# Separation techniques and mass spectrometry for analysis of bioactive components

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## Abstract

Analysis of biological active compounds comprises large number of complex, which trench on many areas of the human live. Bioactive compounds are any compounds, which influence significantly organisms. We used separation techniques and mass spectrometry for determination of specific bioactive compounds, namely antifungal proteins from barley/malt kernels and plant sterols.

This research is focused on relatively small cationic and amphipatic PR peptides, mainly thionins, and nsLTPs, incident in barley grains. These peptides were extracted by various solvents from barley and malt kernels. One-dimensional SDS PAGE and MALDI MS were used for their detection. SDS PAGE and MALDI MS were applied as fast screening tools for observing the relationship between gushing and peptides/proteins. In addition, changes to the proteins expression reflecting the quality of barley and malt kernels, dependent on the variety and conditions of pathogen treatment, for 20 samples originating from a single fast station and the same preceding crop were studied. Finally, it was found by both SDS PAGE and MALDI MS that the presence of basic peptides, presumably hordothionins and non-specific lipid transfer protein type 1, did not correlate with the degree of gushing for malt ($|r| \in \langle 0.07, 0.34 \rangle$), ($|r| \in \langle 0.01, 0.49 \rangle$), respectively.

In second application, a new methodology for chromatographic detection of nonpolar compounds is provided. MALDI MS is a routinely used technique for the analysis of large polar nonvolatile molecules, such as biopolymers and synthetic polymers. However, nonconventional matrices, such as silver ions form readily adducts with sterols, which allows sensitive MS analysis of these nonpolar analytes in positive mode. The MALDI MS detection limit of sterols ($S/N$ = 3) with silver nanoparticles as matrix were about 10 femtomoles per spot. Liquid chromatography of sterols was optimised for their fraction collection and subsequent MALDI MS determination using silver nanoparticles as matrix. Appropriate separation of sterols was obtained in less than 9 minutes on Luna C8 column (150x4.6 mm I.D., 3 µm) with MetOH:water (97:3) as eluent. The experiments were performed on HPLC system with UV optical detection. After splitter, the fractions were collected on MALDI target. The LC effluent was deposited as 4-second fractions with 500 nanoliter drops. MALDI MS signal of separated sterols (~ 15 picomoles injected, splitter 100:1) was two to three orders above LOD determined by MALDI MS of sterol standards. The presented off-line LC – MALDI MS is a viable alternative to GC – MS for analysis of nonpolar compounds.