



**FAIMS: The First Step in Solving  
LC-MS Method Development  
Challenges**

# Method Development Challenges

- Co-eluting interferences
- High background
- Ion fragments poorly
- Long chromatographic run time
- Complicated sample preparation

## Current Solutions

- **Vary MS parameters**
  - Voltages
  - Transitions
- **Vary chromatographic conditions**
  - Column
  - Mobile phase
- **Revisit sample prep**
  - Extraction condition
  - Derivatization

 **Significant time investment; no guarantee of success**

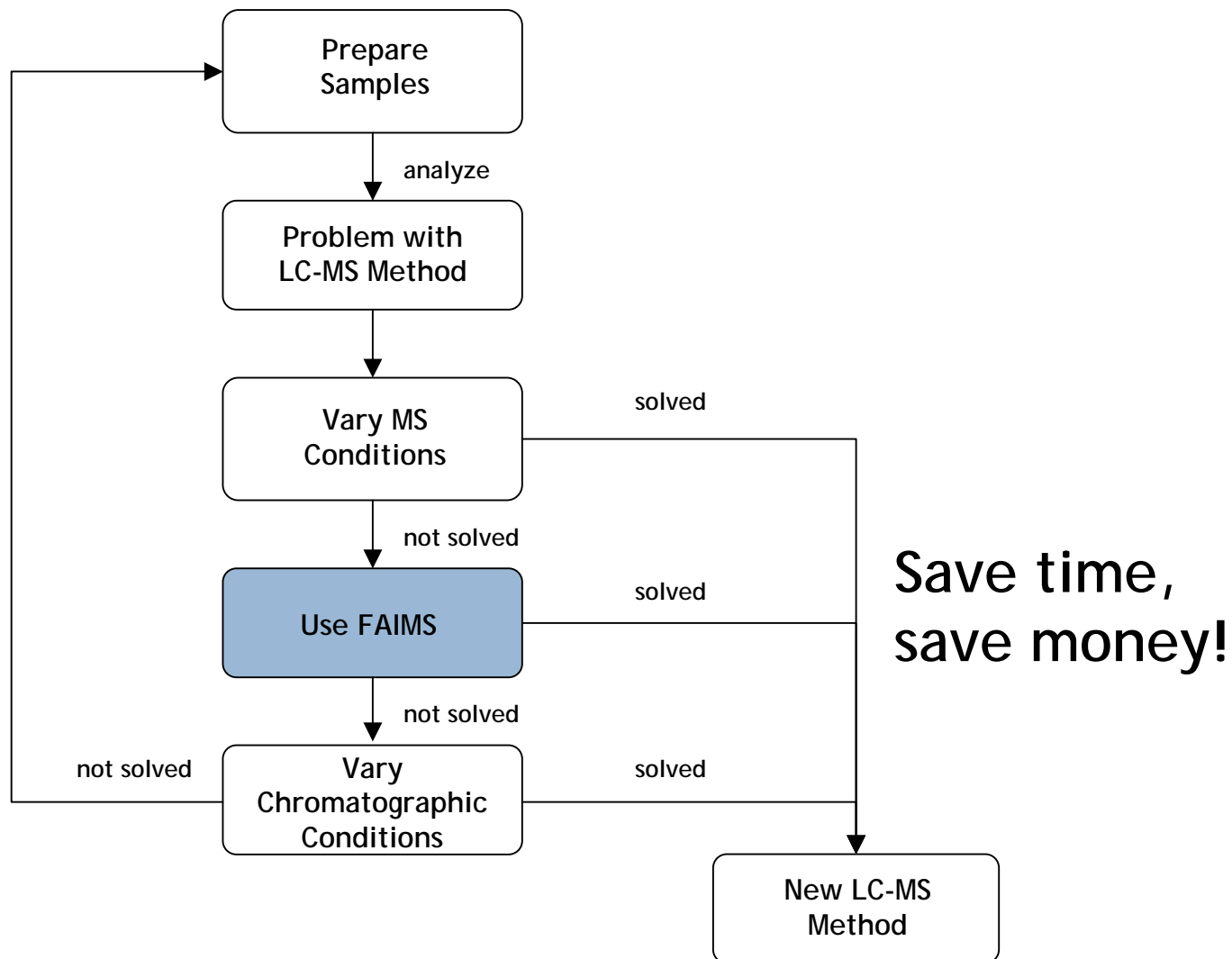
# FAIMS - Get there faster

Get method development results in just a few hours

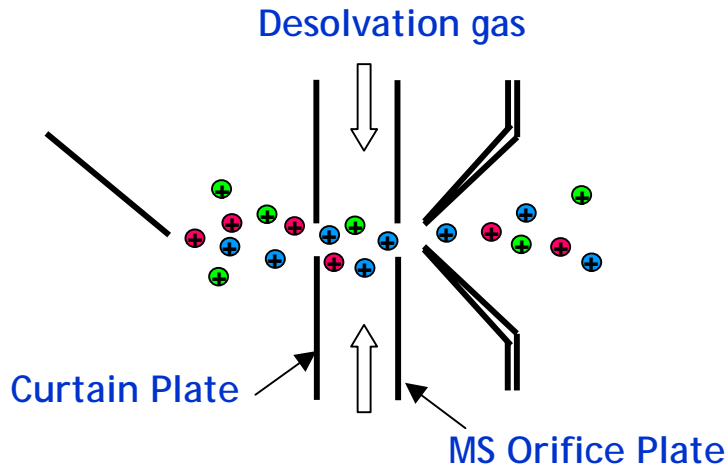
## Easy to Use:

- Install the FAIMS system
- Determine FAIMS condition using reference solutions
- Set FAIMS condition (1 parameter)
- Analyze your samples

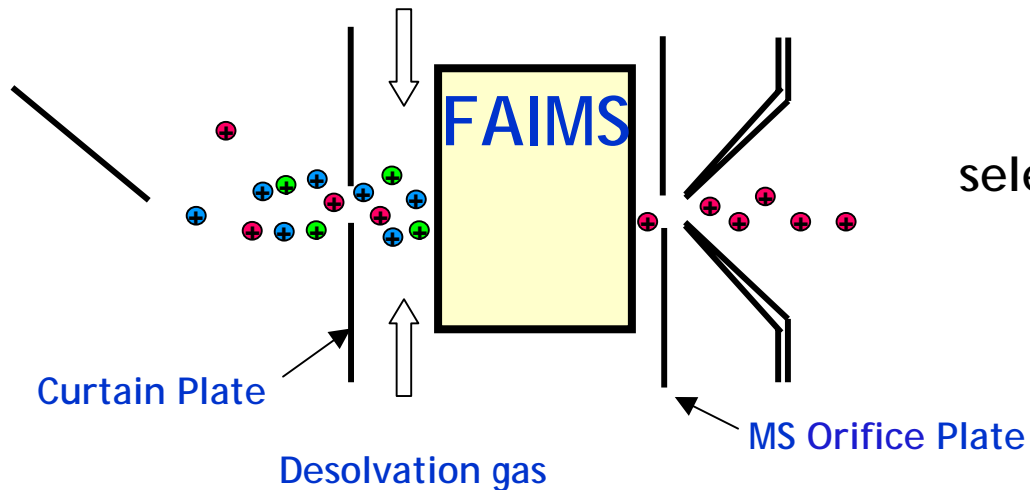
# Add FAIMS to your method development process



