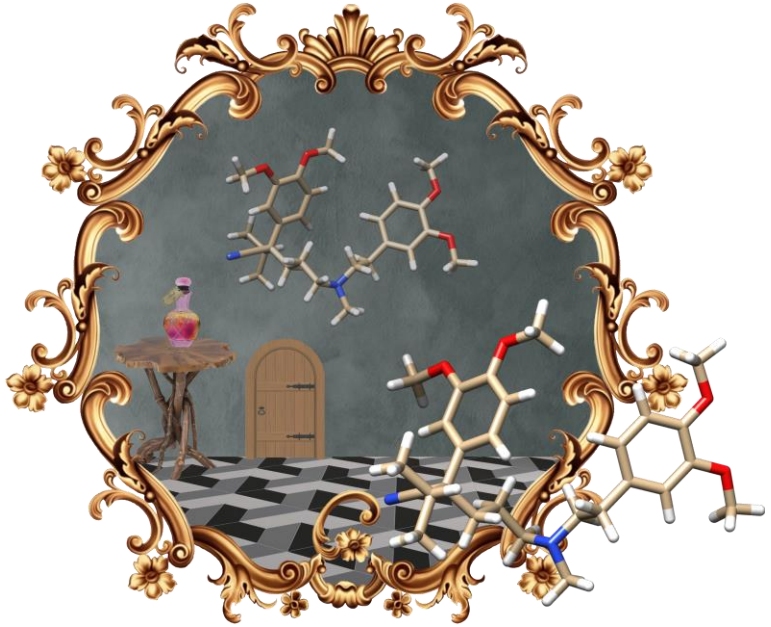
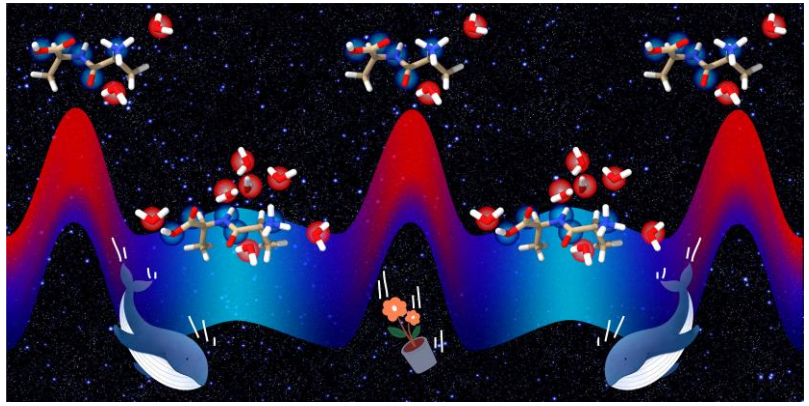
Protonation-Induced Chirality Drives Separations by Differential Mobility Spectrometry

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



2023 Symposium on Chemical Physics



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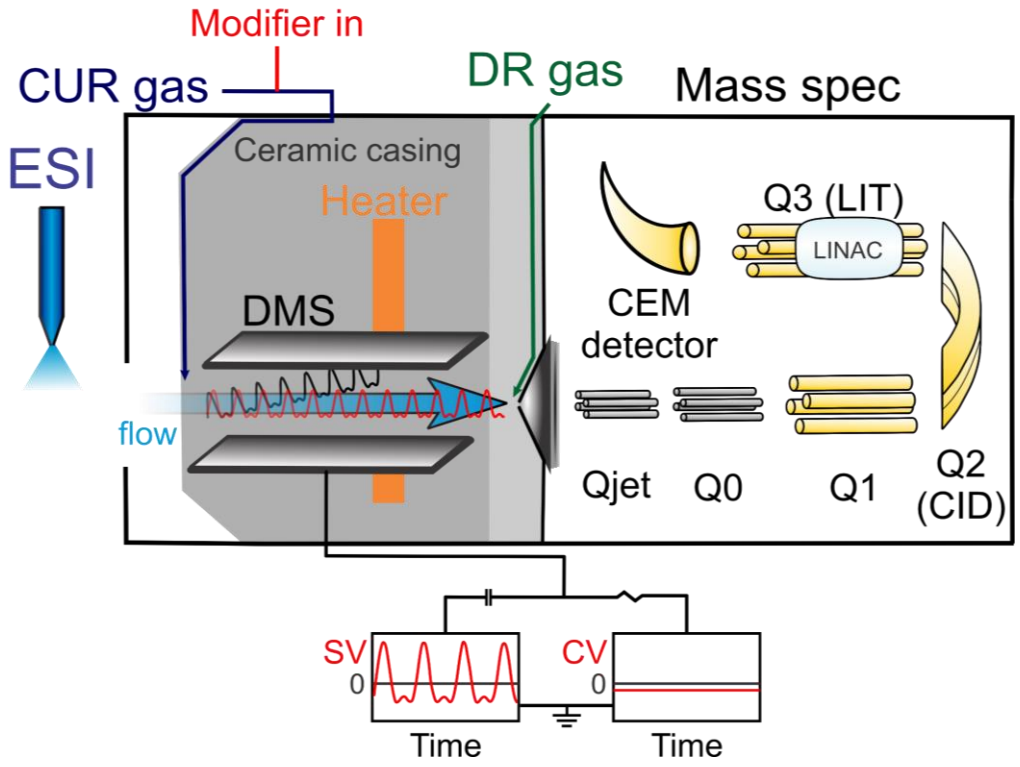
October 3, 2023

What is Differential Mobility Spectrometry (DMS)?

