Short communication

Quantitative determination of trimethylamine in urine by solid-phase microextraction and gas chromatography–mass spectrometry

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Abstract

Trimethylaminuria (fish odour syndrome) is diagnosed from an increase in urinary excretion of trimethylamine with decreased trimethylamine oxide. We report a new quantitative stable isotope dilution gas chromatography–mass spectrometry procedure for the analysis of these metabolites using solid-phase microextraction (SPME). Both polydimethylsiloxane and mixed Carboxen–polydimethylsiloxane SPME fibres were found to be suitable for the headspace extraction of TMA. This new sampling technique could have wide application for the analysis of volatile and semi-volatile compounds by metabolic screening laboratories. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Trimethylamine (TMA) is a volatile amine with a pungent odour of rotting fish. It is produced in the bowel lumen by bacterial degradation of choline, carnitine and trimethylamine oxide (TMAO) present in some salt-water fish [1,2]. After absorption into the portal blood stream it is normally oxidised almost entirely in the liver to non-odorous TMAO and excreted in urine. Normal excretion of TMA is less than 1 µmol/mmol creatinine and of TMAO 50–125 µmol/mmol creatinine [3] (for adults, therefore, less than 10 µmol/day and around 500–1250 µmol/day, respectively). Individuals with the rare inherited disorder trimethylaminuria (fish odour syndrome, McKusick 275700) have reduced capacity to oxidise TMA [2,4–5]. TMA is increased in the breath, sweat, vaginal secretions and urine causing an unpleasant body odour and considerable psychosocial problems [2,6]. The condition is diagnosed from an increase in TMA in urine with a decrease in TMAO to less than 55% of total urine TMA [2]. Carriers of the condition are identified by urine analysis after a TMA loading test [2,7].

It is difficult to analyse TMA accurately at the very low concentrations found in normal urine. The best method available is probably proton nuclear magnetic resonance spectroscopy (NMRS) [3], but few hospital laboratories have access to this for clinical diagnosis. Reported analyses have used GC [8,9] or GC–MS [10,11] coupled with direct headspace analysis or with preliminary concentration by purge and trap sampling [12,13] or steam distillation [14].

In 1990, Pawlisyzn et al. reported a new solid-phase microextraction (SPME) technique for extract-

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ing organic compounds rapidly from a variety of matrices [15]. This uses a modified syringe assembly which houses a fused-silica fibre externally coated with a gas chromatographic stationary phase [16]. After partitioning between the stationary phase and sample matrix, analytes are desorbed into a GC [16] or high-performance liquid chromatograph [17,18]. SPME can be used for headspace (HS) sampling or the fibre can be immersed directly into the sample matrix [16]. Both equilibrium [16] and non-equilibrium [19] sampling modes can be employed. Compared with standard headspace sampling, there is potential for greater sensitivity and reduced sample losses. Although SPME is used widely for analysis of environmental pollutants [20–25], and applications for measuring drugs and volatile compounds in human blood and urine are increasing [26–33], it is not used yet by clinical metabolic diagnostic laboratories. We report an analysis for TMA and TMAO (reduced to TMA) in urine using SPME, which incorporates deuterated TMA as internal standard, for accurate quantification.

2. Experimental

2.1. Materials

Polydimethylsiloxane (PDMS) (100 μm film thickness), polyacrylate (85 μm film thickness) and Carboxen–PDMS (75 μm film thickness) SPME fibres and SPME fibre syringe holders were from Supelco (Poole, UK). Deuterated trimethylamine (D_9.DCl salt) was from Euriso-top (Bâtiment Homére, St. Aubin, France). Titanium(III) sulfate (150 g/l w/v) solution was from Fisons (Loughborough, UK). All other chemicals and analytical reagents were from Sigma (Poole, UK). Acidified water, pH approximately 1.0, was prepared by addition of 6 *M* hydrochloric acid to de-ionised water. Headspace vials (22 ml) with soft silicone rubber seals (20 mm) and aluminium caps were from Alltech Associates (Carnforth, UK).

2.2. Instrumentation

The bench-top GC–MS system was a 5890 series 2 gas chromatograph linked to a 5971A quadrupole mass spectrometer (Hewlett-Packard, Bracknell, UK)

fitted with a Carbowax fused-silica capillary GC column, 30 m×0.25 mm I.D., film thickness 0.25 μm (Alltech). A narrow bore (0.75 mm) SPME injection liner was used (Supelco).