# FAIMS - Where does it fit?

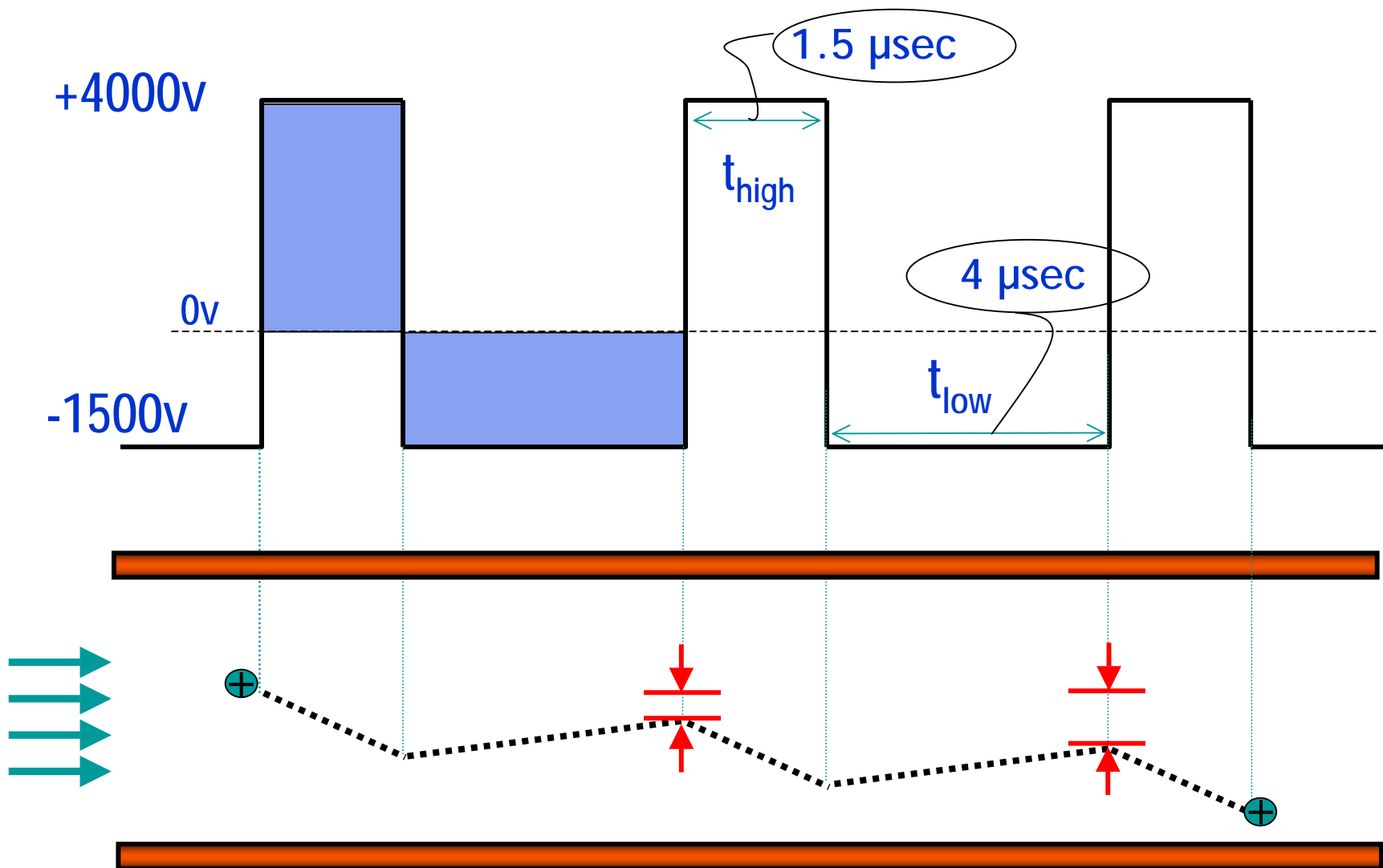


all types of ions transferred to the MS



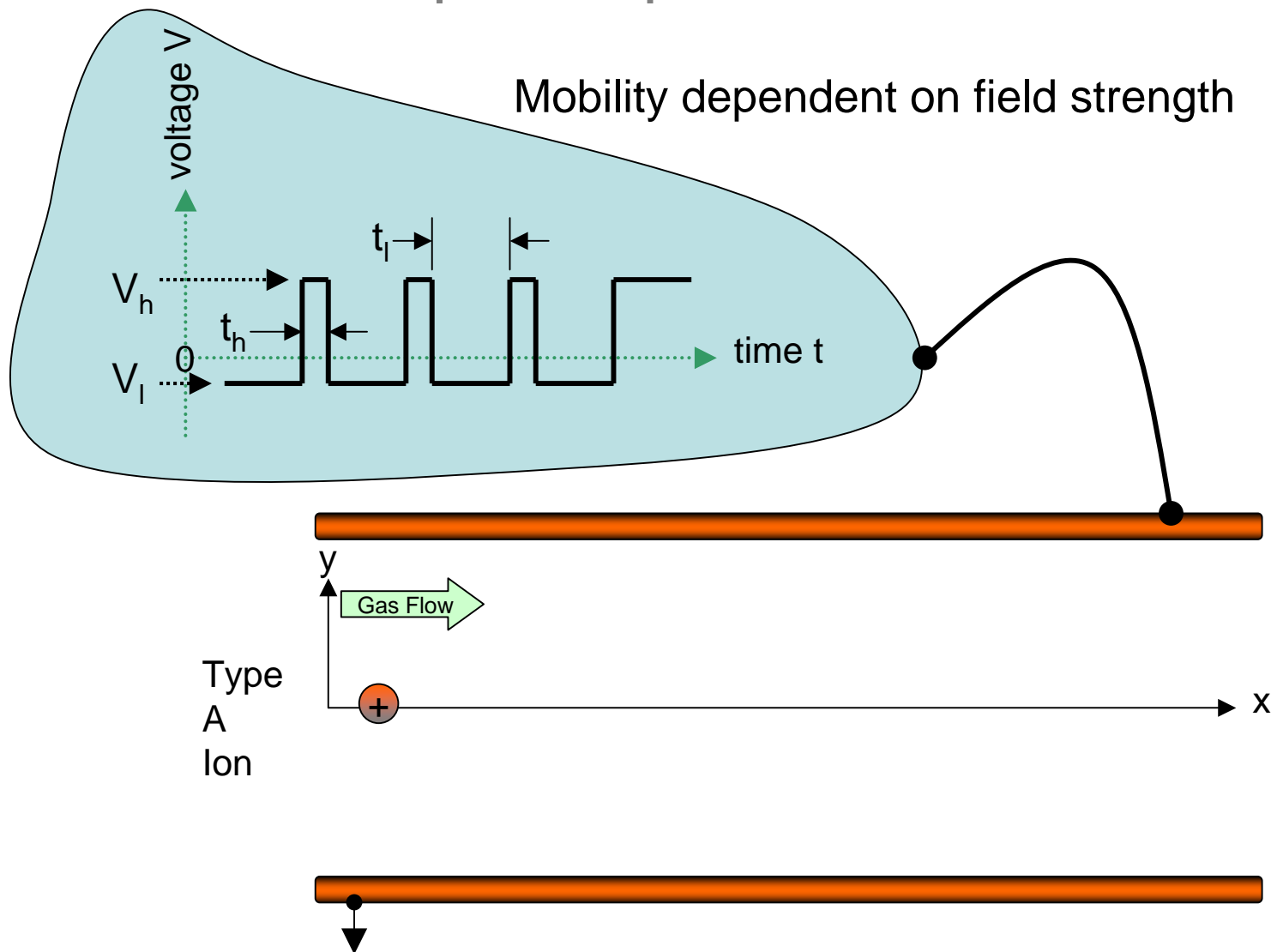
selected ions transferred to the MS

# Asymmetric waveform for FAIMS



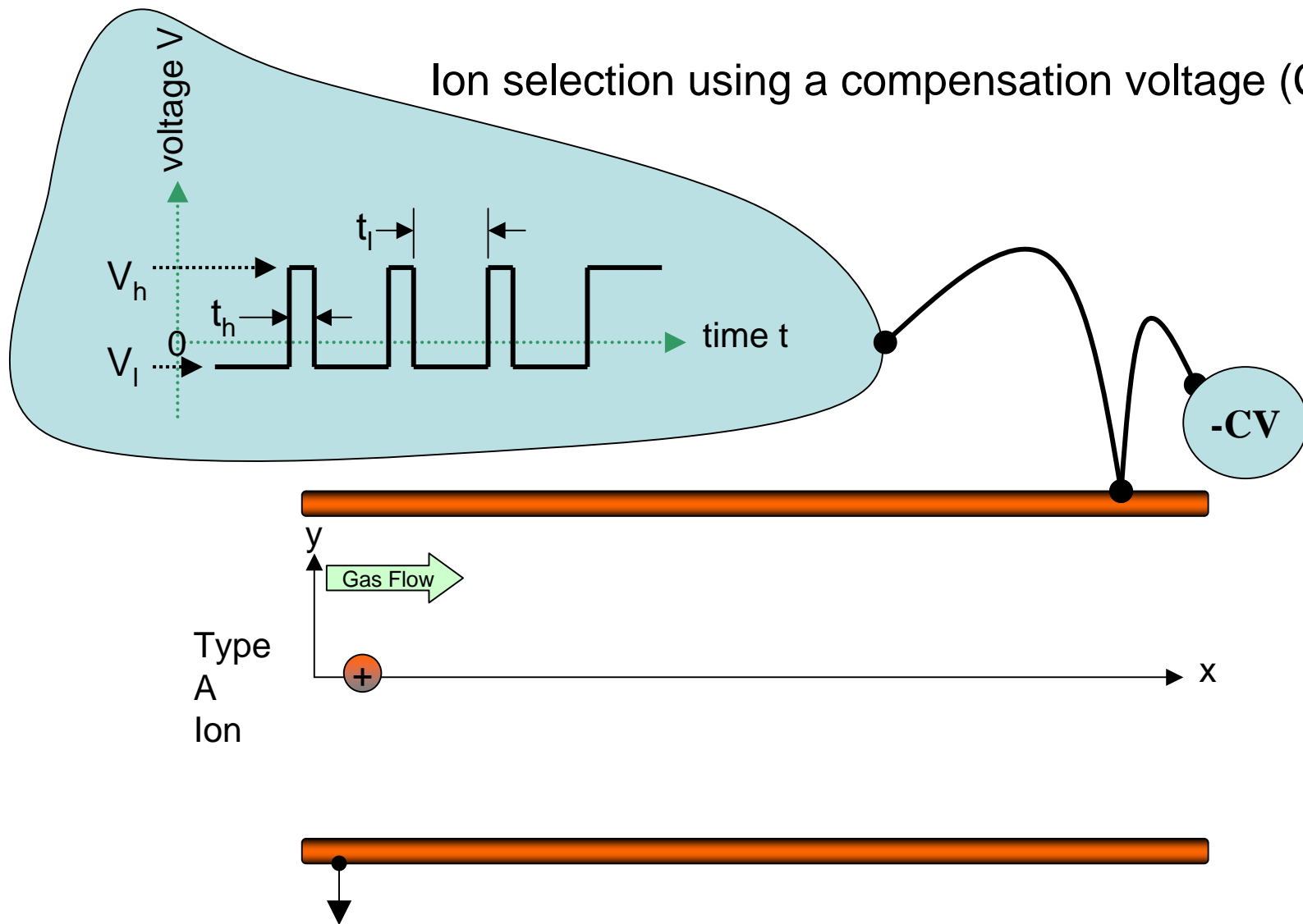
# The parallel plate FAIMS

Mobility dependent on field strength



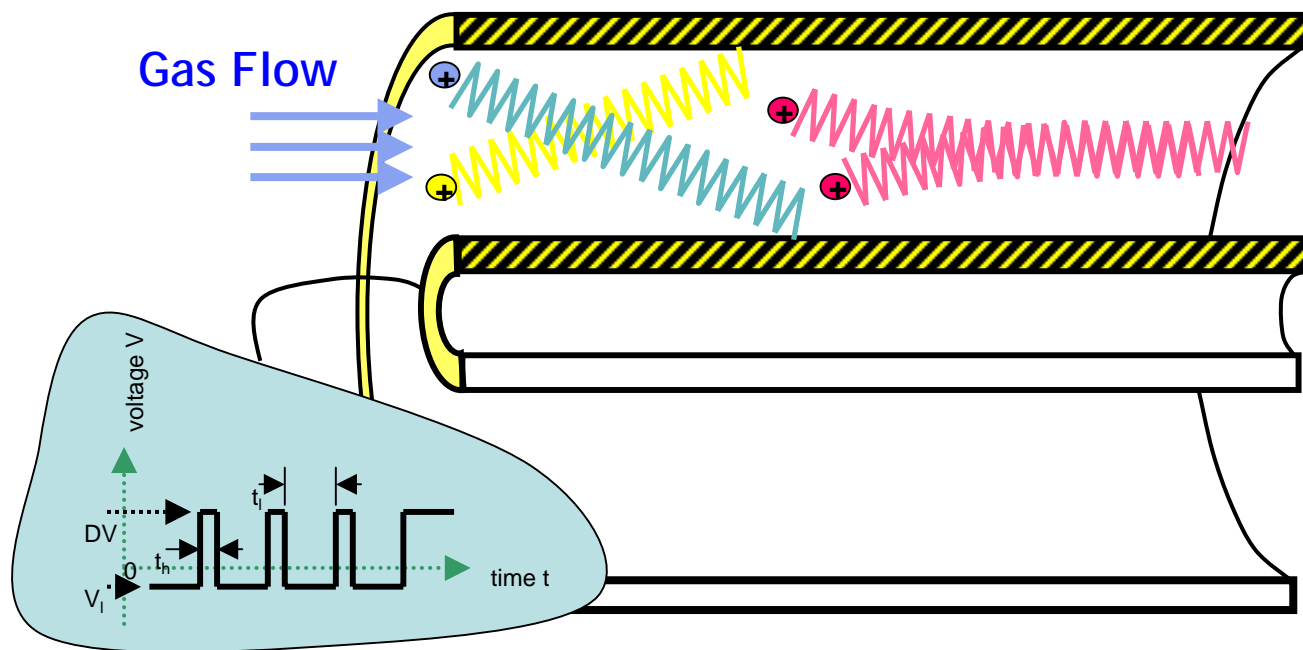
# The parallel plate FAIMS

Ion selection using a compensation voltage (CV)



## FAIMS - How does it work?

- Operates at atmospheric pressure - between API source and MS
- Uses concentric cylindrical electrodes for ion separation.
- Electronic separation - voltages are used to select the  $\oplus$  ion.
- Other ions are discharged to the walls of the electrodes.



