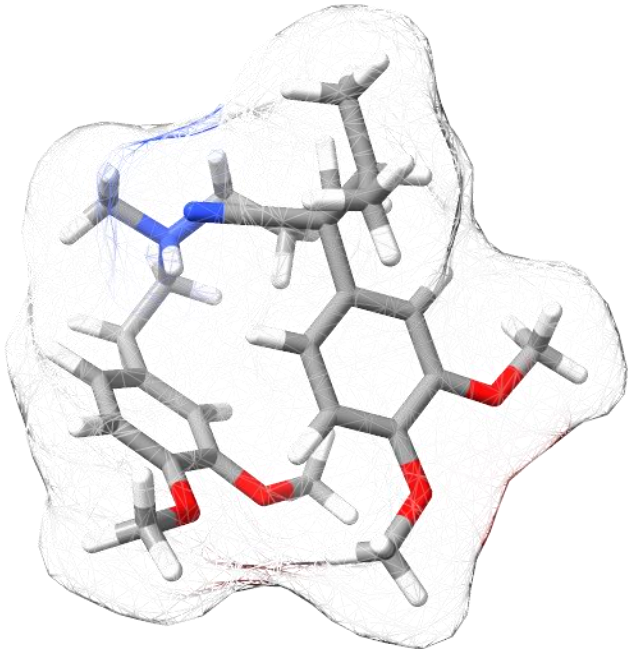
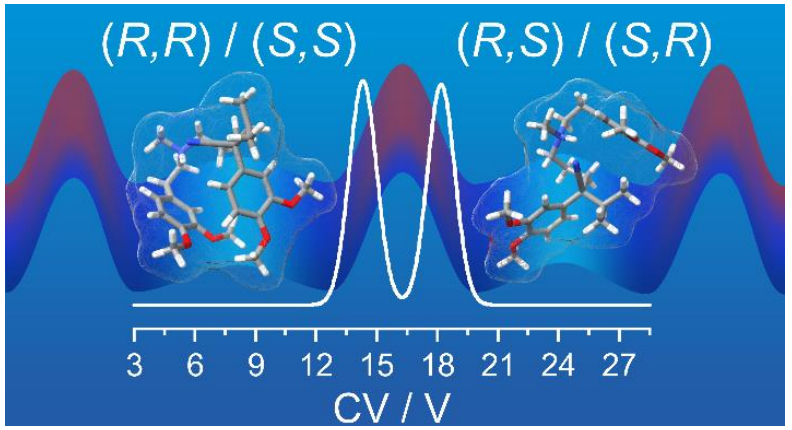
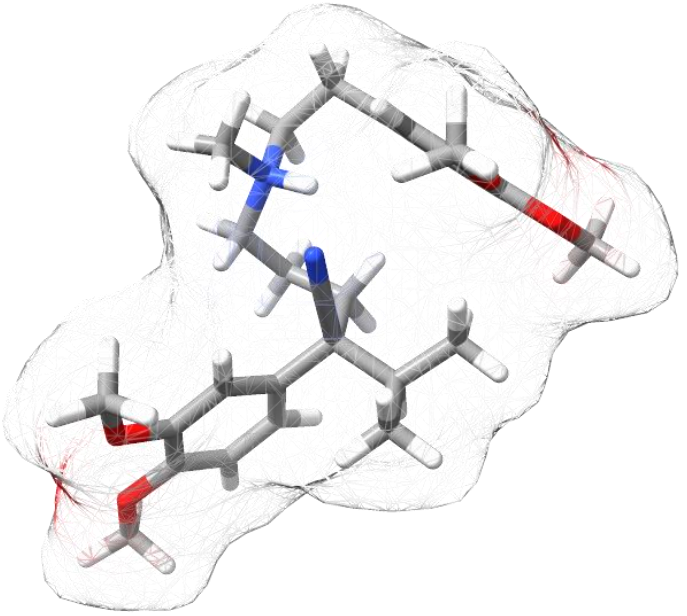
Protonation-Induced Chirality Drives Separation by Differential Mobility Spectrometry

The Curious Case of Verapamil







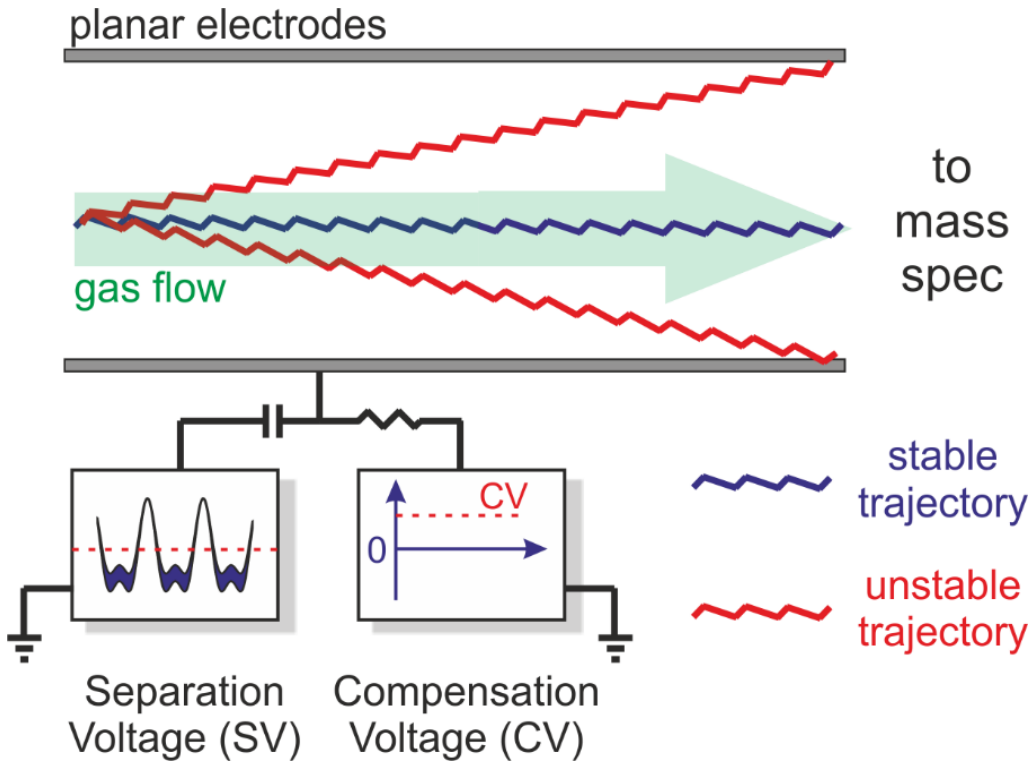
2021 Lake Louise Tandem MS Workshop

Christian Ieritano, J. C. Yves Le Blanc, Bradley Schneider,
Alexander Haack, Justine Bissonnette, W. Scott Hopkins

December 4, 2021

What is Differential Mobility Spectrometry (DMS)?

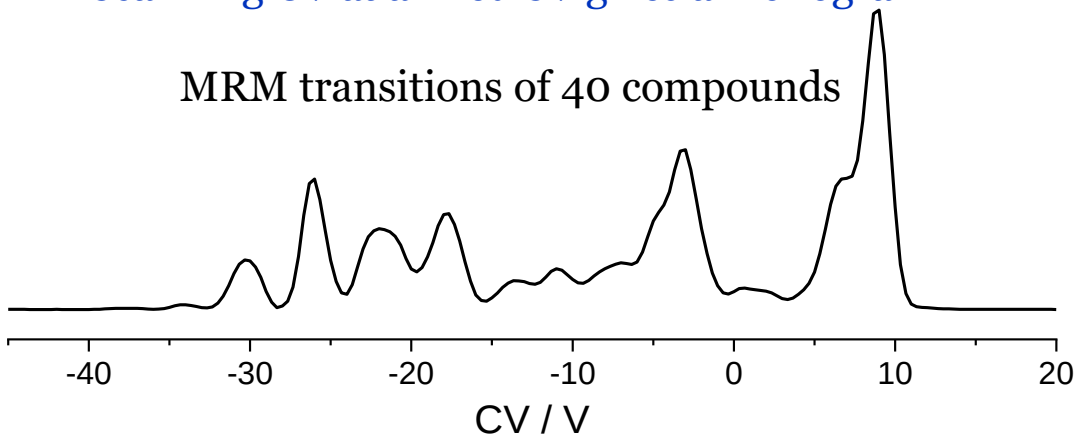
Differential mobility spectrometry (DMS) is a non-linear form of ion-mobility spectrometry



Above the low field limit, ion mobility K varies non-linearly with the field strength

$$K(E) = K(0)[1 + \alpha(E)] \qquad \alpha(E) = \frac{K(E)}{K(0)} - 1$$

Scanning CV at a fixed SV gives an ionogram



For a specific SV, every analyte will elute from the DMS cell at a characteristic CV (1 peak)

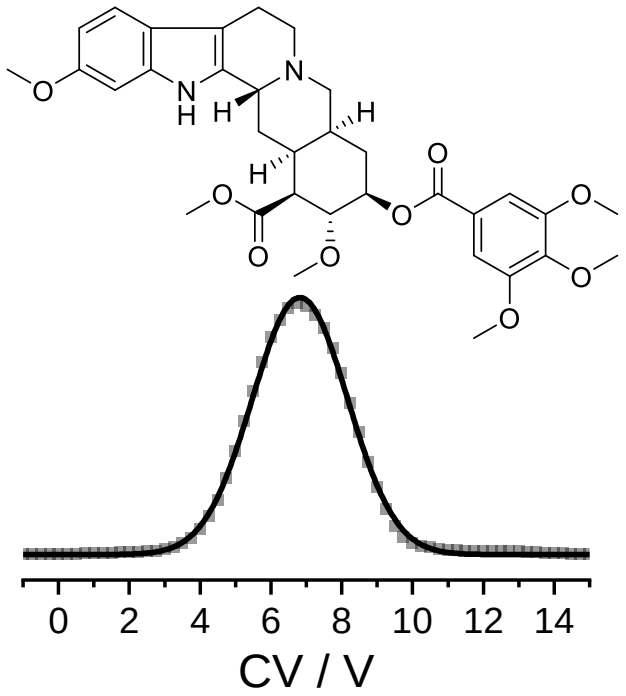
Mol. Phys. **2015**, 113, 3151–3158.

