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Identification of Cyclodextrins by Liquid Chromatography – ESI-Tandem Mass Spectrometry

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1. Introduction

Adulteration of urine is a well known problem in doping analyses. Many products are available that are claimed to eliminate traces of drugs in urine [1, 2]. A substance widely utilized, for instance as a carrier for hydrophobic substances in pharmaceutical products is cyclodextrin. Owing to its ability to form inclusion compounds as a result of the cyclic structure with molecules of molecular masses up to approx. 300 Dalton cyclodextrin is supposed to be suitable for manipulation of urine. Preliminary investigation on adulteration of urine with γ-cyclodextrin was carried out in our laboratory utilizing seven quality control urine samples for anabolic steroids fortified with cyclodextrin. Two of the analysed samples showed decreasing signals in the steroid profile.
Therefore, a fast and reliable method based on Liquid Chromatography – Electrospray Ionisation Tandem Mass Spectrometry was developed enabling the identification of cyclodextrin without time-consuming sample preparation.

2. Material and Methods

Chemicals and materials
Methanol (99%) was purchased from KMF (Lohmar, Germany). Alpha-, β- and γ-cyclodextrin (98%) and lithium chloride (99%) were purchased from Sigma-Aldrich (Steinheim, Germany). All solutions were prepared using deionised water (Water Lab System, Millipore, Eschborn, Germany).

Sample Preparation
Urine samples were fortified with α-, β- and γ- cyclodextrin reference compound, transferred into HPLC vials and 10 µL were injected without further sample preparation.

LC-MS Analyses

All analyses were performed on an Agilent 1100 liquid chromatograph (Waldbronn, Germany) interfaced to an Applied Biosystems API 2000^{TM} triple quadrupole mass spectrometer (Darmstadt, Germany). The LC was equipped with a Macherey-Nagel Nucleosil Carbohydrate column (250 x 4 mm; particle size 10 µm). The chromatographic mobile phase was composed of A: 1 mmol/L lithium chloride and B: methanol. Isocratic elution was employed at 90% B with a total runtime of 7 min at a flow rate of 2.0 mL/min and a post-column split of 1:10.

Positive ionisation was accomplished by means of ESI at an interface temperature of 300ºC. Product ion scans of α-, β- and γ-cyclodextrin were generated using solutions of pure reference compounds at a concentration of 500 µg/mL prepared in methanol-water (9:1, v:v) containing 0.1 mmol/L of LiCl. A 1 mL syringe operated by a syringe pump at a flow rate of 3 µL/min was utilised. In the multiple reaction monitoring mode of the instrument α-cyclodextrin was detected with the ion transitions: *m/z* 979.5→169 (CE=99 eV); *m/z* 979.5→331 (CE=77 eV) and *m/z* 979.5→493 (CE=73 eV), β- cyclodextrin with the ion transitions: *m/z* 1141.3→169 (CE=109 eV); *m/z* 1141.3→331 (CE = 85 eV) and *m/z* 1143.1→493 (CE=77 eV) and γ-cyclodextrin with the ion transitions: *m/z* 1303.3→169 (CE=115 eV); *m/z* 1303.3→331 (CE =97 eV) and *m/z* 1303.3→493 (CE=85 eV). Parameters such as declustering potential, focusing potential and collision energies were optimised for maximum abundance of each ion transition of the analytes. Nitrogen was used as collision gas at a collision cell pressure of 2.93 x 10^{-3} Pa.

3. Results

LC-MS/MS Analyses

The mass spectrometric behaviour of α-cyclodextrin (M_w = 972.9 Da), β-cyclodextrin (M_w = 1135 Da) and γ-cyclodextrin (M_w = 1297.2 Da) after positive electrospray ionisation was studied using the commercially available reference compounds. The lithium adduct ions $[M+Li]^+$ of α, β and γ -cyclodextrin were predominant in full scan analyses. Collisionally activated dissociation (CAD) of the lithium adduct ion ($[M+Li]^+$, *m/z* 979.5, *m/z* 1141.3 and *m/z* 1303.5) of α-, β- and γ-cyclodextrin gave rise to the product ion spectra shown in Figures 2 A-C.

