K51 Quantitative Mass Spectrometric Imaging of Drugs of Abuse in Postmortem Human Brain Tissue

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After attending this presentation, attendees will understand how to perform direct detection and quantitation of drugs of abuse in intact tissues by using deuterated internal standards and matrix-assisted laser desorption/ionization tandem mass spectrometry (MALDI-MS^n).

Direct quantitative mass spectrometric imaging of intact tissue will impact the forensic science community by providing an alternative approach to conventional drug analysis in tissue, which typically involves tissue homogenization, resulting in loss of histological information for drug distribution.

The ability to measure the regional distribution and concentration of drugs of abuse and their metabolites in postmortem brain tissue of chronic human drug users would be an invaluable tool in determining the pharmacological and toxicological actions of the drug of abuse in the human brain. Conventional drug analysis in tissue involves homogenate preparation, followed by extraction and/or derivatization. The extracts are then analyzed by gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS). Sample pretreatments are known to introduce variation in detection. The preparation of tissue homogenate precludes the opportunity to acquire detailed histological information for drug distribution. Mass spectral imaging using MALDI-MS^n provides an alternative approach for the quantitative imaging of drugs of abuse in human brain tissue, while keeping the physical features of the autopsied brain intact.

Tissue samples were excised from the nucleus accumbens (a dopamine-rich area of the striatum) and were snap frozen in liquid nitrogen and stored at -80°C. The drug of abuse and its deuterated analog (internal standard) were spiked onto 20-µm tissue slices using a micropipet. Using an artistic airbrush, MALDI matrix was applied to the tissue. The distribution of the drug of abuse in tissue was imaged using a linear ion trap with intermediate-pressure MALDI source. A MS^n isolation window was selected to include the $[M+H]^+$ ions of both the drug of abuse and the internal standard. An average of ten laser shots per scan was used to produce mass spectra.

Experiments show that ratioing the peak intensities of the analyte and a deuterated internal standard reduces shot-to-shot variability, which is due, in part, to nonhomogeneous crystallization of the matrix on tissue. The MALDI matrix for each drug analysis was chosen based on its ability to ionize the analyte efficiently and to minimize interfering ions. 2,5-Dihydroxybenzoic acid was chosen for the analysis of cocaine and its metabolites; α-cyano-4-hydroxycinnamic acid was determined to be the optimal matrix for the analysis of 6-monoacetylmorphine and morphine.

Brain tissue is a complex sample environment containing a multitude of endogenous lipids and other species that can act as interferants. MS^n methods were developed to increase the selectivity and sensitivity for the target drug analytes in brain tissue. MS^n parameters were optimized for the $[M+H]^+$ ions of cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, 6-monoacetylmorphine, morphine, and the corresponding trideuterated analogs of these species. Instrument software allows for only one isolation window in MS^n experiments, isolating one parent mass (or range of masses) for collision-induced dissociation (CID). This means that MS^n of the target ions of the analyte and internal standard would typically be performed with two separate MS^n experiments. This would increase the response variability and counteract the signal normalizing effects of using an internal standard. Using a 6-amu-wide isolation window centered at a mass-to-charge between the $[M+H]^+$ ions of the drug analyte and its deuterated analog allows for isolation and CID of both ions during a single MS^n experiment. This single isolation method reduces the signal variability inherent with MALDI compared to isolating each ion individually with a 1-amu window (in two alternating MS^n experiments). This method was used to detect and quantitatively image drugs of abuse and their metabolites in postmortem human brain tissue.

This study demonstrated that MS^n increases selectivity, which is critical for differentiating analyte ions from matrix ions and endogenous compounds found in brain tissue. It was also shown that the use of internal standards corrects for signal variability in quantitative MALDI arising from inhomogeneous crystal formation, inconsistent sample preparation, and laser shot-to-shot variability. Using a single MS^n experiment with a wide isolation window to isolate both analyte and internal standard target ions provided improved precision (10-20 times reduction in %RSD) for quantitative imaging studies compared to using two alternating MS^n experiments that isolate the analyte and internal standard target ions separately.

Mass Spectrometric Imaging, Drug Quantitation, Brain Tissue

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