The SV/CV pair is an intrinsic ion property

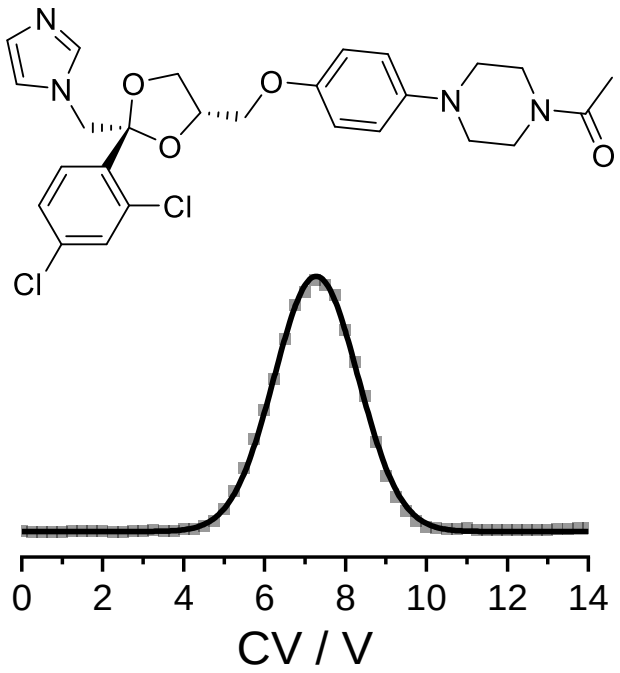
The CV in which an analyte elutes from the DMS cell is an intrinsic ion property

~ 15 years ago, SCIONEX developed a test mixture to verify instrument performance

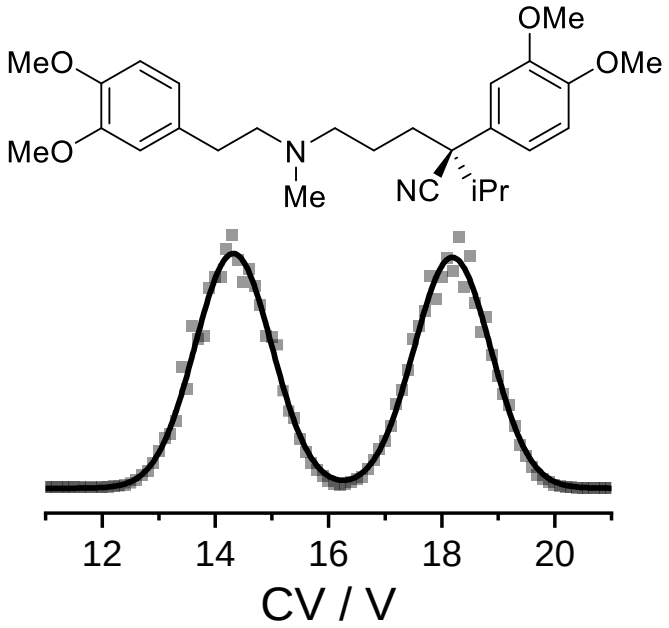
Reserpine



Ketoconazole



...and Verapamil?



Why does Verapamil have two peaks?

This isn't the first time we've seen strange DMS behaviour...

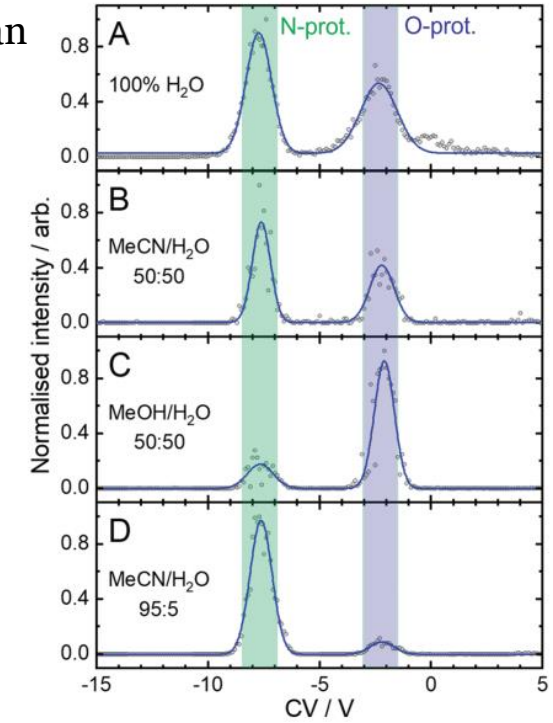
More than one feature in an ionogram **usually** indicates the presence of prototropic isomers

4-aminobenzoic acid

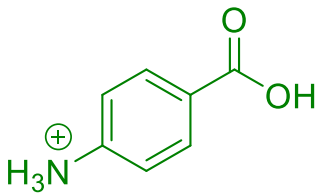
Phys. Chem. Chem. Phys. 2021, 23, 20607

Anal. Chem. 2012, 84, 7857-7864.

