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アプリケーションノート

Illustration of the Selectivity of Collision Cross Section Ion Mobility Screening for the Analysis of Pesticide Residues in Food Using the ionKey/MS System

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Abstract

To explore the improved selectivity of ion mobility collision cross sections (CCS) measurements in combination with iKey microflow-LC technology for screening pesticide residues in food.

Benefits

- Improved selectivity of ion mobility CCS measurements in combination with the iKey Separation Device.
- Enhanced ionization/transmission efficiency, provides higher sensitivity to detect pesticides residues in complex food commodities.
- Removal of matrix suppression with sample dilution and enhanced spectral quality at MRL’ s.
- Use CCS data to provide a higher degree of selectivity in combination with accurate mass measurement.
- Avoid false detections whilst having wider screening windows for retention time, mass accuracy, and filtering with constricted CCS criteria.

Introduction

Pesticide residue analysis in food has become a more challenging task considering the increasing number of compounds and complex food commodities to be monitored at low concentrations with generic extraction procedures. The direct consequences are complex extracts (presence of matrix compounds), for which multiple injections have to be performed while achieving dwell time and duty cycle balance. Screening methods are a practical alternative and full scan high resolution MS (HRMS) offers high specificity and the ability to detect a large number of analytes simultaneously using generic instrumentation parameters. Although, time-of-flight (Tof) mass spectrometry has benefited from higher sensitivity and resolution, it can still be difficult to rapidly and efficiently identify targeted compounds present in a sample containing a large number of co-extracted matrix components.

Full spectra acquisition and accurate mass measurement specificity is well characterized. It is used in combination with time tolerances, isotopic matching, fragment ions/ratios, and response thresholds to help reduce false positive and false negative detections in screening assays. Advances in mass spectrometry have vastly improved sensitivity for full spectral analysis, but further sensitivity enhancements would improve the mass spectral data quality. This is especially important to avoid compromised precursor ion or fragment ion information, and ensure high mass accuracy below the

legislated levels. Improvements in sensitivity using the IonKey/MS System have previously been shown.[1] The ionKey/MS System enables sample dilution to reduce matrix suppression and subsequently increases the overall analyte signal-to-noise values that can be achieved.

In this application note, we explore the use of highly selective collision cross section (CCS) measurements in combination with sensitivity enhancements and reduction of matrix suppression for residue analysis in complex food commodity matrices. Travelling wave ion mobility mass spectrometry (IM-MS) uses a nitrogen buffer gas which enables the measurement of CCS, providing some unique advantages for profiling complex mixtures. It is a combination of high resolution mass spectrometry and high efficiency ion mobility based measurements and separations. IM-MS is a rapid orthogonal gas separation phase technique that that allows another dimension of separation to be obtained within an LC timeframe. Compounds can be differentiated based on size, shape, and charge.

A CCS value is a robust and precise physicochemical property of an ion. It is an important distinguishing characteristic, that is related to its chemical structure and three-dimensional conformation. CCS measurements have been entered into a scientific library within the UNIFI Scientific Information System, which allows the expected and determined CCS values to be utilized to screen and confirm the presence of pesticide residues.[2] Here we present CCS values (derived from ion mobility drift times) as a new parameter that can provide added selectivity and more confidence in identifications. Using the ionKey/MS System in this screening study has shown how CCS can be used to reduce the reliance of screening studies based on retention time. Utilization of CCS measurements enable the application of generic processing parameters to identify targeted compounds, where different chromatographic methodologies have been employed.

