

Using Water Adduction to Differentiate Cannabinoid Isomers in a Quadrupole Ion Trap Mass Spectrometer

Dinuri S. Fernando, Cameron D. Worthington and Gary L. Glish

Department of Chemistry, The University of North Carolina at Chapel Hill, Chapel Hill, NC

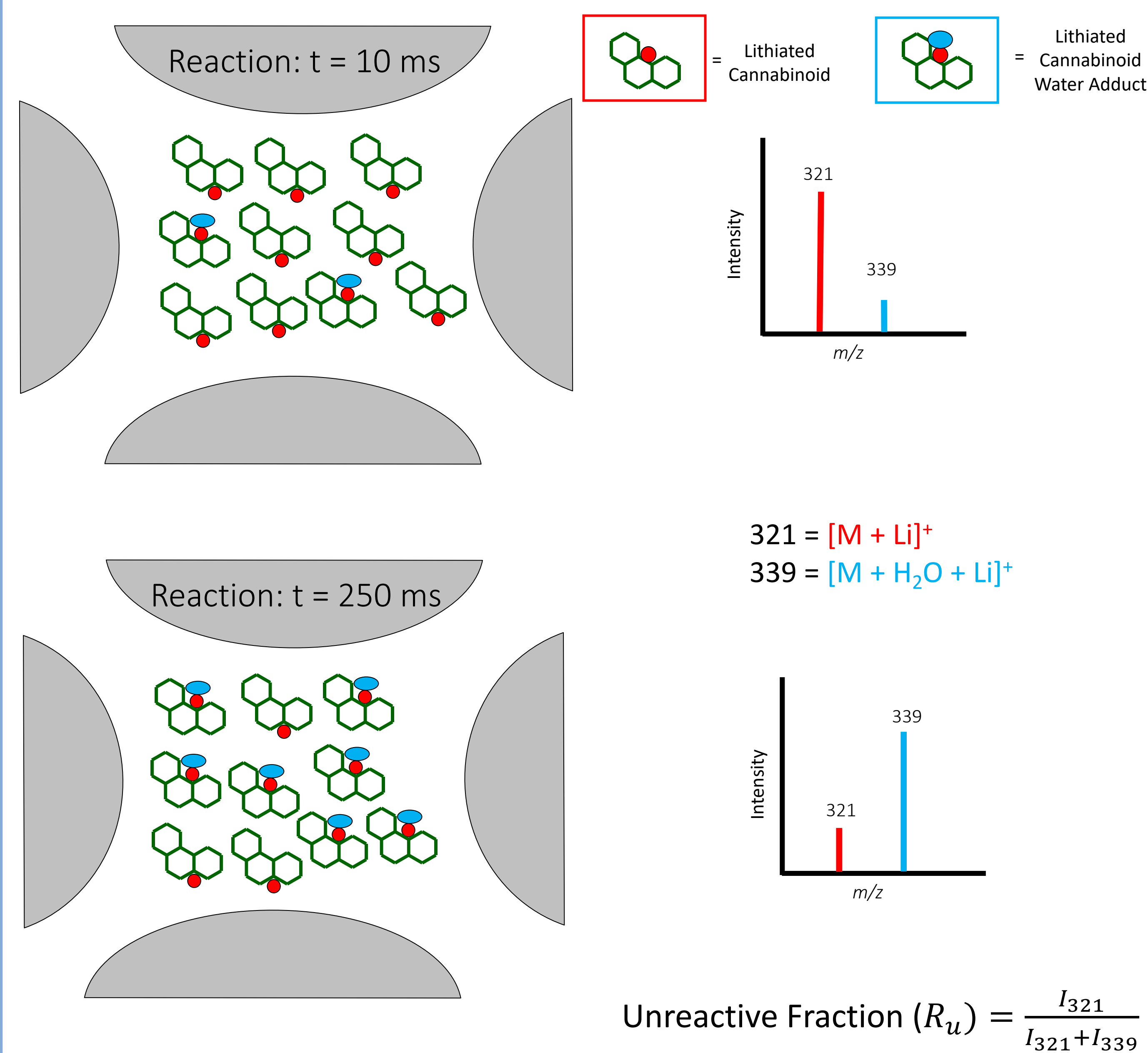


THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

Introduction

The federally illegal psychoactive compound Δ^9 -tetrahydrocannabinol (Δ^9 -THC) is a structural isomer to the following: cannabidiol (CBD), cannabichromene (CBC), Δ^8 -tetrahydrocannabinol (Δ^8 -THC), and Δ^{10} -tetrahydrocannabinol (Δ^{10} -THC), which are all federally legal under US law. CBD and CBC are non-psychoactive structural isomers and show promise for treating chronic pain, inflammation and other conditions. Δ^8 -THC and Δ^{10} -THC are psychoactive and gaining popularity due to federal legality and with a few states criminalizing their sale. Differentiating these structures is challenging due to their isomeric nature. In this work, adduction of water molecules in a quadrupole ion trap mass spectrometer is utilized to differentiate between the aforementioned cannabinoid isomers using direct infusion mass spectrometry.

