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Tissue Imaging of Pharmaceuticals by Ion Mobility Mass Spectrometry

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Abstract

The objective of this study was to examine the distribution of CsA within renal tissues at varying known doses to induce a certain degree of toxicity. In this application note, High Definition Mass Spectrometry (HDMS) was used as an alternative approach for imaging CsA distribution. HDMS is based on travelling wave (T-Wave) technology incorporated into the mass spectrometer.

Introduction

Cyclosporin (CsA) is a drug commonly used as an immunosuppressant that functions as a signal transduction kinase inhibitor; however, CsA has been shown to induce kidney injuries in humans[1]. The objective of this study was to examine the distribution of CsA within renal tissues at varying known doses to induce a certain degree of toxicity.

The traditional approach for MALDI imaging of small molecules, e.g. drug compounds in tissue, utilizes a targeted MS/MS approach followed by mass analysis. This selective strategy provides confirmation of the identity of the drug and enables the molecules to be differentiated from endogenous signals of the same molecular weight. However, some small molecules do not produce satisfactory fragmentation and must therefore be monitored by their intact mass in the MS mode.

Cyclosporin (Figure 1), does not produce intense fragment ions in MS/MS mode and conventional MALDI-TOF MS alone was unable to provide the selectivity required for the analysis.

In this application note, High Definition Mass Spectrometry (HDMS) was used as an alternative approach for imaging CsA distribution. HDMS is based on travelling wave (T-Wave) technology[2] incorporated into the mass spectrometer. Triwave consists of three T-Wave devices, as shown in Figure 2. The first T-Wave (Trap) is used to trap ions during the period when an ion mobility separation (IMS) is being performed in the second T-Wave, thus greatly enhancing the efficiency of the IMS process. The final T-Wave (Transfer) transports the separated ions to the TOF analyzer.

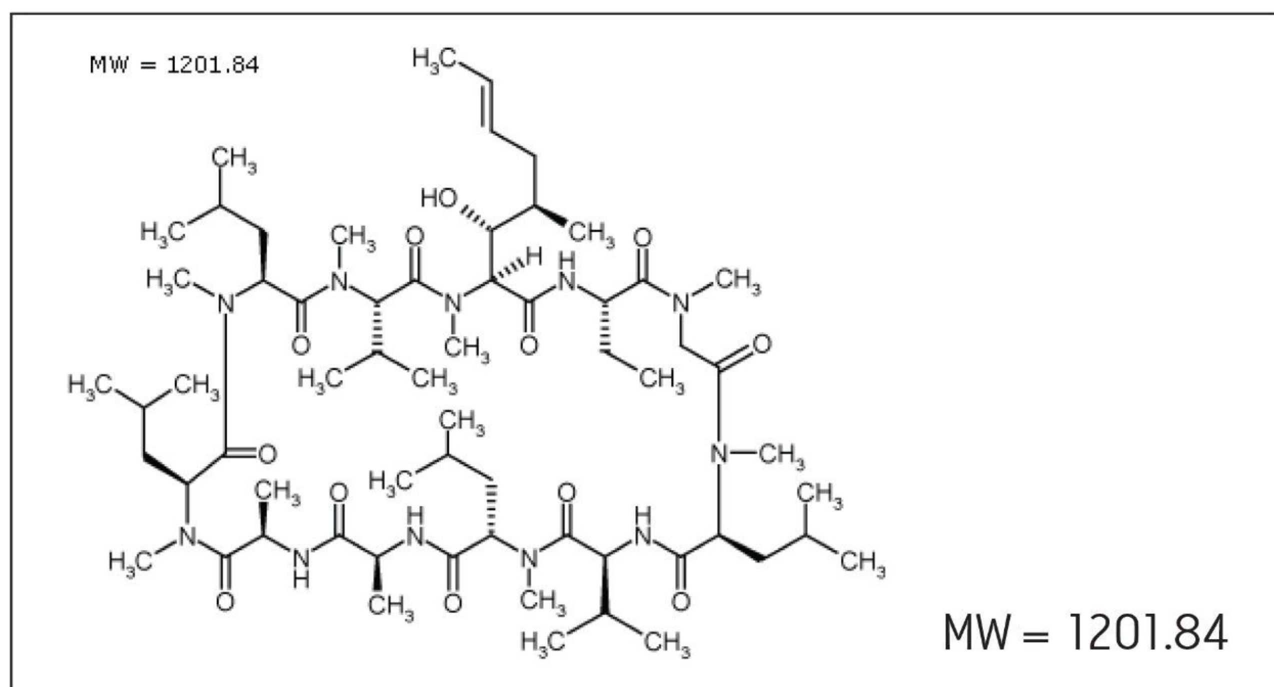

Figure 1. Chemical structure of Cyclosporin (CsA).