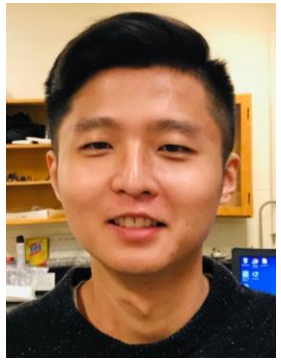
Dr. Neville Coughlan



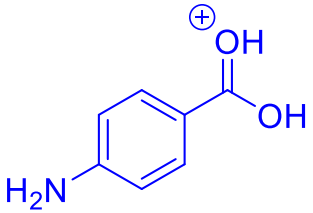
N-Prot



Dr. Weiqiang Fu



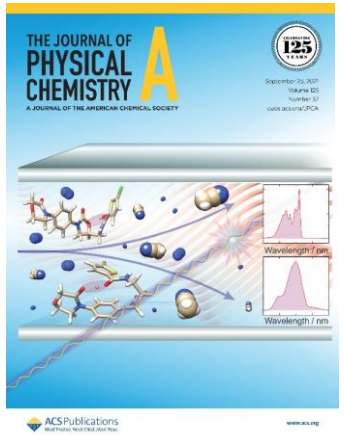
O-Prot

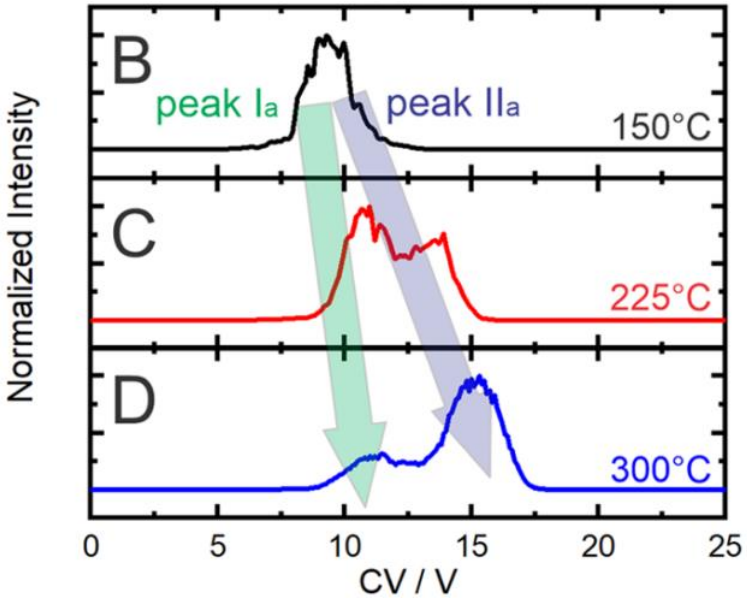


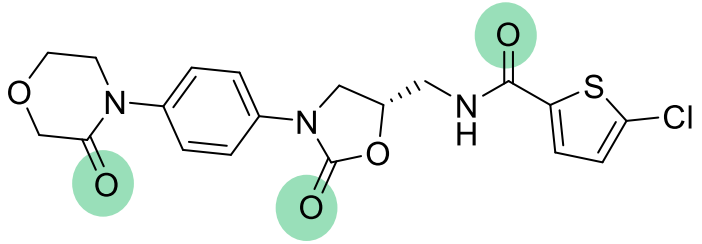
Rivaroxaban

J. Phys. Chem. A. 2021, 125, 8159-8344

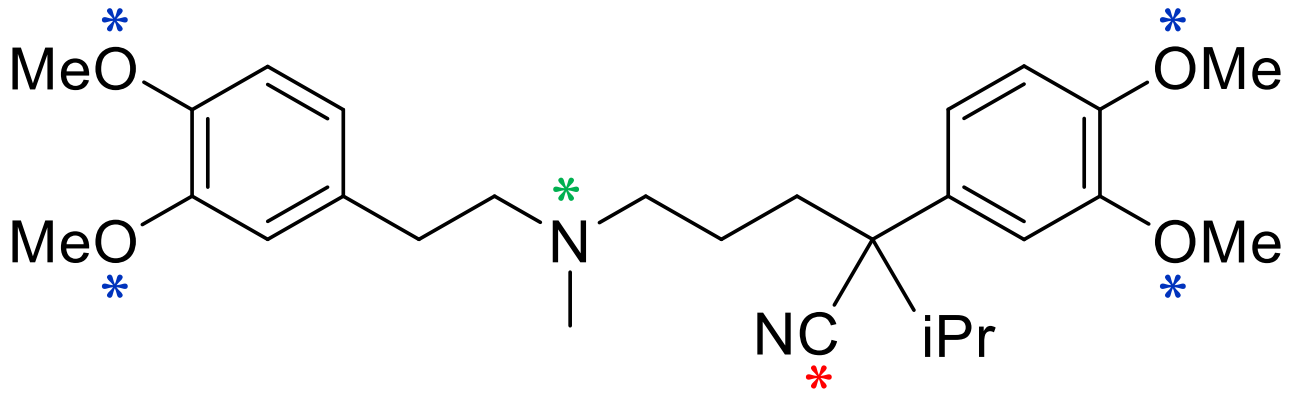
Nour Mashmoushi



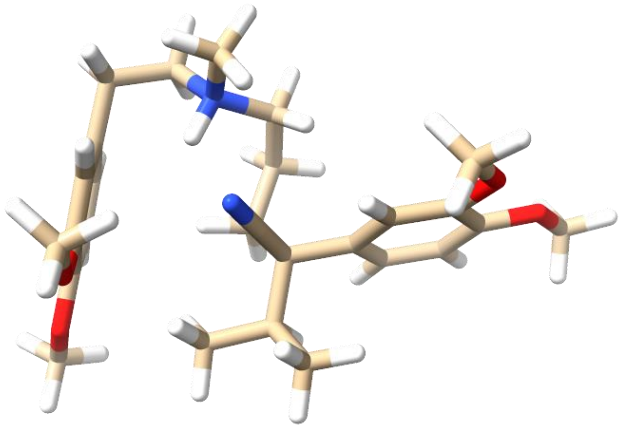




Are we seeing a prototropic isomer of Verapamil?

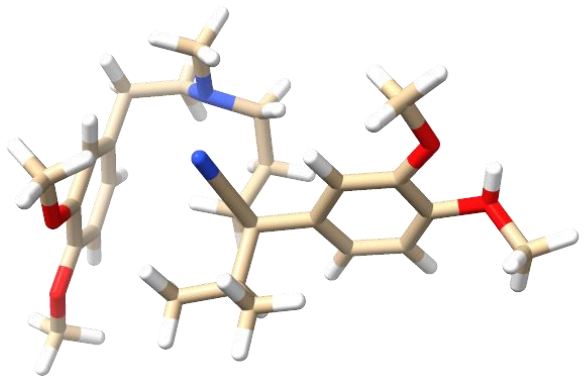


Amino-protonated



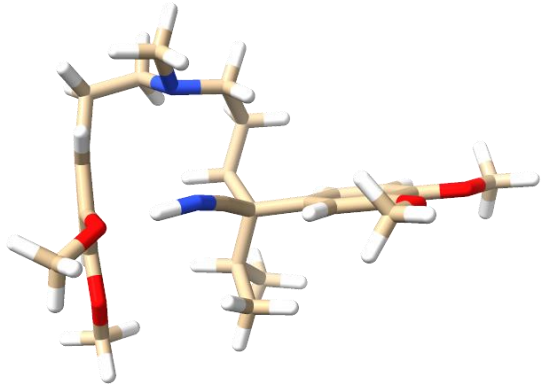
0 kJ mol^{-1}

Methoxy-protonated



110 kJ mol^{-1}

Cyano-protonated

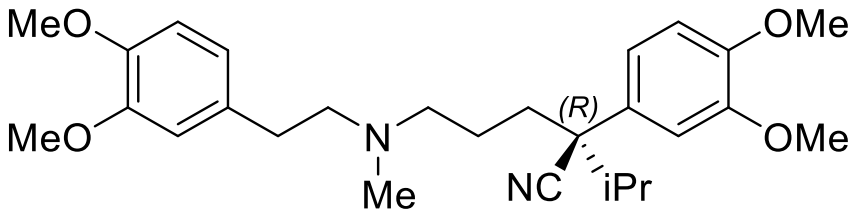


130 kJ mol^{-1}

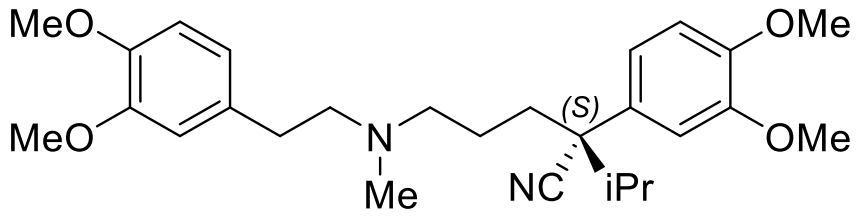
So what gives with Verapamil's dual peaks?

Verapamil is a chiral molecule, so perhaps chirality has something to do with it?

(R)-Verapamil



(S)-Verapamil



Enantiomers (mirror images of one another)

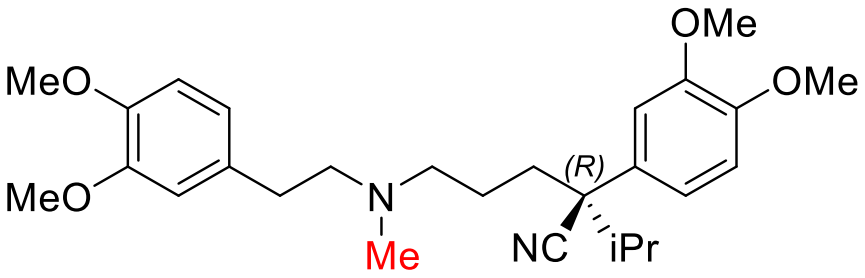
Enantiomers exhibit the opposite stereochemistry at **all** chiral centers

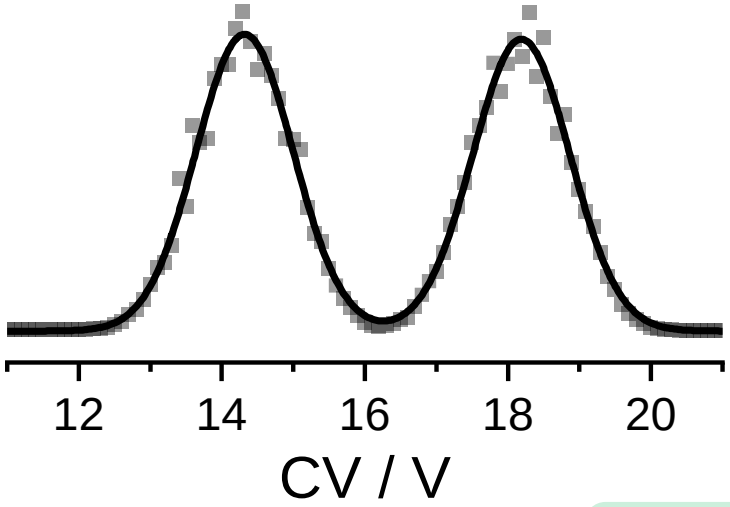
Enantiomers cannot be resolved in an achiral environment or without chiral derivatization

So what gives with Verapamil's dual peaks?

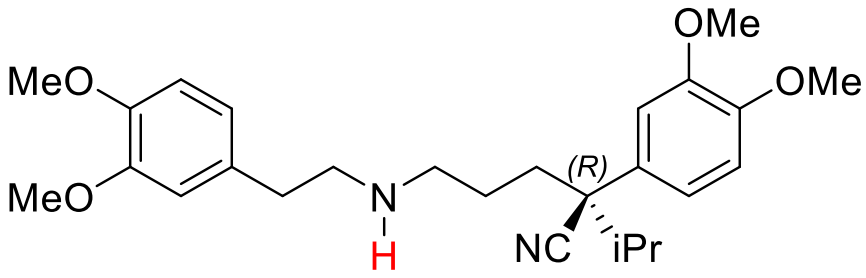
Perhaps looking at the DMS behaviour of Verapamil derivatives will provide some insight?

