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AppNote 5/1994

The Use of a Multi Purpose Sampler for Headspace GC-MS Analysis of Volatile Organic Compounds in Human Urine

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KEYWORDS

Capillary Gas Chromatography, Multi Purpose Sampler, Headspace, GC-MS, Volatiles, Urine

ABSTRACT

A multi purpose sampler (Gerstel MPS), designed for liquid large volume, gaseous and headspace samples was tested for its suitability in the GC-MS analysis of organic volatiles in human urine. Headspace sampling with a volume, temperature and speed controlled gas tight syringe was combined with a temperature controlled cold injection system for cold trapping, enrichment and focussing of analyte. Regular 2 ml GC-vials filled with 1 ml urine were used as headspace sampling vials. A 100 vial autosampler tray was equipped with an additional temperature and heating time controlled pre-heating module for 5 vials.

Preliminary experiments were done with urine samples from our clinical laboratory with a ketone or glucose positive test result. In addition urine samples from healthy volunteers were taken and analysis were done with and without acidifying the urine. The promising results in regard to sensitivity and practicality in a day to day routine together with the cost cutting philosophy of a multi purpose sampler makes this highly automated system very attractive for clinical routine use.

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].

Patternrecognition of profiles [5] and especially the concentration of several ketones [6,12], such as 4-heptanone [8,13] were related to diabetes. A possible relationship 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 be done concerning the instrumentation [23,24].

Next to the GC-MS other selectiv 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 reliable be registered by use of a sulfur detector [25]. Mercaptanes such as methanthiole, ethanthiole, dimethylsulfide and dimethyldisulfide 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, dimethyldisulfide) found in coffee [26].

EXPERIMENTAL

Sample preparation. The urine samples, including those with a ketone and glucose positive test result, were collected from clinical routine laboratory. 2ml GC vials were filled with aliquots of 1 ml acidified urine and analyzed.

Headspace GC-MS with Gerstel MPS Multi Purpose Sampler. Each sample is heated for the same period of time at the same temperature in the pre-heating module (**Figure 1**, 3). 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 (**Figure 1**, 6) and after heating up to the desired temperature transferred to the capillary column in either split or splitless mode.

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 100 2ml standard vials (Hewlett-Packard, Avondale, USA) 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, Avondale, USA).

