Sensitive AP/MALDI multiple reaction monitoring and imaging (MRMi) of acyclovir from tissue

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INTRODUCTION

Mass spectrometry imaging using AP/MALDI has been widely accepted on high resolution MS instruments (example; Orbitrap, QTOF). The ease of inter-changeability has been major draw along with high spatial resolution achievable. **AP/MALDI interfaced with a triple quadrupole MS system brings together the advantages of high sensitivity and MS/MS selectivity with the capability to image surfaces**. This combination will complement the industry preferred LC-MS/MS quantitative workflows with an ability to understand site-specific drug distribution made possible through MSI. In this work, we demonstrate the combined capabilities of AP/MALDI MSI and MRM for imaging acyclovir and its labelled deuterated analogue from tissue samples. Acyclovir is a drug used for treatment of viral infections. Understanding its distribution in tissue is important from a pharmacology and toxicology perspective.

MATERIALS AND METHODS

Sample Preparation:

Calibration standards for pharmaceutical drug analysis were prepared by serial dilutions of acyclovir and acyclovir D4 standards to achieve the working range of 0.1 pg/µL to 100 µg/mL using methanol. MALDI matrix (10 mg CHCA) was prepared in a 50: 50 ratio of methanol- 0.1% TFA in deionized water.

For permanent marker ink test, green, blue, red and black colour markers were streaked on ITO coated glass slide.

For MSI analysis, ITO coated glass slide with the tissue section kept in -80° C was removed and kept in desiccator for 30 minutes for complete drying. Uniformly spray the freshly prepared (80:20; v/v) CHCA matrix solution on the tissue section using the matrix sprayer and let it air dry. An equal volume of sample and matrix was premixed and 1 µL of the mixture was spotted on tissue section placed on ITO coated glass slide and allowed for drying prior to the analysis.

MassTech AP/MALDI system

AP/MALDI (ng) UHR source (MassTech, Inc., Columbia, MD) with a 355 nm Nd: YAG laser source was used for the experiment. Laser energy used for desorption of samples was 80%. The laser fire pattern used was constant speed raster motion. Optimization of parameters such as laser energy, de-clustering potential, collision energy and laser energy was done using reference standard solutions.

SCIEX QTrap 6500+ system

The QTrap 6500+ mass spectrometer was coupled with AP/MALDI to perform MRM analysis for imaging acyclovir at IonSpray voltage floating 1000 V, curtain gas as 10 and interface heater temperature (220°C), De-clustering Potential (DP) as 90 V for acyclovir and 10 V for acyclovir D4 and collision energy (CE) 15 for acyclovir and 14 for acyclovir D4 to achieve fragmentation.

Figure 1. AP/MALDI coupled with SCIEX QTRAP 6500plus system

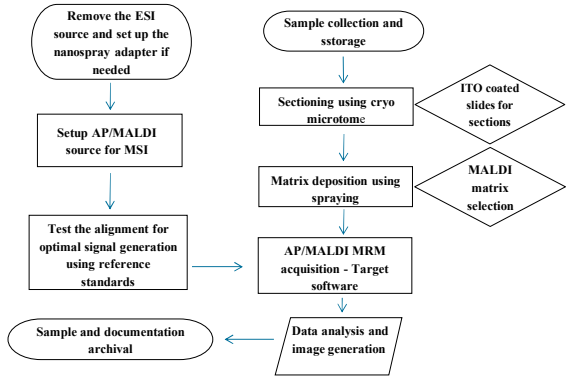

Figure 2. Workflow for mass spectrometry imaging using AP/MALDI ionization source

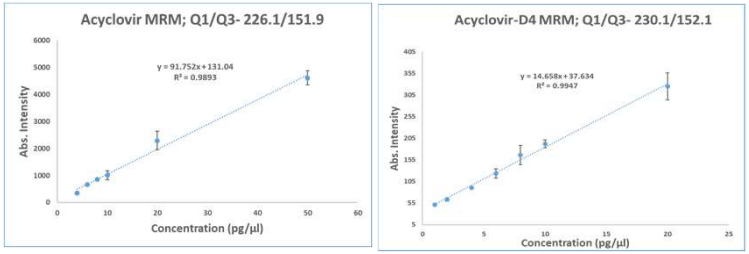

Figure 3. Calibration curves for MRM transition of acyclovir and acyclovir-D4 spotted on stainless still MALDI target plate

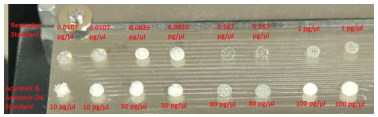

Figure 4. Reserpine aqueous standard and mix of acyclovir and acyclovir D4 standard spotted on ITO coated glass slide

