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Applikationsbericht

Localizing Diazepam and its Metabolite in Rat Brain Tissue by Imaging Mass Spectrometry using MALDI Q-Tof Premier MS

Jose M. Castro-Perez, Kate Yu, John P. Shockcor, Henry Shion, Tasneem Bahrainwala, Hidefumi Kaji, Yasuhiro Yamada

Waters Corporation, Tanabe Seiyaku Co., Ltd.

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Abstract

In this application note, we use diazepam as an example to demonstrate the utility of MALDI TOF MS in this application area. After diazepam was intravenously administered to rats, a study using sliced rat brain tissues was performed. Results obtained showed clear localization of the parent drug and its metabolite.

Introduction

There is increasing interest in the analysis of spatial distribution of small molecules in tissues for drug discovery, disease diagnosis, or biomarker discovery. Localization of the dosed drug and its metabolites are critical

information for understanding the mechanism of target-organ toxicity.

Matrix-assisted laser desorption ionization (MALDI) is a sensitive solid-sampling and soft-ionization technique with extensive applications for the analysis of both large and small molecules. The MALDI mass spectrometry (MS) signal can be easily obtained directly from tissue sections.1 The resulting three-dimensional image becomes very useful for the investigation of localization of dosed drug and its metabolites in tissue.

MALDI imaging provides an alternative to whole-body autoradiography in that there is no need to use a radiolabel to trace the drug and its possible metabolites throughout the organ of interest. This means that substantial savings are made, and, as a consequence, scientists can conduct an imaging experiment in targeted organs earlier on in the discovery process without the need of having a synthesized, radiolabeled new chemical entity.

Combining MALDI with quadrupole time-of-flight (TOF) MS, utilizing the Waters MALDI Q-Tof Premier Mass Spectrometer, offers excellent sensitivity and selectivity for these tissue imaging experiments.

In this application note, we use diazepam as an example to demonstrate the utility of MALDI TOF MS in this application area. After diazepam was intravenously administered to rats, a study using sliced rat brain tissues was performed. Results obtained showed clear localization of the parent drug and its metabolite.

Therefore, MALDI TOF MS proved sensitive, specific, and highly amenable to the image analysis of traditional small molecule drug candidates directly in tissues.