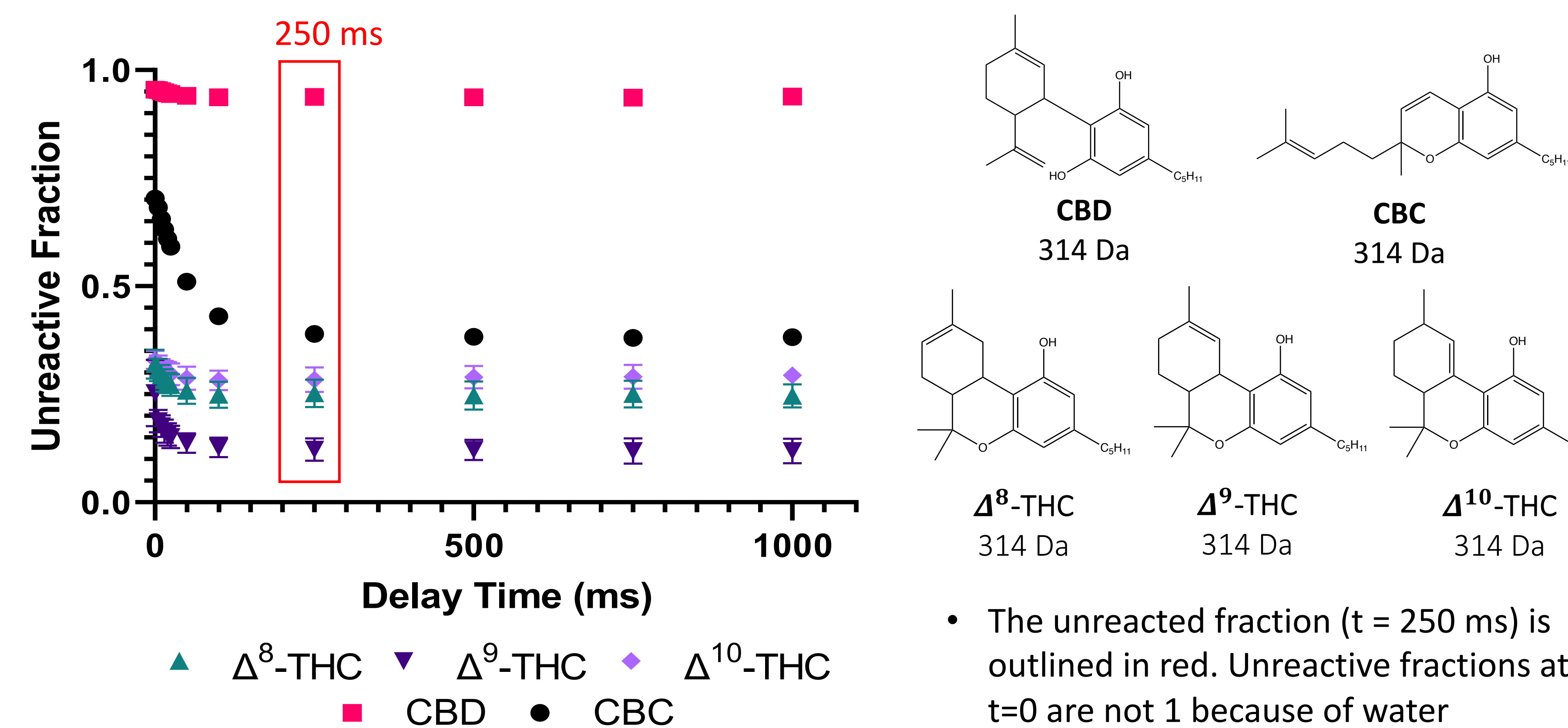
Experimental Setup



Experimental Parameters

- Solvent comp: conc of cannabinoid and salt
10 μM analyte in 95:5 MeOH:H₂O with 200 μM lithium acetate
- Nitrogen drying gas flow rate and temperature were 5 L/min and 250°C, respectively.
- For DIMS experiments, the dispersion field was 42 kV/cm.
- Compensation field was scanned from 50 V/cm to 400 V/cm at a step size of 3.33 V/cm for the DIMS compensation field scans.
- Ion optics experiments scanned declustering potential from 20 V to 200V at step sizes of 20 volts.

