응용 자료

The Simultaneous Acquisition of Exact Mass Data Using Alternating Collision Cell Energy: MS^E

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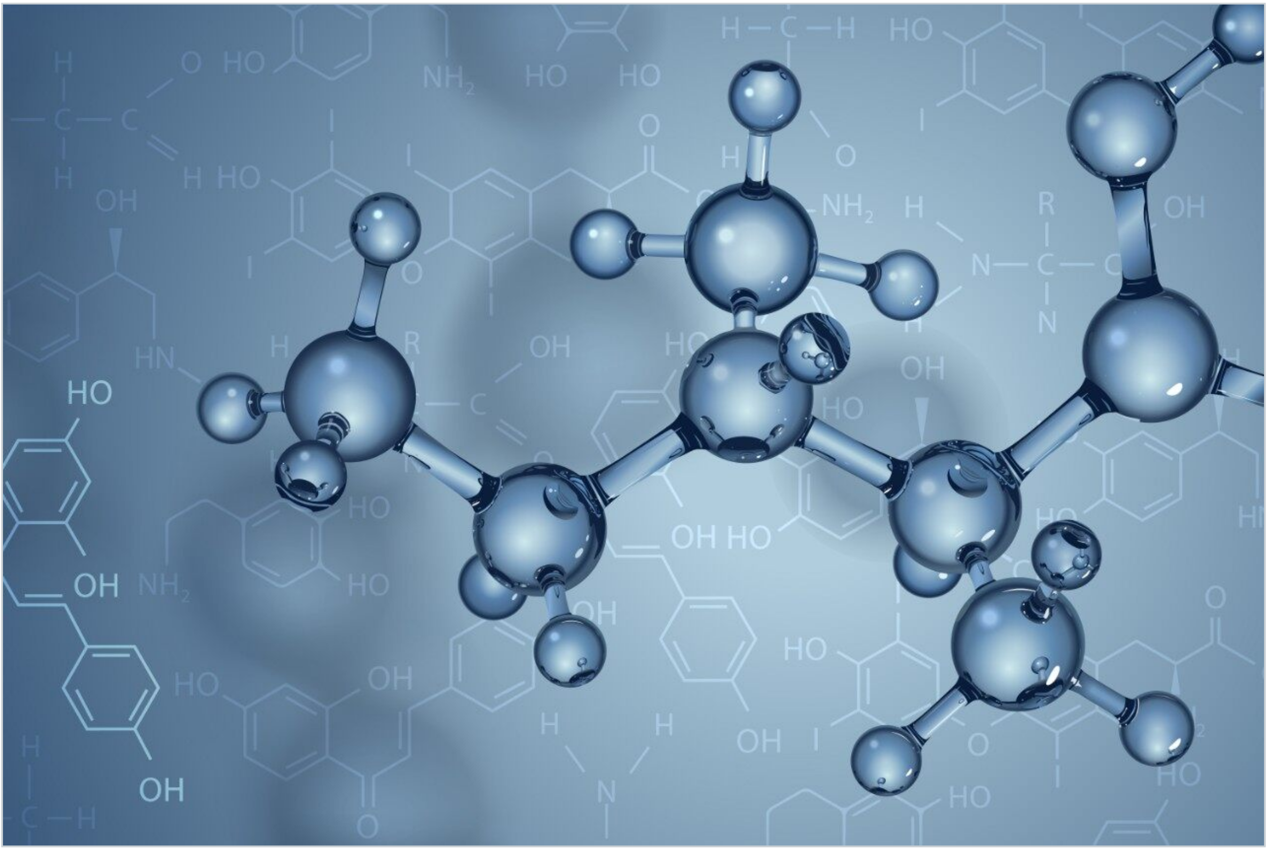

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the simultaneous acquisition of exact mass data using alternating collision cell energy.