Experimental

LC conditions

LC system:	nanoACQUITY UPLC (or the ACQUITY UPLC M-Class System)
Mobile phase A:	A:100% Water (0.1% Formic acid)
Mobile phase B:	100% Acetonitrile (0.1% Formic acid)

Flow rate:	UPLC at 450 µL/min; iKey Separation Device at 1.0 µL/min
Injection volume:	UPLC:5 µL (full loop); iKey Separation Device: 2 µL (full loop)
UPLC column:	ACQUITY UPLC BEH C_{18}, 100 mm x 2.1 mm, 1.7 µm
Column temp.:	30 °C
Separation device:	iKey BEH C_{18} Separation Device, 130Å, 1.7 µm, 150 µm x 100 mm
iKey temp.:	45 °C

Gradient:

Time (min)	Flow rate (µl/min) UPLC	Flow rate (µL/min) ikey	%A	%B
0.0	450	1	98.0	2.0
0.25	450	1	98.0	2.0
12.25	450	1	1.0	99.0
13.00	450	1	1.0	99.0
13.01	450	1	98.0	2.0
13.00	450	1	98.0	2.0
17.00	450	1	98.0	2.0

MS conditions

MS system:	SYNAPT G2-Si
Ionization mode:	ESI+
Mass range:	50 to 1,200 *m/z*
Acquisition rate:	5 spectra/sec
Capillary voltage:	1 kV
Cone voltage:	20 V
Drift gas:	N_2
Collision energy ramp:	10 to 45 eV
IMS wave velocity range:	650 m/s
IMS wave height:	40 V
IMS gas flow:	90 mL/min
IMS duty cycle:	10.8 ms
Lockmass:	*m/z* 556.2766 (Leucine enkephalin)

Sample description

The assay is based on the analysis of solvent standards in addition to matrix samples: mandarin, ginger, leek, and pear extracts, plus matrix matched calibrants.

Sample preparation

Extraction conditions: 10 g of homogenized sample was extracted with 60 mL of 20 mM ammonium acetate in methanol using an Ultra-Turrax device. The crude extract was then filtered and diluted up to 100 mL with 5 mM ammonium acetate in water prior to injection.

An organic mandarin sample was used to produce a matrix matched calibration curve and a previous European ring-test FV-13 sample was analyzed using European Commission proficiency tests for pesticide residues in fruits and vegetables (FV-13 Mandarin Homogenate, 2011).

Spiking protocol

Organic samples were homogenized and 10 g was extracted with 60 mL of 20 mM acetate ammonium in methanol/water (95:5; v/v) solution. Then 5 mL and 3 mL of raw extract were transferred to six volumetric flasks. For the spiking of 0.01, 0.05, and 0.10 mg/kg levels, 50, 250, and 500 µL respectively of a mix solution was prepared containing the targeted pesticides at 0.1 µg/mL. For the higher levels of 0.2, 0.5, and 1.0 mg/kg, a mix solution of 100, 250, and 500 µL respectively was prepared containing the targeted pesticides at 1 µg/mL. Then the final volumes were adjusted to 5 mL with a 5 mM ammonium acetate in water/methanol (90/10;v/v).

Chromatographic method	Starting mass of crop sample	Crop equivalent in the final extract	Spiking concentration (mg/kg)	Solution concentration (ng/mL)	Dilution factor applied during the extraction procedure
ACQUITY UPLC	10 g	0.1 g/mL	0.01	1	x100
ionKey	10 g	0.01 g/mL	0.01	0.1	x1000

Table 1. Spiking concentrations, solution concentrations, and dilution factors applied using the Granby extraction method for UPLC and ionKey comparison.

Matrix comparison

Different injection volumes and sample dilution on column loadings have been used in order to generate extrapolated comparative results (Table 2). For the ionKey/MS System reduction of matrix suppression studies, samples were diluted using 25% water:75% acetonitrile.

Parameter	Chromatographic mode and sample loading details	
	ionKey	UPLC
Injection solvent composition	25 (H_2O):75 (MeCN)	MeOH
Dilution factor applied to the final extract	X1000	X100
Spiking concentration (mg/kg)	1	0.1
Pesticide solution concentration in the final extract (ng/mL)	10 ng/mL	10 ng/mL
Matrix load (ng/mL)	0.01 ng/mL	0.1 ng/mL
Injection volume (µL)	2 µL	5 µL
Loop size (µL) and injection mode	2 µL	5 µL
On column mass (pg)	20 pg	50 pg

Table 2. Parameters used for the direct comparison of the ionKey and UPLC chromatographic systems in matrix extraction.

