Rapid Analysis of Pharmaceuticals and their Metabolites with Atmospheric Pressure Infrared MALDI Mass Spectrometry

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Introduction

The conventional vacuum ultraviolet (UV) matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) has limited utility for the analysis of small molecules due to spectral interferences from the matrix molecules. The application of matrix and the need for vacuum also prevent the possibility of rapid direct analysis. We explored the use of atmospheric pressure (AP) infrared (IR) MALDI MS for the direct analysis of pharmaceuticals and metabolites in biological fluids using their native water content as the matrix.

Method

A Q-TOF Premier time-of-flight (TOF) mass spectrometer was modified with a custom made AP MALDI interface. The ions were produced by a Nd:YAG laser-driven optical parametric oscillator (running at 2.94 μm and 10 Hz) on a stainless steel target and sampled into the mass spectrometer using pulsed dynamic focusing to improve the ion collection efficiency. The method has been successfully used to perform chemical imaging of biomolecules on a tissue surface. (Y. Li, B. Shrestha, and A. Vertes, *Anal. Chem.*, 2007, *79*,523). Here we present the application of AP IR-MALDI MS to the direct rapid detection of small molecules such as formulated drugs, and metabolites. Various common over-the-counter medications and standards formulated as gelatin capsule, syrup, tablet or powder and bodily fluid, such as urine, were directly analyzed with a minimal sample preparation.

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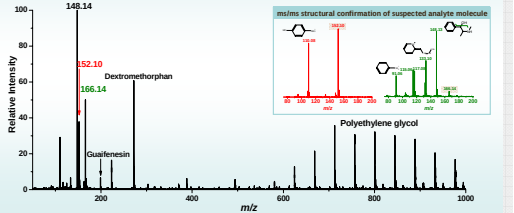
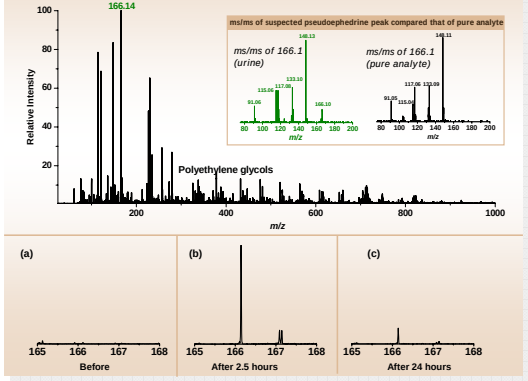
Direct AP-IR MALDI MS of drugs

Mass spectrum of a generic cough medicine formulated as gelatin capsule shows possible molecular ions of all the active ingredients (acetaminophen, dextromethorphan, guaifenesin and pseudoephedrine) and some inactive ingredients (Polyethylene glycol).



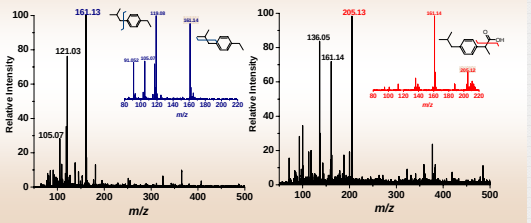
Direct AP-IR MALDI MS of metabolites in Urine

Mass spectra of a few microliters of unprocessed urine collected 2.5 hours after ingesting the drug shows the molecular ions for the metabolites of active ingredients such as pseudoephedrine. The signal of pseudoephedrine (*m/z* 166.1) for urine collected (a) before, (b) after 2.5 hours, and (c) after 24 hours of drug ingestion shows no presence, maximum relative intensity and significantly decreased intensity respectively.



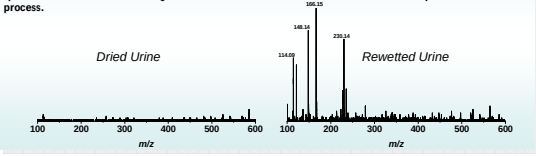
Positive and negative ion modes

Mass spectrum of an ibuprofen tablet sprayed with water showed a fragment of the ibuprofen in positive ion mode and the deprotanated molecular ion in negative ion mode. The ms/ms structural confirmation is shown in the insets.

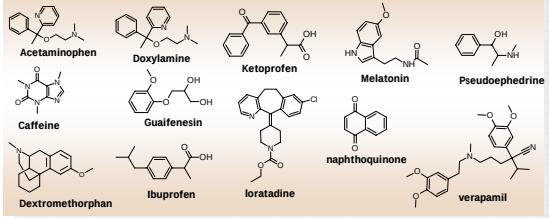


Water as matrix

The importance of water for ionization of metabolites in urine analysis was tested by obtaining the mass spectra of dry urine sample, which did not produce any signal, excluding urea from the urine as a possible matrix. After rewetting the dried residue with water, mass spectra similar to the original urine spectra were obtained indicating that water acted as the matrix for the IR laser desorption/ionization process.

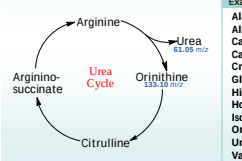


Examples of drugs analyzed



Metabolite study

The mass spectrum of urine also consisted of various other native metabolites in addition to the drug metabolites. For example, two metabolites in the urea cycle, such as urea and orinithine were among the constituents identified in the urine mass spectra. A partial list of the identified metabolites is presented below.

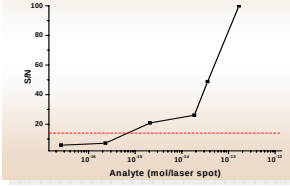


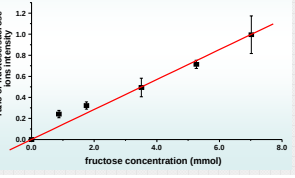
Examples of other Metabolites

- Alanine
- Allantoin
- Carnitine
- Carnosine
- Creatinine
- Glycine
- Histidine
- Homocysteine/Methylcysteine
- Isoleucine
- Ornithine
- Urea
- Valine

Sensitivity

The detection limit was determined to be in the low femtomole range per laser spot based on the signal to noise ratio (S/N) for pseudoephedrine ions as a function concentration.



Quantitative Analysis

Sucrose was used as the internal standard to construct the calibration curve for fructose in water for the AP IR-MALDI. The calibration curve could be used to get the approximate concentration of fructose.