

AXIMA-QIT™

Rapid Characterization of Small
Molecules using MSⁿ on a MALDI
QIT TOF MS

- Characterization of small molecules < 1000 Da by MALDI QIT TOF MS
- Precursor ion selection resolution of up to 1000
- MS/MS and MSⁿ provide diagnostic ions for compound identification

Rapid Characterization of Small Molecules using MSⁿ on a MALDI QIT TOF MS

Introduction

Traditionally, the metabolic fate of pharmaceutical compounds has been investigated using LC/MS/MS. From a well-characterized drug, the possible compounds resulting from metabolism are postulated and these moieties screened for by MRM (multiple reaction monitoring) using a triple quadrupole mass spectrometer with an API ion source. Here, a specific and characteristic fragmentation pattern is monitored (precursor ion to product ion) confirming the presence of a particular ion. This procedure is complicated by the necessity to separate complex samples by liquid chromatography prior to mass spectral analysis. This additional component is time-consuming and requires expertise and costly solvents and columns.

MALDI mass spectrometry has been considered as unsuitable for the analysis of small molecules (i.e. masses less than 1000 Da) due to the complicating matrix related signals and the potential for fragmentation of the target analyte by in-source or metastable decay. To minimize this possible complication, 2,5-dihydroxybenzoic acid (DHB) is considered the matrix of choice. However, we have recently evaluated a matrixless approach with a series of standards and pharmaceutical compounds. This has proven to provide equivalent data to LC/MS in comparative studies.

We have recently extended this review to include analyses performed on a MALDI QIT TOF MS, a novel mass spectrometer incorporating a vacuum MALDI ion source, a quadrupole ion trap and a time-of-flight analyzer. This instrument is capable of both MS and MSⁿ modes of operation in both positive and negative ion polarities. Here, we present data demonstrating the ability to rapidly analyze small molecules on a MALDI QIT TOF MS and generate useful fragmentation data.

Methods

An Axima-QIT™ (Shimadzu Biotech, Manchester, UK) MALDI QIT TOF MS was used in low mass mode for all experiments. MSⁿ experiments were performed on pharmaceutical compounds and drug metabolites using helium as the quadrupole ion trap buffer gas, where ions are trapped using a patented rapid RF start-up method, cooled and ejected into a floated TOF analyzer equipped with a two stage gridless reflectron. DHB (2,5 dihydroxybenzoic acid, 12 mg/ml in 50:50 acetonitrile / 0.1% TFA) was initially utilized as the MALDI matrix and compared with laser desorption ionization (LDI) where no matrix is employed. These analyses were performed using a standard unmodified stainless steel MALDI target, in addition to novel QuickMass targets (NanoHorizons).

Next, MS/MS was performed on the candidate molecules. Prior to MS/MS analysis, precursor ions were isolated using the filtered noise field (FNF) method which ejects all unwanted ions from the trap. Argon was used as the CID gas, although it is possible to use alternative gases if required via an additional gas inlet line designed to allow flexibility of the collision induced dissociation conditions. The resultant fragments were ejected from the trap following cooling and analyzed in the reflectron TOF analyzer.

Standards were prepared at a final concentration of 0.25 mg/ml in 50:50 acetonitrile : water (v/v). Pharmaceutical compounds and metabolites were prepared at a concentration of approximately 1 mg/ml in 50:50 acetonitrile : water. All samples were spotted directly onto the target using a volume of 0.5 µl and an equal volume of matrix added. In the case of matrixless MALDI (LDI), 0.5 µl of the sample solution was spotted and allowed to air-dry. 100 laser shots were acquired using a 25 point raster for each spectrum. Each analysis was undertaken in approximately 30 seconds.

