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Using the Routine Separation Dimension and Identification Criteria of ionKey/MS Ion Mobility to Enhance Specificity in Screening Complex Samples

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Abstract

A novel microflow technique using the ACQUITY UPLC M-Class System and IM-MS that leverages both positive and negative ionization has been developed to analyze the extracts of *Passiflora edulis, alata, caerulea*, and *incarnata.* The profiles determined from the extracts can be used to confirm food commodity authenticity.

Benefits

- Increased sensitivity produced using the ionKey/MS System can extend the dynamic range over which the benefits of ToF full spectral acquisition and ion mobility are used.
- Using the ionKey/MS System ion mobility in positive and negative ion modes, marker flavonoids have been detected at 100 fg/µL.
- The ionKey/MS System ion mobility CCS screening can be used as a viable approach to perform authentication profiling.
- Collision cross section (CCS) values have been generated to enable the routine characteristic assignment for 6-C/8-C flavonoid glycoside isomers (vitexin/ isovitexin) (orientin/isoorientin), which can be used as an additional identification point.

Introduction

Legislative focus has resulted in the expansion of method development to support the analysis of active compounds in and authenticity of functional food products and dietary supplements. For example the European Union’ s Directive 2004/24/EC came into full effect on 30 April 2011. Hundreds of traditional herbal remedies were banned, as the EU directive aims to protect consumers from possible damaging side-effects of over-the-counter herbal medicines. Recent regulations allow only long-established and quality-controlled products to be sold. Manufacturers have to prove that their products have been made to strict standards and contain a consistent and clearly marked dose.

The ionKey/MS System ion mobility mass spectrometry (IM-MS) can provide a route to specific and unambiguous identification at low detection levels. This technique offers some unique advantages for profiling complex mixtures. IM-MS combines high resolution mass spectrometry and high efficiency ion mobility based measurements, with enhanced sensitivity. IM-MS is a rapid orthogonal gas separation phase technique that allows another dimension of separation to be obtained within an LC timeframe. Compounds

can be differentiated based on size, shape, and charge.

A novel microflow technique using the ACQUITY UPLC M-Class System and IM-MS that leverages both positive and negative ionization has been developed to analyze the extracts of *Passiflora edulis, alata, caerulea*, and *incarnata*, shown in Figure 1. The genus *Passiflora* consists of approximately 450 species, a few of which are commercially exploited in functional food products such as teas and juices. These species contain flavonoids, one of the largest and most widespread classes of compounds which possess diverse pharmacological/biological properties. The target marker flavonoids ionize efficiently in positive and negative modes, enabling a positive/negative mode on the ionKey/MS System comparison to be performed. Collision cross section measurements (CCS) can be used to produce routine unequivocal identification of marker flavonoid isomers in complex samples.[1] The profiles determined from the extracts can be used to confirm food commodity authenticity.

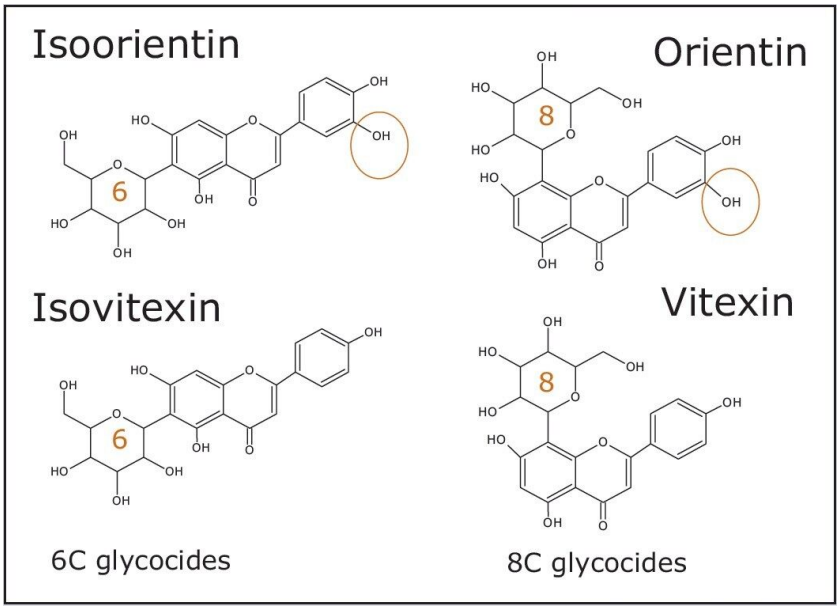

Figure 1. Structures of marker flavonoids profiled using the ionKey/MS System for positive and negative ion mobility mass spectrometry CCS screening.

