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Multi-stage Mass Spectrometry up to MS^4 on a QTof System

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APPLICATION BENEFITS

This application note illustrates how multi-stage mass spectra up to MS^4 can be generated on a SYNAPT® G2 HDMS® System in a single experiment. This experiment avoids the need to trigger on, isolate, and fragment specific ions, as is done in directed MS^n methods. Moreover, the duty cycle remains constant, differing from the performance of ion-trap instruments, where the duty cycle for each transition decreases as the number of monitored ions/ transitions increases.

WATERS SOLUTION

SYNAPT G2 HDMS System

KEY WORDS

T-Wave™ ion mobility, IMS, MS^3, MS^4, structure elucidation

INTRODUCTION

The integration of ion mobility separations (IMS) with high-resolution tandem mass spectrometry offers multiple and distinct capabilities and advantages. First, as an orthogonal dimension of separation, IMS can enhance selectivity in MS and MS^E data sets.[1-3] Second, IMS can serve as a powerful tool for structure elucidation, as indicated by its ability to characterize positional isomers of metabolites from generated collision cross-section measurements.[4-6] Finally, when combined with tandem-MS, IMS enables multi-stage mass analysis.

Here we describe generating multi-stage mass analyses up to MS^4 using a Waters® SYNAPT G2 High Definition® MS (HDMS) System. Multi-stage mass analysis is defined in this application note as follows: MS^2 = 1st generation product ion spectra, MS^3 = 2nd generation product ion spectra, and MS^4 = 3rd generation product ion spectra. Midazolam N-glucuronide was chosen as the test compound and the resultant spectra compared with those obtained on an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific).

EXPERIMENTAL

Sample description

Midazolam N-glucuronide, purchased from Toronto Research Chemicals, was dissolved in methanol, to a final concentration of 20 ng/µL. The solution was infused directly into the mass spectometer at 10 µL/min via the instrument's internal syringe pump.

MS conditions

MS system:	SYNAPT G2 HDMS
Ionization mode:	ESI+
Capillary voltage:	3.0 kV
Cone voltage:	70 V
Extraction cone:	3 V
Trap collision energy (CE):	35 V
Transfer collision energy (CE):	20 V or 40 V
IM gas:	N_2
Wave velocity:	1100 m/s
Wave height:	40 V
IM gas pressure:	3.11 mbar

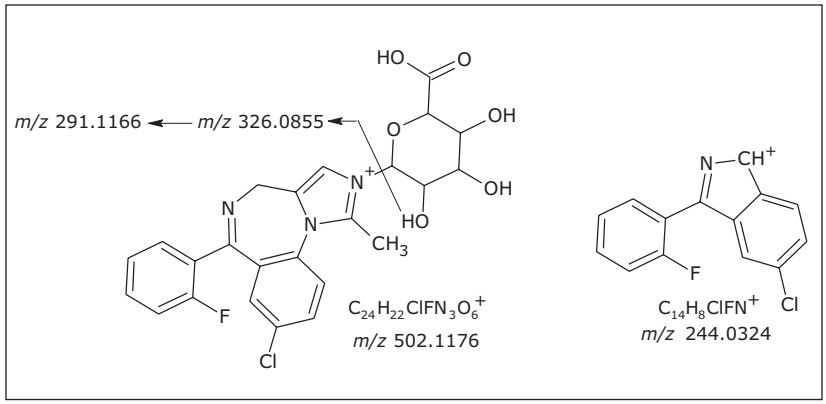

Figure 1. Midazolam N-glucuronide and tentative structure of the main product ions.

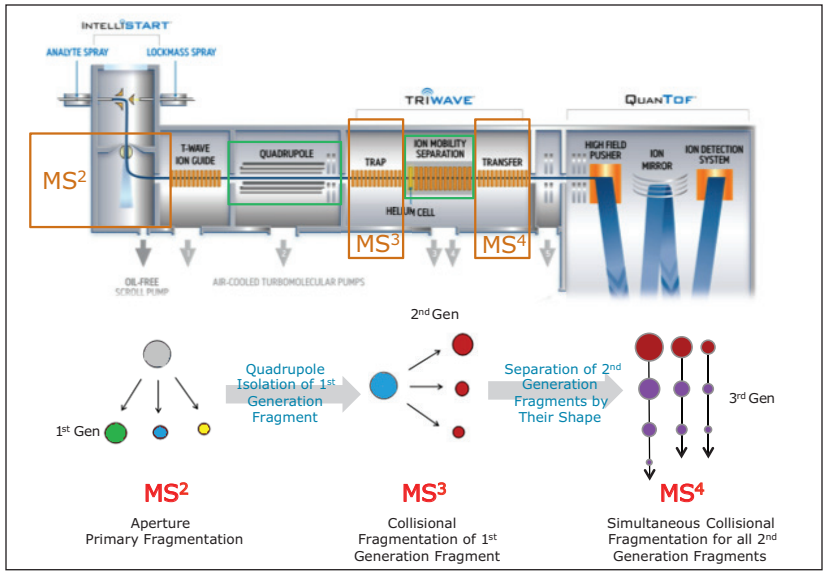

Figure 2. SYNAPT G2 HDMS and generation of MS^3 and MS^4 pathways.

