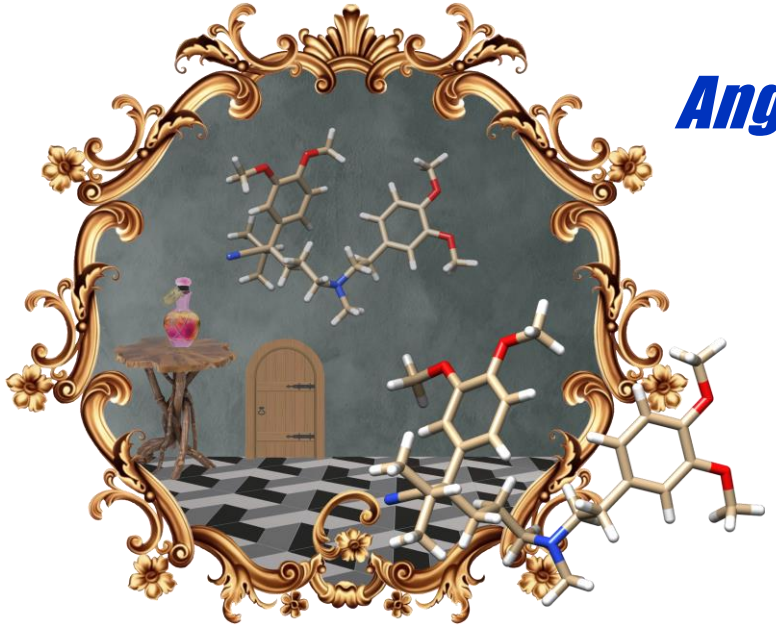
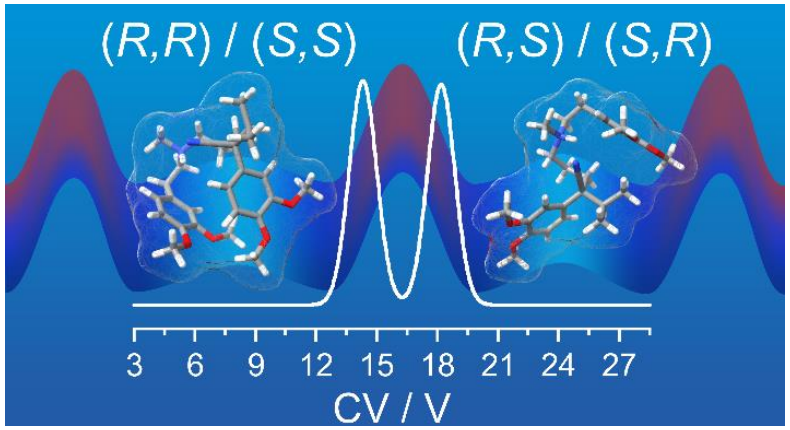
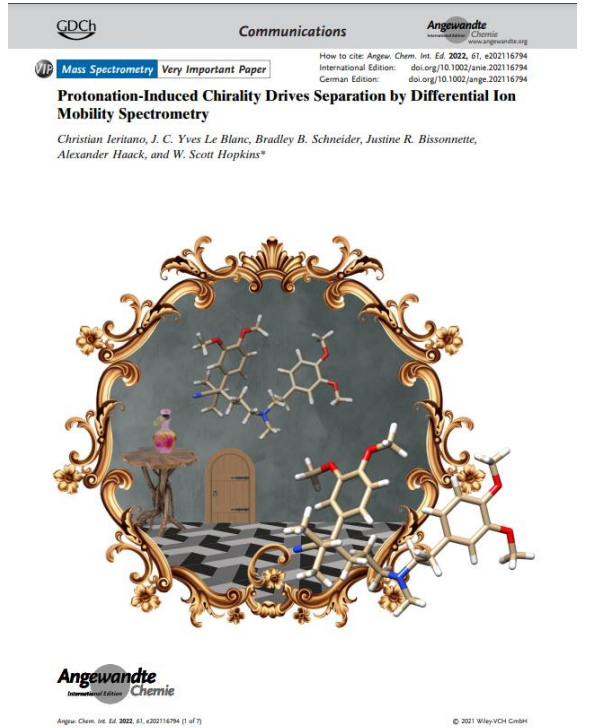
Protonation-Induced Chirality Drives Separation by Differential Mobility Spectrometry






GDCh Communications Angewandte International Edition / German Edition

Mass Spectrometry Very Important Paper

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Protonation-Induced Chirality Drives Separation by Differential Ion Mobility Spectrometry

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

Angewandte International Edition / Chemie

Angew. Chem. Int. Ed. 2022, 61, e202116794 (1 of 7)

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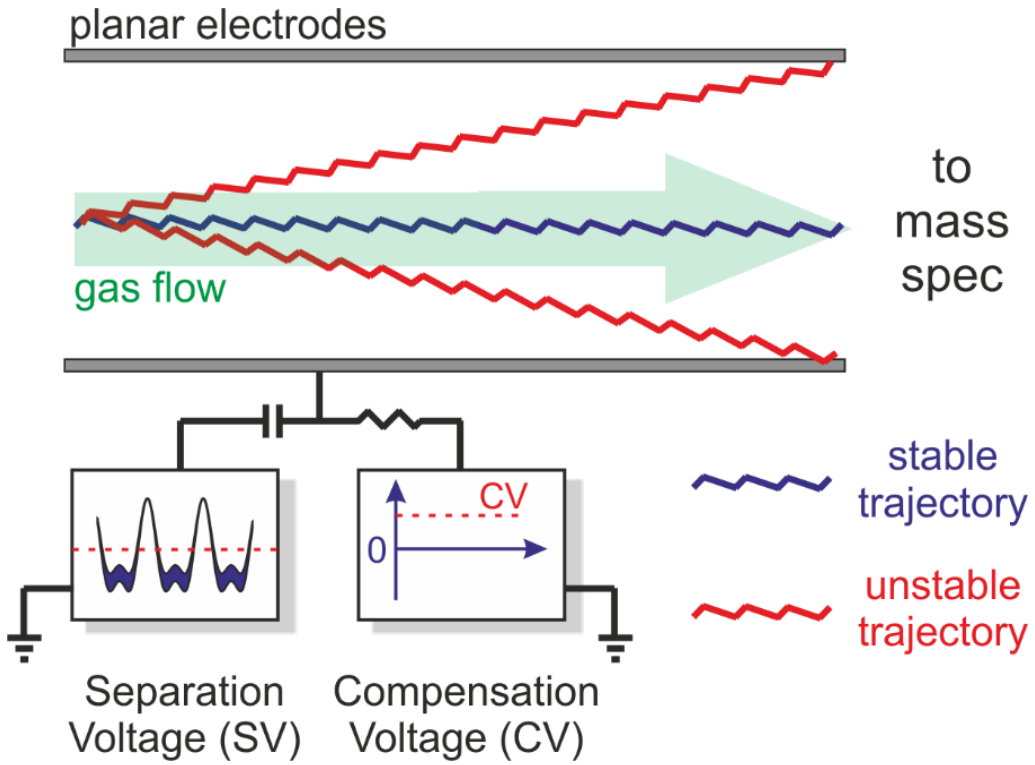
Angew. Chem. Int. Ed. 2022, 61, e202116794

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

June 6, 2022

What is Differential Mobility Spectrometry (DMS)?

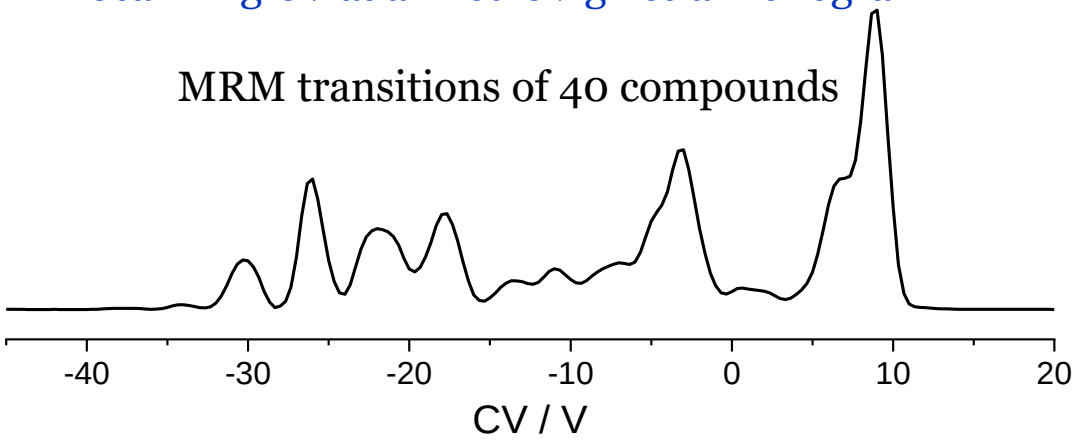
Differential mobility spectrometry (DMS) is a non-linear form of ion-mobility spectrometry



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

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

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

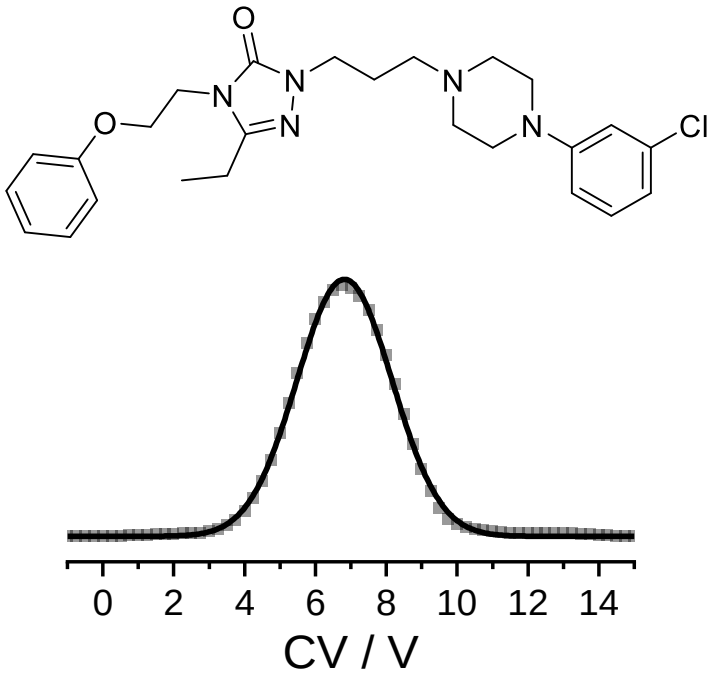
Mol. Phys. **2015**, 113, 3151–3158.