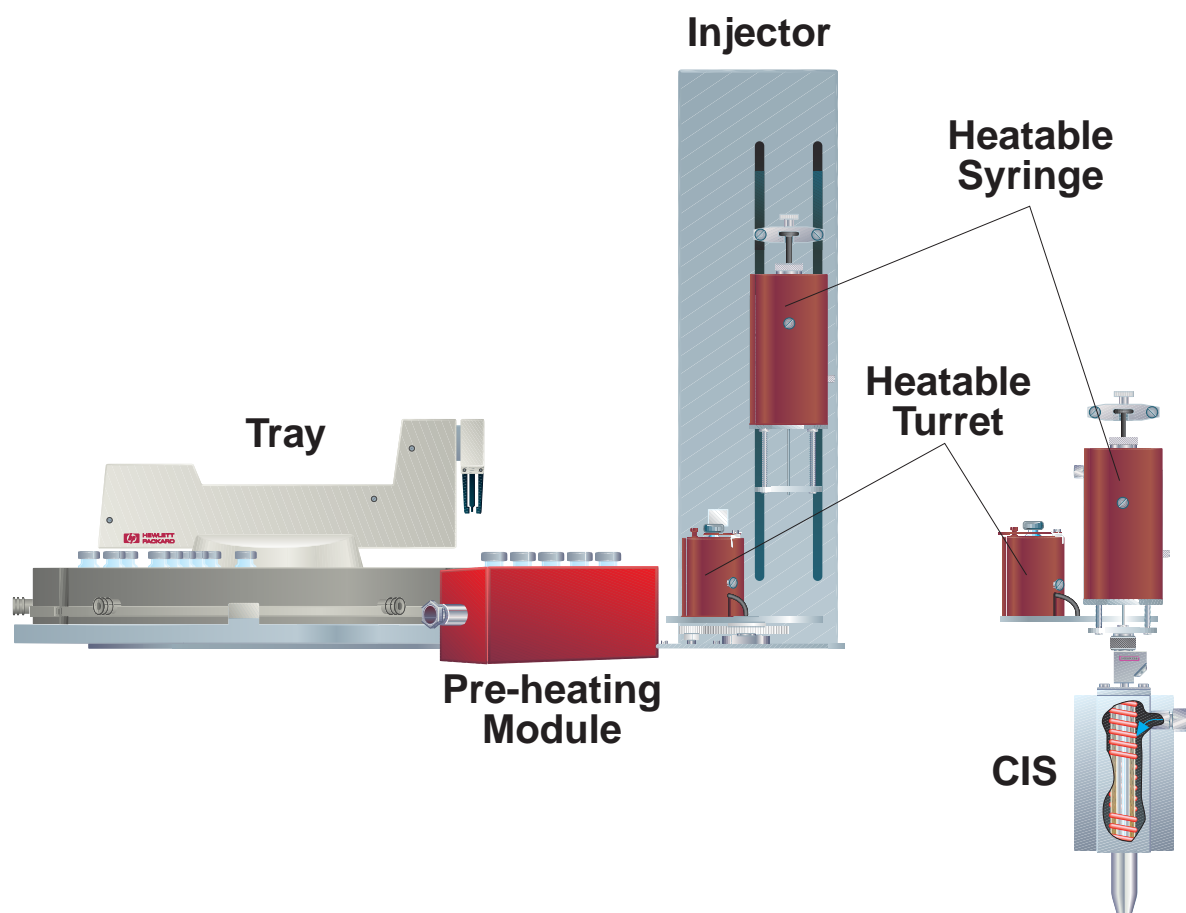


Figure 1. Gerstel Multi Purpose Sampler in standby (left) and injection mode (right).

Analysis conditions.

Columns: CIS-liner 20 mm Carbotrap (Supelco), 20/40 mesh
GC 60 m DB-5 (J&W), $d_i=0,25$ mm, $d_f=0,25$ μ m
Pneumatics: He, $p_i=100$ kPa, split x:30, splitless 0.1-1.1 min
Temperatures: HSS pre-heating module: 70°C (10 min)
HSS turret: 70°C
HSS syringe: 70°C
CIS: 10°C to 300°C with 12°C/s
Oven: 60°C to 100°C with 5°C/min;
to 240°C with 25°C/min
MSD: 280°C
Detector: MSD, scan 10-260 amu

RESULTS AND DISCUSSION

Figure 2 shows the chromatogram from an acidified urine sample of a healthy person. Without acidifying (**Figure 3**) the number of peaks is significant smaller, but on the other hand some peaks become more prominent as is the case for allylthiocyanate. The identified volatiles are listed in **Table I**.

