We have the analyte – but where is the dross?

A systematic approach to investigate the matrix removal during sample preparation

Denise Schimek [1,2], *Gunnar Libiseller* [1], *Anton Mautner* [1], *Kevin A. Francesconi* [2], *Reingard Raml* [1*], *Christoph Magnes* [1]

CONTACT

[1] JOANNEUM RESEARCH Forschungsgesellschaft mbH

HEALTH
Institute for Biomedicine and Health Sciences

Neue Stiftingtalstrasse 2
A-8010 Graz

Phone: +43 316 876-40 00
Fax: +43 316 876 9-40 00

health@joanneum.at
www.joanneum.at/health

[2] Institute of Chemistry-Analytical Chemistry
Karl-Franzens University Graz, Austria

Introduction and Aim

Investigations into sample preparation procedures usually focus on analyte recovery with no information provided about the fate of other components of the sample (matrix) (1-3). Using the example of the drug amitriptyline and three of its metabolites in serum, we track the fate of these trace analytes, while monitoring the undesired matrix compounds using a combination of charged aerosol detection (CAD), LC-CAD, and a metabolomics-based LC-MS/MS approach

The **collective data of**

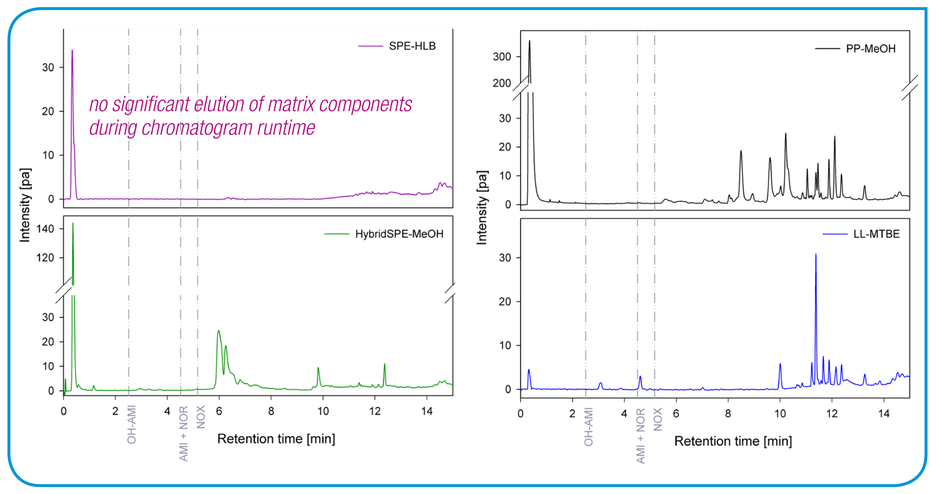
- analyte recovery
- matrix removal
- matrix compound profile

