

Separation without hyphenation using two-dimensional mass spectrometry (2DMS)



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Introduction

In most laboratory applications, mass spectrometers are coupled to chromatography systems that separate the compounds in a sample so that they may be analysed one at a time. Liquid chromatography (LC), gas chromatography (GC) and ion chromatography (IC) are the most common separation techniques to be coupled with mass spectrometry (MS), giving rise to the so-called hyphenated techniques of LC-MS, GC-MS, and IC-MS. However, method development in chromatography is a skilled art, and an unwelcome bottleneck in the analytical workflow. Even in the hands of an experienced operator, separation of all the components in a complex sample is highly unlikely, resulting in co-elution of analytes.

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Tandem mass spectrometers (or MS/MS instruments) are capable of fragmenting precursor ions and mass-analysing the resulting product ions. This is a powerful route to determining both the structure and identity of an analyte. However, it is difficult to analyse mixtures of analytes; the task of determining structure and identity by assigning fragments to a precursor quickly becomes intractable if more than one analyte is present. The problem of co-eluting analytes is overcome in a tandem mass spectrometer by using a quadrupole filter to selectively transmit only one precursor. In a targeted analysis, the analytes of interest are known, and precursor selection may be programmed in advance. For untargeted analysis, more complex methods must be employed. Data dependent analysis (DDA) refers to techniques that dynamically select precursors based on their peak intensities, while data independent analysis (DIA) is an umbrella term for methods that attempt unbiased analysis of all the components in a mixture.

Two-dimensional mass spectrometry (2DMS) is a DIA technique that is unfamiliar to most (even in the field of MS) but has the potential to become a routine and important analytical tool. Its current obscurity is a consequence of its origins in the field of Fourier Transform Ion Cyclotron (FTICR) MS, a highly specialist technique, largely confined to academia and high-end industrial applications. In this note, we show that 2DMS can now be performed using Quadrupole Time-of-Flight (QToF) instruments. These may be found in many analytical laboratories and are widely used for routine high-resolution analyses. Crucially, 2DMS does not require chromatography – the sample mixture is injected by direct infusion. The task of separating out the signals due to all the precursors and their corresponding fragments is performed by the mass spectrometer using a novel Fourier Transform technique.

Interpretation of a 2D mass spectrum

For those new to the technique, a 2D mass spectrum can be confusing, largely as a result of previous exposure to 2D contour plots from LC-MS and other hyphenated techniques. The output from 2DMS can also be displayed as a contour plot (or 3D equivalent) but the orthogonal axes are both m/z axes – one corresponding to the precursor and the other to the fragments. When LC-MS data is displayed in this way, only one axis is m/z , the other is retention time,

and the problem of co-eluting analytes remains.

Correlation is a fundamental concept in 2DMS. There are strong parallels with 2D NMR, a technique that assists structural determination by exposing correlations between spin-spin coupled or nearby nuclei. A 2D mass spectrum reveals correlations between fragments and precursors that allow the structure and identities of all the precursors present to be determined. Figure 1 shows an idealised 2D mass spectrum of five compounds. There is a diagonal line corresponding to precursor ion m/z = product ion m/z . This is called the autocorrelation line. Peaks found on this line represent ions that are unfragmented and are therefore precursors, by definition. Off-diagonal peaks correspond to product ions. All the product ion peaks appearing on the same horizontal line are correlated with the precursor ion peak at the intercept with the autocorrelation line.

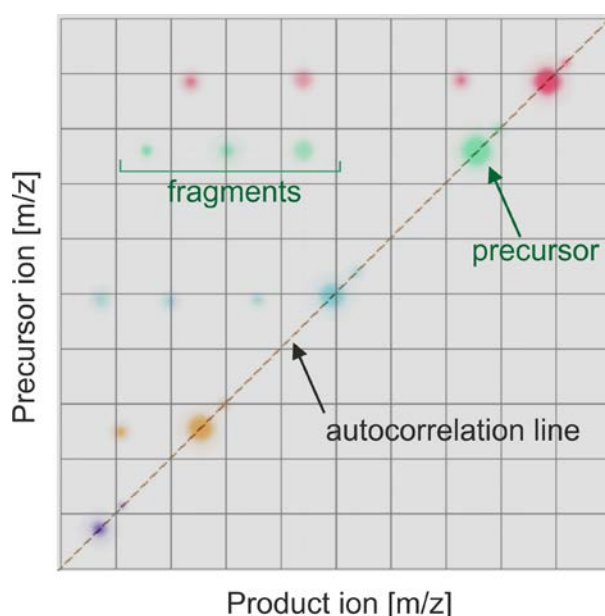


Figure 1: Idealised representation of a typical 2D mass spectrum. Precursors and their associated fragments are colour coded. Intact precursors appear on the diagonal autocorrelation line while correlated product ions are arranged in horizontal lines with the same y-axis value as their parent precursor ion.