Introduction

The characterization and identification of small molecules in the pharmaceutical industry relies heavily upon mass spectrometry. This is due to instrument sensitivity, ease of operation and interfacing with chromatographic techniques such as gas chromatography and liquid chromatography. Modern mass spectrometers generate data in many different ways, including full scan data to obtain precursor ion information, single ion recording (SIR), multiple reaction monitoring (MRM) for high-sensitivity quantitative analysis, and fragment ion analysis using MS/MS.

Structural analysis usually involves a two-stage process; full scan analysis to determine the molecular ion of the analyte in question, followed by fragment ion generation of this analyte using MS/MS. MS/MS experiments are performed on a tandem quadrupole, ion trap, or tandem hybrid quadrupole time-of-flight (Tof) mass spectrometer. During fragment ion generation with a tandem instrument, the first quadrupole is fixed allowing only ions of interest to pass, and the ions are fragmented in the collision cell. The resulting fragments are separated and detected in either the first Tof region or second resolving quadrupole region of the mass spectrometer.

An orthogonal acceleration or oa-Tof instrument has the added benefit of producing elemental composition information on the precursor and fragment ions. Together with the precursor elemental composition data, this is used to deduce the analyte's structural characteristics. In a traditional mode of analysis, acquiring both precursor and production information requires two analytical runs – the first to obtain precursor ion information, and the second to obtain the fragment ion data. This is both time and sample consuming.

An alternative approach to acquire MS and MS/MS is to use data-directed analysis (DDA). In this mode of operation, the MS switches from full scan MS to MS/MS mode when a specific base-peak in the full-scan MS experiment exceeds a set threshold. This typically takes several seconds per peak to acquire the data and has been used successfully to support proteomics studies.

The Waters ACQUITY UltraPerformance LC (UPLC) System has redefined LC-MS, combining the use of porous sub-2 μm particles at pressures in excess of 12,000 psi with elevated mobile linear velocities to generate high peak capacity separations with very sharp peaks 1 to 3 seconds in width. UPLC requires a new approach to MS detection to maximize productivity and minimize sample usage.

The Waters Q-Tof Premier Mass Spectrometer is the latest generation of the revolutionary hybrid quadrupole time-of-flight MS family (Figure 1). This MS is capable of rapid data acquisition at greater than 10 spectra/second – more than adequate for the narrow peaks produced with UPLC-MS. Complementing this capability, the Q-Tof Premier can also perform simultaneous UPLC-MS and UPLC-MS/MS data capture (MS^{E}). MS^{E} is achieved by rapidly switching between a low-energy setting and a high-energy setting (every 150 milliseconds) in the collision cell. In this mode, precursor ions are generated and detected as before in the low-energy regime, while fragment ions are produced in the high-energy function.