Experimental

Mouse kidneys (control, 20 mg/kg, and 80 mg/kg, frozen subcutaneously for seven days) were sectioned at 20 μ m thickness and thaw-mounted onto MALDI target plates. Subsequent sections were acquired for histology

staining and anatomical visualization. Matrix [30 mg/mL of 2,5-dihydroxybenzoic acid (DHB) in 50.0/50.0/0.1 (v/v/v) water/methanol/trifluoroacetic acid] was deposited with a nebulizing spray device (manual nebulizer or ImagePrep (Bruker Daltonics, Bremen, Germany).

The image area was selected using MALDI Imaging Pattern Creator (Waters Corporation, Manchester, UK). Data were acquired using Waters MALDI SYNAPT HDMS System in HDMS positive ion mode over the *m/z* range 100 to 1,500 at an image resolution of 150 x 150 µm and a laser speed of 200 Hz; Figure 2 shows a schematic view. Post acquisition, the ion mobility dimension of the data was evaluated using DriftScope Software. Image reconstruction was performed using BioMap (Novartis, Basel, Switzerland).

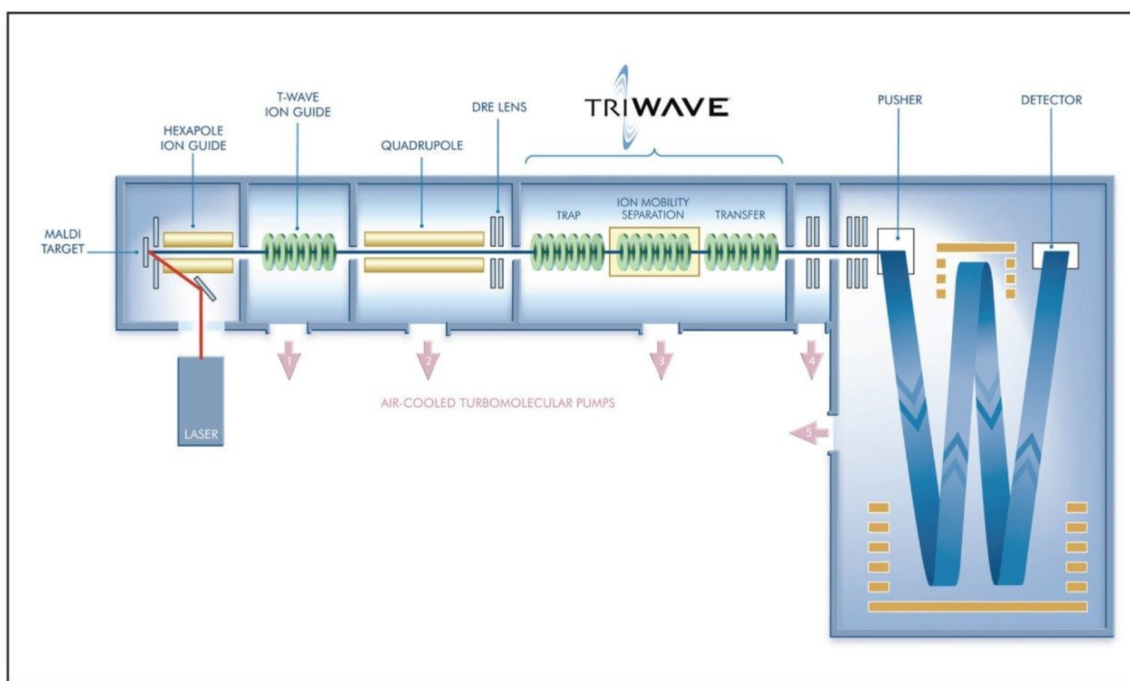

Figure 2. Schematic of the MALDI SYNAPT HDMS.

Results and Discussion

In the mass range of the drug compound, the background ions from the tissue and matrix were intense. Here, the most abundant ion species was the $[M+K]^+$ signal at *m/z* 1240.84; without the selectivity of the ion mobility separation, it was difficult to distinguish drug-related ions.