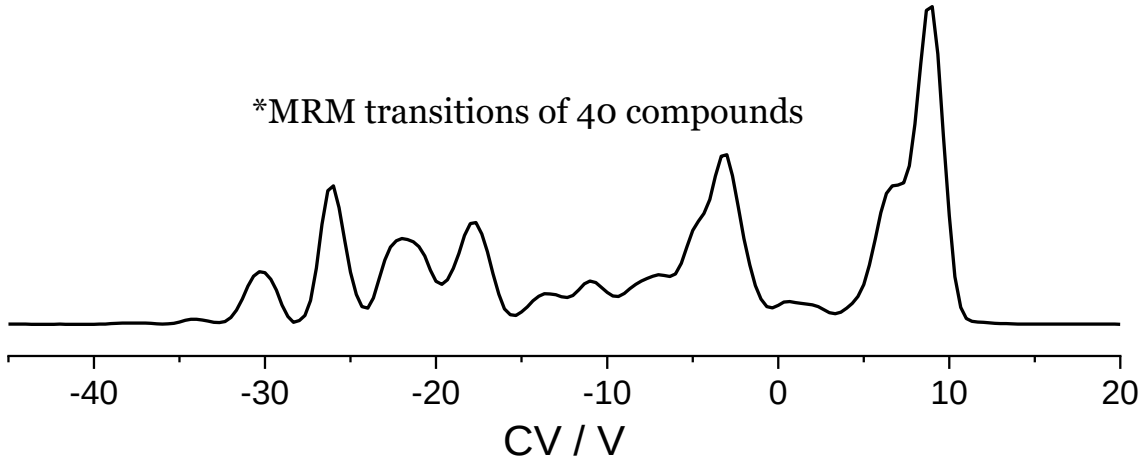
Differential mobility spectrometry (DMS) harnesses the non-linear dependence of an ion's mobility to separate analytes



At high electric field strengths, an ion's mobility changes non-linearly with the applied field

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

Scanning CV at a fixed SV gives an ionogram



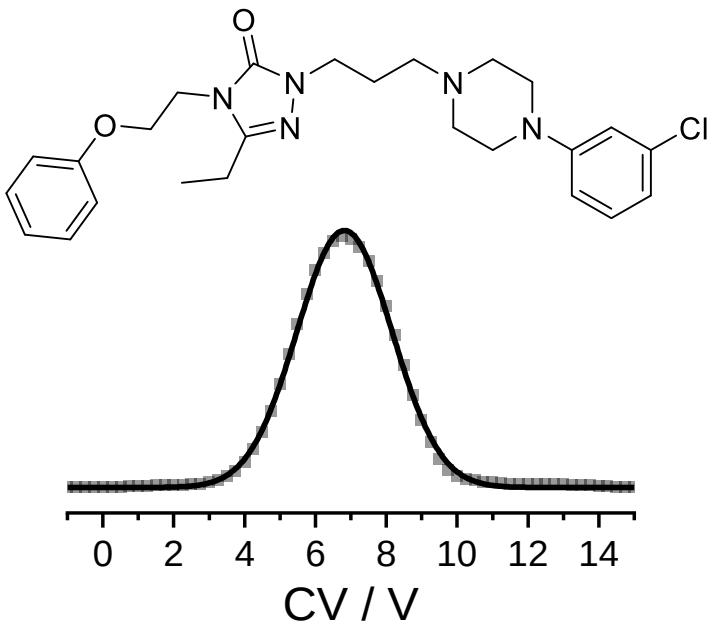
For a specific SV, every analyte will elute from the DMS cell at a characteristic CV related to its alpha function

The SV/CV pair is an intrinsic ion property

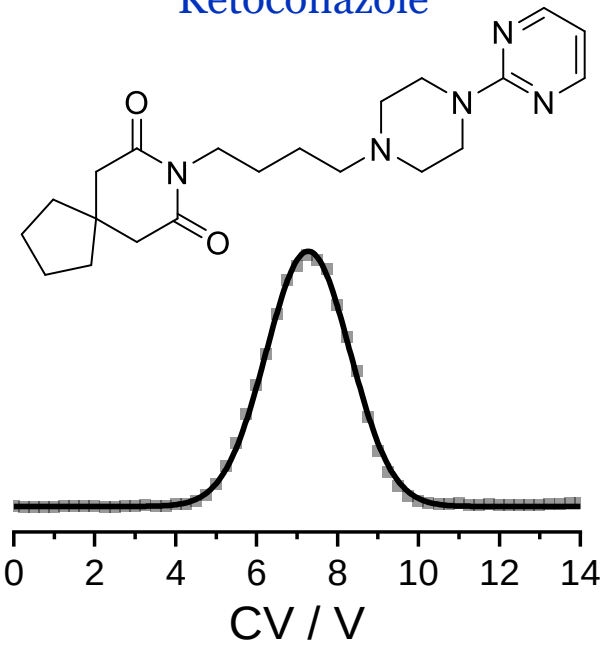
The CV in which an analyte elutes from the DMS cell is analyte specific, and orthogonal to LC

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

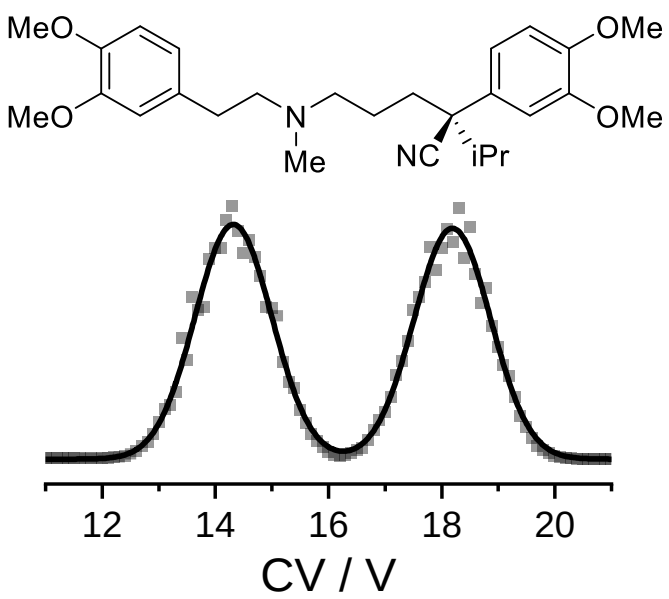
Nefazodone



Ketoconazole



...and Verapamil?



Why does Verapamil have two peaks?