An iKey Separation Device (Figure 1), incorporates a 1.7 µm, ACQUITY UPLC BEH C_{18}, stationary phase in a 150 µm diameter separation channel. The iKey Separation Device temperature was set to 45 °C and the eluent from the separation channel flows directly to an integrated ESI emitter. All microfluidic, gas, and electrical connections are automatically engaged when the iKey Separation Device is inserted into the source enclosure and locked into position.

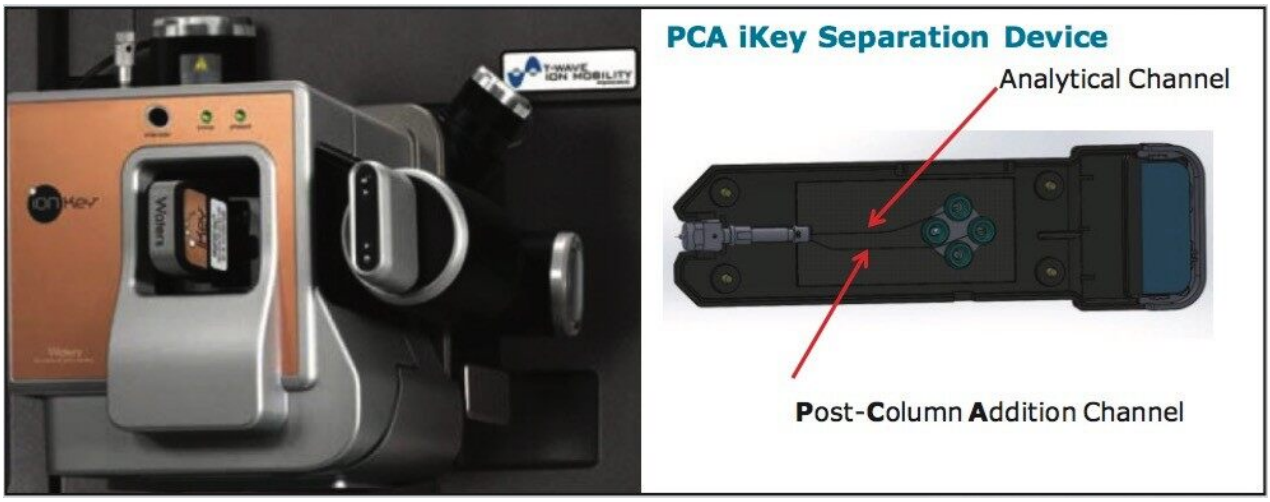

Figure 1. ionKey/MS Source and iKey Separation Device incorporating fluidic/electronic connections and ionization emitter.

IMS calibration

T-Wave ion mobility calibration was performed using previously determined CCS values for polyalanine. Ion

mobility calibration was performed using polyalanine and a travelling wave nitrogen buffer gas. The polyalanine (^{TW}CCS) values were previously generated using an He drift tube derived calibrant species for the CCS calibration process, and software conversion from N_2 to He values were performed. Therefore the collision cross sections described in this study are $^{TW}CCS_{He}$ values.[3] At later stages of the project, in order to develop a CCS screening workflow, Ω_{N2} drift tube derived polyalanine CCS values in positive and negative modes were used for the CCS calibration process, hence $^{TW}CCS_{N2}$ were generated.[4]

Results and Discussion

The study discussed formed part of a project to develop a pesticide CCS screening workflow, where the feasibility of CCS screening was compared across five Waters ACQUITY UPLC I-Class and SYNAPT HDMS Systems. The assay is based on the analysis of sample extracts, matrix matched calibrants (pear, ginger, leek, and mandarin), and quality control samples generated for an EU-RL (European Union Reference Laboratory) proficiency test using the ionKey/MS System. The system was comprised of a nanoACQUITY UPLC System, a SYNAPT G2-Si Mass Spectrometer, an ionKey Source, and the iKey Separation Device which were all controlled using MassLynx MS Software.