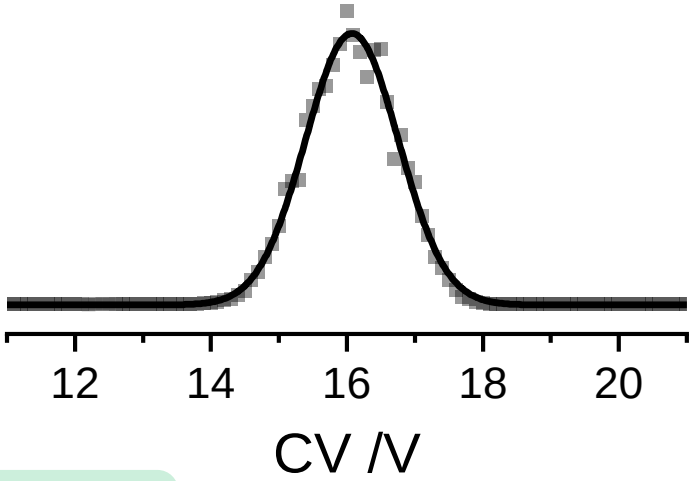
(R)-Verapamil





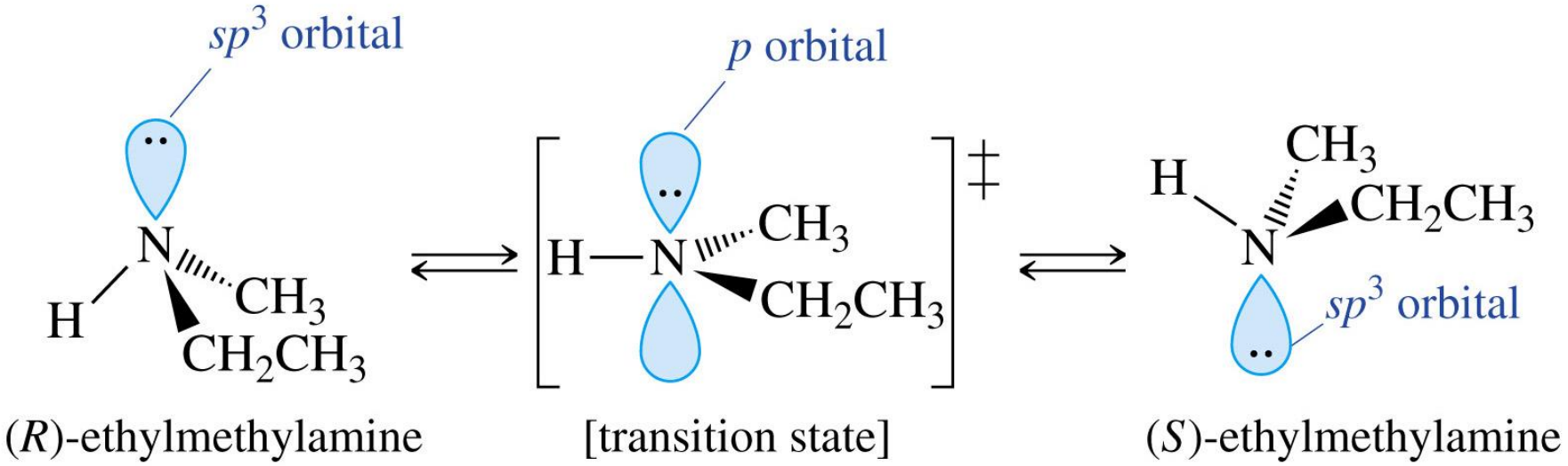
(R)-Norverapamil

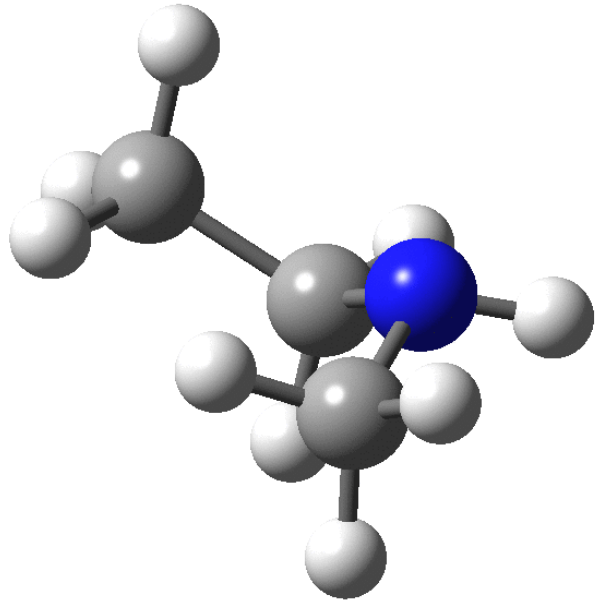




The amino moiety is the key

So what gives with Verapamil's dual peaks?





NH_3
$k = \sim 10^{10}\ s^{-1}$

RNH_2
$k = \sim 10^{8}\ s^{-1}$

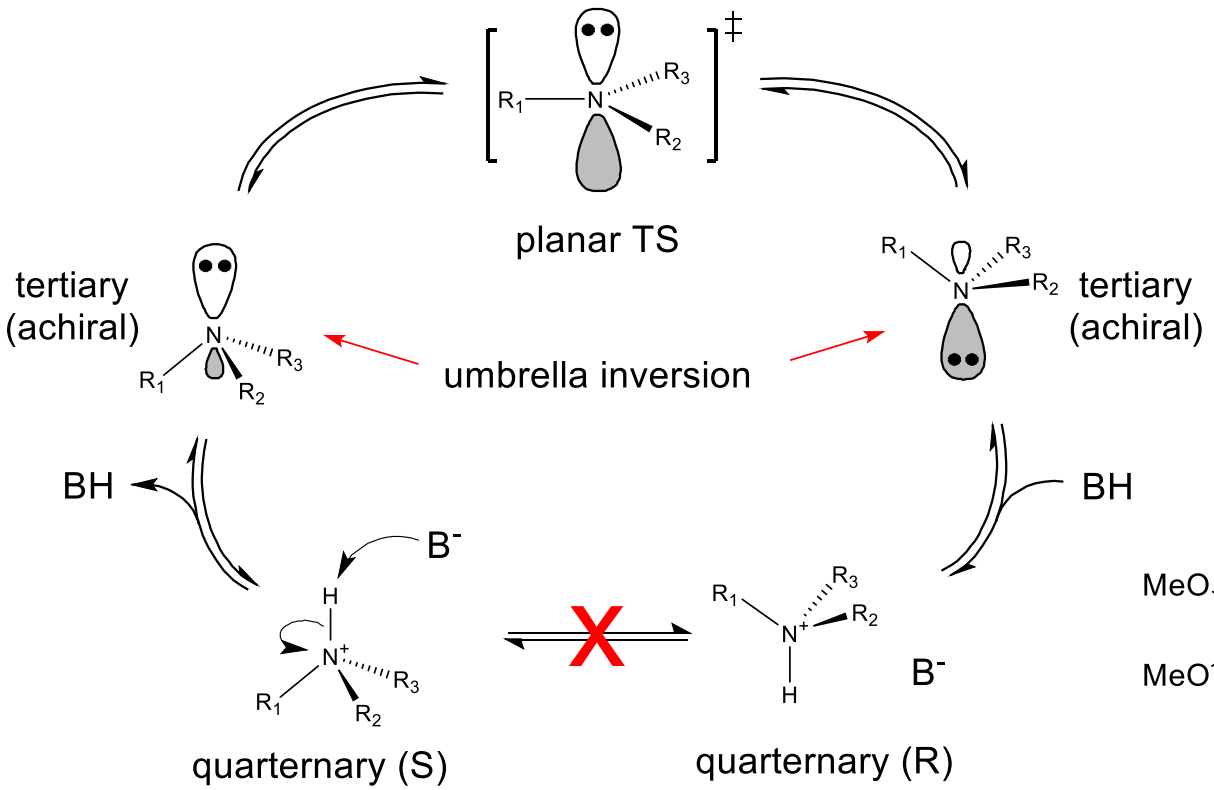
R_2NH
$k = 10^{5} - 10^{7}\ s^{-1}$

R_3N
$k = 100 - 10000\ s^{-1}$

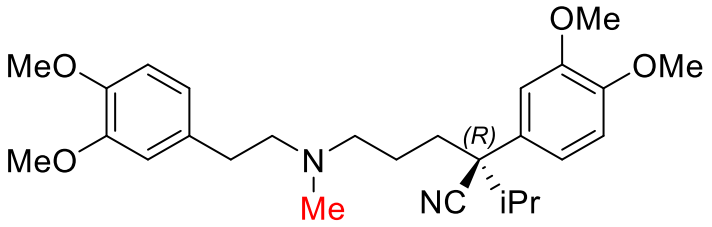
Amines are **not** stereogenic centers

So what gives with Verapamil's dual peaks?