## FAIMS - How does it help you?

- Increases selectivity
- Reduces background
- Increases throughput

# FAIMS - How does it help you?

- Increases selectivity
  - In-source fragmentation
  - Separation of isobaric ions
  - Separation of positional isomers
  - Separation of diastereomers
  - Removal of endogenous interferences
  
- Reduces background
  
- Increases throughput

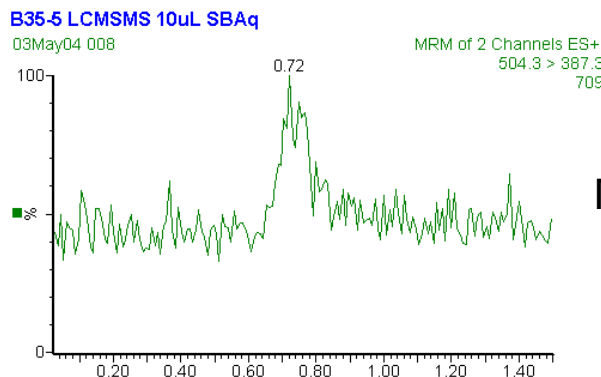


# Increases Selectivity

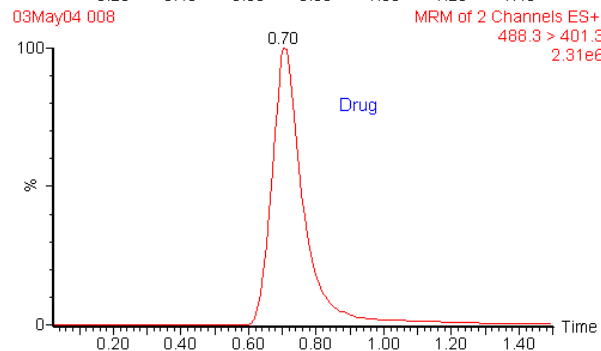
# Problem: In-source fragmentation

LC-MS/MS of a drug (10 $\mu$ L inj. of 5 $\mu$ g/mL solution) - no metabolite present in sample

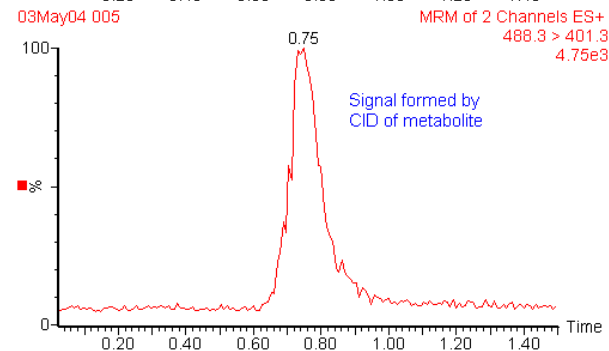
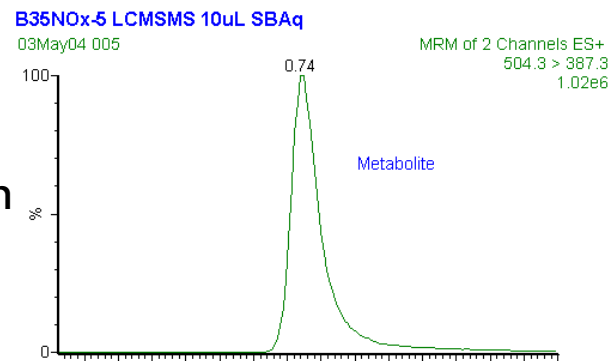
LC-MS/MS of the N-Oxide metabolite (10 $\mu$ L inj. of 5 $\mu$ g/mL solution) - no parent drug present in sample



Metabolite transition

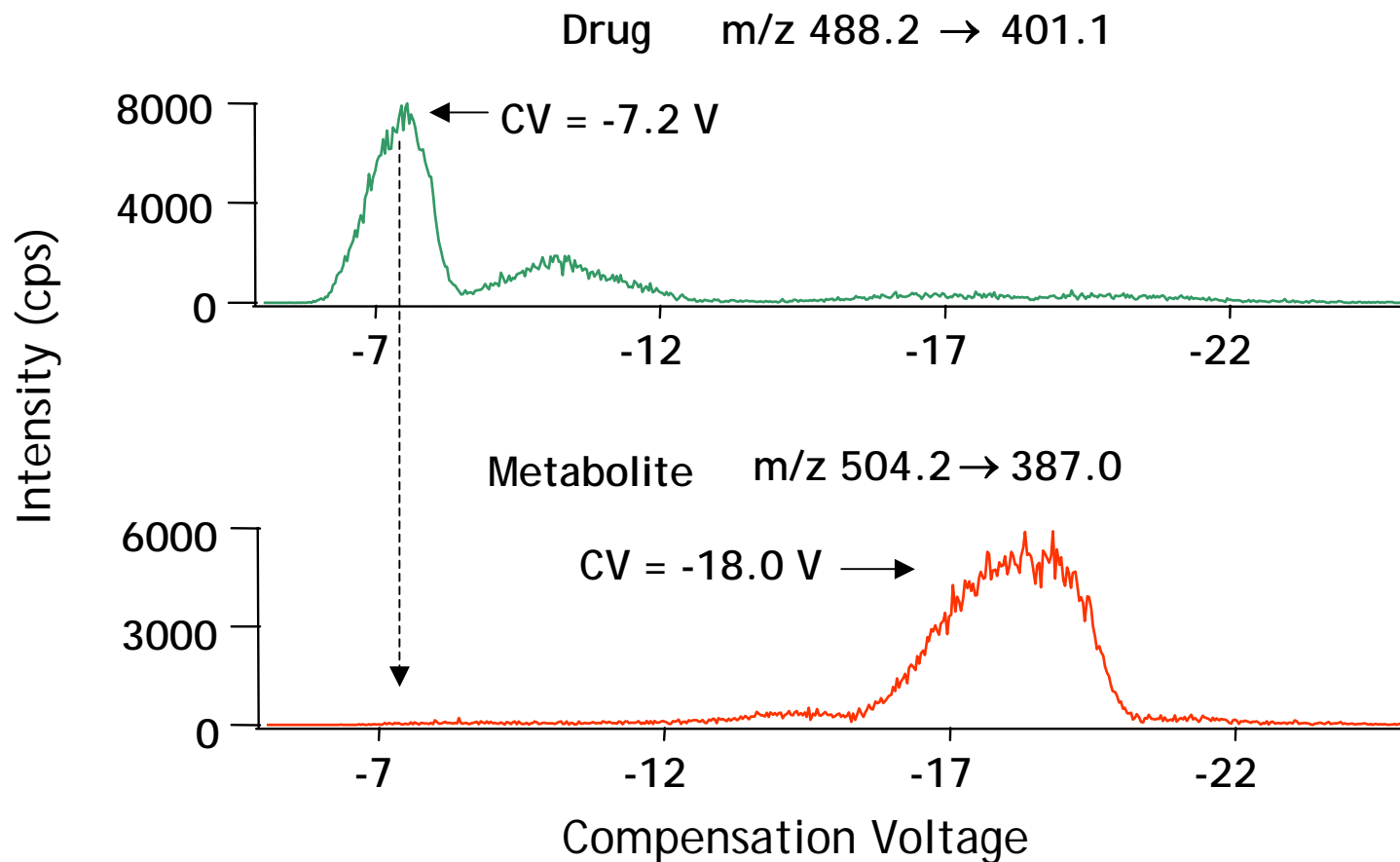


Drug transition



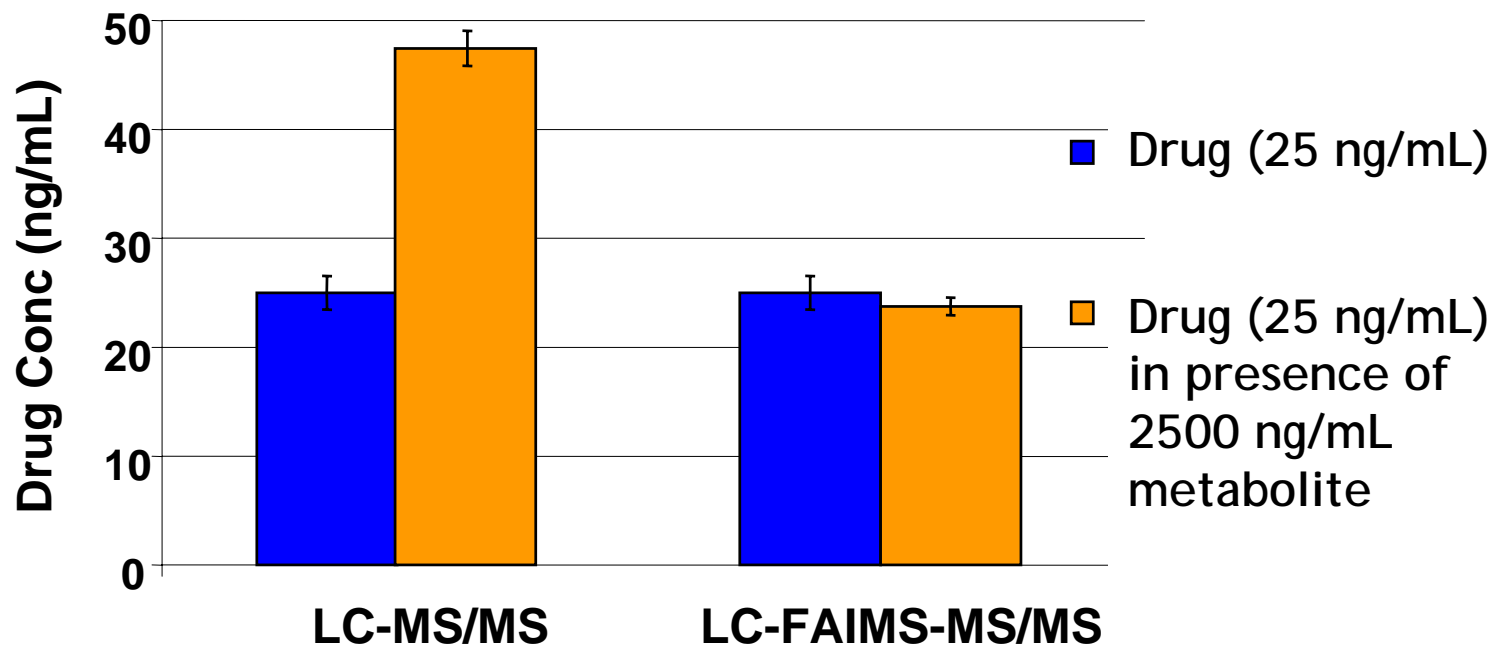
- Drug and metabolite co-elute
- Signal for drug is obtained even when no drug is present in sample
- Will affect measured concentration of drug in sample

# FAIMS-MS/MS separation of drug and metabolite



- FAIMS separates drug and metabolite; eliminates interference formed by CID of metabolite

## Effect of interference on measured drug concentration



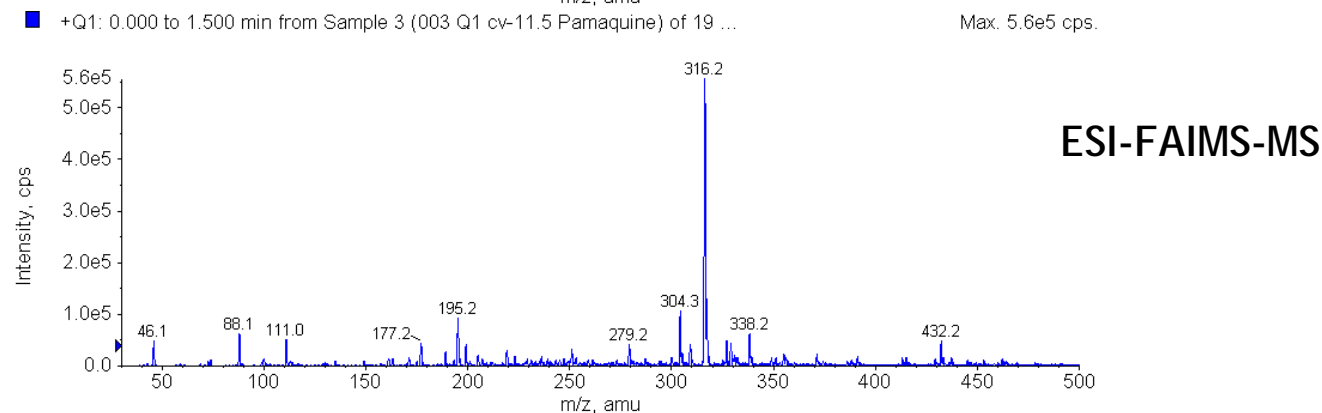
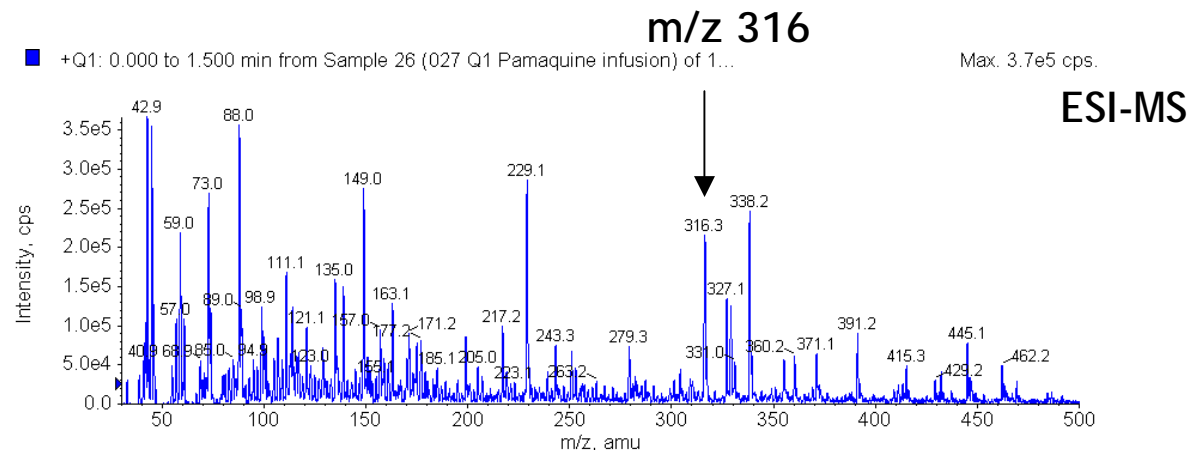
- More accurate quantitation using LC-FAIMS-MS/MS