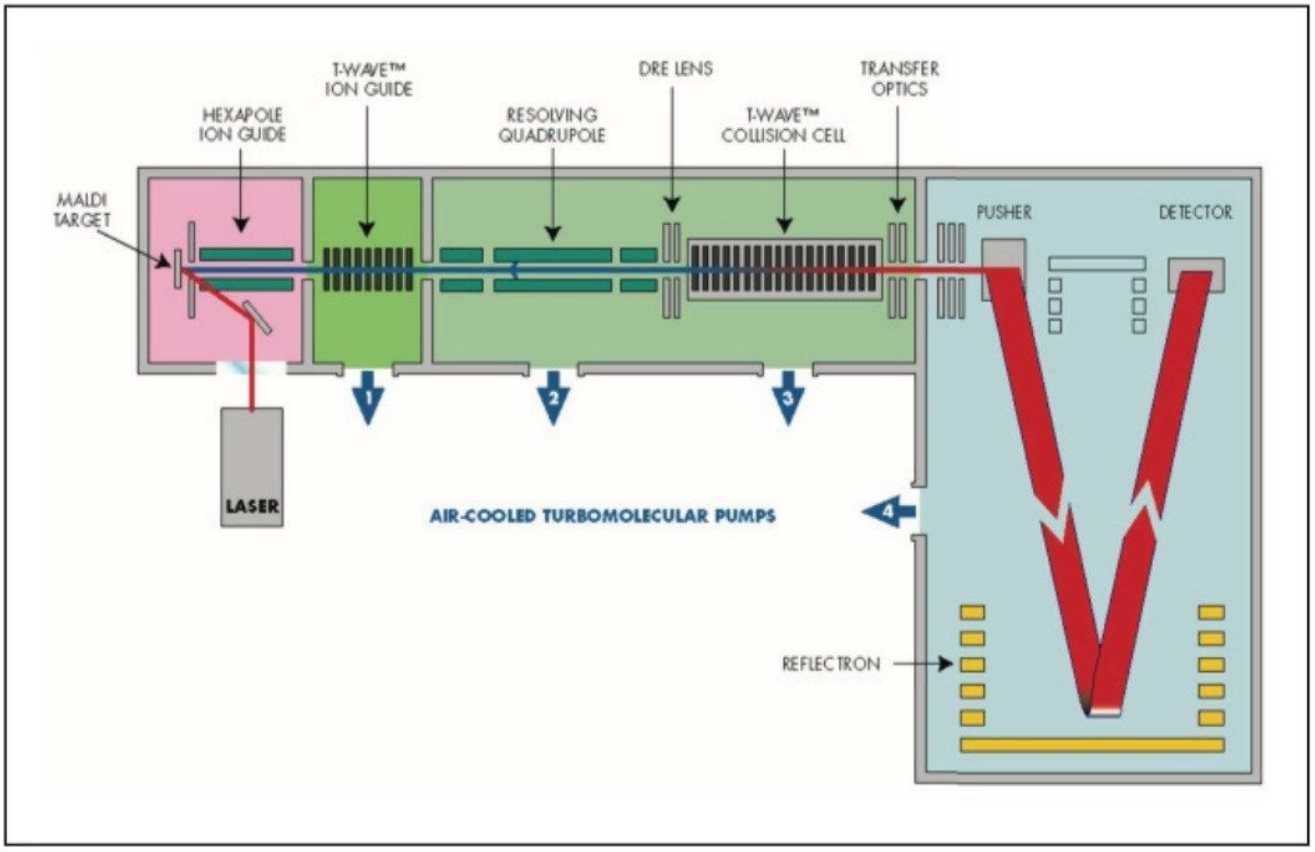

Figure 1. Schematic of the MALDI Q-Tof Premier.

Experimental

Sample Preparation

Diazepam was intravenously administered to seven-week-old male Sprague-Dawley rats at three doses of 100, 30, and 10 mg/kg. Tissue samples were taken five minutes after administration. The isolated brain from the control and dosed rats were frozen by dry ice and embedded in the Tissue-Tek O.C.T. compounds (Sakura Finetek Japan, Tokyo). The tissue was sliced using a Cryostat (Leica CM-3050, Leica Microsystems) at a tissue thickness of 10 micro-m at 18 °C. The slices were mounted onto microscope plates.

The MALDI matrix used was a-cyano-4-hydroxycinnamic acid at 15 mg/mL in 50/50 acetonitrile/water (0.1%

TFA). A TLC sprayer was used to deposit 15 layers of matrix onto the tissue.

MS Conditions

Mass spectrometer:	Waters MALDI Q-Tof Premier
Mass range:	50 to 300 *m/z*
Laser type:	Nd: YAG
Repetition rate:	200 Hz
Collision energy:	25 eV
Gas and collision gas pressure:	Argon (5.30×10^{-3} mBar)
Data acquisition mode:	ESI+ MS/MS with EDC

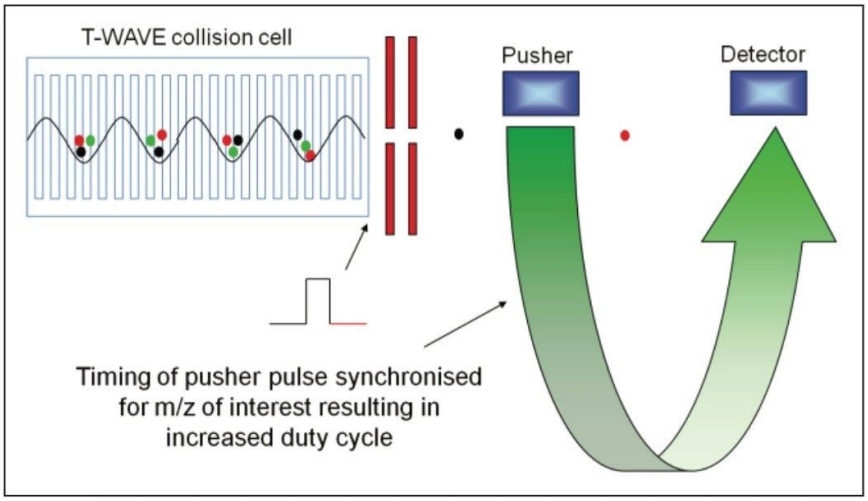

Figure 2. EDC set-up for enhanced MS/MS sensitivity.

Results and Discussion

The concentrations of diazepam and its metabolite, desmethyl diazepam, in rat brain after dosing have been previously determined by LC-MS/MS. The results are shown below.

Dose	Diazepam	Desmethyl Diazepam
10 mg/kg	20.2 µg/g	0.166 µg/g
30 mg/kg	50.5 µg/g	0.595 µg/g
100 mg/kg	266 µg/g	0.080 µg/g

The fragment ions selected for the tissue image were *m/z* 154 (diazepam) and *m/z* 140 (N-desmethyl diazepam). The use of EDC allowed the selected daughter ions at *m/z* 154 (diazepam) and *m/z* 140 (desmethyl diazepam) to be synchronized with the pusher, allowing an increased duty cycle increasing the signal up to five times more than using standard MS/MS conditions.

Figure 3 shows the tissue imaging of the diazepam (*m/z* 154) from the 100 mg/kg dosing, with the MALDI image on the left side and the tissue picture on the right side.