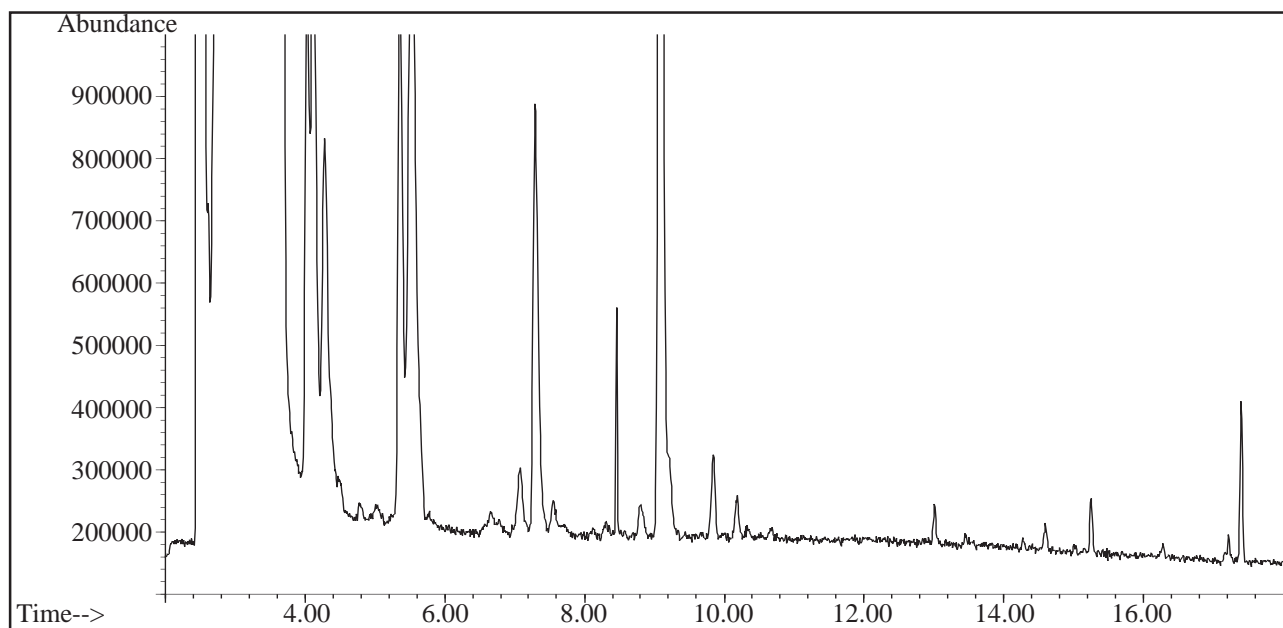


Figure 2. *Chromatogram of acidified urine sample from healthy person.*

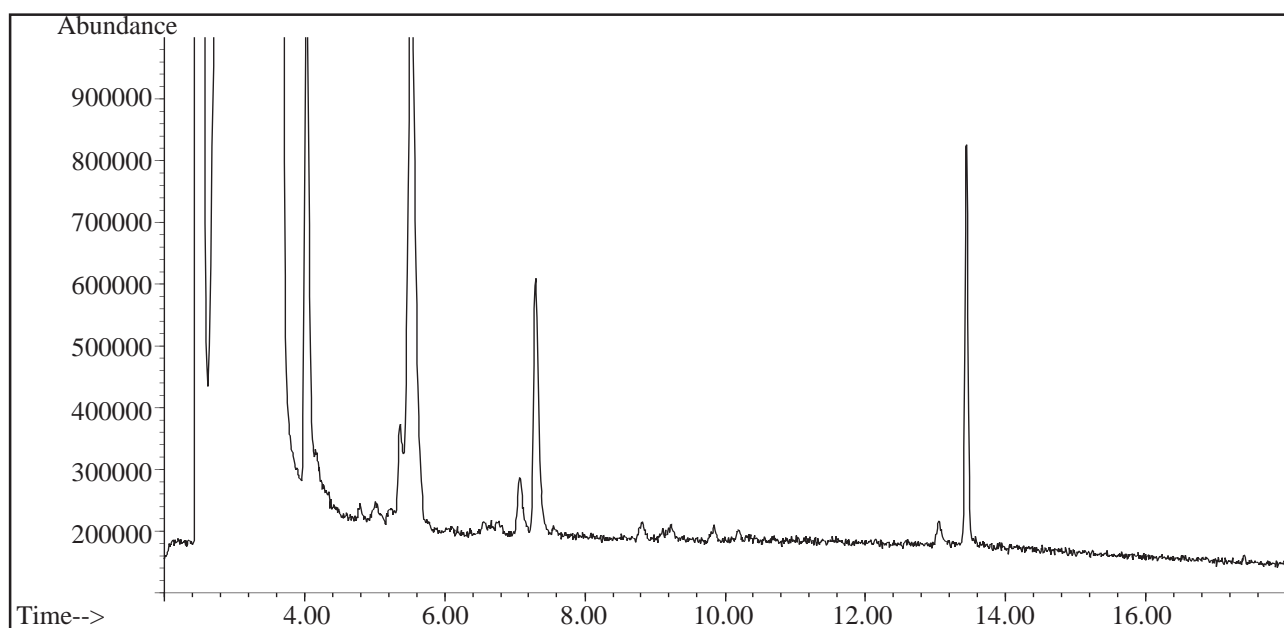


Figure 3. Chromatogram of non acidified urine sample from healthy person.

