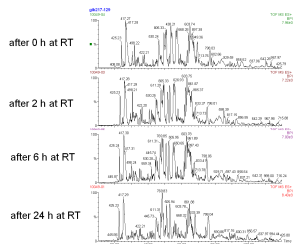


## Overview

- A routine workflow for quantitative analysis of native peptides from CSF and blood as well as from other sources has been developed
- Isobaric labeling with Tandem mass tags (TMT™) has been used for enabling quantification and preserving the native state of the peptidome
- Low abundant CSF peptides can be quantified and characterised using 100 µl sample
- CSF quality is influenced by sample handling (i.e. incubation on the "bench")
- CSF sources differ in their composition (vendors)

## Introduction

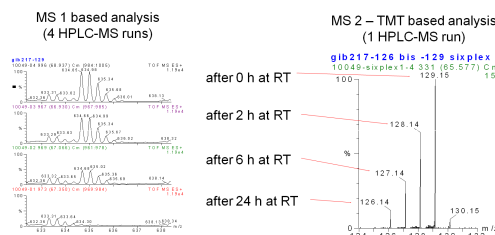
Cerebrospinal fluid (CSF) is frequently studied to explore central nervous disorders because it contains neuropeptides and breakdown products of proteins expressed in brain tissue. The CSF *peptidome*, especially in a "near-to-native" state, contains important carriers of biologically and pathophysiologically relevant information as biomarkers. Therefore, we have developed a new workflow to allow for the quantitative profiling of CSF peptides based on labelling with isobaric tandem mass tag reagents (TMT) under denaturing conditions. It allows for the simultaneous identification and quantitation of peptides under controlled experimental conditions highly preserving quantitative information in combination with a high sample throughput. Here we present this integrated workflow and the study of storage and proteolytic effects in CSF from different sources.



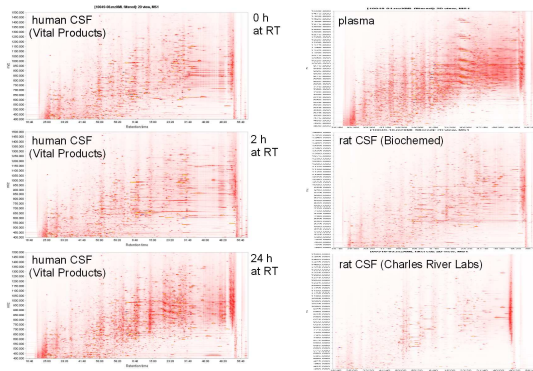
Characterisation of rat CSF samples after different incubation times. The graph shows the base peak chromatogram, which does not exhibit substantial changes.

## Methods

Native polypeptides and peptides in CSF samples from different sources (vendors) were labelled at N-termini and lysine residues using TMT-sixplex reagents under denaturing conditions. In order to monitor preanalytical quality and LC-MS/MS performance, samples were spiked with reference peptides. Some samples were incubated on the bench for 2-24 h at room temperature to investigate the influence of prolonged pre-analytical storage. After mixing samples in a sixplex setup, peptides were enriched and concentrated using RP and SCX procedures. Samples were separated and analysed by capillary LC coupled to an Waters QTOF II. Data were pre-processed, integrated and annotated using a customised bioinformatics workflow.



Analysis of spike Neurotensin in rat CSF for different incubation times performed by means of LC-MS (left) and isobaric labeling and LC-MS/MS analysis (right)



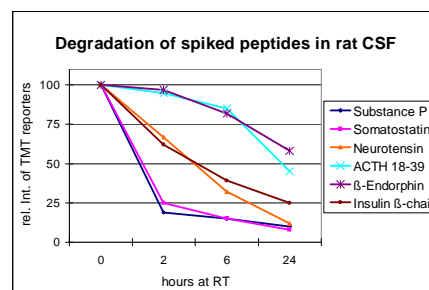
Multiple peptidomics CSF samples run by LC/MS (peptide map) illustrating peptide degradation after incubation as well as differences of CSF sources.

## Results

Because CSF is a biological sample with spatial neighborhood to the brain, dynamic changes in the composition and abundance of the CSF peptidome are likely to relate to aberrant physiological processes. In the context of biomarker discovery, it is essential to obtain reliable and reproducible peptide profiles and to ensure that the variation in signal abundance truly reflects biological differences.

A general issue regarding biological samples and their quality in peptidomic studies is the difficulty to differentiate preparative and analytical artifacts from truly regulated peptides. CSF has a very low peptide concentration. That implies its high susceptibility to pre-analytical artifacts by proteolytic degradation of proteins after sample acquisition. The purpose of this study was to investigate the influence of incubation time of CSF for the quantitative analysis of the CSF peptidome. We investigated sample quality and stability of the peptidome of various CSF samples with respect to defined spike landmark peptides during incubation and proteolytic degradation.

Our peptidomic workflow allows for the detection and quantitation of >1000 entities in rat and human samples, which enabled us to define appropriate landmarks for the assessment of CSF sample quality. A volume of 100 µl per individual sample is sufficient for an analysis.



## Conclusions

The analysis of native peptides in CSF is challenging due to its high complexity and sensitivity towards artificial proteolytic cleavage of abundant proteins. Therefore, we have developed a novel peptidomics workflow enhancing the quantitative measurement of peptides by using TMT labeling. Since the initial steps are labeling and mixing of clinical samples the native peptide pattern is conserved throughout the whole workflow.

Our results demonstrate the importance of carefully conducted quality controls to be able to manage analytical artefacts from biological effects. By extracting quality control peptide landmarks, we provide a means for enhancing the quality of differential peptidome analyses in general. Furthermore, we show the complexity of the native CSF peptidome and provide a basis for the establishment of a corresponding peptide database of native peptides. Blood contaminations should be avoided, which is particularly difficult in rat CSF samples, where exceptionally low volumes are present and the procedure is invasive to obtain CSF.

**It can be shown that spiked peptide hormones are highly susceptible towards degradation. However, the majority of peptides in CSF appear not to be largely changed upon prolonged incubation. In addition, it can be shown that the source (vendor) of CSF determines its quality. In particular, it appears that blood-born peptides largely alter the general peptide pattern in some CSF preparations.**

## References

- Schulz-Knappe P, Schrader M, Zucht HD: The Peptidomics Concept Comb Chem High Throughput Screen – 2005, Vol 8: 697-704
- Proteomics in Drug Research (Methods and Principles in Medicinal Chemistry: Ed. by Marcus K, Stühler K, Warscheid B, Hamacher M, van Hall A, Meyer HE, Mannhold R, Kubinyi H, Folkers G: John Wiley & Sons 2006, in press; contributed chapter: "Peptidomics technologies and applications in drug research" by Schrader M, Budde P, Rose H, Lamping N, Schulz-Knappe P, and Zucht HD
- Lamerz J, Selle H, Scapozza L, Cramer R, Khamenia V, Kellmann M, Zucht HD: Correlation-associated networks of the human cerebrospinal fluid. Proteomics, Vol. 5 2005: 2789-2798 and title page
- Zucht HD, Lamerz J, Khamenia V, Schiller C, Appel A, Tammen H, Selle H: Datamining Methodology for LC-MALDI-MS based Peptide Profiling Comb Chem. & High Throughput Screen.: 2005, Vol 8: 717-723