The SV/CV pair is an intrinsic ion property

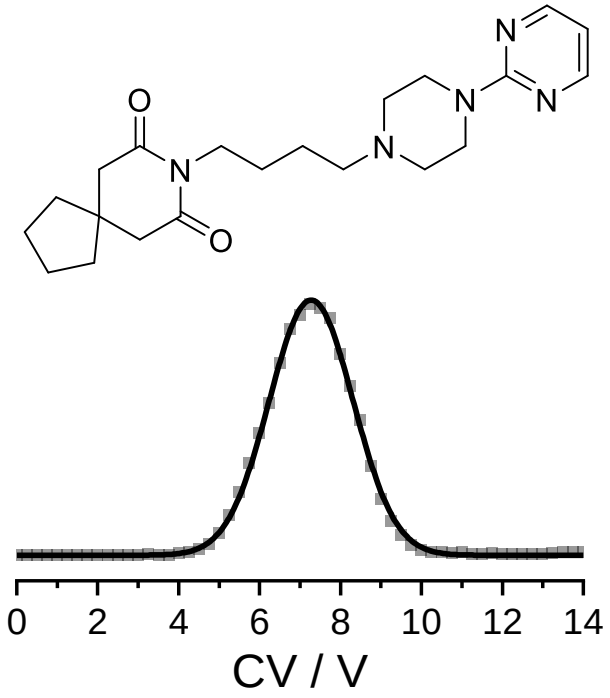
The CV in which an analyte elutes from the DMS cell is analyte specific, and orthogonal to LC

~ 15 years ago, SCIEX was looking at performing drug metabolite quantitation without LC

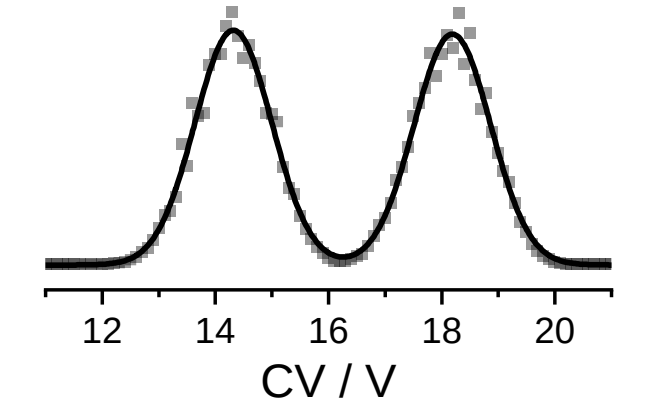
Nefazodone



Ketoconazole



...and Verapamil?



Why does Verapamil have two peaks?

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

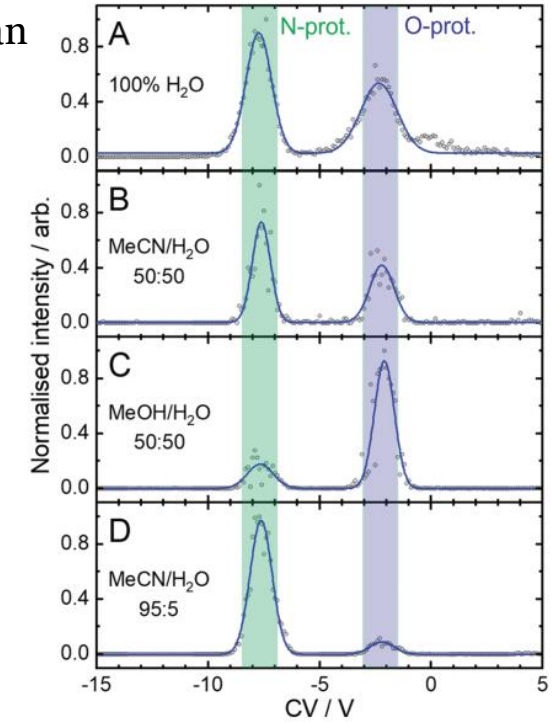
More than one feature in an ionogram **usually** indicates the presence of prototropic isomers

