QUICK NOTE

Pushing the Limits of High-Resolution Ion Mobility (HRIM) for Catechin Epimer Analysis

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The Objective

To demonstrate the utility of High-Resolution Ion Mobility (HRIM) to separate known epimers in green tea extracts.

The Challenge

Green tea is a beverage of broad interest due in large part to the presence of polyphenols in the leaves of *Camellia sinensis*.[1] The beneficial effects of green tea are attributed to the presence of number catechins, including non-esterified (+)-catechin (C), (-)-epicatechin (EC), (-)-gallocatechin (GC) and (-)-epigallocatechin (EGC) and the esterified (-)-catechin gallate (CG), (-)-gallocatechin gallate (GCG), (-)-epi- catechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG).[2] The four catechins that account for the majority of the components of green tea include EGCG, ECG, EGC, and EC with the potential of conversion to the non-epimeric forms during tea leave processing.[3,4] As a result, it is necessary to evaluate epimerization during various physical processing to improve the nutritional value of green tea products. Although the catechin profile in green tea has been extensively studied, method agnostic characterization of the full catechin profile remains limited, representing a shortcoming in the study of health benefits of various epimers and processing impacts on epimerization.

The Answer

The application of HRIM to food science provides several advantages including the separation of isobaric and isomeric compounds, potential increases in method sensitivity by reducing background noise, and the addition of collision cross section (CCS) as a complementary identifier to mass spectra. A seven-minute liquid chromatographic (LC) method was compared to a two-minute flow injection analysis (FIA) method on an Agilent 1290 and 6545XT Q-TOF equipped with the MOBIE® HRIM system from MOBILion Systems to analyze the standard catechin mix shown in Figure 1.

The Key Takeaway

The MOBIE® HRIM platform separates epimers found in green tea extracts and provides additional confidence in compound determination through the use of CCS measurements to more effectively characterize nutritional value.

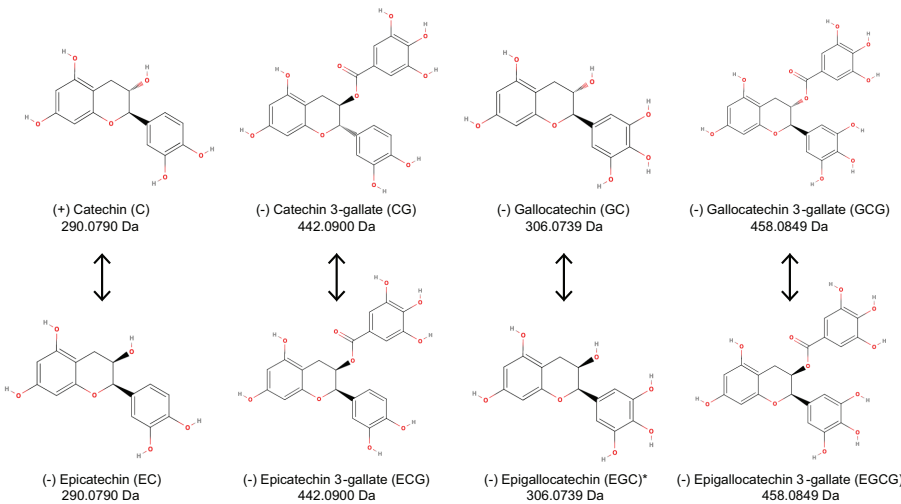

Figure 1. The primary catechins found in green tea extract and their isomeric forms. *EGC is the only compound shown not commercially available in the standard mix and was not included in the present study.

Figure 2A demonstrates the separation of the seven catechins present in the standard mix represented by the normalized base peak chromatogram (BPC). The ion mobility of each standard compound was extracted and the resulting extracted ion mobiligrams (EIMs) displayed in Figure 2B highlight the ability of HRIM to reveal distinct arrival times (measured in milliseconds) of the epimeric catechin pairs in the mobility dimension less C and EC. Each compound has a unique arrival time in the mobility dimension based on size, shape, and charge, which can be converted into collision cross section values. For C/EC, the measured CCS values differ by only 0.01%, while CG/ECG and GCG/EGCG differ by 0.30% and 0.61%, respectively (see Table 1). The HRIM system from MOBILion Systems has previously demonstrated capability to resolve compounds with 0.5% CCS difference or greater, supported by the empirical data presented here. The same standard mix of catechins was then analyzed using a two-minute FIA-HRIM method and the resulting normalized EIMs are shown in Figure 2C. Partial separation of the largest epimeric pair, GCG/EGCG, was maintained with a 3.5-fold reduction in acquisition time.

