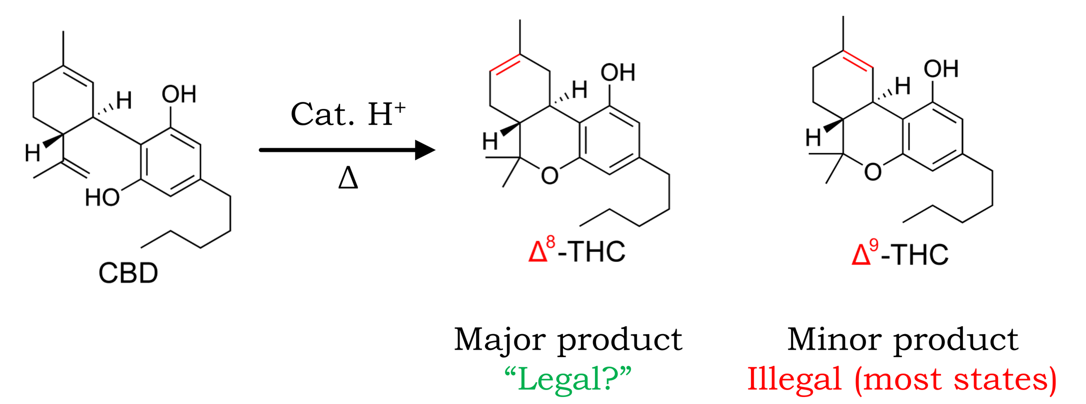
Argentination: A Silver Bullet for Cannabinoid Analysis by Differential Mobility Spectrometry

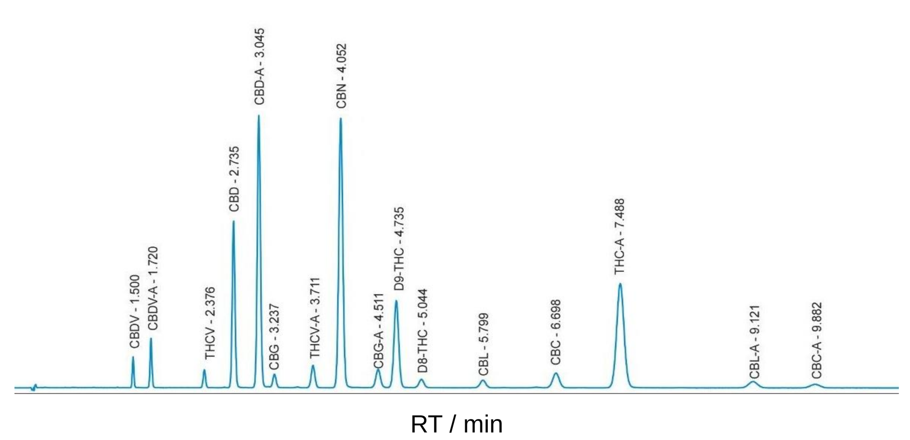
Christian Ieritano, Patrick Thomas, W. Scott Hopkins*
University of Waterloo, 200 University Ave W., Waterloo, ON, N2L 3G1

The Δ8-THC problem

The 2018 amendment to the Farm Act in the US legalized all components and derivatives of the cannabis plant so long as its Δ9-THC content does not exceed 0.3 % by weight.[1] This amendment created an interesting situation since heating CBD, which is now legalized, produces Δ8-THC, a less potent but still psychoactive derivative of Δ9-THC. Because the Farm Act only regulates Δ9-THC content and explicitly states that cannabinoid derivatives are protected *(i.e,* Δ8-THC), significant debate surrounding Δ8-THC's legality has ensued.



Several methods exist to separate/quantify Δ8-THC products,[3] although despite fast analysis times via LC (< 5 min), the separation is still limited by the elution time.