## Problem: Interfering isobaric ions

### Example: Pamaquin/Oxycodone/Clonazepam

- Ions are isobars with similar fragment ions
- Detection of these ions could be achieved by:
  - Chromatographic resolution
  - Selection of alternate fragment ion
  - Different collision energy
  - Increased precursor ion specificity
  - MS<sup>3</sup>
  - FAIMS

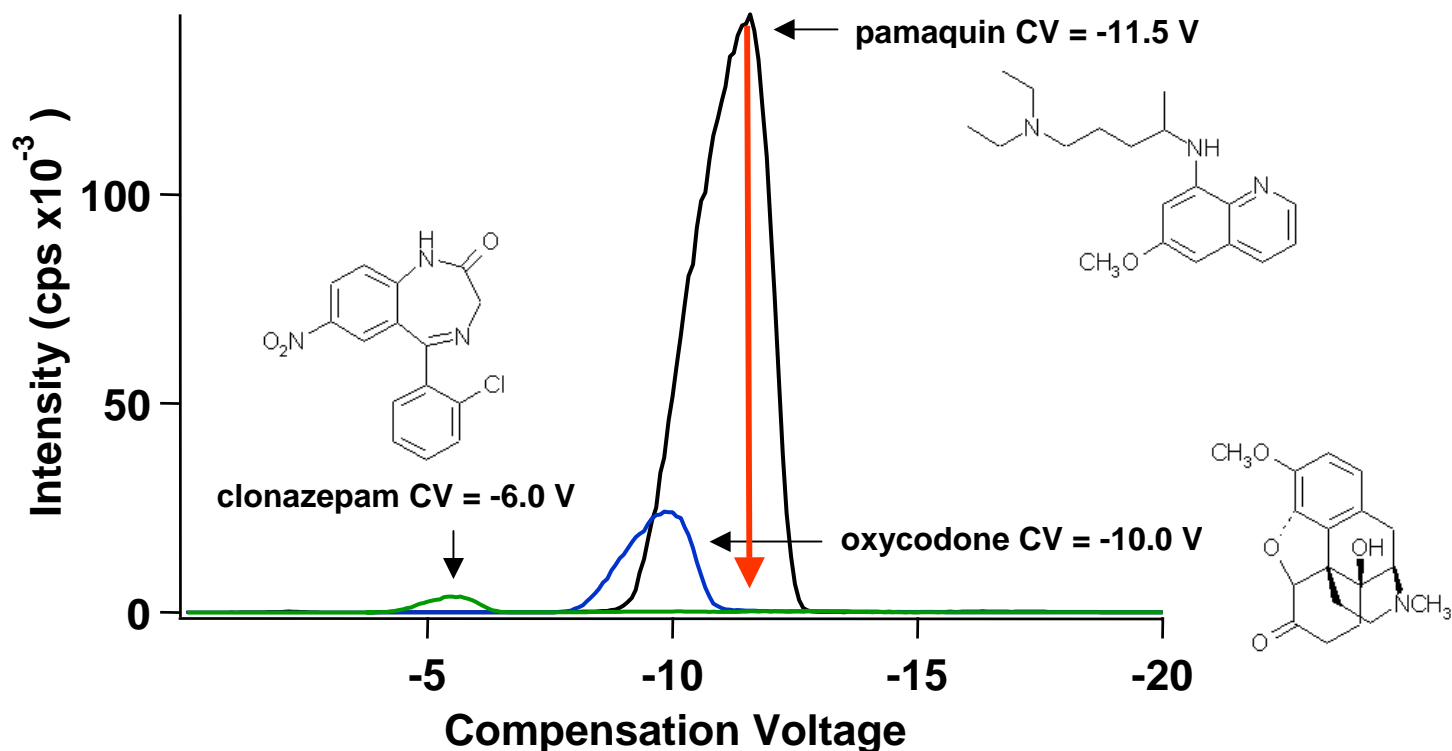
# Pamaquin: Full scan single quadrupole MS



- ESI-MS spectrum: Many intense background ions
- ESI-FAIMS-MS spectrum: Pamaquin most intense ion, background dramatically reduced

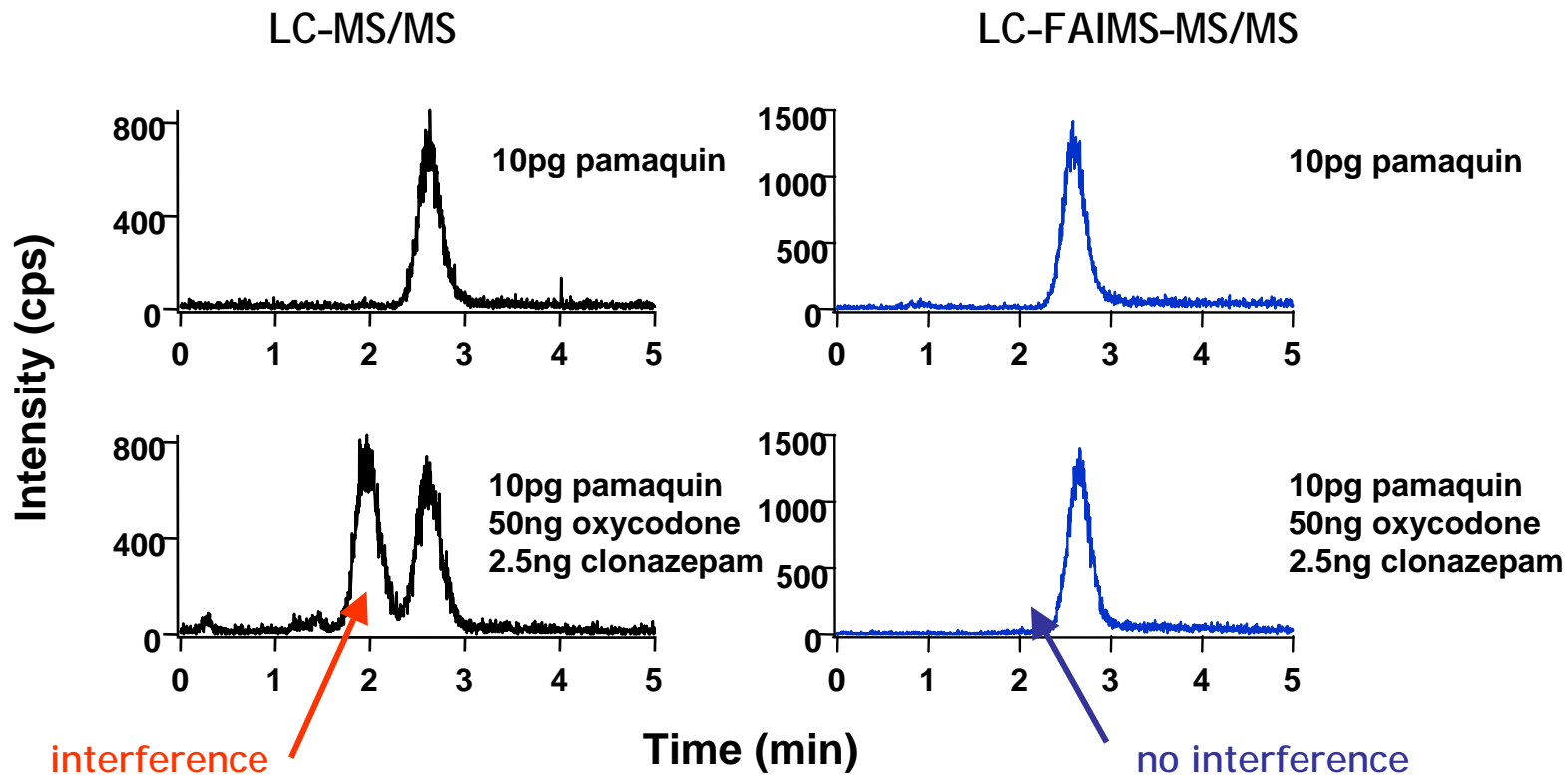
# FAIMS separation of pamaquin/oxycodone/clonazepam

Infusion ESI- FAIMS-MS/MS  
(SRM optimized for each)



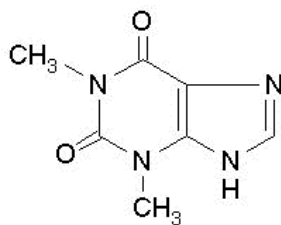
- FAIMS separates the isobaric ions pamaquin, oxycodone and clonazepam

## Representative chromatograms for pamaquin with and without interferences

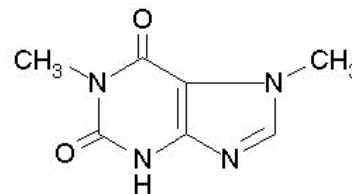


- Using FAIMS, no interference of oxycodone or clonazepam on pamaquin
- Using FAIMS, signal for pamaquin increased