Experimental

LC conditions

LC system:	ACQUITY UPLC M-Class
Mobile phase A:	Water (0.1% Formic acid)
Mobile phase B:	Acetonitrile (0.1% Formic acid)
Flow rate:	2.0 µL/min
Injection volume:	5 µL (full loop)
Separation device:	iKey BEH C_{18} PCA Separation Device, 130A, 1.7 µm, 150 µm x 50 mm (p/n 186007580) iKey BEH C_{18} Separation Device, 130A, 1.7 µm, 150 µm x 50 mm (p/n 186007256)
Separation device temp.:	40 °C

Gradient:

Time (min)	Flow rate	%A	%B
0.0	2	99.0	1.0
1.0	2	99.0	1.0
3.0	2	90.0	10.0
5.0	2	70.0	30.0
13.0	2	1.0	99.0
15.0	2	1.0	99.0
15.1	2	99.0	1.0
17.0	2	99.0	1.0

MS conditions

MS system:	SYNAPT G2-S*i*
Ionization mode:	ESI^+ and ESI^-
Capillary voltage:	3 kV (+) and 2.6 kV (-)
Sample cone voltage:	30 V
Lock mass and CCS:	Leucine enkephalin, $[M+H]^+$ =556.2766 and $[M-H]^-$ =554.2620
Acquisition range:	50 to 1,200 *m/z*
Acquisition rate:	10 spectra/sec
Collision energy ramp:	30 to 70 eV
Resolution:	20,000 FWHM (Res mode)

Default IMS parameters

IMS T-Wave velocity ramp:	Start: 1,000 m/s, End: 300 m/s
IMS T-Wave pulse height:	40 V
IMS gas flow:	90 mL

For positive ion electrospray, an iKey Separation Device (p/n: 186007256) was used. The iKey Separation Device, shown in Figure 2, incorporates a 1.7 µm, ACQUITY UPLC BEH C_{18}, stationary phase in a 150 µm diameter separation channel. 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 Seperation Device is inserted into the source enclosure and locked into place. For negative mode, the Post Column Addition (PCA) iKey Separation Device (p/n: 186007580) was used. The PCA iKey Separation Device incorporates an additional channel, enabling post column addition of solvent. The make up solvent was configured to be delivered from channel A of the MS system fluidics for this study.

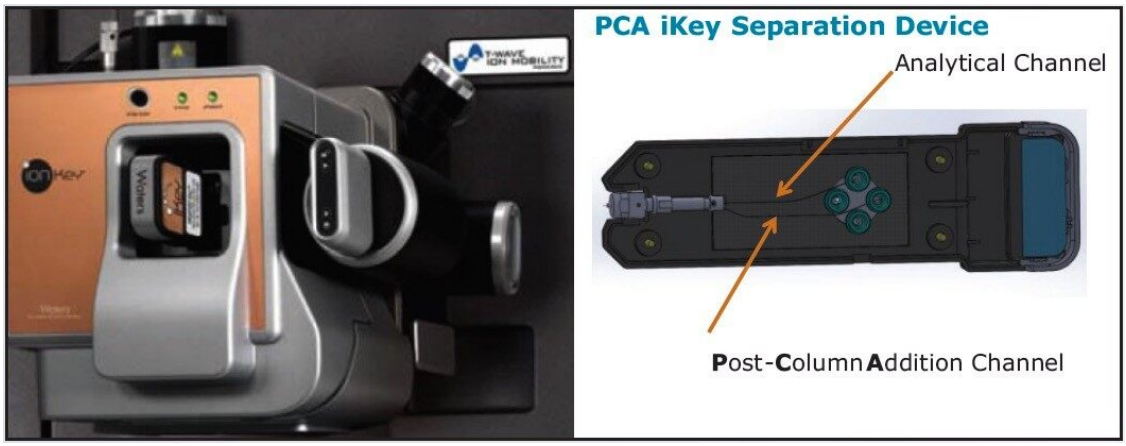

Figure 2. ionKey Source and PCA iKey Separation Device incorporating fluidic/electronic connections with ionization emitter.

