AN OVERVIEW OF THE PRINCIPLES OF MS^E, THE ENGINE THAT DRIVES MS PERFORMANCE

MS^E is the only method of data acquisition that allows you to:

- Get exact-mass precursor and fragment ion spectra from every detectable component in your samples
- Identify and quantify in a single analysis
- Access the full benefits of UltraPerformance LC® and mass spectrometry in a single experiment
- Use simple, generic methods

INTRODUCTION

When your organization relies on your ability to deliver key information to tight deadlines, what are the consequences of using an analytical method that gives you incomplete data?

Research goals or company profitability can hinge on your capacity to solve complex scientific problems quickly, communicate the results effectively, and take critical decisions confidently. The key to delivering all the information you need to make this possible is an analytical method that acquires all the data you need, all the time.

The key requirements of a comprehensive quantitative and qualitative analytical workflow are:

1. **Selectivity in the analytical method.** Selectivity is obtained by a combination of efficient chromatographic separation and high resolution mass spectrometry. UltraPerformance LC delivers the highest levels of chromatographic separation and produces very narrow chromatographic peaks. In order to maintain high analytical selectivity, this requires the mass spectrometer to acquire data extremely rapidly without compromising its resolving power.

2. **High performance full-scan MS detection.** High sensitivity and in-spectrum dynamic range is required from the mass spectrometer, so that every detectable sample component, regardless of concentration, is recorded with the correct isotopic pattern and exact-mass. This allows automated software to propose a shortlist of identities for the molecules of interest.

3. **Comprehensive product ion spectra.** To determine molecular structures, exact-mass fragmentation spectra are needed for every detectable sample component.

4. **Quantitative accuracy.** The mass spectrometer must deliver a wide linear dynamic range to correctly determine analyte concentrations in the sample. In addition it must provide consistent, fast data acquisition rates to allow reproducible, accurate, and precise quantitative results.

5. **Simple methodology.** Methods used must be rapidly implemented and deliver high quality results consistently.

6. **Rapid delivery of meaningful information.** Intelligent informatics is needed to interrogate data and provide results quickly and confidently.

Traditional approaches require the use of multiple analytical methods and often more than one type of mass spectrometer. The costs and inefficiencies of such an approach can limit the productivity of a busy laboratory.

Waters' MS^E is a simple, generic method that enables you to meet these requirements in a single analysis.[1,2]

THE THREE STAGES OF MS^E

There are three distinct steps in the MS^E process.

Stage 1: Separation

The more complex the sample the greater the selectivity required from the analytical system. Chromatographic resolution is an essential component of selectivity and with Waters' Universal Ion Source Architecture, state of the art UltraPerformance LC[3] or Capillary GC[4,5] can be employed to separate sample components with a high degree of efficiency. This allows scientists to maximize the selectivity, sensitivity and speed of their analytical methods. Figure 1 demonstrates the improvements in chromatographic resolution (selectivity), peak height (sensitivity), and run time (speed) of UltraPerformance LC over HPLC methods.

Waters® SYNAPT® High Definition MS™ Systems also allow ion mobility separation to be employed as part of the MS^E method, either independently or nested within chromatographic separation.[6,7]

MS^E terminology is determined by the separation techniques employed, so that terms such as UPLC®/MS^E, GC/MS^E, and HDMS^E refer to the use of UltraPerformance LC, capillary gas chromatography, or ion mobility respectively as part of the experiment.