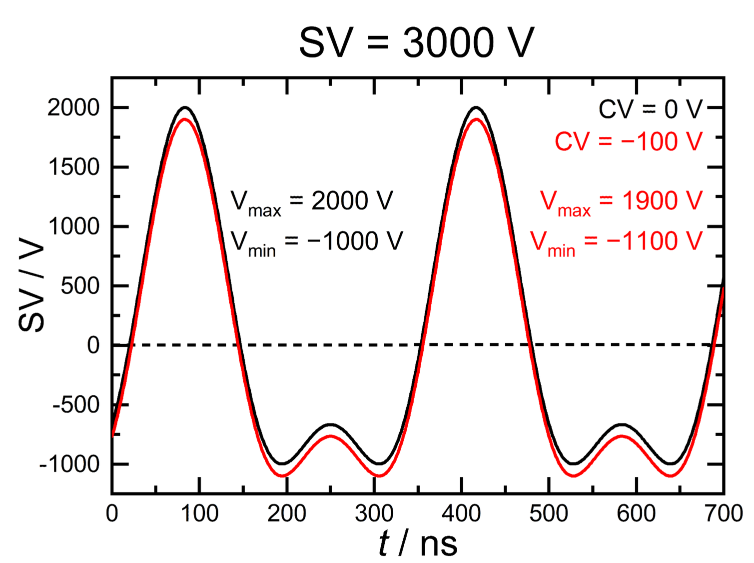


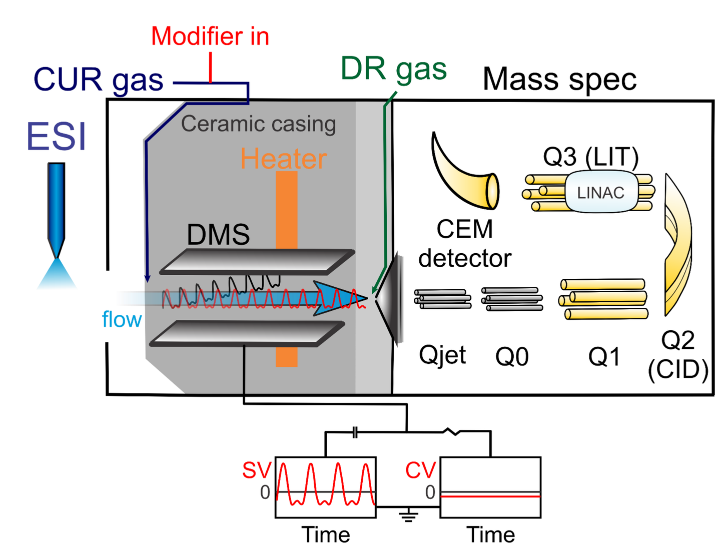
Can DMS achieve equivalent separation power?

Since Δ8-THC products are unregulated, those that are being sold as a "legal" alternative contain several unintended byproducts from the synthesis. These include novel cannabinoids with no known information on how they impact human health.[2]

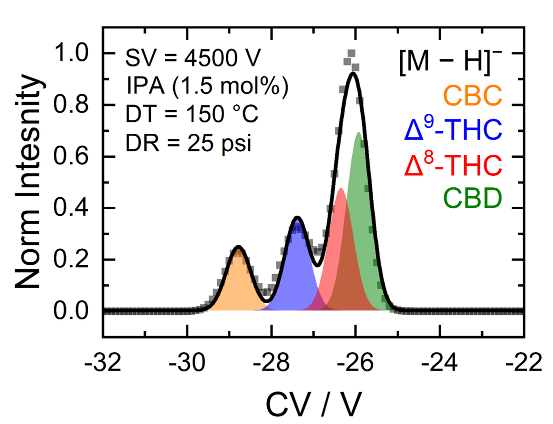
Cannabinoid analysis seldom employs ion mobility

To date, < 5 studies have been conducted that employ ion mobility for cannabinoid separation. We propose the use of DMS-MS^2 to instantaneously separate and quantify a series of 7 cannabinoids, 5 of which are isobaric. Analytes are introduced to the DMS by electrospray ionization (ESI) with transmission being monitored by MRM.