Initially, ion mobility data was acquired using the ionKey/MS Source, for a series of solvent standard mixtures. These were utilized to generate retention time information and CCS measurements for the iKey pesticide library within UNIFI. These measurements were subsequently used to enable the correct identification of the pesticide residues in the matrix matched samples and proficiency samples. The results were compared to those previously obtained, where analysis was performed using conventional UPLC with ion mobility MS. Previous studies have shown the benefits of CCS screening, including spectral cleanup, avoidance of false positives, and discovery of pesticide protomers.[5-7]

Using the ionKey/MS System, when comparing to the previous UPLC-IM-MS study, the results have shown gains in both sensitivity and signal-to-noise with excellent linearity correlation coefficients obtained for the majority of matrix matched calibrants ($r^2 \geqslant 0.95$). Gains in sensitivity have enabled matrix dilution to be performed, and the detection of 1 pg/µL on column to be obtained, where both precursor ion and ion mobility product ions have been obtained as shown in Figure 2 for tetraconazole. Figure 3 shows example linearity plots and correlation coefficients obtained for pesticides in the mandarin matrix matched samples analyzed using the ionKey/MS System ion mobility.

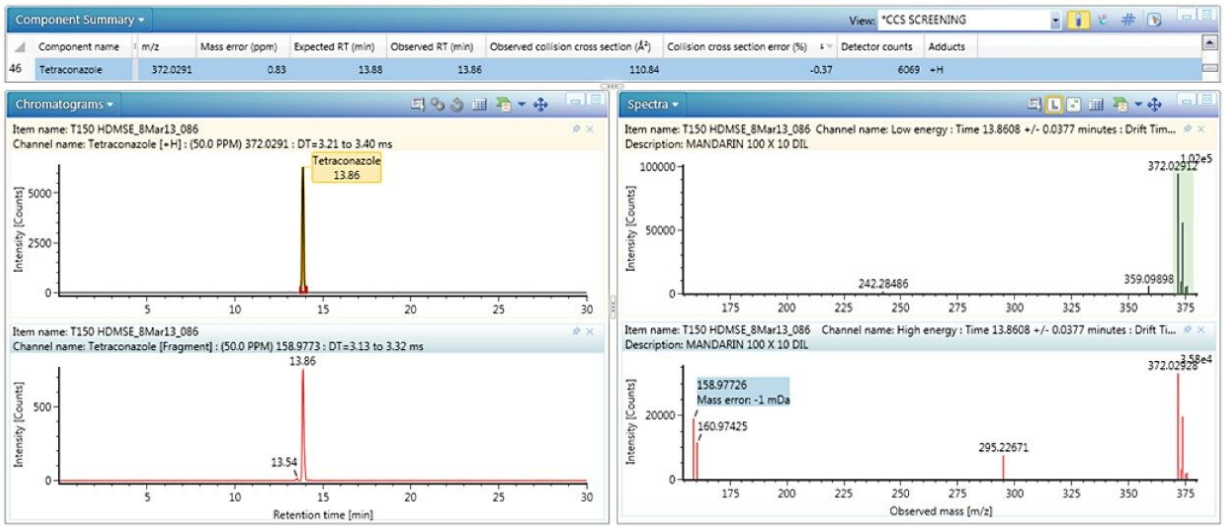

Figure 2. UNIFI Software Component Summary showing the response obtained for pesticide tetraconazole at 1 pg/µL using the ionKey/MS System with ion mobility. Retention time and drift time aligned precursor ion and ion mobility product ion spectra are presented, with corresponding extracted mass chromatograms. Observed CCS values and CCS errors are presented along with retention time.