Results and Discussion

The ionKey/MS System ion mobility screening was performed in positive and negative modes to analyze the hydromethanolic extracts of *P.incarnata, P.edulis, P.caerulea*, and *P.alata*. In a previous study using UPLC and ion mobility, the extracts were diluted 40:1.[2] For the analysis performed it has been possible to dilute the samples further to 400:1. Also separate aqueous high purity standard solutions (0.1 pg/µL to 10 pg/µL) of isovitexin, vitexin, isoorientin, and orientin were used to determine sensitivity and linear response. The iKey Separation Device gradient employed initial conditions at 99% aqueous. Using a post column addition solvent (IPA), enabled a single voltage of 2.6 kV to be employed throughout the chromatographic gradient, while maintaining stable spray conditions and stable ionization of the analytes exiting the iKey Separation Device. In Figure 3 the complexity of the samples analyzed is illustrated. The base peak ion chromatogram illustrates the conventional view of the complexity of the sample profiled. A series of flavonoid isomers have been selected to clearly illustrate the chromatographic performance obtained using both iKey Separation Device designs, as shown in Figure 4. The ionKey/MS System’ s positive and negative mode extracted exact mass chromatograms for a series of isomeric flavonoids determined to be present in 400:1 diluted *Passiflora edulis* extract are presented. The chromatographic profile obtained is shown to be comparable using the iKey Separation Device and PCA iKey Separation Device.

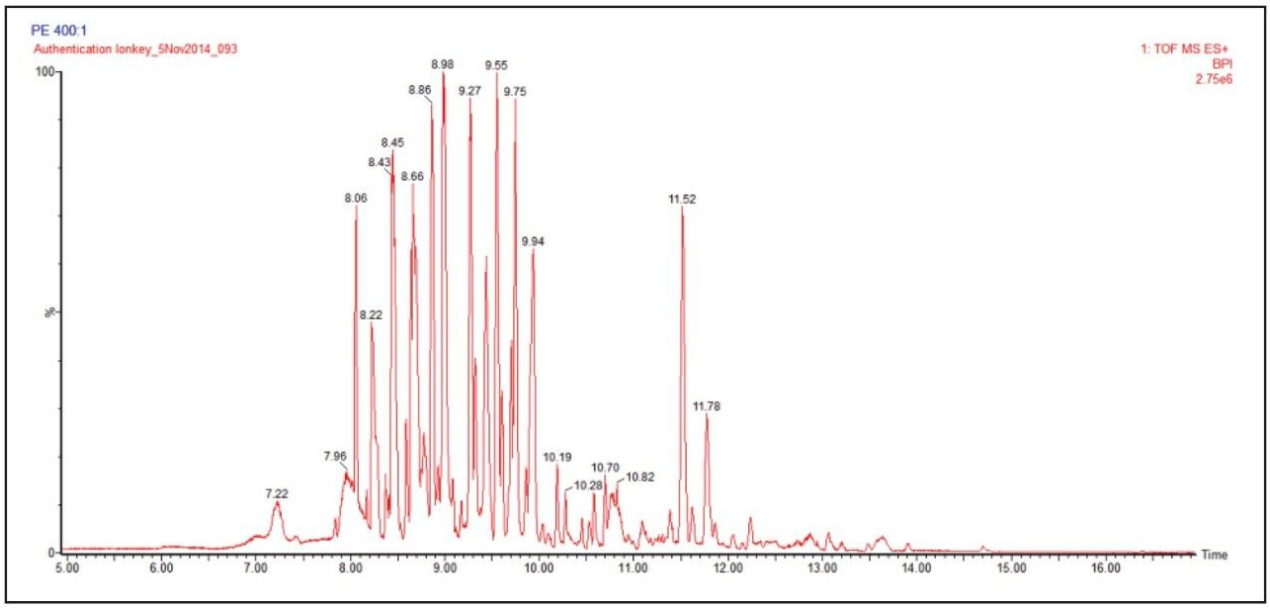

Figure 3. The ionKey/MS System ion mobility positive mode base peak ion chromatogram obtained for analysis of 400:1 diluted Passiflora edulis extract. The base peak ion chromatogram illustrates the conventional view of the complexity of the sample profiled.

