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Methods for the detection of tuaminoheptane

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Introduction

Since January 2007, the list of prohibited substances established by the World Anti-Doping Agency includes the sympathomimetic compound tuaminoheptane (2-heptylamine, 1-methyl-hexylamine).[1] It belongs to the class of primary amines with sympathomimetic activity that are commonly employed in the treatment of nasal obstruction and reduced air flow or as conjunctival decongestant by local application to the mucous membranes of the eye. Tuaminoheptane is the active component, for example, of the topical intranasal drug Rhinofluimucil, which is available over-the-counter in several European countries.
In this study, different methods for the detection of tuaminoheptane in urine samples using GC-MS and LC-MS-MS are described.

Experimental

Sample preparation for GC-MS

Five mL of urine specimens were extracted into TBME at pH 14 (addition of 0.5 mL of 5M potassium hydroxide), and converted into Schiff-bases using 0.1 mL of methanolic solutions (5%) of formaldehyde, acetaldehyde, benzaldehyde or acetone. In order to use conventional derivatisation with MSTFA and MBTFA, samples were evaporated and subjected to respective agents yielding the corresponding N-TMS and N-TFA derivatives.

Sample preparation for LC-MS/MS

Native urine was enriched with the internal standard (200 ng/mL of d_5-isoxsuprine) and subjected to LC-MS/MS analysis without further treatment.

GC-MS analysis

Analyses were conducted using the following equipment:

Agilent 5890/5973 GC-MSD, column: HP-5MS, length 24m, I.d.0.25mm, film thickness 0.25µm, temperature program: 85°C for 0.7 min – 28°C/min to 250°C – 35°C/min to 330°C. EI and full scan analysis (*m/z* 40-400, 4 scans s^{-1}).

LC-MS/MS analysis

All analyses were performed on an Applied Biosystems API 4000 Qtrap equipped with a M&N Pyramid column (2x50 mm, particle size 3µm). The flow rate was 400 µL/min and solvents used were A: 5mM ammonium acetate (pH 3.5) and B: acetonitrile. The gradient was 100%A at 0 min decreasing to 0%A within 4 min. Ion transitions for tuaminoheptane and the ISTD were 116 – 72, 116 – 43, 116 – 41, and 307 – 150, respectively.

Results

GC-MS analysis

With formaldehyde, acetaldehyde and benzaldehyde a complete conversion into the corresponding Schiff-base was accomplished within 20 min. In contrast to the aldehydes, acetone yielded only 89% of the desired product.

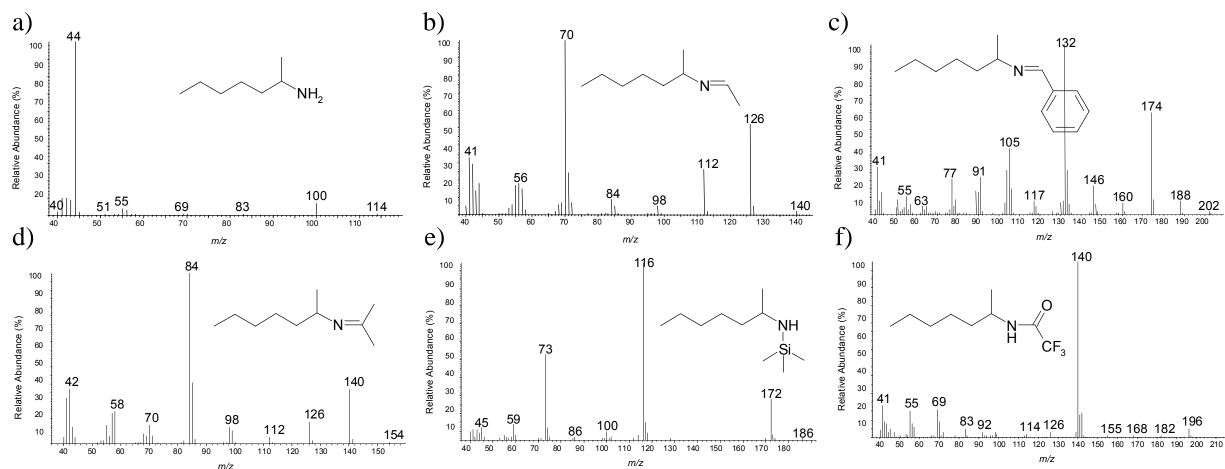

Figure 1: EI mass spectra of a) tuaminoheptane, b) tuaminoheptane-acetaldehyde, c) tuaminoheptane-benzaldehyde, d) tuaminoheptane-acetone, e) tuaminoheptane-TMS, and f) tuaminoheptane-TFA.

EI-MS spectra of six derivatives are illustrated in Figure 1: a) tuaminoheptane, b-d): Schiff-bases of acetaldehyde, benzaldehyde and acetone, respectively, e) tuaminoheptane-TMS, and f) tuaminoheptane-TFA. The improved fragmentation behaviour of the derivatives provides

characteristic ions that allow the unambiguous identification of the target analyte. Exemplarily, the extracted ion chromatograms of a blank specimen (a) and urine samples spiked with 1 µg/mL of tuaminoheptane (b) or collected 17.5 h post administration of 3 mg of tuaminoheptane (c) are shown in Figure 2 after derivatisation with acetaldehyde.