Figure 3 shows a comparison of the DriftScope 2D plots obtained from the control kidney and the 80-mg/kg-dosed kidney, zoomed around the $[M+K]^+$ ion. The red circle indicates the position of the ion species from CsA in the dosed kidney data. In addition, the drift time of the ion was different from the drift time of the interfering background ions to enable specific selection of CsA. Therefore, it is possible to extract very specifically the CsA $[M+K]^+$ ion species from the DriftScope 2D plot and recreate the ion-image.

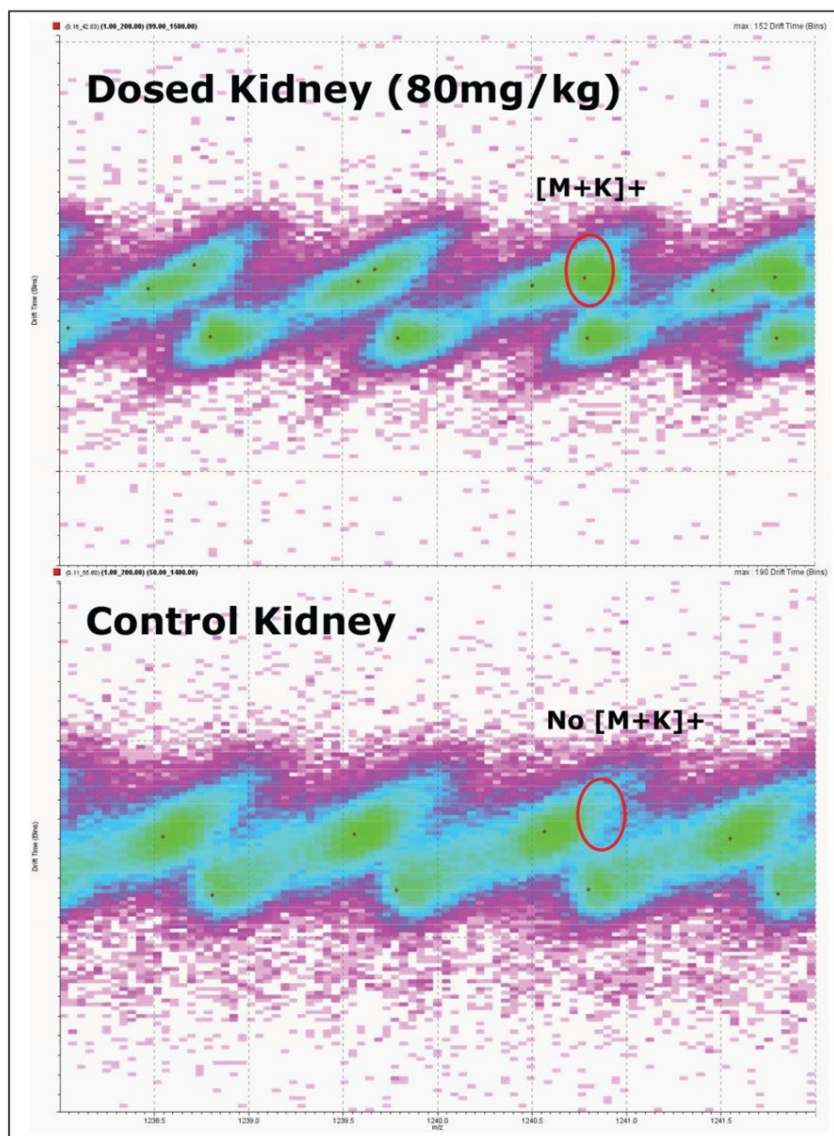

Figure 3. DriftScope 2D plots of the control and 80-mg/kg-dosed tissue sections.

Figure 4 shows the mobilogram (drift time versus intensity plot) of ion *m/z* 1240.8 in the dosed tissue, again showing the presence of two species at the same *m/z* value, each with different mobility. The mass spectrum for each species can be extracted and the mass spectrum on the left-hand side represents the interference species, whereas the mass spectrum on the right-hand side corresponds to the Cyclosporin drug.