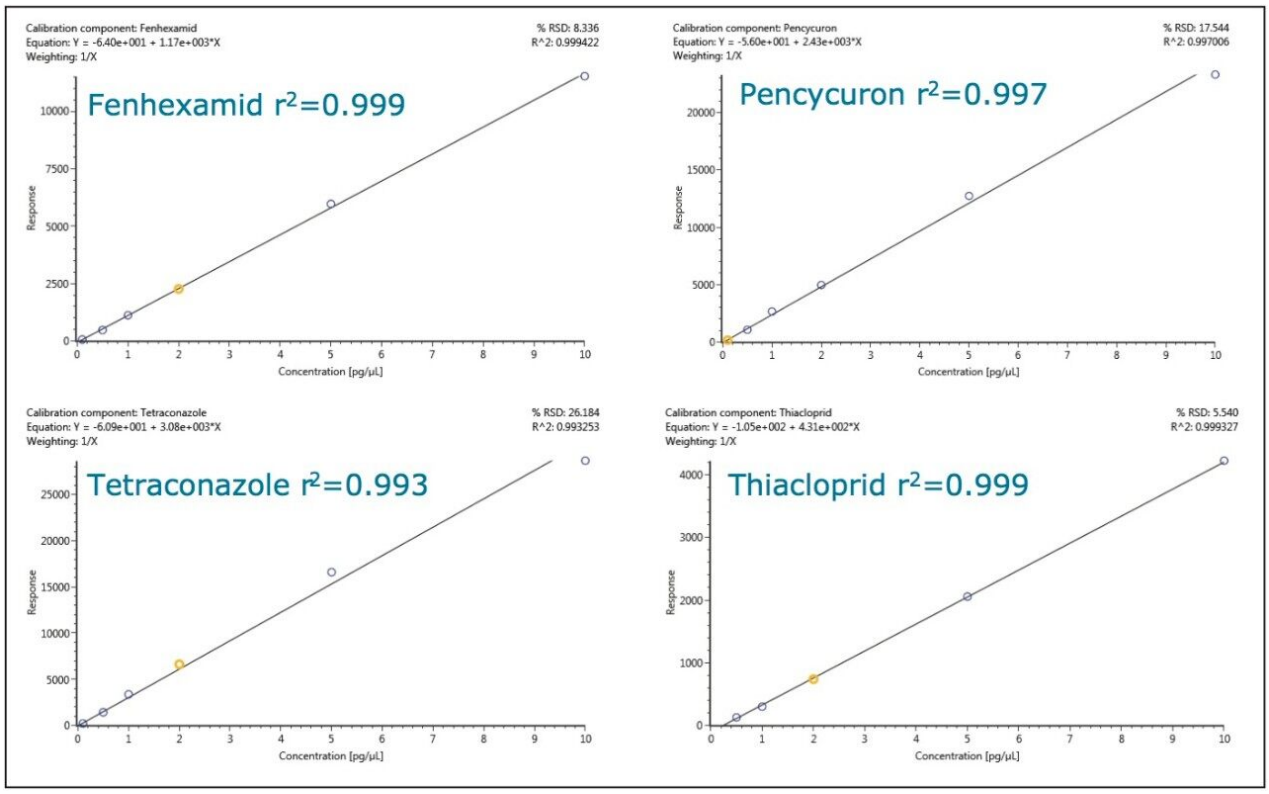

Figure 3. Correlation coefficients obtained for pesticides in mandarin matrix matched sample analyzed using the ionKey/MS System with r^2= 0.99 (0.1 to 10 pg/µL).

A major point of discovery during the ionKey/MS System CCS screening study came from using the added selectivity of CCS measurements obtained during UPLC ion mobility pesticide residue assays. In this study, the previously determined CCS values were used as an identification point to rapidly determine the retention times of the pesticide solvent standards and identify the residues present in a previous proficiency sample. The same chromatographic gradient was employed for the ionKey and UPLC chromatography. However the resultant retention times were not the same; hence it was necessary to create an applicable pesticide library in UNIFI containing the iKey Separation Device retention times. The conventional approach would require initial manual data interrogation to generate the iKey Separation Device retention time library. In this case the previously developed UPLC CCS values could be utilized, where the selectivity of CCS could be used to identify the analytes of interest, in combination with precursor ion mass. The ionKey/MS System ion mobility data was screened using an accurate mass measurement tolerance of 10 ppm and a CCS tolerance of 10% for the target residues. Since the iKey Separation Device retention times were not known, a 30 minute retention time window was applied, i.e. the same time as the chromatographic run.