RESULTS AND DISCUSSION

The geometry of the SYNAPT G2 System appears in Figure 2 together with the mechanisms by which multistage MS spectra can be obtained. Briefly, MS^3-like spectra can be generated in two ways. They can be generated by in-source fragmentation, followed by selection of the first-generation product ion in the quadrupole and fragmentation in the trap or transfer collision cell. Alternatively, the spectra can be generated by quadrupole precursor-ion selection, fragmentation in the trap collision cell, separation of the product ions by IMS, and subsequent fragmentation in the transfer region. Combining the latter approach with in-source fragmentation using a higher cone voltage results in MS^4-like spectra.

Figure 3 illustrates all stages of CID fragmentation performed using the SYNAPT G2 HDMS in ESI positive-ion mode. Figure 3A shows the complete mass spectrum of midazolam N-glucuronide (*m/z* 502) at a cone voltage of 40 V. Increasing the cone voltage to 70 V induces in-source dissociation of the N-glucuronide, resulting in the formation of the aglycone at *m/z* 326 (Fig. 3B). Quadrupole selection of this aglycone ion and fragmentation in the trap, or in the transfer, T-Wave collision cells results in an MS^3 spectrum (Fig. 3C). MS^3 like spectra can alternatively be obtained via quadrupole precursor ion selection, fragmentation in the trap collision cell, separation of the product ions by ion mobility separation and subsequent fragmentation in the transfer region (spectra not shown). Combining the latter approach with in-source fragmentation using a higher cone voltage (as applied in this example to produce the aglycone ion) results in MS^4-like spectra (Figs. 3D and E).

Because the MS^4 product ions are generated after the ion mobility separation, the precursor and product ions can be aligned on the basis of their drift time (Fig. 2). Such alignment allows the extraction of MS^4-like spectra from the two-dimensional plot of *m/z* versus drift time or from the ion mobility "drift time" chromatograms (mobilograms).