4-aminobenzoic acid

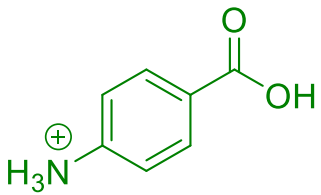
Phys. Chem. Chem. Phys. 2021, 23, 20607

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

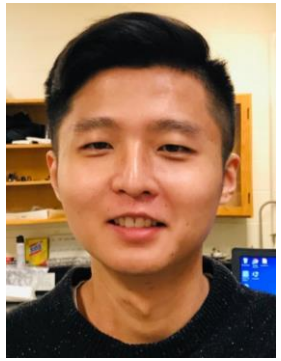
Dr. Neville Coughlan



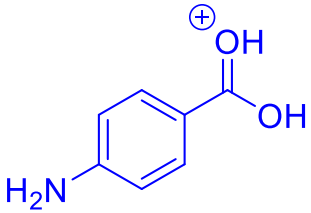
N-Prot



Dr. Weiqiang Fu



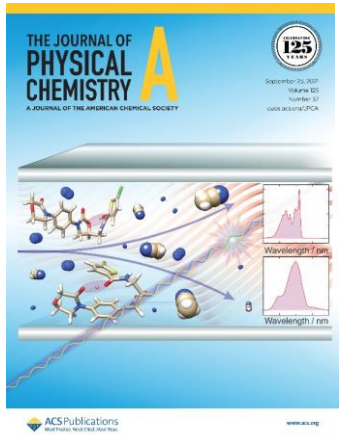
O-Prot

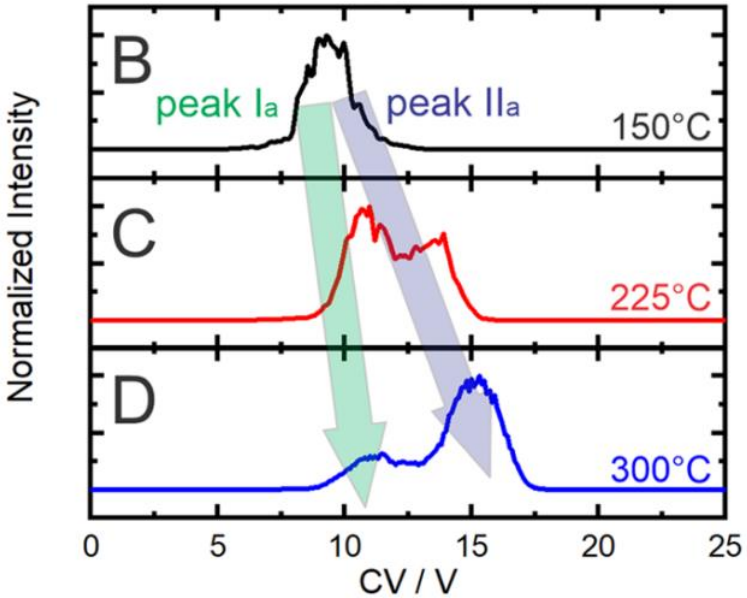


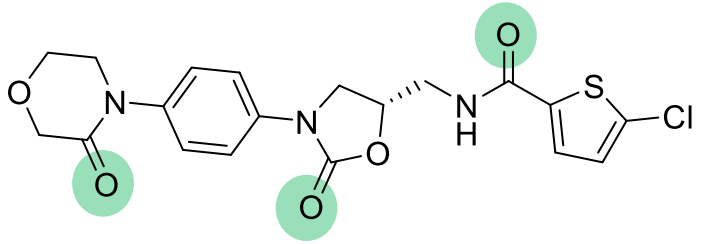
Rivaroxaban

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

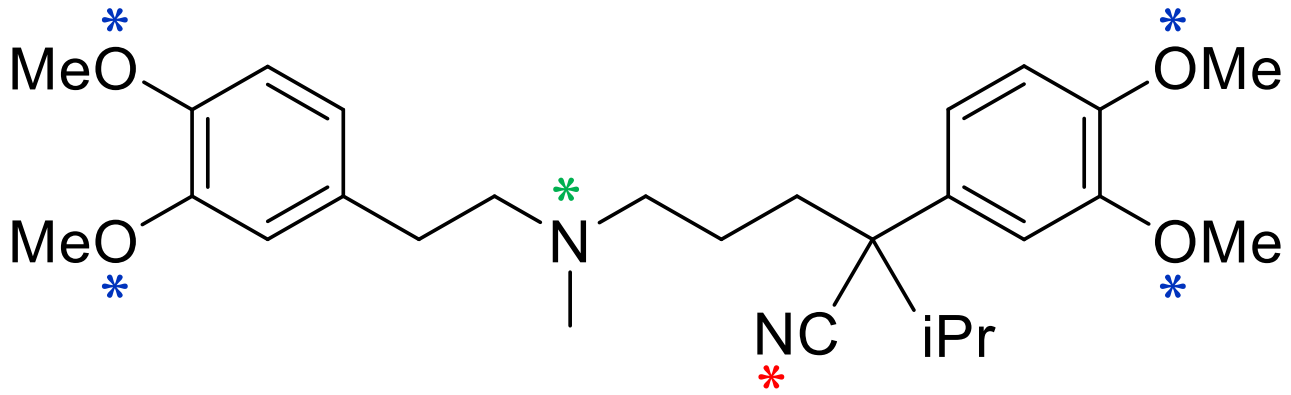
Nour Mashmoushi



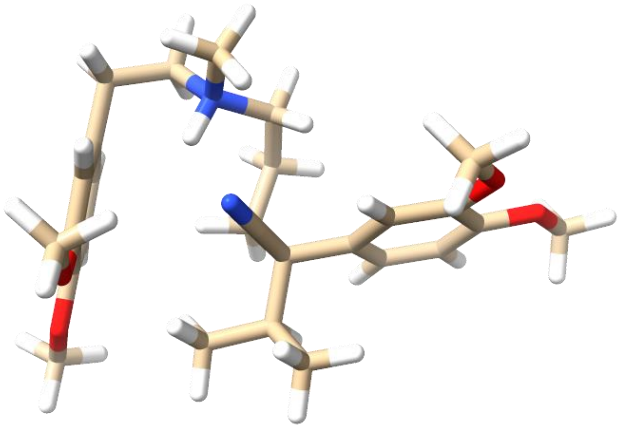




Are we seeing a prototropic isomer of Verapamil?

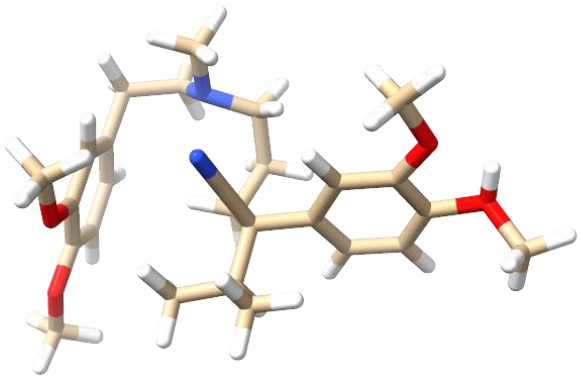


Amino-protonated



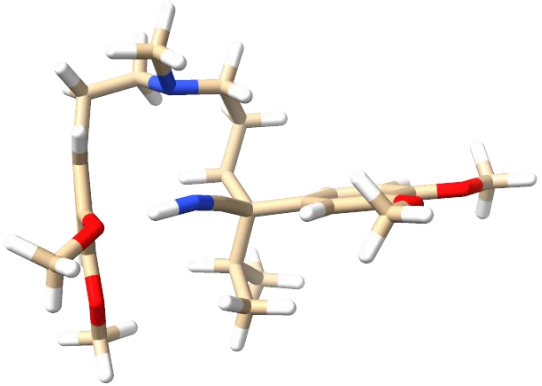
0 kJ mol^{-1}

Methoxy-protonated



110 kJ mol^{-1}

Cyano-protonated

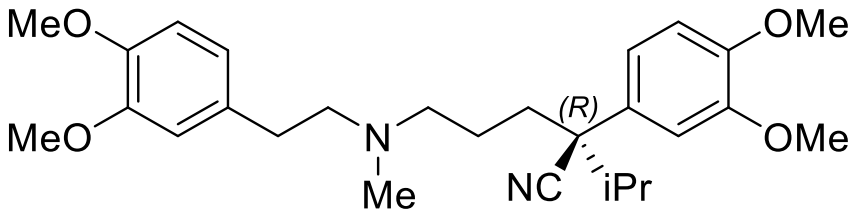


130 kJ mol^{-1}

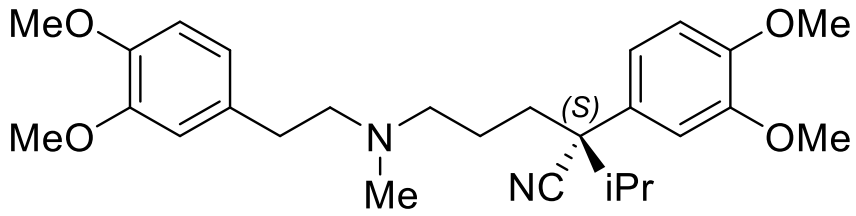
So what gives with Verapamil's dual peaks?

Verapamil is sold as a **racemate**, so perhaps **chirality** has something to do with it?

(*R*)-Verapamil



(*S*)-Verapamil



Enantiomers (mirror images of one another)

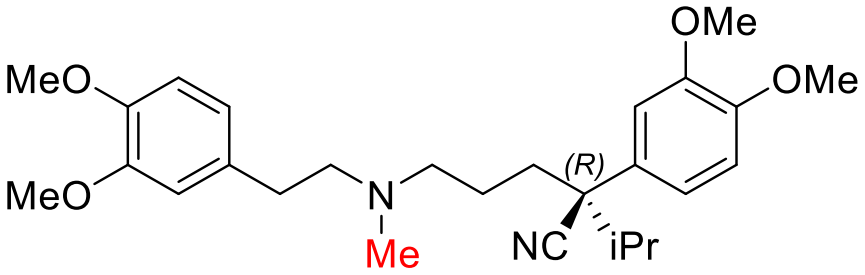
Enantiomers exhibit the opposite stereochemistry at **all** chiral centers

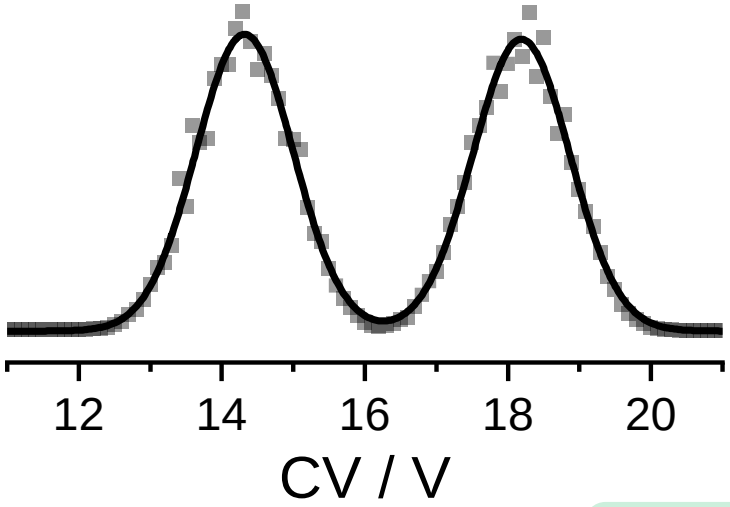
Enantiomers cannot be resolved by DMS **without chiral derivatization**

So what gives with Verapamil's dual peaks?

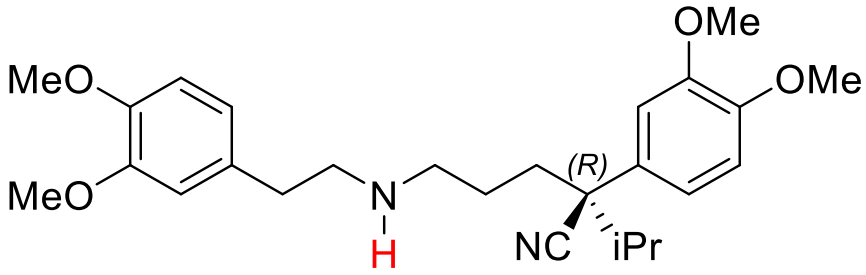
Perhaps looking at the DMS behaviour of Verapamil derivatives will provide some insight?

