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ionKey/MS Ion Mobility: A New Approach to Authentication and Routine Screening of Ginsenoside Isomers in Functional Food Products

Michael McCullagh, Ramesh Rao, John Chipperfield, and David Douce
Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

- Novel approach for authentication profiling.
- Unique, non-targeted screening workflow to determine the presence of ginsenoside markers, including pairs of ginsenoside isomers.
- Data processing capability of the UNIFI® Scientific Information System can routinely be used to enable characteristic assignment for ginsenoside isomers.
- ionKey/MS System ion mobility $^{TW}CCSN_2$ screening has the potential to produce significant cost saving with reduced consumption of solvents and high purity standards.
- Significant cost savings through reduced consumption of solvents and high purity standards.

WATERS SOLUTIONS

ionKey/MS System

ACQUITY UPLC® M-Class System

Post Column Addition (PCA) iKey™ Separation Device

Waters® Ion Mobility Mass Spectrometry Systems

UNIFI Scientific Information System

KEY WORDS

microflow, ginsenoside isomers, CCS, ion mobility, spectral clean up, enhanced sensitivity, authenticity, functional food, dietary supplements

INTRODUCTION

The potential improvements that can be obtained using microflow liquid chromatography and mass spectrometry in a positive ion mode assay to analyze pesticides in food commodities have previously been discussed.[1] The benefits of using the ACQUITY UPLC M-Class System with ion mobility mass spectrometry (IM-MS) to authenticate and routinely screen ginsenoside isomers in functional food products has also been previously reported.[2] The Post Column Addition (PCA) iKey Separation Device can be used to add solvent after chromatographic separation. This enables much more analytical flexibility including the ability to increase sensitivity for negative mode at microflow and flow rates that use gradients containing a high percentage of aqueous solvent. The addition of organic solvent (in this case IPA), via the post column addition flow path improves the sensitivity of the assay.

The analysis of ginsenosides in food commodities is a suitable assay to explore the feasibility of analyzing complex samples using the PCA iKey Separatiuon Device (p/n 186007580) in negative mode, since the targeted ginsenosides ionize efficiently in negative mode. Simultaneously IM nitrogen-based traveling wave collision cross section ($^{TW}CCSN_2$) screening reproducibility can be explored in combination with time-of-flight mass spectrometry's full spectral acquisition. Utilizing microflow chromatography for this application area has many potential benefits, since recent legislative focus has prompted new methods for the analysis of active compounds in these products. While the growing global popularity of nutraceutical and functional food products continues to increase, for the European Union, current legislation aims to protect consumers from possible damaging side effects of over-the-counter herbal medicines and functional foods that are intended to deliver therapeutic benefits. EU Directive 2004/24/EC, came into full effect on 30 April 2011.

Ginseng is one of 11 species of slow-growing perennial plants with fleshy roots, that belong to the genus Panax of the family Araliaceae. The most abundant forms of ginseng Panax ginseng (Korean Ginseng), *P. Japanoicus*, and *P. Quinquefolium* (American Ginseng) grow in North America. Ginseng is believed to offer different therapeutic benefits. Ligor et. al. discussed CNS stimulant activity, hypogylcemic properties, and the sedative effects of American Ginseng.[3]

For each species it is believed that the ginsenoside and polysaccharides content are responsible for the biological activity of products produced from the roots and leaves of ginseng species. Figure 1 shows the structures of the ginsenosides screened in this assay, which are part of a diverse group of steroidal saponins.