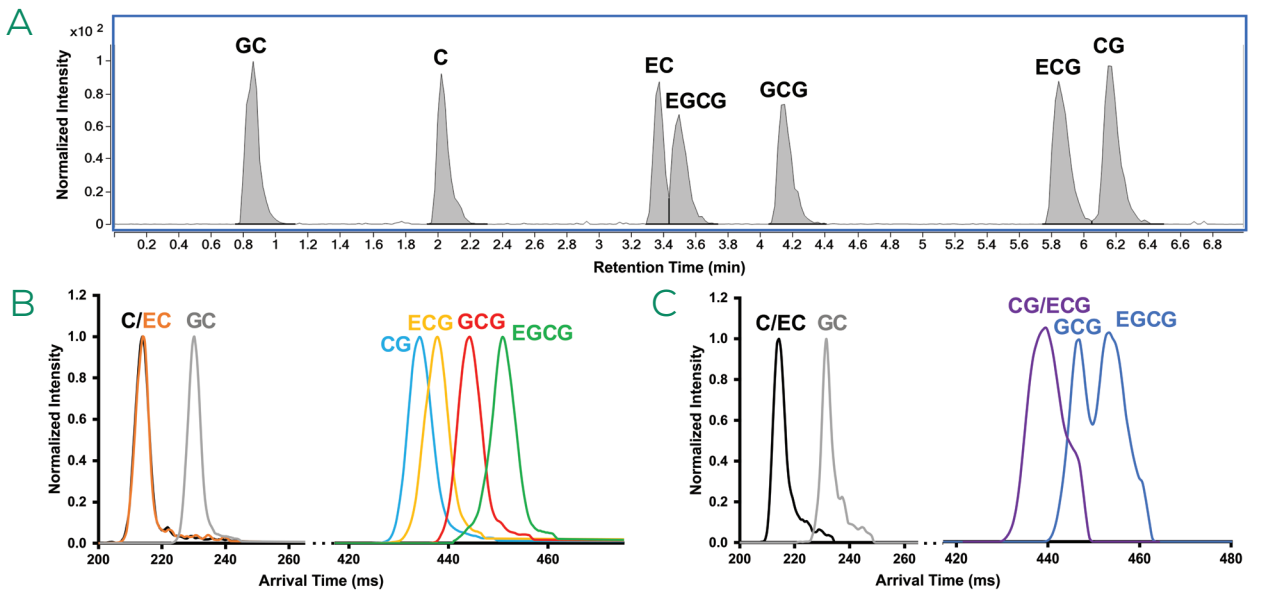

Figure 2. (A) Normalized BPC for the identified catechins using a seven-minute LC gradient. (B) Extracted ion mobiligrams of the individual compounds from (A) highlighting unique arrival times for each. (C) Total ion mobiligram of the same catechin standard mix analyzed using FIA. Abbreviations: C, catechin; EC, epicatechin; GC, gallocatechin; CG, catechin gallate; ECG, epicatechin gallate; GCG, gallocatechin gallate; EGCG, epigallocatechin gallate.

The unique arrival times and derived CCS values of the standard catechin mix are compiled in Table 1. From five technical replicates, the %RSD of the CCS values were less than or equal to 0.01% for all catechins in the standard mixture, indicating a high degree of repeatability using the MOBIE HRIM system. As CCS values are inherent to an ion's size, shape, and charge, these highly reproducible values can increase the confidence of identification of these compounds across matrices and method parameters.

Compound	*m/z*	Arrival Time (ms)	CCS (Å²)	CCS %RSD
Catechin (C)	289.0712	213.82	154.86	0.010
Epicatechin (EC)	289.0712	213.86	154.88	0.007
Gallocatechin (GC)	305.0661	230.19	158.10	0.008
Catechin gallate (CG)	441.0822	434.24	196.93	0.009
Epicatechin gallate (ECG)	441.0822	437.53	196.93	0.003
Gallocatechin gallate (GCG)	457.0771	444.12	197.93	0.005
Epigallocatechin gallate (EGCG)	457.0771	451.02	199.14	0.004

Table 1. Compound name, *m/z*, arrival time in milliseconds, CCS in square angstroms, and %RSD for all included catechins (n = 5).

Summary

HRIM is a gas phase separation technology that provides an additional dimension of separation based on size, charge, and structure. This work demonstrates the benefit of incorporating HRIM as an orthogonal technique to LC and HRMS, yielding deeper insights into the separation and characterization of green tea extract epimers. For epimeric pairs of catechins, the additional dimension and high degree of reproducibility of CCS values result in greater confidence in compound identification.

References

1. Nain, C.W., et al., *The Catechins Profile of Green Tea Extracts Affects the Antioxidant Activity and Degradation of Catechins in DHA-Rich Oil. Antioxidants* (Basel), 2022. **11**(9).

2. Kah Hui, C., et al., *Catechin profile and hypolipidemic activity of Morinda citrifolia leaf water extract.* Heliyon, 2020. **6**(6): p. e04337.

3. Park, J.E., T.E. Kim, and K.H. Shin, *Quantitative Analysis of Four Catechins from Green Tea Extract in Human Plasma Using Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry for Pharmacokinetic Studies.* Molecules, 2018. **23**(4).

4. Liu, X., et al., *Reactivity of flavanols: Their fate in physical food processing and recent advances in their analysis by depolymerization.* Compr Rev Food Sci Food Saf, 2021. **20**(5): p. 4841-4880.