Basic principles and instrumentation

2DMS is implemented on a QToF instrument by repurposing the quadrupole as an ion trap. This is simply accomplished by alternating the voltages applied to lenses either end of the quadrupole between low and high values to trap and release ions. Ions in a quadrupole oscillate or orbit as if trapped in a potential well. The frequency of this oscillation (known as the secular frequency) is dependent on the m/z value of the ion. Light ions oscillate at high frequencies; heavy ions oscillate at low frequencies. Ions of a particular m/z may be resonantly excited. For example, if an m/z 100 ion has a secular frequency of 200 kHz, applying a 200 kHz waveform across two opposing quadrupole rods will excite all m/z 100 ions to higher orbits but leave ions of, say, m/z 150 unaffected. Consequently, a broadband pulse comprising many frequencies with varying amplitudes will excite different ions to different orbital radii. These pulses can be specifically constructed to sinusoidally modulate the ion orbital radii with pre-determined encoding frequencies.

A necessary component of 2DMS is the coupling of precursor ion radius modulation with a radius-dependent fragmentation technique. If the fragmentation efficiency depends on the precursor ion orbital radius, then the fragment yield will modulate at the same frequency as the precursor signal; precursors and their fragments become inextricably linked by their common modulation frequency. Collision induced dissociation (CID) and ultraviolet photodissociation (UVPD) are examples of radius-dependent fragmentation methods that can be implemented in a quadrupole. During data acquisition, ion signals are seen to modulate. The modulation frequencies can be recovered in post-processing by applying the Fourier transform to every ion signal. Construction of a 2D spectrum starts by plotting modulation frequency on the y-axis and the m/z values recorded by the ToF analyser on the x-axis. A precursor ion and its correlated product ions have the same modulation frequency and therefore the same y-axis value, resulting in peaks arranged in horizontal lines. The y-axis is then converted to m/z (of the parent precursor) using the known relationship between encoding frequency and m/z .

2DMS is currently available as a retrofitted upgrade kit for Bruker QToF instruments. Kits for instruments supplied by

other manufacturers are expected to be available soon. A bespoke electronics unit is required to supply radio frequency (RF) waveforms and excitation pulses to the four quadrupole rods. No further modifications are required if CID is the chosen fragmentation method. UVPD requires further adaptations to allow a laser beam to be directed along the axis of the quadrupole.

Applications

Analyses of a simple mixture of active pharmaceutical ingredients (APIs) by traditional MS and 2DMS are compared in Figures 2 and 3, respectively. Using traditional MS (Q1 operated in RF-only mode so that all precursors are transmitted), the precursors are easily identified when CID

is inactive but many additional product ion peaks appear when the collision energy is 20 eV. Clearly, interpretation of this data would be challenging if the identities of the compounds were unknown. When 2DMS is applied to the same mixture, peaks corresponding to the intact precursors are seen on the diagonal autocorrelation line. Fragmentation by CID results in the appearance of product ion peaks lying on horizontal lines passing through the precursor peak positions. Interpretation of the data is now straightforward. For example, product ion peaks 1-5, as well as the precursor peak on the same line, can be used to confirm or deduce the presence of ranitidine in the mixture. The empirical formula for each ion can be determined with high confidence as the x-axis coordinate of every peak retains the mass accuracy of the ToF analyser (~2 ppm).

While manual data analysis is possible for simple mixtures, in most cases, automated peak picking is used to generate lists of product ion peaks belonging to each precursor found on the autocorrelation line. The components of the mixture can then be determined using spectral databases

The data generated is unbiased, complete, and easily interrogated, both manually and by software.

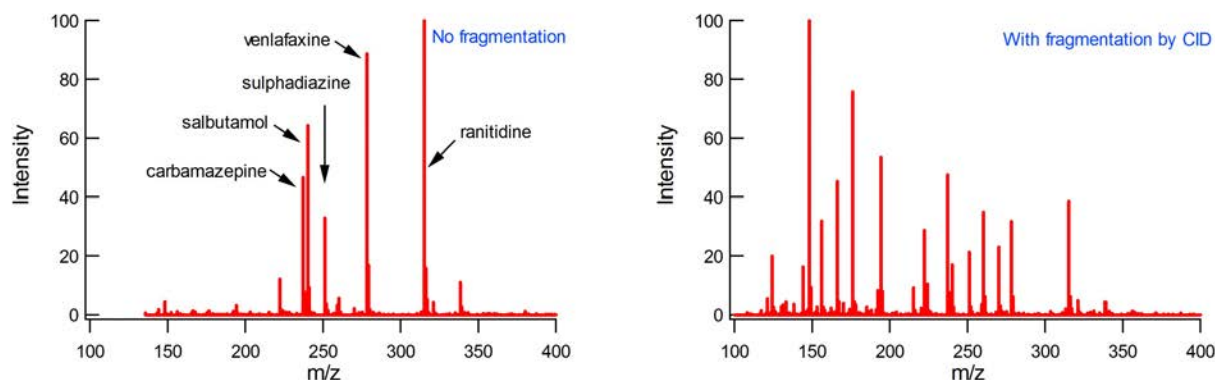


Figure 2: A mixture of pharmaceuticals analysed using traditional mass spectrometry (Q1 in RF-only mode). When the components are fragmented by CID, interpretation of the spectrum is very challenging as it is unclear which product ion peaks belong to each of the precursors.