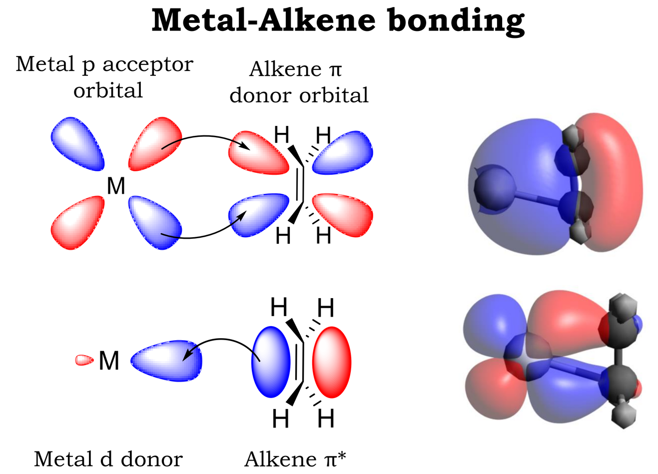


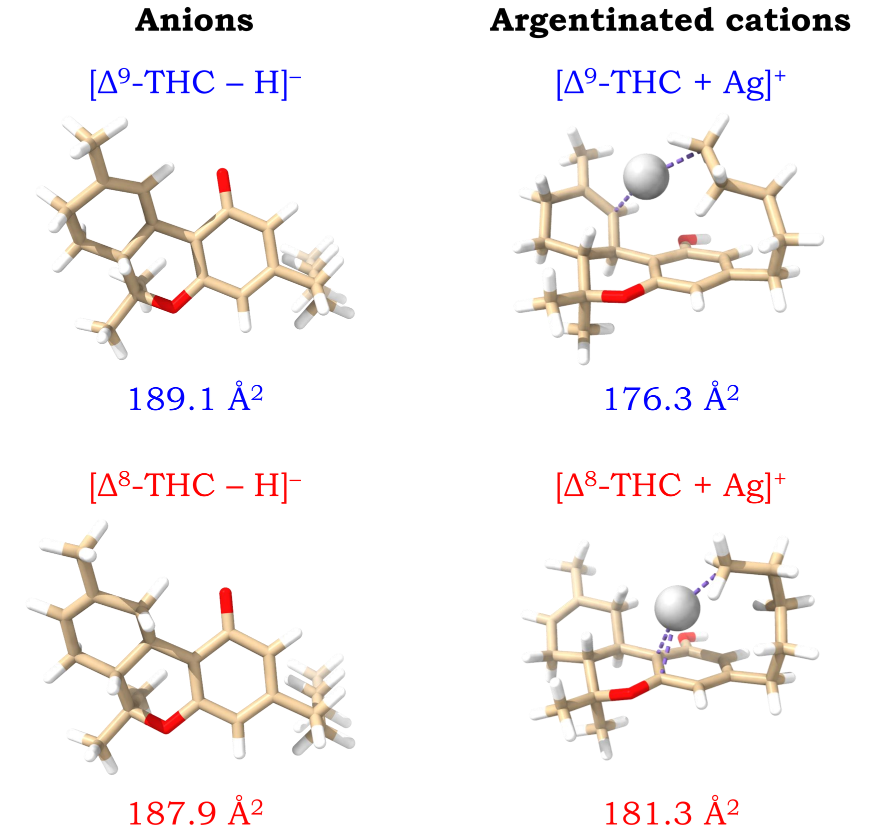


When the cannabinoids are monitored as deprotonated adducts, Δ8-THC and CBD unfortunately coelute, necessitating an alternative strategy is needed. Since isobaric cannabinoids differ in the placement of double bonds and cyclic moieties, argentination can enhance structural differences owing to their proclivity to coordinate π-electrons. Structural differences driven by argentination could afford separation by DMS and/or linear IMS techniques as per *in silico* evaluations of the argentinated adduct CCSs.



