THE BENEFITS OF GAS-PHASE COLLISION CROSS-SECTION (CCS) MEASUREMENTS IN HIGH-RESOLUTION, ACCURATE-MASS UPLC/MS ANALYSES

The rotationally-averaged collision cross-section (CCS) represents the effective area for the interaction between an individual ion and the neutral gas through which it is travelling. CCS is an important distinguishing characteristic of an ion in the gas phase, being related to its chemical structure and three-dimensional conformation. CCS affects the mobility of an ion as it moves through a neutral gas under the influence of an electric field and ions may be separated accordingly using ion mobility spectrometry (IMS).[1] CCS values may be measured experimentally using IMS. CCS values may also be estimated computationally if the 3D structure of the molecule is known.[2]

Figure 1 illustrates the separation of ionic species achieved by IMS. In this example, two ions of equal mass and charge, but different three-dimensional conformation, will travel through an IMS device at rates dependent on their mobilities and emerge at different times (drift times). The ion with a more compact three-dimensional conformation has a shorter drift time through the IMS device because its smaller CCS value gives it a higher mobility than its neighbour with a more open three-dimensional conformation.

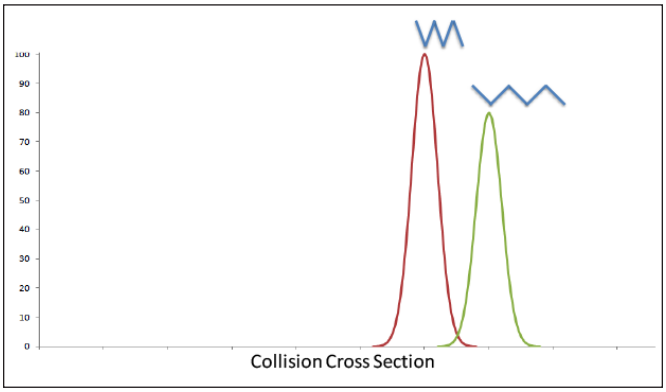

Figure 1. The CCS value of an ion may be determined using its drift time through an IMS device.

By calibrating the IMS device using ions of known CCS, the drift times measured during subsequent analyses of unknown species may be converted directly to CCS values.

The Waters® SYNAPT® G2-S*i* HDMS™ Mass Spectrometer incorporates high-efficiency T-Wave™ ion mobility separations and enables CCS values to be measured routinely as an integral part of high-resolution, accurate-mass LC/MS experiments.[3] This allows CCS to be used alongside the traditional molecular identifiers of precursor ion accurate mass, fragment ion accurate masses, isotope pattern, and chromatographic retention time as a confirmation of compound identity or as an indicator of compound structure.

CCS FOR MAXIMIZING ANALYTICAL PEAK CAPACITY AND SELECTIVITY

The selectivity of an analytical method depends on the ability of the analytical system to resolve sample constituents from each other. Often, a sample may contain many thousands of individual components.

Chromatography and mass spectrometry are techniques typically used to resolve sample constituents. The resolving power of modern UltraPerformance LC® (UPLC®) instruments and high resolution mass spectrometers, when combined, deliver very high levels of analytical selectivity but many analyses either cannot make full use of chromatographic separations (MALDI[4] or direct analysis techniques such as DART, DESI, and ASAP[5]) or demand levels of selectivity that cannot be provided even by the combination of state of the art LC/MS systems.

In such cases T-Wave ion mobility separation provides an additional dimension of resolution that works orthogonally to both chromatography and mass spectrometry to multiply the peak capacity and selectivity of an analytical method.[6] By incorporating CCS based separations into an analytical method it is possible to distinguish analyte from matrix interferences or even resolve structural isomers[7], conformers, and protomers.[8] For MALDI Imaging experiments, where samples may be complex and chromatographic separation is not possible, CCS based separation provides the selectivity required to accurately and confidently determine the spatial distribution of important molecular species.[9]

CCS FOR CONFIRMING COMPOUND IDENTITY

Because CCS measurements are undertaken in the gas phase, remotely from the ion source, CCS values are unaffected by sample matrix and are consistent between instruments and across a range of experimental conditions. The precision of CCS measurements obtained with T-Wave IMS can be used in combination with other molecular identifiers to increase the confidence of compound identifications.

As an example, Figure 2 shows the average measured CCS values from a range of pesticides analysed in a variety of different sample extracts, compared with the values measured in solvent standards. The results show that CCS may be measured within 2% of the expected value regardless of the nature and complexity of the analytical sample.