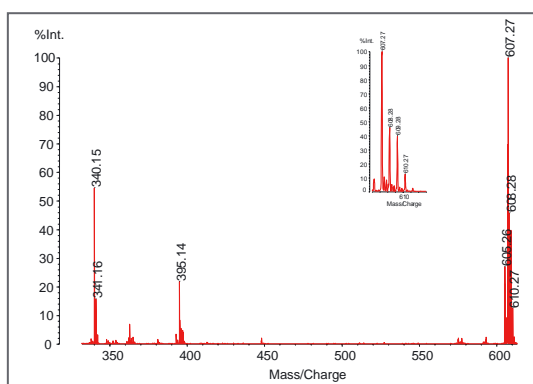


Figure 1. Reserpine and papaverine standard solutions

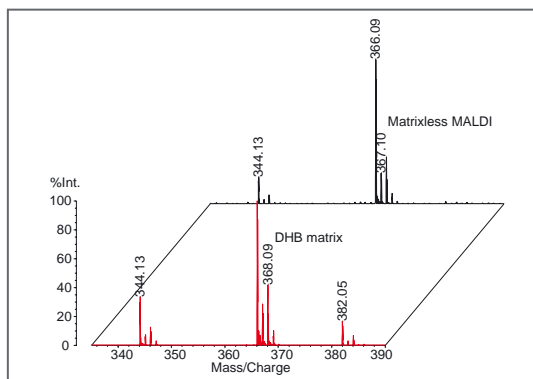


Figure 2. Pharmaceutical compound 1, MS positive ion mode

Results

Initially, a standard sample consisting of reserpine and papaverine was analyzed to evaluate the efficacy of the technique. Both compounds were easily distinguished as intense ions in both the DHB and matrixless LDI analyses, the latter being shown in Figure 1. Similar intensities were observed for both preparations, however, the lower mass range was complicated by matrix related signals in the case of the DHB preparation. Following on, a series of pharmaceutical compounds were analyzed in the same manner using both methods of preparation and examples of typical spectra are shown in Figures 2 and 3. Again, in the majority of cases, either protonated molecular ions or alkali metal adducts (Na^+ or K^+) were observed. Mass accuracy obtained for all analyses in MS mode was found to be < 0.1 Da.

Next, the quadrupole ion trap was utilized to isolate individual species with high precursor ion selection resolution (up to 1000). The filter noise field (FNF) method was used to isolate the reduced reserpine protonated molecular ion at m/z 607. It is possible to isolate ions with different precursor ion selection resolution, stepping from a resolution of 70 to 1000 with intermediate steps of 250 and 500 resolution, permitting selection of a single isotope as demonstrated in Figure 4. The MS/MS spectrum of reduced reserpine is shown in Figure 5. The major product ion observed is at 395 Da, which corresponds to the cleavage position indicated in Figure 6.