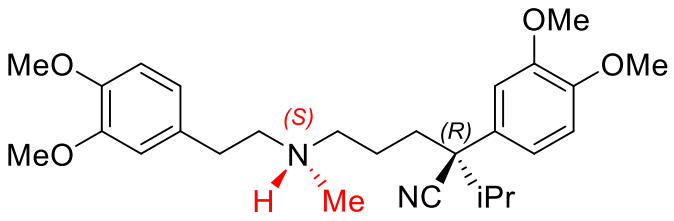
However, Verapamil exists as a **desolvated, protonated** ion in the DMS cell

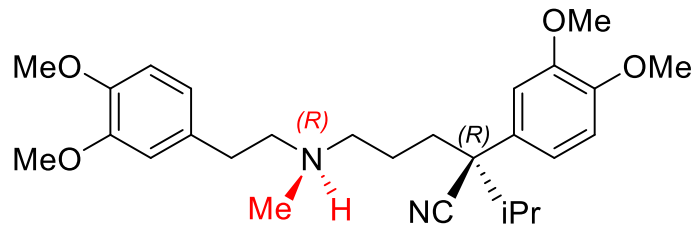


(R)-Verapamil



Protonation during ESI



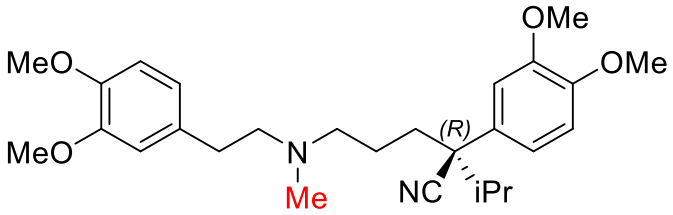


Diastereomers differ in the configuration of **at least one, but not all** stereocenters

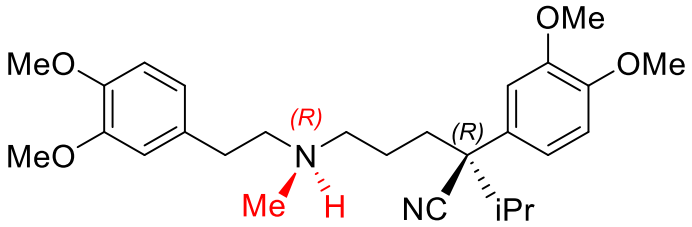
So what gives with Verapamil's dual peaks?

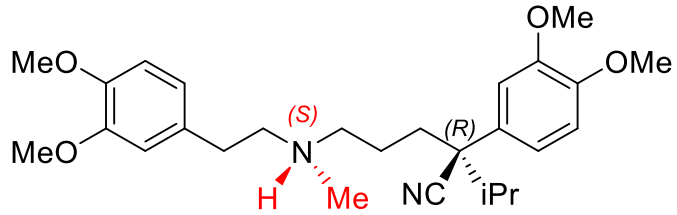
What happens with a **racemic** mixture of Verapamil?

(R)-Verapamil



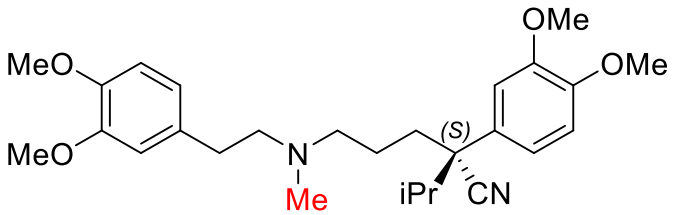
Protonation during ESI



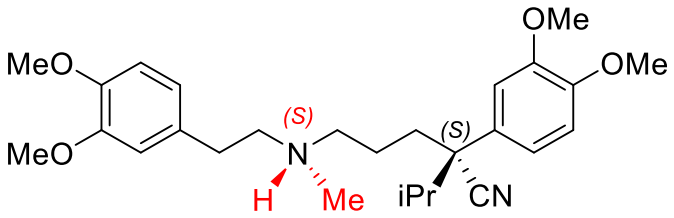


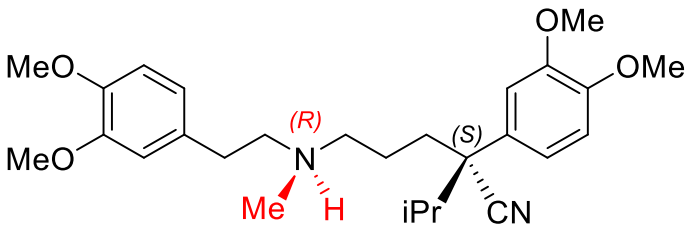
(R,R) and (S,R) Verapamil diastereomers

(S)-Verapamil



Protonation during ESI





(S,S) and (R,S) Verapamil diastereomers

Diastereomers differ in the configuration of **at least one, but not all** stereocenters

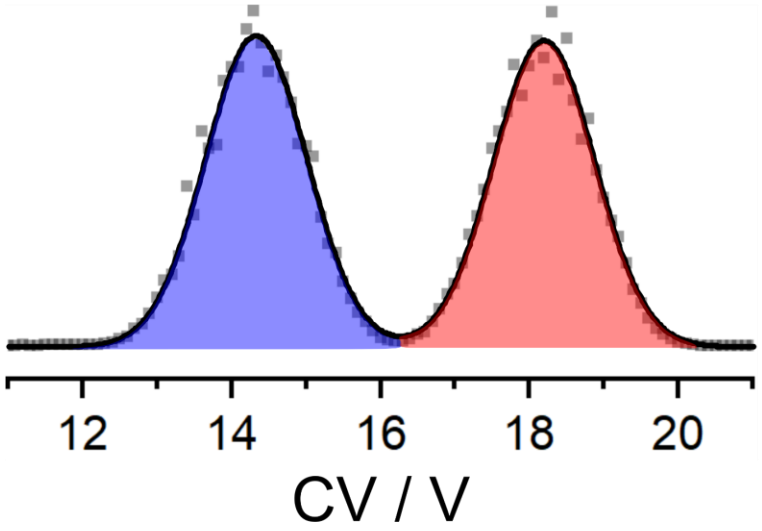
Of the 4 possible stereoisomers, 2 pairs are enantiomeric

Protonation-induced chirality drives the separation

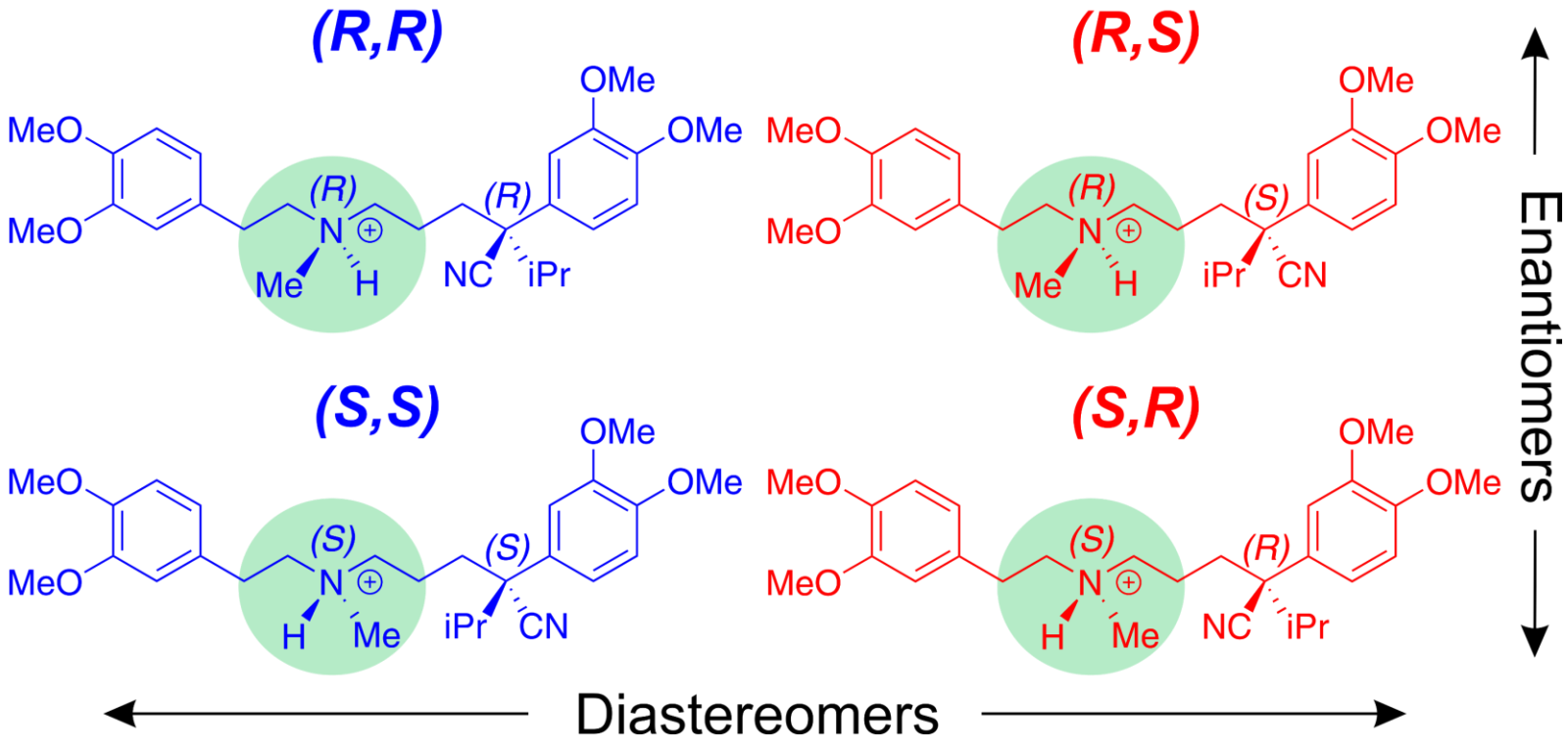
A (±)-Verapamil

SV = 4500 V T_{bath} = 50°C

Resolving gas **on** (10 psi)



