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FRIEDRICH-ALEXANDER UNIVERSITÄT ERLANGEN-NÜRNBERG

NATURWISSENSCHAFTLICHE FAKULTÄT

ODORANTS IN HUMAN URINE – STRUCTURAL ELUCIDATION AND QUANTITATIVE DETERMINATION

Maria Wagenstaller[1)] and Andrea Buettner[1,2)]

1) Department of Chemistry and Pharmacy, Emil-Fischer-Center, University of Erlangen-Nuremberg, 91052 Erlangen, Germany

2) Department of Sensory Analytics, Fraunhofer Institute for Process Engineering and Packaging (IVV), 85354 Freising, Germany

Aim of the study

The aim of this study was to identify characteristic odorants in native human urine. Characterization of the odorous substances was carried out by means of a combinatory approach involving chemo-analytical and human-sensory tools.

Introduction

The volatile and odorous profile of human urine may be a rich source of physiological information [1] and may increase our understanding of metabolism and excretion processes of low-molecular weight compounds originating for example from dietary or endogenous sources [2]. Odorants in human urine may furthermore play an important role as semiochemicals in human communication as it is common in the animal kingdom [3].

Experimental

Samples

Urine was collected in 100 mL amber glass bottles. The urine samples were either processed directly after donation or frozen at –80°C until analysis. Donors were volunteers exhibiting no known illnesses at the time of examination. Participants were allowed to maintain a normal diet.

Enzymatic hydrolysis

Urine samples were either processed directly or subjected to enzymatic deglucuronidation. The glucuronidase-assay was carried out by adding acetate-buffer solution and β-glucuronidase to the urine samples. Samples were stirred for 15 h at 37°C.

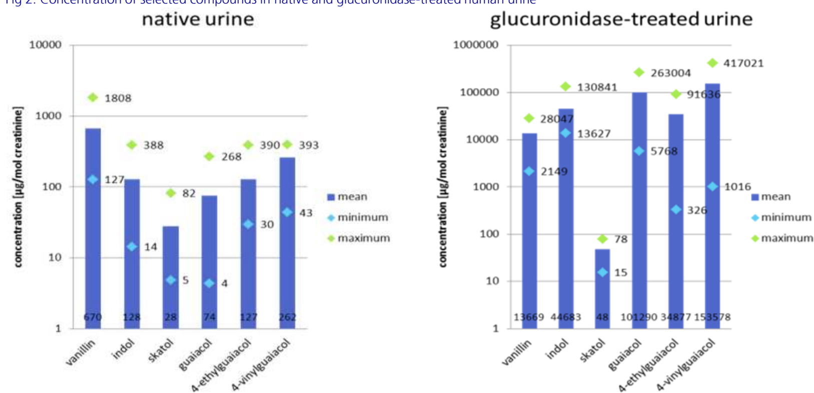
Enrichment of odour-active compounds and analysis

After addition of dichloromethane (50% v/v) to the native or hydrolyzed samples, the solutions were equilibrated by stirring (30 min) and subsequently subjected to Solvent Assisted Flavour Evaporation (SAFE; [4]), solvent extraction of the distillate with dichloromethane, and concentration by Vigreux-distillation and micro-distillation. The extracts were analyzed by HRGC-O and 2D-HRGC-O/MS (fig. 1). Identification of volatiles was based on comparison of retention indices and odour quality with reference compounds on two columns of different polarity and by MS-EI.

Aroma Extract Dilution Analysis (AEDA):

Flavour Dilution (FD) factors of the aroma compounds were determined by AEDA on the original extracts (2 µL, FD=1), and stepwise dilutions (1+1, v/v) with DCM using HRGC-O on DB-FFAP and DB-5.

Fig 2: Concentration of selected compounds in native and glucuronidase-treated human urine



Stable isotope dilution analysis

Enrichment of odour-active compounds was achieved according to the procedure described on the left, but after addition of dichloromethane (50% v/v) isotopic labeled standards were added to the native or hydrolyzed samples.

2-Dimensional High Resolution Gas Chromatography-Olfactometry/Mass Spectrometry (2D-HRGC-O/MS):

Analyses were performed with a system consisting of two gas chromatographs type 3800 (Varian, Darmstadt, Germany), coupled with a Saturn 2200 mass spectrometer (Varian) and ODP sniffing ports (Gerstel, Mülheim an der Ruhr, Germany) (fig. 1).

Results

Based on retention indices, odour qualities and intensities, and MS-spectra in comparison with references a total of 14 odorants could be detected in the majority of the untreated urine samples, while 6 odorants were only present in a few samples. The majority of the identified substances were found in human urine for the first time, amongst them some potent odorants also found in food, such as 2-acetyl-1-pyrroline [5].

Huge variations in the odorous profiles, both inter-individual and intra-individual, could be observed, although inter-individual variations were larger.

After deconjugation 26 compounds were identified in the majority of samples while 9 compounds were only found in a minority of samples. Predominantly a series of phenols were present at increased concentrations in the glucuronidase-treated samples while the concentration of some other hydroxyl-containing compounds did not increase (fig. 2 and 3) [5].

Discussion

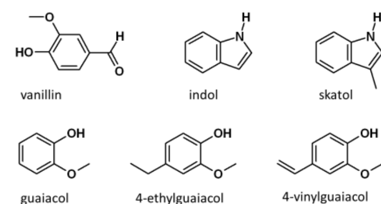
It was shown that application of this approach led to the successful detection of a range of substances, a series of them being identified for the first time as urine constituents of healthy adults.

These findings offer the possibility to further explore changes of odorous urinary constituents.

These results form a basis for identifying changes in the odorous profile of urine that may be due to disease, metabolism or nutritional effects.

Furthermore, some of the identified compounds could act as human semiochemicals, which might be of further interest. 2-Acetyl-1-pyrroline, for instance, has been proposed as a pheromone-like substance in tiger urine, thereby being important for reproduction [6].

fig. 3: Structures of selected compounds quantified in native as well as in glucuronidase-treated human urine



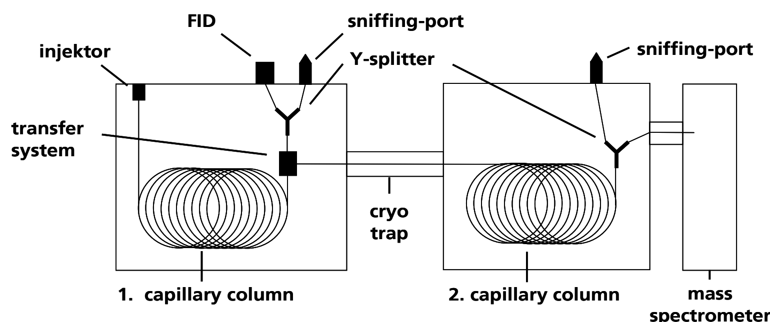


Fig. 1: Schematic drawing of a two-dimensional gas chromatographic-olfactometric system in combination with a mass spectrometer (2D-HRGC-O/MS) as employed in these experiments

Acknowledgements

This study was financed by the German Federal Ministry of Education and Research (BMBF). The authors are exclusively responsible for the contents of the publication.

We are grateful to Fraunhofer IVV, Freising, Germany, for support of our scientific work.

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