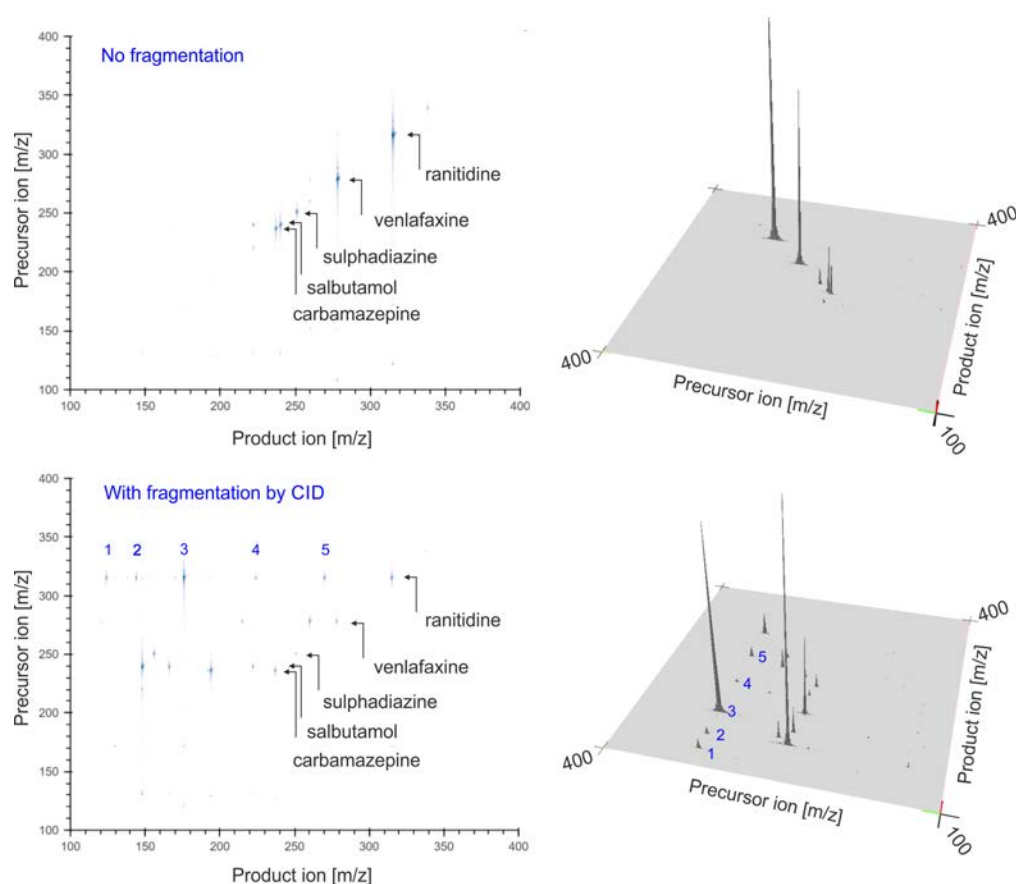


Figure 3: Analysis of the same pharmaceutical mixture using 2DMS. Precursor ion peaks are found on the diagonal autocorrelation line and peaks corresponding to their respective fragments are located along separate horizontal lines. Structural elucidation and precursor identification are now straightforward. For example, peaks 1-5 are all fragments of ranitidine.

or common software tools. While broad overviews of the entire mass range such as those in Figure 3 are instructive, zoomed-in views can yield a great deal of additional detail. As the chemical noise is also separated in two dimensions, it is possible to identify peaks of interest with intensities varying over five orders of magnitude.

There are numerous application areas and scenarios that are likely to benefit from the application of 2DMS:

- 2DMS can be used to rapidly identify unknown or unexpected contaminants in applications ranging from food safety to industrial process control, providing early warning of unexpected pesticides or reaction byproducts, for example.
- Rather than replace chromatography, 2DMS can also augment traditional targeted method development. A survey scan using 2DMS provides a list of all precursors present and prominent fragments that can then be used for selected reaction monitoring (SRM).
- A 2DMS spectrum provides a complete historical record of the sample composition. The data can be retrospectively examined for components that were not of interest at the time of analysis but later become significant.
- For complex samples, complete structural elucidation and identification of all components remains a formidable task and is often unnecessary. A 2DMS spectrum has a prodigious peak capacity and is consequently data rich. Statistical methods can be applied to extract nuanced information such as the country of origin or the flavour profile of foods and foodstuffs without assigning every peak in the spectrum.
- Additional fragments may be generated if the UVPD option is implemented. CID generally favours low energy fragmentation routes whereas unusual and/or higher energy pathways can be accessed by UVPD. For some compound classes, these additional fragments provide important structural information, such as the position of double bonds in lipids.

Conclusions

2DMS is an emerging tool for mainstream laboratory applications. Existing QToF instruments with modest specifications can be upgraded to perform this powerful DIA technique. Here, the principle of 2DMS has been demonstrated with a simple mixture but much more complex samples can also be analysed. The data generated is unbiased, complete, and easily interrogated, both manually and by software.