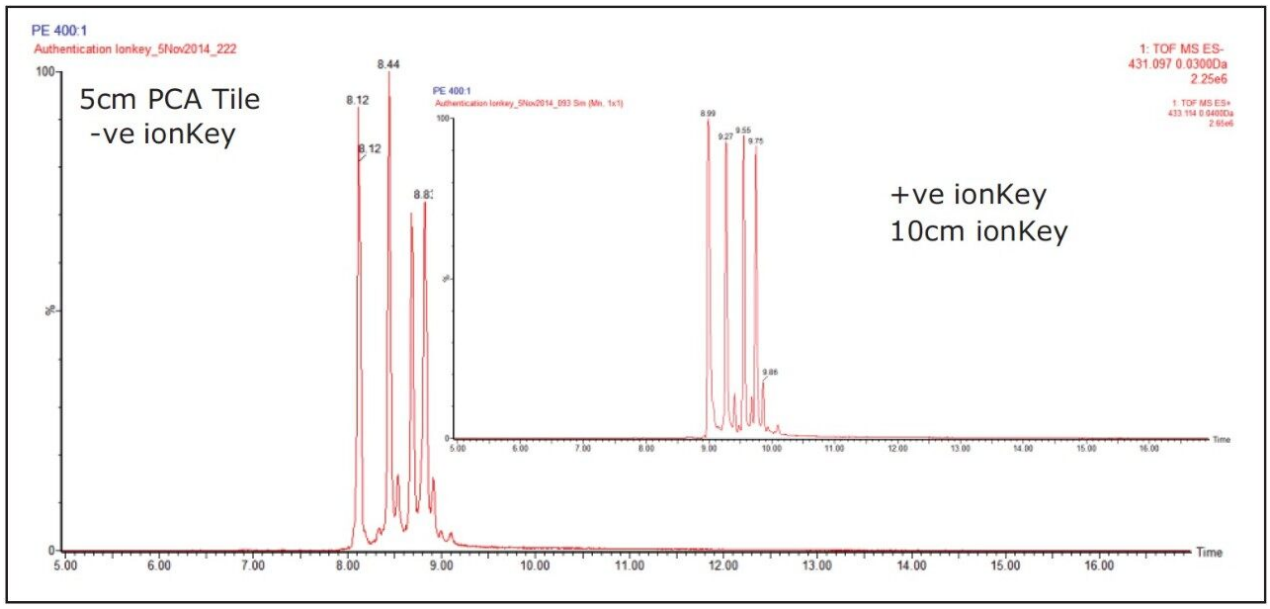

Figure 4. The ionKey/MS System ion mobility positive and negative mode extracted exact mass chromatogram for a series of isomeric flavonoids determined to be present in 400:1 diluted Passiflora edulis extract. The chromatographic profile obtained is shown to be comparable using conventional and PCA microfluidic tiles.

Figure 5 shows that the linear response obtained for orientin and vitexin solvent standards (0.1 pg/µL to 10

pg/µL) using positive and negative mode on the ionKey/MS^{E} System is equivalent. Excellent correlation coefficients of R^2>0.99 have been acquired over three orders of dynamic range. The data shows the potential of using the ionKey/MS System with benefits of full spectral acquistion attained using time-of-flight mass spectrometry at low detection levels; in this case at 100 fg/µL.

