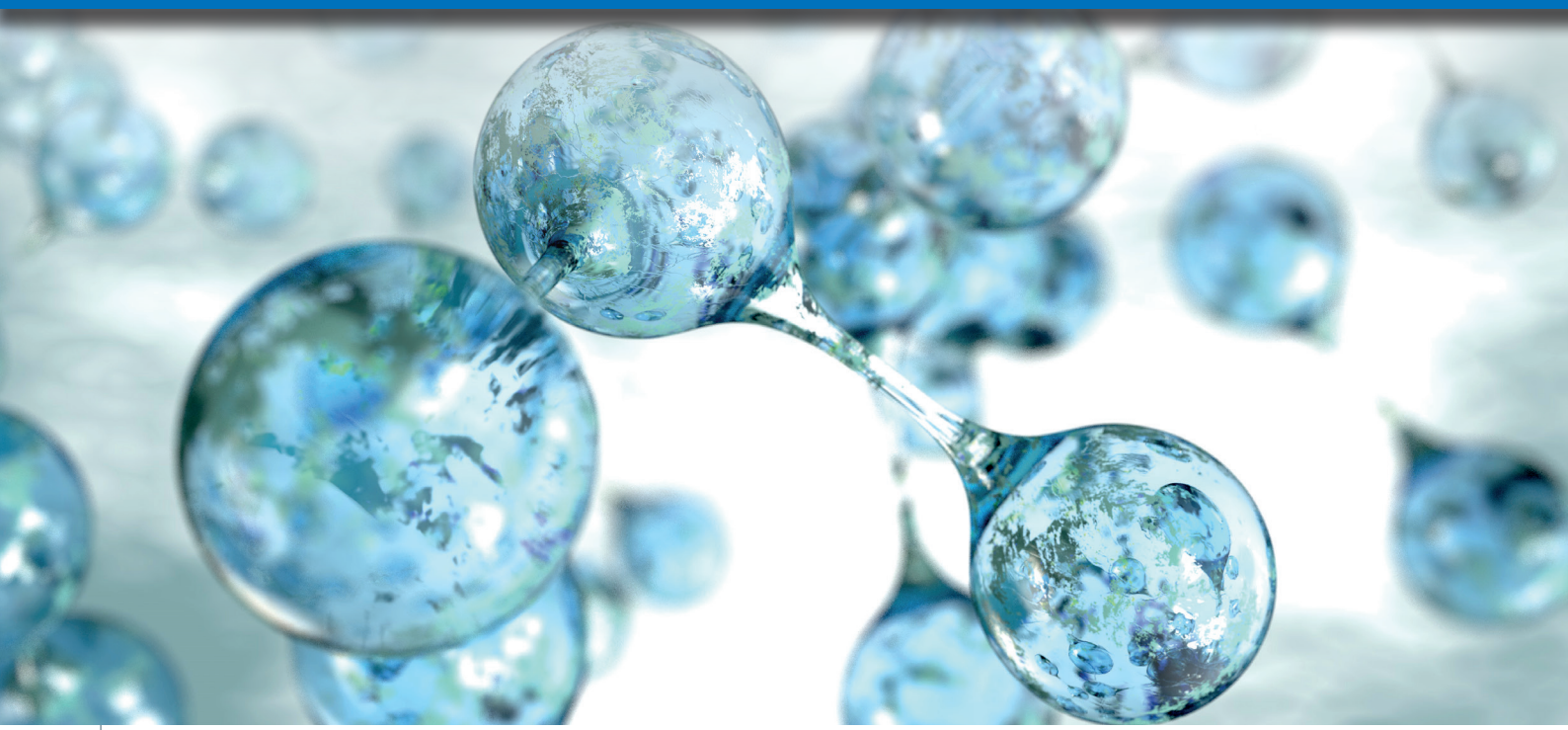

TIMS enabled quantification of small molecules in MALDI Imaging

timsTOF fleX was used to demonstrate quantitative mass spectrometry imaging on several drug compounds.

