

THE DEVELOPMENT OF DIRECT INFUSION MASS SPECTROMETRY FOR METABOLOMICS

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Metabolomics has led to the development of new strategies in analytical chemistry. The emphasis is no longer on the accurate, precise and sensitive analysis of specific compounds, but on the comprehensive measurement of as many compounds as possible in the shortest possible time. The increase in the number of analytes and decrease of analysis time will both compromise the accuracy and precision of analysis of each individual analyte, in comparison to the optimal analytical method for that particular analyte. Moreover, many of the compounds are not yet identified, which confounds accurate and precise analysis. The aim of analysing both known and unknown compounds demands that in addition to quantitative information, qualitative information will also be recorded to facilitate the identification of known compounds and the classification of unknowns. Our aim was to develop a direct infusion mass spectrometry method that enables the rapid collection of both qualitative and quantitative data on as many as possible analytes in a biological extract. We chose a linear ion trap mass spectrometry for its ability to rapidly collect fragmentation data and for its large trap size, maximising the dynamic range.

A fast method was developed to directly infuse raw biological extracts into a linear ion trap mass spectrometer, using the ion trap to isolate and fragment as many ions as possible from the extract. Simple extraction methods were used to extract large numbers of samples. From the extract 40 μl was infused with a flowrate of 10 $\mu\text{l min}^{-1}$ into a flow of 100% MeOH at 190 $\mu\text{l min}^{-1}$. Full mass spectra were collected for the first 1.5 min after the start of infusion and thereafter fragmentation was performed in data-dependent mode on the most abundant ions for a period of 6 minutes or until the intensity of the ions was below the threshold. The full mass spectra were analysed by multivariate statistics to determine discriminating ions, and the fragmentation data facilitated rapid classification or identification of these ions. The full mass spectrum yielded for many ions sufficient precision to discriminate quantitative differences. Due to the short analysis time the method is extremely useful for the analysis of large sample sets.

We successfully applied the method for the chemotaxonomic analysis of range of strains of *Neothyphodium lolii*. This is an endophytic fungus living in the intracellular spaces of grasses and the analyses were carried out on grass seeds, infected with different fungal strains. We were able to classify the 22 different strains on the basis of their secondary metabolites within the background of the more abundant grass metabolites. In a separate experiment we determined in duplicate quantitative differences in concentrations of metabolites in 200 individual plants in, which were the progeny of a cross between ryegrass cultivars. From the total mass spectrum over 25% of peaks were measured with sufficient precision for quantitative trait analysis and mapping on the chromosome. The developed methodology showed excellent performance for the determination of qualitative differences, while the performance on a quantitative level was far better than expected.