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Application 4:

Identification of odorous compounds in urine after the consumption of asparagus (*Asparagus officinalis*)

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The young shoots of *Asparagus officinalis* are described as a delicacy since the time of the ancient Roman Empire. In the 18th century the first reports appear that the consumption of asparagus correlate with the production of malodorous urine smelling like "rotten cabbage". Nevertheless different studies showed that there are two different categories of "excretors" and "non-excretors" [1]. Scientists remain still divided why people have different urinary response to eating asparagus.

One theory is based on the thesis that about half of the population have a gene that enables about fifty percent of the population to break down the sulphur containing amino acid methionine (concentration in asparagus 240 - 310 mg/kg [2]).

The second theory says that everyone digests asparagus the same way, but only about half of us can smell the specific compounds formed during the digestion of asparagus.

This study was performed to provide a very fast and simple method for the identification of odorous substances in urine to prevent the changes in the composition of the volatile fraction as it can happen with normal methods described in the literature (solvent extraction, head space at elevated temperatures..)

Collecting the samples

Several volunteers donated urine samples just before the consumption of approximately 400 gram fresh asparagus, prepared just by cooking in salted water. In intervals of about one hour during the first three hours the total amount of urine was measured and 10 mL were put into a screw vial (see SPME method).

SPME Method

For the identification of the volatile compounds a simple and rapid method was developed. 10 mL urine were put into a 30 mL head space vial with an 1 cm magnetic stirring bar. The vial is closed with a screw cap with a PTFE lined rubber septum and put in heated metal block at 40°C (see Figure 1.). This metal block was especially design for

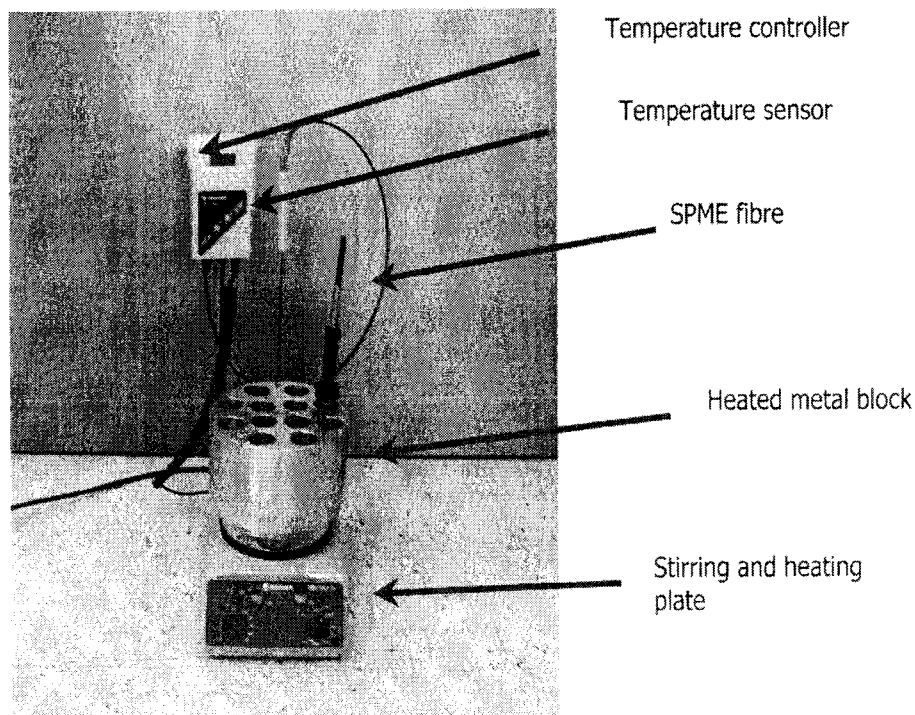


Figure 1. Apparatus for the SPME sample preparation

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SPME sample preparation. Due to the big thermal mass of the massive metal block the temperature can be very constant. Holes in the size of the headspace vials were drilled in the metal block for perfect fit to guarantee a good heat exchange between the metal block and the vial. After 5 minutes the SPME fibre (75 μ m Carboxen/PDMS, conditioned according to the instructions of the manufacturer) was exposed to the headspace of the urine sample for additional 20 minutes. The sample was stirred with 150 rpm during the extraction process to enhance the sensitivity. Afterwards the fibre was directly desorbed in the heated injector of the GC-MS system. The samples were analysed immediately after collecting, or stored for period of maximum 6 hours in a refrigerator at 4°C. This in combination with an extraction temperature of maximal 40°C (closely to the inner temperature of a human body) should minimize the possible formation of artefacts.