Abstract

timsTOF fleX was able to separate near-isobaric ions by their ion mobilities, significantly improving targeted compound specificity and sensitivity for quantitation in a complex molecular environment. Higher charge state ions such as dimers were also separable by TIMS, allowing tracking of these ions that might affect the linear dynamic range. Results also show that a combination of parallel accumulation and selective elution of ions by PASEF and matching quadrupole isolation improves sensitivity.

Keywords:
Small molecule, mass spectrometry imaging, drug, timsTOF fleX, quantification, mimetic tissue

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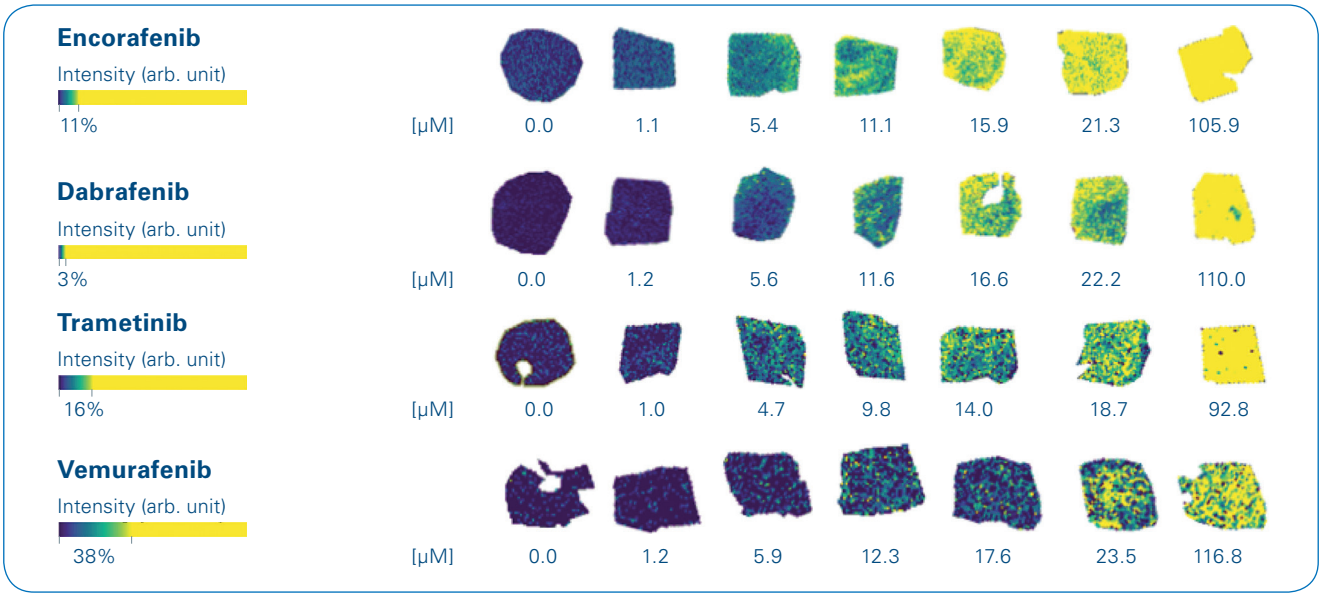

Figure 1: Drug quantitation by MALDI Imaging of four BRAF inhibitor drugs from mouse brain tissue homogenate.

Introduction

Quantification mass spectrometry imaging (qMSI) remains a challenging but necessary aspect of MALDI Imaging. Numerous factors such as the prevalence of isobaric endogenous compounds, chemical background from tissue matrix, and ion suppression often leads qMSI to have low reliability [1]. However, to fully realize MALDI Imaging as a versatile and highly applicable technique, quantification results must be accurate and reliable. This is especially important in pharmacology, where the distribution of drugs, their metabolites in tissue is as important as the absolute quantification [2, 3]. The concentration of drugs present in disease sites determines the efficacy of the dosage and the impact of side effects, which in turn also illustrates the efficiency of any drug delivery methods [3, 4].

Pharmacology research is guided by determining the pharmacokinetics and the pharmacodynamics of the drug, and such studies often require screening large cohort of samples [3, 4]. Four key factors – speed, sensitivity, spatial resolution, and specificity often determine the efficiency of a qMSI method [2]. Here we capitalize on the unique capabilities of timsTOF fleX to tackle all these factors and demonstrate timsTOF fleX as an effective MALDI-qMSI workhorse.

