

APPLICATION OF A MASS SPECTRUM IMAGING SYSTEM FOR THE IDENTIFICATION OF RELEVANT MEDICINAL DRUGS MONOGRAPHED IN THE EUROPEAN PHARMACOPOEIA

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In the European Pharmacopoeia several European and Chinese herbal drugs are monographed. Each monograph describes different methods for the identification of the drug material as well as for the quality assurance. Foreign matters like heavy metals can be found as impurities. But also the active ingredients may occur in too low quantities.

In the China Academy of Chinese Medical Sciences in Beijing a new imaging mass spectrometry system is established. In order to clarify the spatial distribution of the main constituents in a herbal drug, Matrixassisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) or imaging mass spectrometry (imaging MS) is a powerful tool. This technique has been applied to intact plant tissues or thin slices which could reveal insights into the biological processes taking place. This imaging approach is a promising technique for rapid evaluation and identification of medicinal constituents in plant tissues. For example, MALDI-MSI measurements can reliably illuminate the spatial distribution of small molecules in cryodissected immature barley (*Hordeum vulgare*) grains and tobacco (*Nicotiana tabacum*) roots, barley grain and tobacco root sections.

Sample preparation is a crucial step in producing reproducible and reliable mass spectral images. The quality of the images greatly depends upon factors such as tissue embedding medium, slice thickness, MALDI matrix, and matrix application technique.

Liquid chromatography (LC)-MS coupled to MALDI-MSI can be employed to obtain both spatial information and confident identifications of endogenous metabolites.

MS/MS experiments can be performed directly on the tissue with MALDI-MSI or on tissue extracts with LC-MS and used for the validation of the metabolite identification. This protocol provides a simple method to map endogenous metabolites in herbal drugs which can be adapted and applied to MSI of small molecules in various tissue types and biological systems. MALDI-MSI allows for direct analysis of intact tissues that enables sensitive detection of analytes in single organs and even single cells.

MALDI-MS imaging is a direct and easy-to-use technique for the determination of target molecule localization without any marker. This method can be applied as a new screening method for raw plant material or plant cells like callus which possess a high pharmacological activity, a new quality evidence system for food, and a new evaluation method for plant cultivation or cell culture. Mass spectrometric imaging (MSI) is a powerful analytical tool that can provide spatial information of analytes within intact slices of biological tissue samples. The standard workflow can be easily modified for different tissue types, molecular species, and instrumentations.

High resolution MS (HRMS) can be performed in the positive or negative ionization mode and with normal or reversed phase LC depending on the analytes of interest.

Once an accurate mass is obtained with high resolution LC-MS, the resulting mass can be searched with several databases to obtain identification. MS/MS data can be collected and the characteristic fragmentation pattern of the analyte of interest can be compared to standards, literature spectra, or theoretical fragmentation patterns. The sample preparation is the most critical step in the MSI workflow. Embedding the tissue unevenly will cause sectioning to be difficult or not possible in some cases. The section size and adequate equilibration time are crucial to maintain the tissue integrity and to avoid folding and tears. Selection of matrix and application technique will play a role in determining the types of analytes to be detected, the spatial resolution, and the reproducibility of the results. Using a combination of matrices or application techniques could provide complementary results. MSI experiments produce an abundance of data and can be incredibly time consuming to analyze. Overall, MALDI-MSI offers unique advantages for obtaining spatial information of many compounds simultaneously within a single experiment that can be extremely useful for the untargeted analysis of small molecules and other compounds with many biological applications.

In the frame of the described project this method should be applied to relevant European and Chinese drugs monographed in the European Pharmacopoeia eg Rad Liquiritiae (*Glycyrrhiza glabra*) and Herba Centellae asiaticae (*Centella asiatica*).

This powerful technique does not require a high amount of solvents as some chromatographic methods do and can therefore also be regarded as “green technology”. The method will be validated and the reliability will be tested. As conclusion of the project it should be evaluated if the results obtained by this method are equivalent to these of other methods described in the European Pharmacopoeia so that the technique can be suggested to be added or even to replace other methods.