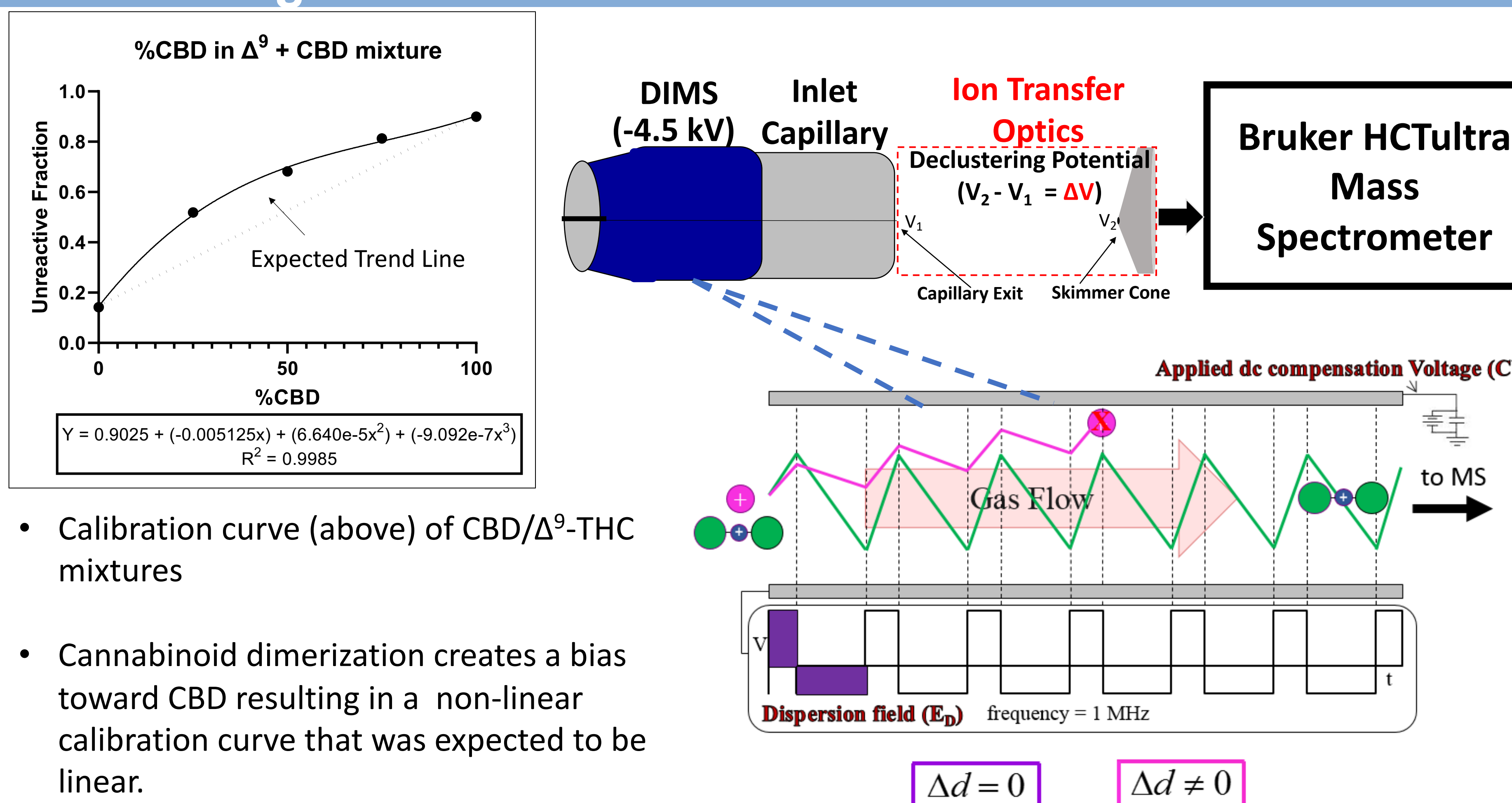
Water Adduction with Isomeric Cannabinoids



Cannabinoid	Relative Rate	Unreacted Fraction @ 250ms
CBD	4.530 \pm 0.710	0.9375 \pm 0.0062
CBC	70.83 \pm 2.390	0.3892 \pm 0.0149
Δ^8 -THC	66.22 \pm 4.170	0.2519 \pm 0.0317
Δ^9 -THC	120.3 \pm 9.870	0.1222 \pm 0.0261
Δ^{10} -THC	53.00 \pm 17.44	0.2840 \pm 0.0289

- The unreacted fraction ($t = 250 \text{ ms}$) is outlined in red. Unreactive fractions at $t=0$ are not 1 because of water adduction during electrospray ionization.
- The unreactive fractions were calculated using the equation:
$$R_u = \frac{I_{321}}{I_{321} + I_{339}}$$
- Plotting $\ln(R_u)$ vs. delay time yields a linear plot for pseudo-first order reaction kinetics.
- The slope of the linear fit applied to each plot is the relative reaction rate.