Experimental

To perform qMSI, a mouse brain homogenate was prepared by combining control mouse brains. The use of a tissue homogenate removes potential heterogeneity in desorption/ionization efficiency that can be observed between different anatomical regions of the brain. Serial dilutions of each drug were prepared in DMSO and spiked into the tissue homogenate ranging from a high 100 µM to low 1 µM. The spiked homogenates were spun down, and the vials frozen. Since the concentration of DMSO can affect desorption/ionization efficiency, the final volume of each drug dilution was between 1-2% of the total homogenate volume. A 40% gelatin tissue microarray mold with 1.5 mm size wells was frozen at -20°C. Using a tissue punch, the frozen homogenate was transferred from the vial to the wells. In total, seven wells of varied drug concentrations were used. The drug tissue mimetic was then sectioned at 10 µm thickness and thaw-mounted onto ITO coated slides. The slides were placed inside a vacuum desiccator for 10 mins and a TM-Sprayer was used to apply a matrix composition of 2,5-dihydroxybenzoic acid (DHB) (160 mg/mL) in 70:30 MeOH:0.1% TFA with 1% DMSO. Spray parameters consisted of a flow rate (0.05 mL/min), spray nozzle velocity (1200 mm/min), spray nozzle temperature (75°C), nitrogen gas pressure (10 psi), track spacing (2 mm) and a two-pass spray. The applied matrix coating was recrystallized at 85°C using 5% acetic acid.

The timsTOF fleX was operated in positive ion mode, and the ESI source was used to define the parameters for the ion transfer funnels, quadrupole, collision cell, and focus pre-TOF for each drug using direct infusion. The ESI method was then translated to the MALDI method for qMSI. Pixels with dimensions of 50 µm^2 were analyzed with 100 laser shots, using the single-shot laser mode. SCILs and TIMS viewer were used for visualization of imaging data.

