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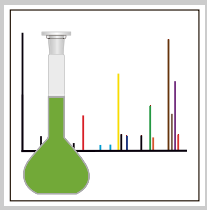

Highlights of Analytical Sciences in Switzerland

Division of Analytical Sciences

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Multimode Separation for Metabolomics and Complex Environmental Samples

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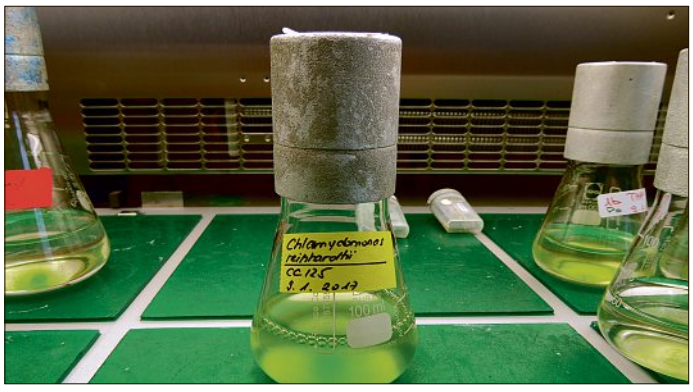
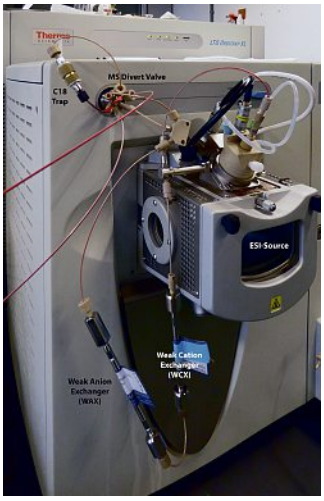
To understand how organisms, for instance green algae, react to external stimuli, such as drugs, environmental pollutants or other stressors, it is necessary to monitor changes in their metabolome. Additionally, it is very often unknown what chemical in the environment caused an adverse effect on the physiological level. In both situations, the analytical method used should capture and analyze the 'universe of chemicals', since depending on the pH, both the endogenous metabolites and the environmental chemicals range from ionic and very polar to lipophilic with log K_{ow} greater than five. While a mixed-mode solid phase extraction allows this multitude of chemicals to be captured, there is today only a limited combination of different separation mechanisms available in one chromatographic run. By combining a C18 trap with two analytical columns, consisting of a mixture of C18 and weak anion (WAX) or cation exchange material (WCX), four separation mechanisms become available during the same run, including hydrophobic interaction (HILIC) at the beginning of the run. First, the injected sample is loaded onto the C18 trap which retains the non-polar part of the chemicals, while ionic and very polar material is passed on to the analytical columns. The gradient starts with 97% acetynitrile (ACN) and aqueous NH_4HCO_3 (3 mM) and ends at 10% ACN and 30 mM aqueous NH_4HCO_3. HILIC conditions are maintained down to 70% ACN followed by ion exchange chromatography given with the increasing ionic strength of the two eluents. At 21 min. the MS divert valve switches and now guides the eluents through the C18 trap, which starts the reversed phase chromatography with again increasing organic content. The chromatogram shows a nice separation of a standard mixture of 18 compounds, detected with a triple quadrupole mass spectrometer in multiple reaction monitoring mode.

The combination of two commercially available mixed-mode ion exchange/reversed phase columns together with a trap column allows separating complex chemical mixtures of metabolites and environmental pollutants in one run.



Batch culture of the green algae *Chlamydomonas reinhardtii*, grown in an incubator.



Front end of an Orbitrap MS with the trap column and the two ion exchange columns clearly visible.

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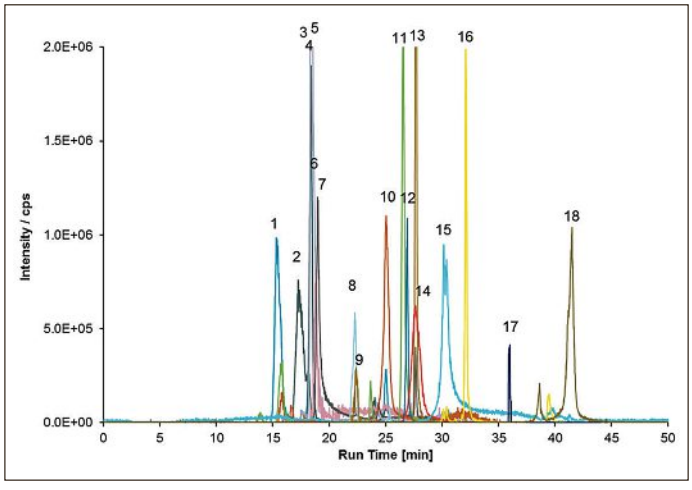

Multimode separation chromatogram obtained from 18 standard compounds on a triple quadrupole mass spectrometer (multiple reaction monitoring): 1 phenylalanine, 2 ascorbic acid, 3 galacturonic acid, 4 glutamic acid, 5 cystine, 6 hexanoic acid, 7 glutathione, 8 glucose-1-phosphate, 9 glutathione disulfide, 10 lysine, 11 tryptophan, 12 sucralose, 13 fluconazole, 14 arginine, 15 cysteine, 16 clotrimazole, 17 tocopherol, 18 dodecyl sulfate.

Can you show us your analytical highlight?
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