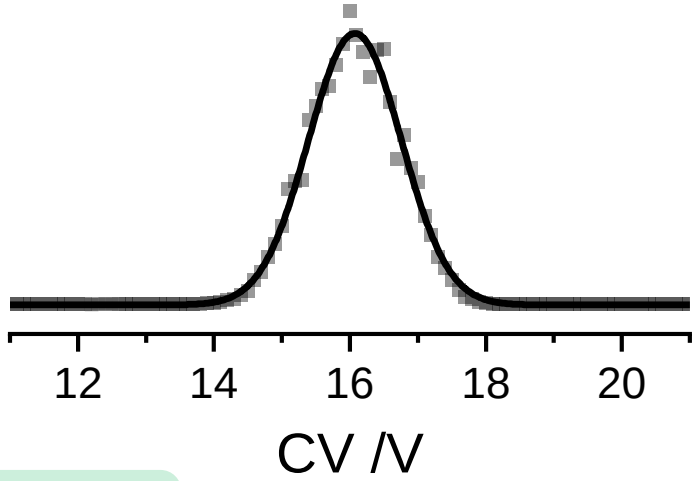
(*R*)-Verapamil





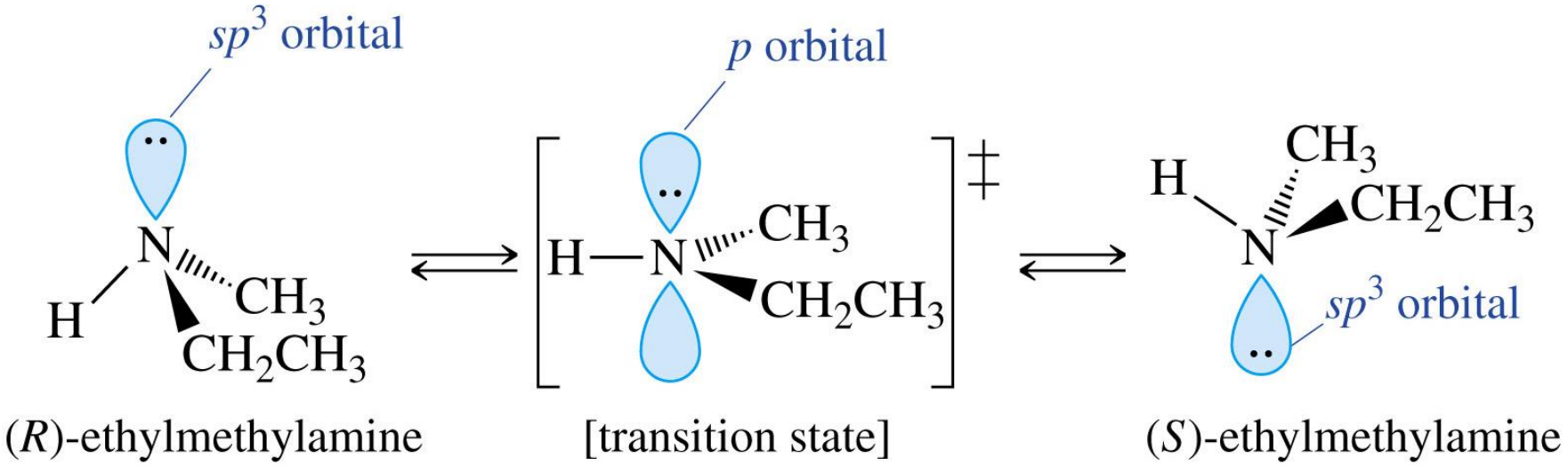
(*R*)-Norverapamil

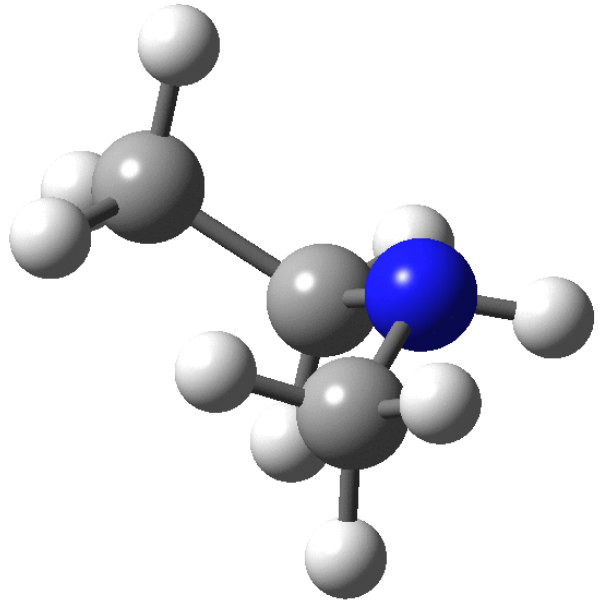




The **amino** moiety is the key

So what gives with Verapamil's dual peaks?





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

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

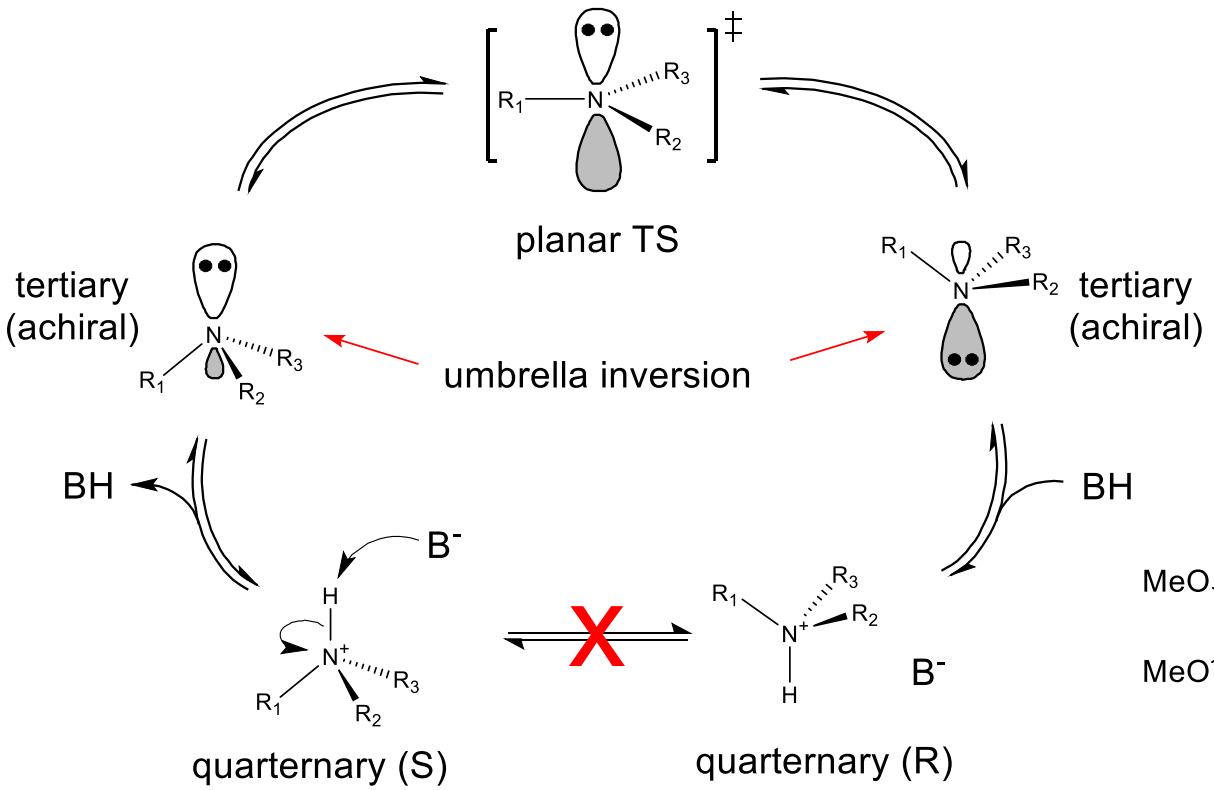
R_2NH
$k = 10^{5} - 10^{7}\ s^{-1}$

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

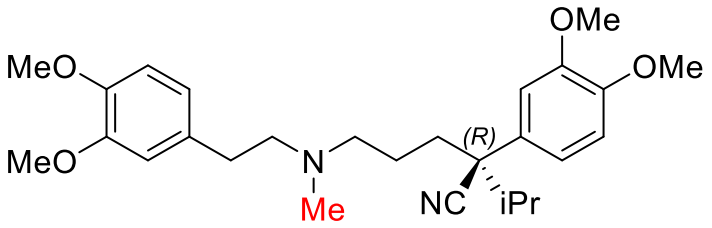
Amines are **not** stereogenic centers, but…

So what gives with Verapamil's dual peaks?

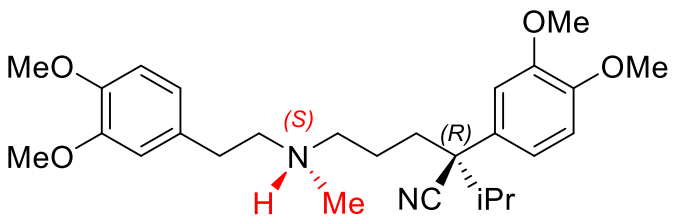
Verapamil exists as a **desolvated, protonated** ion in the gas-phase

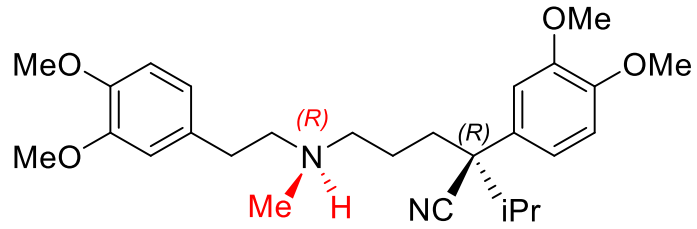


(R)-Verapamil



Protonation during ESI yields 2 diastereomers



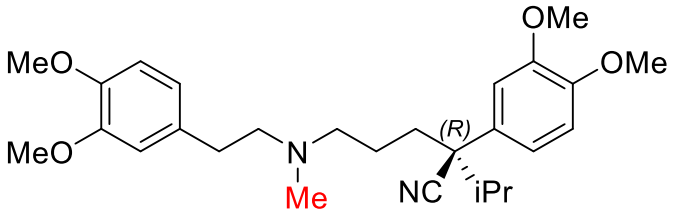


Diastereomers differ in the configuration of **at least one, but not all** stereocenters

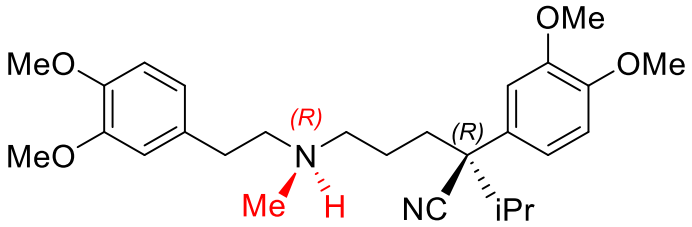
So what gives with Verapamil's dual peaks?

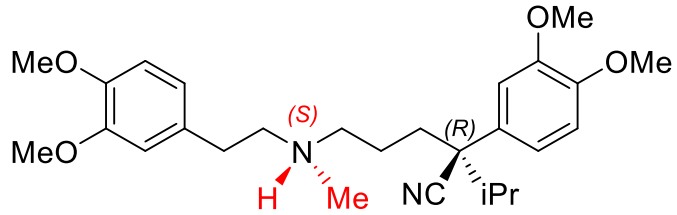
What happens with a **racemic** mixture of Verapamil?