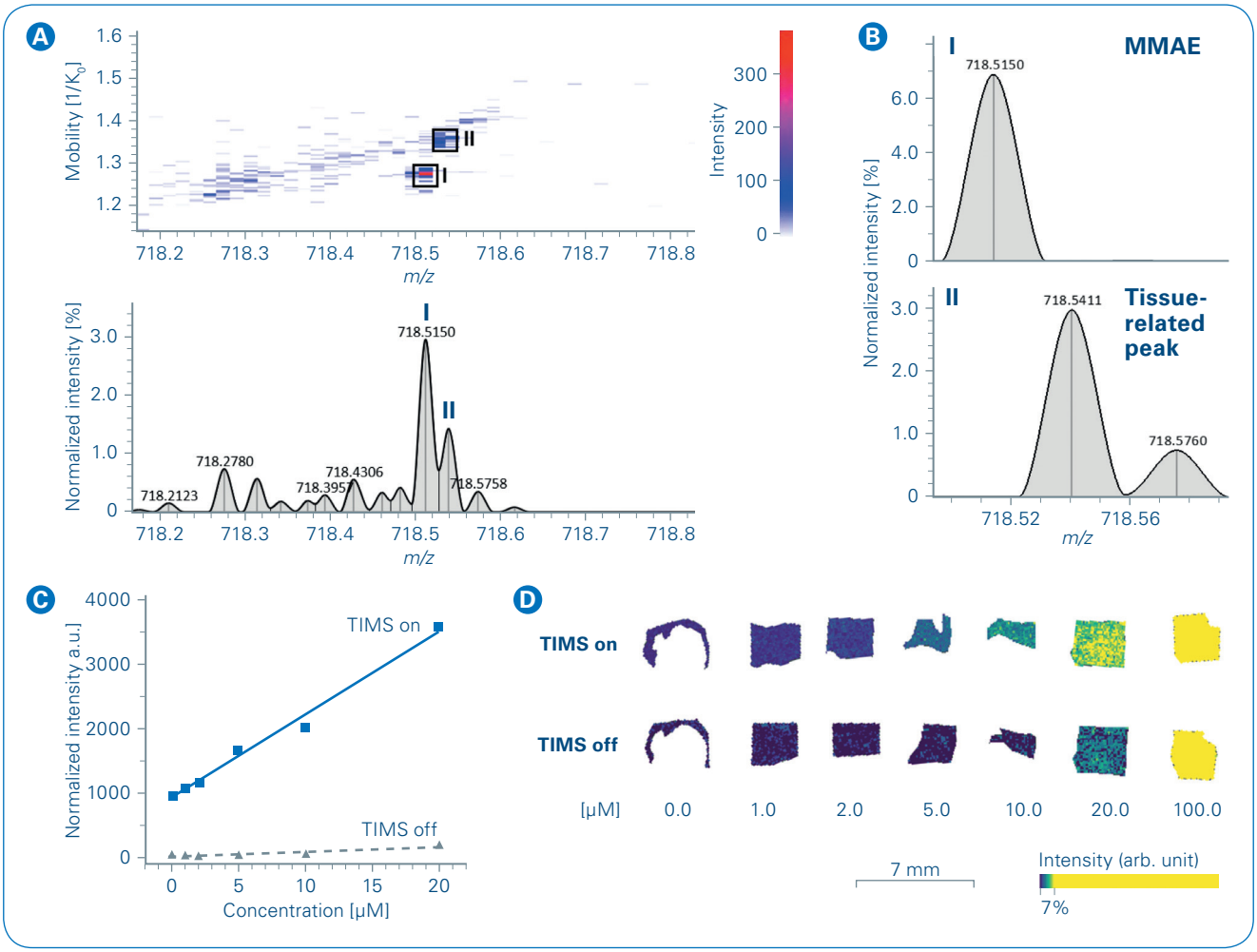

Figure 2: (A) Heat map of 100 µM MMAE revealed two distinct reduced mobility clusters for near isobaric species. Corresponding collapsed mass spectrum encompassing the entire reduced mobility range. (B) Selected regions of interest extracted from the heat map corresponding to I. MMAE ($1/K_o$ = 1.1 – 1.3) and II. tissue-related peak ($1/K_o$ = 1.3 – 1.5) revealed separation of near-to isobaric species in the mass spectrum. (C) Quantitation of MMAE using both TIMS on and off. (D) Corresponding ion images of individual concentrations in the tissue mimetic model.