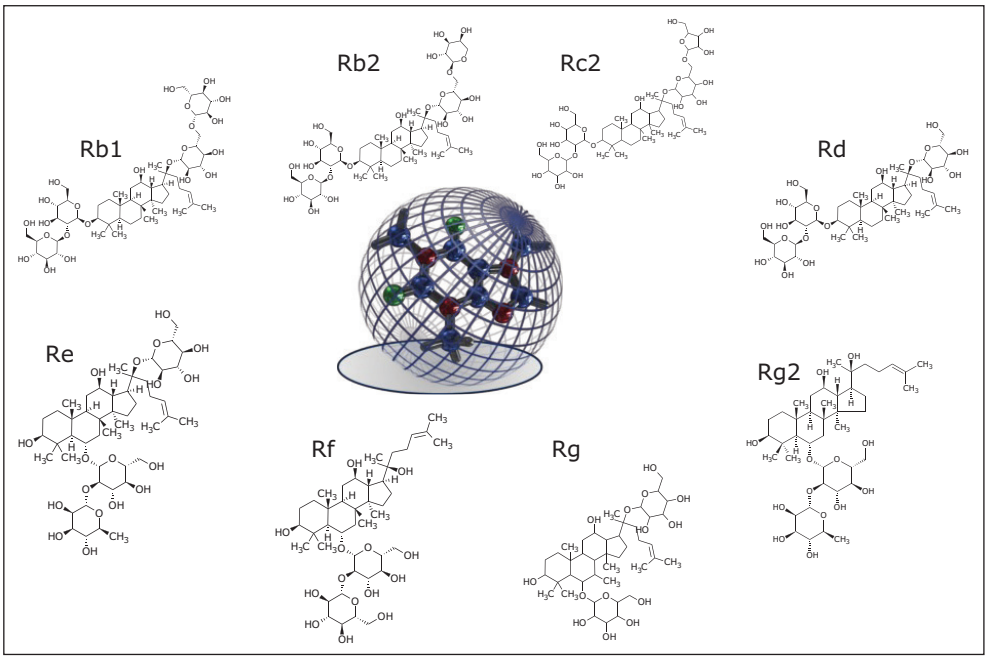

Figure 1. Illustration of rotating three-dimensional conformation of an ion and average collision cross section (CCS) (shadow). Structures of the ginsenosides profiled using microflow UPLC ion mobility mass spectrometry and CCS screening.

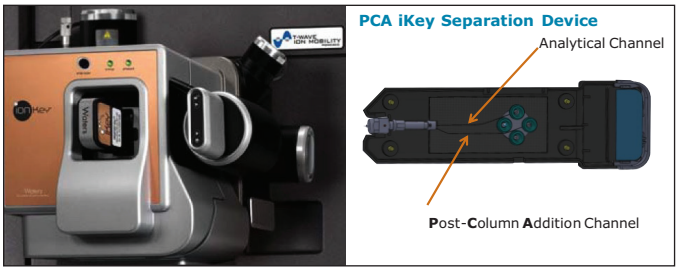

Figure 2. ionKey/MS Source and PCA iKey Separation Device incorporating fluidic/electronic connections and ionization emitter.

Ion mobility 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. ionKey/MS IM-MS is a combination of high resolution mass spectrometry and high efficiency ion mobility based measurements with UPLC® separations that offers some unique advantages for profiling complex mixtures. In this application note, we investigate the use of IM separation in combination with microflow chromatography to provide a route to specific and unambiguous identification of ginsenosides, where a PCA iKey Separation Device has been used to perform the assays using negative mode ionization.

A PCA iKey Separation Device (p/n 186007580), shown in Figure 2, 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 40 °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 in place. The PCA iKey Separation Device incorporates an additional channel that enables post column addition of IPA solvent. The makeup solvent was configured to be delivered from channel A of the MS system fluidics for this study.

