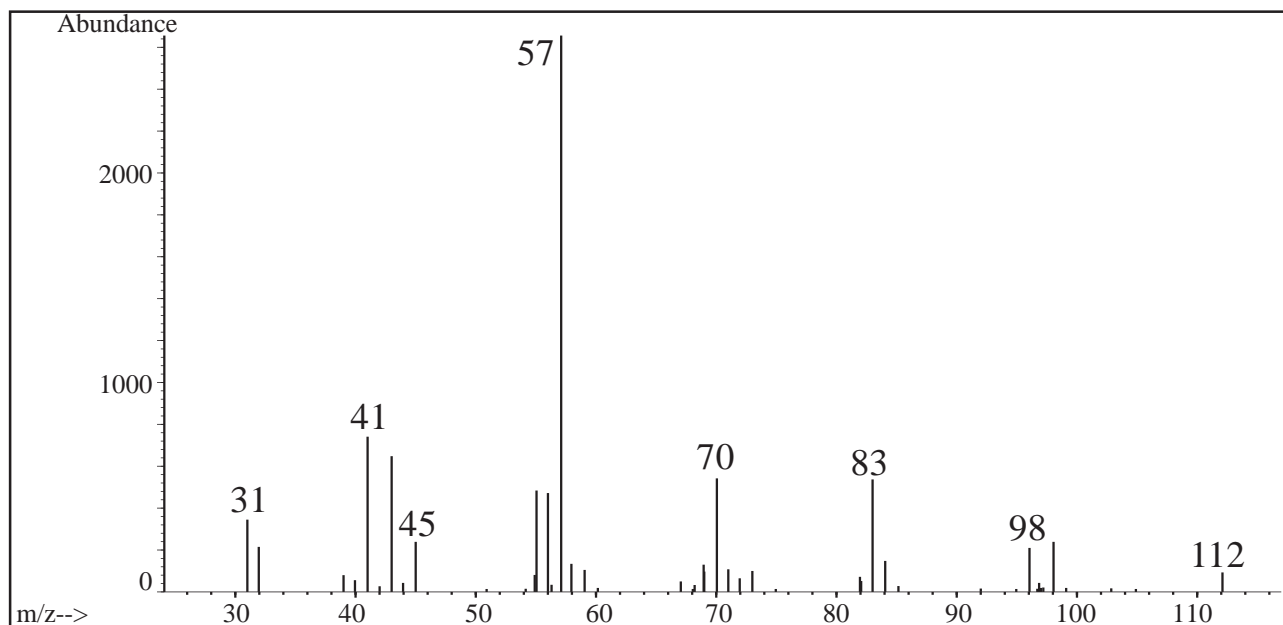


Figure 7. Mass spectrum of 2-ethyl-1-hexanol (RT 10.2 min).

CONCLUSION

With the method described organic volatiles in human urine and plasma can now be easily analyzed. Applications are metabolic profile studies in a qualitative as well as quantitative way. For 4-heptanone as one example high precision and sensitivity of the method was shown. First results from human studies under current investigations show that the plasticizer DEHP is the origin of elevated 4-heptanone concentrations in urine and plasma of patients receiving intravenously applied infusions.

The high practicality in a day to day routine together with the cost cutting philosophy of a multi purpose sampler makes this automated system very attractive for clinical routine use in analysis of organic volatiles.

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