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

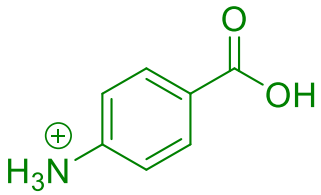
More than one feature in an ionogram **usually** indicates the presence of prototropic isomers

4-aminobenzoic acid

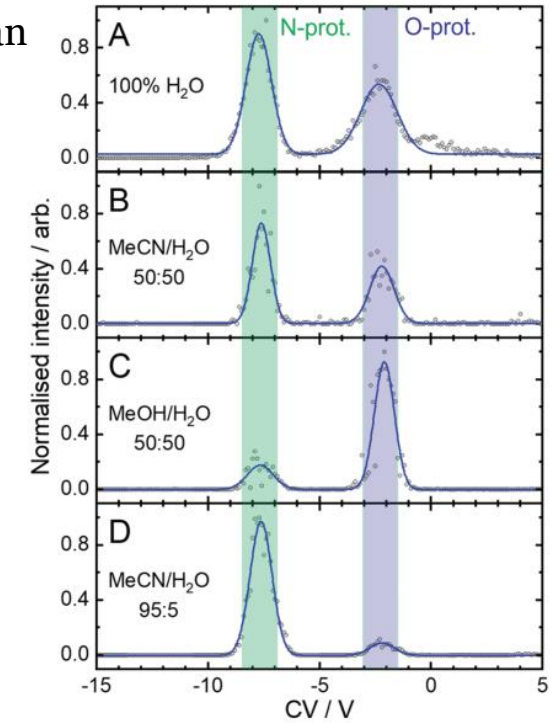
Phys. Chem. Chem. Phys. 2021, 23, 20607

Dr. Neville Coughlan

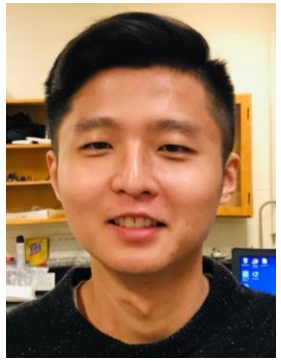
N-Prot



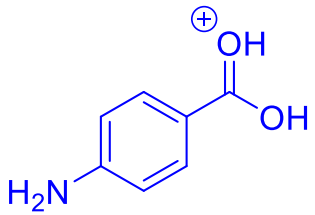
5 kJ mol^{-1}



Dr. Weiqiang Fu

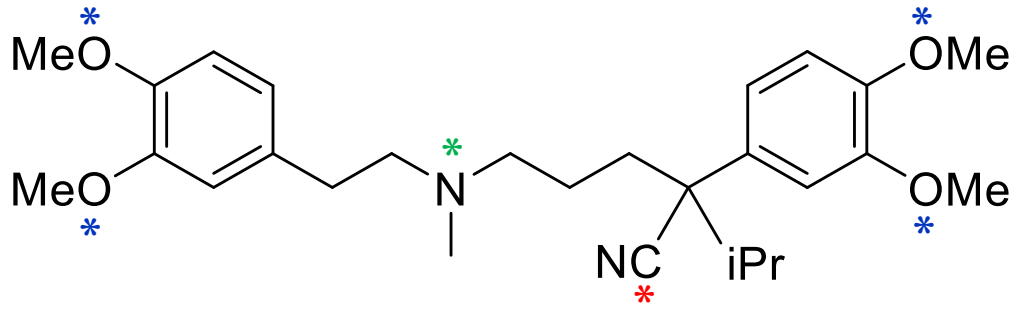


O-Prot

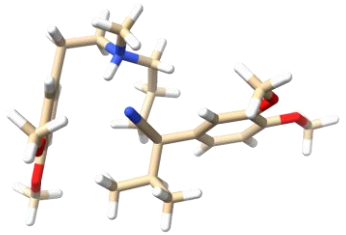


0 kJ mol^{-1}

Are we seeing a prototropic isomer of Verapamil?

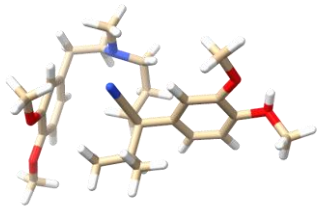


Amino-protonated



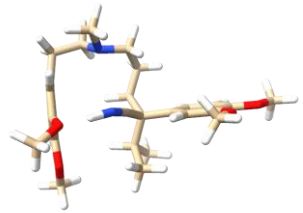
0 kJ mol^{-1}

Methoxy-protonated



110 kJ mol^{-1}

Cyano-protonated



130 kJ mol^{-1}

...not likely

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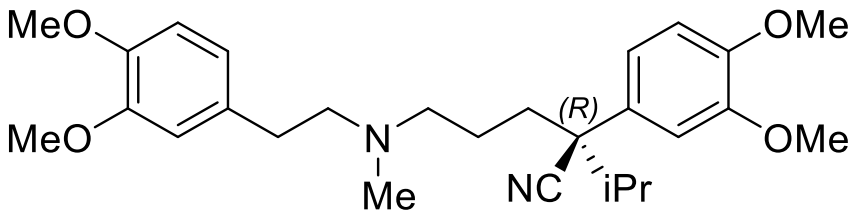
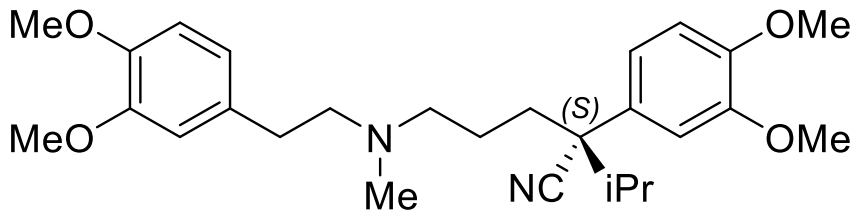
So what gives with Verapamil's dual peaks?