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)

Gradient:

Time (min)	Flow rate	%A	%B
0.00	2	97.0	3.0
1.00	2	97.0	3.0
3.00	2	95.0	5.0
5.00	2	85.0	15.0
13 .00	2	1.0	99.0
15 .00	2	1.0	99.0
15.10	2	97.0	3.0
17.00	2	97.0	3.0

Flow rate:	iKey at 2.0 µL/min
Injection volume:	1 µL (full loop)
Separation device:	iKey BEH C_{18} PCA Separation Device, 130Å, 1.7 µm, 150 µm x 50 mm (p/n 186007580)
Separation device temp.:	40 °C

MS conditions

MS system:	SYNAPT® G2-S*i*
Ionization mode:	ESI-
Capillary voltage:	2.6 kV
Sample cone voltage:	30 V
Lockmass:	Leucine enkephalin
LockCCS:	$[M-H]^-$ =554.2620
Acquisition range:	50 to 1200 *m/z*
Acquisition rate:	10 spectra/sec
Collision energy ramp:	30 to 70 eV
Resolution:	0,000 FWHM (Res Mode)
Default IMS parameters:	IMS T-Wave™ velocity ramp: Start=1000 m/s End=300 m/s
IMS T-Wave pulse height:	40 V
IMS gas flow:	90 mL

Sample description

Korean ginseng tea (extracted into 20 mL of H_2O x 10 dilution), ginkgo biloba+red panax extract (x10 dilution), red panax extract (x10 dilution), and ginsenoside standards (100 pg/µL).

RESULTS AND DISCUSSION

A PCA iKey Separation Device, coupled with IM-MS was successfully used to profile ginsenosides Rb1, (Rb2, Rc), (Rd, Re), (Rf, Rg1), and Rg2. IPA at 1 µL/min flowed into the PCA channel and a MS ES voltage of 2.6 kV was applied. IM-MS was used to generate $^{TW}CCS_{N_2}$ values, precursor ion accurate mass, accurate mass mobility product ions, and retention times. Using $^{TW}CCS_{N_2}$ measurements can increase targeted screening specificity.

A ginsenoside CCS scientific library within UNIFI was previously generated.[2] This allows the expected and previously determined CCS values to be utilized to screen and confirm the presence of isomeric flavonoid markers. Three extracts: gingko biloba+red panax, red panax, and Korean ginseng were screened against the library in order to determine their presence, and unequivocally identify isomeric ginsenosides. CCS values (derived from ion mobility drift times) are used as an identification parameter that can distinguish ginsenoside isomers, as well as to profile unknowns.

In Figure 3, the ionKey/MS System IM negative mode base peak ion chromatogram obtained for analysis of 10:1 diluted Korean ginseng tea extract is shown. Figure 3 depicts a conventional view of the complex sample profiled. However, in Figure 4 the ionKey/MS System IM negative mode plot of the drift time (ion mobility resolution), versus the retention time for 10:1 diluted Korean ginseng extract is presented. Leveraging the unique software functionality of UNIFI.

Figure 4 visually illustrates how an orthogonal separation to the chromatographic separation is achieved with ion mobility, and the increased peak capacity that is possible. The retention time region between 5 and 14 minutes in Figure 4 shows there are a large number of compounds that are now resolved, compared to the same region on the conventional base peak ion extracted mass chromatogram shown im Figure 3.

The ion mobility data viewer in UNIFI enables investigative interaction with acquired ion mobility data. UNIFI incorporates many easy-to-use features, such as Zoom to Component and Bookmark, that enable the same investigative interrogation of data to be applied across many acquisitions. It is possible to select any one of these components and generate the drift plot, mass spectrum, and extracted mass chromatogram.