was used to assess the effectiveness of each sample preparation method

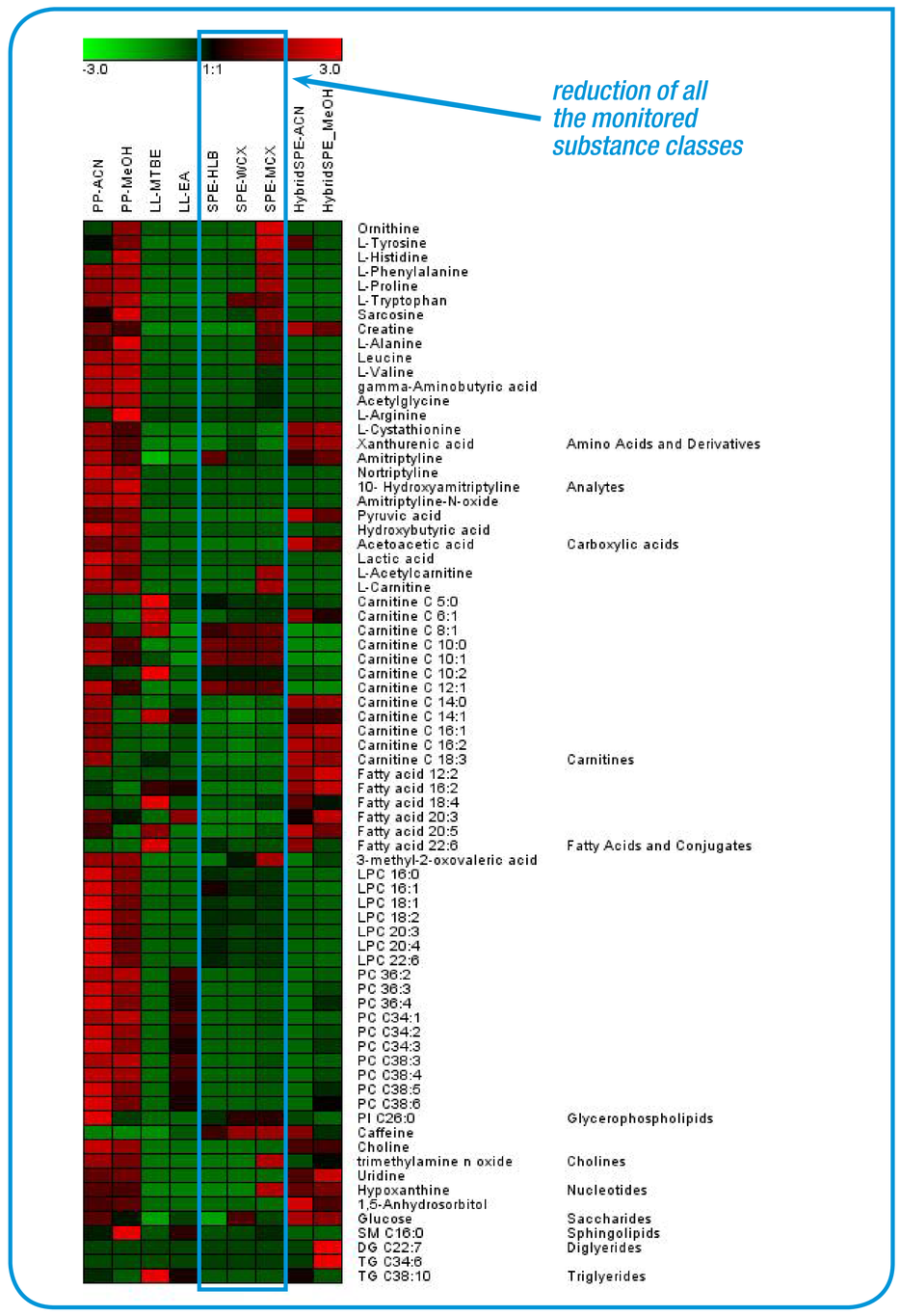
Results and Discussion

Sample preparation method	Remaining matrix [µg/mL]	AMI Recovery [%]	NOR Recovery [%]	OH-AMI Recovery [%]	NOX Recovery [%]
PP-ACN	1092 ± 10	113 ± 17	92 ± 4	89 ± 15	96 ± 13
PP-MeOH	1878 ± 21	78 ± 3	69 ± 5	123 ± 10	102 ± 11
LL-MTBE	239 ± 25	53 ± 6	48 ± 4	94 ± 17	27 ± 4
LL-EA	242 ± 8	24 ± 5	22 ± 5	34 ± 9	150 ± 7
SPE-HLB	**123 ± 32**	**79 ± 7**	**68 ± 2**	**64 ± 4**	**42 ± 2**
SPE-WCX	**72 ± 2**	**76 ± 5**	**45 ± 4**	**37 ± 4**	**35 ± 5**
SPE-MCX	**48 ± 13**	**73 ± 2**	**25 ± 1**	**61 ± 6**	**62 ± 6**
HybridSPE-ACN	1500 ± 220	66 ± 8	72 ± 4	64 ± 7	35 ± 5
HybridSPE-MeOH	2100 ± 380	81 ± 12	77 ± 12	86 ± 8	51 ± 12

Matrix load remaining (determined by FIA-CAD) and analyte recovery (LC-MS) after serum has been processed with various sample preparation methods. Data represent mean ± SD, n=3



Elution of the matrix compounds by HPLC-CAD retention times of the analytes are: Hydroxyamitriptyline (OH-AMI 2.5 min), Amitriptyline + Nortriptyline (AMI+NOR 4.5 min), and Amitriptyline-n-oxide (NOX 5.2 min).



Heatmap, arranged by compound classes, reflecting the clean-up efficiency of different sample preparation techniques (x-axis) with matrix compounds (y-axis)

color-code:
green: decreased
red: increased

compared to centered and scaled mean value for each metabolite

Conclusion

All SPE methods showed very good overall performance

- HLB-SPE showed better **analyte recoveries** than the cation-exchange SPE materials.
- The SPE methods recorded by far **the lowest levels of total recovered matrix**
- The HPLC-CAD data revealed no significant elution of matrix components during chromatogram runtime, which indicates an **overall good clean-up**
- The heatmap revealed an efficient **reduction of all the monitored substance classes**

The CAD is an effective tool which showed **considerable differences** of the **sample preparation methods** and enables fast matrix monitoring. The metabolomics profiles of **eleven compound classes**, comprising 70 matrix compounds showed a **trend** of compound class removal of each sample preparation strategy.

References

[1] Bylda, C., Thiele, R., Kobold, U. & Volmer, D. The Analyst 139, 2265-76 (2014)

[2] Yang, Y. et al. Journal of chromatography. A 1300, 217-26 (2013).

[3] Yuan, M., Breitkopf, S.B., Yang, X. & Asara, J.M. Nature protocols 7, 872-81 (2012)

Acknowledgement

This work was supported financially by the Österreichische Forschungsförderungsgesellschaft FFG

Project IDT4Brain

Experimental

Sample preparation method: Precipitation methods (PP) (acetonitrile + 1% formic acid, methanol at -80°C); liquid-liquid extractions (LL) (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/MS 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 0.4 mL/min; injection volume 20 µL.

Matrix clean-up: *HPLC-Charged aerosol detector (CAD) method;* Chromatographic conditions: Identical with those used for the MS measurements described above. The HPLC effluent was split post-column and augmented with an auxiliary flow with inverse gradient conditions from Dionex P680 HPLC Pump; Detector: CAD - Corona ultra RS Detector (Thermo); *Untargeted metabolomics-based HILIC HPLC/HRMS method;* Chromatographic conditions: Phenomenex Luna NH2 column (2.0 mm x 150 mm, 3 µm) & water/acetonitrile 19:1 containing ammonium acetate (20 mmol/L at pH 9.45) (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.