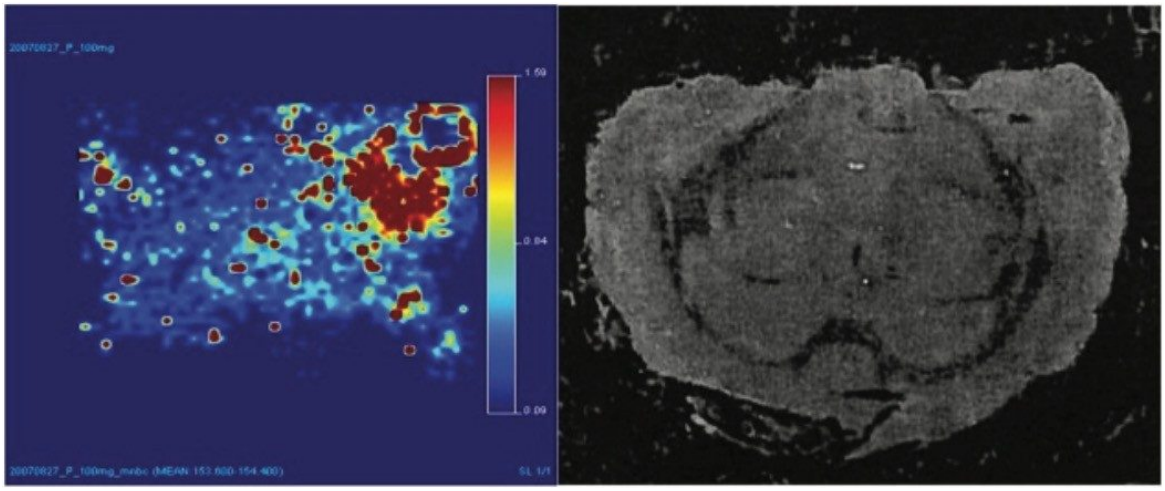

Figure 3. Rat brain tissue image for the 100 mg/kg dose.

The 100 mg/kg imaging showed an area of increased concentration on the top right corner of the brain with sparse spots of the drug in the tissue. This last finding was confirmed by autopsy of the animal, as the high dose lead to the death of the animal, with cerebral hemorrhage leading to the burst of blood vessels and leakage of the drug in the brain. As a result, no metabolite imaging was obtained for this dosage level.

Figure 4 shows the images corresponding to the 30 mg/kg dose. Figure 4A shows the image belonging to the parent drug. At this dosage, the drug has migrated to the lower right part (different distribution compared with the 100 mg/kg dose) with no sign of drug leakage through the vessels; the animal showed no sign of acute toxicity. Figure 4B shows imaging of the metabolite, N-desmethyl diazepam. The localization of the metabolite was not as confined to one region as the drug but more delocalized throughout the entire tissue.

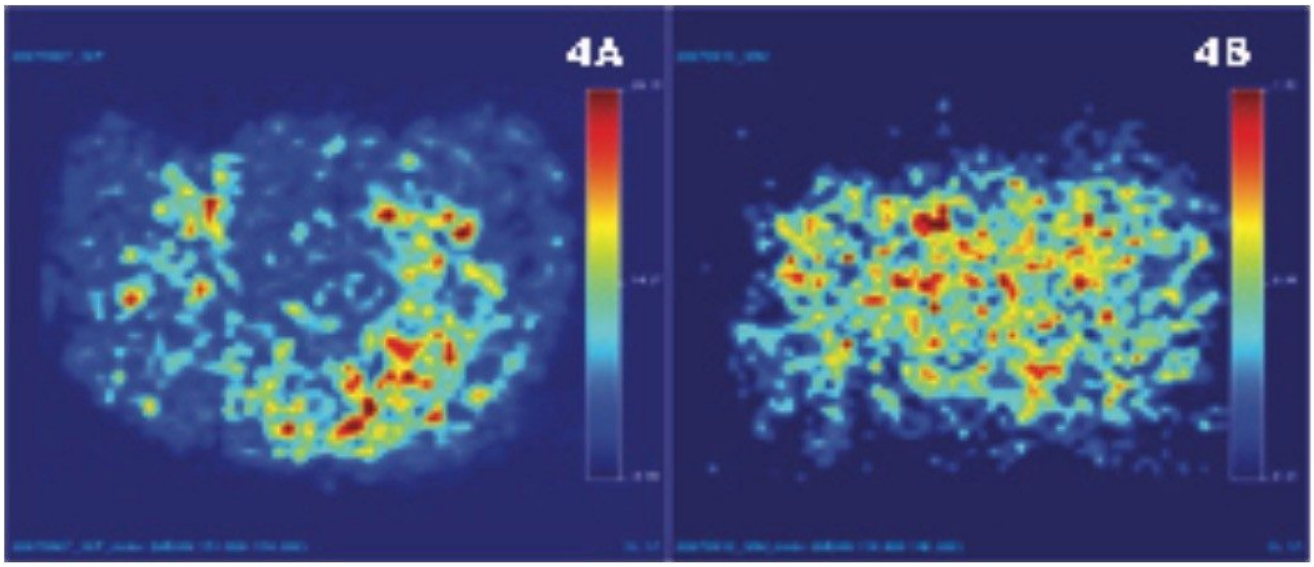

Figure 4. Rat brain tissue images for the 30 mg/kg dose.