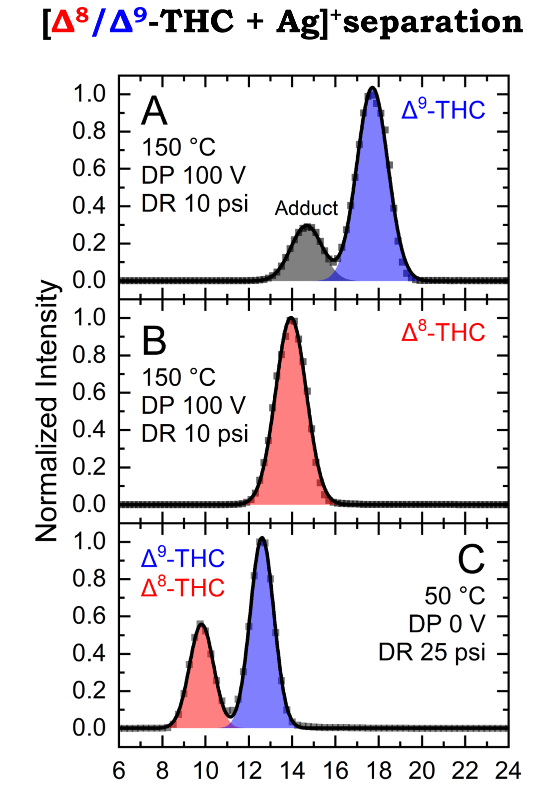
Metal-Alkene bonding





ΔCCS = 1.2 Å^2   **ΔCCS = 5.0 Å^2**

[Δ8/Δ9-THC + Ag]+separation

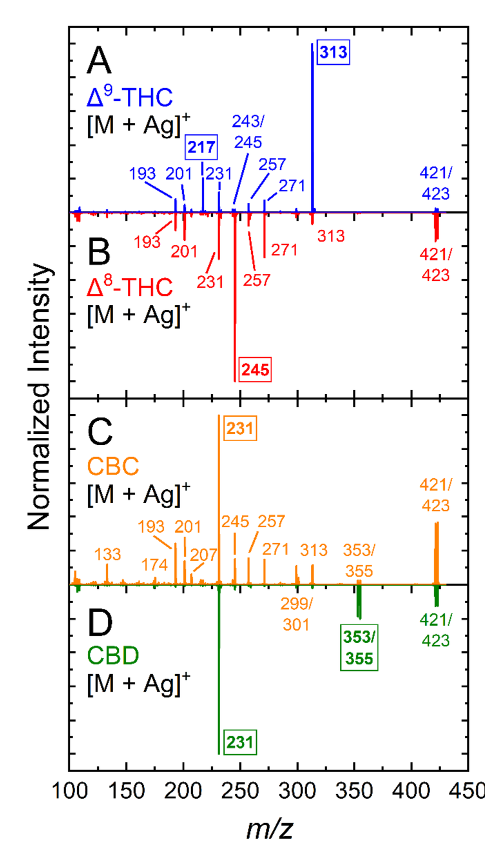


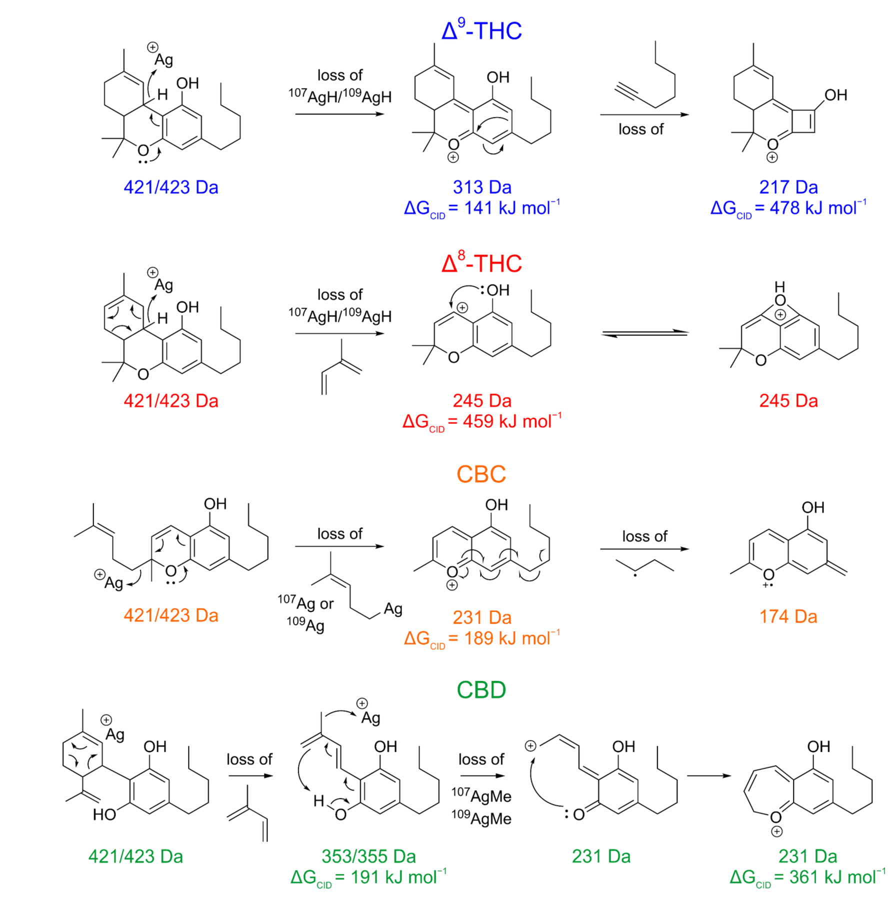
Detection of adducts is mitigated by minimizing DT and DP