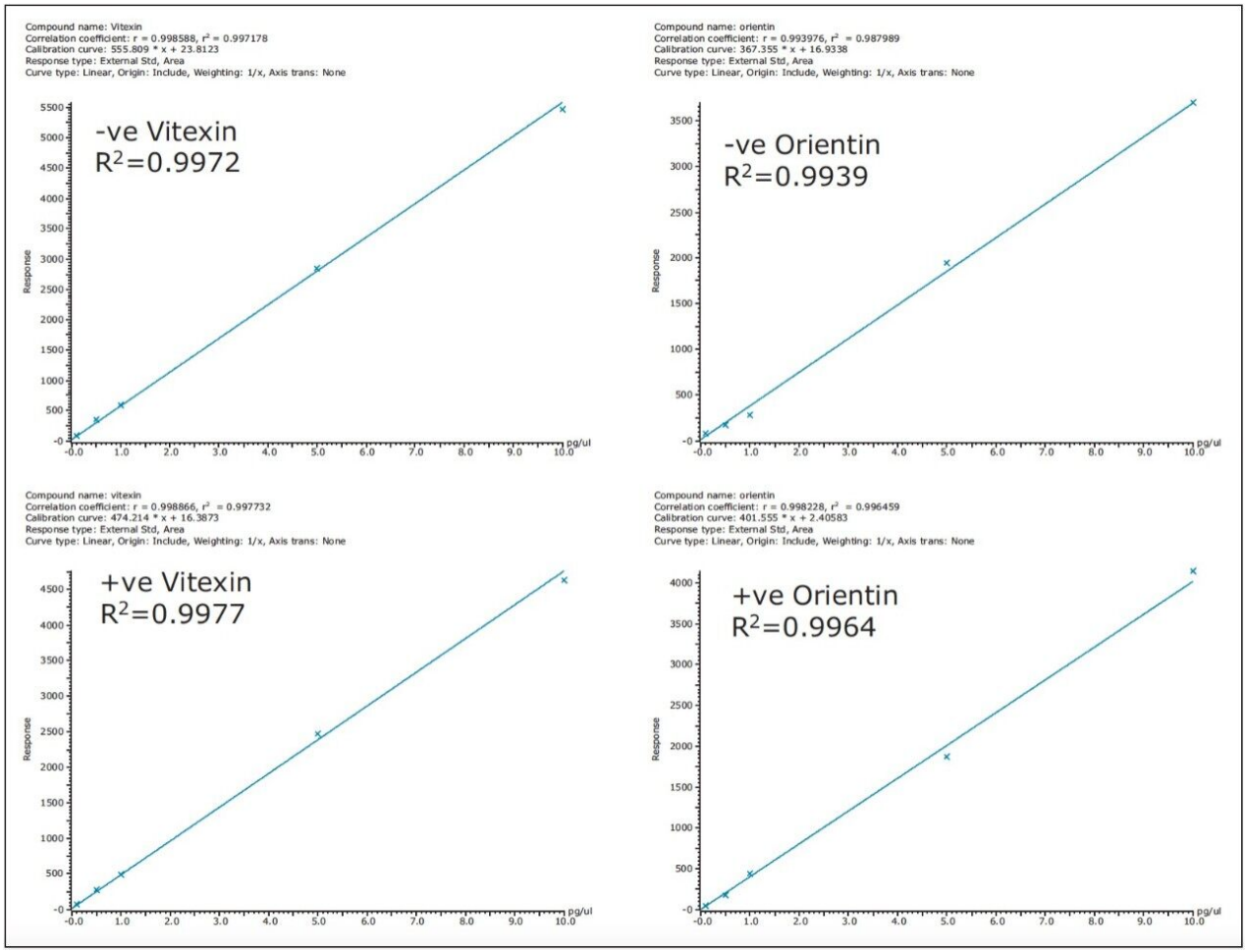

Figure 5. Illustration of linearity obtained for orientin and vitexin solvent standards (0.1 pg/µL to 10 pg/µL) using positive and negative mode on the ionKey/MS System. Correlation coefficients of r^2>0.99 have been obtained.

Utilizing the extended functionality of the SYNAPT G2-Si, it shown in Figures 6 and 7 that using positive and negative mode the ionKey/MS System ion mobility IM-MS,^{E} it is also possible to retain sensitivity as illustrated for isovitexin solvent standard over the concentration range 0.1 pg/µL to 10 pg/µL.