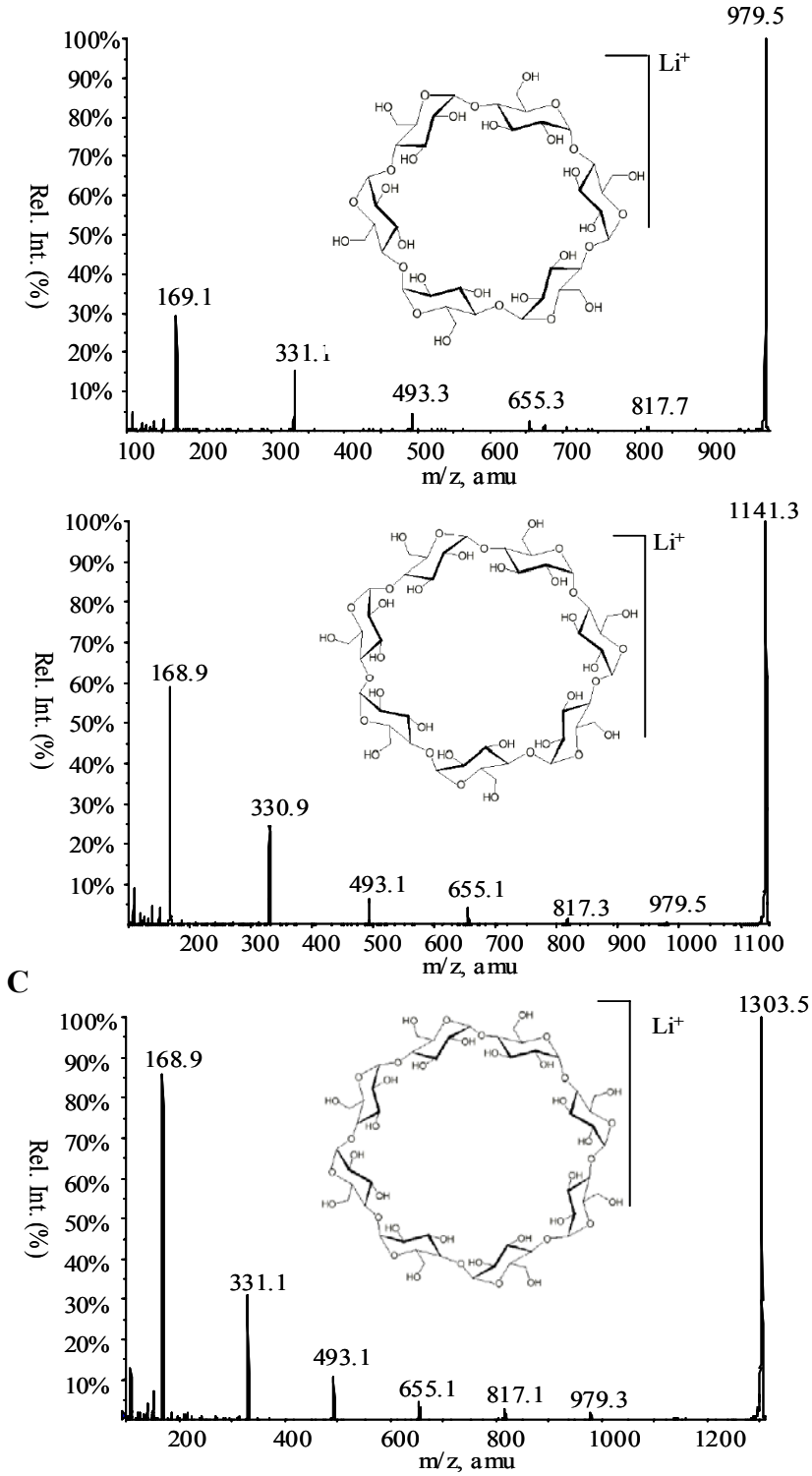

Figure 2: ESI product ion spectra of the lithium adduct ions $[M+Li]^+$ of A: *m/z* 979.5 of α-cyclodextrin, B: *m/z* 1141.3 of β –cyclodextrin and C: *m/z* 1303.5 of γ-cyclodextrin