The results obtained can be seen in Figure 4, where for the EU RL proficiency test sample FV-13, 81 residues

have been detected under these screening parameters. Thereafter the processed data was filtered using a 2% CCS measurement tolerance and a response threshold of 150 counts; hence the ionKey/MS System retention times for the pesticide solvent standards were rapidly determined, as well as the residues present in the FV-13 proficiency sample.

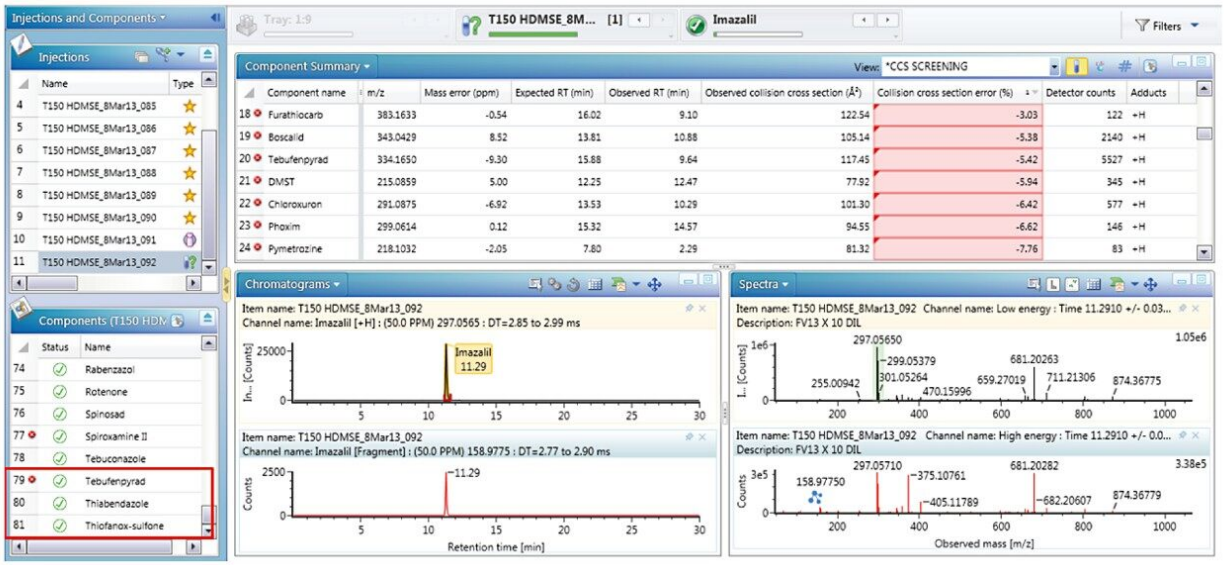

Figure 4. ionKey/MS System ion mobility results for EU RL proficiency test FV-13 pesticide residue screen using an accurate mass measurement tolerance of 10 ppm and CCS tolerance of 10% for the target residues. To determine the unknown iKey Separation Device retention times, a 30 min retention time window was applied, 81 observations made (highlighted in red).

In the preliminary study, those compounds where CCS values had not been determined and entered into the UNIFI library were removed using the CCS filter. Using this approach, it was possible to rapidly determine the presence of the expected eight detected pesticide residues in the sample analyzed, as can be seen in Figure 5. The initial 81 analytes observed using wide tolerance parameters of 10 ppm and a 30 min retention time window, was reduced to 9 when a tolerance filter using the selectivity of CCS was applied to the processed data. There was no requirement to reprocess the data.

