Field asymmetric waveform ion mobility spectrometry combined with mass spectrometry for the analysis of anabolic steroids

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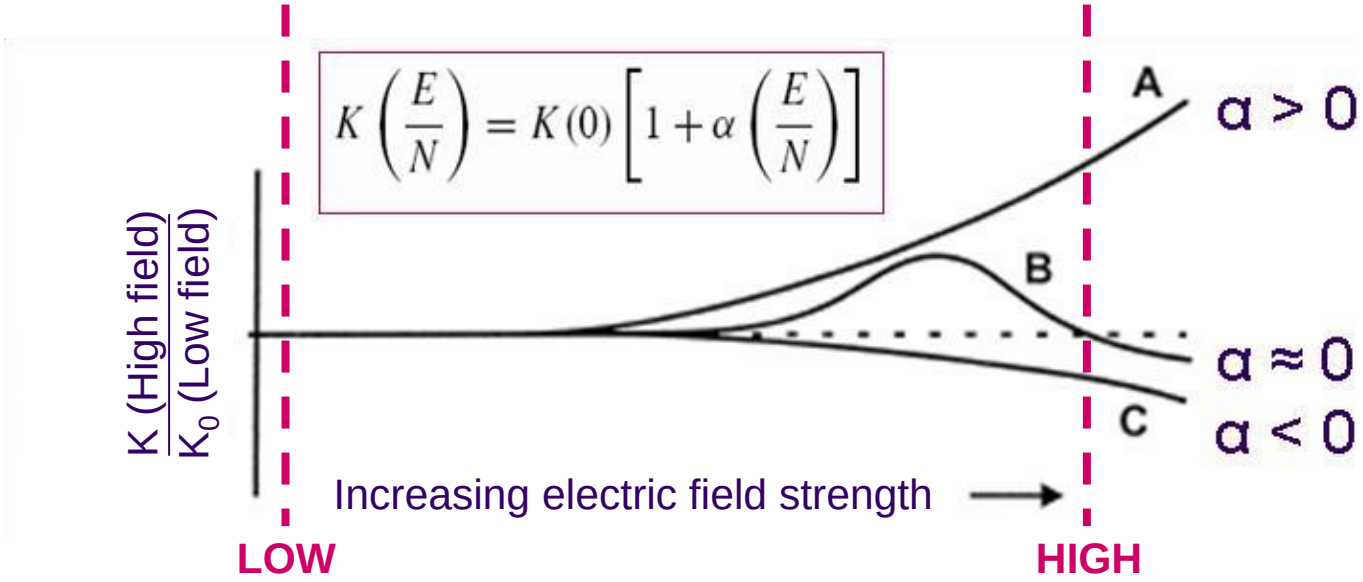
Outline

- Aims
- What is FAIMS?
- FAIMS-MS and LC-FAIMS-MS instrumentation
- FAIMS-MS separation of steroid metabolites – sulfates and glucuronides
- LC-FAIMS-MS analysis of steroids in urine matrix