2.3. Sample preparation and SPME procedure

All the SPME fibres were conditioned by inserting them into the GC injector according to the manufacturer's instructions. TMA was released into headspace vapour using a method based on Al-Waiz et al. [8], but with deuterated TMA as internal standard (I.S.). A 2 ml amount of urine, aqueous standard or aqueous calibrant (15.7 μmol/l), 3 ml of acidified water, approximately 2 g of anhydrous potassium carbonate, 1 ml of aqueous deuterated I.S. (56.8 μmol/l), a magnetic stirrer and (finally) six potassium hydroxide pellets were placed into a glass vial which was sealed and crimped quickly. The vial was mounted on a magnetic stirrer submerged in a water bath maintained at 50°C and the contents stirred continuously. The septum was pierced with the SPME needle guide, the SPME fibre exposed and headspace vapour sampled for 15.0 min. Extracted compounds were desorbed for 2 min in the injector of the GC–MS (splitless mode, split valve closed for 2 min) for analysis using single ion monitoring under the following conditions: helium carrier gas at 1 ml/min, interface transfer line 280°C, injector 250°C; oven temperature programme 40°C (5 min) ramped at 25°C/min to 180°C (0 min). The column was heated to 180°C to ensure that there was no carry over of the co-extracted material between analyses. Ions were monitored at m/z 58 and 66 ($(M-1)^+$ and $(M-2)^+$) and m/z 59 and 68 (molecular ions) for unlabelled and deuterated TMA, respectively. Retention times were 1.70 min for unlabelled TMA and 1.78 min for deuterated TMA. The detector signals were collected, integrated and recorded using an HP Chemstation (Hewlett Packard). TMA concentration was calculated from the 58/66 peak area ratios of tests and calibrant (analysed in duplicate).

Total TMA (free TMA and TMAO) in urine was analysed in a two-stage procedure based on Al-Waiz et al. [8] in which TMAO is first reduced to TMA in acid solution using titanium(III) sulfate. A 100 μl amount of urine or aqueous TMAO standard, 900 μl of acidified water and 200 μl titanium(III) sulfate

were sealed in a headspace vial and incubated in a
heating block at 30°C for 30 min. Working on ice,
the vials were decapped in turn, and 2 ml acidified
water, 1 ml I.S. (deuterated TMA, 56.8 μmol/l), 2.0
g potassium carbonate, a magnetic stirrer, and six
potassium hydroxide pellets were added. The vials
were re-sealed and TMA analysed as above. The
reduction procedure is reported to achieve 98–99%
chemical reduction of TMAO to TMA [8]. Using
conventional headspace GC analysis (without SPME)
we found previously that reduction was complete by
30 min and obtained recoveries of TMAO from urine
of 100–117% at three concentrations (unpublished
observations).

3. Results

Sharper chromatographic peaks for TMA were
obtained with a Carbowax capillary GC column than
with a non-polar column (BPX 5, SGE, Milton
Keynes, UK). Experiments showed that the poly-
acrylate SPME fibre did not extract TMA efficiently.
Peak areas of TMA and deuterated TMA were only
0.005% of those for the polydimethylsiloxane fibre,
which was subsequently used for the study. TMA
was completely desorbed from the fibre within 2 min
with no carry over between samples. Exposure of the
fibre to the headspace vapour for 15 min at 50°C
produced a satisfactory MS response at physiological
TMA concentrations, with acceptable throughput of
samples.

3.1. Linearity and sensitivity

The analysis was linear for TMA in aqueous
solution over the range 0.8 to 157 μmol/l ($y =
0.0103X - 0.0099$; $R = 0.9986$; $SD = 0.0318$, $n = 9$)
and sensitive to at least 0.8 μmol/l, and for TMAO,
after reduction to TMA, from 14.9 to 956 μmol/l
($y = 0.0016X + 0.0228$; $R = 0.9991$; $SD = 0.0251$; $n =
7$) and sensitive to at least 14.9 μmol/l.

3.2. Within-batch imprecision

Coefficient of variation (C.V.%) for aqueous solu-
tions of TMA at three concentrations was 6.1 to
9.9% and for unspiked urine with a very low TMA
concentration was 12.2% (Table 1).

Table 1
Within-batch imprecision of TMA analysis of three aqueous
solutions and one sample of unspiked urine. 2 ml of aqueous
standard or 2 ml of normal urine was analysed for TMA as
described in the text

TMA (μmol/l)		C.V. (%)[a]	n[b]
Mean	1 SD		
Aqueous standards			
95.2	5.8	6.1	7
50.6	5.0	9.9	7
10.7	1.0	9.3	7
Unspiked urine			
2.4	0.3	12.2	8

[a] C.V.% = coefficient of variation %.
[b] n = number of observations.

3.3. Recovery

Recovery of TMA added to normal urine (TMA
concentration 8.5 μmol/l) at three concentrations
was: 84% for 7.2 μmol/l spike, 75% for 31.4 μmol/
l and 85% for 125.6 μmol/l spike.

3.4. TMA and total TMA (TMA+TMAO) in normal adult urine

Table 2 shows results for urine collected from 10
normal adults on an unrestricted diet (8×24 h urine
collections into hydrochloric acid and two fresh
random samples).

