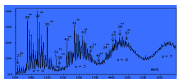
DETECTION OF VOLATILE METABOLITES OF HIGH MOLECULAR WEIGHT IN URINE BY ATMOSPHERIC PRESSURE IONIZATION-MASS SPECTROMETRY



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OVERVIEW

Fenn and colleagues have proposed a method of charging vapors by mixing them with an electrospray cloud for analysis in an atmospheric pressure source mass spectrometer (MS).[1,2] After initial success analyzing breath vapors,[3] we investigate here urine volatiles.

INTRODUCTION

Because urine contains a wide stream of metabolic wastes, it provides an attractive non invasive fingerprint of the biochemical activity of a subject. Indeed, nowadays urine analysis is part of routine clinical examinations. The array of compounds present in urine expands from simple inorganic molecules to proteins. Mass spectrometric analysis of a single target molecule in such a complex matrix usually involves time consuming procedures. The analysis of volatiles released from its surface represents a welcome simplifying approach. However, current analysis of volatiles is non-trivial. Usually, due to the low concentration in air (ppm-ppt), preconcentration steps are required. We report here the direct detection and identification in situ of volatile metabolites of high molecular weight.

METHODS

The entrance of a QTOF (Qstar from Sciex) was modified to hold a closed chamber, which supports an electrospray source facing the MS sampling orifice. 5.5 L/min of ambient air (+ 0.5 L/min CO_2) are driven in and out the chamber and its mass spectrum (blank) continuously monitored. The blank is humidified, as we have noted that humidity modifies the signal of many background peaks. When placing a urine sample (~15 mL; ~37º C; ~3 cm² surface) in close vicinity to the inlet of the sampling tube open to the lab atmosphere, its vapors are drawn in, charged by the ES cloud, and mass analyzed (Figure 1). The experiment was carried out in positive and negative mode.

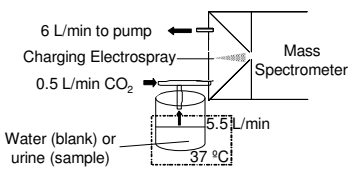

Figure 1. Sketch of the experimental set-up used for the direct analysis of volatiles in urine.

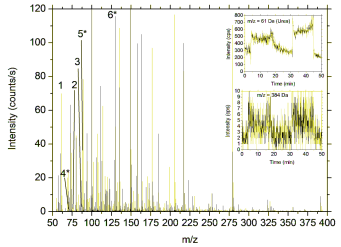

Figure 2. Positive urine spectrum (blank subtracted). The inset shows the corresponding single ion monitoring (SIM) trace for urea (top) and for the heaviest vapor clearly seen at 384 Da. The steps are produced when placing the urine sample close to the entrance of the ionization chamber. 1: Urea; 2: Amino-2-propanol; 3: Piperidine; 4*: 1-Pyrroline; 5*: 4-Aminobutanal; 6*: octylamine.*Compounds tentatively identified.

RESULTS

Figure 2 shows the resulting urine's mass spectrum after subtraction of the background spectrum. The spectrum is taken in positive ionization mode using an acidified solution in the charging ES (molecular weight + 1). We detect vapors up to molecular weights approaching 400 Da. The insets in Figure 2 show SIM traces for the peaks at 61 and 384 Da. The two steps observed correspond to the moment when the sampling tube is changed from the headspace of the water flask to the urine one. The latter was the heaviest one cleanly observed above the background. The peak appearing at 61 Da was assigned to urea by collision induced dissociation (CID).

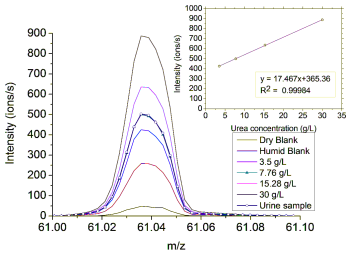

Figure 3. Urea peak measured from urea solutions in water and from a urine sample. Note also the signal increase when moisturizing the ambient air. The inset shows the corresponding calibration curve with a detection limit of ~ 2 g/L. The inferred urea concentration in urine is ~ 7.8 g/L (37 ppt in vapor phase for an ideal solution).

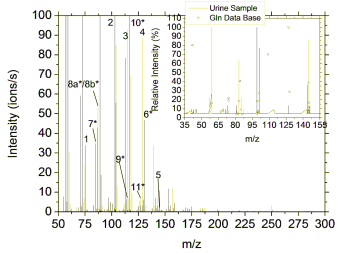

Figure 4. Resulting spectrum in negative mode after blank subtraction. The heaviest compound appears at 250 Da. 1: Glycolic acid; 2: 2-Hydroxybutanoic acid; 3: 3-Methyl-2-oxobutanoic acid; 4: 4-Methyl-2-oxopentanoic acid; 5: Glutamine *; 6*: Ornithine; 7*: 2 or 3 Methylbutanal; 8a*: Pyruvic acid; 8b*: Butanoic acid; 9*: Heptanal; 10*: Hydroxypentanoate; 11*: 1-Octanal. *Compunds tentatively identified. The inset shows the CID spectrum at 145 Da and the one for glutamine from the database.

In order to estimate the concentration of urea in urine we measured the urea vapors coming from solutions of known concentration. Figure 3 shows the mass spectrum for their corresponding urea peak (61 Da) and the one obtained for a urine sample. One can estimate a value of about 7.8 g/L, in accordance with typical concentrations in urine (3-30 g/L). The estimated urea concentration in vapor phase, according to its vapor pressure (1.2×10^{-5} Torr at 25 ºC), is ~ 37 ppt for an ideal solution. The detection limit is about 2 g/L (~ 10 ppt).

With the aim of widening the family of vapors investigated, we carried out the same experiment in negative mode. In this case we electrosprayed a basified solution, leading to deprotonated species, mainly acids. Figure 4 displays the resulting spectrum after subtracting the background. At 145 Da we identify at least two partially overlapped peaks, one of them is most probably associated to glutamine according to its CID pattern (inset Figure 4).

CONCLUSIONS

- ES ionization of volatiles is efficient enough to permit the detection of metabolites *in situ* from the headspace of a urine sample expanding to masses approaching 400 Da.
- Ambient air (blank) should be moisturized to match the charging probability of background and urine vapors.
- Important metabolites as for example urea have been identified by CID, among several others.
- Urea concentration has been quantified to a value of ~ 7.8 g/L (~ 37 ppt in vapor phase), in accordance with typical values. The detection limit is about 2 g/L (~ 10 ppt).

REFERENCES

[1] Whitehouse, C.M.; Levin, F.; Meng, C.K.; Fenn, J. B. *Proc. 34th ASMS Conf. Mass Spectrom. All. Top.* Denver (CO) USA, 1986, 507.
[2] Fuerstenau, S.; Kiselev, P.; Fenn, J. B. *Proc. 47th ASMS Conf. Mass Spectrom. All. Top.* Dallas (TX) USA June, 1999 (ThOE 3:00).
[3] Martínez-Lozano, P.; Fernández de la Mora, *J. Int. J. Mass Spectrom.* 2007, 265, 68–72.