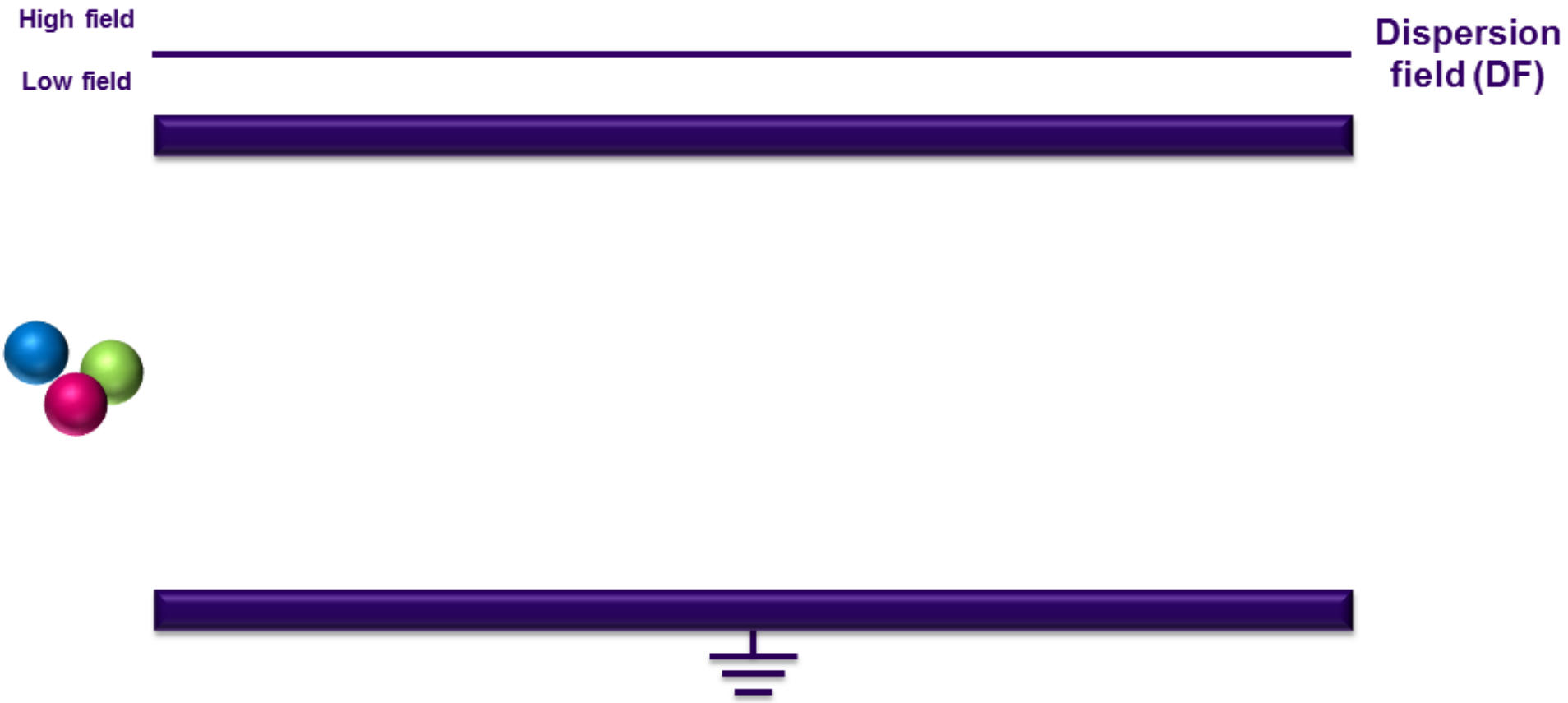
What is FAIMS?

- Field asymmetric waveform ion mobility spectrometry
- Separation of ions based upon their non-linear relationship between mobility and increasing electric field strength
- **Ion separation based upon differential mobility**