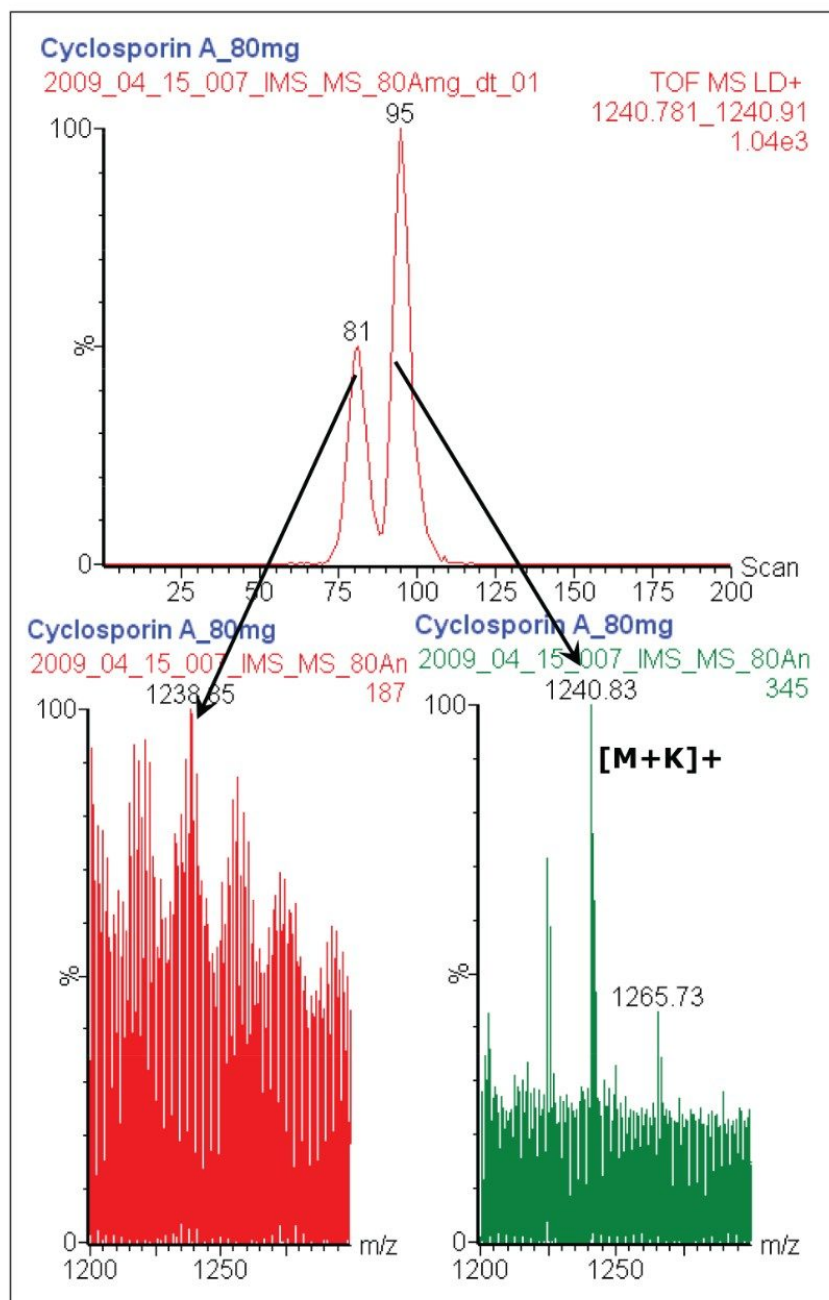

Figure 4. Top: Mobilogram of m/z 1240.8. Bottom: Extracted MS spectra with specific drift time from each species.

Figure 5 illustrates the effect of ion mobility on the MALDI ion images. Images of control, low, and high CsA-dosed renal tissues are shown. Each panel contains the ion image prior to and after drift time selective

extraction of the CsA signal from the DriftScope data, together with the corresponding histology image. For each reconstructed image, the same *m/z* range was selected. The matrix ion was used for normalization purposes.

