Mass Spectrometry-Based Imaging Approaches to Spatial Localisation of Drugs in Tissue

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Overview

Aim

To compare two approaches to imaging mass spectrometry for localisation of a drug, AZ-1, in rodent tissue samples. Important experimental considerations such as spatial resolution, time of acquisition and fit-for-purpose assessment were evaluated.

Methods

Matrix-assisted laser desorption/ionisation (MALDI) and liquid extraction surface analysis (LESA), coupled to a Waters Synapt G2 HDMS travelling wave ion mobility mass spectrometer.

Results

AZ-1 was successfully identified in dosed tissue slices by means of LESA-MS/MS and MALDI-MS/MS experiments. Extra selectivity of an MS/MS experiment was required to identify the drug compound, due to the complex ion mixture present in tissue sections.

Conclusions

Whilst both techniques can be used to analyse tissue sections, method choice will ultimately depend on the compound of interest within the tissue and spatial resolution required for the experiment.

Introduction

Knowing the distribution of a drug in target tissue can provide important biological information during the drug development process. Traditional methods used to look at drug localisation are limited due to their dependence on labels, either fluorescence tags or radioactivity (e.g. quantitative whole body autoradiography (QWBA)) to detect the drug in the tissue. A number of mass spectrometry-based imaging approaches have been described for localisation of drug compounds and metabolites in tissue, which provide data complementary to existing imaging techniques.

Matrix-assisted laser desorption/ionisation (MALDI) imaging was pioneered by Caprioli *et al.* in 1997[1]. Since then, MALDI imaging has been used for a number of different application areas, including localisation of drug and metabolite compounds in rodent tissue[2].

More recently, an electrospray-based technique for imaging has been described, termed liquid extraction surface analysis (LESA), which uses a chip-based robotic nanoelectrospray platform (TriVersa NanoMate, Advion)[3]. This technique brings an extraction solvent into contact with the surface of a sample (e.g. tissue section) in a pre-defined position held by the NanoMate. Analyte compounds are then extracted from the surface and the solvent is sprayed through an ESI chip into the mass spectrometer. The schematic shown in Figure 1 explains this process more clearly.

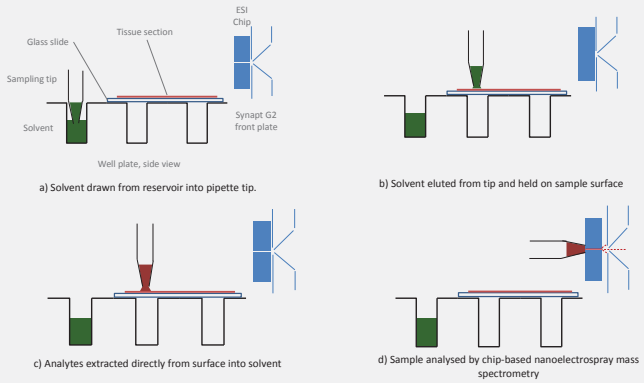

Figure 1. Schematic of liquid extraction surface analysis technique[3]

Liver tissue sections from rats dosed with a drug, AZ-1, were analysed using these two approaches. AZ-1 contains one chlorine atom, providing a characteristic isotope pattern of the parent drug ion at *m/z* 254 and 256 with an intensity ratio of 3:1 respectively. This isotopic ratio is also present in fragments of the drug which retain the chlorine atom. A previous QWBA study completed by AstraZeneca in Sweden showed distribution of the drug in kidney and liver.

Materials and Methods

Sample preparation

Control and dosed rat liver sections were sectioned to 40 µM and 12 µM thickness and thaw-mounted onto glass microscope slides for subsequent analysis by LESA-MS and MALDI-MS respectively. Tissue sections were frozen and stored at -80˚C and brought to room temperature under vacuum prior to analysis.

AZ-1 was spotted onto glass slides alongside tissue at 0.5 mgml^{-1} as a standard in both MALDI and LESA experiments.

Liquid Extraction Surface Analysis

40 µm thick liver sections from dosed and negative control rats were analysed using a NanoMate 100 system (Advion BioSciences, Inc. Ithaca, NY, USA) coupled to a Synapt G2 HDMS mass spectrometer (Waters Corporation, Manchester, UK). A nano-ESI voltage of 1.55 kV and gas pressure of 0.5 psi was applied in all experiments. Extraction solvent for all experiments was 1 µl of 50 % acetonitrile, 50 % water, 0.1 % formic acid, and was held on the tissue surface for 5 seconds before infusion into the mass spectrometer. Data were acquired in positive ion MS/MS mode between 50 and 300 *m/z*. An ion mobility separation step was included in each experiment with a wave velocity of 800 m/s and wave height of 40.0 V prior to fragmentation of the parent drug ion *(m/z* 254). Collision energy of 23 V was applied after mobility separation in the transfer cell of the mass spectrometer.

Figure 2. 40 µm thick dosed liver section after LESA analysis.

Figure 3. 12 µm thick dosed liver section spotted with matrix for profiling.

Matrix-Assisted Laser Desorption/Ionisation

Profiling: 0.5 µl of 15 mgml^{-1} α-cyano-4-hydroxycinnamic acid matrix in 50 % acetonitrile, 50 % water, 0.05 % trifluoroacetic acid was spotted on dosed liver sections as shown in Figure 3. Control tissue sections were also spotted. Each spot was analysed using a MALDI Synapt G2 HDMS mass spectrometer operated in positive ion mode. Fragmentation of *m/z* 254 was achieved with a collision energy of 20 V, and resulting MS/MS data acquired between 50-300 *m/z*.