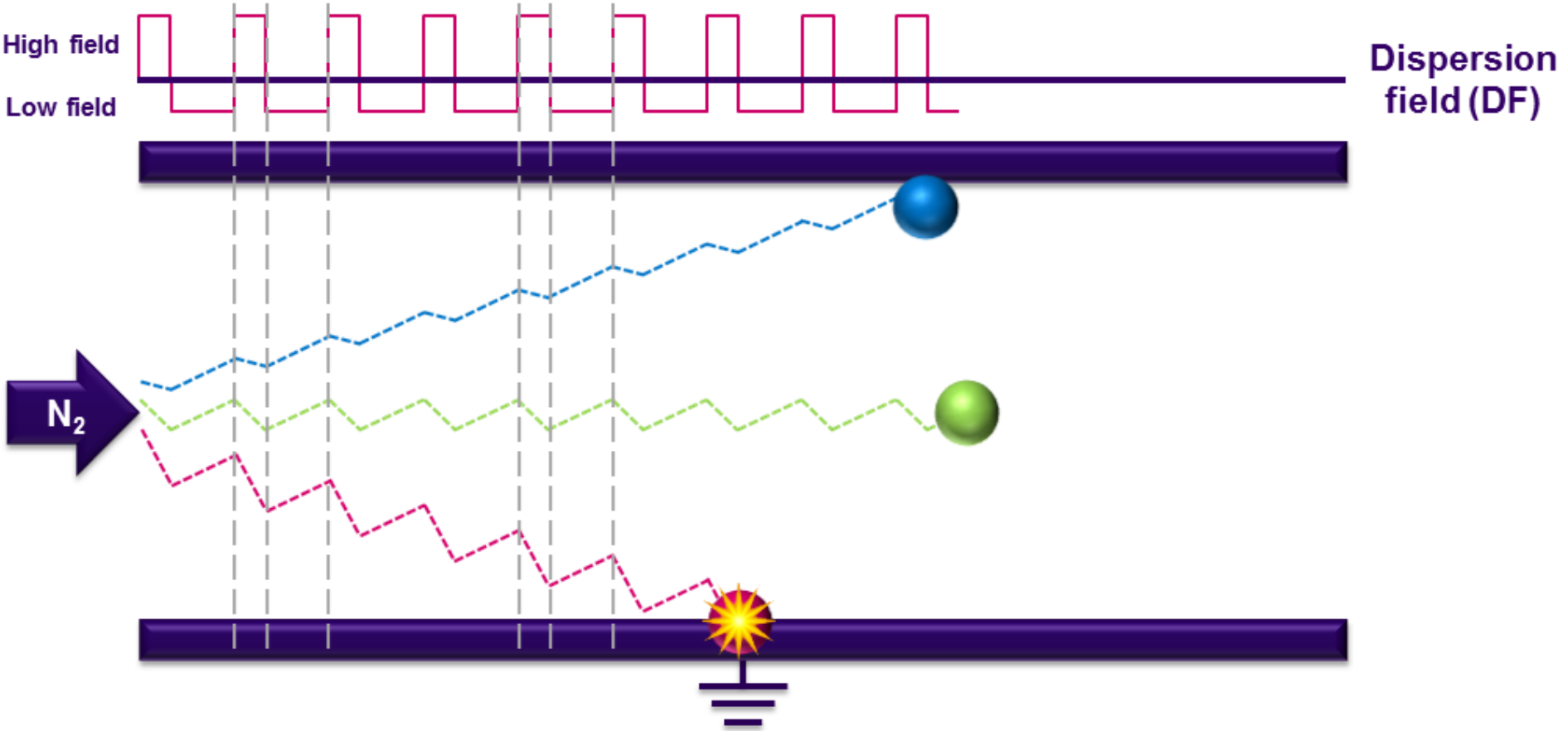


[Purves R W, Guevremont R, Anal. Chem. 