QUICK NOTE

Revealing Novel Compounds in the Biochemical Hemp Haystack Using High-Resolution Ion Mobility

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The Objective

To demonstrate the ability of high-resolution ion mobility (HRIM) to enhance existing chromatographic methods and simplify complex sample analysis.

The Challenge

The ongoing quest for sustainable and effective therapeutic agents has increasingly pointed researchers towards the vast pharmacopeia of natural extracts.[1] The development of medicines from natural extracts presents a myriad of challenges, beginning with the complex task of novel compound identification. Owing to the immense chemical diversity and complexity within these extracts, pinpointing bioactive compounds, let alone characterizing their precise structures and biological functions, can be akin to finding a needle in a biochemical haystack. Analytical challenges, such as the difficulty of isolating individual compounds in sufficient quantities for testing and the lack of authentic standards for comparative purposes, further complicate the development process, raising the bar for accurate and reliable therapeutic development from natural sources. The presence of isomers – molecules with identical molecular formulas but different structural or spatial arrangements – in natural extracts adds an additional layer of complexity as isomers can significantly impact bioactivity and compound identification efforts. As a representative example to demonstrate the utility of high-resolution ion mobility, we analyzed 28 cannabinoids found in Cannabis sativa, an herbaceous species known to offer a rich tapestry of possibilities, encompassing both psychoactive and non-psychoactive agents with potential applications spanning from pain management to neuroprotection.

The Answer

The application of HRIM to the analysis of natural extracts 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 as a complementary identifier to mass spectra. A simple and rapid liquid chromatography method was used to analyze 28 different cannabinoids including 15 isomers using an Agilent 1290 LC, an integrated MOBILion Systems MOBIE instrument, and an Agilent 6546 Q-TOF (Figure 1).

The Key Takeaway

The MOBIE® platform provides an additional dimension of separation that reveals size and mass relationships to help unravel the complexity of natural extracts.

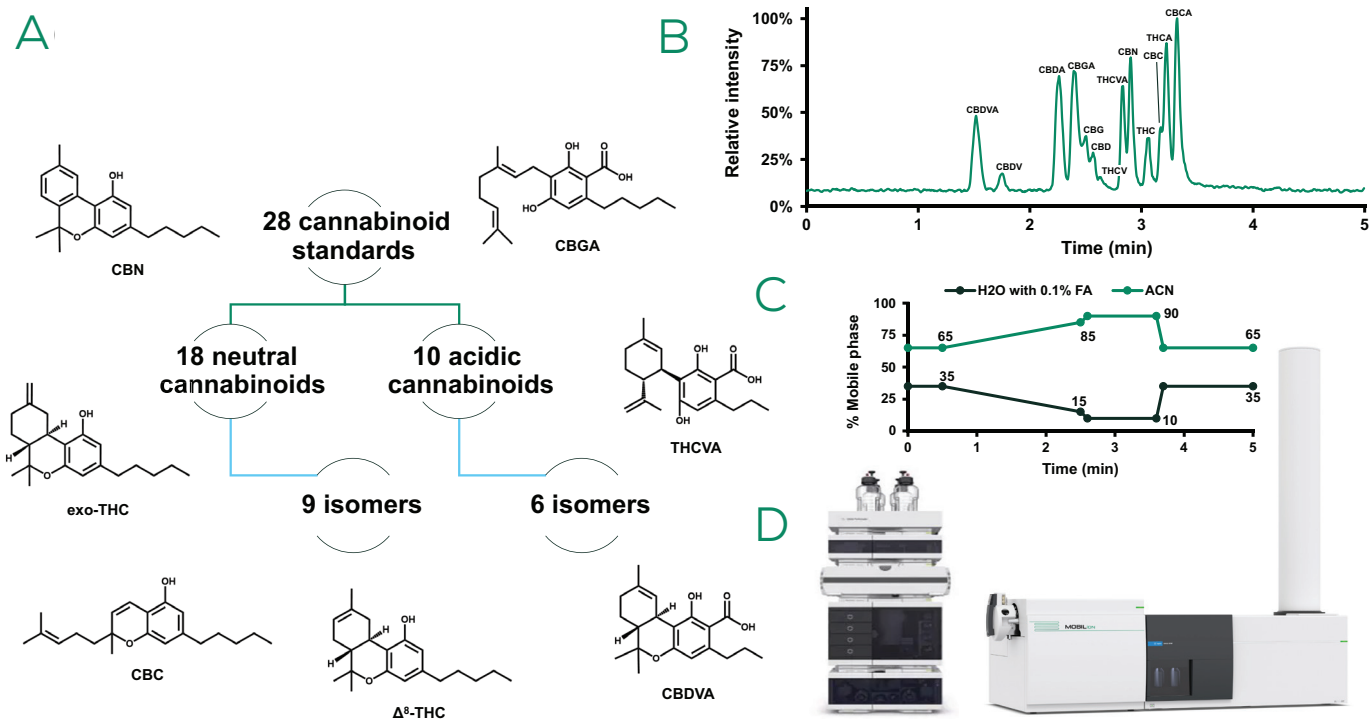

Figure 1. (A) Representative cannabinoid structures with numbers indicating compounds included across neutral and acidic classifications as well as known isomers. (B) total ion chromatogram (TIC) using the gradient shown in (C) and the Agilent 1290 LC, the MOBILion MOBIE HRIM system, and the Agilent 6546 Q-TOF (D) used for analysis.