Figure 5 shows the images corresponding to the 10 mg/kg dose. Figure 5A shows the image corresponding to diazepam. At this dose level, the localization of the parent drug was in the upper central part of the brain. Figure 5B shows the image corresponding to N-desmethyl diazepam. Similar to the 30 mg/kg dose, the metabolite was delocalized throughout the tissue, only at a lower concentration level.

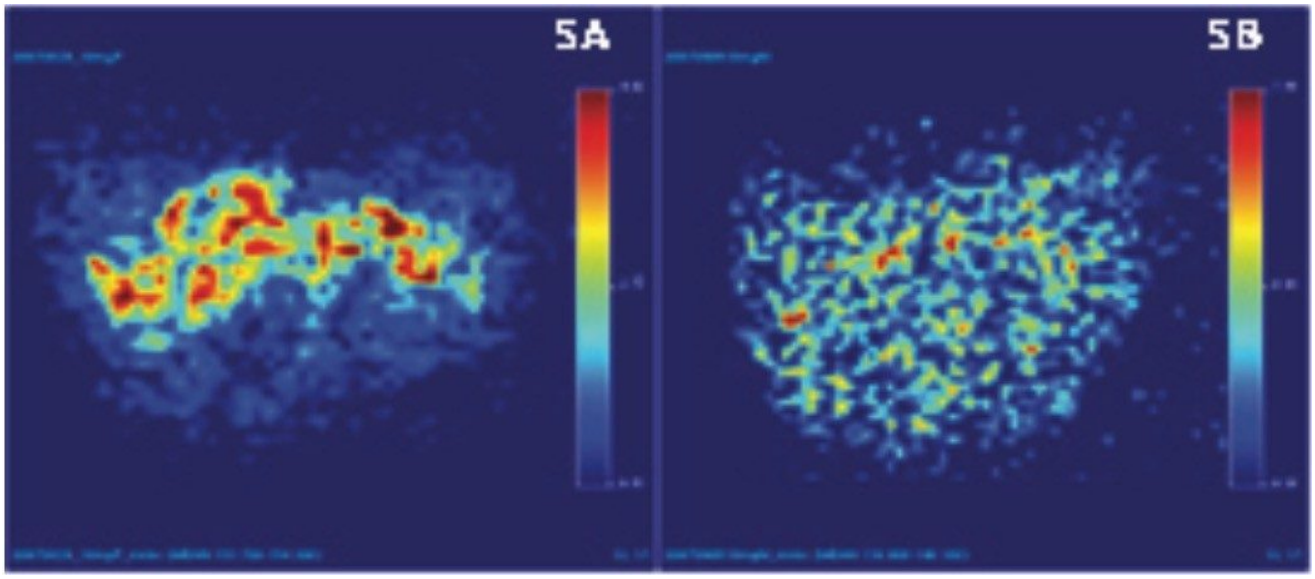

Figure 5. Rat brain tissue images for the 10 mg/kg dose.

The localization of the drug (diazepam) was noticeably different for all the concentrations analyzed. An explanation for this may be rationalized by the fact that since the blood-brain barrier penetration speed of diazepam is very fast, it is thought that localization immediately after administration is dependent on blood flow rate.

In other words, since the blood flow-dependent distribution is seen immediately after administration of diazepam, the distribution of high concentrations appears in the region where the blood flow rate is fast, and the distribution of low concentration is shown in the region where blood flow rate is slow.

In addition, it is reported that flow rate varies within regions of the brain, and the speed of fast regions is about five times that of the slow region. The reason that localization of the distribution for the N-desmethyl diazepam metabolite was not seen is that the penetration speed of blood brain barrier was slower than the parent drug.

Conclusion

MALDI TOF MS using the MALDI Q-Tof Premier Mass Spectrometer proved sensitive, specific image analysis of

traditional small molecule drug candidates directly in tissues. MALDI imaging is a powerful technique used to visualize the localization of drug and metabolite in biological tissues. This particular approach, using EDC, provided enough sensitivity to monitor the drug and metabolite at low levels.

References

1. Stoeckli M., Chaurand P., Hallahan D., Caprioli R. *Nature Med*. 2001; 7 (4), 493–6.

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