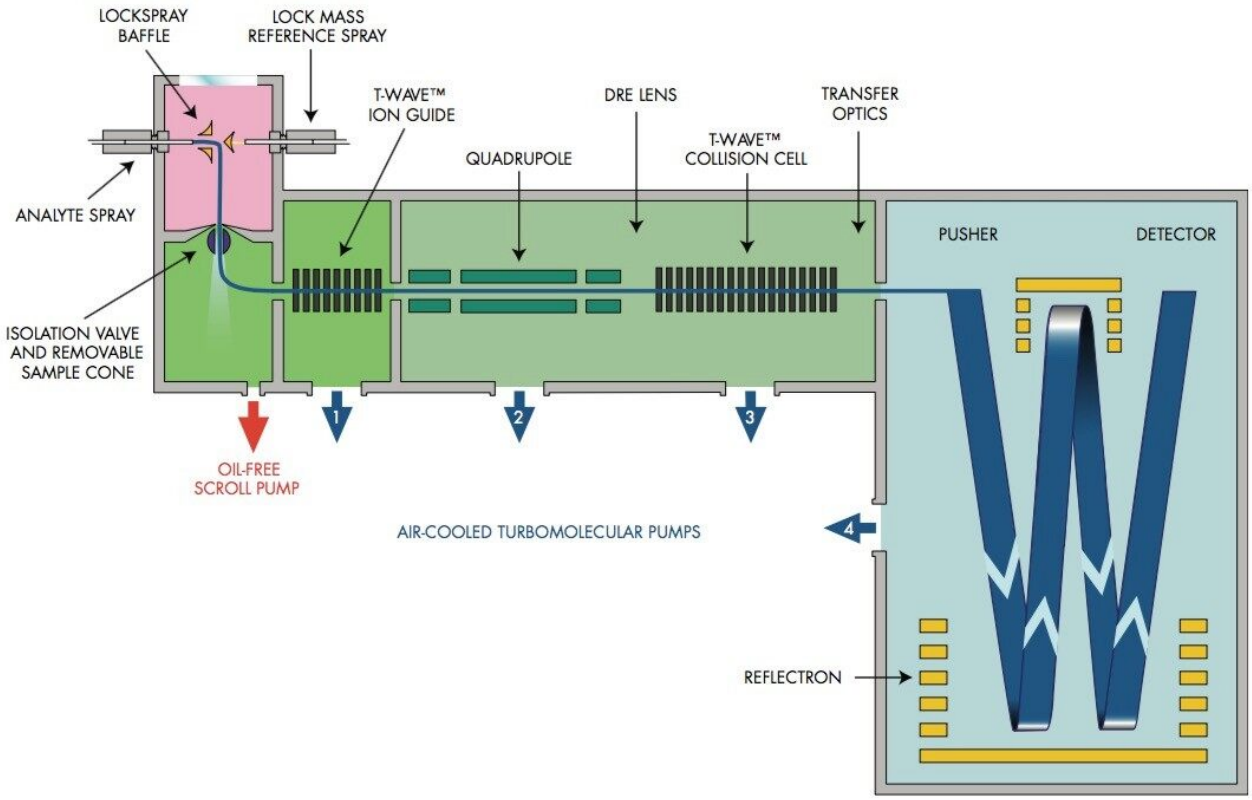

Figure 1. Waters Q-Tof Premier Mass Spectrometer schematic.

MS^{E} has four major benefits over conventional MS/MS or DDA analysis:

1. No need to perform a second analysis to generate MS/MS data

2. Both precursor and product ion data are acquired under exact mass conditions
3. Data is acquired at a sufficiently fast rate to accommodate narrow UPLC peaks
4. No need for prior knowledge of the ions to be fragmented

Experimental

UPLC Conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm, 1.7 µm
Flow rate:	500 µL/min
Injection volume:	5 µL
Gradient:	0 to 95% B, 0 to 9 min, curve 4, where A = 0.1% aqueous formic acid, and formic acid
Column temp:	40 °C

MS Conditions

MS system:	Waters Q-Tof Premier Mass Spectrometer
Ionization:	Positive ion ESI
Range:	80 to 800 *m/z*, centroid mode
Acquisition rate:	0.1 sec, inter-scan 0.05 sec
Collision energy 1:	10 eV

Collision energy 2:	25 eV
Capillary voltage:	3 kV
Cone voltage:	70 V
Cone gas:	0 L/hour
Source temp.:	120 °C
Desolvation gas:	400 L/hour
Desolvation temp.:	300 °C

Results and Discussion

Chromatography

One of the major advantages of MS^E acquisition is the ability to simultaneous acquire precursor and product ion information in one analytical run. During acquisition, it is critical that the mass spectrometer acquires data at a sufficient rate to maintain data fidelity across the chromatographic peaks.

The data displayed in Figure 2 shows the low-collision energy and high-collision energy data of control rat urine. We can see that there is both a qualitative and quantitative difference in the UPLC-MS chromatograms, demonstrating the difference in the information obtained from the low- and high-collision energy data. Also, the chromatographic definition is maintained across the entire chromatogram. This is ideal for the acquisition of high-quality accurate mass measurements and for semi-quantitative tasks such as impurity and metabolite identification.