Gas chromatography-mass spectrometry

GC:	HP GCS system with mass selective detection
Injector:	Solit/splittless, mode splittless
Injector temperature:	270°C
Carrier Gas:	Helium purity 99,99%, 0,9 mL/min, constant flow
Oven:	-30°C (1 min), with 8°C to 250°C (hold for 5 min.)
GC Column:	HP 5 MS, 30 m, 0.25 mm i.d., 1 μ m film thickness
Interface:	direct, 280°C
Ionisation:	EI, 70 eV
Acquisition mode:	Scan, scan range 20-200 amu, 2.5 amu/s
Data acquisition:	HP Chemstation

To optimise the chromatographic parameters (peak shape, resolution) the initial oven temperature was set to -30°C by cooling the oven with liquid nitrogen an a special insert for SPME for the injector (0.75 μ m inner diameter, distributed by Supelco) was used. This enables even the separation of very volatile compounds like gases (H₂S, COS) without loss of chromatographic performance due to dead volume and desorption characteristics.

Figure 2. shows a chromatogram obtained from a sample 2 after consumption of asparagus. The main peaks in the chromatogram were identified as sulphur containing peaks. Besides there are some of these group of substances detectable only in trace amounts, shown in the zoomed area of the chromatogram (see Figure 3.).

Identification of compounds

Positive identification was done by comparison of the obtained mass spectra and a commercially available spectra data base (Wiley275k) and calculation of the retention indices using the algorithm according to *van den Dool & Kratz* [3]. A retention index database for aroma active compounds is under construction on our institute.

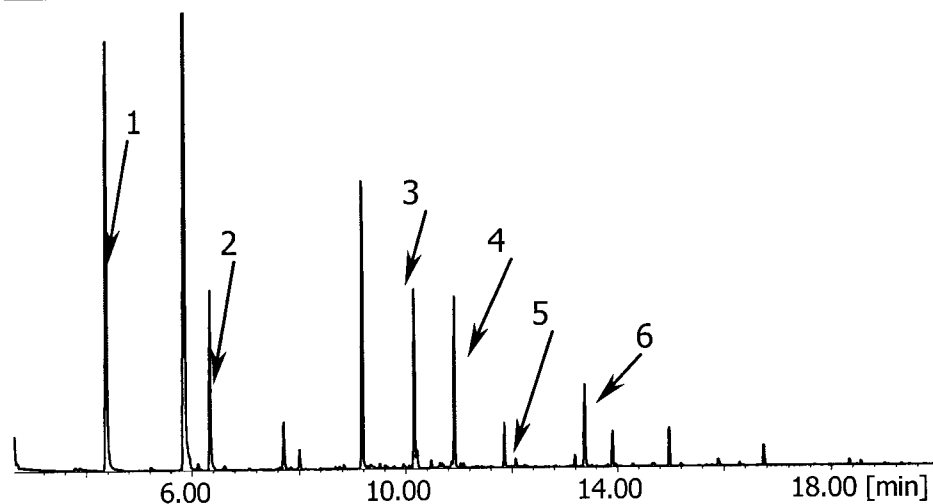


Figure 2. Chromatogram of urine sample, list of identified compounds see Table 1.

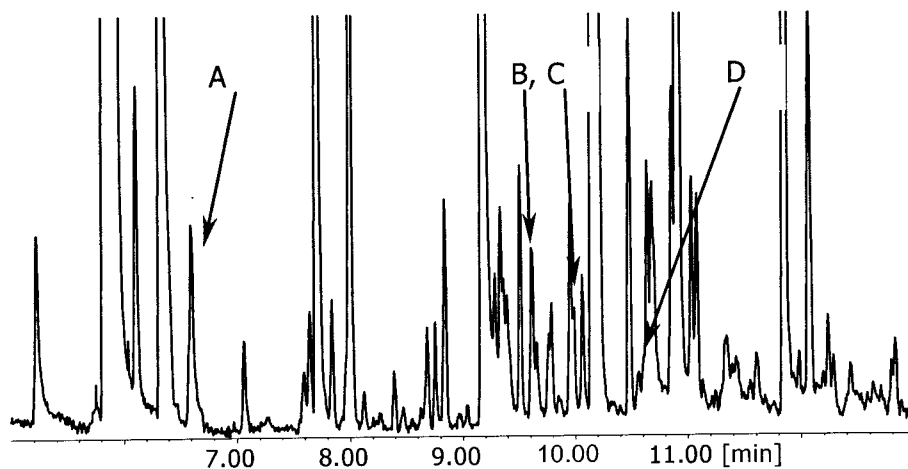


Figure 3. Zoomed chromatogram of urine sample, list of identified compounds see Table 1

12 different sulphur containing compounds were identified by using this technique. A list of these substances is given in Table 1. with the threshold values in water and air, when available. Some of these compounds show very low thresholds and most of these compounds have described odour properties like "sulphurous, rotten egg, rotten cabbage, cooked cabbage".

