Detection of Drug Metabolites in Urine by NMR-based Hyphenation

Introduction

The presence of highly concentrated drugs and their metabolites in body fluids can easily lead to the assignment of „wrong" biomarkers in NMR based Metabonomics approaches when the spectra of a control group are compared to the spectra of patients suffering from a certain disease and are therefore treated with drugs.

The isolation and identification of drug metabolites can be achieved by using modern hyphenation techniques, i.e. LC-SPE-NMR/MS and LC-cut-NMR/MS. While LC-SPE-NMR covers moderate- to unpolar metabolites, LC-cut-NMR/MS allows isolation of extremely polar water soluble metabolites.

Experimental

Chromatography HPLC-SPE-NMR

Agilent 1260 HPLC; flow rate 0.5mL/min; injection volume 50uL; column Phenomenex Kinetex EVO C18 250x4.6mm 5µm; A: Water 0.1% CDOOD, B: acetonitrile 0.1% CDOOD: 0min 2%B, 5min 2%B, 40min 40%B, 55min 100%B, 60min 100%B; UV@220nm, 254nm

Chromatography HPLC-cut tube-NMR

Agilent 1260 HPLC; flow rate 0.2mL/min; injection volume 50uL; column Phenomenex Kinetex EVO C18 250x4.6mm 5µm; A: D2O, B: acetonitrile 0.1% CDOOD: 0min 0%B, 40min 0%B, 55min 100%B, 60min 100%B; UV@220nm, 254nm

MS

MicrOTOF (Bruker Daltonic, Bremen, Germany) operated in pos. and neg. ESI; scan range: 50-1000 m/z; Calibrant: Na-acetate.

NMR

Bruker AVANCEIII 500MHz; CPPTCI (with cryofit 30uL for LC-SPE-NMR); LC1DCWPS multiple solvent suppression with 128 scans after transfer of time slices with CD_3CN. Parameter sets CMC-se for structure elucidation

Software

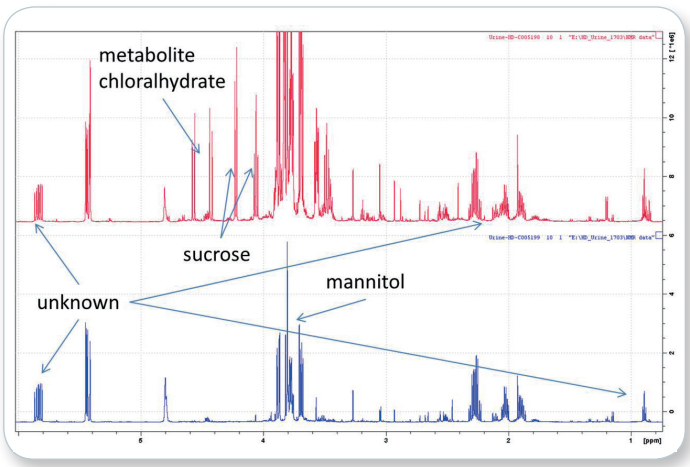
Chromatography: Hystar 3.2 (Bruker Daltonics); MS: Microtof control (Bruker Daltonics); NMR: Topspin 3.5 (Bruker Biospin); CMC-se for structure elucidation (Bruker Biospin)