## Problem: Separation of positional isomers

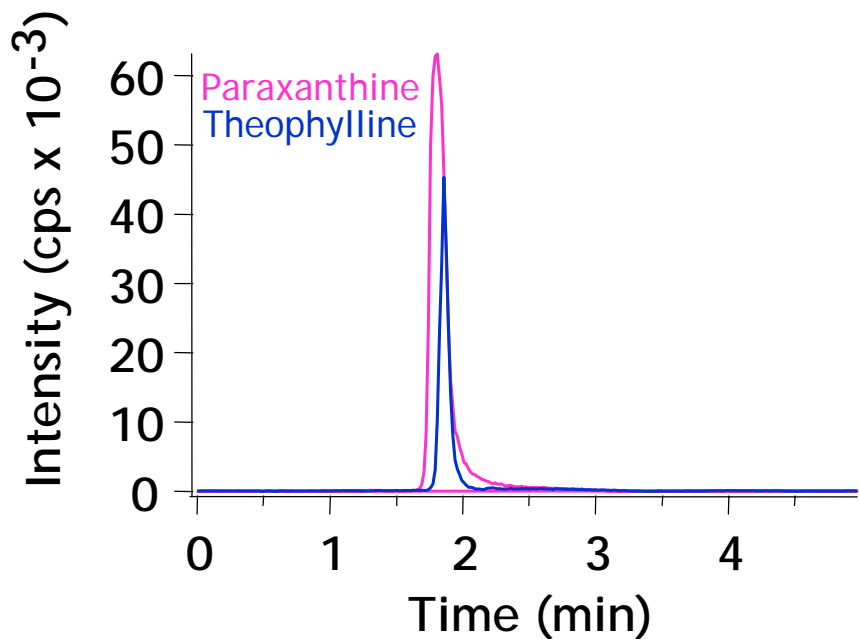


theophylline

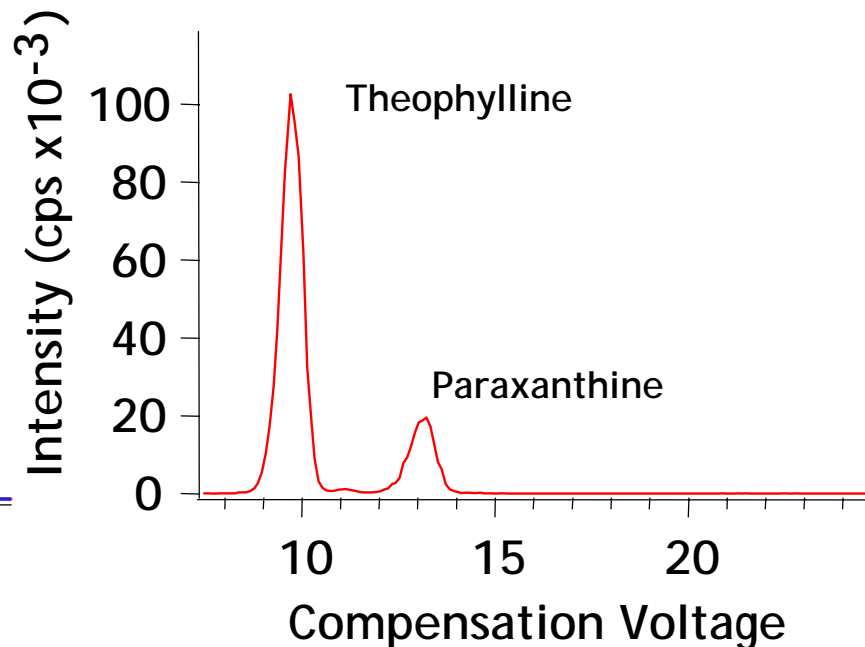


paraxanthine

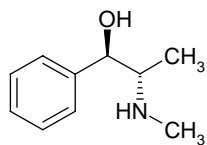
LC-ESI-MS analysis - ions co-elute



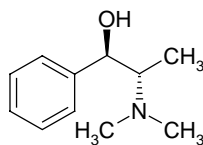
ESI-FAIMS-MS - ions separated



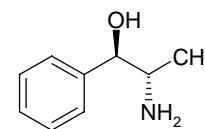
# Problem: Separation of diastereomers



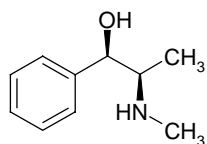
**(-)-ephedrine (E)**



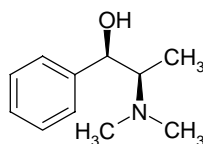
**(-)-methylephedrine (ME)**



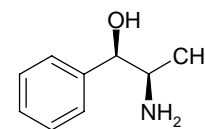
**(-)-norephedrine (NE)**



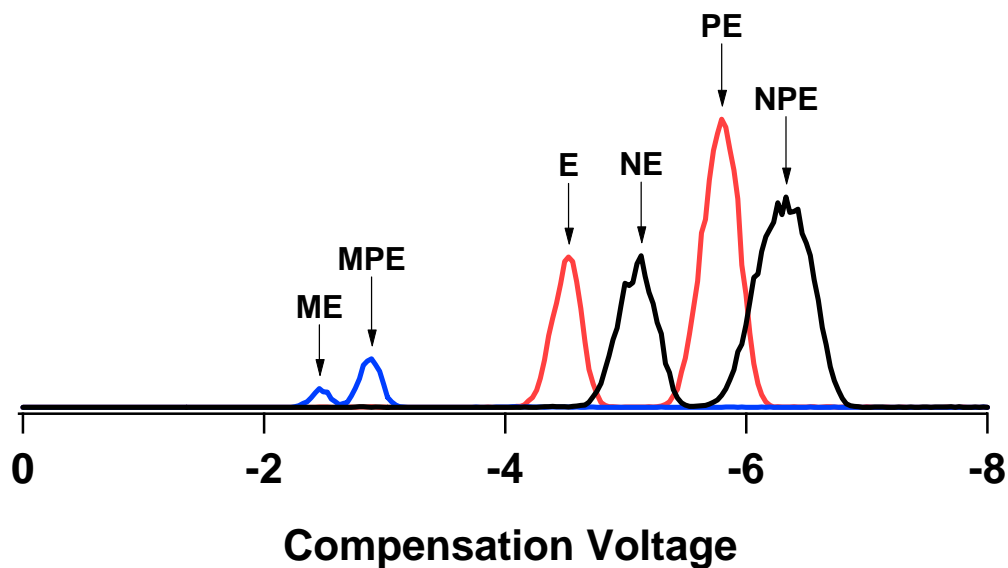
**(+)-pseudoephedrine (PE)**



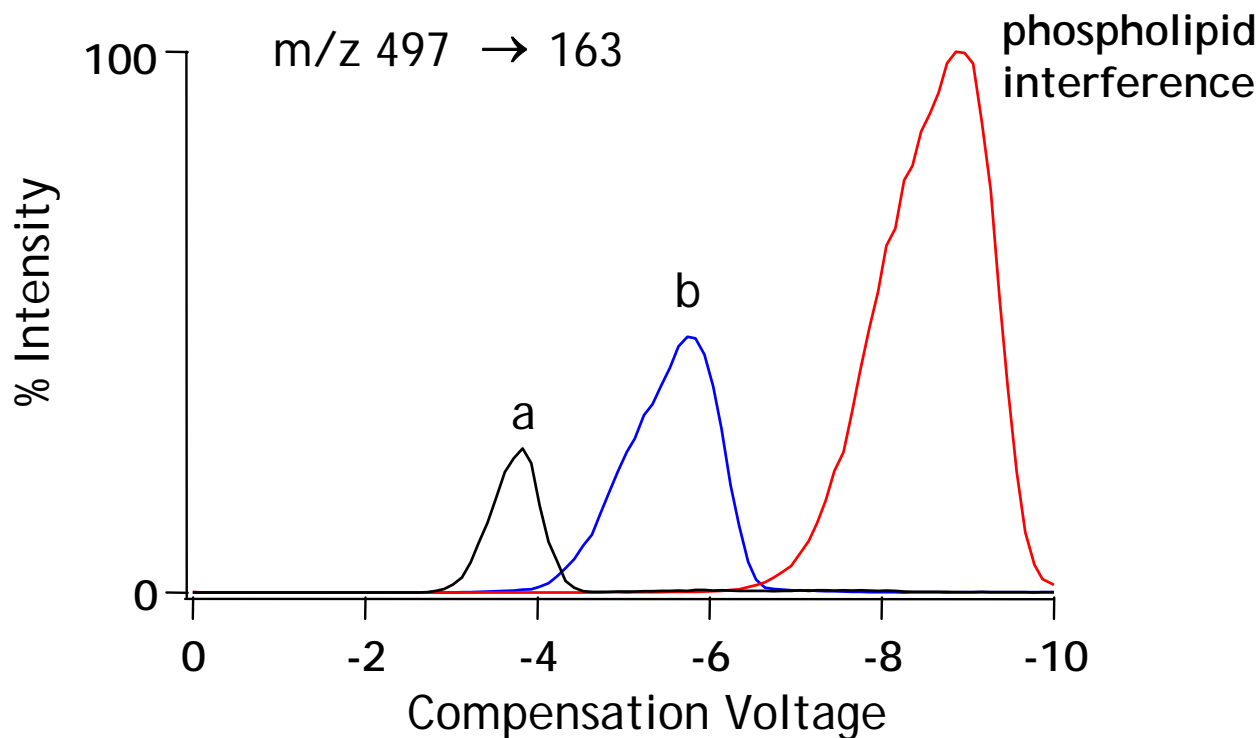
**(+)-methylpseudoephedrine (MPE)**



**(+)-norpseudoephedrine (NPE)**



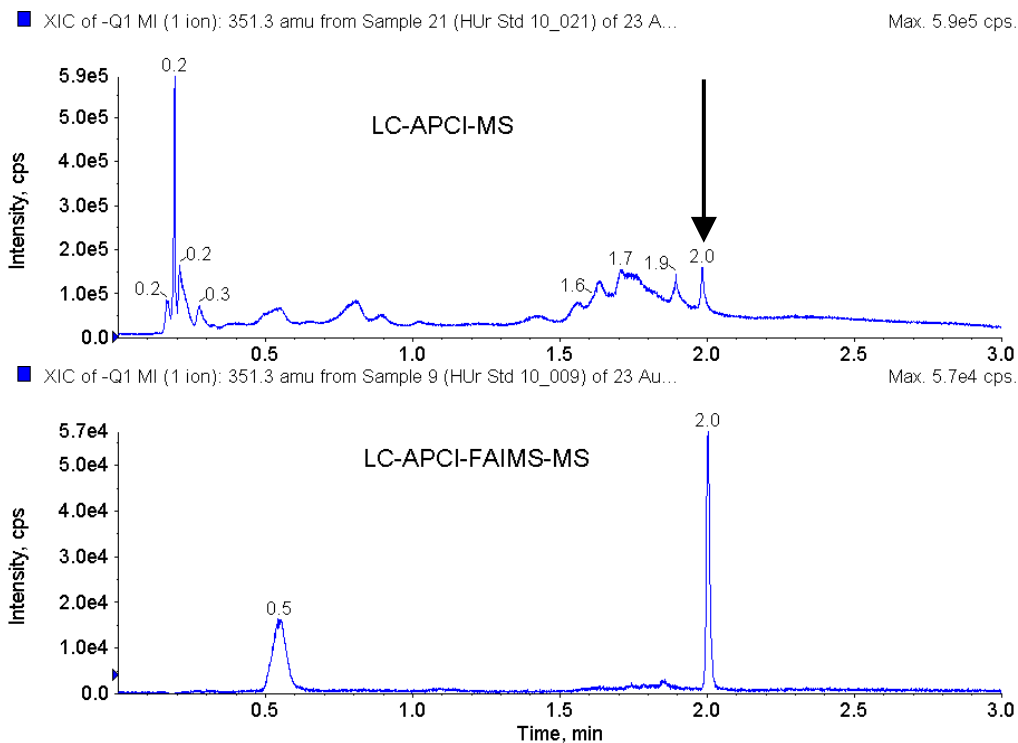
## Problem: Separation of diastereomers in presence of an endogenous interference



- Diastereomers a and b are difficult to separate chromatographically, and the endogenous compound makes the background high
- FAIMS allows the diastereomers to be analyzed in the presence of the endogenous interference

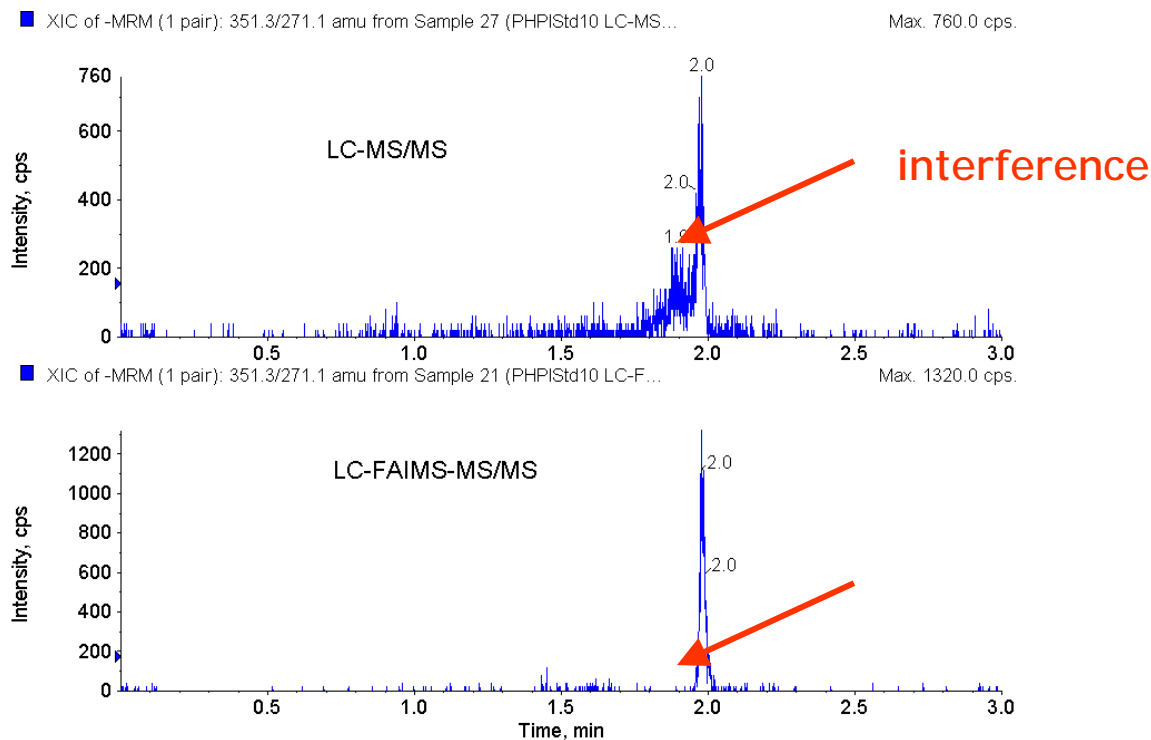
# Problem: Removal of endogenous interference