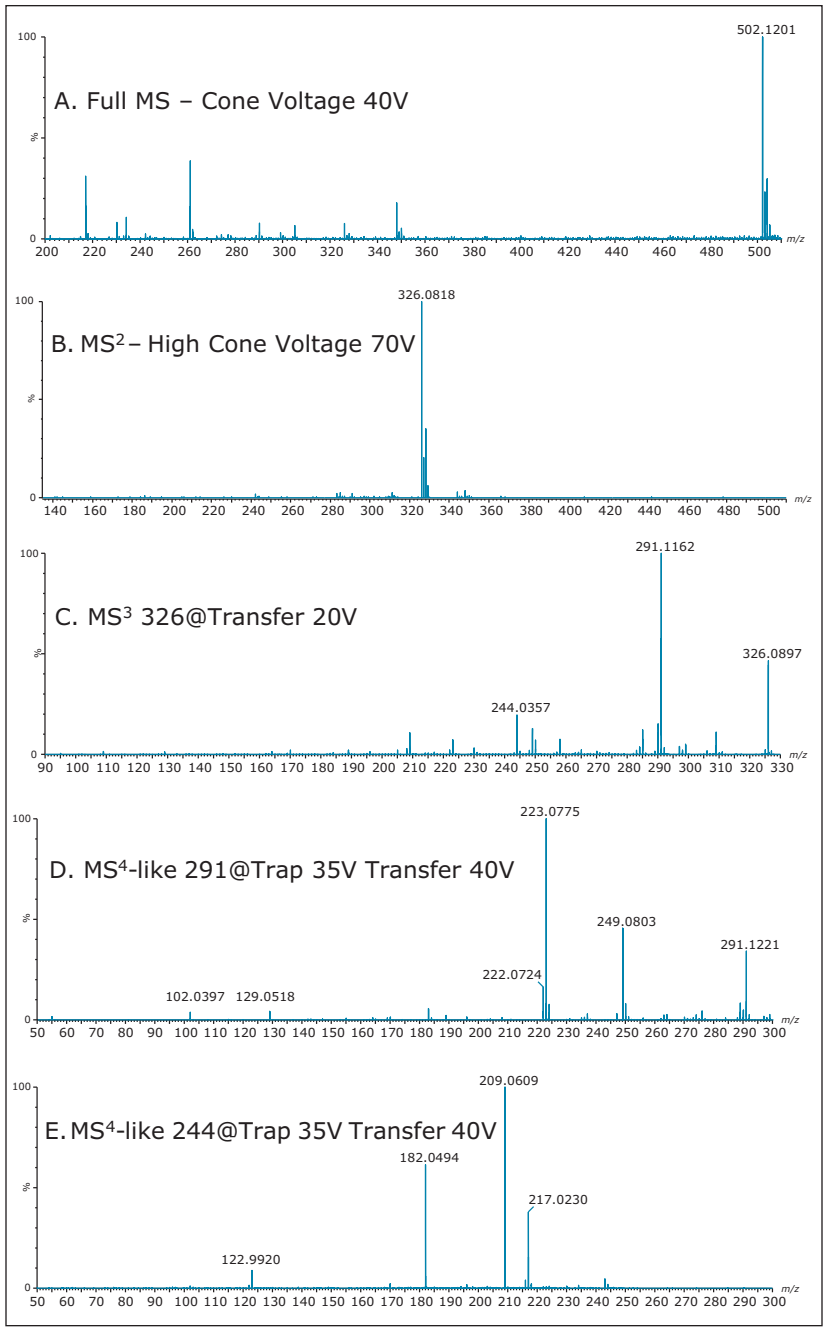

Figure 3. Multi-stage mass spectrometry up to MS^4 on the SYNAPT G2 HDMS.

Mobilograms of the total ion current and main MS^4 precursor ions appear in Figure 4. Spectra can be generated from this plot as they are for conventional LC-MS data. Where the precursor ions are not fully separated in IMS, a partial separation and drift-time alignment of the extracted ion chromatograms can suffice to indicate whether product ions are assignable to a specific precursor ion.

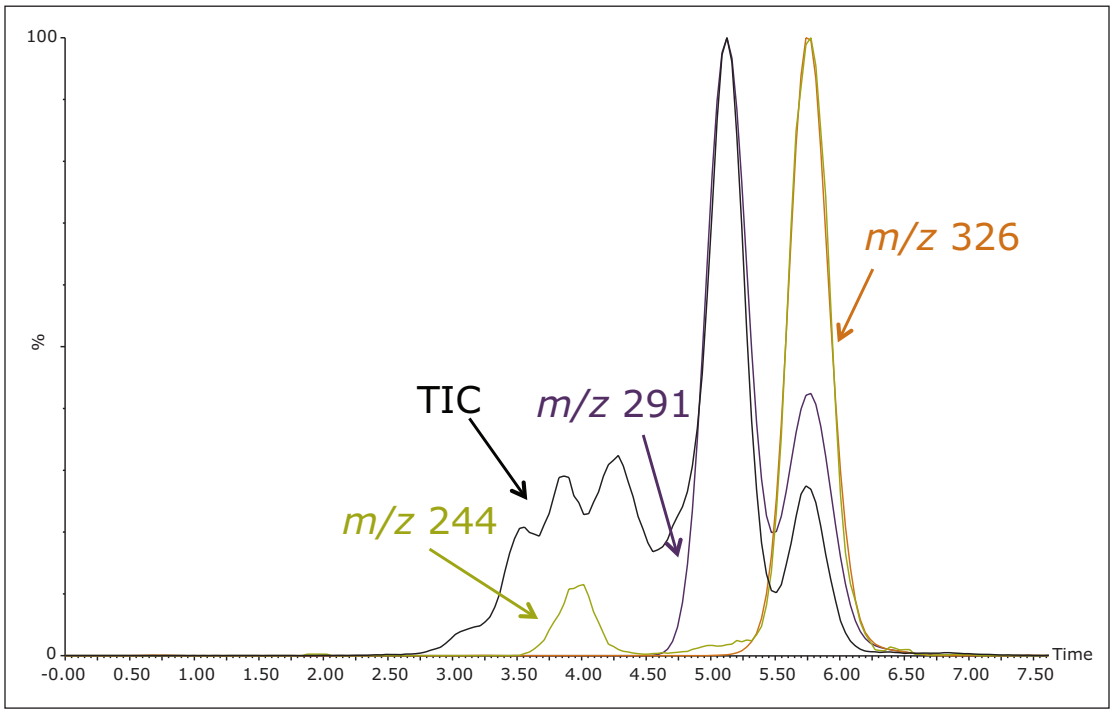

Figure 4. Overlay of the ion mobilograms of the total ion current (TIC) and MS^4 precursor ions 326, 291, and 244.