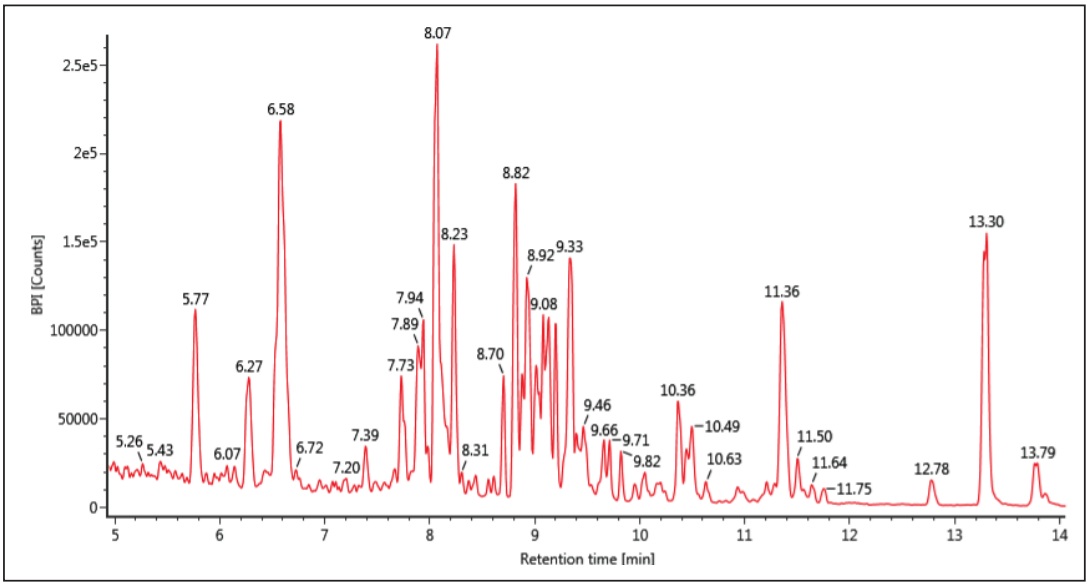

Figure 3. ionKey/MS System IM negative mode base peak ion chromatogram obtained for analysis of 10:1 diluted Korean ginseng extract.

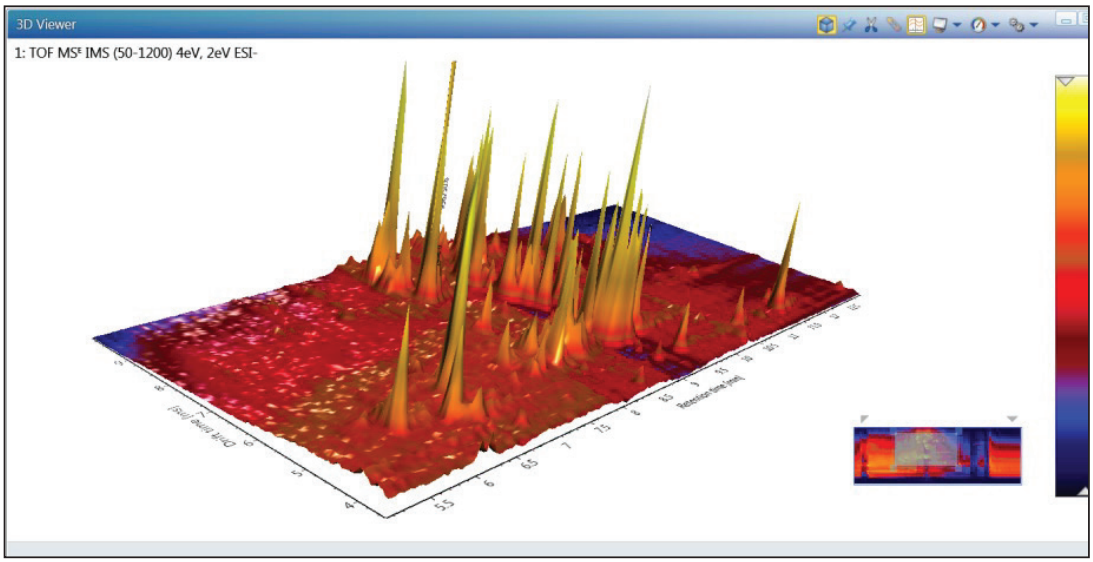

Figure 4. ionKey/MS System ion mobility negative mode plot of drift time (ion mobility resolution) versus retention time for 10:1 diluted Korean ginseng extract.