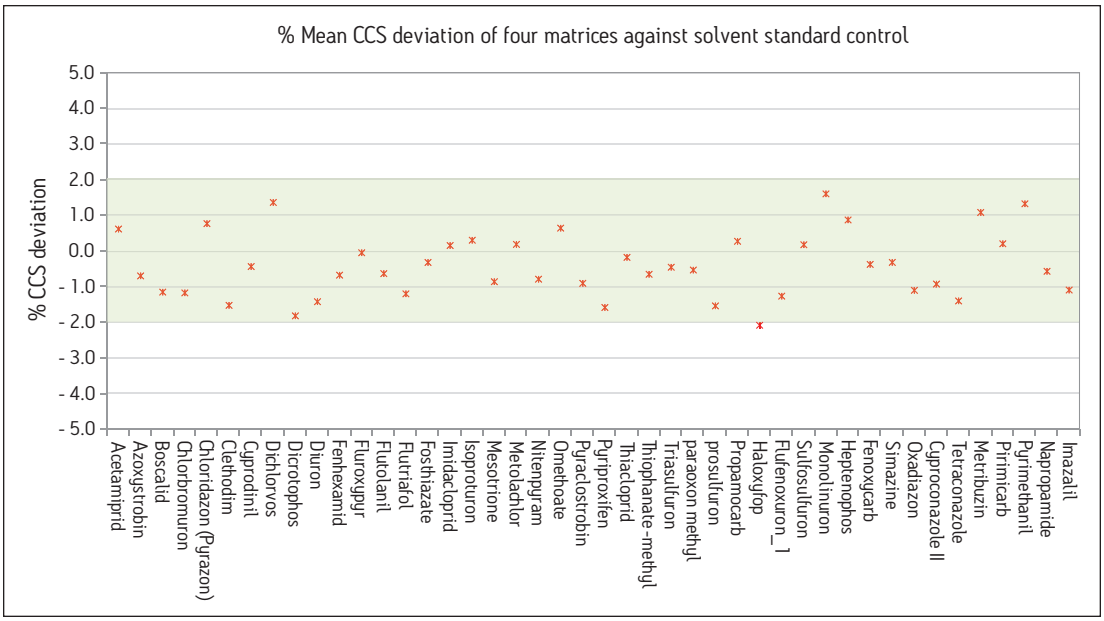

Figure 2. Average measured CCS values for a wide range of pesticide compound classes over a range of sample matrices deviate by less than 2% from CCS values measured using solvent standards.

Large numbers of pesticides can be screened for in high-resolution, accurate-mass LC/MS experiments but often, components in the sample matrix give rise to signals that can obscure the signal from a residue present in the sample (false negative detection) or can be mistaken for pesticide residues not present in the sample (false positive detections). The number of false negative and false positive detections can be minimised by choosing appropriate mass accuracy and retention time tolerance windows and by filtering the results based on the presence of expected fragment, adduct or isotope confirmatory ions. The CCS values of pesticides may be used as an additional, orthogonal means of filtering the data to significantly reduce the proportion of false positive and false negative detections.

A spiked extract of mandarin fruit, produced for a European Union Reference Laboratories (EURL) proficiency test, was analysed in a blind study using a Waters SYNAPT mass spectrometer. The data were searched against a library of approximately 480 pesticide compounds. Approximately 50,000 sample components were observed and filtering these components according to chromatographic retention time, the presence of expected fragment ions and a 5 ppm mass tolerance gave 9 identifications, with 1 false negative and 2 false positive results. Increasing the mass tolerance to 10 ppm gave 10 identifications with 2 false positive results. Applying a CCS tolerance of ± 2%, as a further filter to the results reduced the number of identifications to 8 as shown in Figure 3. The 8 identifications obtained using CCS as an extra, orthogonal data filter were all correct and, in this particular case, the false positive and false negative identifications were removed.

	Without CCS	Without CCS	With CCS
m/z tolerance (+/-)	5 ppm	10 ppm	10 ppm
rt tolerance (+/-)	2.5%	2.5%	2.5%
CCS tolerance (+/-)	–	–	2%
Correct IDs	7	8	8
False Negatives	1	0	0
False Positives	2	2	0

Figure 3. EURL proficiency test sample results: Pesticide screening without using CCS shows a number of false positive and false negative identifications. For this study, using CCS information removes false positive and false negative identifications.

The use of CCS as an additional, orthogonal means of filtering the data effectively allows a more balanced set of tolerance criteria to be applied to compound screening experiments, which results in more confident compound identifications.

CCS AS AN INDICATOR OF MOLECULAR CONFORMATION

Theoretical CCS values determined by molecular modelling may be compared directly with experimentally derived CCS values obtained by T-Wave ion mobility separations, in order to yield useful information on the structures and shapes of large proteins/protein complexes, peptides, organometallic complexes and small

molecules; information that can provide unique insights into important biological and chemical processes.

The use of T-Wave IMS has enhanced knowledge in diverse areas of scientific research, from mapping the size distribution in oil and petroleum samples[10] to revealing the molecular architecture of multi protein complexes[11,12] and to rapidly determining sites of biotransformation in drug metabolism studies.[13,14]

These types of studies are able to provide useful scientific information far more rapidly and efficiently than by more traditional techniques. Often, using CCS to probe molecular structure can yielded scientific advances not possible by other means.

CONCLUSION

The ability to separate ions by T-Wave IMS and measure their CCS values as an integral part of high-resolution, accurate-mass LC/MS experiments allows significant increases in the peak capacity and resolution of analytical methods. It enables the separation of isomers, conformers and protomers that cannot be separated by other means. It also provides a unique, orthogonal identifier for target analytes in screening experiments and can be used to probe the structures and conformations of diverse types of molecule. All these experiments can be carried out on the Waters SYNAPT G2-S*i* HDMS Mass Spectrometer, enabling significant increases in the performance of analytical methods and delivering unique capabilities not available on conventional mass spectrometers.

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