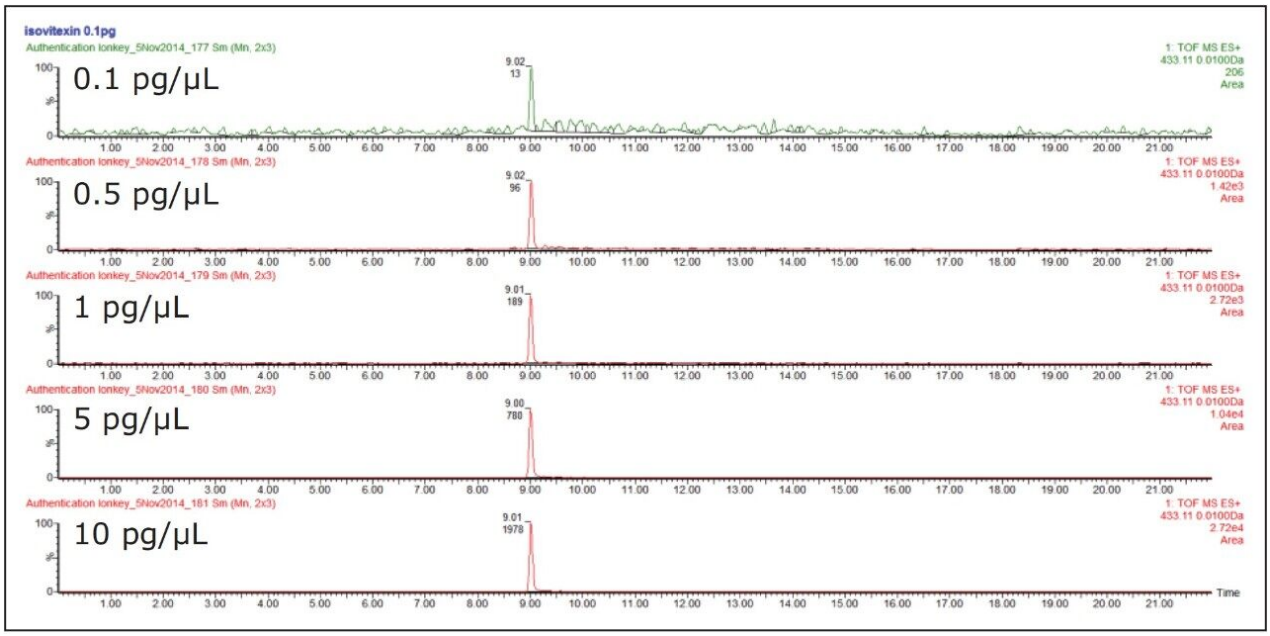

Figure 6. Illustration of the sensitivity, peak area response, and chromatographic integrity obtained for isovitexin using positive ion mode on the ionKey/MS System ion mobility (HDMS^E DRE).