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

(*R*)-Verapamil



(*S*)-Verapamil



Enantiomers (non-superimposable mirror images)

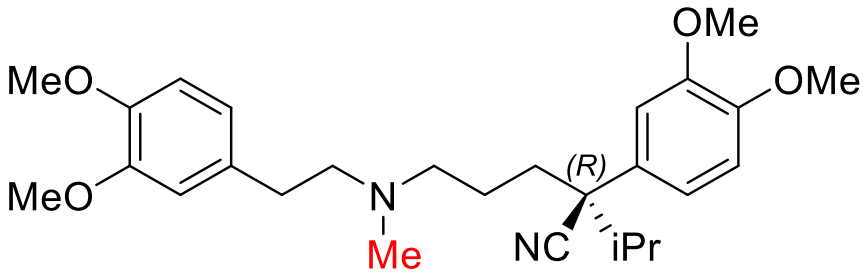
Enantiomers exhibit the opposite stereochemistry at **all** chiral centers

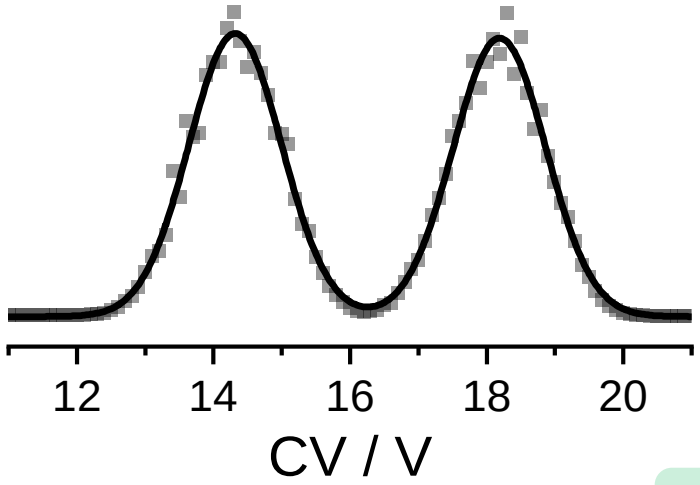
Enantiomers cannot be resolved by DMS **without chiral derivatization**

So what gives with Verapamil's dual peaks?

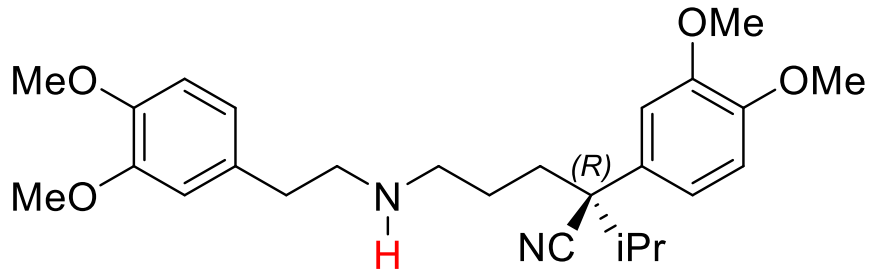
Perhaps looking at the DMS behaviour of Verapamil derivatives will provide some insight…