Results

Liquid Extraction Surface Analysis

The arrival time distribution of the *m/z* 254 ion (parent drug), and the MS/MS spectrum are shown below in Figure 4 for standard drug, dosed and control tissue. The MS/MS spectrum from AZ-1 standard shows a major fragment at *m/z* 208, with a corresponding Cl^{37} isotope at *m/z* 210.

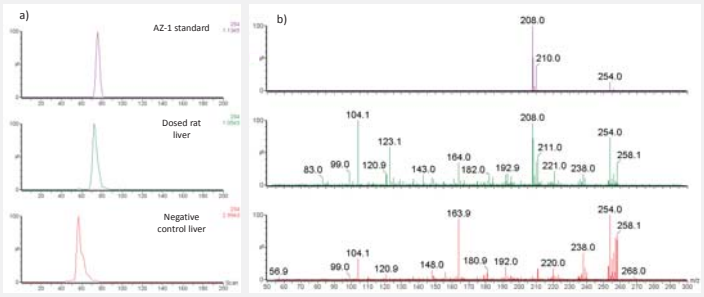

Figure 4. a) Arrival time distribution of *m/z* 254 ion. b) MS/MS of *m/z* 254 after mobility separation.

MS/MS spectra of fragmentation of the *m/z* 254 ion in dosed tissue and drug standard show the major fragment ion and chlorine isotope at *m/z* 208 and 210. These ions do not appear in the MS/MS spectrum from control tissue. This indicates AZ-1 is being detected in the dosed tissue section when analysed by means of the LESA method.

Comparison of the arrival time distributions of the *m/z* 254 ion through the mobility cell provides confirmation that the drug is being detected from tissue. The *m/z* 254 ion from the drug standard and from dosed tissue have the same mobility, whilst the mobility of *m/z* 254 from control tissue is different, with an earlier arrival time.

Matrix Assisted Laser Desorption/Ionisation

A profiling experiment was conducted on dosed and control tissue samples. MS/MS spectra for each sample are shown below in Figure 5. The spectra for drug standard and dosed tissue show the presence of the major drug fragment at *m/z* 208 and Cl^{37} isotope at 210 indicating presence of AZ-1. In control tissue, although there is a peak at *m/z* 208, there is no corresponding Cl^{37} isotope peak, and ion counts are very low compared to the dosed tissue sample.

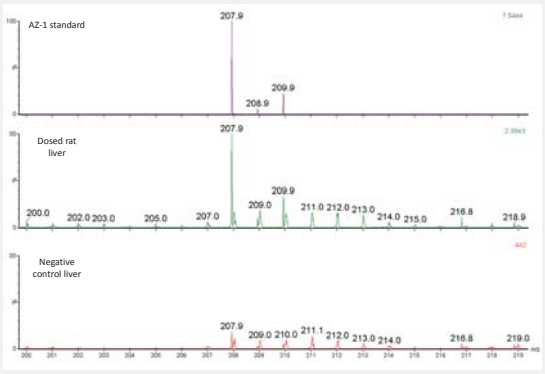

Figure 5. MS/MS of *m/z* 254 from drug standard, dosed tissue section and control tissue section, spotted with matrix in a profiling experiment.

Experimental Considerations

The spatial resolution of LESA is dependent on the size of the solvent droplet held on the tissue surface. On average, this was around 1-2 mm in diameter. MALDI, however, can achieve spatial resolution on the micrometre scale, dependent on matrix crystal size and laser diameter.

Spatial resolution has an impact on experimental acquisition time. Data acquisition across a theoretical tissue area of 90 mm^2 by LESA-MS, with a 3 minute acquisition time per position would take around 1.5 hours. A MALDI imaging experiment on the same tissue area with spatial resolution of 75 µm and 1 second scan time would take around 7.5 hours. Currently, there is no software available to convert LESA-MS data across a tissue surface into a format suitable for creating an ion intensity map. This limits the possibility of creating an image for LESA-MS data in the same way as with a MALDI-MS imaging experiment, where the software and processing tools are already established.

Conclusions

- Both LESA-MS and MALDI-MS can be used to ionise AZ-1 from dosed tissue sections.
- MS/MS experiment was required to confirm identify the drug compound from tissue samples.
- Ion mobility separation can add an extra level of selectivity to an imaging experiment.
- MALDI can achieve much higher spatial resolution than LESA, but there is an increased acquisition time associated with this.
- Current software capabilities do not easily allow LESA data to be converted into an ion intensity map of tissue sections.
- LESA could be viewed as fit-for-purpose where spatial resolution is not a primary concern.
- Whilst both techniques can be used to analyse tissue sections, method choice will depend on the compound of interest within the tissue and spatial resolution required for the experiment.

Future Work

The experimental methods described above will be extended to imaging experiments on dosed tissue to determine the localisation of AZ-1 and its metabolites within liver. The applicability of both LESA-MS and MALDI-MS to tissue samples dosed with other drugs, and other tissue types will also be addressed.

References

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2. **Khatib-Shahidi, S., M. Andersson, *et al.*** (2006). *Analytical Chemistry* **78**(18): 6448-6456.
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