

AXIMA™

Analysis of Small Molecules by MALDI-MS in the Absence of Matrix using QuickMass™ Targets (‘Matrixless MALDI’)

- Analysis of small molecules by MALDI-MS in the absence of MALDI matrix
- Applicable to full automation (sample preparation and analysis) for increased sample throughput
- Good mass accuracy (<0.1 Da) demonstrated using external calibration
- Disposable targets designed for single use - carry-over is eliminated

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Introduction

Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been shown to be a key analytical tool for the analysis of biomolecules^{1,2}. The high sensitivity and speed of MALDI-MS analyses have contributed to its rapid development and widespread acceptance.

Whilst MALDI is primarily used for the analysis of biomolecules (proteins, peptides, oligonucleotides etc.), many other types of sample are amenable to this ionization technique. These include synthetic polymers³, carbohydrates, lipids and petrochemicals.

More recently, the high throughput capabilities of MALDI-MS have prompted the evaluation of this technique for the analysis of small organic compounds. Currently, the most common approach for the analysis of small molecule compound libraries uses electrospray mass spectrometry (ES-MS) with flow injection sample introduction. Without an HPLC column to perform sample clean up, samples must be of sufficient purity and dissolved in solvents compatible with ES-MS. MALDI is more tolerant to buffers and salts than ES and, as a result, extensive sample purification may not always be necessary.

Interference from matrix-related signals and the potential for fragmentation (in-source/metastable) of the target analyte could potentially limit the use of MALDI-MS for this application. To minimize the risk of the aforementioned problems, 2,5-Dihydroxy benzoic acid (DHB) is considered the MALDI matrix of choice.

Here, we describe the analysis of small molecule samples (<700 m/z) by MALDI-MS in the absence of MALDI matrix using QuickMass™ targets (see figure 1). Sample solutions are simply deposited in discrete locations on the QuickMass™ targets and allowed to dry. Examples of small molecule samples taken from a pharmaceutical compound library, prepared using the QuickMass™ targets, and analyzed on an AXIMA-CFR™*plus* MALDI TOF-MS instrument are presented.

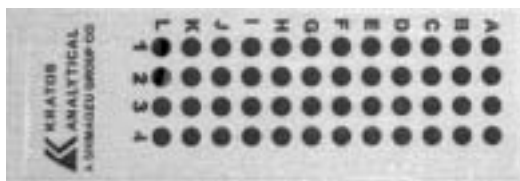


Figure 1. QuickMass™ target

Methods

The samples were dissolved in HPLC grade acetonitrile and diluted using HPLC grade water as required. An aliquot of sample solution was deposited onto a QuickMass™ target and allowed to air dry.

Samples were analyzed using an AXIMA-CFR™*plus* MALDI-TOF MS in positive-ion reflectron mode using INTELLIMARQUE™, a fully automated analysis procedure. The pulsed extraction (P.Ext) value was set to 500. Each spectrum consisted of 100 shots, acquired using a 25-point raster.

Samples were calibrated using a 2-point (external) calibration procedure using papaverine and reserpine. The standards were prepared and analyzed in the same manner as the samples.

For comparison, several of the samples were prepared with DHB as the MALDI matrix and deposited onto an unmodified stainless steel MALDI target. An aliquot of each sample solution was mixed (on-target) with an equal volume of matrix solution (DHB: 12.5 mg/ml in 50/50 acetonitrile/0.1 % (v/v) trifluoroacetic acid (TFA)) and allowed to air dry. Calibrants and samples prepared in DHB were analyzed as described above.

Results

The patented QuickMass™ technology was developed by NanoHorizons (www.nanohorizons.com) for the analysis of small molecule samples using MALDI-MS instrumentation in the absence of MALDI matrix. Experiments are on-going to determine the exact mechanism of ionization in the absence of matrix but it is believed to be via point heating resulting in desorption of the analytes from the target surface.

The QuickMass™ targets consist of a nanometer-thick layer of metal deposited onto a glass microscope slide. Prior to deposition of the metal layer, the entire glass slide may be coated with a hydrophobic film to minimize spreading of deposited samples, which may contain high levels of organic solvents. The metal layer is subsequently deposited as discrete spots on the glass slide onto which sample/calibrant solutions are deposited. The pitch density of the QuickMass™ targets is the same as that of a 384-well microtitre plate/MALDI target, allowing deposited samples to be easily located using LAUNCHPAD™ software (see figure 1).