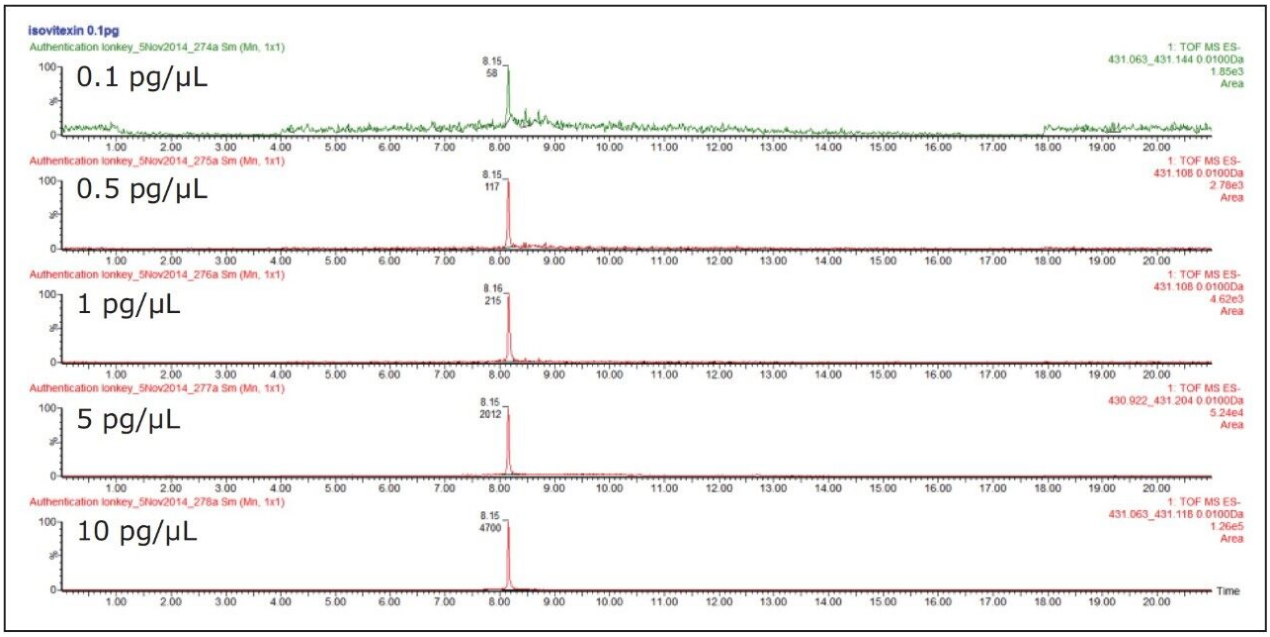

Figure 7. Illustration of the sensitivity, peak area response and chromatographic integrity obtained for isovitexin using negative ion mode on the ionKey/MS System ion mobility (HDMS^E DRE).

Under the chromatographic conditions used, vitexin and isovitexin coelute. In negative ion mode both the

6C glycoside isomers (isoorientin/orientin) and the 8C glycoside isomers (isovitexin/vitexin), can be separated using ion mobility. They can also be identified from their CCS values obtained using nitrogen based travelling ion wave mobility ($^{TW}CCSN_2$).

Hence it is possible to generate the individual calculated concentrations for two isomeric species (isovitexin/vitexin) that coelute at the same retention time using ion mobility, as shown in Figure 8. For the first time, two coeluting isomeric species have been quantified using precursor ion selection. This has been acheived because the flavonoids are ion mobility separated, which could not be acheived using only the selectivity of mass accuracy. In addition, the UNIFI Software Component Summary is presented where a mass accuracy of <1 ppm has been obtained, as well as CCS measurements within 0.21% of the expected $^{TW}CCSN_2$ values.