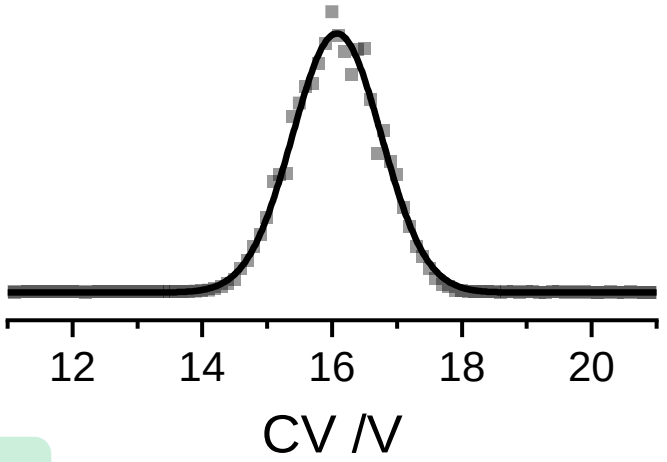
(*R*)-Verapamil





(*R*)-Norverapamil

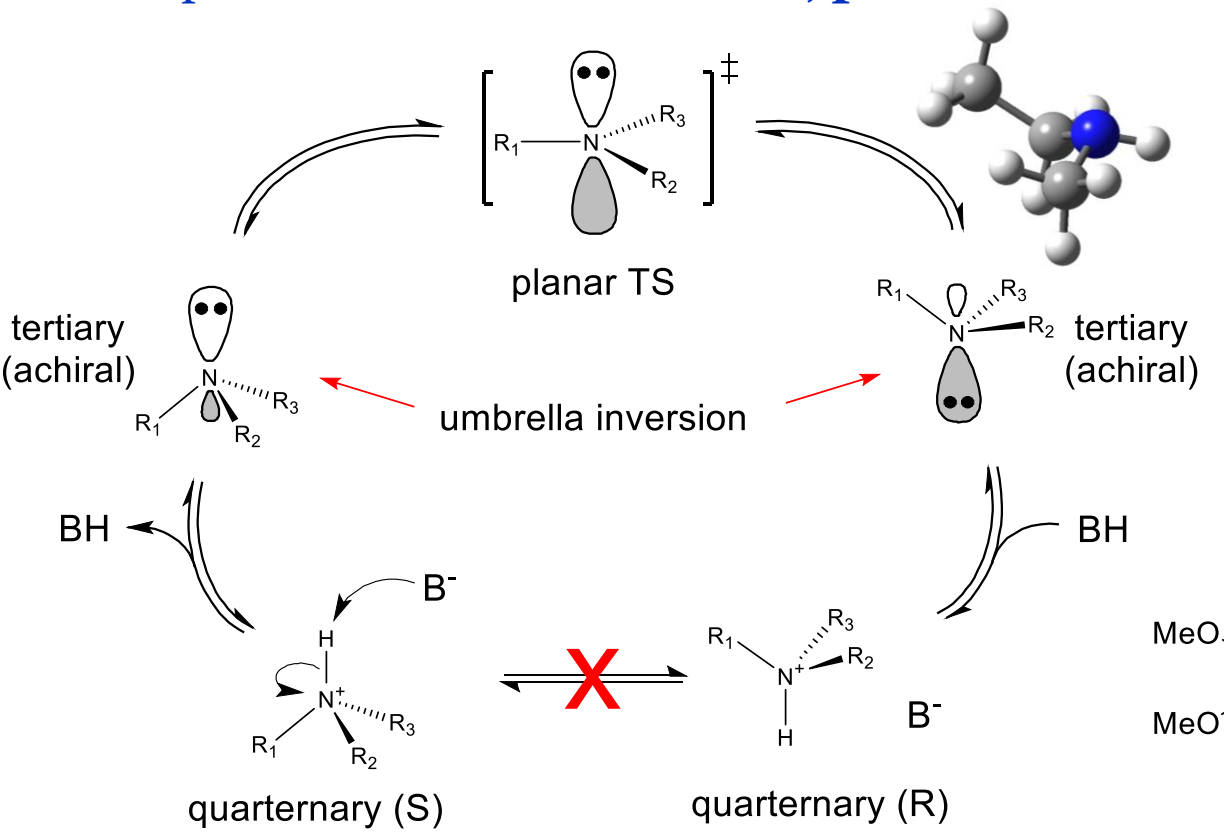




The **amino** moiety is the key

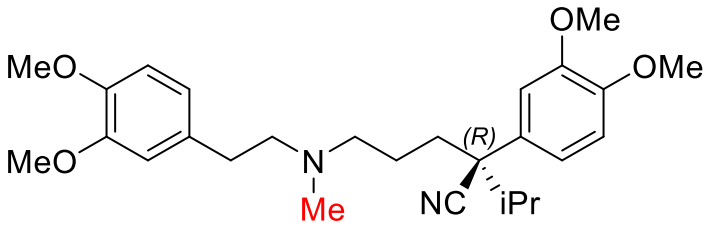
So what gives with Verapamil's dual peaks?

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

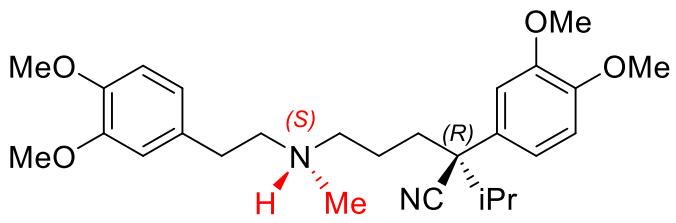


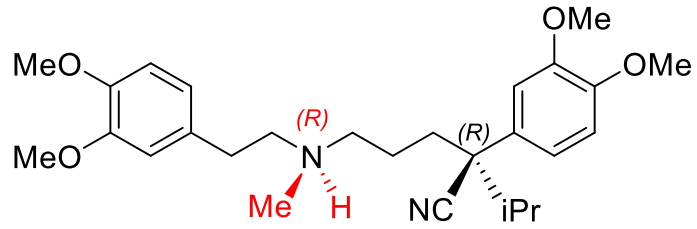
Solution phase equilibration should yield a 1:1 ratio S:R quaternary amine configuration

(R)-Verapamil



Protonation during ESI yields 2 diastereomers



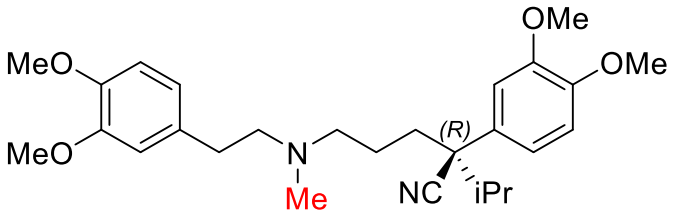


Diastereomers differ in the configuration of **at least one, but not all** stereocenters

So what gives with Verapamil's dual peaks?

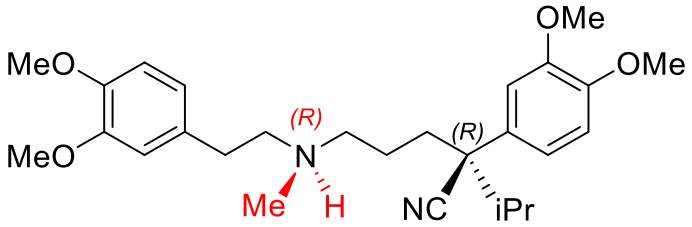
What happens with a **racemic** mixture of Verapamil?