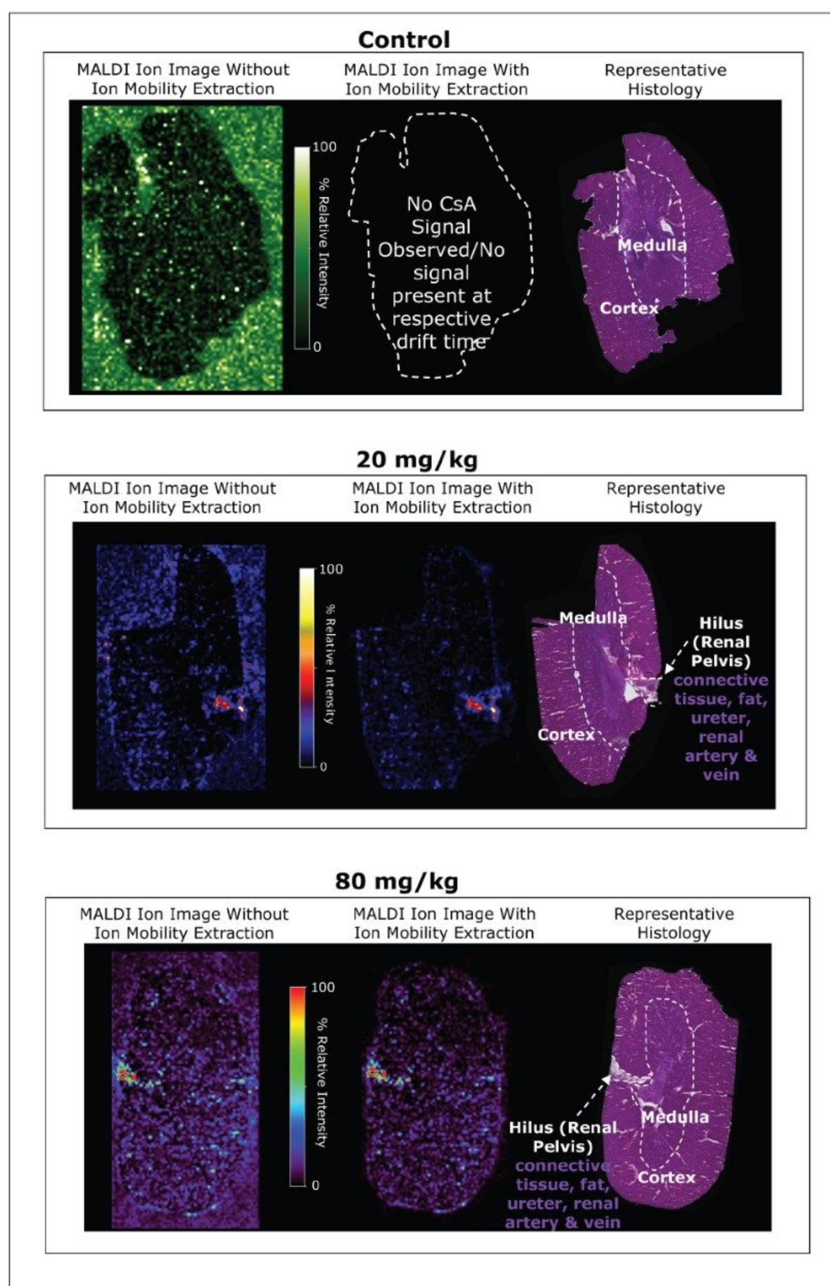

Figure 5. CsA ion reconstituted images of control, 20 mg/kg, and 80 mg/kg-dosed kidney, with and without ion mobility separation, compared to the histology image.

The ion mobility 2D plot from the control sample confirms that no signal corresponding to CsA is present

endogenously in renal tissues, but the image reconstructed from the *m/z* value (without incorporating drift data) corresponding to CsA reveals the distribution of the background ions present at that *m/z* value. The ion images shown from dosed tissue, before and after the use of DriftScope to isolate the analyte, demonstrate the added selectivity provided by ion-mobility separation. The 20-mg/kg dose was near the lower limit of detection for CsA.

Biological Discussion

Images from the 80-mg/kg and the 20-mg/kg CsA-dosed samples illustrate CsA’ s distribution to the renal medulla, cortex, papilla, and hilus. Less drug is present in the 20-mg/kg sample, but shows a similar distribution pattern to the 80-mg/kg sample.

The drug was more highly concentrated in the hilus region than in the cortex or the medulla. The hilus region contains the renal pelvis where concentrated urine, containing the drug to be excreted, accumulates prior to its passage to the bladder. The renal artery within the hilus region may also contribute to the higher drug concentrations. The renal artery carries blood into the kidney where it is filtered and then exits through the renal vein.

Conclusion

- The advantage of applying IMS as a first-dimension separation of ions prior to time-of-flight (TOF) mass analysis for imaging pharmaceuticals in tissues was demonstrated in this study.
- In the case of pharmaceutical compounds that do not give satisfactory MS/MS fragmentation, the selectivity of the traditional approach, where only mass analysis is taken into account, can be poor. In the case of CsA, the drug was confounded by unresolved background ions. The control image highlights the amount of interfering signal at the same *m/z* as CsA.
- Incorporation of ion mobility separation prior to TOF mass analysis in an imaging experiment enabled the true visualization of CsA distribution in the renal tissues, without interfering signal obstruction.

References

1. Kahah BD. Transplantation Proceedings, 2009; 41: 1423-1437.
2. Giles K, Pringle S, Worthington K, Little D, Wildgoose J, Bateman R. Rapid Commun. *Mass Spectrom.*, 2004; 18: 2401-2414.

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