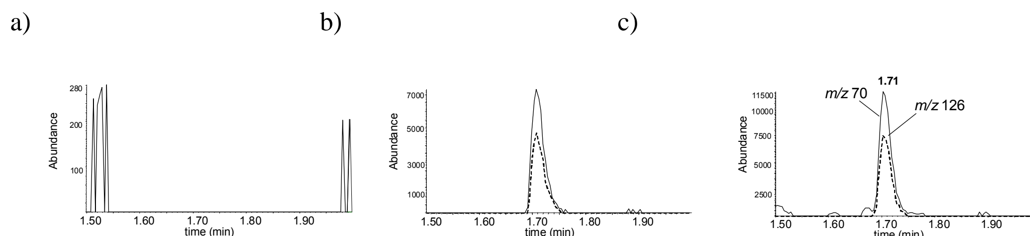

Figure 2: Extracted ion chromatograms of a) blank urine sample, b) spiked urine specimen containing 1µg/mL of tuaminoheptane and c) administration study urine sample after extraction and derivatisation with acetaldehyde and analysis using GC-MS.

LC-MS/MS analysis

Aliquots of spiked human urine samples (50 ng/mL and 500 ng/mL) as well as an administration study urine sample were measured by LC-MS/MS as depicted in Figure 3. The MRPL is readily accomplished using the simple and fast analysis of native urine, and detection limits of 50 ng/mL are feasible. Also administration study urine specimens provide unambiguous test results using three diagnostic ion transitions.

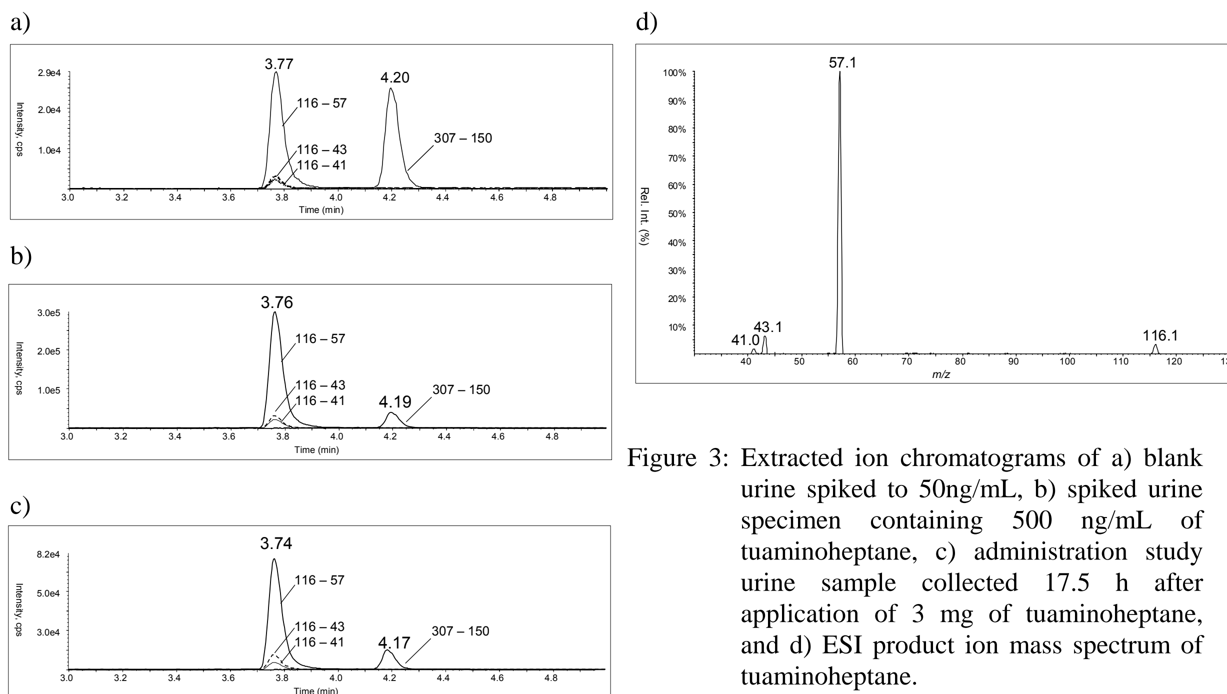

Figure 3: Extracted ion chromatograms of a) blank urine spiked to 50ng/mL, b) spiked urine specimen containing 500 ng/mL of tuaminoheptane, c) administration study urine sample collected 17.5 h after application of 3 mg of tuaminoheptane, and d) ESI product ion mass spectrum of tuaminoheptane.

Discussion and conclusion

No data on the metabolism of tuaminoheptane was found in the literature. Possible glucuronicacid conjugates did not occur to a considerable extent.[2] Tuaminoheptane was implemented into an existing screening procedure using an GC-MS system equipped with an additional nitrogen-phosphorus detector as analytical instrument.[3] Modifying selected parameters enables the detection of the drug in human urine without derivatisation. However, derivatisation improves gas chromatographic and mass spectrometric properties of the target analyte. The retention time was prolonged, peak tailing was reduced and mass spectrometric information considerably improved providing diagnostic fragment ions under EI conditions.[1] The identification with LC-MS/MS needs very little effort of sample preparation.[4] All the methods for the detection of tuaminoheptane enables to confirm concentrations according to the minimum required performance limit of 500 ng mL^{-1} requested by the World Anti-Doping Agency (WADA).[5]

References

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