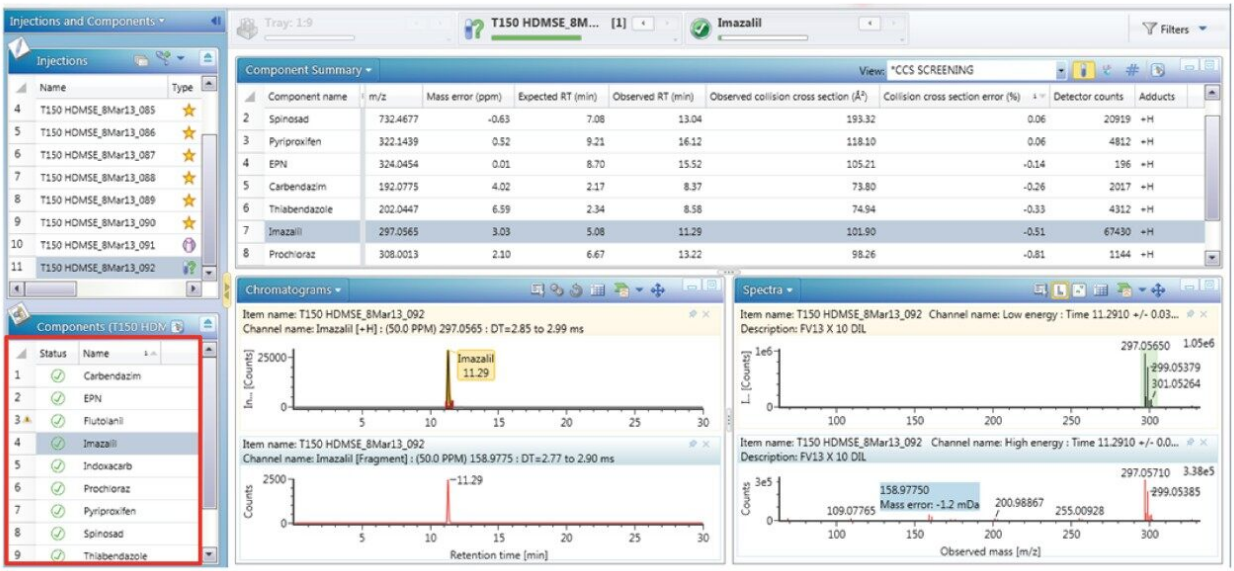

Figure 5. IonKey/MS System ion mobility results for EU RL proficiency test FV-13 pesticide residue screen. A data filter comprising 2% CCS error and threshold response was applied with nine observations made (highlighted in red).

Using mixtures of solvent standards, it was possible to rapidly determine the retention times using ionKey chromatography. Once retention times were determined, it was possible to filter the data using a 0.2 min retention time tolerance window, a 10 ppm mass accuracy tolerance. and a CCS error tolerance of 2%. No false positive or negative detections occurred for the expected eight detected compounds. This clearly shows the benefits and selectivity that can be provided using CCS measurement, where the pesticides have been identified based on their accurate mass and collision cross section. The expected retention times for the observed residues in proficiency sample FV-13 are of the order of 7 minutes different to those observed. For example, different chromatographic retention times compared for imazalil (UPLC 5.08 /ionKey 11.29 mins) and thiabendazole (UPLC 2.34/iKey 8.58 mins) were obtained. The power of CCS selectivity was confirmed from its utilization to determine retention times to be entered into the ionKey pesticide screening library in UNIFI.

The benefits of ion mobility selectivity are further illustrated in Figures 4 and 5, where imazalil has been selected and the precursor ion and fragmentation spectra are presented. For Figure 4, the retention time aligned fragmentation spectra are presented at 11.29 minutes, which incorporates a large number of chromatographically coeluting components. However, in Figure 5, where the retention time aligned and ion mobility drift time aligned data is selected (11.29 mins/2.92 ms), it can be seen that resolution provided by ion mobility results in highly selective data. The spectra have effectively been “cleaned up”, because the components that were chromatographically coeluting with imazalil, are now ion mobility resolved. As a result, it is possible to generate ion mobility specific product ions for all analytes detected in the acquisition.