3.5. TMA analysis with the Carboxen–PDMS fibre

During the course of this study a new type of
SPME fibre (75 μm film Carboxen–PDMS) became
available. This was reported recently to have high
sensitivity for ethanol [26]. In preliminary inves-
tigations 1 ml of aqueous TMA (62.8 μmol/l) was
incubated with 1 ml of deuterated TMA (56.8 μmol/
l) with the standard procedure. For the polydi-
methylsiloxane (PDMS) fibre peak areas for the 58
and 66 ions were 7.9 and 9.2%, respectively, of those
for the Carboxen/PDMS fibre. The 58/66 ion ratios
were similar for the two fibres. The Carboxen/
PDMS fibre was therefore approximately 12 times
more sensitive for TMA. With serial dilutions of
TMA over the range 6.3 to 0.01 μmol/l, detection
was demonstrated to 0.01 μmol/l, using the standard
procedure. Estimates of the TMA concentration in a

Table 2
Urine TMA and total TMA of 10 normal adults. Free TMA and free TMA with TMA released by reduction of TMAO (total TMA) were analysed in urine as described in the text

	TMA (μmol/l)	Total TMA (μmol/l)	TMA (μmol/24 h)	Total TMA (μmol/24 h)	Percentage TMA of total TMA
Median	4.2	438	7.5	754	0.8
Range	1.4–11.0	208–2768	2.3–26.5	385–5741	0.3–1.8
Number	10	10	8	8	10

normal urine sample were 2.4 μmol/l with the PDMS fibre and 2.3 μmol/L with the Carboxen–PDMS fibre.

4. Discussion

Proton NMRS is the only procedure available currently in which urine TMA and TMAO can be analysed simultaneously without prior extraction [3,34]. In all other methods, free TMA and TMA produced by reduction of TMAO must be released from solution in urine by alkalinisation to a pH above the pKa (9.8) of TMA [35], and then transferred to a GC or GC–MS for analysis. Some reported methods have used standard headspace sampling with GC using packed columns [8,9,35], with triethylamine [9] or isopropylamine [8] as I.S. Al-Waiz et al.[8], who have the greatest experience of patients with trimethylaminuria worldwide [2] injected 2 ml of headspace vapour and reported detection to 0.2 μmol/l, linearity from 3.4 to 169 μmol/l and between-batch imprecision (C.V.%) 3.2–6.9%. However, in this laboratory, using similar procedures [8], with direct headspace sampling and quantification by GC–MS (using triethylamine as I.S.), we obtained less impressive results. Imprecision for urine TMA analysis was 12–13% (C.V.%) and recoveries were 65–84%.

Deuterated TMA has been used as I.S. by King et al. [10] and Treacey et al. [11] with headspace GC–MS. King et al. achieved detection to 2 μmol/l, and percentage recovery of 92–104% of TMA added to urine. Treacy et al. found linearity over the working range but did not present other validation data. Dorland et al. [14] reported a GC procedure sensitive to 5 μmol/l with a nitrogen/phosphorus detector but used complex sample preparation.

Others have used cumbersome purge and trap methods [12,13].

The SPME procedure performed well, was easy to carry out and required little equipment other than a GC–MS, which is now standard instrumentation in clinical laboratories concerned with diagnosis of inherited metabolic disorders. The concentrations found for TMA and TMAO (Table 2) in normal urine using SPME were comparable with those reported for NMRS (TMA<1 μmol/mmol creatinine, TMAO 50–125 μmol/mmol creatinine [3], equivalent to around 10 μmol and 500–1250 μmol/24 h, assuming creatinine excretion of 10 mmol/24 h) and GC–MS with stable isotope dilution (TMA up to 20 μmol/l (5.6 μmol/mmol creatinine) [10] and 11.9$\pm$6.2 μmol/mmol creatinine (mean$\pm$SD) [11]). Using headspace GC, Al-Waiz et al. reported values for normal adults of 20.3$\pm$20.9 μmol of TMA/24 h and 505.6$\pm$329.7 μmol of TMAO/24 h (mean$\pm$SD) [8] with TMAO accounting for 93.6$\pm$1.6% of total TMA excreted [7]. SPME would be a useful method for metabolic laboratories for diagnosis of trimethylaminuria. With the Carboxen–PDMS fibre, an extremely sensitive assay is possible. Because a stable deuterated standard is used for quantification, sample equilibration times could be reduced to allow for higher throughput of samples by a screening laboratory.

The potential value of SPME for analysis of volatile compounds in human blood and urine is becoming recognised. Recent applications include estimation of ethanol [26], solvents of abuse [27], anaesthetics [28], therapeutic drugs [29,30] and semi-volatile chemicals used industrially (chlorophenols [31], aromatic amines [32] and polynuclear aromatic hydrocarbons [33]). Introduction of the new Carboxen–PDMS fibre has extended the range of compounds that can be sampled and lowered the detection limits for a number of analytes. There is

considerable scope for use of this technique in the setting of a diagnostic metabolic clinical laboratory. The procedure can be used for profiling or, with addition of suitable deuterated internal standards, for accurate quantification of specific metabolites.

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