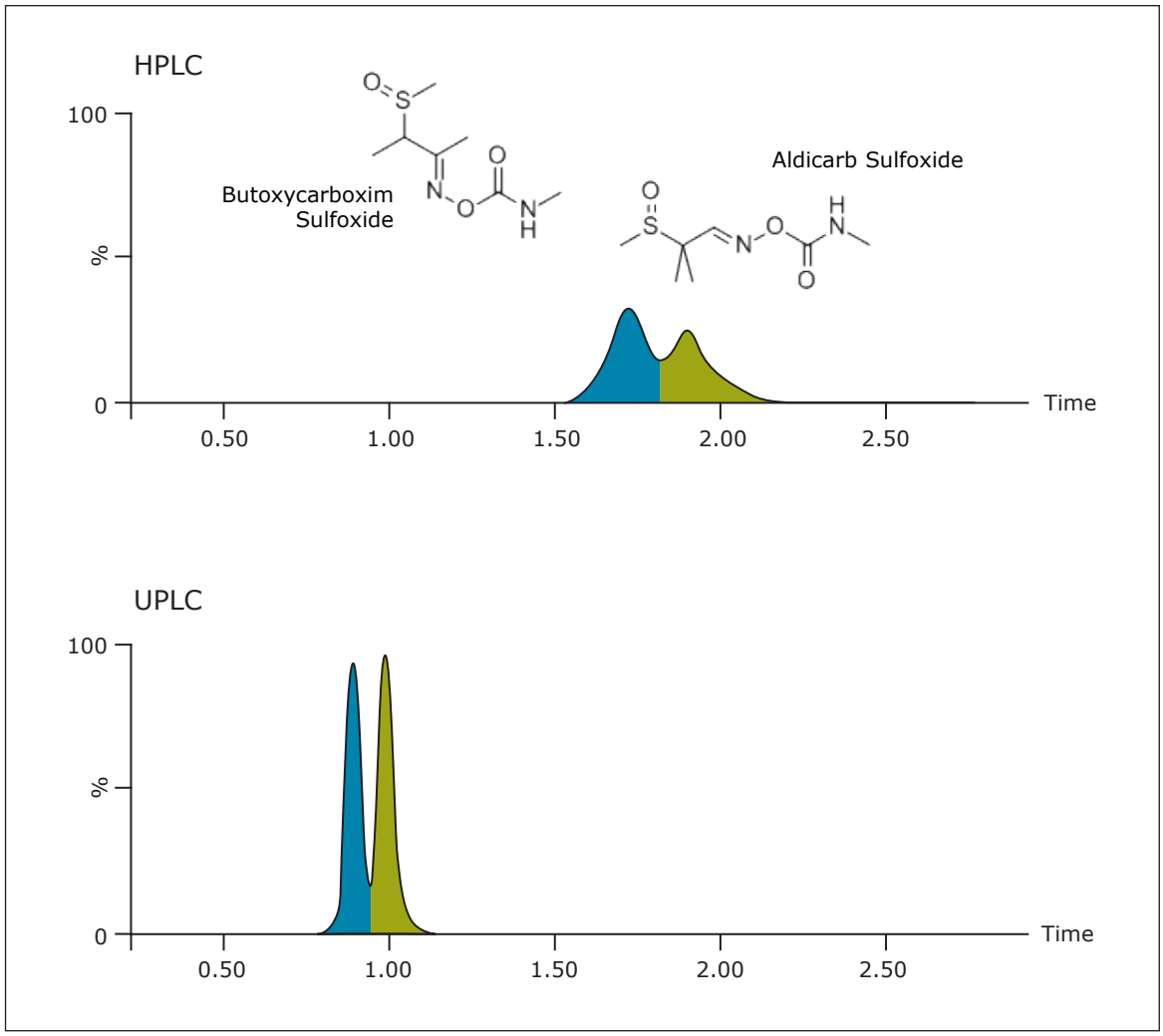

Figure 1. The upper chromatogram shows the two isomeric compounds Aldicarb Sulfoxide and Butoxycarboxim Sulfoxide separated by HPLC chromatography. The lower chromatogram shows these compounds separated by Waters UltraPerformance LC, demonstrating clear improvements in sensitivity, selectivity, and speed of analysis.

Stage 2: Generation of a complete MS dataset

After separation, the sample component peaks arrive at the mass analyzer in very narrow time windows so the mass spectrometer must be able to generate spectra very quickly while maintaining high spectral resolution, sensitivity, exact-mass measurement and in-spectrum dynamic range. Waters QuanTof™ Technology enables the mass spectrometer to meet these challenges successfully.[8]

The identities of the peaks of interest are not known at the beginning of the experiment so, in order to avoid missing key information, data is required on every detectable sample component. In order to eliminate the need for a second or third analysis, both precursor and fragment ions must be generated by the mass spectrometer simultaneously while securing enough data points across each component peak to ensure correct peak integration and quantitative accuracy. This is achieved by the MS^E method, the mechanics of which are described in Figure 2.

With few parameters to adjust, MS^E is simple to set up, requires a minimum of method development to implement, and needs no prior knowledge of the sample components. No pre-selection of ions occurs prior to fragmentation so no data is lost. The instrument literally records "all the data all the time".