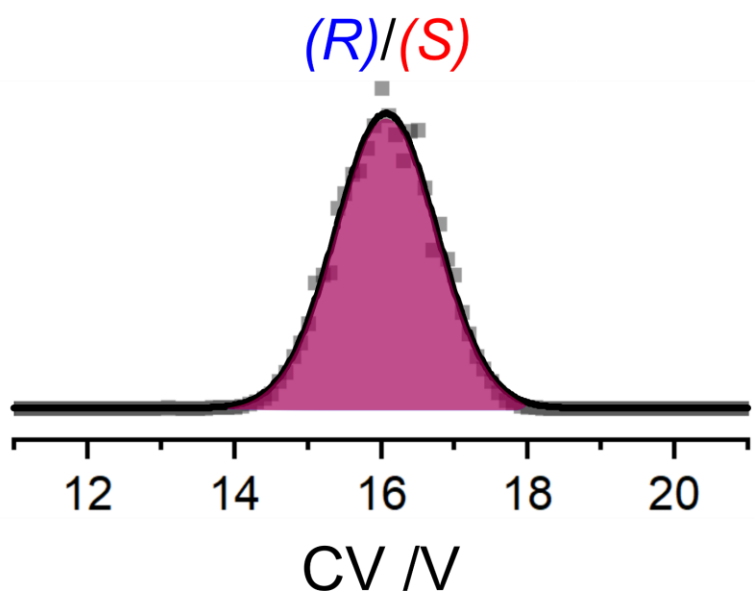
B Protonation yields an additional stereocenter



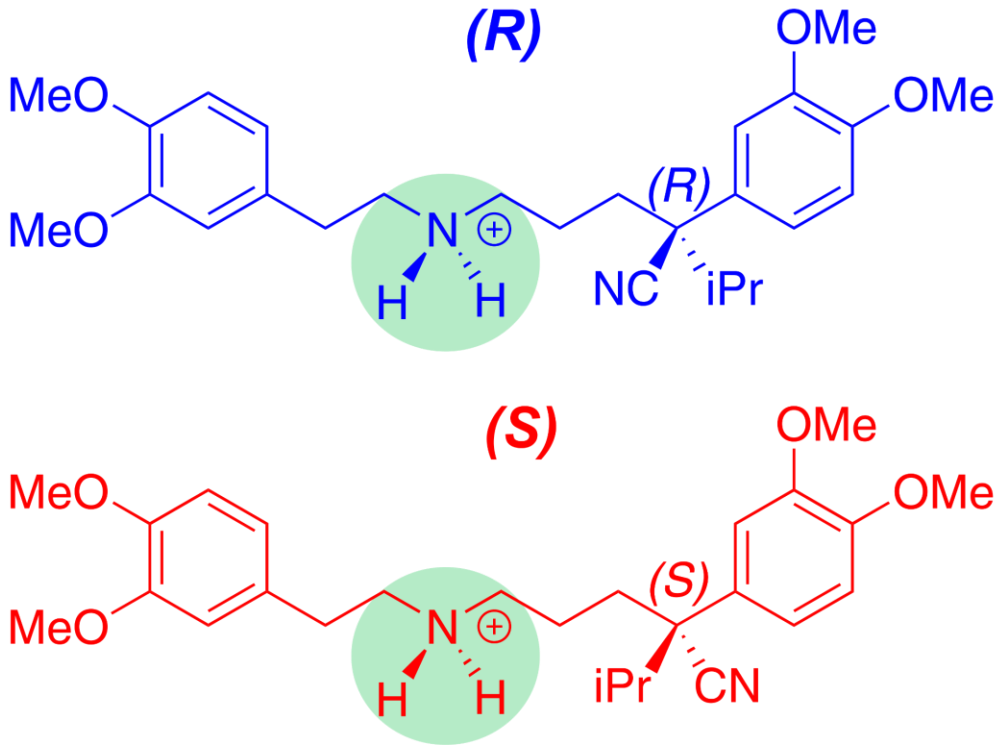
Protonation-induced chirality drives the separation

A (±)-Norverapamil

SV = 4500 V T_{bath} = 50°C
Resolving gas **on** (10 psi)



B Protonation does not affect stereochemistry

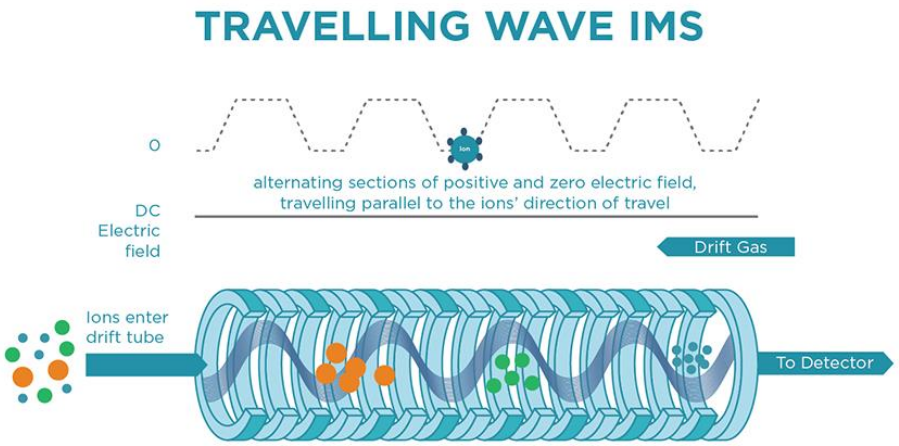


How can we justify the hypothesis?

Experimentally

Protonation-induced diastereomers exhibit different DMS behaviour, implying that their low-field mobility is also different

Experimentally measure the low-field ion mobility by linear IMS (Synapt G2-Si)



$$K(0) = \frac{\sqrt{18\pi}}{16}\frac{ze}{\sqrt{\mu k_b T}}\frac{1}{\boldsymbol{\Omega}}\frac{1}{N}$$

Ω – Collision Cross Section

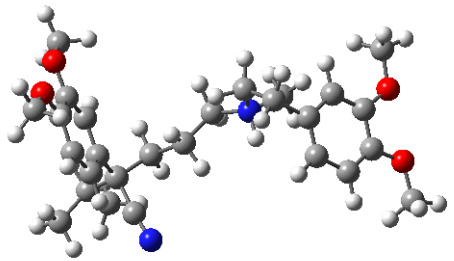
Figure courtesy of Owlstone Medical.

Computationally

Map the PES of *N*-protonated verapamil

Modified AMBER forcefield

(R,R) *(R,S)*
(S,S) *(S,R)*



Refine low-energy structures from PES search with high-level DFT calculations

ωB97X-D/def2-TZVPP

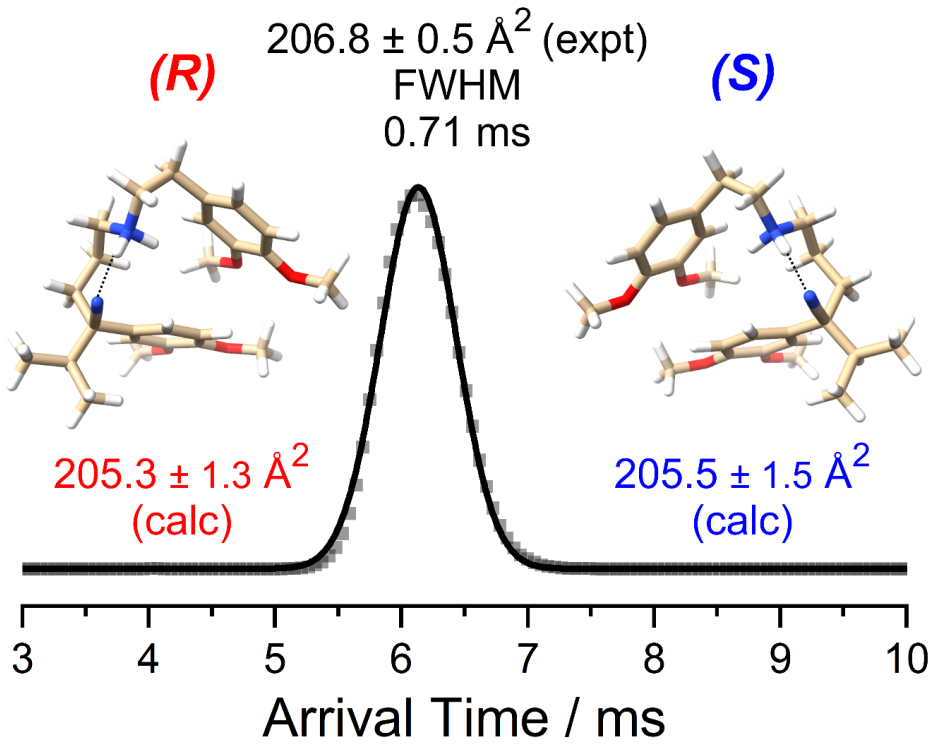
Use DFT structures and partial charges to calculate CCSs via MobCal-MPI