## Estradiol-3-Sulfate (APCI)



- Many peaks present in LC-APCI-MS chromatogram - difficult to determine which is estradiol-3-sulfate
- Major peak in LC-APCI-FAIMS-MS is estradiol-3-sulfate

# Estradiol-3-Sulfate (TurbolonSpray)



- LC-MS/MS of estradiol-3-sulfate in dilute urine shows endogenous interference present
- FAIMS removes interference, quantitation is more accurate

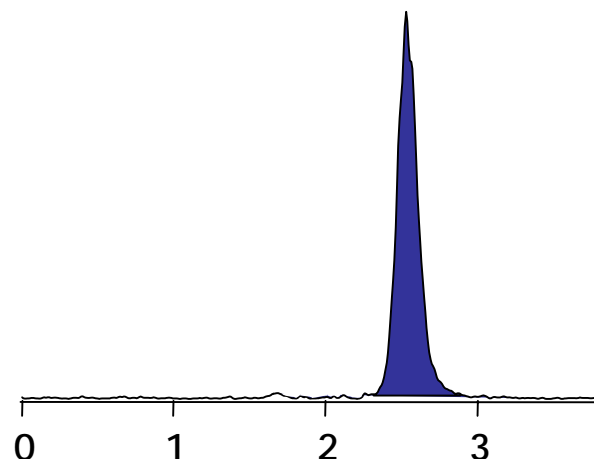
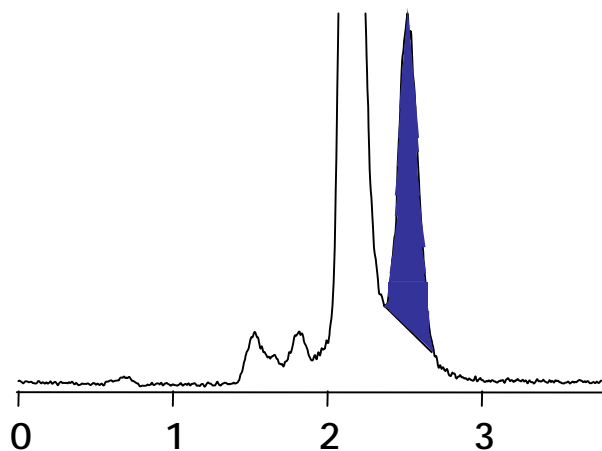
## Problem: Removal of endogenous interference

### 4-Hydroxyisoleucine in rat plasma

30 pg on column

LC-MS/MS

LC-FAIMS-MS/MS



- LC-MS/MS of 4-hydroxyisoleucine in plasma shows endogenous interference present; 0.1 amu resolution (i.e., H-SRM) still has interference
- FAIMS removes interference, quantitation is more accurate
- Chromatographic run time can be reduced

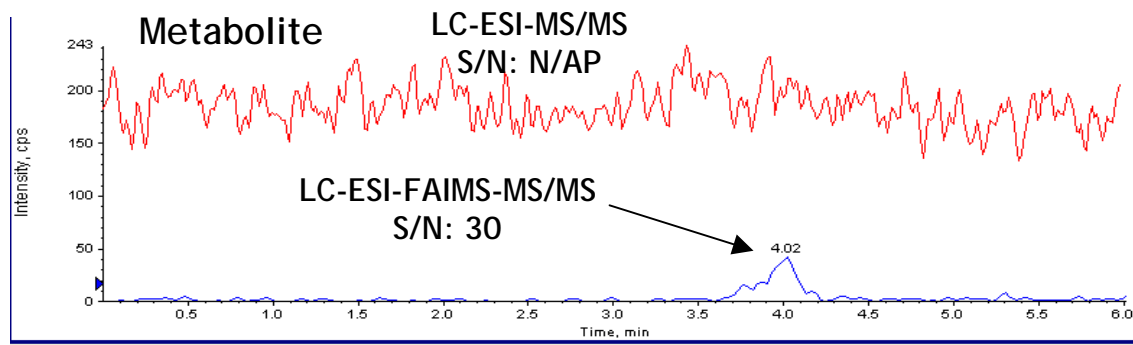
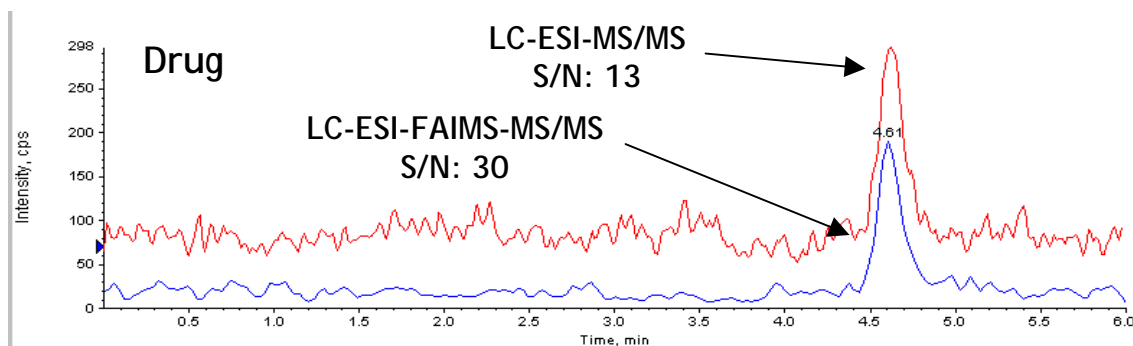
## FAIMS - How does it help you?

- ✓ Increases selectivity
- Reduces background
- Increases throughput

# Problem: High background

## LC-MS/MS vs. LC-FAIMS-MS/MS

50 pg/mL of drug + metabolite in plasma



- Drug shows a S/N improvement of ~2:1 with FAIMS
- Without FAIMS, metabolite is not detected

## FAIMS - How does it help you?

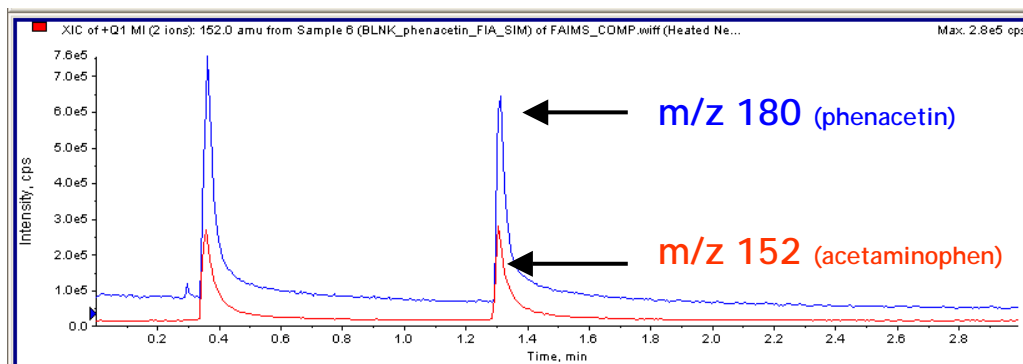
- ✓ Increases selectivity
- ✓ Reduces background
- Increases throughput

## Problem: Long analytical run time

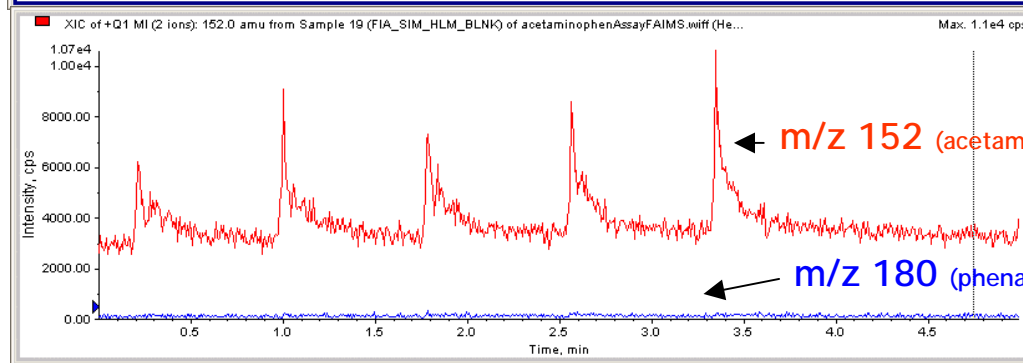
### Acetaminophen Cytochrome P<sub>450</sub> 1A2 Marker Assay

- Monitors the metabolism of phenacetin to acetaminophen
- Detect acetaminophen in the presence of a large excess of phenacetin
- Two challenges:
  - High background in liver microsome blank at the m/z for both phenacetin and acetaminophen
  - Phenacetin undergoes CID at inlet to mass spectrometer to form acetaminophen, affecting quantitation

# FIA-SIM of human liver microsome blank after protein precipitation



APCI-MS

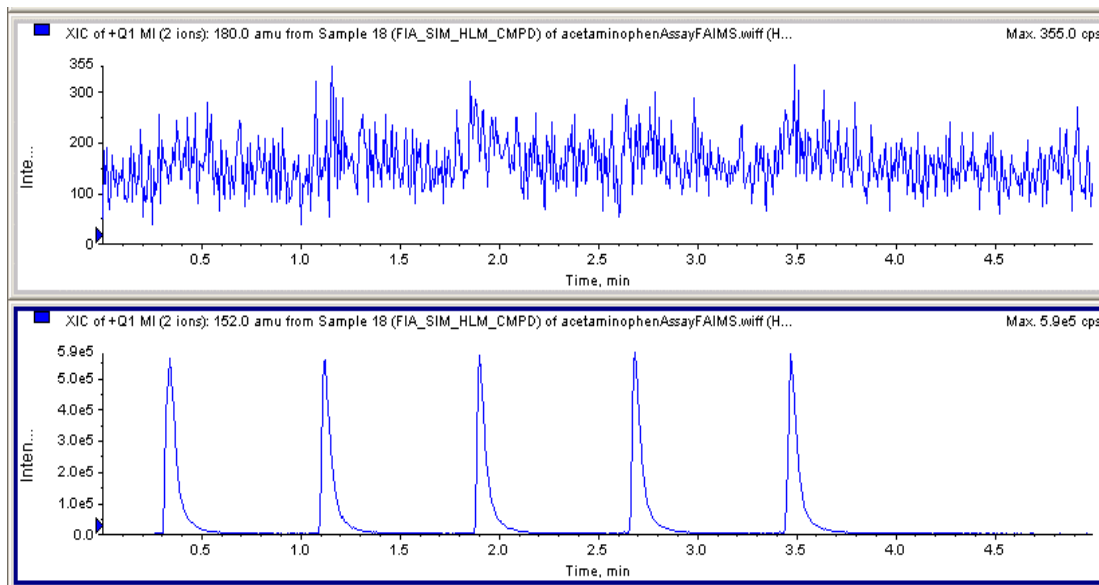


APCI-FAIMS-MS

- High chemical background by APCI-MS; chromatography used
- FAIMS removes chemical background; chromatography not needed

