TARGETED METABOLOMICS ANALYSIS OF AMINO ACIDS, ACYLCARNITINES, SUGAR, GLYCEROPHOSPHOLIPIDS AND SPHINGOLIPIDS FROM CEREBROSPINAL FLUID USING A STANDARDIZED FIA-ESI-MS/MS METHOD

Manfred Uhr1, Christian Namendorf1, Ralf Bogumił2, Matthias Keller2, Sascha Dammeyer2
1Max Planck Institute of Psychiatry, Munich, Germany 2BIOCRATES Life Sciences AG, Innrain 66, A-6020 Innsbruck, Austria

Introduction
The composition of cerebrospinal fluid (CSF) can provide biochemical insights into central nervous system disorders. Therefore metabolomics studies of CSF could result in the identification of biomarkers for disease, disease progression or response to therapy.

We have evaluated and optimized the AbsoluteIDQ™ Kit, a product validated for human plasma, for the analysis of human CSF. The AbsoluteIDQ kit is based on a targeted metabolomics approach and can simultaneously identify and quantify a large number of endogenous metabolites in plasma. The method has been optimized using pooled human CSF and has been further tested using patient samples.

Method Optimization
The AbsoluteIDQ p150 kit (Biocrates Life Sciences AG) was used for all experiments.

Optimization of Sample Load
Since the concentration of most metabolites in CSF is significantly lower compared to plasma the amount of CSF added onto the filter paper in the upper kit plate has been optimized. The addition of 30 µL CSF gave the best results regarding ease of preparation and coefficient of variation (Standard volume for plasma is 10 µL). Addition of significant higher amounts (60 µL) resulted in significantly elevated ion suppression. For details consult application note 1003-1 from the manufacturer.

Results
Coefficient of Variation (CV) and Limit of Detection (LOD)
For pooled human CSF, intra-day CVs of the different metabolite classes were compared (Figure 1). Mean CV values were below 10% for amino acids and therefore comparable to plasma. For acylcarnitines, phosphatidylcholines and sphingomyelins CVs were slightly higher due to significantly lower concentrations, but they were still below 20%.

The LODs of the different metabolites and their median concentration values in pooled human CSF were also determined. The number of metabolites that exhibit values above LOD are given in Table 1.

Metabolite Concentrations in CSF
The mean concentrations of acylcarnitines were in general about 5-20 times lower when compared to plasma. All amino acid concentrations in CSF were in the µM range and clearly above LOD. The hexose concentration was found to be 3.4 mM in accordance with values from the literature.

Phosphatidylcholine and glycerophospholipids concentrations in CSF were for most analytes in the nanomolar range and significantly lower when compared to plasma concentrations. Despite the relatively low concentrations of these metabolites, 45 lipids were above LOD. In certain disease states there is a significant increase in the number of lipids in the CSF, we would therefore expect to find more lipids above LOD.

To further investigate the performance of the kit at the lower lipid concentrations observed a dilution series of pooled human plasma was conducted. Generally, good linearity was observed for most glycerophospholipids and sphingolipids in the concentration ranges found in pooled human CSF as demonstrated in Figure 2.

Analysis of patient samples/CSF analytics
CSF and serum samples from patients with psychiatric and neurological diseases were analyzed. Both concentration sets were interpreted with respect to the blood/CSF barrier function, and important information about the relationship between CSF and serum metabolite concentrations could be found, which are a prerequisite for the comparison of disease groups.

Conclusion
- The AbsoluteIDQ kit validated for human plasma can be – with minor adaptations – successfully applied for studies using human CSF.
- Compared to plasma, significantly lower concentrations were found especially for the glycerophospho- and sphingolipids. However, 45 of these lipids were above LOD and should give valuable information about the lipid status.
- In summary, the data reveal clear evidence for the high potential of targeted metabolomics analysis of CSF and its impact on the study of CNS disorders.