Verapamil "separates" by linear IMS

TWIMS suggests that verapamil's ATD consists **of more than one** configuration (based on peak FWHM)

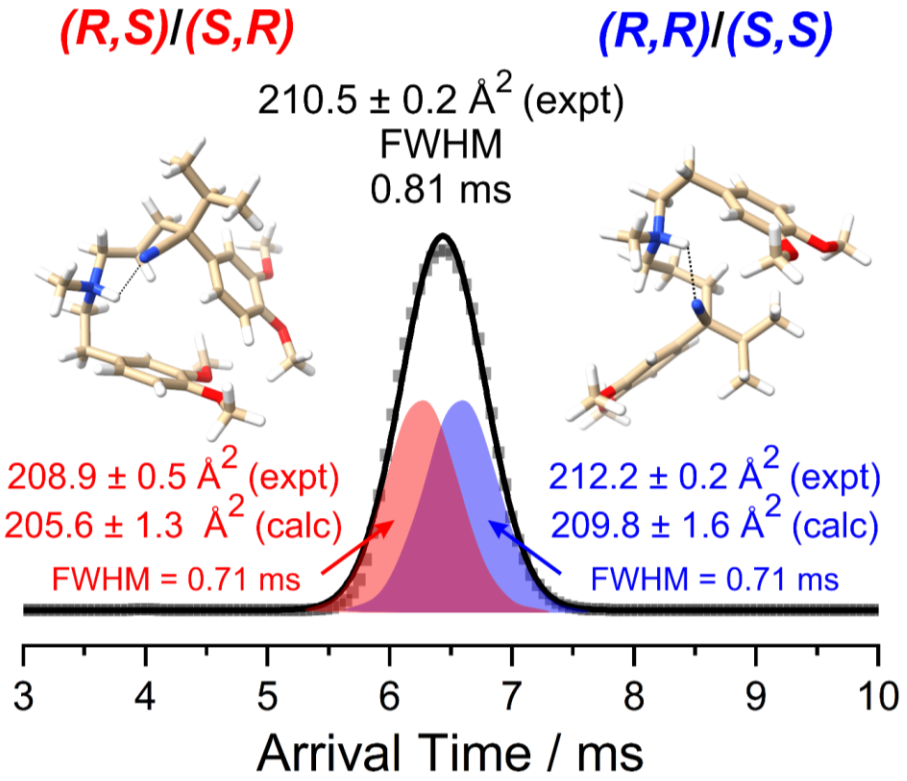
(±)-Norverapamil

Protonation does not affect chirality. ATD is "1 compound"



(±)-Verapamil

Protonation-induced diastereomers form upon protonation

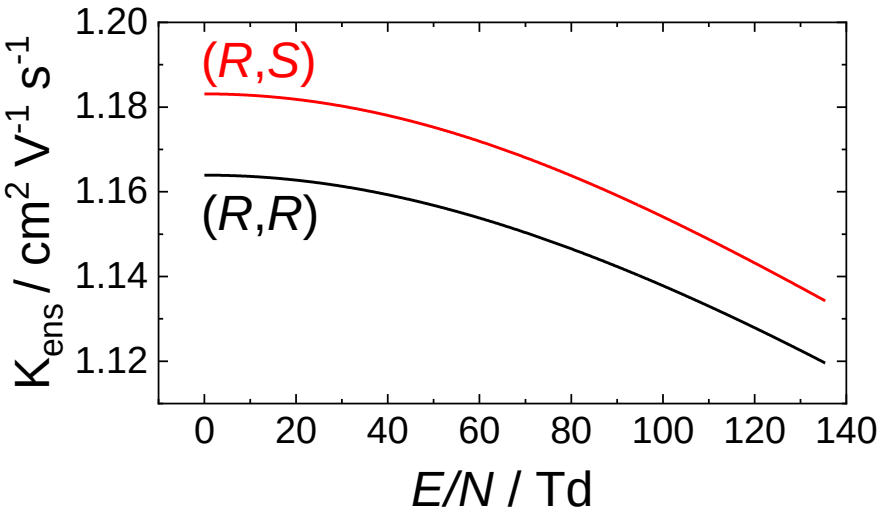


In silico DMS modelling reproduces verapamil separation

1. Calculation of the ensemble mobility (K) using 2T theory (first-order)

$$T_{eff} = T_{bath} + \frac{m_{bath} \cdot v_D^2}{3k_b} \qquad v_D = K \cdot E$$

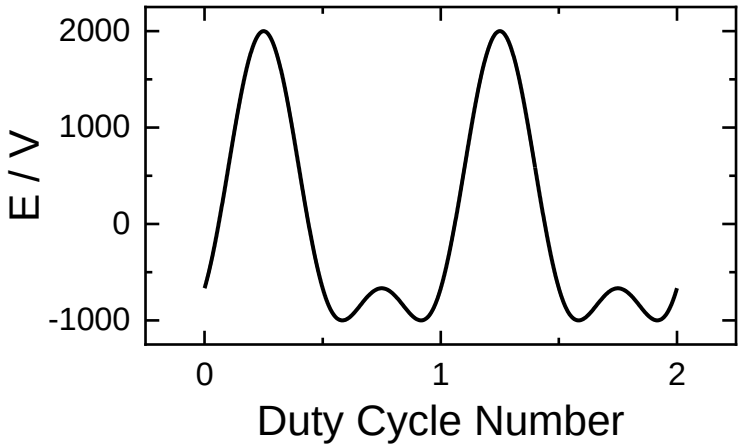
$$K = \frac{\sqrt{18\pi}}{16} \frac{ze}{\sqrt{\mu k_b T_{eff}}} \frac{1}{\Omega} \frac{1}{N} \qquad \langle K \rangle_{ens} = \sum_i K_i \cdot p_i$$



2. Calculation of optimal CV at a given SV

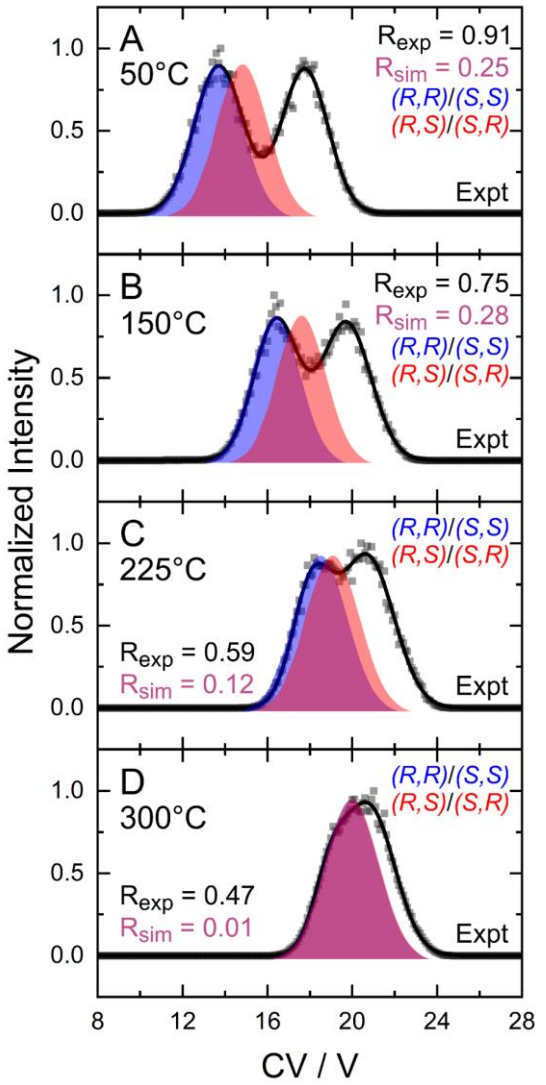
$$\alpha(E) = \frac{\langle K(E) \rangle_{ens}}{\langle K(0) \rangle_{ens}} - 1$$

$$E(t) = \frac{SV}{d}\left(\frac{2}{3}\sin(\omega t) + \frac{1}{3}\sin\left(2\omega t - \frac{\pi}{2}\right)\right)$$