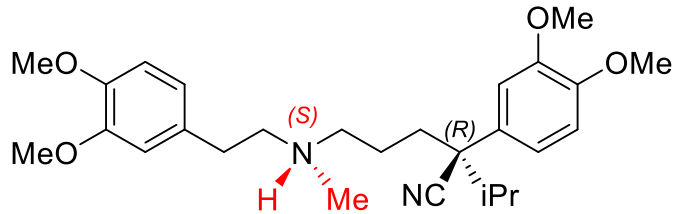
(R)-Verapamil



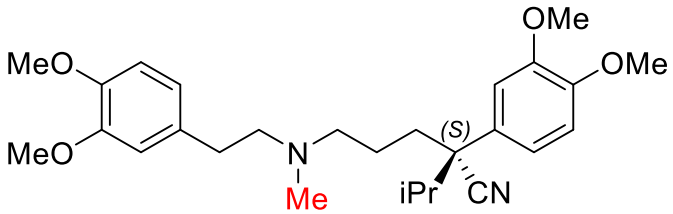
Protonation during ESI

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



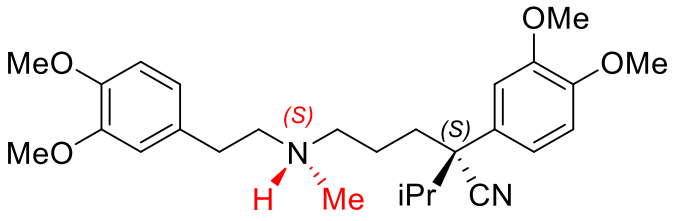


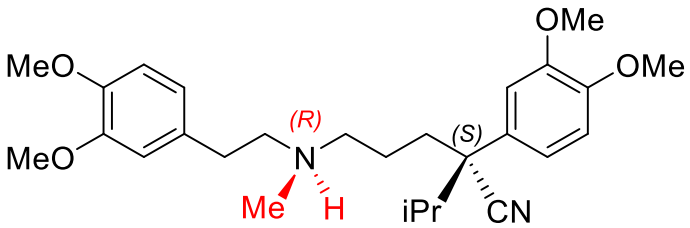
(S)-Verapamil



Protonation during ESI

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





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

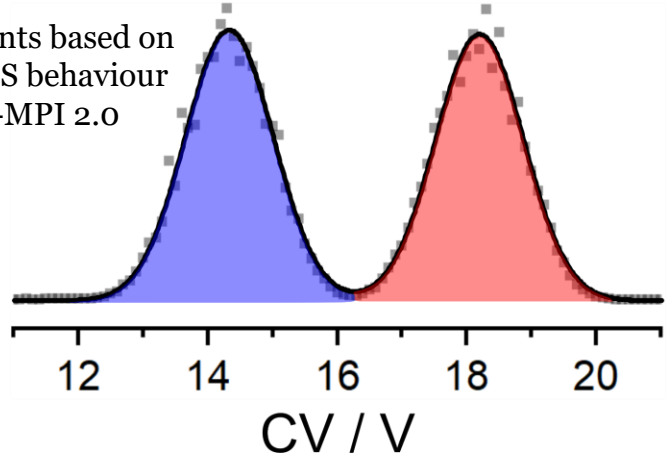
Protonation-induced chirality drives the separation

A (±)-Verapamil

SV = 4500 V T_{bath} = 50°C

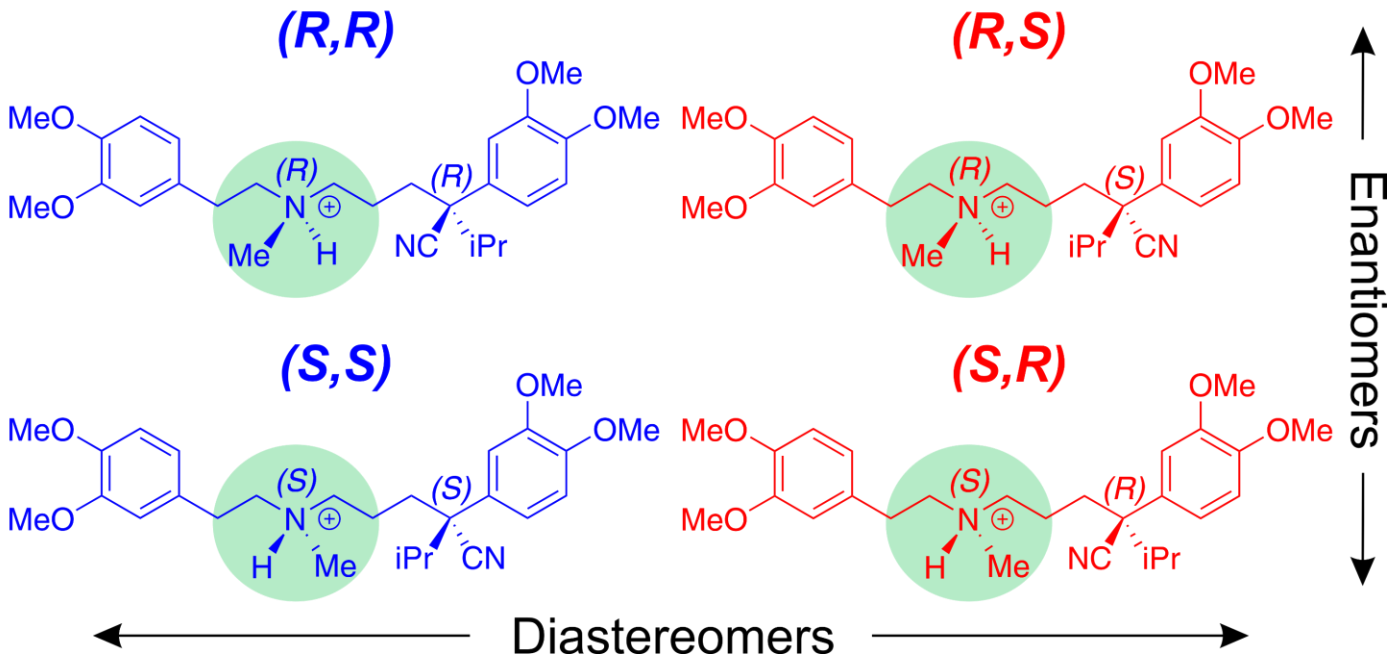
Resolving gas **on** (10 psi)

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



Peak assignments based on modelling DMS behaviour in MobCal-MPI 2.0

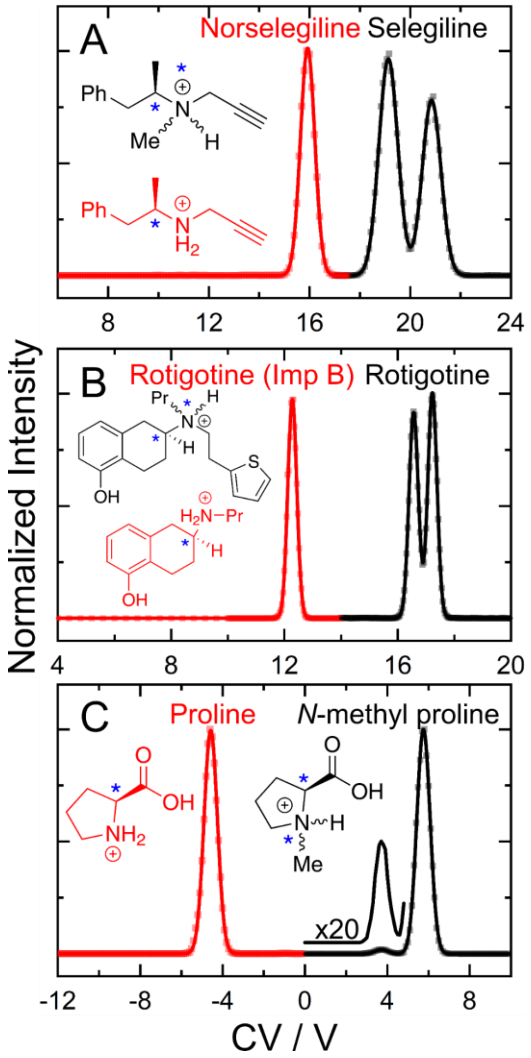
B Protonation yields an additional stereocenter



