High-throughput quantification and unambiguous identification for metabolomics

Laan, T. van der

PROPOSITIONS

High-throughput quantification and unambiguous identification for metabolomics

1. Reversed phase chromatography is an effective technique to rapidly remove ion suppression. (Chapter 2)

2. High performance solid-phase extraction columns allow for the trapping and separation of metabolites in a comprehensive and high-throughput fashion. (Chapter 3)

3. SWATH acquisition allows for a more selective fragmentation than MS^{ALL} with regards to the quantification of structural isomers and identification of unknown metabolites. (Chapter 4)

4. Directed two-dimensional chromatography allows for a better purification of metabolites in comparison with one-dimensional or fixed two-dimensional chromatography. (Chapter 5)

5. *"The wide range of polarities and chemical diversity of metabolites poses a challenge for extracting all metabolites or even all metabolites in a subset and failure will provide a biased outcome and an incomplete view of the present metabolites."* (de Raad et al. Curr. Opin. Chem. (2016)) Extraction selectivity causes a challenge for comprehensive metabolic profiling. This challenge can be overcome by including multiple orthogonal extractions in series and analyzing the non-extracted fraction.

6. *"The only way to process DIA-MS/MS based metabolomic data is to perform mathematical deconvolution of fragment ions and their association with precursor ions."* (Fenaille et al. J. Chromatogr. A (2017)) Deconvolution is a powerful tool for DIA-MS/MS. However, it often fails in case of wide fragmentation windows and severe coelution emphasizing the importance of selective DIA fragmentation and sufficient chromatographic separation.

7. *"In the most favorable case, in which the retention times in the two dimensions are completely independent, we speak of orthogonal separations."* (Pirok et al. Anal. Chem. (2019)). For global profiling, it is indeed important that the retention time in two dimensions are completely independent. For a more targeted approach, the most favorable orthogonality is achieved when the retention times of the remaining impurities from the first dimension differ to a different extent than the analyte.

8. *"Although faster throughput can be attained with ballistic gradients on ultrashort columns at pressures around 1000 bar, the lower plate number is not sufficient to efficiently separate complex mixtures and, in particular, compositional isomers that are frequently found in natural extracts."* (Fuhrer *et al.* Anal. Chem. 2011). Complex mixtures can efficiently be separated on ultrashort columns and ballistic gradients when multiple orthogonal columns are serially coupled. In combination with mass spectrometry, unresolved structural isomers can be distinguished by ion mobility and MS/MS experiments.

9. Bioanalysis is like a pair of glasses for society; it reveals aspects of life that were previously hidden from our sight. (Tom van der Laan)

10. The speed of the leader determines the rate of the pack. (D. Wayne Lukas)

11. Almost all aspects of life are engineered at the molecular level, and without understanding molecules we can only have a very sketchy understanding of life itself. (Francis Crick)

12. Research is formalized curiosity. It is poking and prying with a purpose. (Zora Neale Hurston)

Tom van der Laan