As seen in Figure 2A, the combination of retention time (RT) and mass-to-charge (*m/z*) provides separation of most cannabinoids analyzed. However, three regions highlighted with red boxes indicate clusters of isomeric compounds where the sole use of chromatography and mass spectrometry is limited. In contrast, Figure 2B includes the use of the HRIM dimension and illustrates the powerful combination of RT and the ion mobility derived collision cross section (CCS) to separate the same three previously noted isomeric compound clusters, including two isomers of 7-OH-CBD and 6-OH-CBD (Figure 2B, Box 3).

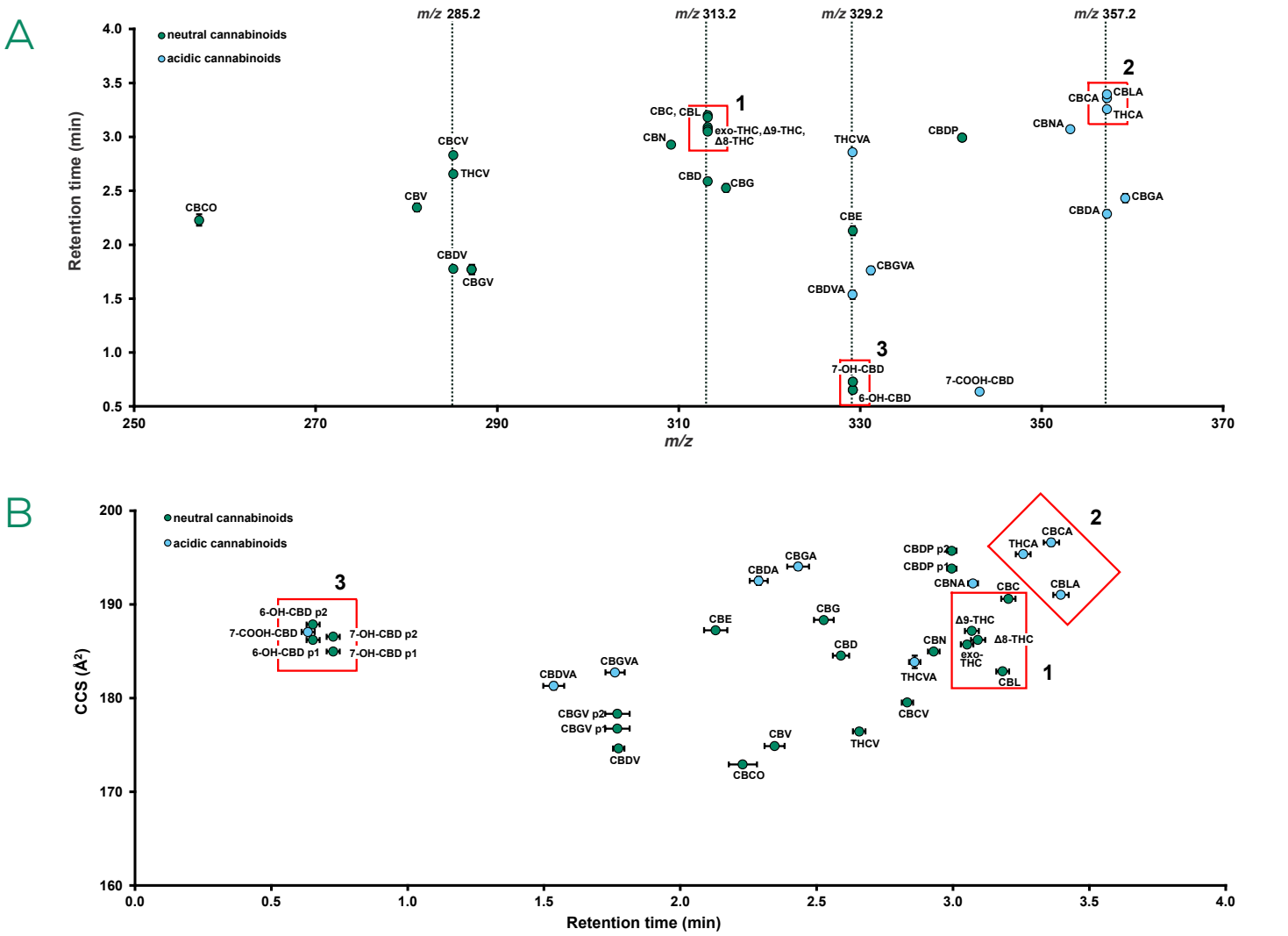

Figure 2. (A) Retention time vs. *m/z* for all included compounds. Red boxes indicate three areas where the combination of retention and MS are unable to fully resolve similar compounds. (B) However, by combining retention time and CCS with MS in a multidimensional separation approach, the red boxes highlight enhanced separation of the previously clustered compounds. The additional isomers contained within Box 3 were only separated via mobility and further illustrate the powerful combination of LC and HRIM.

