We have the analyte – but where is the dross?

A systematic approach to investigate matrix removal during sample preparation

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Background & Aim

Analyzing a complex biological sample is often challenging. The matrix can cause several problems during HPLC/HRMS measurement such as short column life or ionization suppression effects. But matrix removal is never the focus of method development which usually monitors only the recovery of the analyte [1-3].

To assess the effects of matrix removal by using a systematic approach with amitriptyline and three of its metabolites in human serum as model compounds.

We performed

- **most common sample preparation techniques**

and determined the

- **analyte recovery**
- **matrix clean-up**

Conclusion

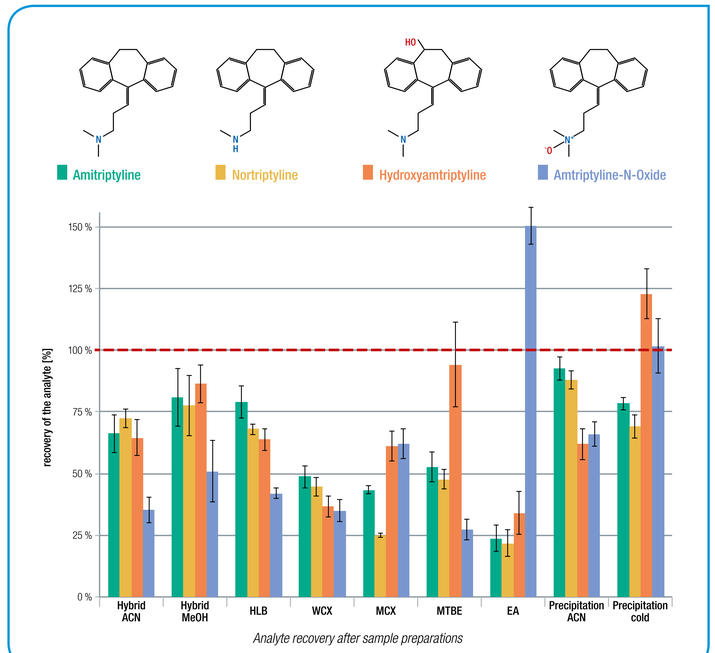
This combination of ...

- quantitative analysis and
- untargeted metabolomics

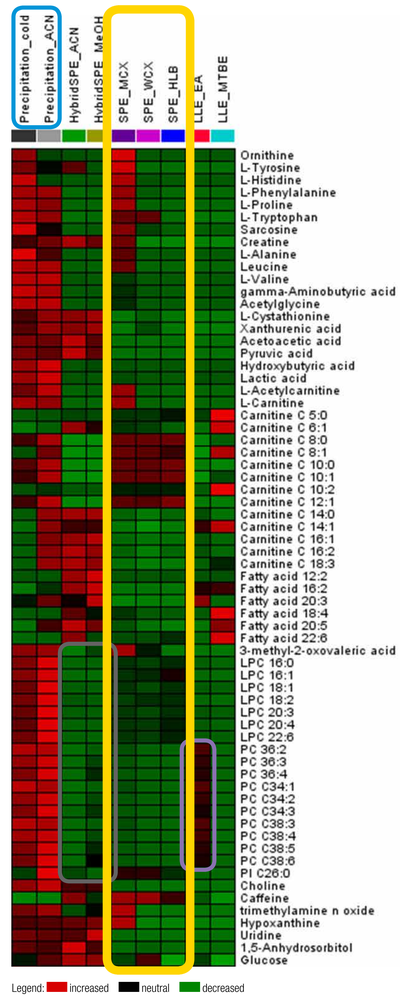
is an **innovative tool** which gives insight into what really happens to the matrix during clean-up.

In this study, which determined amitriptyline and its metabolites in human serum, this combined approach clearly showed that SPE HLB material is the best choice resulting in a very good clean-up and good recoveries for all 4 analytes.

Results and Discussion



Analyte recovery after sample preparations



Heatmap of the matrix compounds

Precipitation methods

- Most matrix components remained
- When using cold methanol precipitation, carnitines and fatty acids were reduced

SPE

- SPE reduced most matrix compounds
- Except for strong cationic exchange (MCX) SPE which retained amino acids and lipophilic compounds

Phospholipid removal SPE

- Reduced phospholipids
- Amino acids and small molecules were also removed
- Very good recoveries for all 4 analytes

LLE with ethyl acetate

- Ethyl acetate extracted phospholipids
- Not the extraction medium of choice for serum
- 150 % recovery for Amitriptyline-N-oxide!
- Low recoveries for other analytes (between 20 – 30 %)

Experimental

Sample preparation methods

Precipitation methods *(acetonitrile + 1 % formic acid, methanol at – 80 °C)*; liquid-liquid extractions LLE *(MTBE (alkaline), ethyl acetate)*; solid-phase extractions (SPE) mixed mode *reversed-phase material (HLB), weak cationic exchange material (WCX), strong cationic exchange material (MCX); phospholipid-removal hybrid-SPE (in-well precipitation and SPE material selective for phospholipids)*

Analyte recovery

Reversed-phase HPLC / HRMS method: *Chromatographic conditions:* Phenomenex Kinetex reversed-phase C18 column (2.1 mm x 50 mm, 2.6 µm) & water + 0.1 % formic acid (A) and acetonitrile + 0.1 % formic acid (B) under gradient elution conditions: 0 – 7 min, 10 – 40 % B; 7 – 11 min, 40 % B; 11 – 13 min, 40 – 70 % B, 13 – 14 min 70 – 10 % B. Flow rate was 200 µL/min; injection volume 20 µL.

Matrix clean-up

Untargeted metabolomics-based HILIC HPLC / HRMS method: *Chromatographic conditions:* Phenomenex Luna NH_2 column (2.0 mm x 150 mm, 3 µm) & 20 mmol/L ammonium acetate + 5% acetonitrile (pH 9.5) (A) and acetonitrile (B) under gradient elution conditions: 0 – 15 min, 85 – 0 % B; 15 – 20 min, 0 % B; 20 – 22 min, 0 – 85 % B; 22 – 37 min 85 % B. Flow rate was 150 µL/min and post-column flow was split 1 : 20 before coupling to the mass spectrometer; injection volume 10 µL.

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