(R)-Verapamil

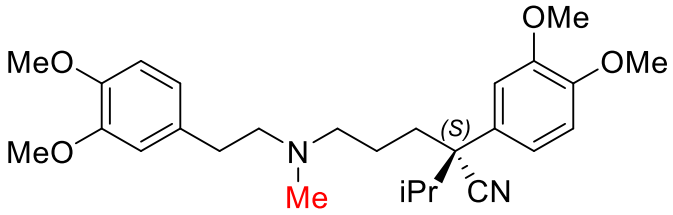


Protonation during ESI

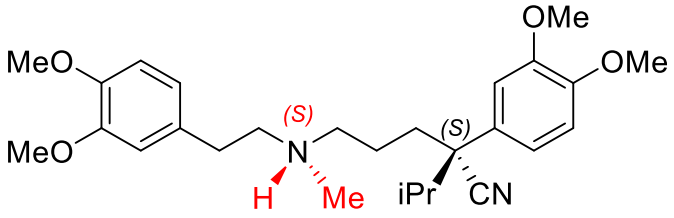


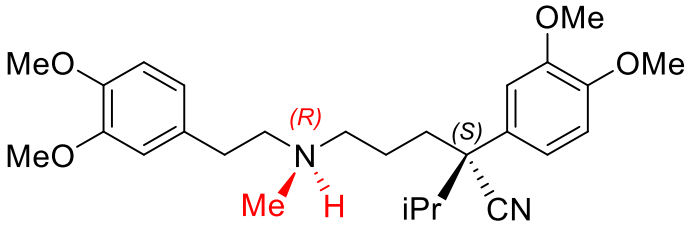


(S)-Verapamil



Protonation during ESI





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

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

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

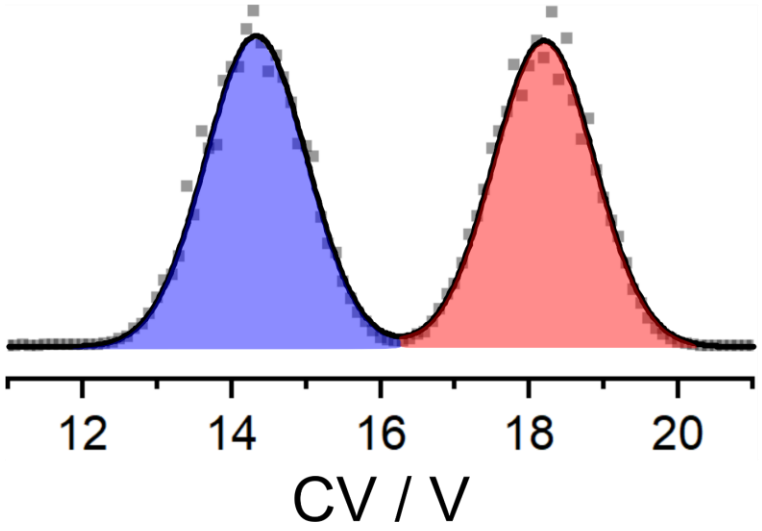
Of the 4 possible stereoisomers, **2 pairs are enantiomeric**

Protonation-induced chirality drives the separation

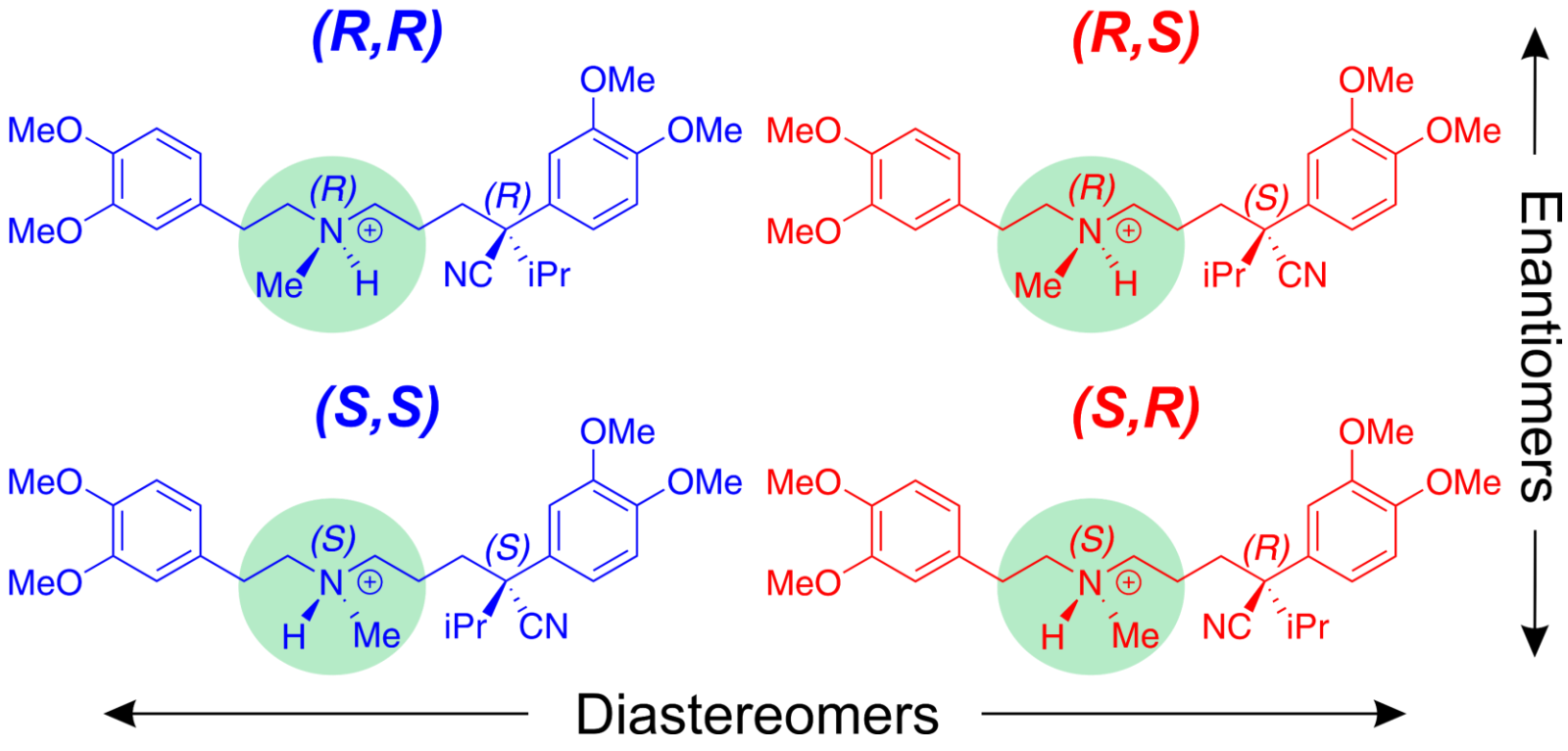
A (±)-Verapamil

SV = 4500 V T_{bath} = 50°C

Resolving gas **on** (10 psi)



B Protonation yields an additional stereocenter

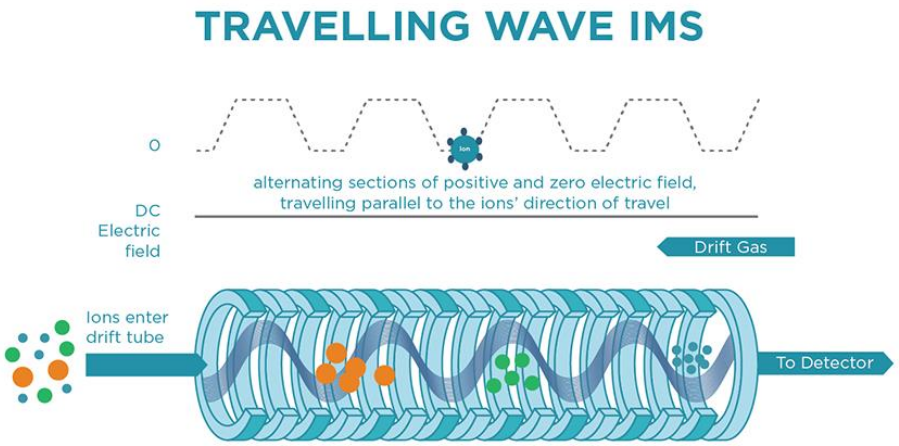


How else can we justify the hypothesis?

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

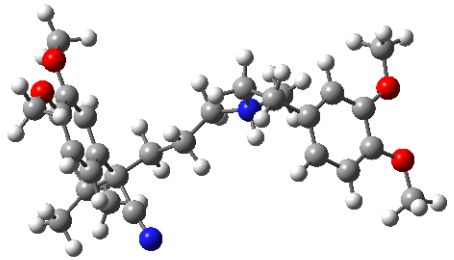
Figure courtesy of Owlstone Medical.