The QuickMass™ targets offer several advantages over stainless steel MALDI targets:

- (i) The absence of MALDI matrix simplifies the spectra obtained. Matrix-related signals, which may interfere with the low mass analyte signals, are eliminated. Additionally, sample preparation time is decreased due to the fast evaporation times of sample solutions containing high levels of organic solvents.
- (ii) Sample homogeneity is improved in the absence of matrix. For example, it is widely known that samples prepared using DHB form non-homogeneous sample/matrix layers. As a result, analysis times are increased as the user must search the sample spots for positions containing analyte signals (sweet spots). The improved sample homogeneity makes the targets ideal for high throughput analyses.
- (iii) Following analysis, the targets containing the deposited samples can be archived allowing re-analysis of the same sample at a later date.
- (iv) The targets are disposable and have been designed for single use. Therefore, problems associated with carry-over resulting from incomplete cleaning of the targets between analyses are eliminated.

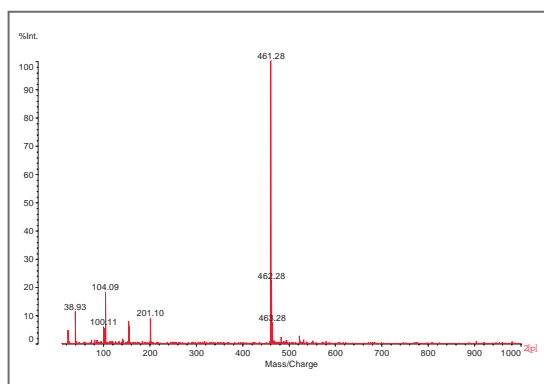


Figure 2. MS spectrum obtained for pharmaceutical sample 1 using a QuickMass™ target (expected $[M+H]^+$ = 461.22)

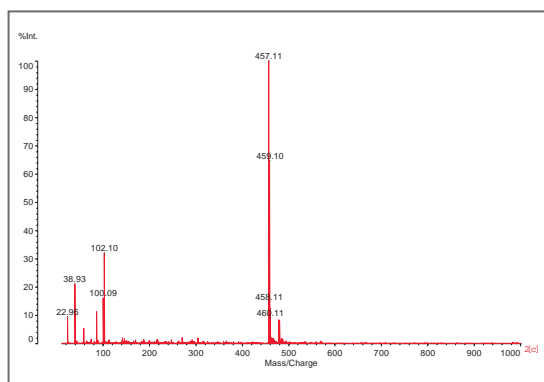


Figure 3. MS spectrum obtained for pharmaceutical sample 2 using a QuickMass™ target (expected $[M+H]^+$ = 457.09)

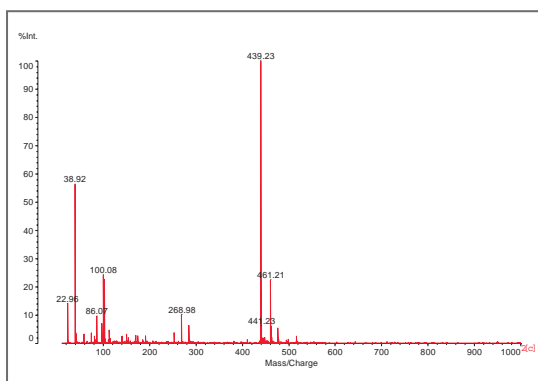


Figure 4. MS spectrum obtained for pharmaceutical sample 3 using a QuickMass™ target (expected $[M+H]^+$ = 439.18)

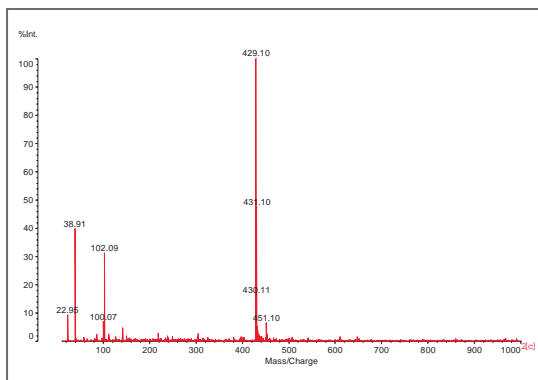


Figure 5. MS spectrum obtained for pharmaceutical sample 4 using a QuickMass™ target (expected $[M+H]^+$ = 429.08)

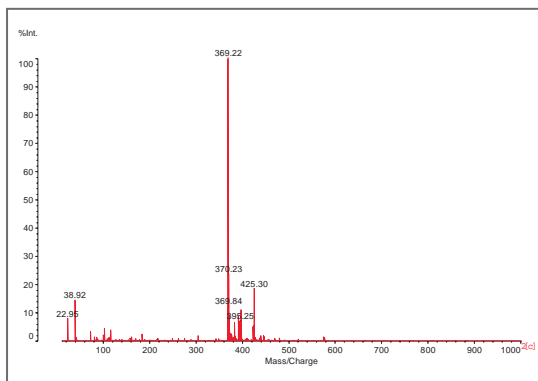


Figure 6. MS spectrum obtained for pharmaceutical sample 5 using a QuickMass™ target (expected $[M+H]^+$ = 369.20)