Using ionKey/MS System in combination with ion mobility it has been possible to obtain precursor ion and mobility product ion spectra for 2 pg on column loadings for pencycuron, as shown in Figure 6. Accurate mass measurement and diagnostic ion mobility product ions provide confidence in identification. However, it can be seen that pencycuron has also been detected at 200 fg on column. Confidence can still be had where only a mono isotopic peak has been observed, because CCS (0.51%) provides an additional information point to the 0.86 ppm mass measurement error determined. The improved ionization efficiency, increases in sensitivity, and improved sensitivity achieved on a Q-Tof mass spectrometry platform coupled to ionKey has been shown.[1]

Figure 6. Accurate mass measurement (<1 ppm) , precursor ions, ion mobility product ions, and collision cross sections (<1%) for confidence in identification, for pencyuron detected at 2 pg and 200 fg on column.

An example of the increase in S/N (x4) and response (x3) using the ionKey/MS System with the SYNAPT G2-S*i* System is presented in Figure 7 for indoxacarb, as observed in EU RL proficiency sample FV-13. The response gains take into account the injection volumes and x10 sample dilution performed for the ionKey/MS System pesticide residue analysis performed. With its enhanced selectivity and sensitivity, this approach has the potential to be used to review and confirm whether suspected MRL violations may have occurred.

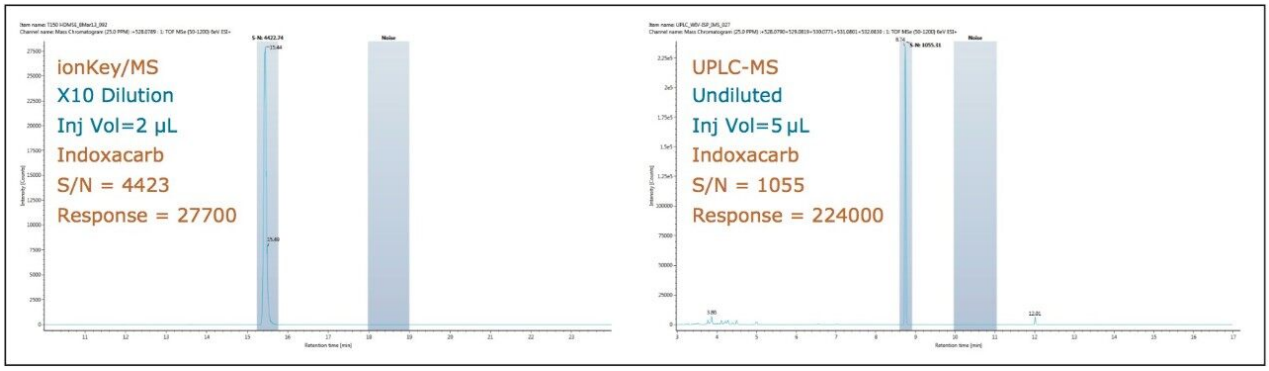

Figure 7. Comparison of S/N and response for UPLC-MS and the ionKey/MS System for indoxacarb, where X4 S/N and X3 response improvements have been obtained over UPLC using the ionKey/MS System.

Conclusion

The ionKey/MS System with ion mobility offers some unique advantages for profiling complex matrices:

Spectral cleanup.

Collision cross section measurements provide unique selectivity and added confidence in identifcation.

- Ion mobility selectivity has been illustrated, where accurate mass measurement and CCS measurement have been used to successfully detect pesticide residues in previous EU RL proficiency test sample FV-13.
- Sensitivity gains and improved transmission and ionization efficiency of the ionKey Source have enabled mass measurement of pencycuron with CCS determination providing an additional identification point for monoisotopic peak information at 200 fg on column.
- Linearity for the pesticides using matrix matched standards, produced correlation coefficients of >r^2= 0.95.
- For the analyst, this advanced ionKey Technology, brings the benefits of microfluidic chromatography to the required “routine use” platform in combination with routine ion mobility screening.

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720005427, January 2016

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