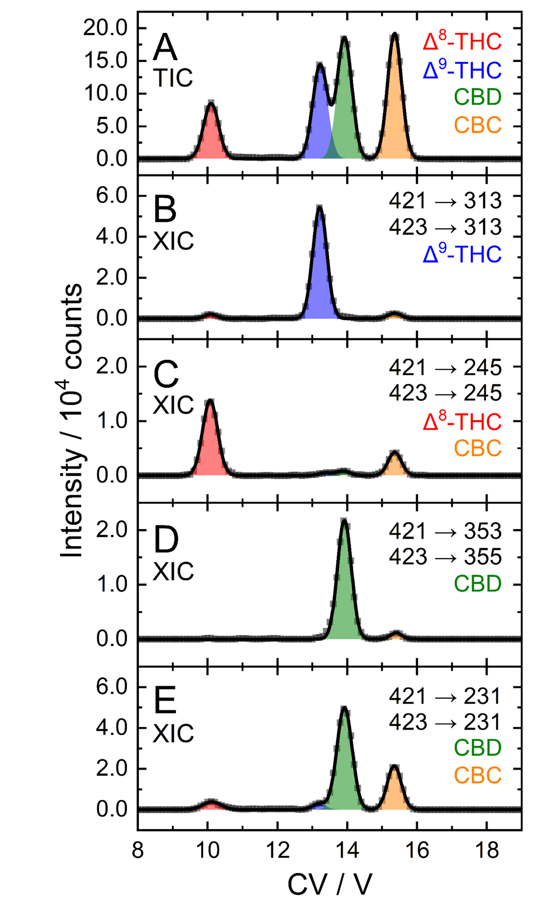
Cannabinoid argentination induces unique fragmentation behaviour via CID

While optimizing MRM transitions for the argentinated cannabinoids, it was noticed that silver adduction promotes unique fragmentation pathways that are not possible if cannabinoids are monitored as protonated *(i.e.,* [M + H]+) or deprotonated *(i.e.,*[M – H]−) species. When coupled with DMS, cannabinoids can easily be quantitated via standard addition.

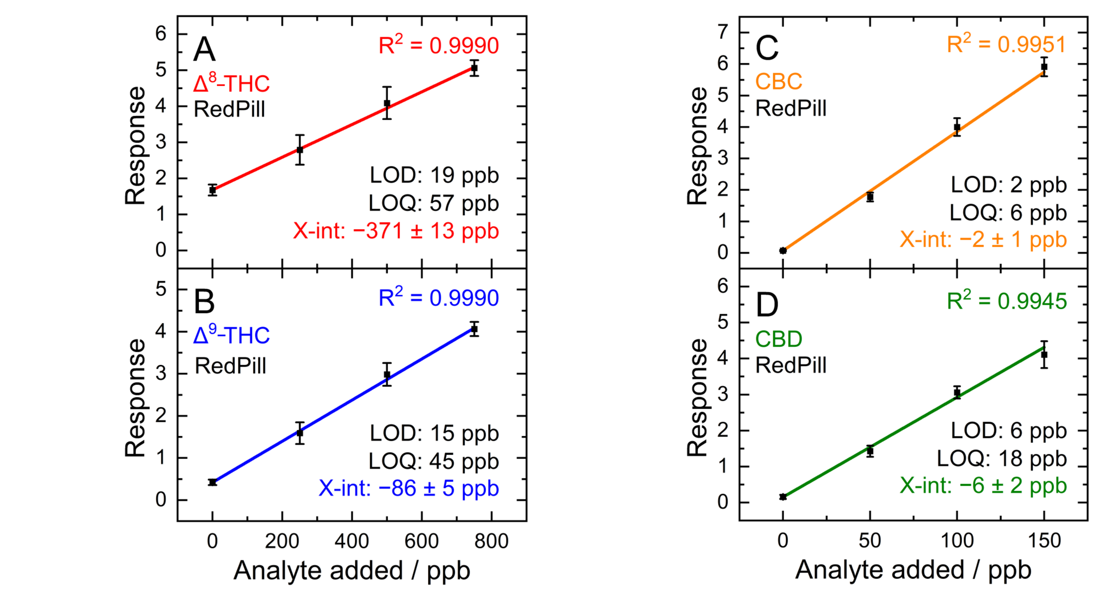
Argentination promotes unique cannabinoid fragmentation pathways