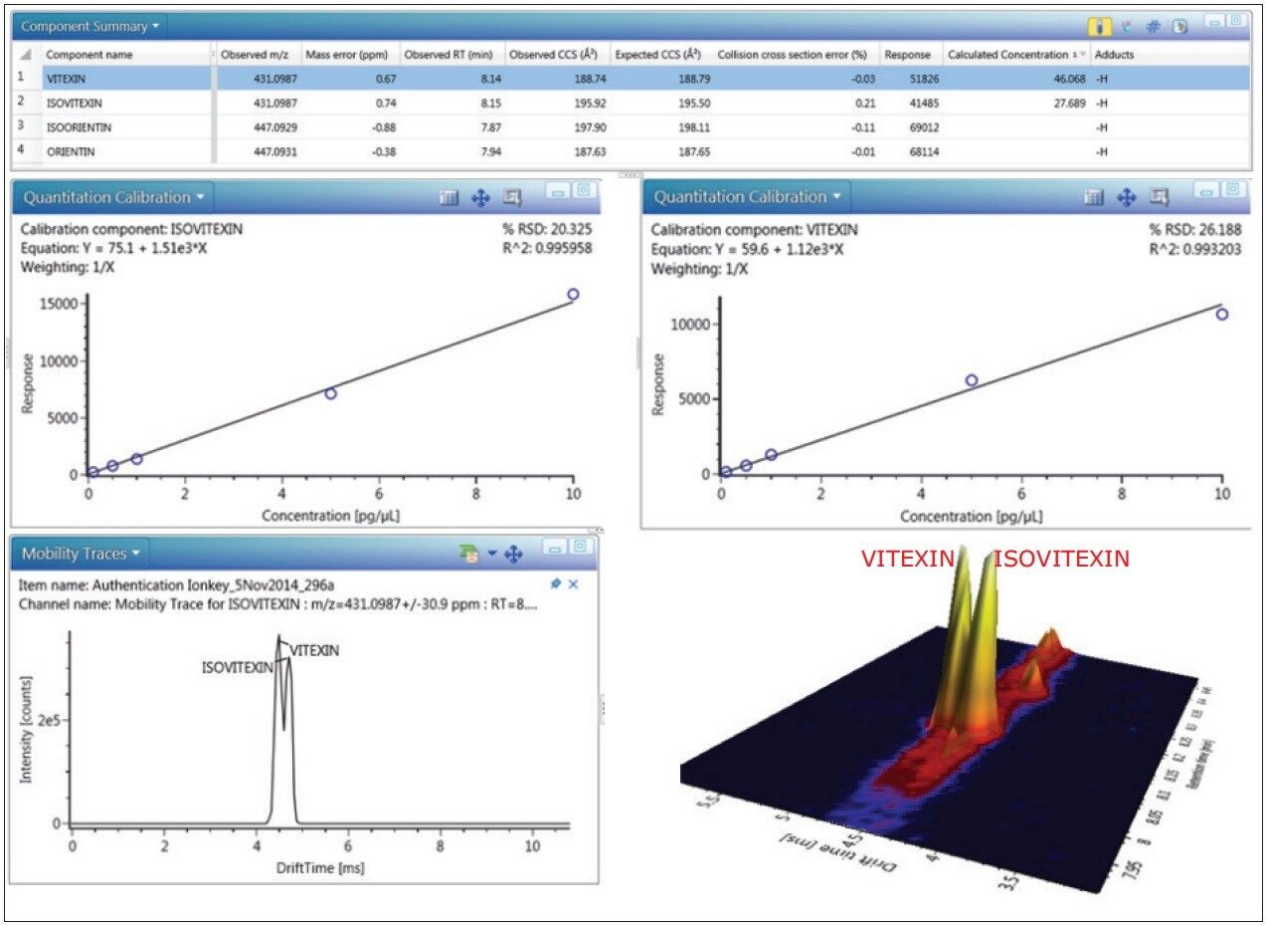

Figure 8. UNIFI Component Summary for Passiflora incarnata, illustrating coeluting vitexin and isovitexin linearity, with mass measurement error <2ppm. CCS errors <0.5% are presented. The ion mobility trace and ion mobility data viewer 3D plot of drift time versus retention time are also presented.

The flavonoid library was created within the UNIFI Scientific Library 17 months before this assay was

performed, illustrating the reproducibility and precision that can be obtained using ion mobility. The combination of the ionKey/MS System and ion mobility offers the potential of high selectivity, specificity, and sensitivity, as well as practical aspects such as reduced solvent and sample consumption. Using $^{TW}CCSN_2$ as an additional identification parameter also has the potential to reduce the reliance on retention time based confirmations and hence the need to purchase expensive high purity standards (1 mg vitexin 98% pure= £144.50, and 1 mg isovitexin 98% pure= £144.50).

Conclusion

- Using positive and negative mode on the ionKey/MS System, comparable linearity and sensitivity has been illustrated for flavonoid solvent standards (0.1 pg/µL to 10 pg/µL). Correlation coefficients of r^2 >0.99 have been obtained.
- The ionKey/MS System has been used to screen and profile flavonoid markers in complex extracts of *Passiflora edulis, alata, caerulea*, and *incarnata*.
- In negative mode the isomeric 6C glycosides (isoorientin/oreintin) and 8C glycosides (isovitexin/vitexin) were distinguished using collision cross section measurements.
- CCS measurements within 0.5% have been obtained routinely, when compared to a $^{TW}CCSN_2$ library produced more than a year earlier.
- For the first time, chromatographically coeluting isobaric flavonoids have been separated using ion mobility mass spectrometry, enabling the individual calibration curves for isovitexin and vitexin to be obtained.
- The ionkey/MS System ion mobility offers potential cost savings as an analytical approach, through the reduced consumption of solvents and expensive high purity standards where $^{TW}CCSN_2$ can reduce the reliance on retention time confirmation.

References

1. The use of collision cross section measurements (CCS) in food and environmental analysis. Waters Technical Note 720005374en, 2015.
2. M McCullagh, K Neeson, J Goshawk, C A M Pereira, J H Yariwake, C Carver, and D Douce. Using the routine separation dimension and identification criteria of UPLC ion mobility to enhance specificity in

profiling complex samples. Poster shown at ASMS Baltimore, Waters Library Number: PSTR134803078. June, 2014.

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