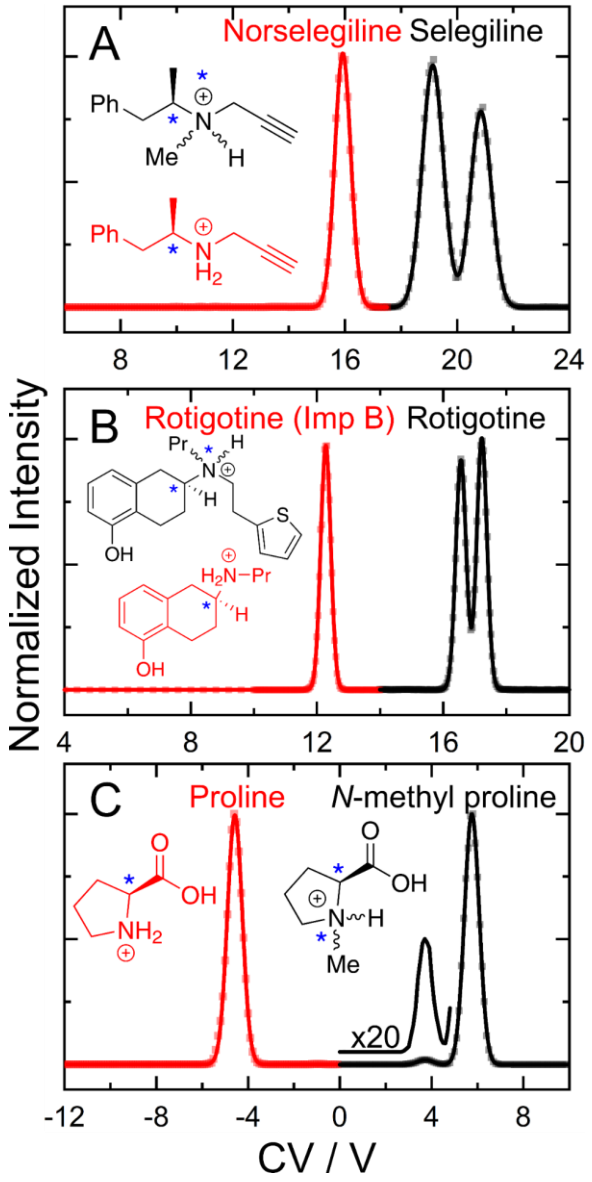


$$\boldsymbol{CV = -\frac{\langle \alpha(E(t)) \cdot E(t) \rangle_{wf}}{1 + \langle \alpha(E(t)) \rangle_{wf} + \langle \alpha'(E(t)) \cdot E(t) \rangle_{wf}} \cdot d}$$

SV = 4500 V



Is protonation-induced chirality a general phenomenon?



3 additional cases with a parent molecule and "achiral" metabolite

Why are there intensity differences between the two peaks?

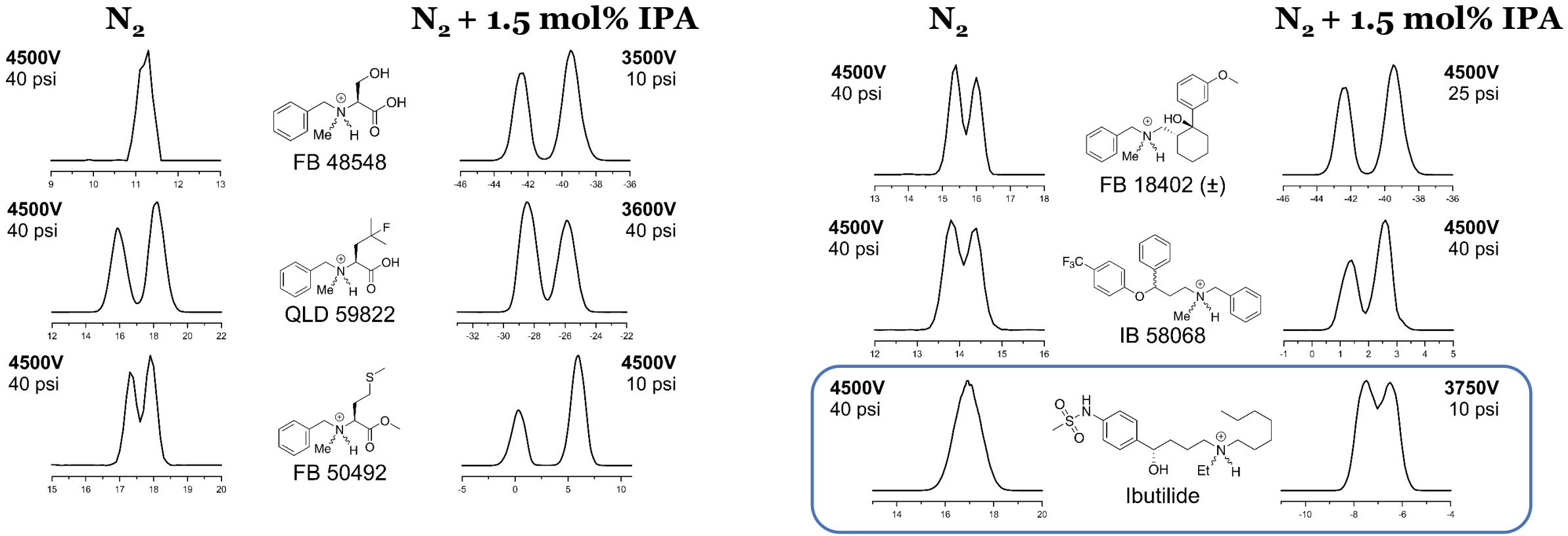
1) Energy differences between the protonation induced diastereomers

2) Does steric hindrance impede nitrogen inversion in the solution phase?

3) Does a more stable solution-phase diastereomer get kinetically trapped during the droplet evaporation portion of ESI?

Is protonation-induced chirality a general phenomenon?

6 other molecules susceptible to protonation-induced chirality are separable by DMS

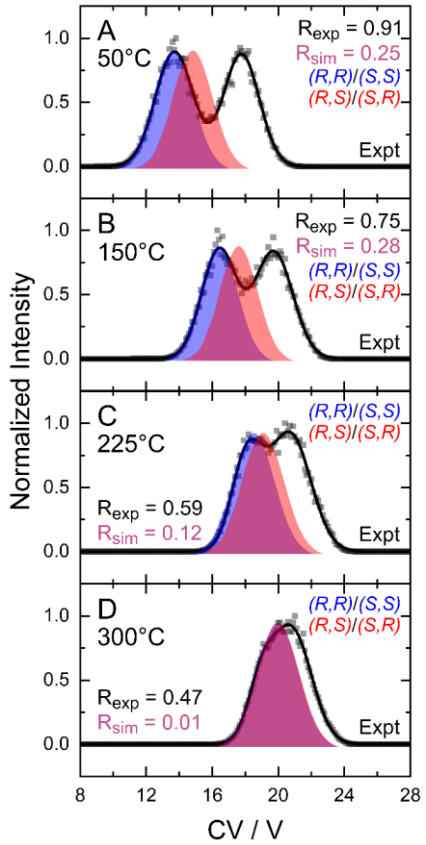


Seeding the DMS carrier gas with IPA was required to fully resolve some compounds

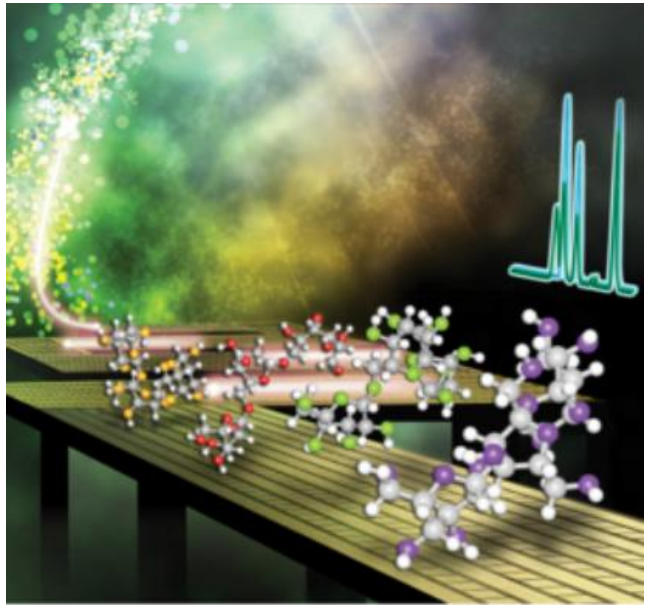
Where can we go with this?

Three criteria need to be met to observe protonation-induced chirality:

1. The molecule must possess a **permanent stereocenter**
2. The molecule must possess a **stereogenic precursor** susceptible to protonation (e.g., tertiary amine)
3. Once protonated, the **diastereomers must not interconvert** and must **preserve structural differences**



Things to watch out for experimentally



J. Am. Soc. Mass Spectrom. **2021**, 32, 1126-1137.

Verapamil is a standard for calibrating CCSs in drift-tube IMS and TWIMS

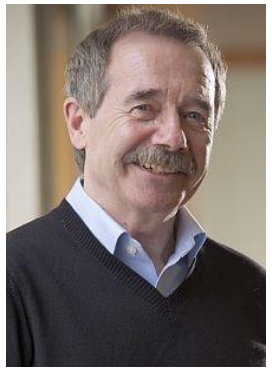
Waters Synapt G2-Si: $R_p \sim 60$

SLIMS, Cyclic-IMS: $R_p > 150$

Acknowledgements

PhD Committee

Prof. W. Scott Hopkins



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Collaborators, Resources, and Funding