No.	Con
*	Hyd
*	Carb
1	Met
2	Dim
A	Carb
B	Met
C	E-M
3	Dim
D	3-M
4	S-M
5	1-P
6	Dir

* n

1,2,... C

A,B,... C

n.a. d

14000

12000

10000

rel. Peakarea

8000

6000

4000

2000

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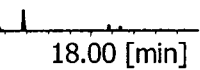
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Table 1. List of identified compounds

No.	Compound	Formula	Threshold Air [µg/m ³] [4,5]	Threshold Water [µg/L] [4,5]
*	Hydrogensulfide	H ₂ S	1-30	10
*	Carbonoxidesulfide	COS	n.a.	n.a.
1	Methanethiol	CH ₃ SH	n.a.	0.02-0.2
2	Dimethylsulfide	CH ₃ SCCH ₃	3-25	0.3-3
A	Carbondisulfide	CS ₂	50-100	n.a.
B	Methylpropylsulfide	CH ₃ SC ₃ H ₇	n.a.	n.a.
C	E-Methylthio-1-propene	CH ₃ SC ₃ H ₆	n.a.	n.a.
3	Dimethyldisulfide	CH ₃ SSCH ₃	3-20	0.2-7
D	3-Methylthiophene	C ₅ H ₆ S	n.a.	n.a.
4	S-Methyl-2-propenthioate	CH ₃ SC ₃ H ₃ O	n.a.	n.a.
5	1-Propene-3-isothiocyanate	C ₃ H ₅ NCS	n.a.	400
6	Dimethyltrisulfide	CH ₃ SSSCH ₃	0.1-7	0.01

* not shown in chromatogram
1,2,... Compounds shown in chromatogram 1
A,B,... Compounds shown in chromatogram 2
n.a. data not available from literature



ounds see Table 1.



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using this technique.
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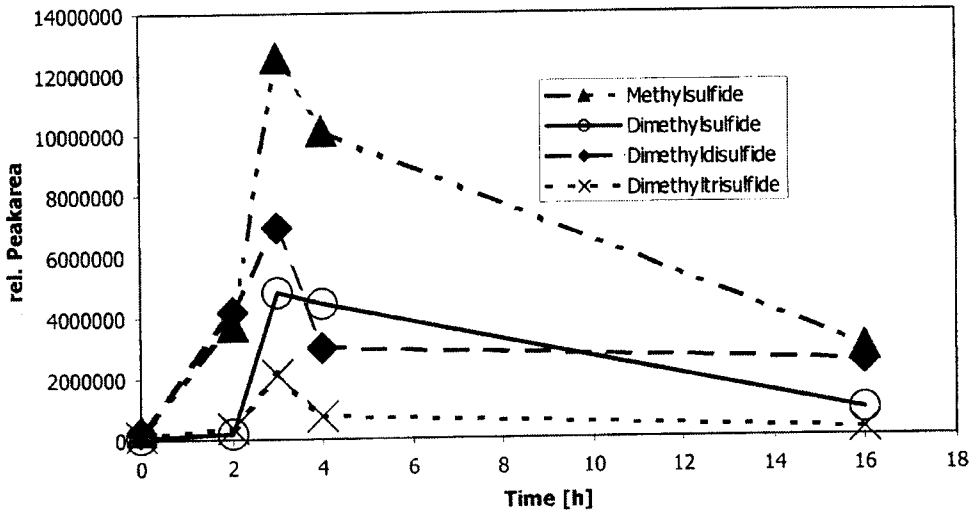


Figure 4. Time dependence of some compounds

Excretion of sulphurous compounds

Figure 4. shows the excretion behaviour of the homologous series of methylsulfides. None of these compounds were present in the first sample. Normally the first "asparagus

smell" can be detected after a short period of only 10 or 15 minutes. During a period of several hours the excreted amount increases and reach a maximum at about 3 hours after consumption. Then there is a steady decrease, but even after 16 hours there are still detectable amounts of all these compounds present, still detectable by the human nose.

Conclusion

The use of SPME in combination with GC-MS offers a very sensitive and rapid method for the determination of odour active compounds in the microgram/litre range. It was possible to identify 12 different sulphur containing compounds with very low thresholds down to the nanogram/litre range. With a minimum of sample preparation steps several analysis can be performed on a day.

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