Cannabicyclovarin (CBLV) is a minor, non-psychotropic cannabinoid in the cannabicyclol class. CBLV has not been extensively studied, though a recent paper highlighted its production via a synthetic route.[2] In the absence of an available standard for CBLV, we instead used the CCS and *m/z* results from the 28 included standards to generate a scatterplot as shown in Figure 3. Referred to as a "conformational space map," this two-dimensional representation provides a visual depiction of the relative size and mass of each included compound. Further, we used the relative position of cannabichromenic acid (CBCA), cannabichromene (CBC), and cannabichromevarin (CBCV) to predict the likely location where CBLV would be seen (Figure 3, red circle), based on similar structural differences between cannabicyclolic acid (CBLA), cannabicyclol (CBL), and CBLV.

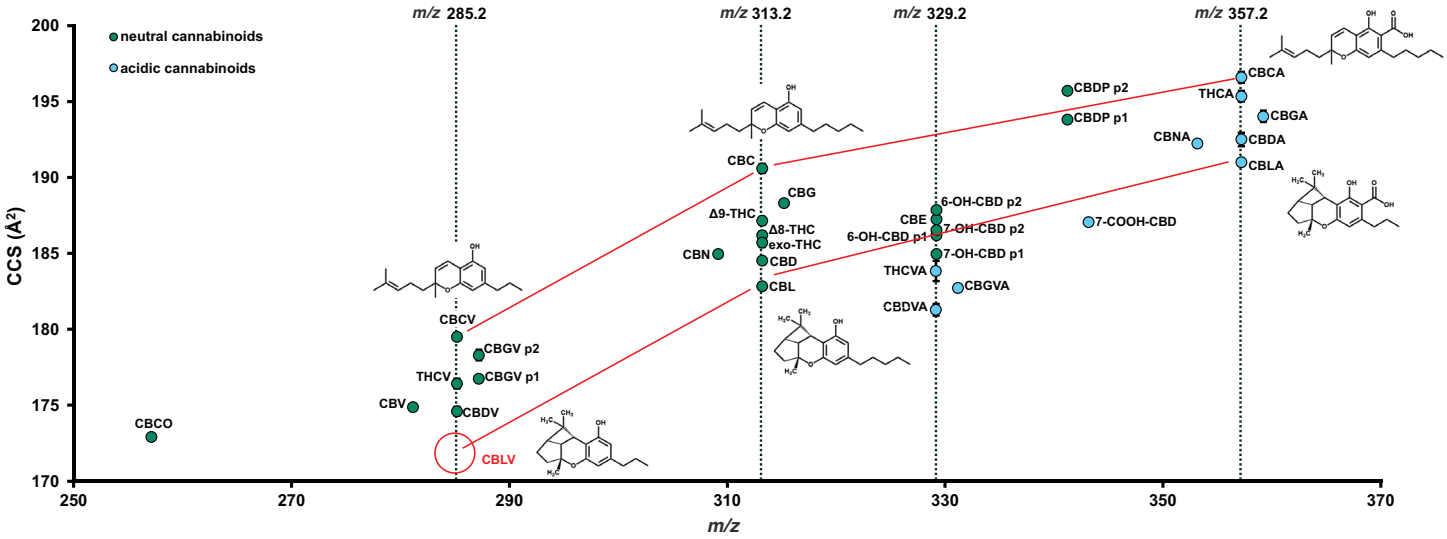

Figure 3. The conformational space map generated by plotting CCS vs. *m/z* provides a visual representation of the relative size and mass of each compound. The top red line was drawn from the precursor CBCA to the derivate CBC and the structurally similar CBCV. The bottom red line was drawn from the precursor CBLA to the derivate CBL. Based on the associated structural differences of a three-carbon vs. five-carbon tail and a decarboxylation process, a red dashed line was drawn to the predicted position of the structurally related CBLV, where a standard was unavailable for analysis.

Summary

HRIM is a gas phase separation technology that provides an additional dimension of separation based on size, charge, and structure. It is partially orthogonal to LC and HRMS, yielding deeper insights into complex samples. For isomer determination, the HRIM dimension leverages the overall compound structure, resulting in greater confidence in compound determination when fragmentation patterns are complex or lack specificity. A two-dimensional visual database (i.e., a conformational space map) can be generated and used to estimate the location of compounds of interest even in the absence of available standards.

References

1. Newman, D.J. and G.M. Cragg, *Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019*. Journal of Natural Products, 2020. **83**(3): p. 770-803.
2. Li, X. and Y.R. Lee, *Efficient and novel one-pot synthesis of polycycles bearing cyclols by FeCl3-promoted [2 + 2] cycloaddition: application to cannabicyclol, cannabicyclovarin, and ranhuadujuanine A*. Org Biomol Chem, 2014. **12**(8): p. 1250-7.