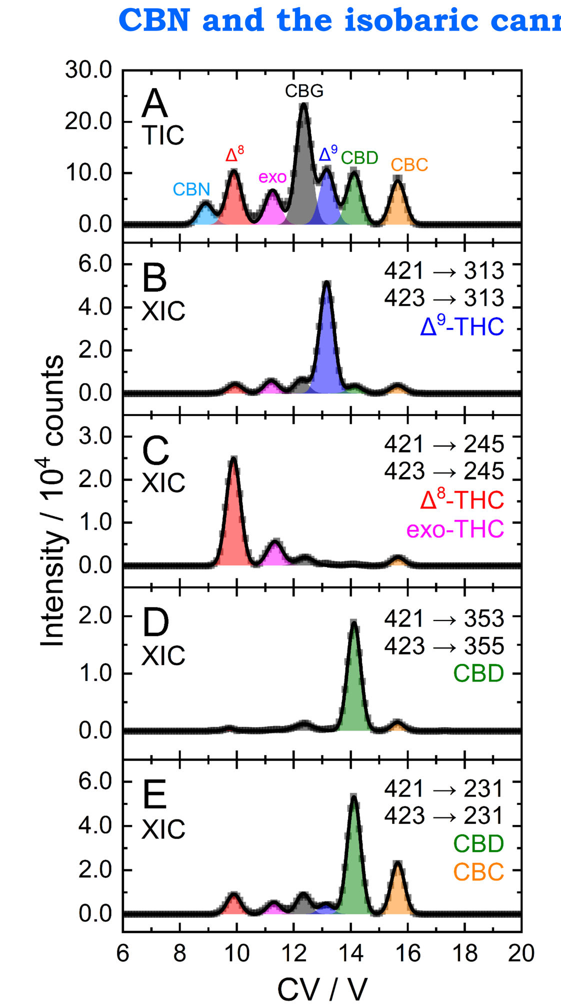
Quantitation of the RedPill cannabis extract (CBD-A internal std.)