Sample

NMR buffered urine sample. Injection volume 50µL

Results

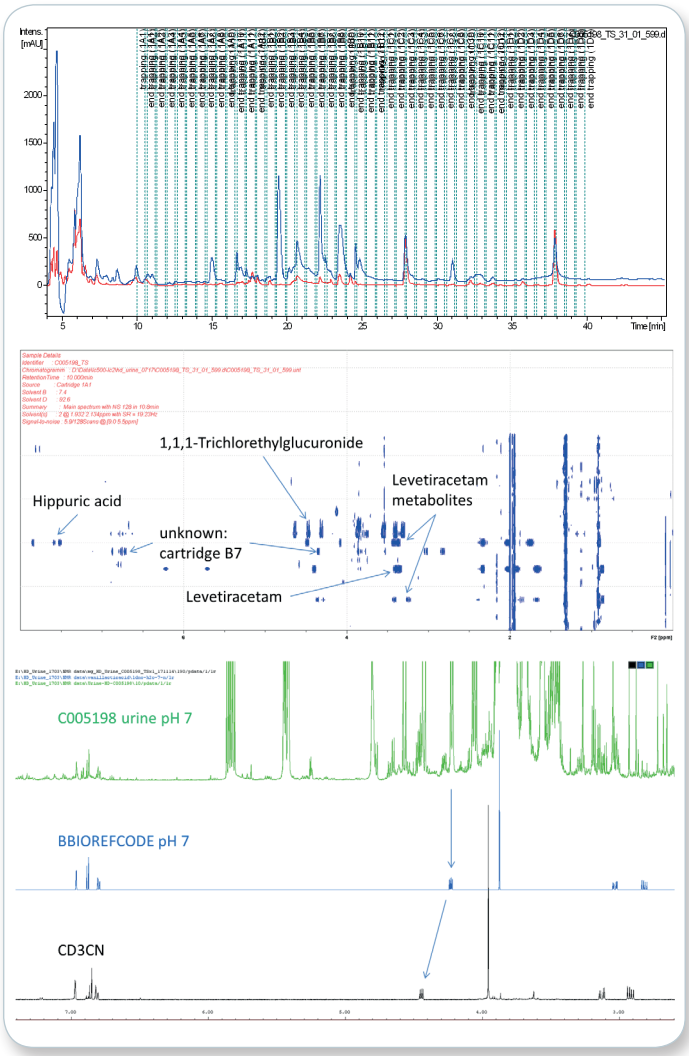
A typical example of a urine sample obtained during routine screening is shown below. The high concentrations of exogenous metabolites are clearly visible.



Urine spectra showing high concentration of drug metabolites disturbing statistical analysis.

Unpolar metabolites detected by HPLC-SPE-NMR/MS

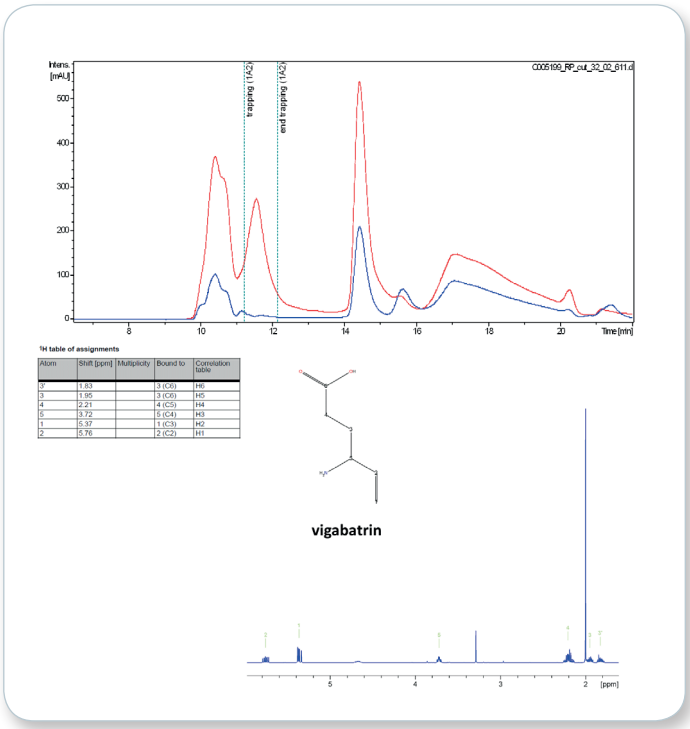
The time-slice LC-SPE-NMR/MS chromatogram and the corresponding pseudo onflow NMR chromatogram reveal a number of highly concentrated drug metabolites together with an unknown endogenous metabolite identified as vanillactic acid.



Time slice LC-SPE-NMR/MS chromatograms and pseudo onflow NMR chromatogram of the urine sample shown above. Identification of vanillactic acid by comparison with the proton spectrum obtained from the BBIOREFCODE.

Polar metabolites using HPLC-cut-NMR/MS

Polar metabolites can be isolated from urine samples by HPLC fractionation using the selection port of the Spark Prospekt 2 ACE (MS data not shown). The peak eluting between 11 and 12 min has been guided directly into a 3mm NMR tube using a peek capillary for further NMR data acquisition.



HPLC cut of unknown polar compound using 100% D_2O with an RP column. CMC-se result reveals the presence of vigabatrin in the urine, a drug used in the treatment of epilepsy.

After acquisition of a proton, edited HSQC and HMBC spectrum, the CMC-se software calculated the most probable structure which is consistent with the structure of the drug Vigabatrin, which is used in the treatment of epilepsy.

Conclusions

LC-SPE-NMR/MS and LC-NMR/MS can be used to isolate and identify structures of drug- and endogenous metabolites in urine. Both polar and unpolar compounds can be isolated and identified using this technique in an efficient way. The importance of this ability enables quantification in urine, as soon as compounds are identified.

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