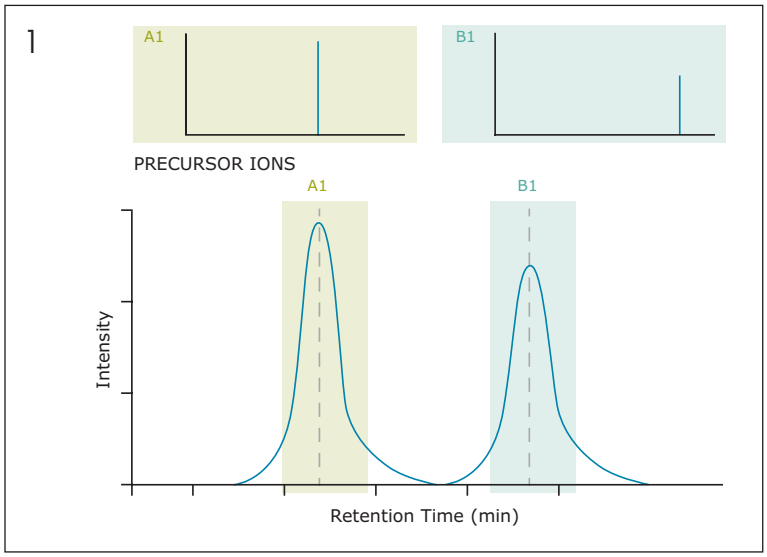

Figure 2. The Tof mass spectrometer rapidly and continuously cycles between two states.

*In state 1, all the ions are transmitted from the ion source, through the collision cell (**low collision energy so that no fragmentation occurs**) to the mass analyzer and recorded as a precursor ion spectrum.*

*In state 2, all the ions are transmitted from the ion source, through the collision cell (**ramped collision energy to generate maximum information from fragment ions**) to the mass analyzer and recorded as a fragment ion spectrum.*

Stage 3: Alignment of spectra and data interpretation

Fragment ion spectra are assigned to their associated precursor ion peaks so that all the information necessary to identify each compound of interest is collated and readily available. This is accomplished with advanced software algorithms that profile each chromatographic peak and determine their retention times. Precursor and fragment spectra are then aligned according to retention times and linked together.[9] This is illustrated graphically in Figure 3.

Baseline chromatographic resolution is not required as the software is able to separate spectra belonging to co-eluting peaks. Figure 4 indicates how clean spectra are generated from co-eluting peaks.

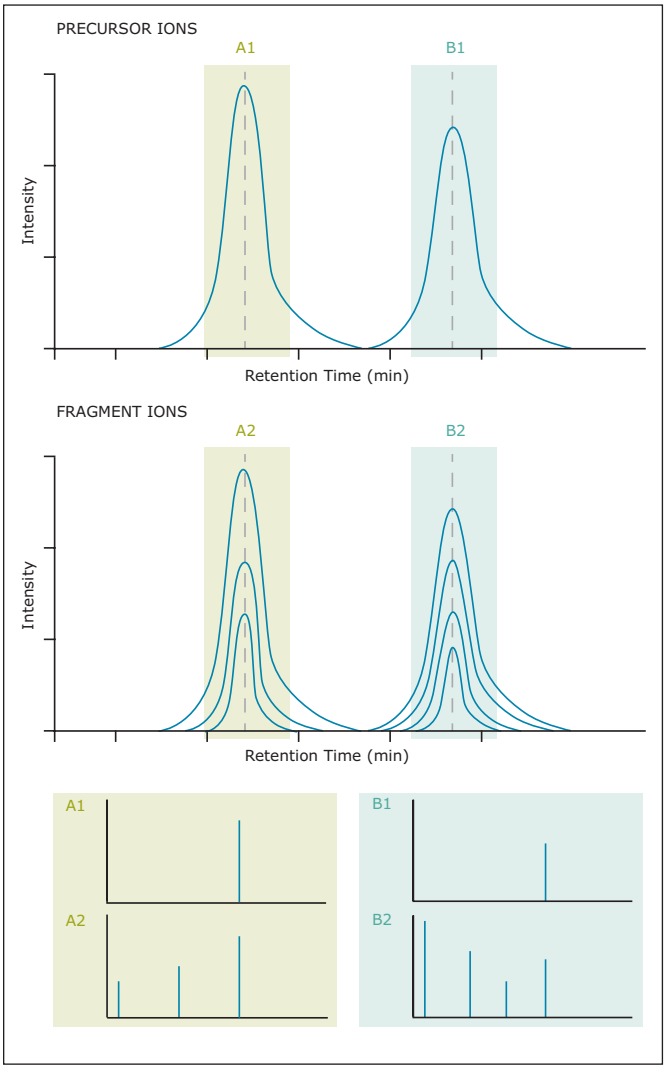

Figure 3. Software algorithms deconvolute the data into separate spectra. The precursor ion spectrum of each component is then aligned with its corresponding fragment ion spectrum by retention time.