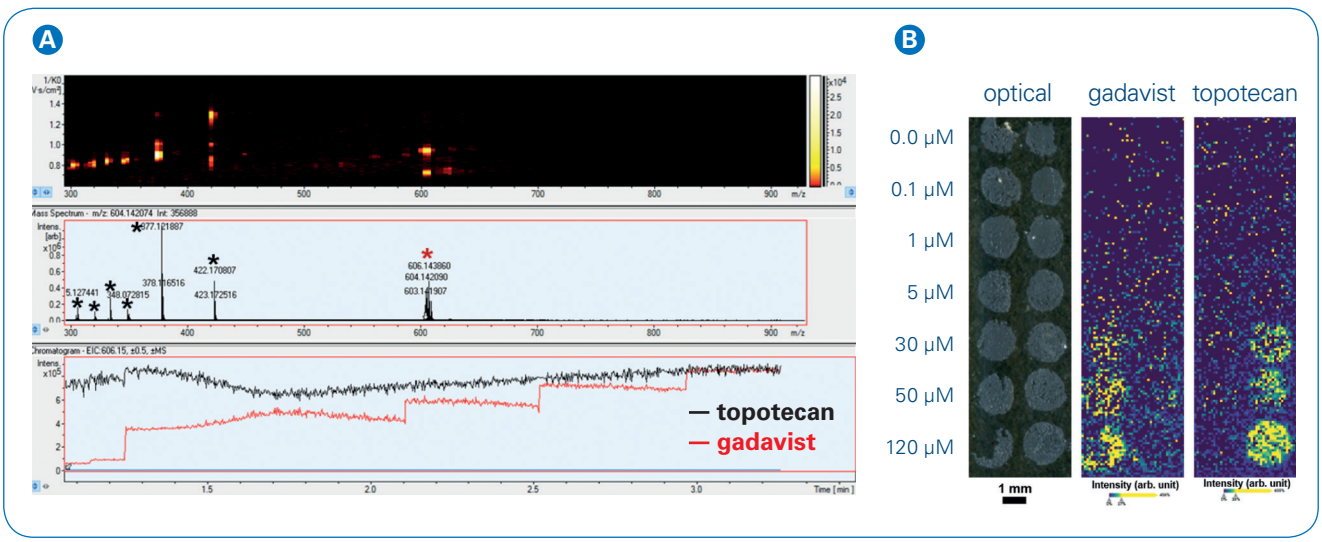

Figure 3: (A) For a co-dosage drug experiment of topotecan (black) and gadavist (red) fine adjustments of the ion optics needed to be performed due to the wide mass range that needed to be covered. The parent peak of each drug ion was monitored over time until both drug detection were equally sensitive.

Results and Discussion

Four BRAF inhibitor drugs were used initially as standard compounds to demonstrate increased signal intensity along with increasing concentrations spiked into mouse brain tissue. Encorafenib (*m/z* 540.1578), dabrafenib (*m/z* 520.1087), trametinib (*m/z* 616.0807) and vemurafenib (*m/z* 490.0764) belong to an important group of targeted therapeutics used for the treatment of melanoma [5].

All four compounds show visible detection in concentration within the low µM range. The highest sensitivity observed was for encorafenib and trametinib with detection limit as low as 1.1 µM, while vemurafenib was detected with the least sensitivity.

timsTOF fleX differentiated two isobaric ions in MALDI-qMSI by exploiting their distinctive ion mobilities, as shown in Figure 2. Monomethyl auristatin E (MMAE) was detected as *m/z* 718.5150, which was close to an endogenous tissue compound at *m/z* 718.5411. Without TIMS on, the peaks from both ions would have a significant overlap (Figure 2a). The extra dimensionality offered by TIMS allows detection with far higher specificity on a targeted compound within a complex mass spectrum profile than without, not unlike separation by chromatography in traditional LC/MS methods. Furthermore, the built-in