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

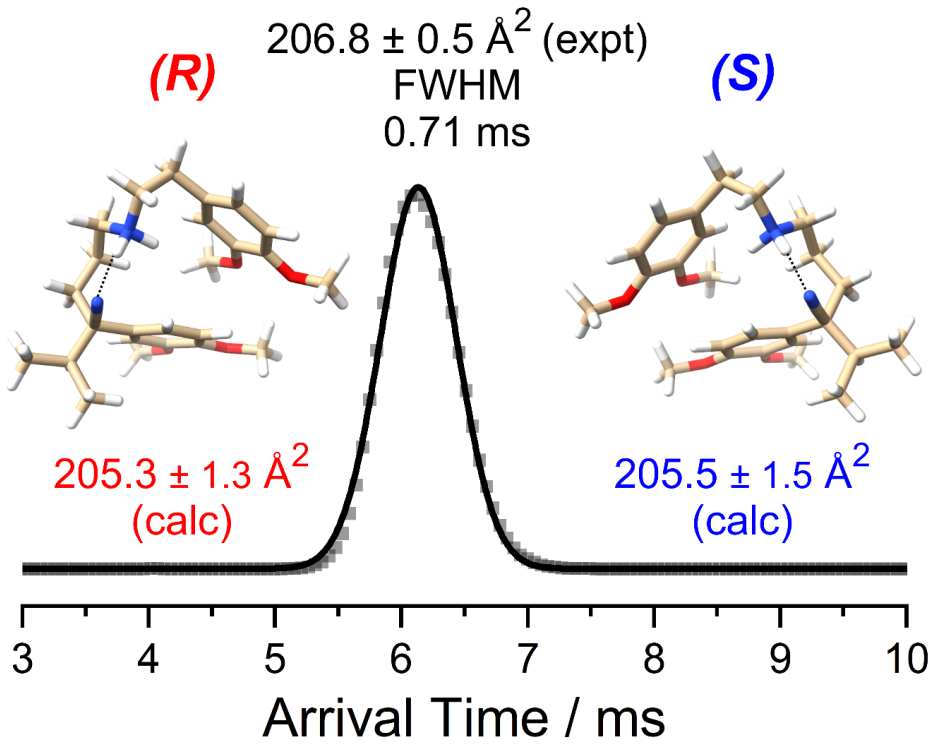
Use DFT structures and partial charges to calculate CCSs via MobCal-MPI

Verapamil "separates" by linear IMS

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

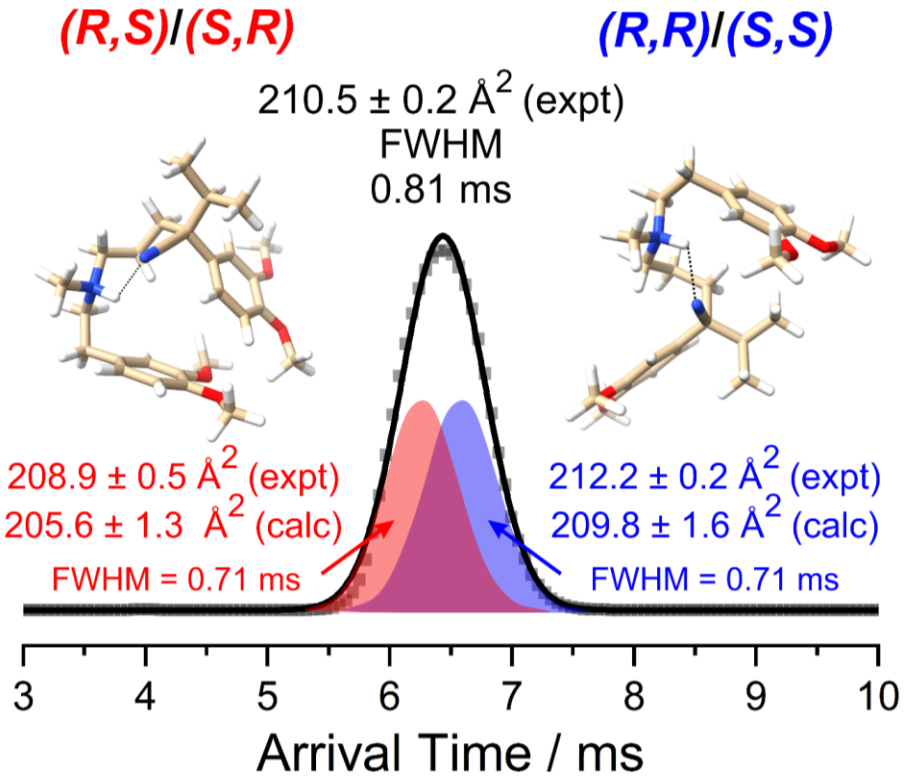
(±)-Norverapamil

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



(±)-Verapamil

Protonation-induced diastereomers form upon protonation



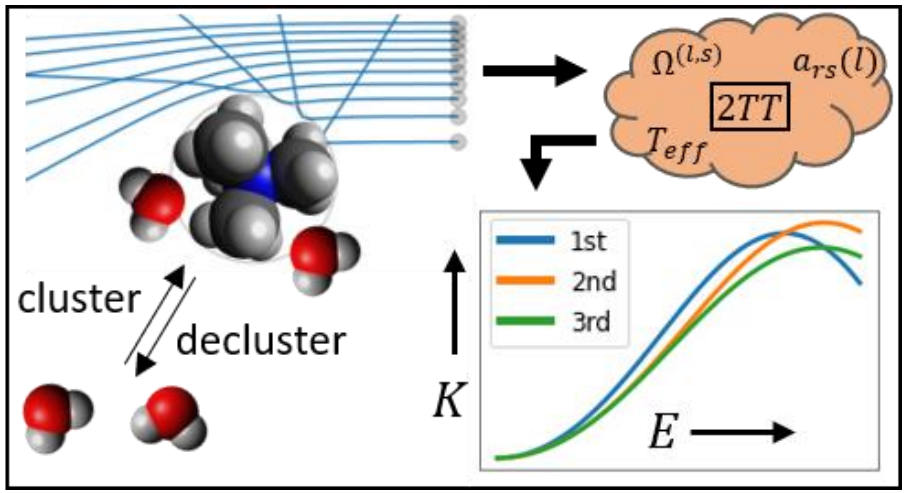
In Silico modelling of DMS behaviour

In silico models of Differential Mobility Using Higher Order Two-Temperature Theory

Dr. Alex Haack

@ahaack91

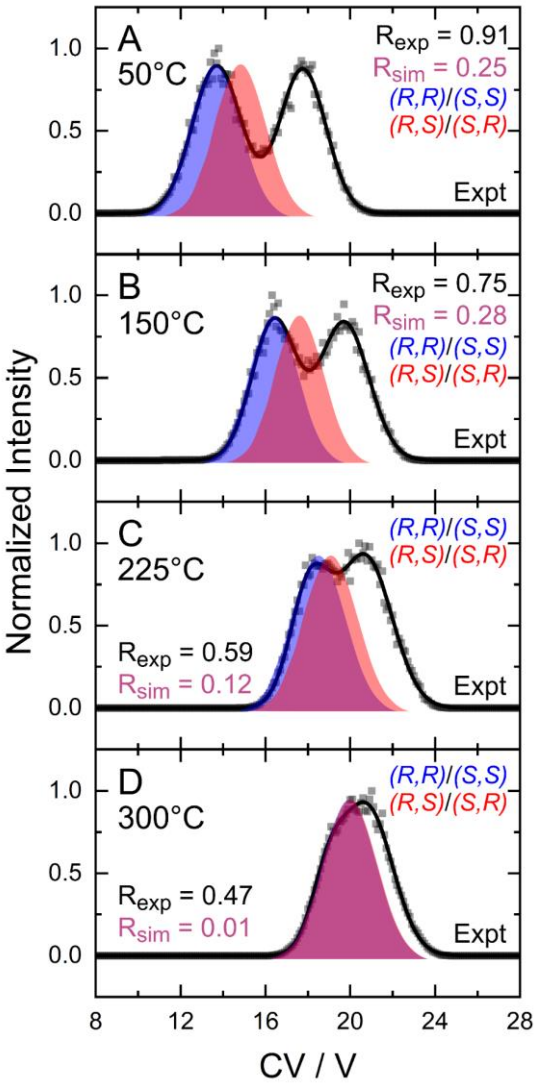
***J. Am. Soc. Mass Spectrom.*, 2022, 33, 535-547**



ThP 261

On the Conformational Changes of Tripeptides in Differential Mobility Spectrometry

SV = 4500 V



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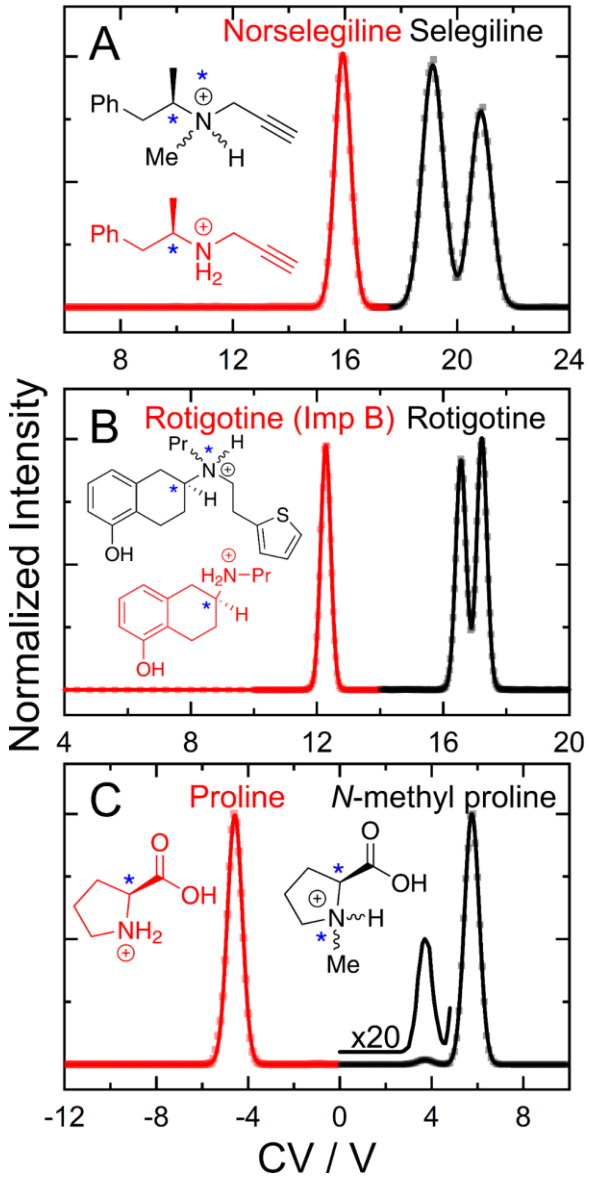
Is protonation-induced chirality a general phenomenon?