The true complexity of the profiled sample is illustrated when both ion mobility and UPLC chromatographic resolution are combined. Ginsenoside isomers Rg1 *m/z* 845.4897 (green) and Re *m/z* 991.5484 (brown) in Figure 5 are chromatographically coeluting at 8.058 minutes. Figure 5 shows the combined precursor and fragmentation spectra data of the two coeluting ginsenoside components.

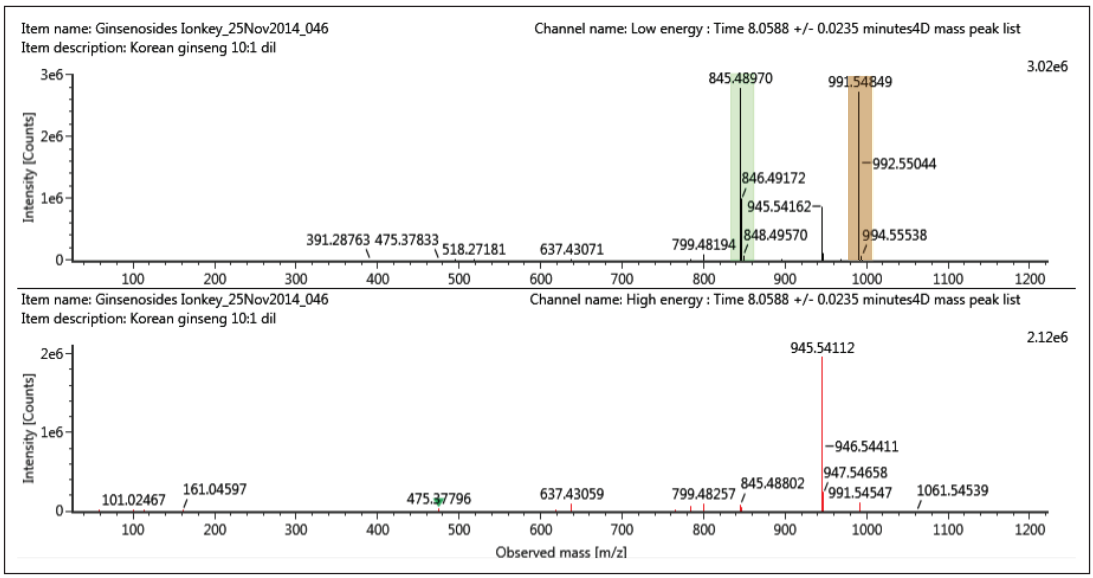

Figure 5. Retention time aligned precursor and fragmentation spectra for Rg1 ginsenoside at m/z 845.4897 (in green) coeluting with Re ginsenoside at m/z 991.5484 (in brown).

In Figure 6 the single component retention time aligned and drift time aligned ion mobility product ion spectrum for the ginsenoside isomer Rg1 is presented, further illustrating the utility of ion mobility separations.