1999, 71, 2346-2357]

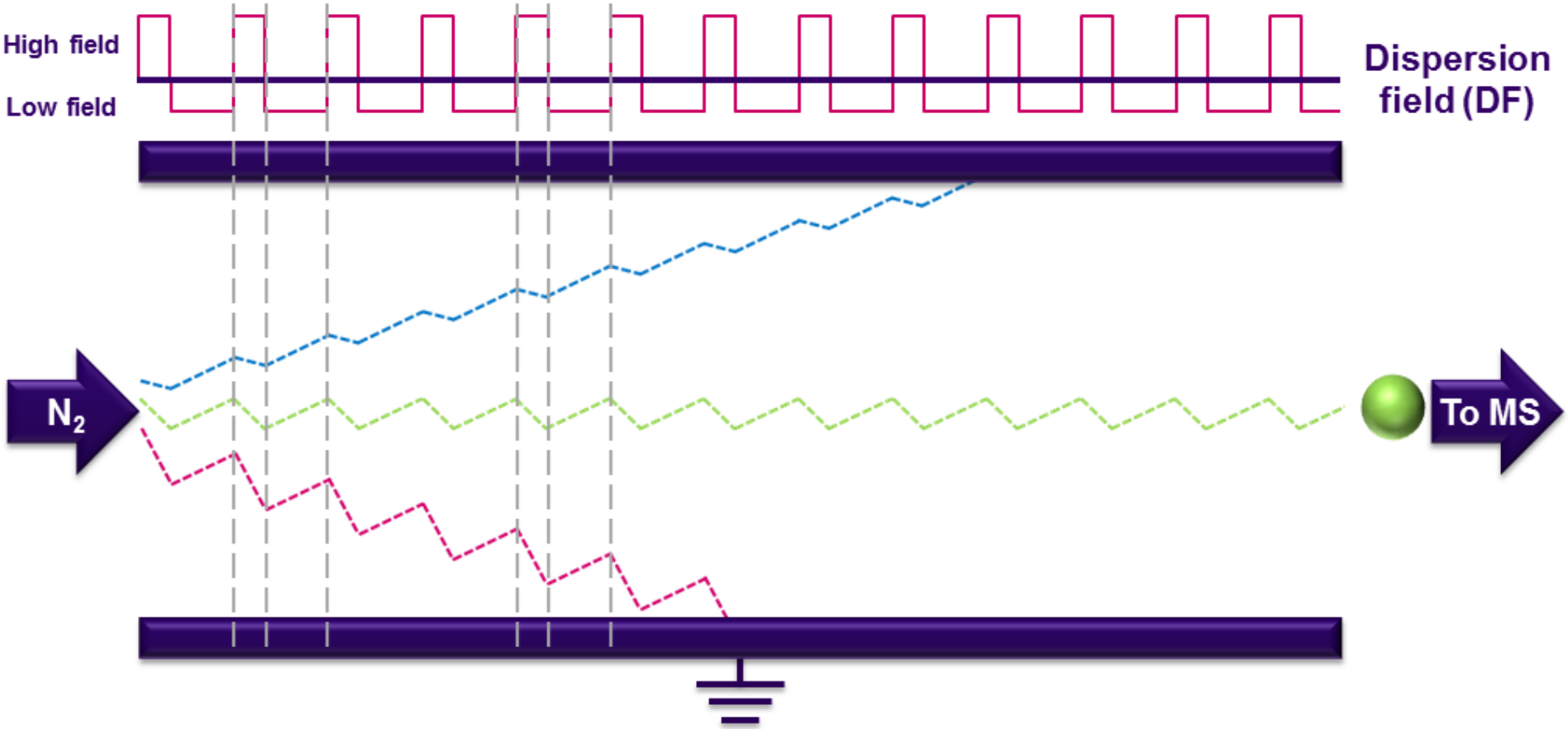
What is FAIMS?