We tested 12 additional compounds that were commercially available and susceptible to protonation-induced chirality

Why are there intensity differences between the two peaks?

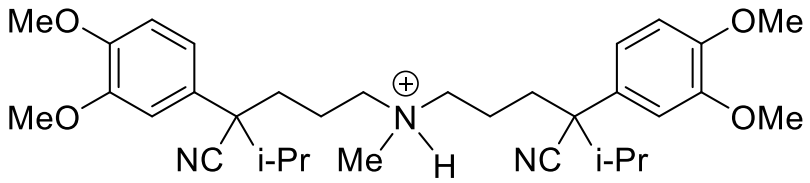
1) Energy differences between the protonation induced diastereomers

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?

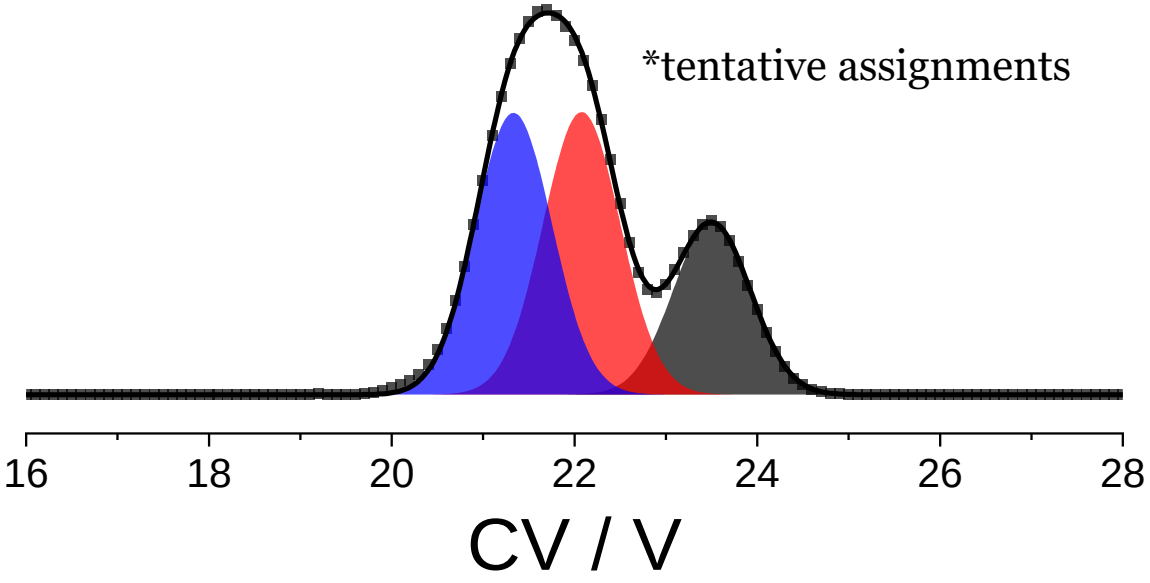
How complicated can protonation-induced chirality get?

Verapamil Impurity *N*

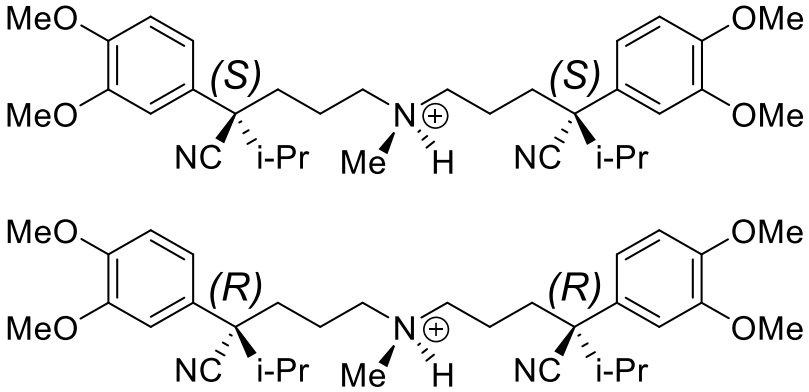


Internal plane of symmetry, so no chirality

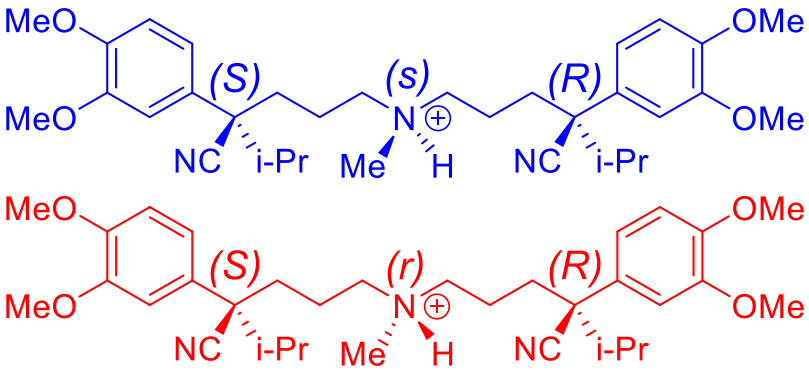
….. Or so we thought



Expected single peak (2 enantiomers)



But then we learned about pseudo-asymmetric centers!
Or what I like to call "conditional chirality"

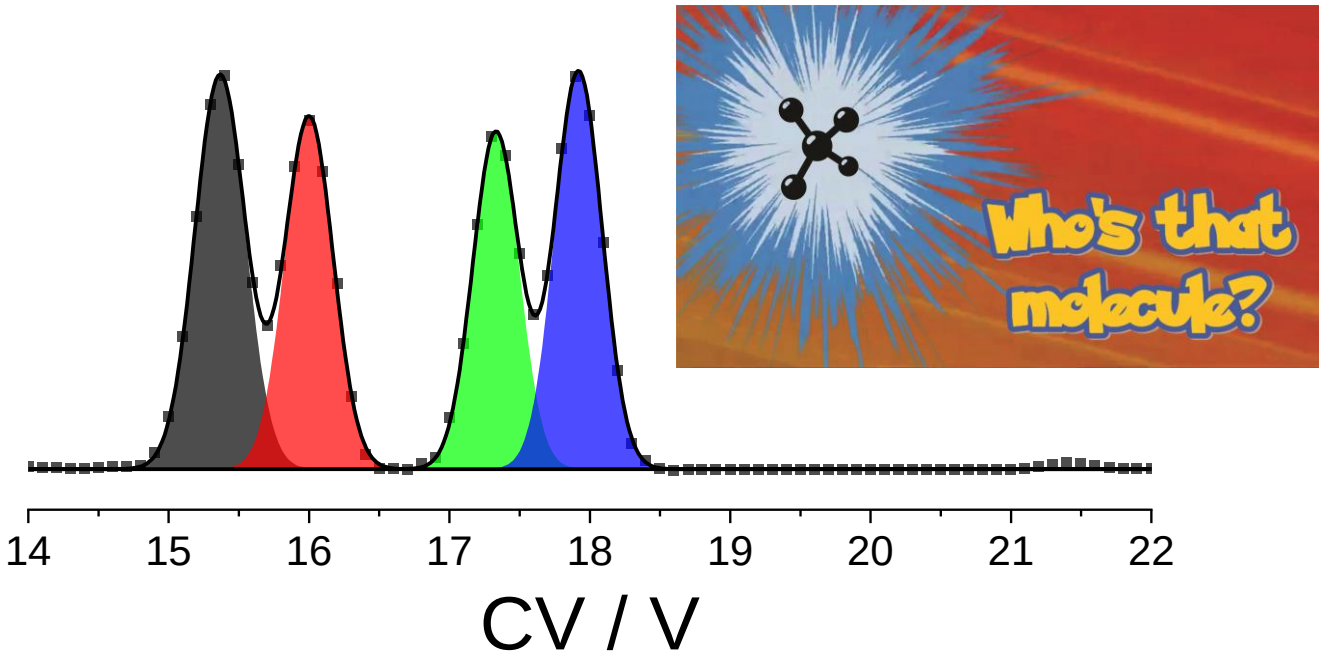


Where can we go with this?

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

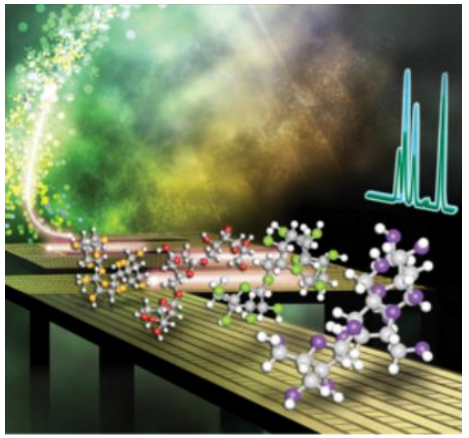
1. The molecule must possess a **permanent stereocenter**
2. The molecule must possess a **stereogenic precursor** susceptible to protonation (e.g., tertiary amine)
3. Once **protonated, the diastereomers must not interconvert and must preserve structural differences**

Crazy chirality!