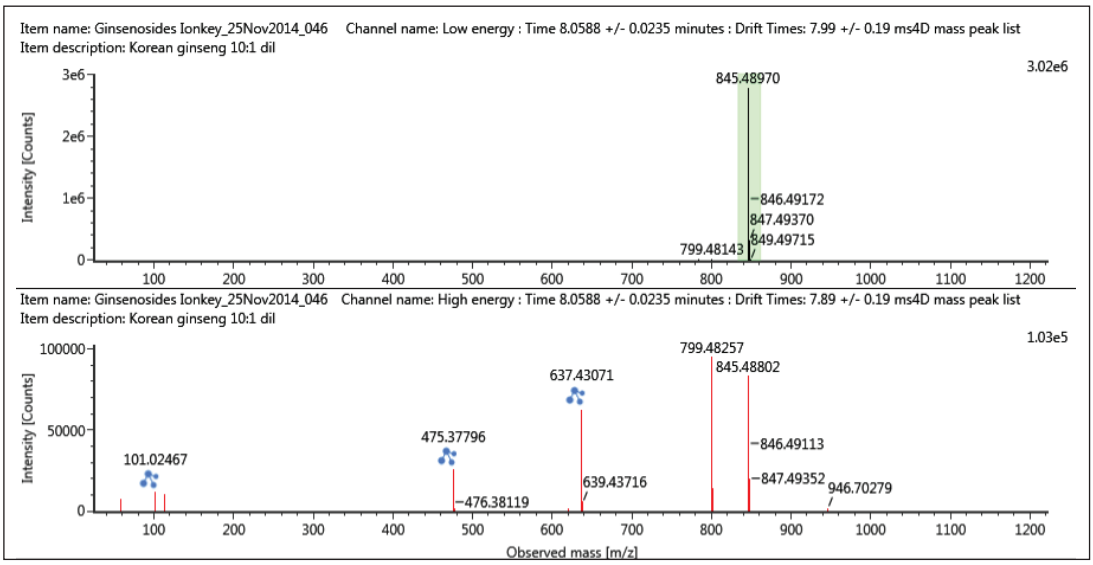

Figure 6. Retention time (8.058 mins) and drift time (7.99 ms) aligned precursor and ion mobility product ion spectrum for Rg1 ginsenoside at m/z 845.4897 (in green).

In Figure 7 the ion mobility separated ginsenoside Re:A and ginsenoside Rg1:B mobility peaks are presented. Here, the single component precursor/ion mobility product ions are obtained, even though they chromatographically coelute with other compounds present in the complex ginseng extract. $^{TW}CCSN_2$ measurements can increase confidence in identification. The results are summarized in the UNIFI Component Summary shown in Figure 8, where the values obtained for the profiling of Korean ginseng are presented. For the marker ginsenoside isomer pairs (Rb2, Rc), $^{TW}CCSN_2$ measurements of 355.24 Å^2/344.50 Å^2, (Rd, Re), 328.31 Å^2/323.46 Å^2, and 301.60 Å^2/292.03 Å^2 (Rf, Rg1) were obtained. The $^{TW}CCSN_2$ measurement errors were typically <2%, when compared to the study performed using UPLC-IM-MS in 2013.[2] This further confirms that it is possible to confidently distinguish, the marker isomer pairs of ginsenosides from the extracts of the specified products analyzed, using $^{TW}CCSN_2$ measurements.