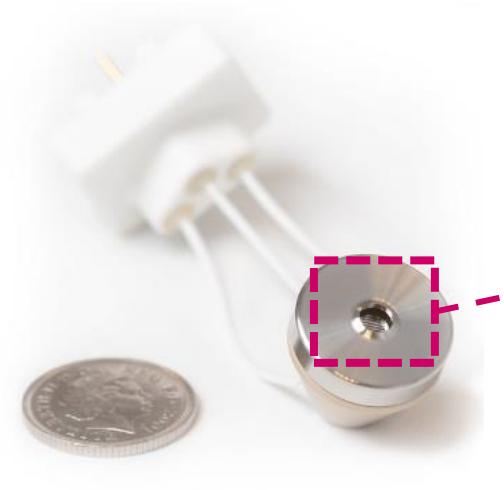
What is FAIMS?



What is FAIMS?

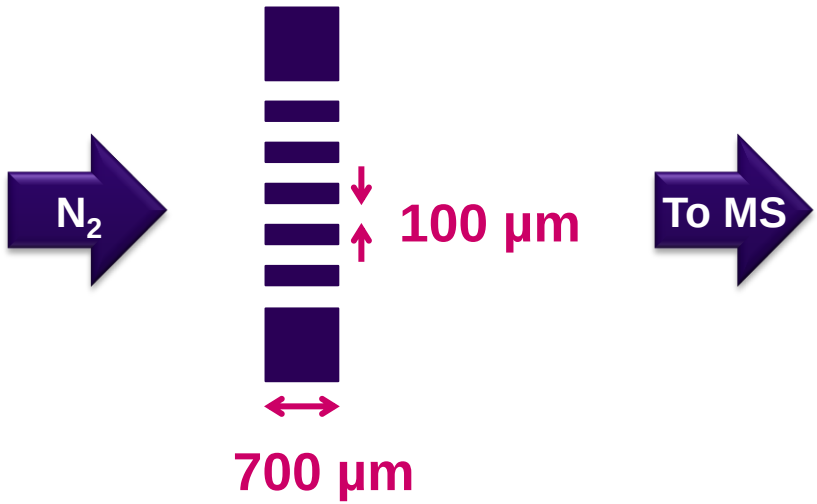


Owlstone chip-based FAIMS



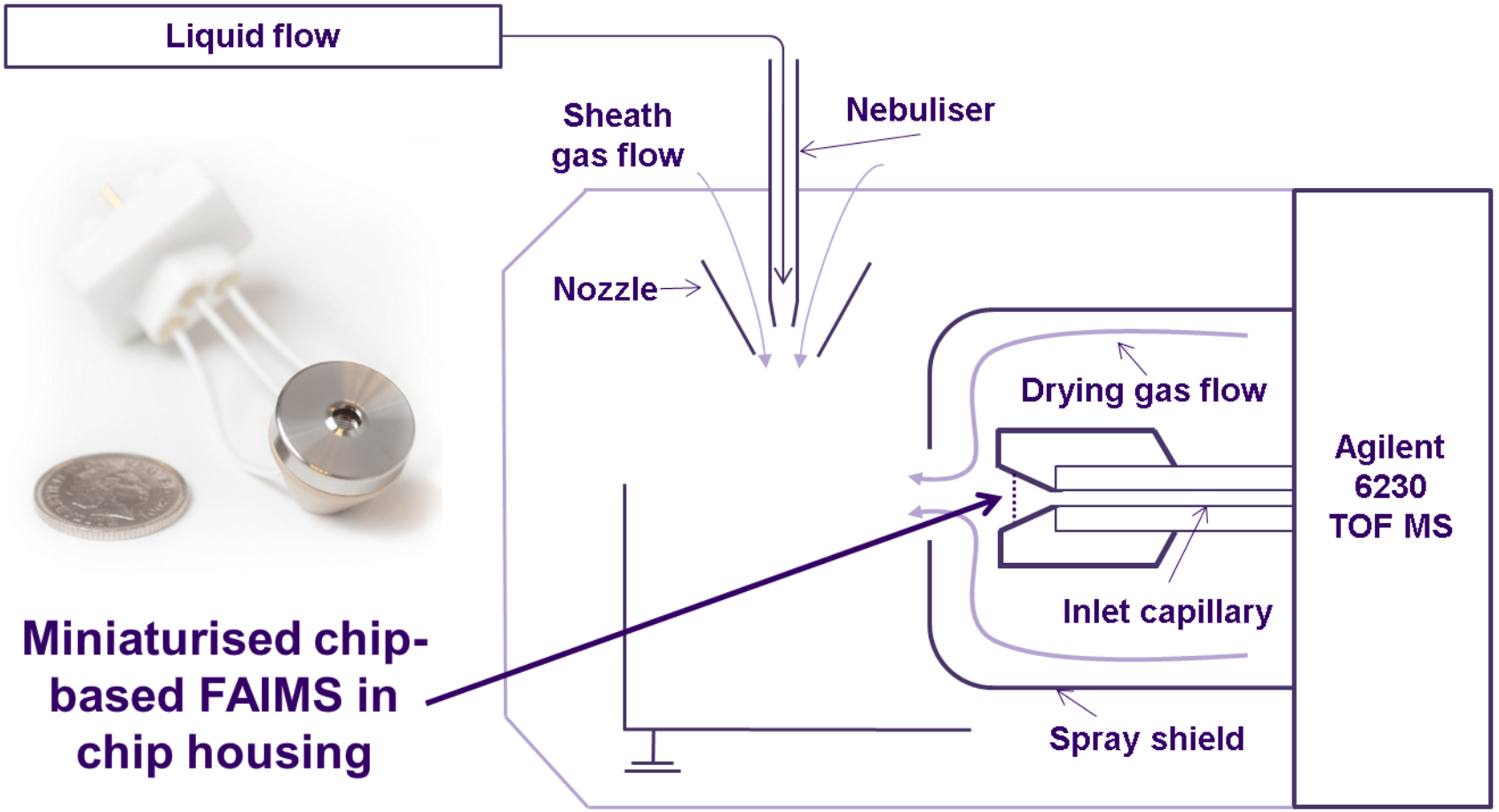
Miniaturised chip-based FAIMS in chip housing