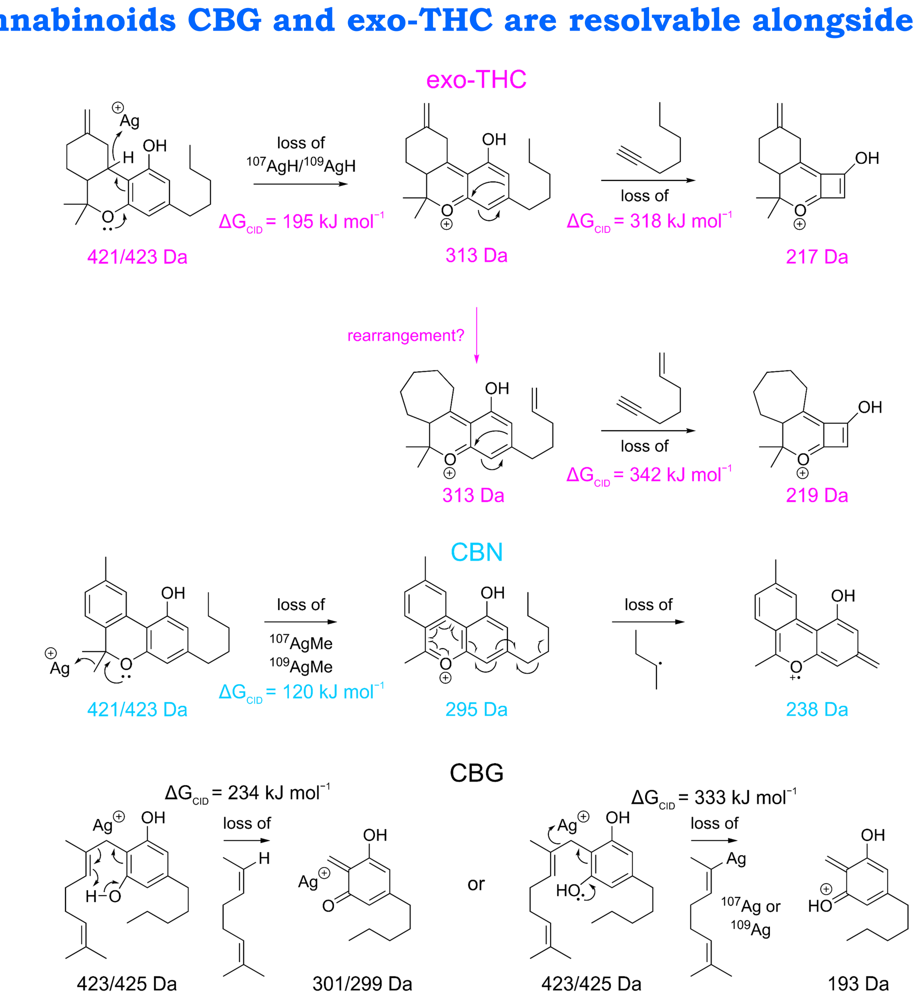


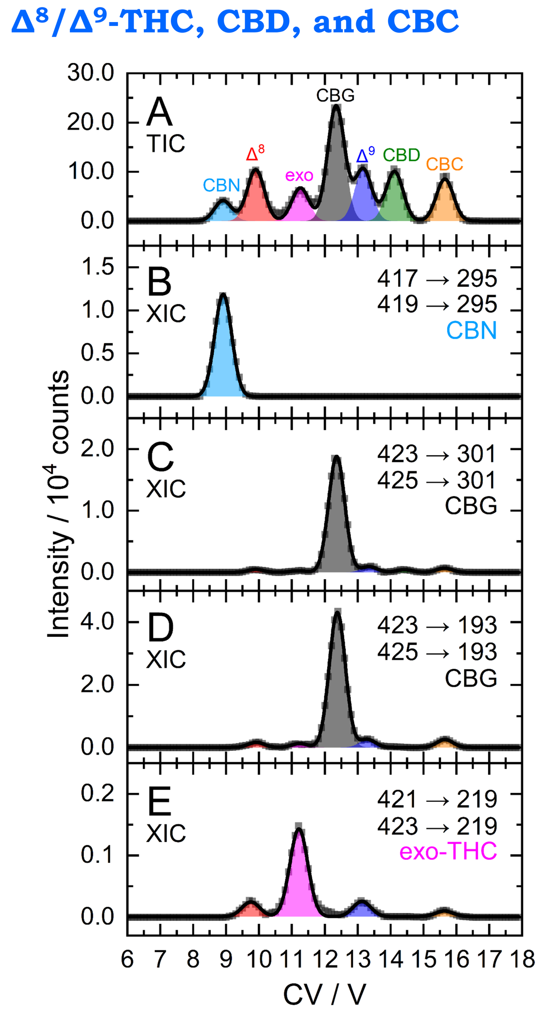
Analyte	*Stated conc. / mg mL^{-1}*	*Measured conc. / mg mL^{-1}*	*LOD / ppb*	*LOQ / ppb*
Δ8-THC	19	18.6 ± 0.5	19	57
Δ9-THC	3.8	4.3 ± 0.3	15	45
CBD	< 1	0.3 ± 0.1	6	18
CBC	Not stated	0.1 ± 0.03	2	6