The mobilograms in Figure 4 demonstrate that at the drift time of the *m/z* 244 precursor ion (3.86 ms), other ions are co-eluting. Therefore, a drift-time alignment of the extracted ion mobilograms at 3.86 ms and subtraction of the background was applied, producing the MS^4 spectrum of *m/z* 244 on the Synapt G2 HDMS. Figure 5 shows corresponding midazolam N-glucuronide spectra obtained on an LTQ-Orbitrap mass spectrometer using standard precursor-ion selection. A comparison of the two data sets (Figs. 3 and 5) shows the spectra to be generally comparable.

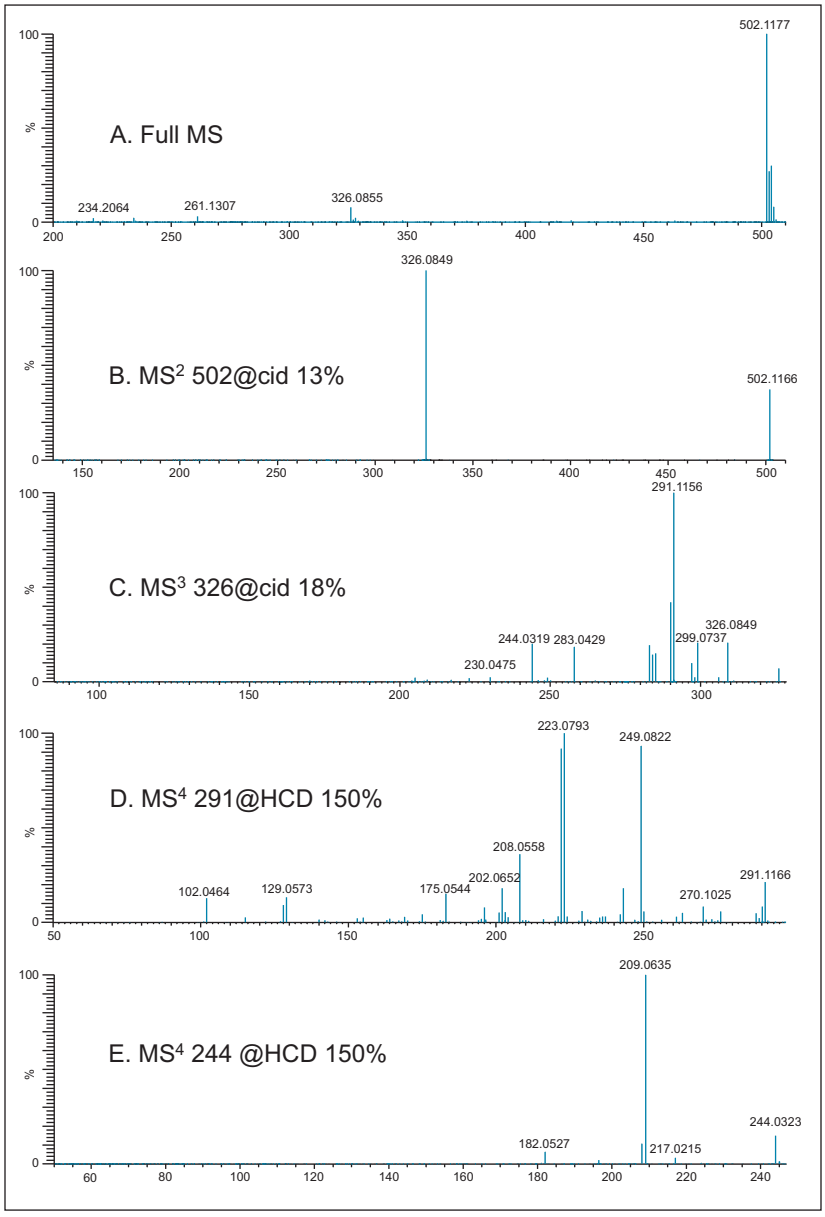

Figure 5. Multi-stage mass spectrometry up to MS^4, as performed on the LTQ-Orbitrap.

CONCLUSIONS

This example demonstrates the application of multi-stage mass spectrometry up to MS^4 on a SYNAPT G2 HDMS System using quadrupole selection in combination with ion mobility separation.

The interpretation of the MS^n spectra obtained via ion mobility separation is assisted by using the drift-time relationship between an ion and its downstream fragments.

The product ion spectra obtained were comparable to those obtained on an LTQ-Orbitrap.

The demonstrated approach generates, in one step, product ion spectra and ion trees in a generic manner using a fast scan speed, which is compatible with modern chromatographic approaches.

References

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