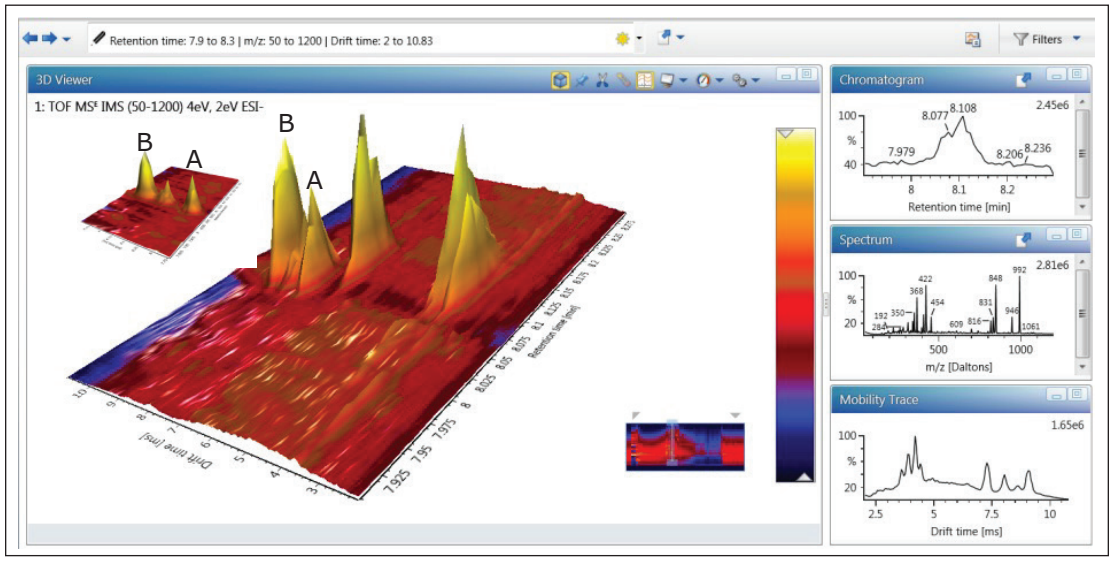

Figure 7. Illustration with expanded view of ion mobility separated ginsenoside Re:A and ginsenoside Rg1:B that are chromatographically coeluting.

This approach offers unique selectivity for profiling complex mixtures. The results obtained clearly show the benefits of using CCS measurements and the combined peak capacity of ionKey/MS System with IM. Coeluting analytes and isomers have been resolved, as well as unequivocally identified in the three extracts profiled. In addition, it is possible to acquire the cleaned up mobility specific product ion spectra, that are mobility resolved from coeluting components. This approach has the potential to change the scope of authentication profiling.

The added confidence in making identifications using $^{TW}CCSN_2$ measurements has the potential to reduce the need to use expensive high purity standards, where assay confirmation relies on retention time and accurate mass measurement. The cost of purchasing 10 mg of each standard for the assay performed totalled £2483.00, a significant cost undertaking. Cost savings across many application areas can be made.

	Component name	Observed m/z	Mass error (ppm)	Observed RT (min)	Observed CCS (Å^2)	Expected CCS (Å^2)	Collision cross section error (%)	Response	Adducts
1	GINSENOSIDE Rb1	1153.5980	-2.71	8.94	351.21	357.18	-1.67	10061	+HCOO
2	GINSENOSIDE Rb2	1123.5864	-3.70	9.13	355.24	361.50	-1.73	16580	+HCOO
3	GINSENOSIDE Rc	1123.5869	-3.26	9.02	344.50	350.36	-1.67	13319	+HCOO
4	GINSENOSIDE Rd	991.5475	-0.81	9.34	328.31	333.12	-1.44	63194	+HCOO
5	GINSENOSIDE Re	991.5485	0.17	8.06	323.46	329.11	-1.72	117685	+HCOO
6	GINSENOSIDE Rf	845.4902	-0.19	8.81	301.60	306.13	-1.48	58490	+HCOO
7	GINSENOSIDE Rg1	845.4897	-0.84	8.06	292.03	296.15	-1.39	111075	+HCOO
8	GINSENOSIDE Rg2	829.4946	-1.12	9.04	296.63	300.54	-1.30	14448	+HCOO

Figure 8. UNIFI Component Summary obtained for Korean ginseng extract. CCS measurements have been obtained within 2% of the ginsenoside CCS library created November 2013.

CONCLUSIONS

- UPLC and ion mobility mass spectrometry have been used to screen and determine ginsenoside Rb1, (Rb2, Rc), (Rd, Re), (Rf, Rg1), and Rg2 phytochemical makeup in Korean ginseng, gingko biloba+red panax, and red panax extracts.
- Isomeric ginsenosides have been differentiated using IM-MS and $^{TW}CCS_{N_2}$ measurements.
- $^{TW}CCS_{N_2}$ screening can be used to profile sample makeup and uniquely differentiate ginsenoside isomer composition, increasing confidence that ginsenosides are not missed due to chromatographic coelution.
- $^{TW}CCS_{N_2}$ measurements of <2% have been obtained routinely, when compared to a library produced in October 2013.
- The ionKey/MS System offers a significant improvement in sensitivity, a reduction in matrix effects, and cost savings through the reduced consumption of solvents and high purity standards.

References

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34 Maple Street
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