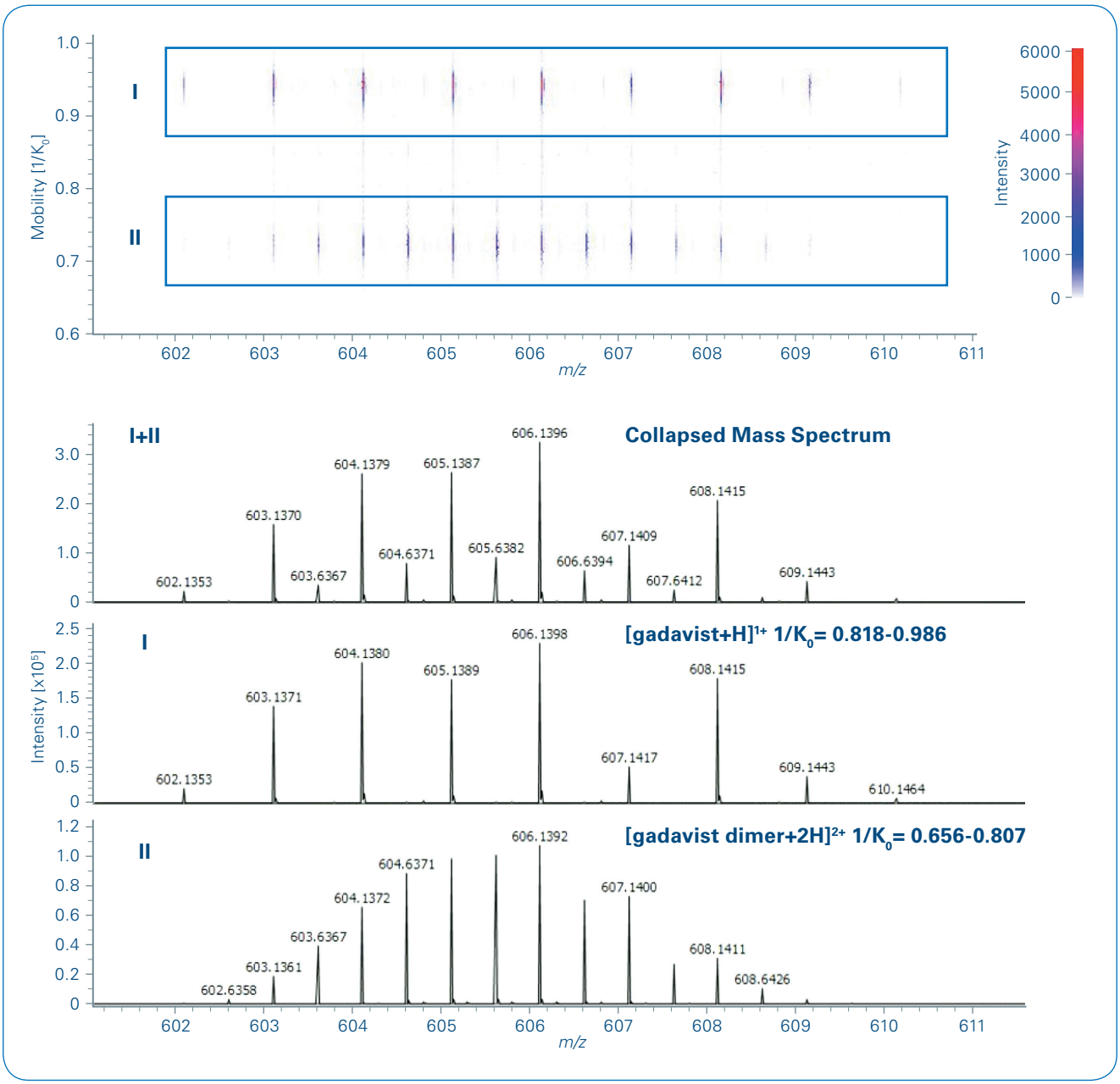

Figure 4: TIMS separation deconvolutes different charge states of gadavist. Gadavist and a gadavist-dimer were both detected and could be observed in the collapsed mass spectrum.

PASEF function enabled with the TIMS measurement and the matching quadrupole isolation also offers inherently higher sensitivity by eliminating the chemical background. This is achieved by only allowing the mobility filtered MMAE ion package to elute into the TOF region, with far less interference from other endogenous tissue compounds during detection. This produces a more reliable calibration curve for qMSI, as seen in Figure 2c.

In a co-dosing study of topotecan and gadavist summarized in Figure 3, direct infusion of drug solution by ESI was used to optimize drug detection by adjusting the voltages of the ion transfer funnels, quadrupole, collision cell, and focus pre-TOF. This defined method was then applied to the MALDI Imaging experiment to achieve similar sensitivity for co-dosed compounds. When evaluating the co-dosed MALDI Imaging data, two isobars having different ion mobilities were observed at the *m/z* of gadavist, seen in Figure 3a.

Gadavist, a contrast agent used for MRI was detected at two different ion mobilities. A closer examination of the *m/z* of each ion mobility, shown in Figure 4, reveals that the MALDI data contains gadavist ions present as isobaric $[M_2+2H]^{2+}$ and $[M+H]^{+}$. In MALDI, analyte cluster ions are not unknown and appear to be correlated with abundance. This can prove problematic for quantitation measurements as the linear relationship between ion intensity and concentration will begin to deviate as dimerization becomes significant, and acts as a soft upper limit to the linear dynamic range. The ability of timsTOF fleX to clearly separate intensity of monomers from dimers further adds to the confidence and accuracy of qMSI measurements made with this platform.

Conclusion

- Bruker's timsTOF fleX was able to separate isobaric ions based on ion mobility in MALDI Imaging mode, offering high specificity on targeted compound for improved quantitative imaging.
- PASEF and quadrupole isolation improved sensitivity on targeted compound significantly by eluting only targeted ions into qTOF region.
- timsTOF fleX can also separate isobaric ions of higher charge states from the monomer allowing observation of dimers that can affect the calibration curve's linear dynamic range.

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