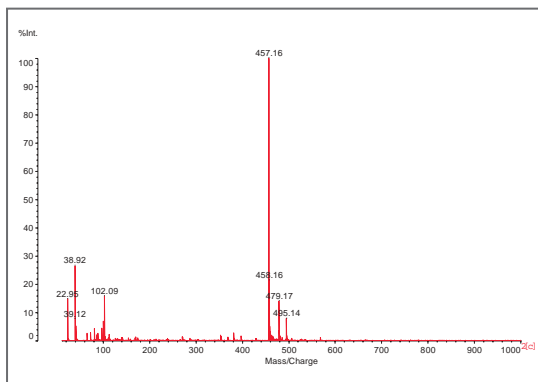


Figure 7. MS spectrum obtained for pharmaceutical sample 6 using a QuickMass™ target (expected $[M+H]^+$ = 457.15)

Using the QuickMass™ target, signals were obtained for a high percentage of the samples analyzed consistent with ES-MS (see figures 2-7). This is based on analyses performed solely in positive-ion mode. It may be possible to increase the success rate by analyzing samples in both positive- and negative-ion modes. Good mass accuracy was obtained (<0.1 Da) allowing confirmation of the presence of target compounds.

In addition, experiments were performed to demonstrate the use of the QuickMass™ targets for high throughput analyses (see figures 8-9). Several samples were re-analyzed using a method in which a total of 8 shots were acquired per sample. 24 samples were analyzed and processed in total (including calibration standards) in a time of ~2.5 min, equivalent to ~6 sec/sample. Even under these high throughput acquisition conditions, the mass accuracy obtained was <0.2 Da.

Conclusion

- signals were obtained corresponding to the expected target mass for a high percentage of samples which was consistent with ES-MS (positive-ion mode only)
- good mass accuracy (<0.1 Da) was obtained under normal acquisition conditions, allowing confirmation of the presence of the target compounds
- analysis and sample preparation - although not demonstrated in this application note - are amenable to automation for increased sample throughput. Use of the QuickMass™ targets for high throughput analyses was demonstrated, with analysis times of ~6 sec/sample (equivalent to > 10,000 samples per day)

Figure 8. MS spectrum obtained for pharmaceutical sample 8 using a QuickMass™ target under high throughput analysis conditions (expected $[M+H]^+ = 449.19$)

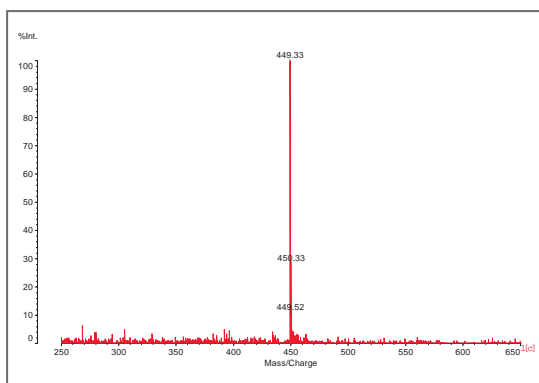
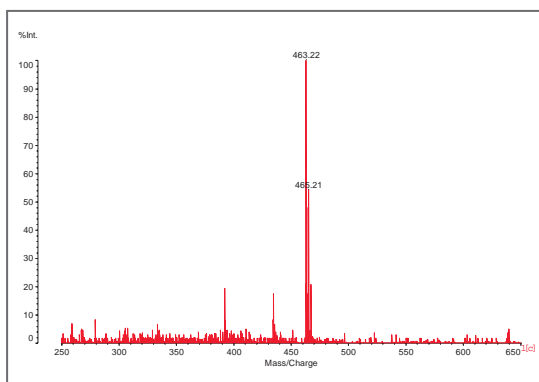


Figure 9. MS spectrum obtained for pharmaceutical sample 9 using a QuickMass™ target under high throughput analysis conditions (expected $[M+H]^+ = 463.04$)



REFERENCES

1. K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida and T. Yoshida, *Rapid Commun. Mass Spectrom.*, 2, (1988), 151-153
2. M. Karas and F. Hillenkamp, *Anal. Chem.*, 60, (1988), 2299-2301
3. Application Note: Analysis of Polymers using Higher Resolution MALDI TOF-MS (Shimadzu Biotech)

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