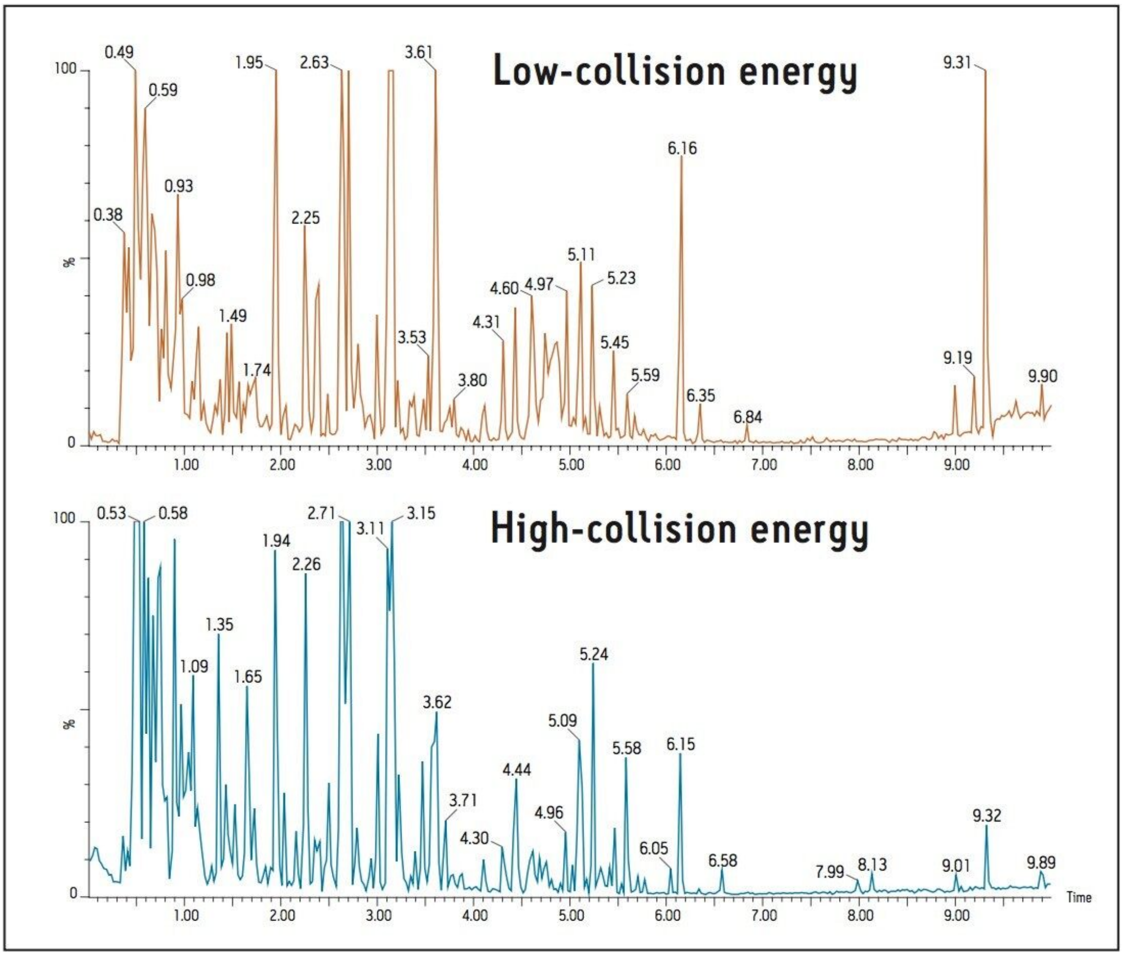

Figure 2. UPLC/oa-Tof TIC chromatogram.

The spectral data obtained from the MS^E experiment for the peak eluting with a retention time of 2.25 minutes is shown in Figure 3.