Consecutively, *m/z* 979.5, *m/z* 1141.3 and *m/z* 1303.3 eliminate glucose (-H_2O) subunits (-162 Da) to the resulting product ions. The overlayed extracted ion chromatogram in Figure 3 represents three blank urine sample fortified with α-, β- and γ- cyclodextrin at concentrations of 500 µg/mL. An aliquot of 10 µl of each urine sample was injected into the LC-MS system

without further sample preparation. The ion transitions *m/z* 979.5→169 for α-cyclodextrin, *m/z* 1141.3→169 for β-cyclodextrin and *m/z* 1303.3→169 for γ -cyclodextrin are depicted in the chromatogram.

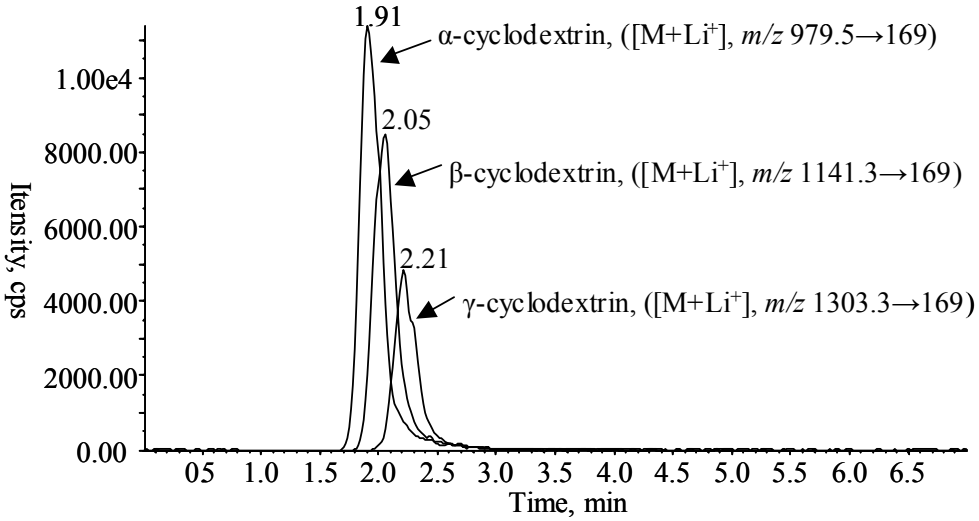

Figure 5: Overlayed extracted ion chromatograms of three blank urine samples fortified with α-, β- or γ- cyclodextrin (conc. 500 µg/mL each).

4. Discussion

Gamma-cyclodextrin is supposed to be the most effective cyclodextrin concerning adulteration of urine owing to its ability to form inclusion compounds with larger molecules (up to 300 Da). An appropriate concentration of cyclodextrin for the manipulation of urine is assumed to be located in the lower milligram/mL range. The presented preliminary results demonstrate the capability of the approach to detect α-, β- and γ-cyclodextrin in human urine with a lower limit of detection of approx. 50 µg/mL without time consuming sample preparation.

The presence of cyclodextrin in urine after oral administration is improbable due to the cleavage of β- and γ-cyclodextrin by α-amylases and missing intestinal absorption of the native molecule [3]. Thus, this method can be used to detect urine manipulation with cyclodextrin.

References

[1] WU AHB et al, Adulteration of urine by „Urine Luck“. Clinical Chemistry 1999; 7: 1051-1057

[2] Scholer A, The effect of urine manipulation on Substance Abuse Testing. http://www.iatdmct.org/adulteration.htm (Accessed Feb. 2005)

[3] Gröger et al, Cyclodextrine. http://www.science-forum.de/download/cyclodex.pdf (Accessed Feb. 2005)