In short, chiral derivatization of an analyte is achieved solely by protonation during ESI

Is protonation-induced chirality a general phenomenon?

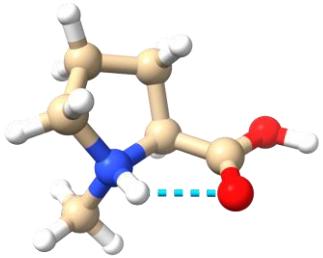
12 commercially available compounds were tested for their susceptibility to protonation-induced chirality

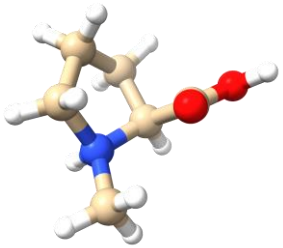


Why are there intensity differences between the peaks corresponding to the protonation-induced diastereomers?

1) Energy differences between the protonation induced diastereomers

[*N*-methyl proline + H]$^+$
IMHB
0 kJ mol^{-1}





[*N*-methyl proline + H]$^+$
No IMHB
20 kJ mol^{-1}

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

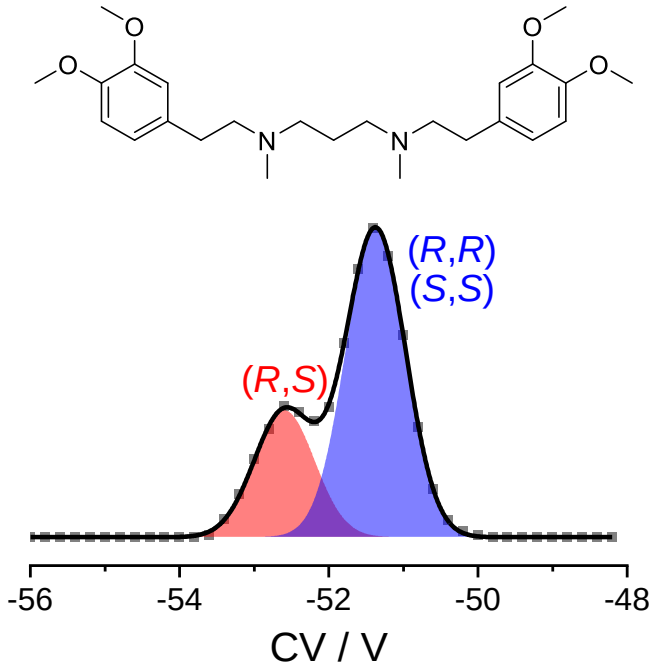
3) Does a more stable solution-phase diastereomer get kinetically trapped during the droplet evaporation portion of ESI?

Where can we go with this?

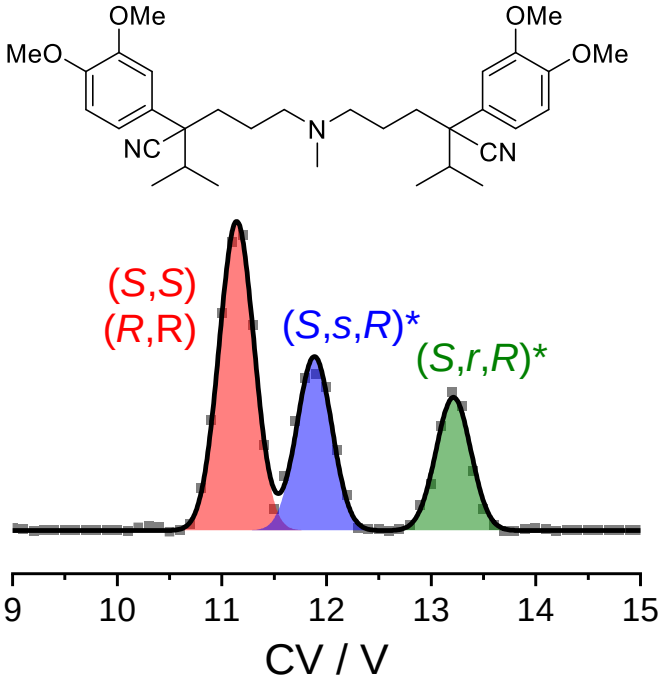
Three criteria need to be met to observe protonation-induced chirality:

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

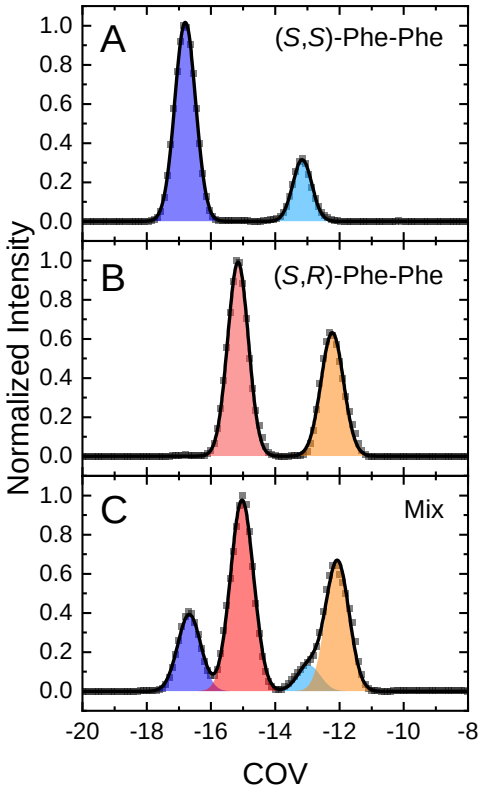
Chirality induced solely by protonation

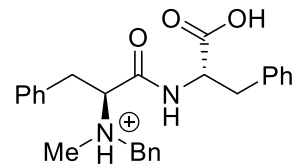


Separation of pseudo-asymmetric centers

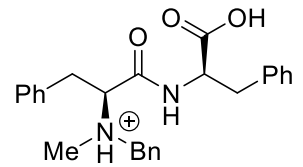


Screening for epimerization during chemical synthesis





Exact Mass: 417.22

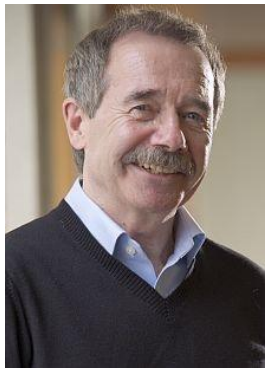


Exact Mass: 417.22

Acknowledgements

PhD Advisory Committee and Examiners

Prof. W. Scott Hopkins



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& many others!

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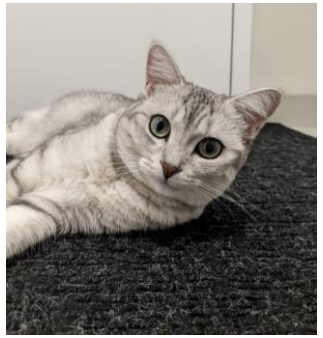


Dr. Freya J. Margaret

Dr. Francis J. Keats and Dr. Elizabeth J. Keats

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)

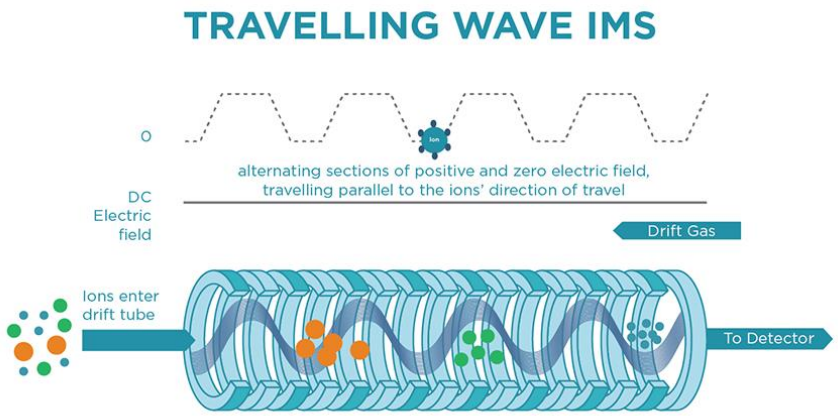


Figure courtesy of Owlstone Medical.

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

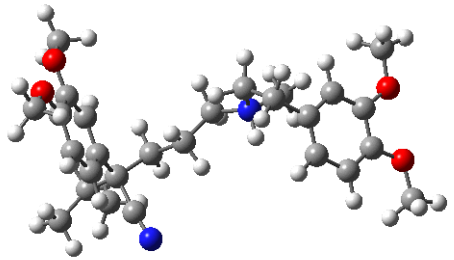
Ω – Collision Cross Section

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-D3/def2-TZVPP

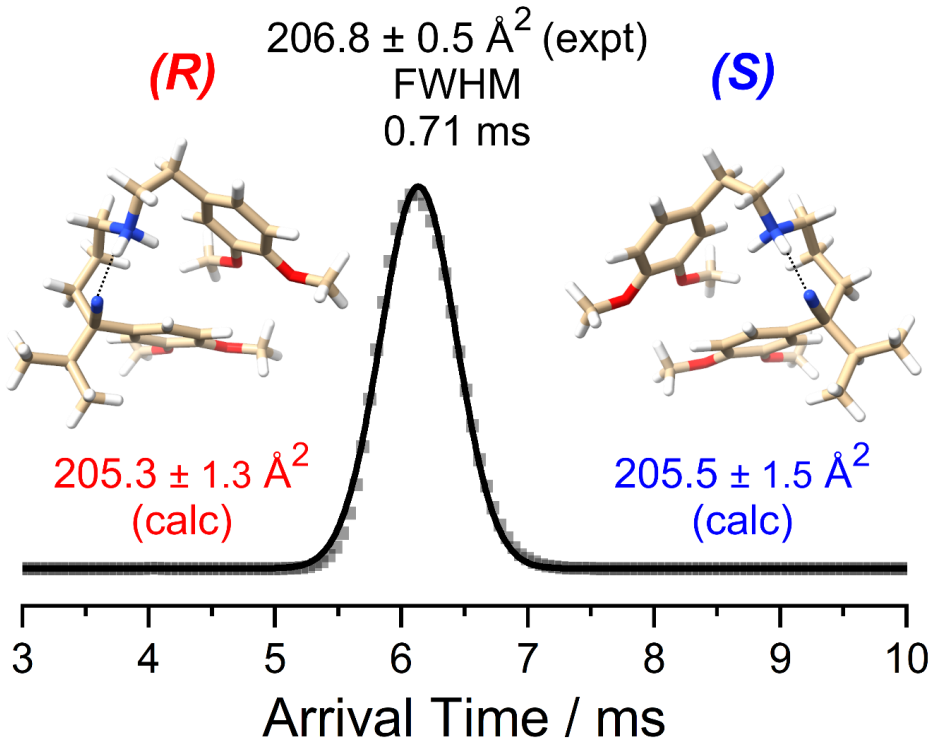
Use DFT structures and partial charges to calculate CCSs via MobCal-MPI 2.0 (**Chapter 6**)

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