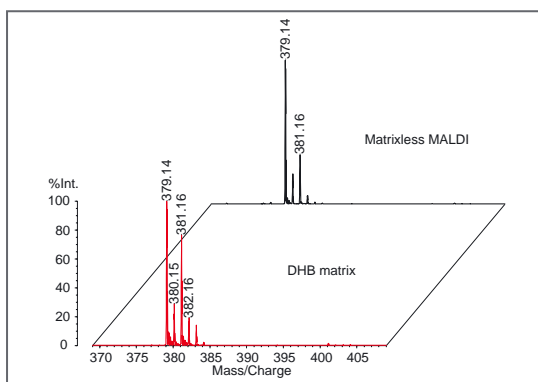


Figure 3. Pharmaceutical compound 2, MS positive ion mode

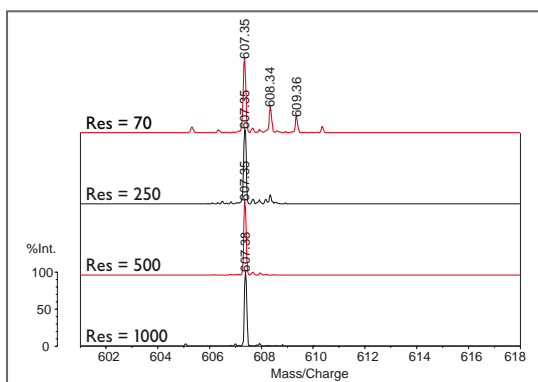


Figure 4. Precursor ion selection resolution

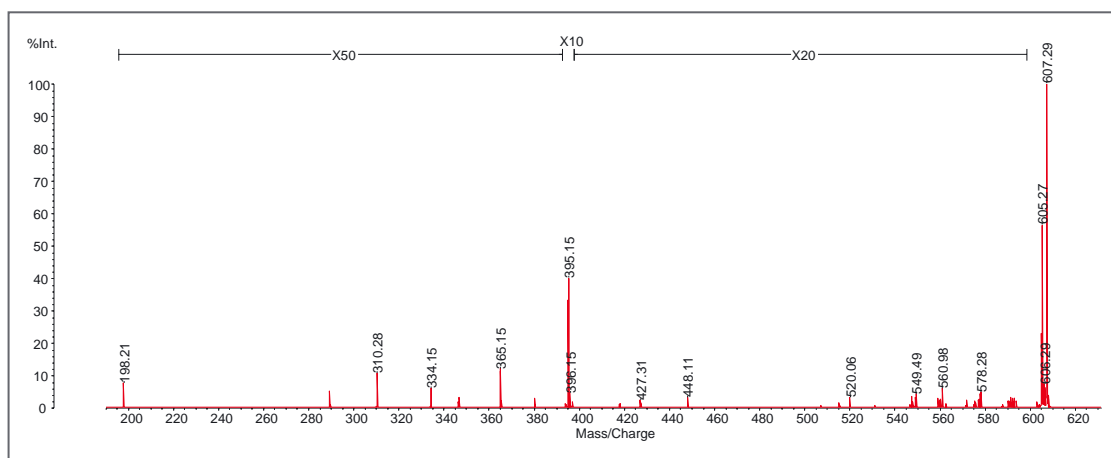


Figure 5. MS/MS of reduced reserpine

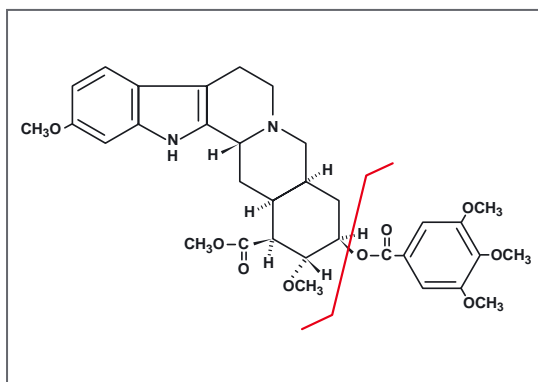


Figure 6. Structure of reserpine indicating cleavage position

A typical pharmaceutical drug metabolism sample was analyzed in positive ion low mass mode. The MS spectrum is shown in Figure 7. The protonated compound is observed at m/z 552. Metabolites of this compound are also observed at m/z 538 (demethylation) and m/z 568 (oxidation).

The “parent” compound and the proposed oxidized compound were both selected for MS/MS, the resultant spectra are displayed in Figures 7B and C. It may be seen that the two spectra contain common ions and ions which vary. For example, the diagnostic ion at m/z 524 appears in the oxidized MS/MS spectrum but not in the “parent” compound MS/MS spectrum, where an ion 16 Da less is observed at m/z 508.

MS³ was also performed on the ion observed in both MS/MS spectra at m/z 201. The spectrum is shown in Figure 7D. It is clear that MALDI MSⁿ is capable of providing valuable structural information for low molecular weight species.

Conclusion

- MALDI is a viable technique for the analysis of small molecules
- Matrixless MALDI for low molecular weight compounds generated less complicated spectra containing less background noise
- MS analyses can provide accurate molecular weights, with mass accuracy of < 0.1 Da
- Signals were obtained corresponding to the expected target mass for a high percentage of samples
- Precursor ion selection resolution of up to 1000 was demonstrated providing extremely specific ion isolation for MS/MS experiments
- MS/MS provided diagnostic ions for compound identification equivalent to those used for MRM scanning on a triple quadrupole system
- MALDI QIT TOF MS was capable of MSⁿ experiments providing useful structural information
- A significant reduction in analysis time (less than 30 seconds per sample) was observed when compared with LC/MS techniques

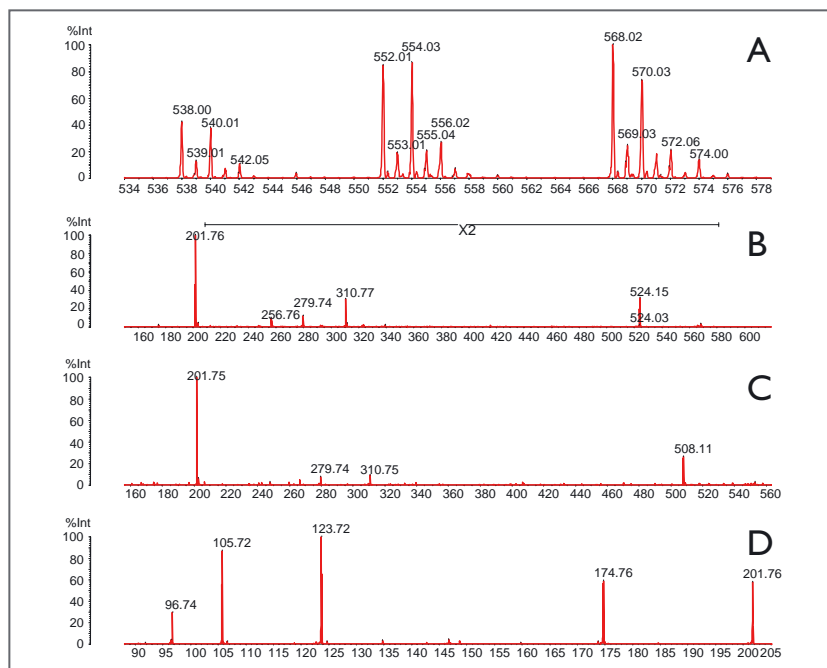


Figure 7. Drug metabolism sample:

A: MS spectrum

B: MS/MS of m/z 568, oxidized compound

C: MS/MS of m/z 552, parent compound

D: MS³ of m/z 201

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