Parallel pairs of electrodes



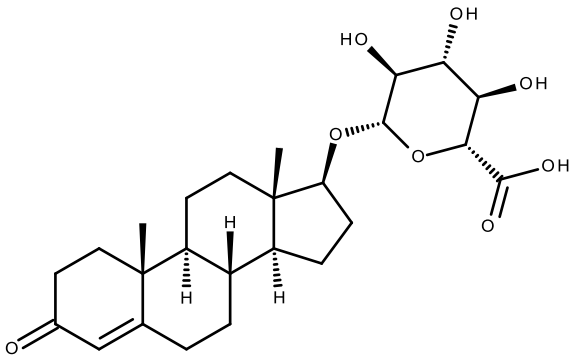
Side view of FAIMS chip

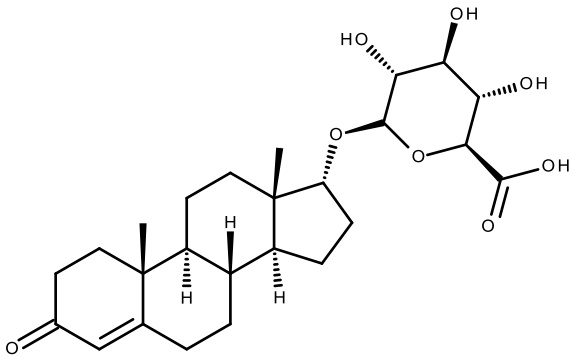
Owlstone chip-based FAIMS



Targeted steroid compounds

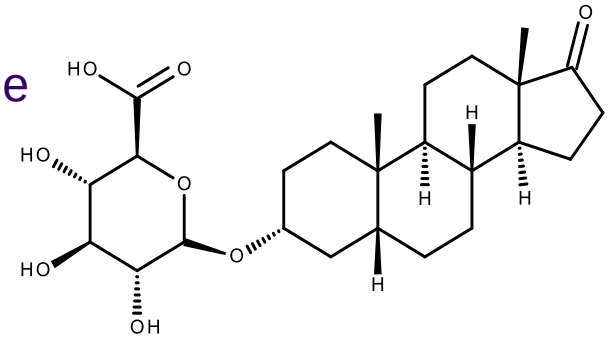
Testosterone glucuronide
TG
MW 464

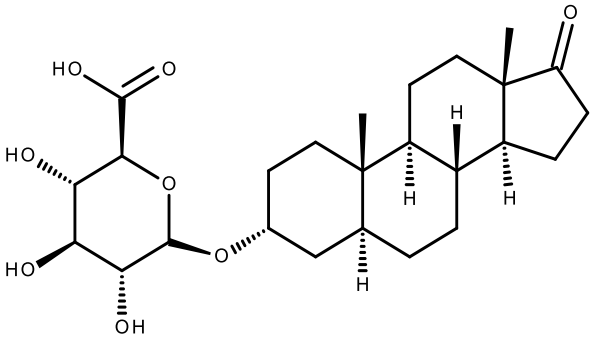




Epitestosterone glucuronide
ETG
MW 464

Etiocholanolone glucuronide