# Human liver microsomes after protein precipitation using FIA-SIM

5  $\mu\text{M}$  Acetaminophen, 80  $\mu\text{M}$  Phenacetin



phenacetin

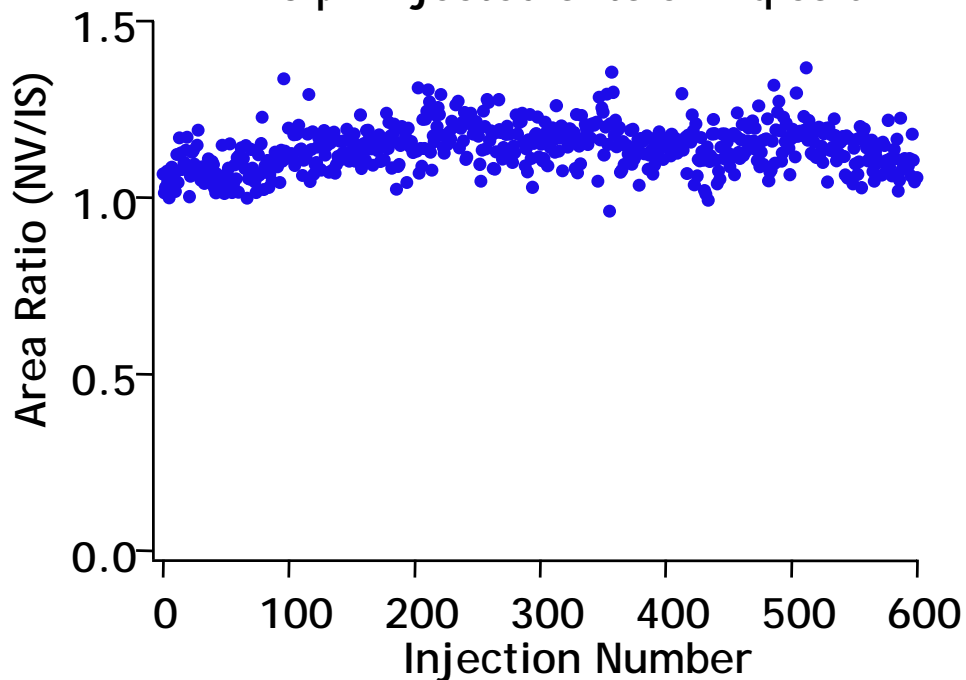
acetaminophen

- FAIMS excludes phenacetin from the acetaminophen analysis
- 5-minute LC separation is not required
- Analysis time is reduced from 5 minutes to 1 minute

# FAIMS - Robustness for batch analyses

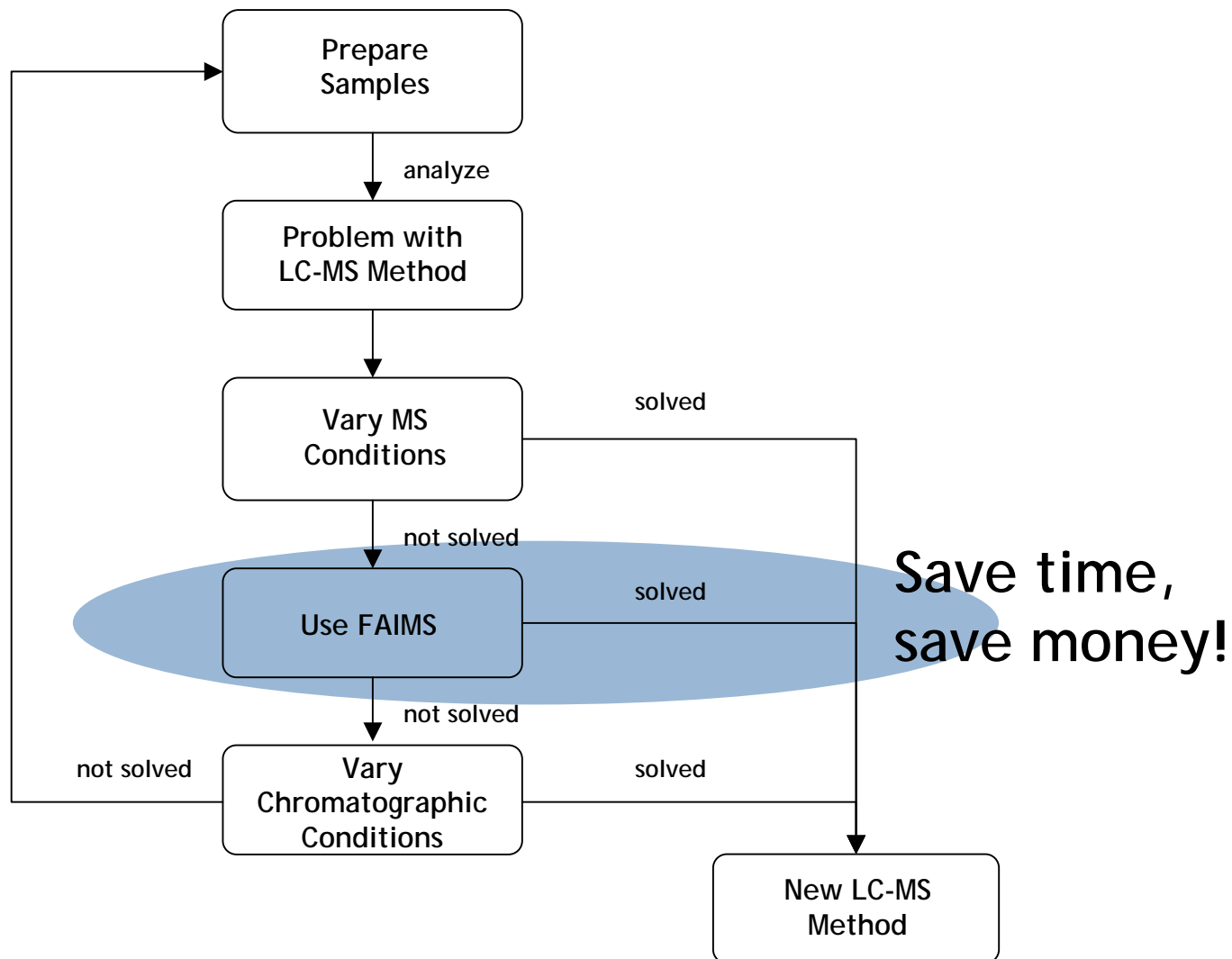
FAIMS system run for 54 hours analyzing 1 ng/mL norverapamil (analyte) and verapamil (IS) in urine

Spiked urine diluted 1:3 with 0.1% formic acid in water (final conc. 250 pg/mL)  
10  $\mu$ L injected onto SB-Aq column