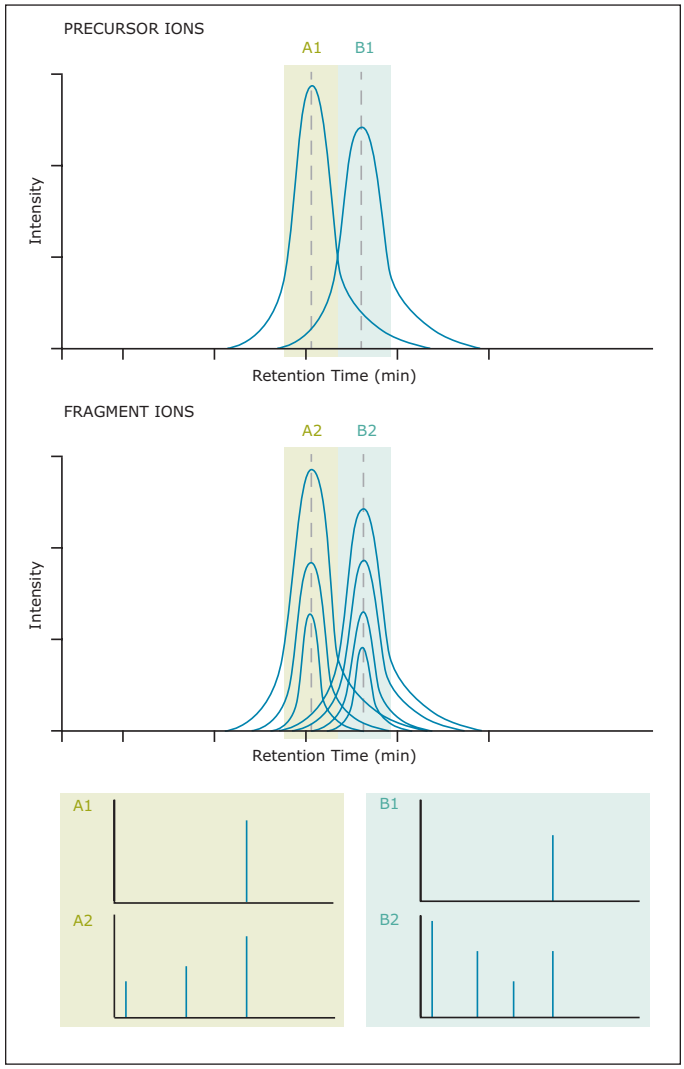

Figure 4. Even when chromatographic peaks co-elute, the deconvolution algorithms are able to align the spectra by retention time and produce separate spectra for each component.

Once this comprehensive dataset is generated it must be interrogated to determine the presence and concentrations of relevant molecules. This data is stored as a complete digital record of the sample and is available for re-interrogation without the need to reanalyze the sample.

Waters informatics takes advantage of the MS^E technique to deliver productivity benefits in areas as diverse as biopharmaceutical characterization, metabolite identification, metabolomics/ lipidomics profiling, impurity identification, forensic toxicology, food testing, environmental analysis, chemical materials characterization, and proteomics studies.[10-15]

CONCLUSIONS

A single analytical technique that provides "all the data all the time" is capable of significantly increasing the productivity of analytical laboratories. Essential components of such a technique include:

- The ability to separate the components of a complex sample as efficiently as possible, with capillary GC, UltraPerformance LC, or ion mobility separation techniques
- Waters QuanTof Technology, which produces data of the highest quality rapidly enough to keep pace with the fastest, most efficient chromatographic separations
- The MS^E method of data acquisition that records exact-mass precursor and fragment ion information while simultaneously obtaining accurate quantitative profiles from every detectable component in the sample
- The simplicity of MS^E, which allows rapid method development with reproducible results
- Software that intelligently interrogates the comprehensive MS^E dataset for quantitative and qualitative information and presents it for review in a simple, intuitive manner

MS^E is central to the implementation of comprehensive quantitative and qualitative analytical workflows and has been fundamental in increasing the productivity of organizations that rely on efficient and effective laboratory services to drive their success. To learn more visit **www.waters.com/mse**.

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