DMS-MS^2 can separate isobaric cannabinoid mixtures quantitatively

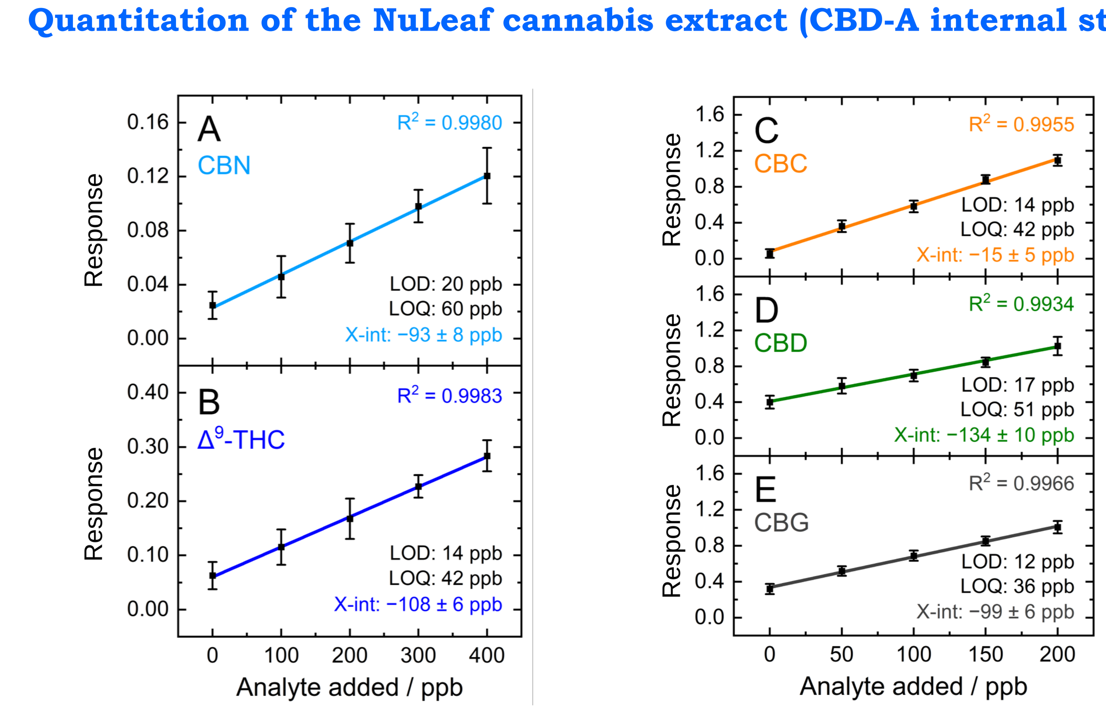
CBN and the isobaric cannabinoids CBG and exo-THC are resolvable alongside Δ8/Δ9-THC, CBD, and CBC







Quantitation of the NuLeaf cannabis extract (CBD-A internal std.)



Analyte	*Stated conc. (mg mL^{-1})*	*Measured conc. (mg mL^{-1})*	*LOD / ppb*	*LOQ / ppb*
Δ9-THC	13.6	14.5 ± 0.8	14	42
CBD	12.0	17.8 ± 1.3	17	51
CBN	12.0	12.4 ± 1.0	20	60
CBG	12.0	13.2 ± 0.8	12	36
CBC	12.0	2.1 ± 0.6	14	42

Concluding remarks

The separation of seven cannabinoids (Δ8-THC, Δ9-THC, CBD, CBC, exo-THC, CBN, and CBG), five of which are isobaric, was achieved by DMS in a pure N_2 environment when the analytes were detected as argentinated species *(i.e.,* [M + Ag]+). Upon optimization of the MRM workflow to monitor analyte transmission through the DMS cell, it was discovered that the argentinated cannabinoids exhibit distinct fragmentation pathways. This finding was unexpected, as prior work suggested that the protonated and deprotonated forms of cannabinoids generate indistinguishable product ions from CID. Incorporating DMS into a tandem-MS workflow enabled the (near)-baseline separation of Δ8-THC, Δ9-THC, CBD, CBC, exo-THC, CBN, and CBG, and thus, facilitated their quantitation within two commercial cannabis extracts with LODs/LOQs that parallel detection as deprotonated species.

References and acknowledgements

1 Agriculture Improvement Act of 2018.
2 Meehan-Atrash, J.; Rahman, I. *Chem Res Toxicol.* **2022**, *35* (1), 73–76.
3 McRae, G.; Melanson, J. E. *Anal. Bioanal. Chem.* **2020**, *412* (27), 7381–7393.