organic volatile	RT (min)	U 1	U 2	U 3	U 4
methanthiole	3,5	+			+
trimethylamine	3,8		+		
acetone	4,1	+	+	+	+
dihydro-3-methyl-2,5-furandione	4,1				+
dimethylsulfide	4,35		+		+
2-butanone	5,3	+	+	+	+
hexane	5,5	+	+	+	+
2-pentanone	7,3	+	+	+	+
2,5-dimethylfuran	7,9	+			
2-hexanone	8,8			(+)	+
dimethyldisulfide	9,1		+	(+)	+
toluene	9,9		+	+	+
3-hexanone	10,2				+
hexanal	10,7	+			
4-heptanone	13,0	+	+	+	+
allylthiocyanate	13,4			+	

RT: retention time

Urine samples: U 1 = ketone positive
 U 2 = glucose positive
 U 3 = normal
 U 4 = normal, acidified

Table I. Identified volatiles in urine.

Applicability. 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 4 to 8** show ion chromatograms (m/z 43, gained from total ion chromatogram) of urin specimen from different persons. The ion m/z 43 was chosen to monitor the following ketones (**Figures 4 to 8**): 2-propanone (a), 2-butanone (b), 2-pentanone (c) and 4-heptanone (d).

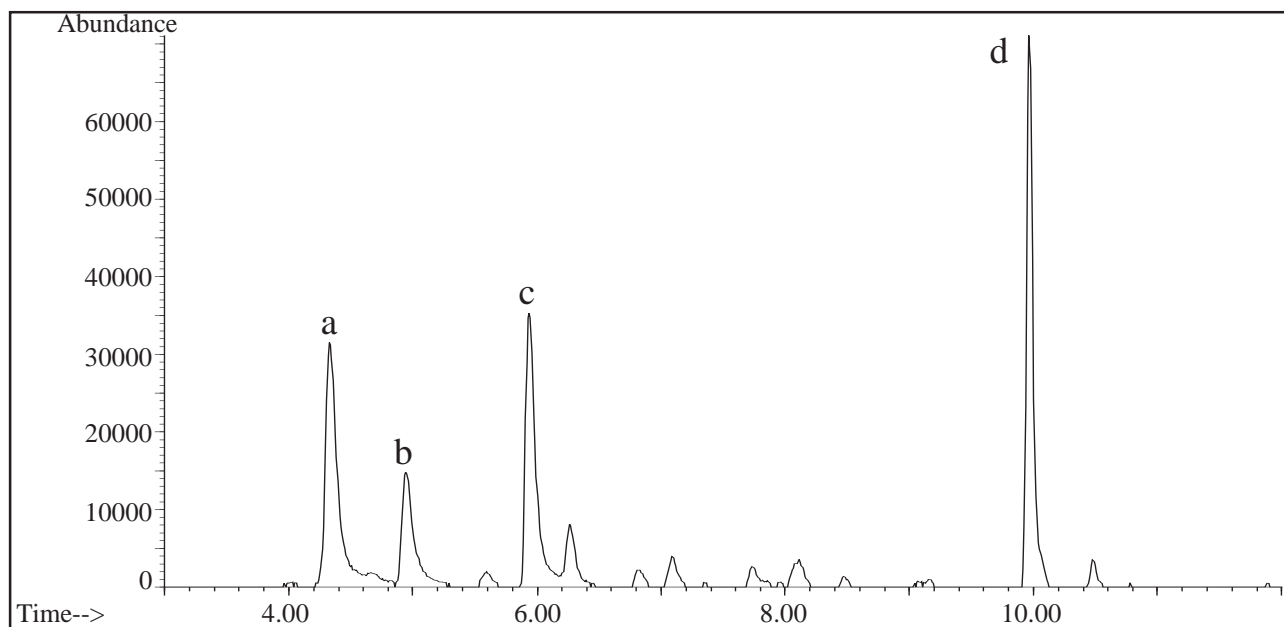


Figure 4. *Healthy person.*

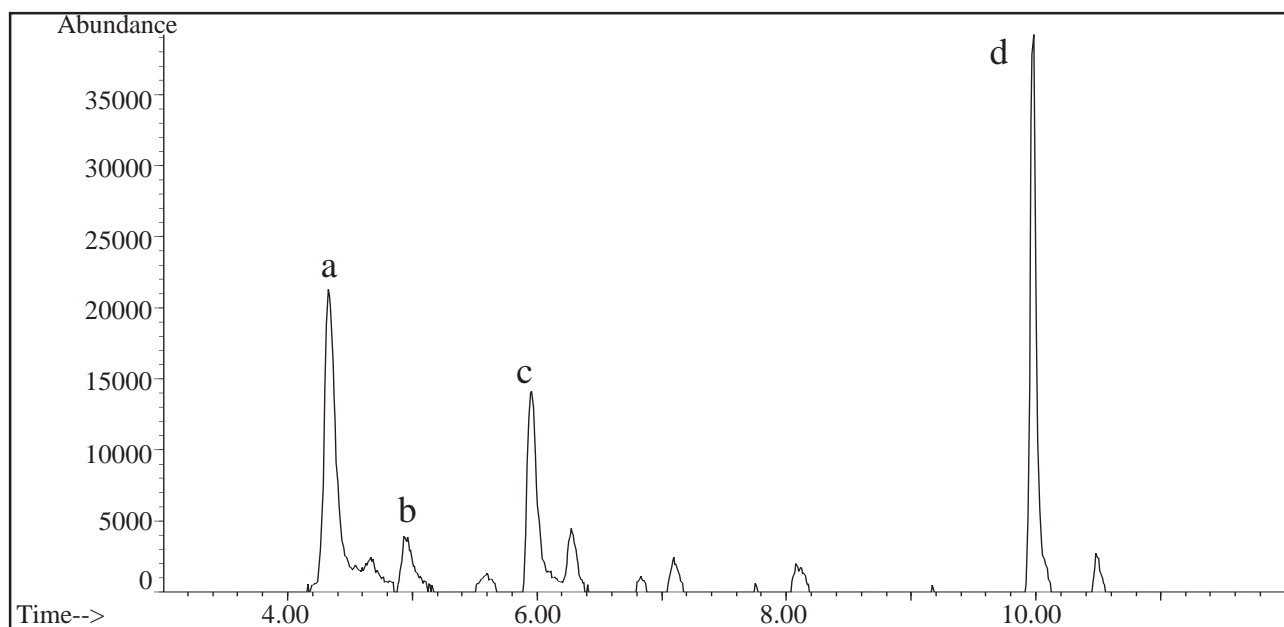


Figure 5. *Person with diabetes mellitus, normoglycaemic.*

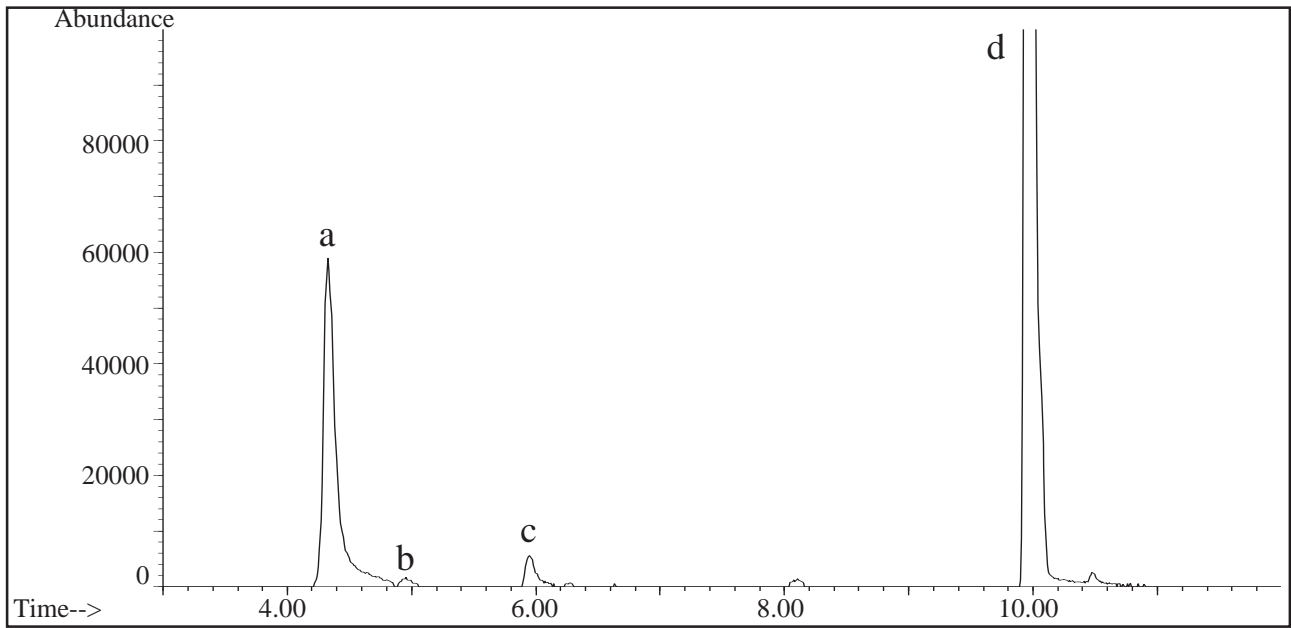


Figure 6. *Person with diabetes mellitus, renal failure, pancreatic tumor, liver abscess.*