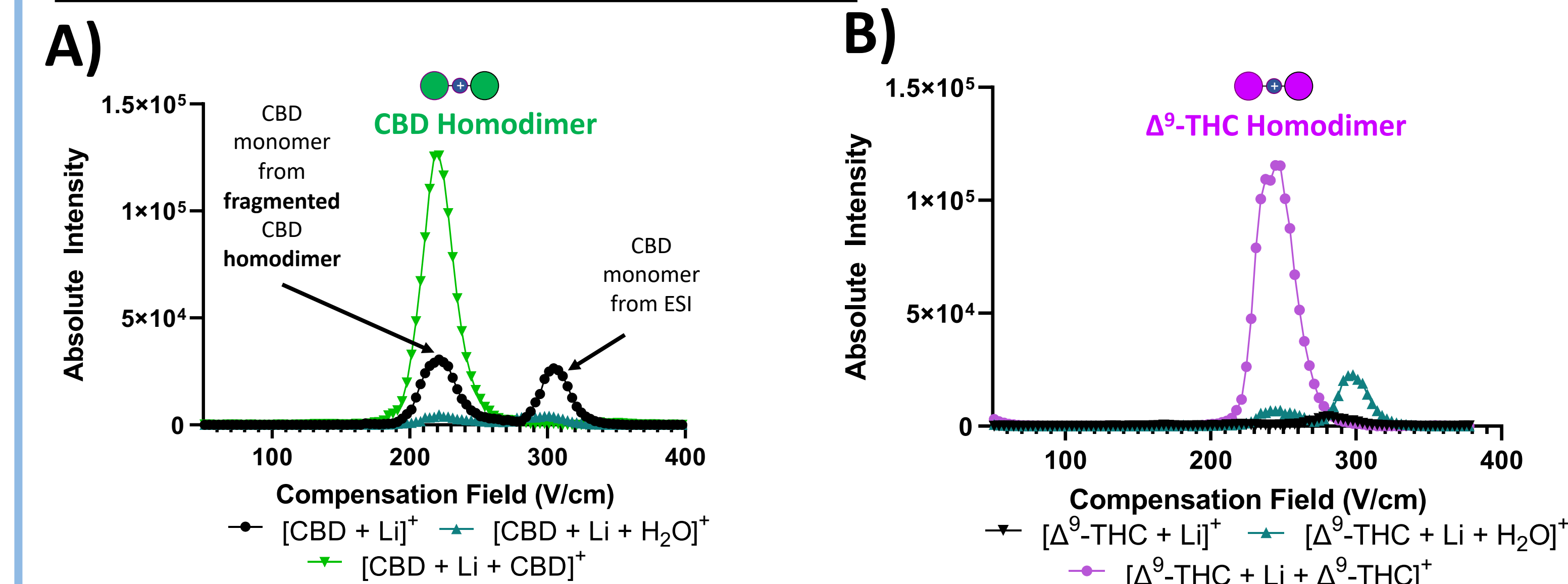
Creating a Calibration Curve for CBD and THC Mixtures