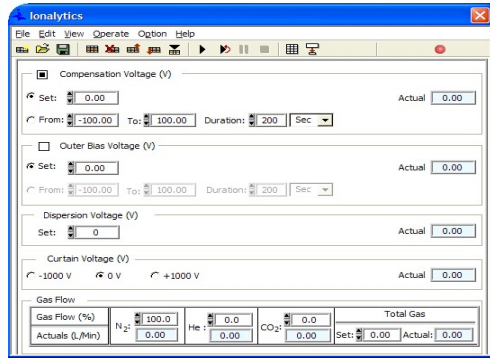


- 600 injections over a 54 hour period gave an RSD of 6.3%

# FAIMS could save you months

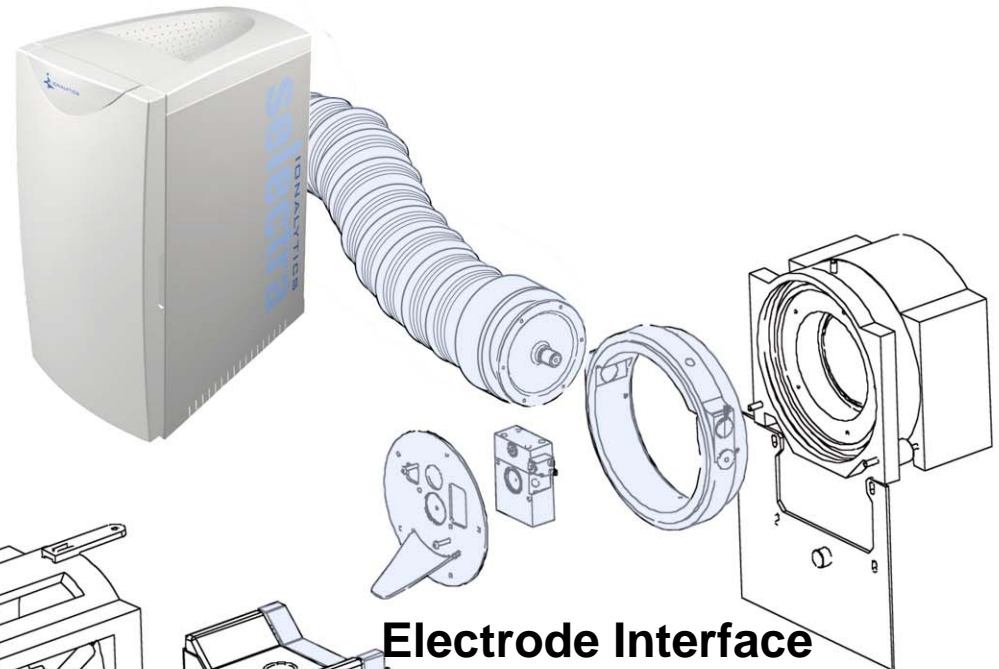


# Assembly on an AB/Sciex API 3000

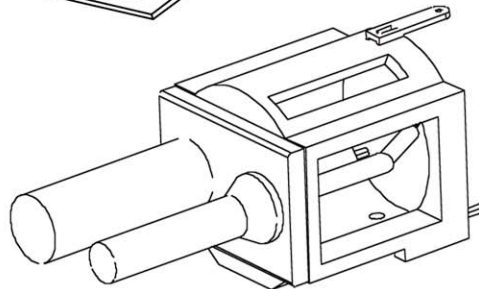


**Control Software  
Integrated with  
Analyst 1.4.1**

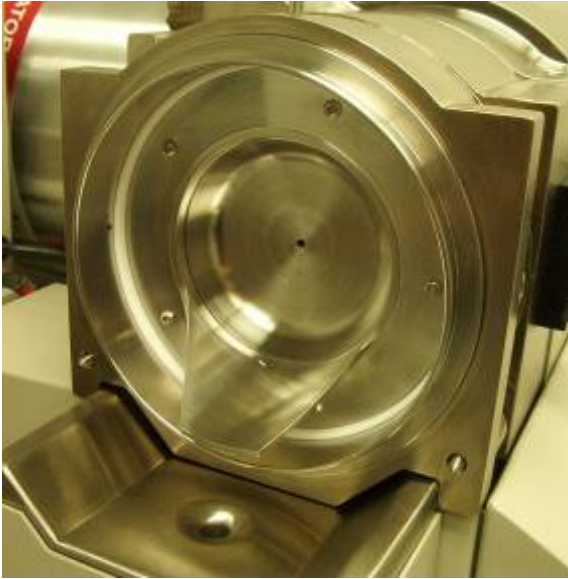
**Waveform Generator**



**Electrode Interface  
Kit**



# FAIMS on the API 3000

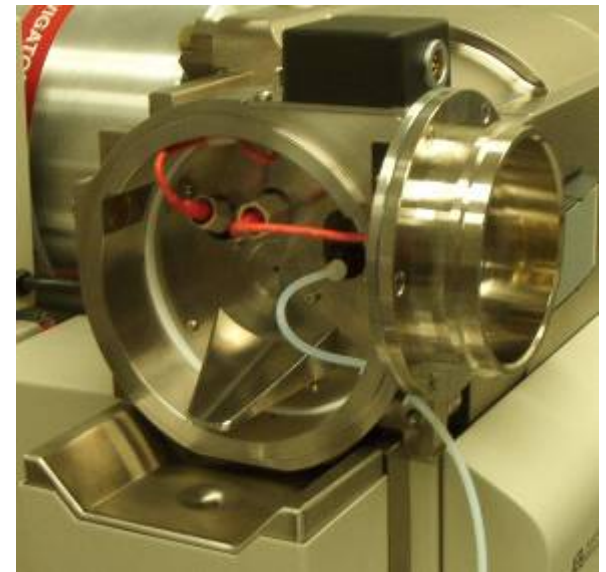


conventional  
configuration

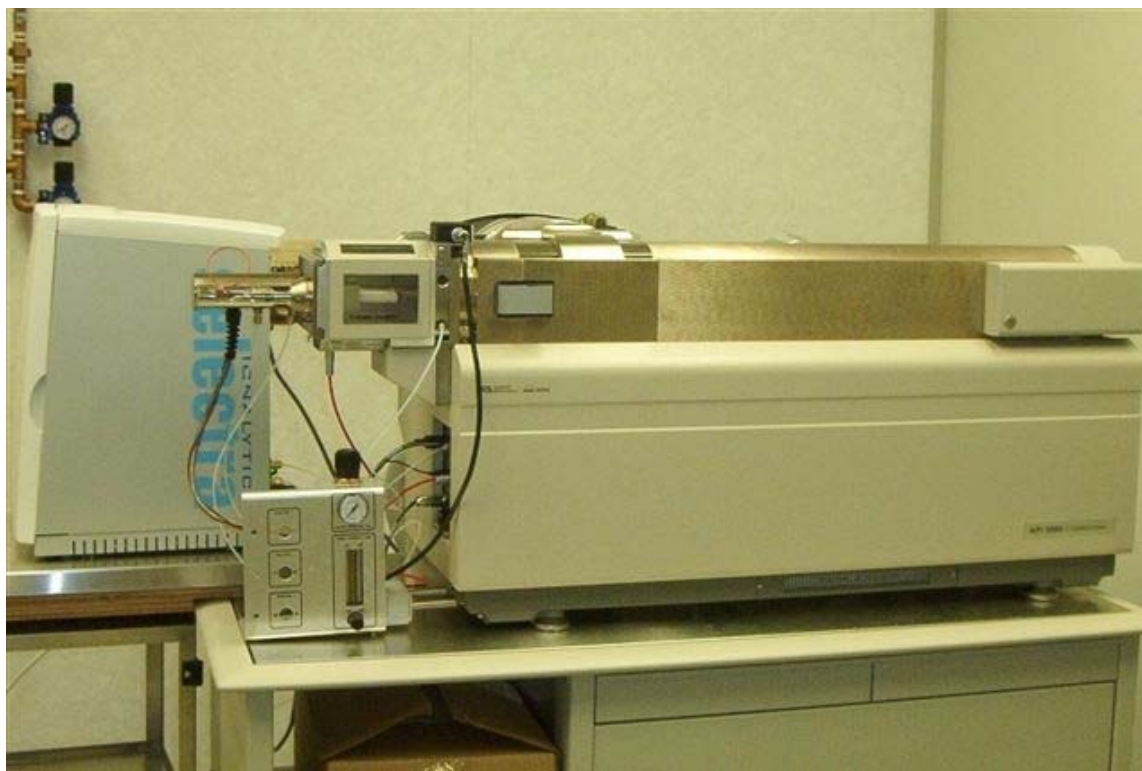


FAIMS  
electrodes

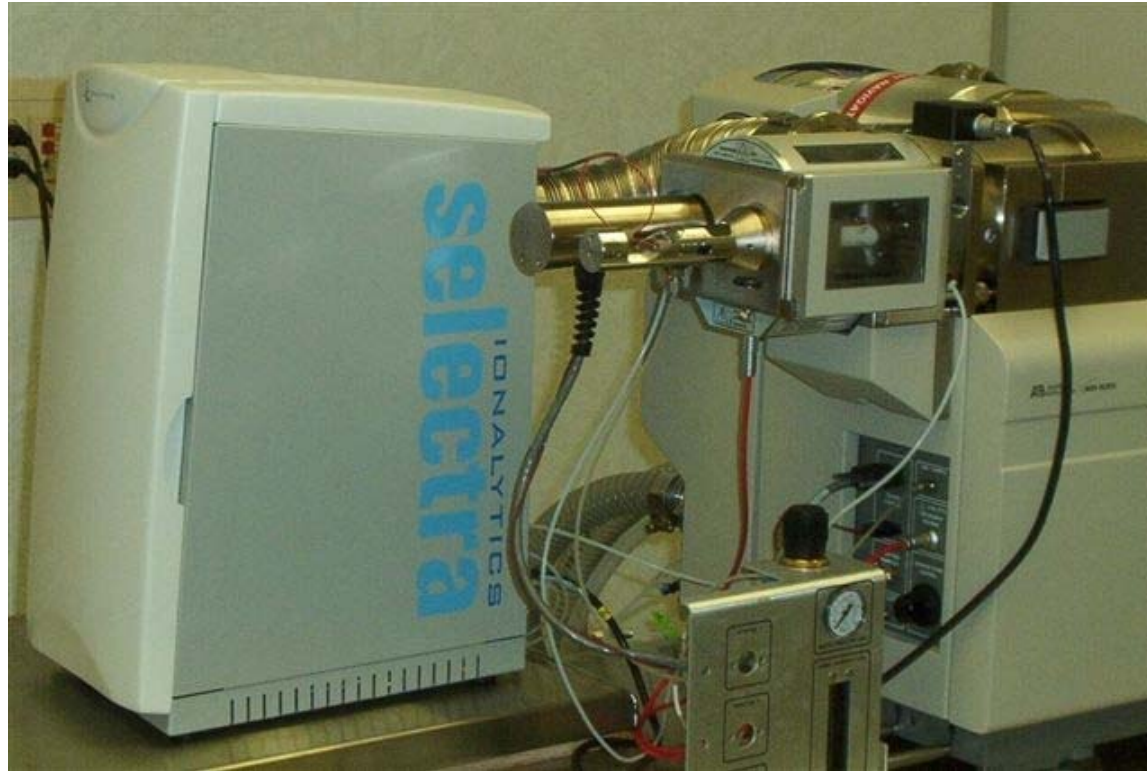
FAIMS  
configuration



## Selectra on the API 3000



## Selectra on the API 3000



# Acknowledgements

Beata Kolakowski

David Barnett

Garnet McRae

James Kapron

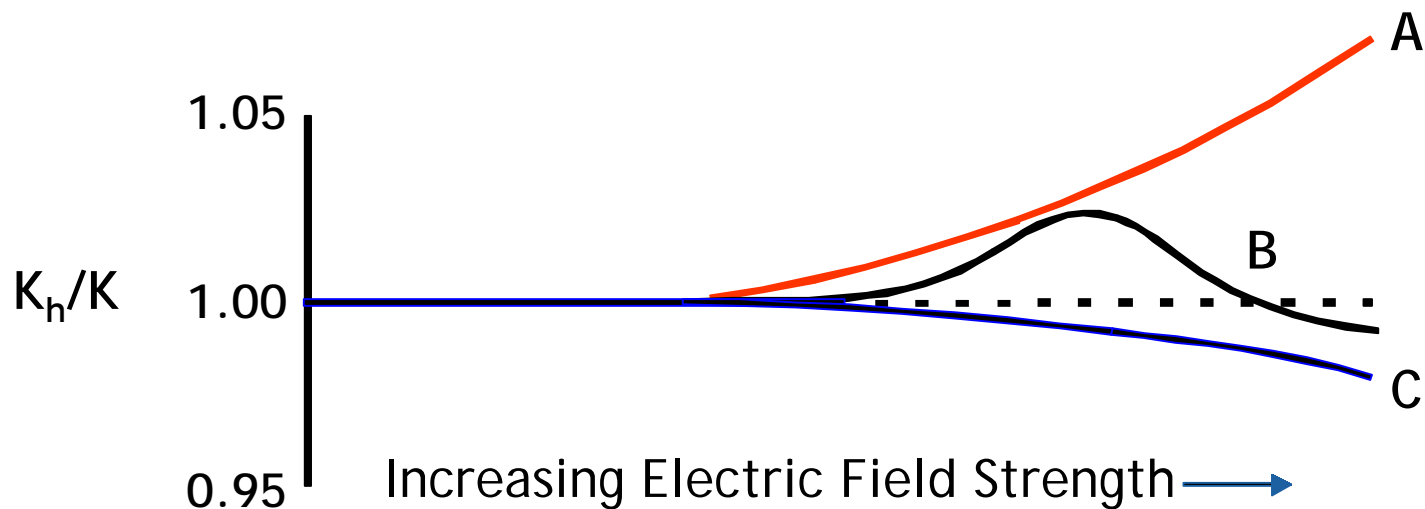
Randy Purves

Roger Guevremont

Unny Thekkadath

# Basis of FAIMS Operation

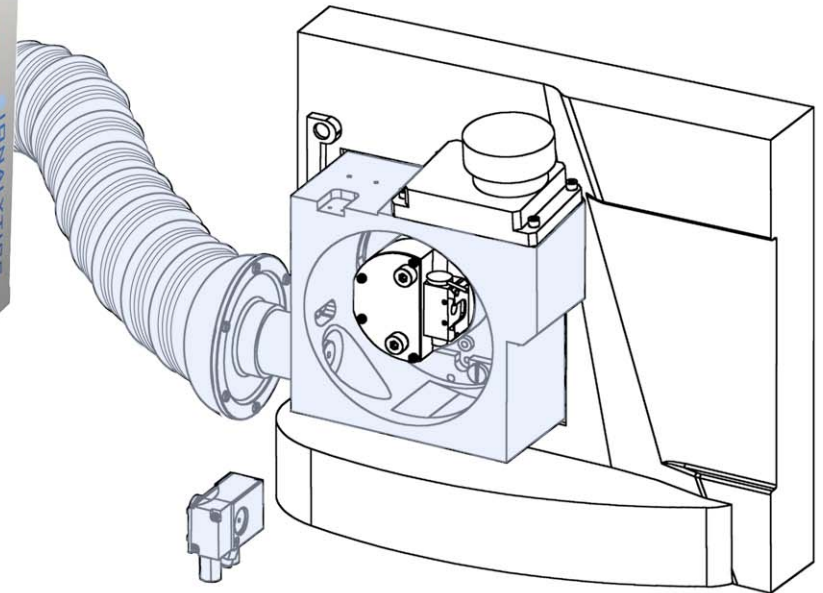
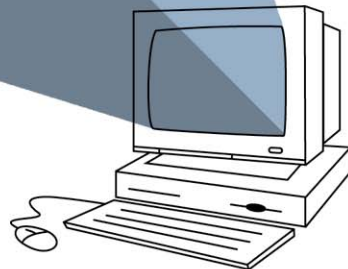
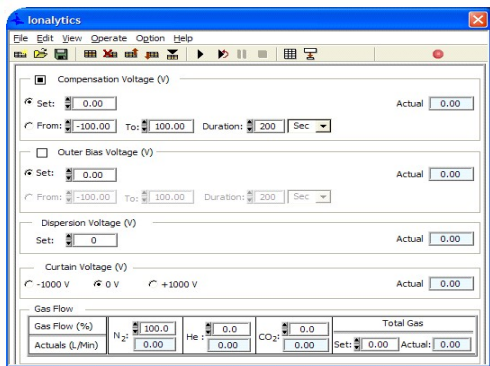
- exploits differences in an ion's mobility resulting from an alternating high ( $K_h$ ) and low ( $K$ ) electric field



# Assembly on a Waters Micromass Quattro micro

## Waveform Generator

## Control Software



## Electrode Interface Kit