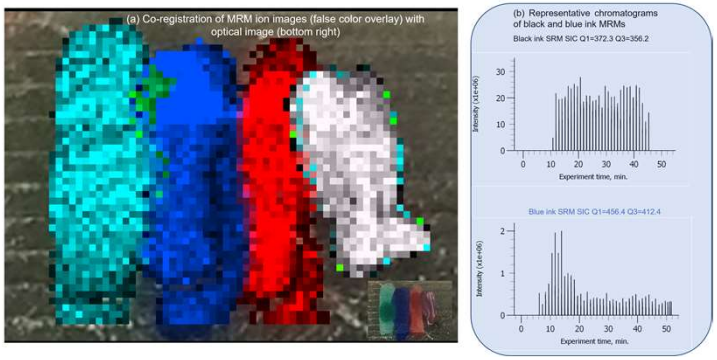

Figure 5. AP/MALDI MRM MSI reconstructed ion images of permanent marker inks co-registered with optical image and (b) selected ion chromatograms of black and blue permanent marker inks. Image generated using Mozaic software from Spectroswiss Sarl.

Sr. No	Concentration (pg/µl)	Acyclovir			Acyclovir-D4		
		MRM Q1/Q3- 226.1/151.9		Average of Abs. intensity	MRM Q1/Q3- 230.1/152.1		Average of Abs. intensity
		Abs. intensity			Abs. intensity		
		Spot 1	Spot 2		Spot 1	Spot 2	
1	10	122	162	142	34	36	35
2	50	613	693	653	80	74	77
3	80	851	984	917.5	113	126	119.5
4	100	1276	1307	1291.5	166	170	168

Table 1. Absolute intensities of acyclovir and acyclovir-D4 MRM transitions observed on ITO coated glass slide

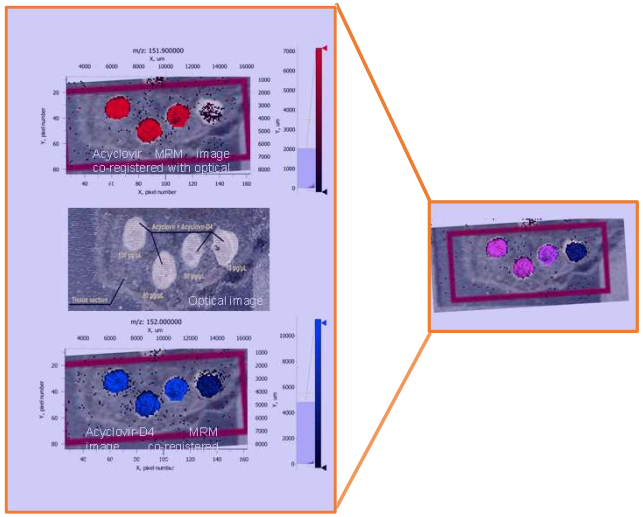

Figure 6. Acyclovir standard dilutions spiked on tissue section on ITO coated glass slide and reconstructed image of spiked acyclovir & acyclovir D4 on ITO coated tissue slide. Image generated using Mozaic software from Spectroswiss Sarl.

CONCLUSIONS

1. AP/MALDI MRM of reserpine & acyclovir from MALDI target plate showed linearity across a wide concentration range.
2. The optimized instrument optical setting was subsequently used for AP/MALDI MRMi of the permanent markers on the ITO coated glass slide – a simple and quick check for MSI using AP/MALDI
3. Four different concentrations of the acyclovir from 10 pg/µL to 100 pg/µL - were spatially resolved with AP/MALDI MRMi with the relative intensities of the reconstructed images correlating with the respective known concentrations.
4. The spatial ion intensities decreased with the spiked analyte concentration. The MRM transitions helped identify, confirm, and image the acyclovir and acyclovir D4 in a single step using the AP/MALDI MRMi analysis.
5. AP/MALDI MRMi using the Q1/Q3 transition of an isotopically labelled analogue along with the drug within the same acquisition demonstrated the potential of the imaging methodology for comparative investigations across tissue samples or different ROIs.
6. AP/MALDI MRMi is a highly selective, sensitive, and cost-efficient targeted imaging approach beyond the currently practiced HRMS imaging methods

REFERENCES

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2. AP/MALDI Sciex QTOF 6600 imaging Protocol2020 (https://apmaldi.com/main/wp-content/uploads/bsk-pdf-manager/2020/06/AP-MALDI_Sciex_QTOF_6600_Protocol2020-2.pdf)

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