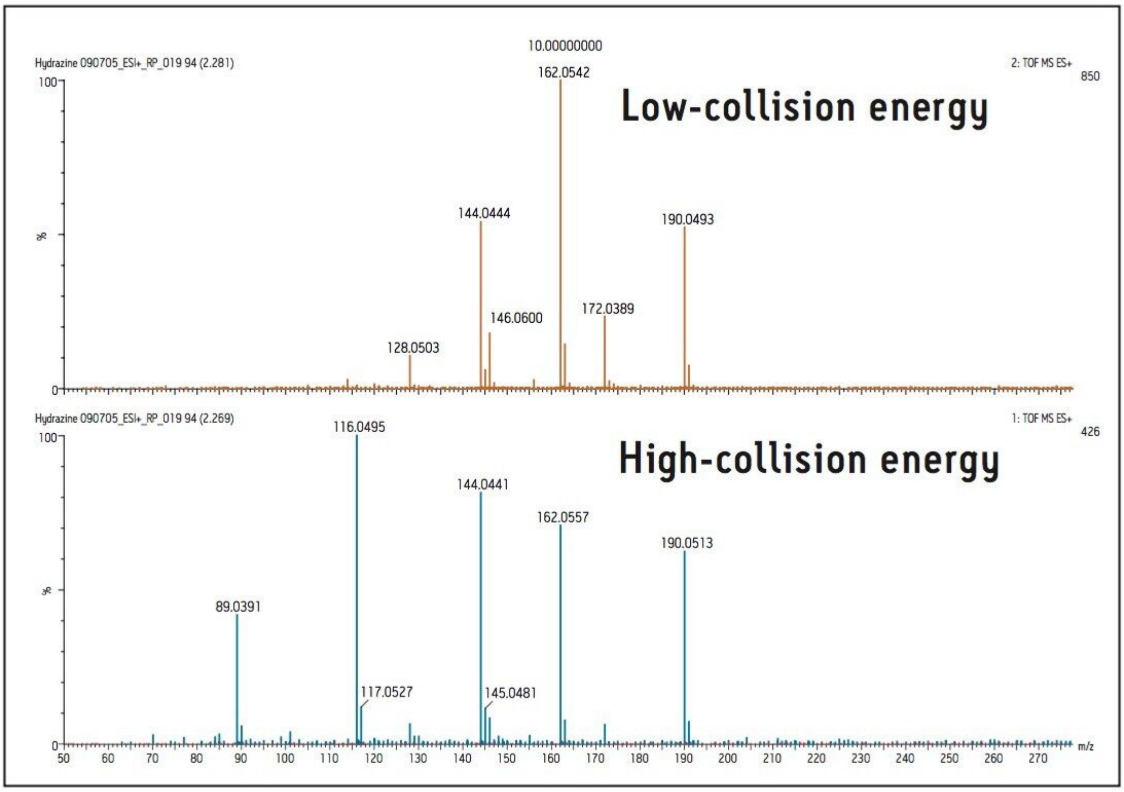

Figure 3. High-low collision energy of kynurenic acid.

Kynurenic acid

This peak at 2.25 minutes is known to be kynurenic acid, part of the lysine degradation pathway and effected by hepatic toxins such as hydrazine. This results in an increase in 2-amino-adipate, as hydrazine blocks the transamination of 2-amini-adipate to 2-oxoadipate. The low-collision energy data produced a base peak with a mass of 190.0508, for an elemental composition of $C_{10}H_8NO_3$ and a mass accuracy of 2.1 ppm. The high-collision energy data generated the ions *m/z* 162.0506 (consistent with the loss of CH_2O) and *m/z* 144.0397 (corresponding to the further loss of H_2O).

Xanthuric acid

The capability of simultaneously generating precursor and product ion information is shown by the analysis of the authentic standard of xanthuric acid (Figure 4) using MS^E. The low-collision energy data produced a precursor ion *m/z* 206.0451. The high-collision energy data generated fragments of *m/z* 188.0355, 178.0505 and 160.0392.