Experimental considerations

Verapamil is a standard for calibrating CCSs



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

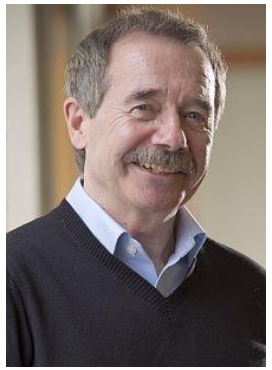
Synapt G2
R_p ~ 60

SLIMS, Cyclic-IMS
R_p > 150

Acknowledgements

PhD Committee

Prof. W. Scott Hopkins



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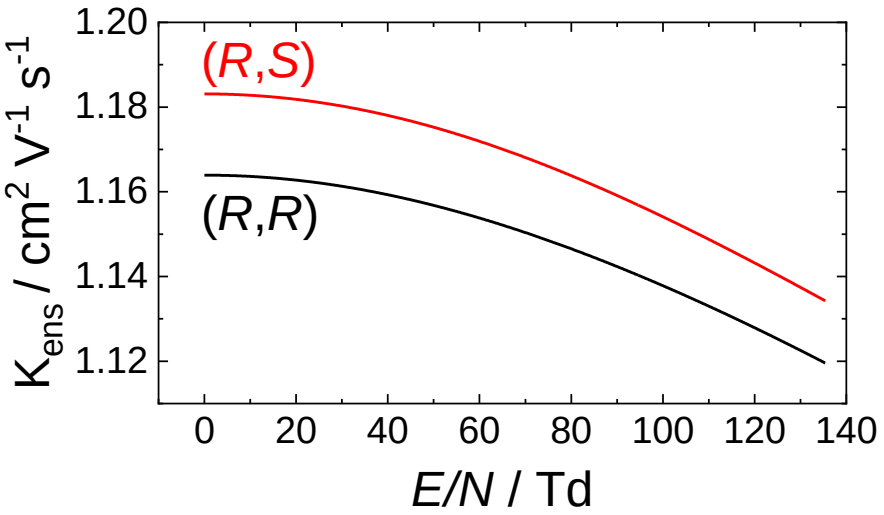
In silico DMS modelling reproduces verapamil separation

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

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

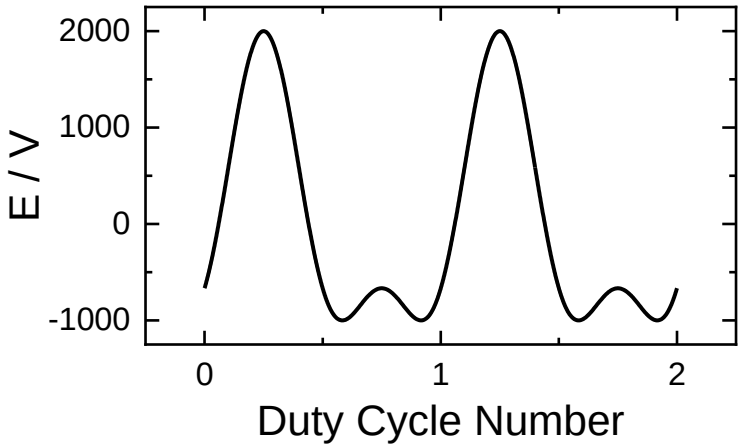
$$K = \frac{\sqrt{18\pi}}{16} \frac{ze}{\sqrt{\mu k_b T_{eff}}} \frac{1}{\Omega} \frac{1}{N} \qquad \langle K \rangle_{ens} = \sum_i K_i \cdot p_i$$



2. Calculation of optimal CV at a given SV

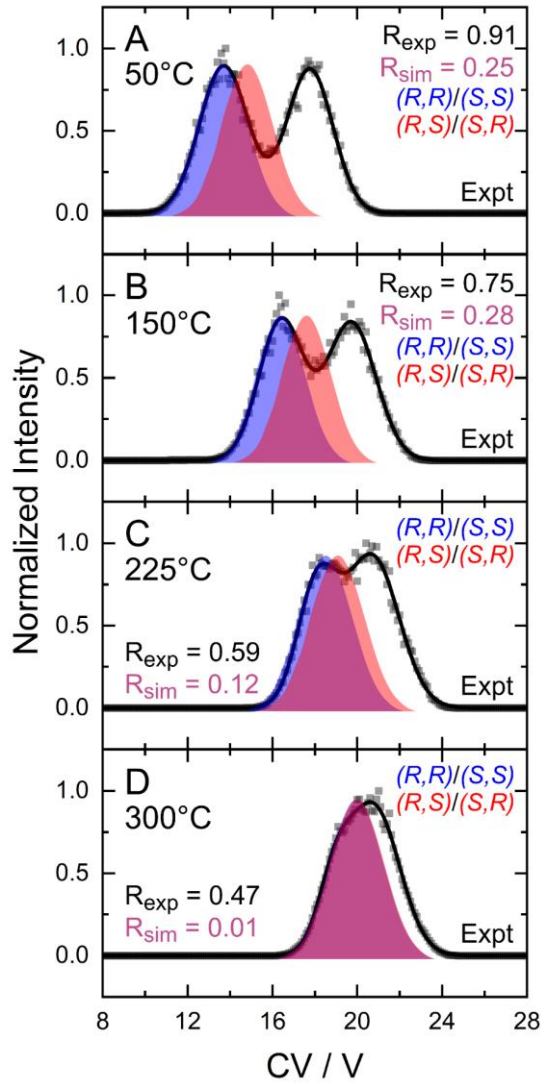
$$\alpha(E) = \frac{\langle K(E) \rangle_{ens}}{\langle K(0) \rangle_{ens}} - 1$$

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



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

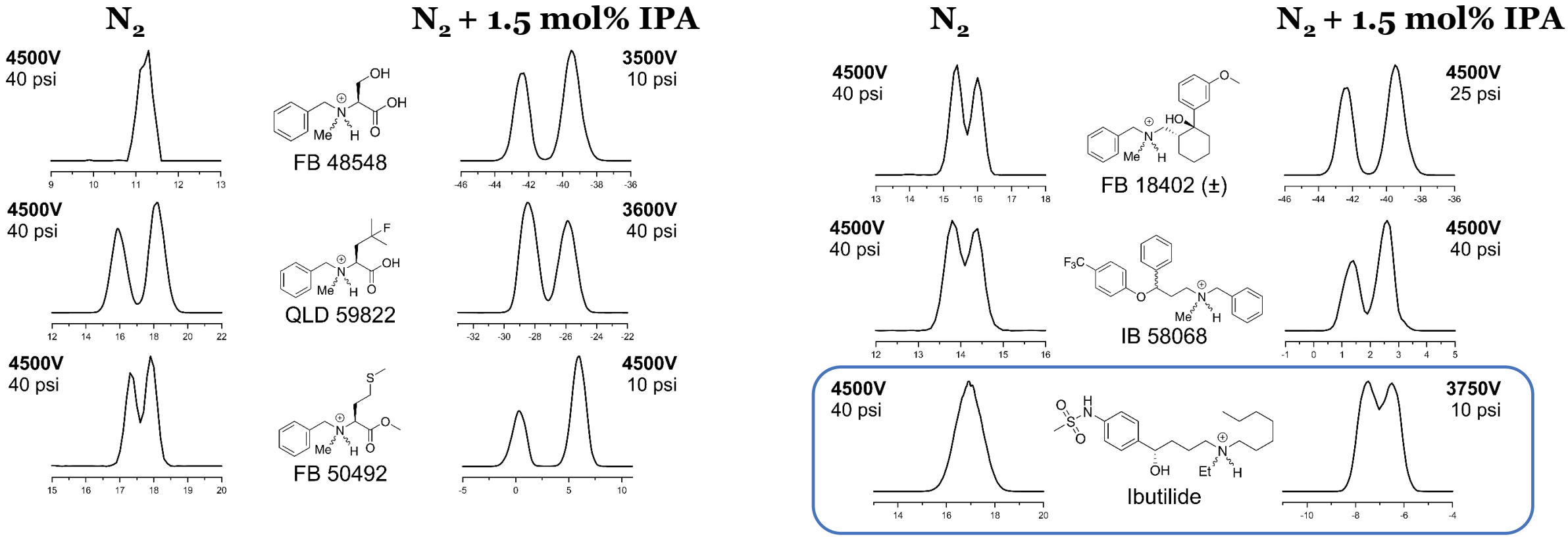
SV = 4500 V



J. Am. Soc. Mass Spectrom. **2019**, 30, 2711–2725.

Is protonation-induced chirality a general phenomenon?

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

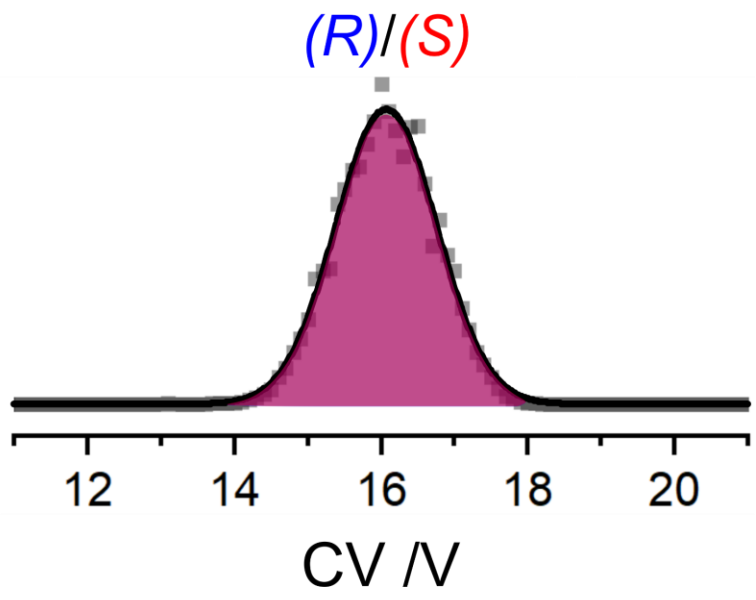


Seeding the DMS carrier gas with IPA was required to fully resolve some compounds

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