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Direct analysis of drugs and their metabolites by atmospheric pressure infrared matrix-assisted laser desorption ionization mass spectrometry

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Introduction

Mass spectrometry is a well established tool for the analysis of the pharmaceuticals and their metabolites. Many of the traditional ion sources, however, slow down the analysis due to the need for extensive sample preparation.

Atmospheric pressure (AP) infrared (IR) matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) was successfully applied to the rapid and direct detection of formulated drugs and their metabolites in unprocessed urine.

Instrumentation

A Q-TOF Premier (Waters Co.) mass spectrometer was modified by replacing the electrospray source with a custom made AP-MALDI interface. To improve the ion collection efficiency, the ions produced by a Nd:YAG laser-driven optical parametric oscillator (running at 2.94 μm) were sampled into the mass spectrometer using pulsed dynamic focusing [1].

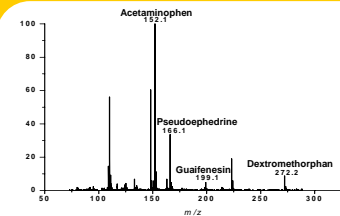
Conclusions

We have demonstrated that AP-IR-MALDI mass spectrometry [2] has potential applications in the direct semi-quantitative analysis of drugs and their metabolites as well as in pharmacokinetics investigations.

References

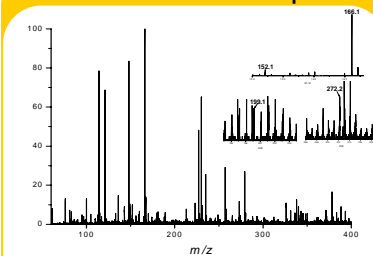
- [1] Tan, P. V.; Laiko, V. V.; Doroshenko, V. M. *Anal. Chem.* 2004, 76, 2462.
[2] Li, Y.; Shrestha, B.; Vertes, A. *Proceedings of the 54-th ASMS Conference on Mass Spectrometry and Allied Topics*, Seattle, WA, 2006.

Direct MS of medicine



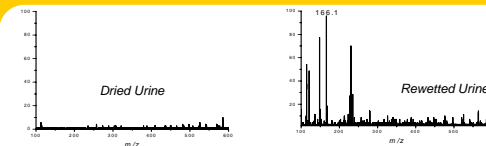
AP-IR-MALDI mass spectrum of a common generic cough medicine formulated as gelatin capsule acquired in 1 second (10 laser shots). The molecular ions of the active ingredients; acetaminophen, dextromethorphan, guaifenesin and pseudoephedrine are easily detected.

Direct MS of urine sample



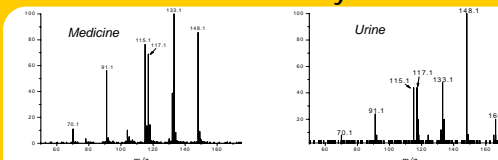
A few microliters of the unprocessed urine collected at different times after drug ingestion were analyzed directly without drying, extraction or any other preparation steps. Molecular ions and some metabolites of all the active ingredients and an inactive ingredient, PEG, were detected in the direct MS analysis of the drug.

Water as a matrix



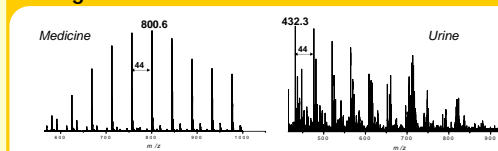
The dried urine sample did not produce any signal. Thus, we excluded that the urea from the urine acted as a matrix. The mass spectra obtained after rewetting the sample spot with water were similar to the original urine mass spectra. This indicated that water played an important role, perhaps as a matrix, in the IR laser desorption ionization process.

Structural Identification by MS/MS



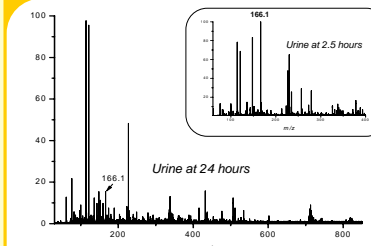
The structural identification of the individual ions produced by AP-IR-MALDI can be obtained by MS/MS. For example, the MS/MS of pseudoephedrine in the medicine is compared to the MS/MS of the suspected pseudoephedrine peak in urine. The spectra are nearly identical.

Drug additives



Inactive ingredients, such as PEG, were also detected in the direct MS analysis of the drug (left panel) and the urine sample (right panel).

Time dependence of metabolites in urine



Compared to the urine collected at 2.5 hours, the relative intensity of pseudoephedrine ions (m/z 166.1) significantly decreased in the sample taken 24 hours after the ingestion of the medication.

Quantitation and sensitivity

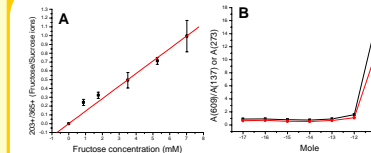


Figure A: Sucrose ion was used as the internal standard to construct the calibration curve for the fructose ion in water. The calibration curve can be used to get the approximate concentration of the fructose ions.

Figure B: Sequentially diluted reserpine (MW 608) solutions were mixed with DHB (matrix) to measure the sensitivity of the AP-IR-MALDI interface. The sensitivity was found to be in the low picomole range.