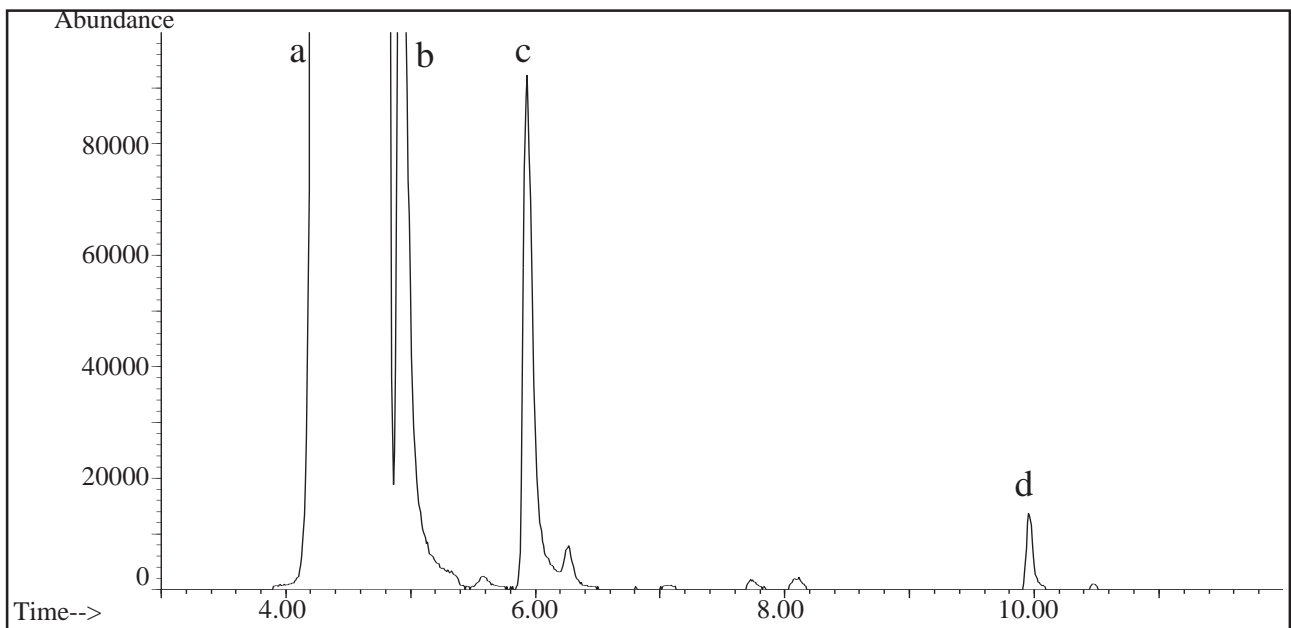


Figure 7. *Healthy person, starving.*

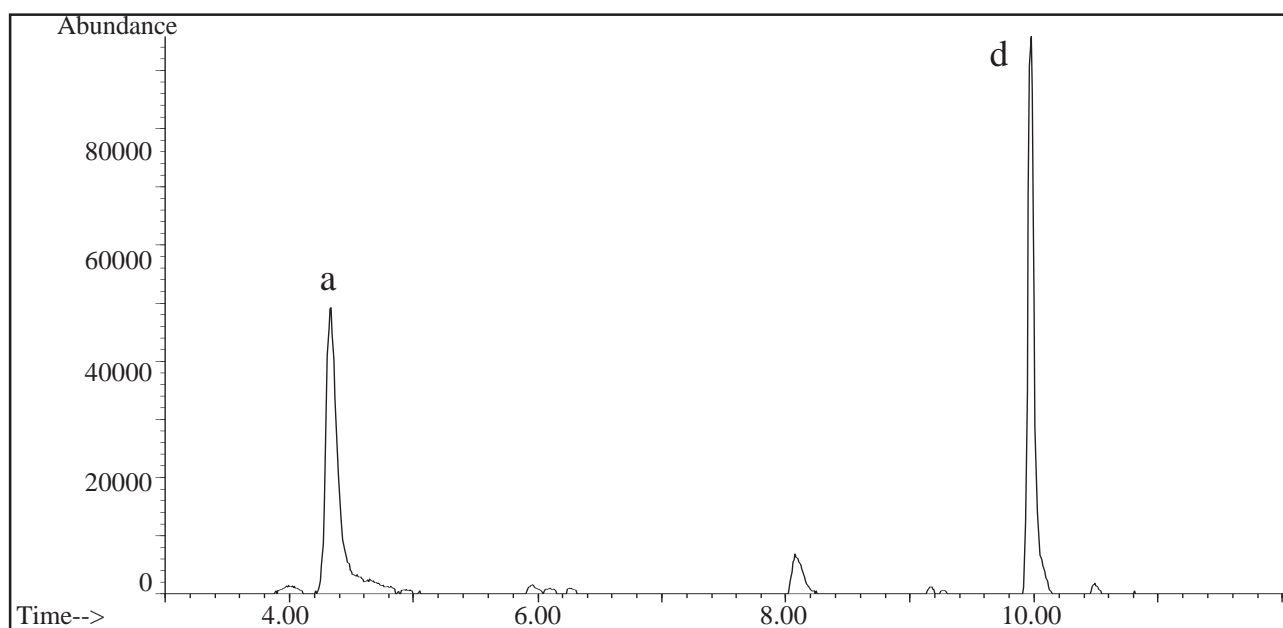


Figure 8. *Person with sepsis, lactic acidosis.*

Reproducibility. For the evaluation of the reproducibility three different urines were chosen and each of them analyzed 10 times. Standard deviation was calculated for the 4-heptanone content in the samples using the area counts obtained for ion m/z 43. The results can be seen in **Table II**.

Sample	mean (area counts)	standard deviation (area counts)	standard deviation (%)
Urine 1	2323276	± 70454	3.0
Urine 2	25971515	± 891562	3.3
Urine 3	793437	± 39437	4.9

Table II. *Reproducibility.*

Although this preliminary experiments are promising, further work has to be done in optimizing operating conditions of the headspace sampling system. The volume of the urine sample needed, sample preparation (pH, salt), temperature and time of preheating, removal of water as well as optimizing the GC-conditions are parameters which are under further investigation.

CONCLUSION

Preliminary experiments with the new multi purpose sampler showed a fairly good sensitivity for the urine samples analyzed and further studies are done in regard to increasing sensitivity by multiple sampling, optimizing operating conditions and sample preparation. Precision of the method has to be shown by additional experiments, but the so far promising results in regard to sensitivity and practicality in a day to day routine together with the cost cutting philosophy of a multi purpose sampler makes this highly automated system very attractive for clinical routine use.

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