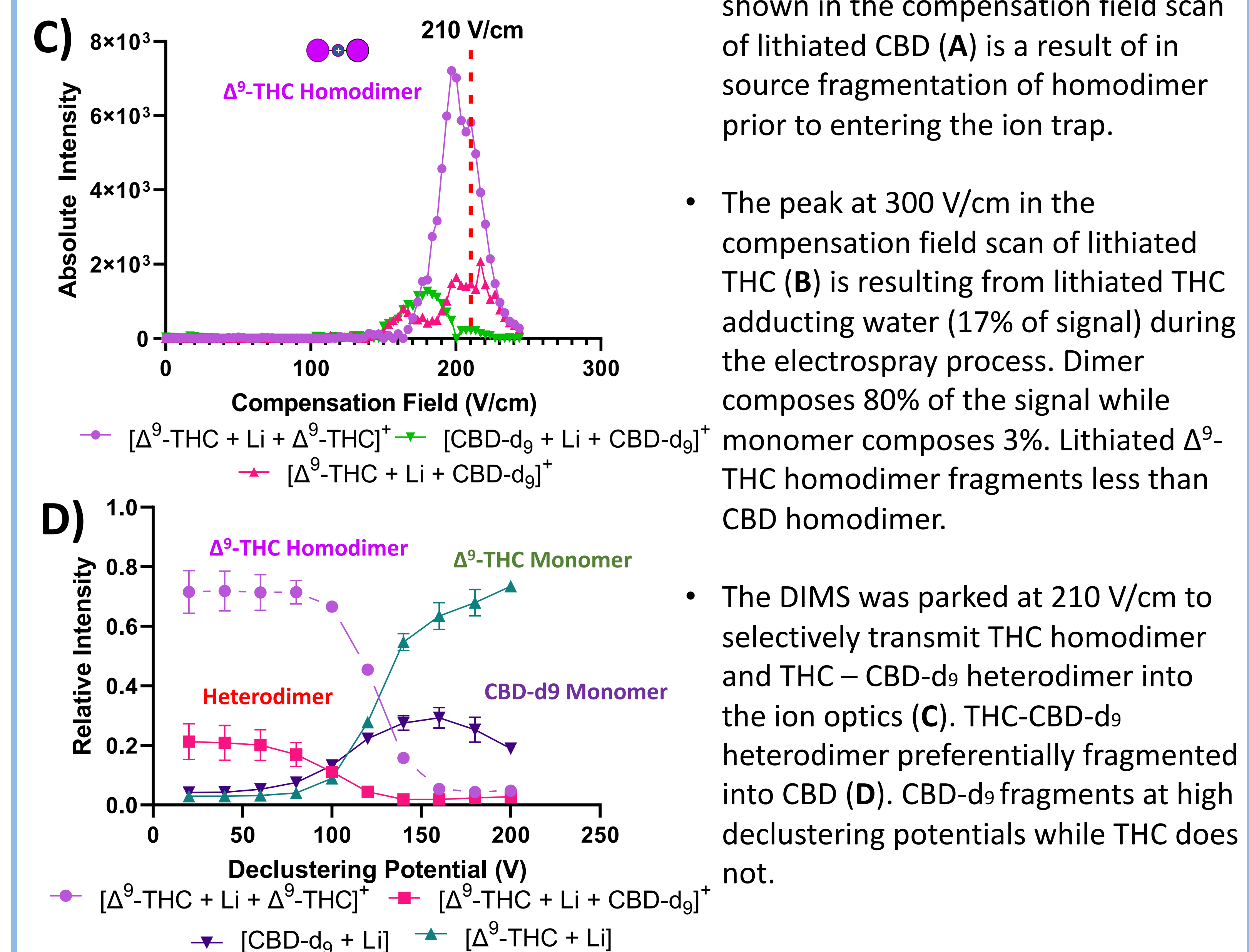
- Calibration curve (above) of CBD/ Δ^9 -THC mixtures
- Cannabinoid dimerization creates a bias toward CBD resulting in a non-linear calibration curve that was expected to be linear.

Impact of Cannabinoid Dimers

Compensation Field scans of CBD and Δ^9 -THC



Ion Optics Fragmentation



- The $[\text{CBD} + \text{Li}]^+$ peak at 220 V/cm shown in the compensation field scan of lithiated CBD (A) is a result of in source fragmentation of homodimer prior to entering the ion trap.
- The peak at 300 V/cm in the compensation field scan of lithiated THC (B) is resulting from lithiated THC adducting water (17% of signal) during the electrospray process. Dimer composes 80% of the signal while monomer composes 3%. Lithiated Δ^9 -THC homodimer fragments less than CBD homodimer.
- The DIMS was parked at 210 V/cm to selectively transmit THC homodimer and THC – CBD- d_9 heterodimer into the ion optics (C). THC-CBD- d_9 heterodimer preferentially fragmented into CBD (D). CBD- d_9 fragments at high declustering potentials while THC does not.

Summary

- A water adduction ion molecule reaction with lithiated cannabinoids in a quadrupole ion trap mass spectrometer is demonstrated. The cannabinoids used in this study, except Δ^8 - and Δ^{10} -THC, can be differentiated by the relative rate they adduct water and by their unreacted fractions.
- Calibration curves created to differentiate cannabinoids were expected to be linear. Instead, the calibration curves were biased toward CBD's unreacted fraction. Shown using DIMS, cannabinoid dimer formation and fragmentation is skewing mixture calibration curve data.