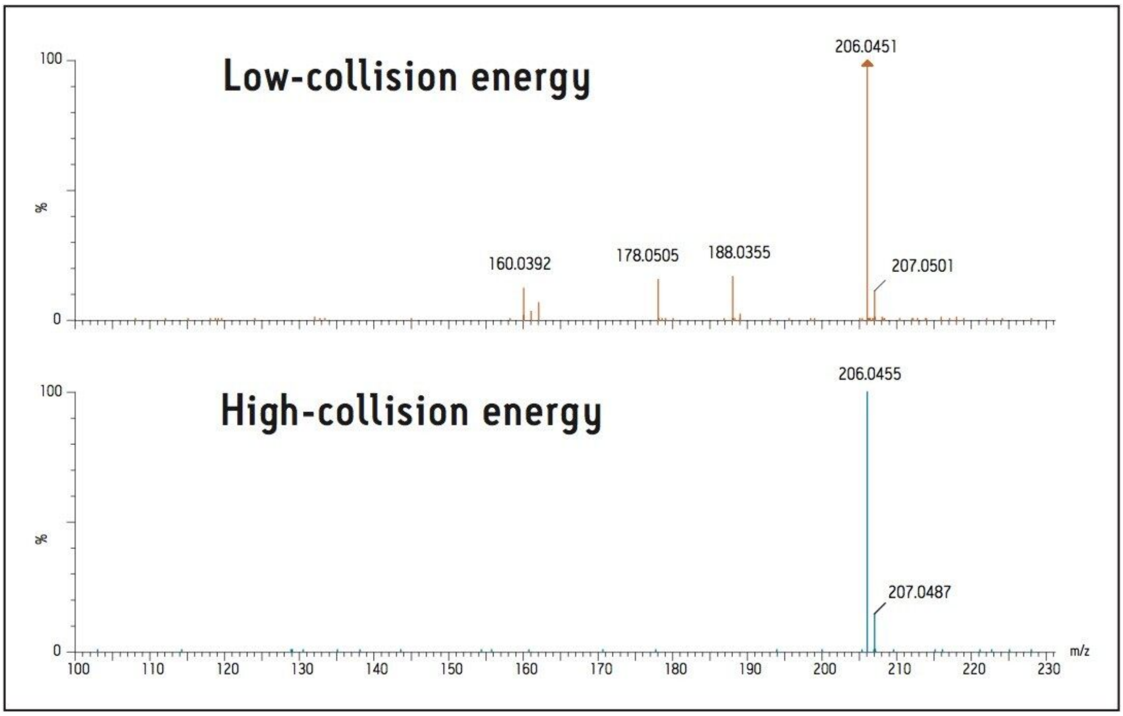

Figure 4. High-low collision energy of xanthuric acid.

The possibility of ion cross-talk is negated with the Q-Tof Premier's T-Wave collision cell. Unlike a conventional quadrupole collision cell, T-Wave utilizes a series of charged plates and a superimposed RF signal to transport the ions through the collision cell. This allows for the fast clearance of the collision cell, facilitating rapid collision energy switching without the worry of ions carrying over from one measurement to the next.

Conclusion

The Q-Tof Premier Mass Spectrometer has been specifically designed to complement the ACQUITY UPLC System. The unique design of the Q-Tof Premier allows MS data to be captured with alternating low- and high-collision cell energy. This MS^E approach, when used to acquire precursor and product ion information, has the additional benefits of obtaining both types of data one analytical run. Unlike conventional MS/MS, both the precursor and product ion data are acquired in accurate mass mode so that elemental composition information to be generated from both sets of data.

A further advantage of MS^E is that neutral loss information from a comparison of the two alternating collision energy scans can be obtained, eliminating the need for any further experimentation.

This novel mode of operation removes the need for time-consuming re-analysis to obtain both MS and MS/MS data. MS^E maximizes the productivity of the UPLC-MS instrumentation platform by significantly increasing sample throughput.

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720001597, February 2007