O7: Analysis of grape composition through MS-based metabolomics: solvent extraction protocol optimisation

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Optimisation of metabolite extraction from plant/fruit samples for holistic metabolite profiling is difficult, because an unknown number of different molecular classes coexist in diverse concentration ranges. UPLC-TOF-MS is a very powerful tool that offers high sensitivity and efficiency over a broad dynamic range, resulting in the detection of thousands of features and wide metabolome coverage. If applied to both RP and HILIC, UPLC-TOF-MS can profile molecules of varying properties and polarity: highly polar metabolites and lipophilic substances are measured using a single instrumental platform. If required, the application of SPE can provide fractionation of metabolites, however a number of metabolites may appear in both load/wash and elution fractions. Hence the application of SPE in metabolomics should include profiling of all fractions to avoid the loss of information. This presentation aims to focus on these considerations and the strategies used to address them.
Optimal solvent conditions for grape sample preparation were investigated for the purpose of metabolite profiling studies, with the aim of obtaining as many features as possible with the best analytical repeatability. Mixtures of water, methanol and chloroform in different combinations were studied as solvents for the extraction of ground grapes.
The experimental design used a two stage study to find the optimum extraction medium. The extracts obtained were further purified using solid phase extraction and analysed using a UPLC full scan TOF MS with both reversed phase and hydrophilic interaction chromatography. The data obtained were processed using data extraction algorithms and advanced statistical software for data mining. We found that mixtures of three solvents (water, methanol, chloroform) in certain ratios can provide effective extraction and analytical robustness. A fairly broad optimal area for solvent composition could be identified, containing approximately equal amounts of methanol and chloroform and up to 20 % water. Since the water content of the samples was variable, the robustness of the optimal conditions suggests these are appropriate for large scale profiling studies for characterisation of the grape metabolome.