ECG
MW 466

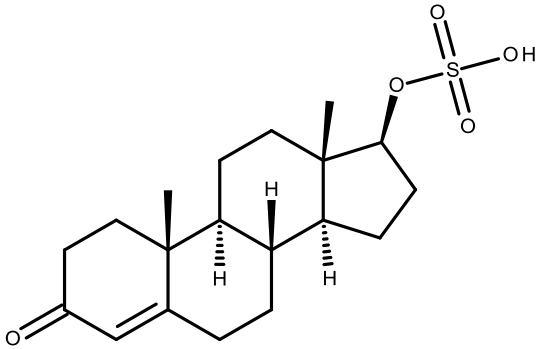


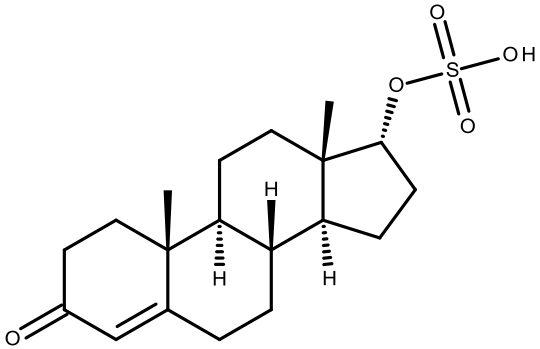


Androsterone glucuronide
ADG
MW 466

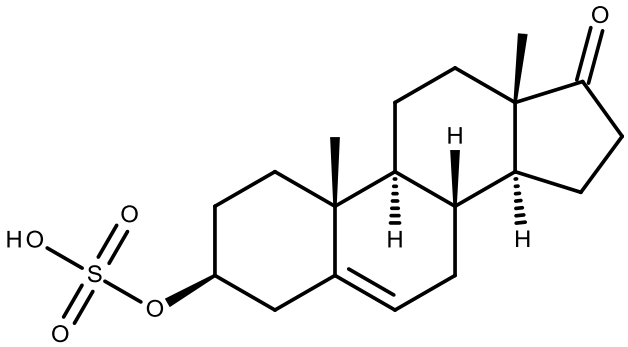
Targeted steroid compounds

Testosterone sulfate
TS
MW 368



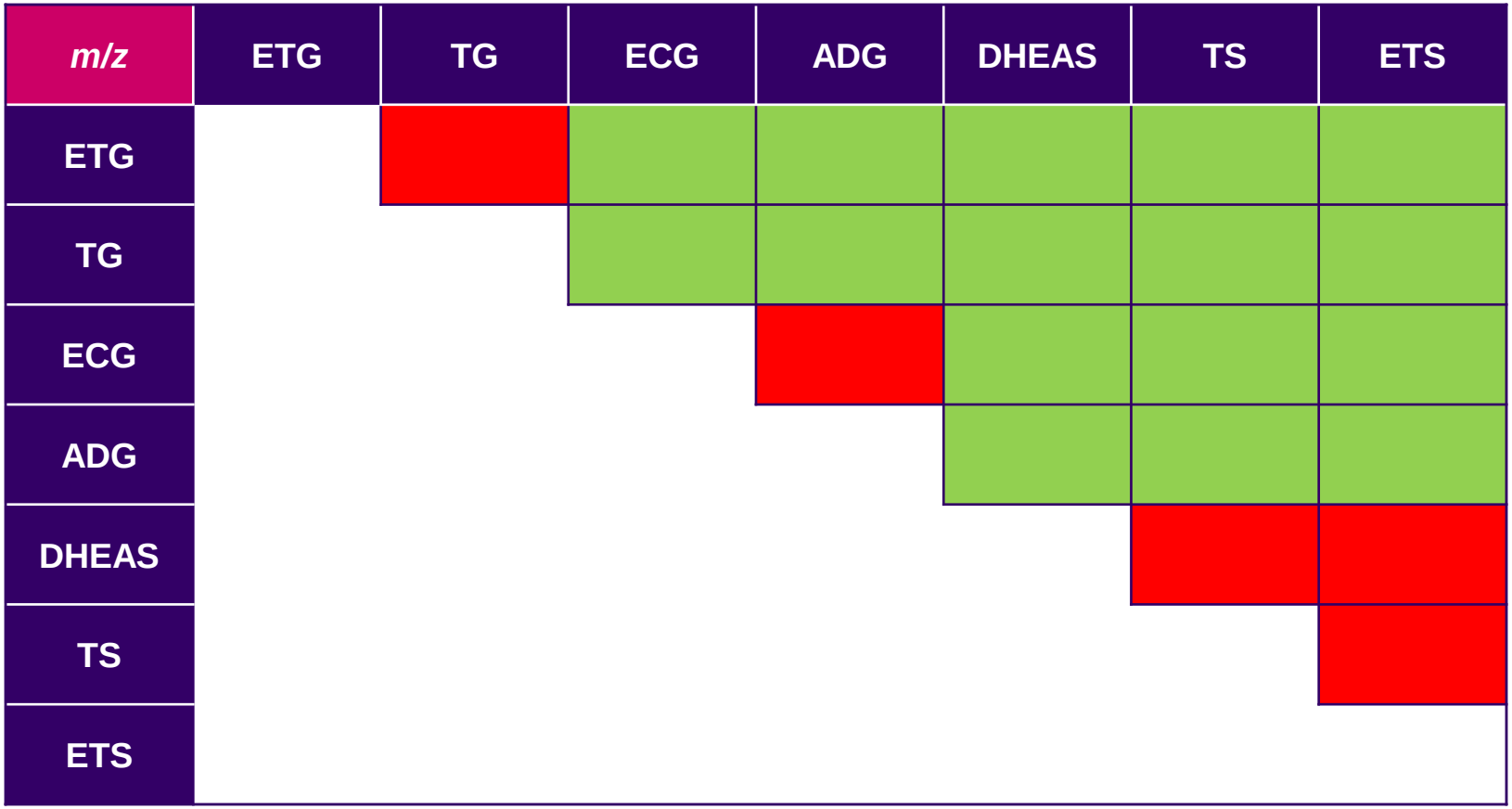


Epitestosterone sulfate
ETS
MW 368



Dehydroepiandrosterone sulfate
DHEAS
MW 368

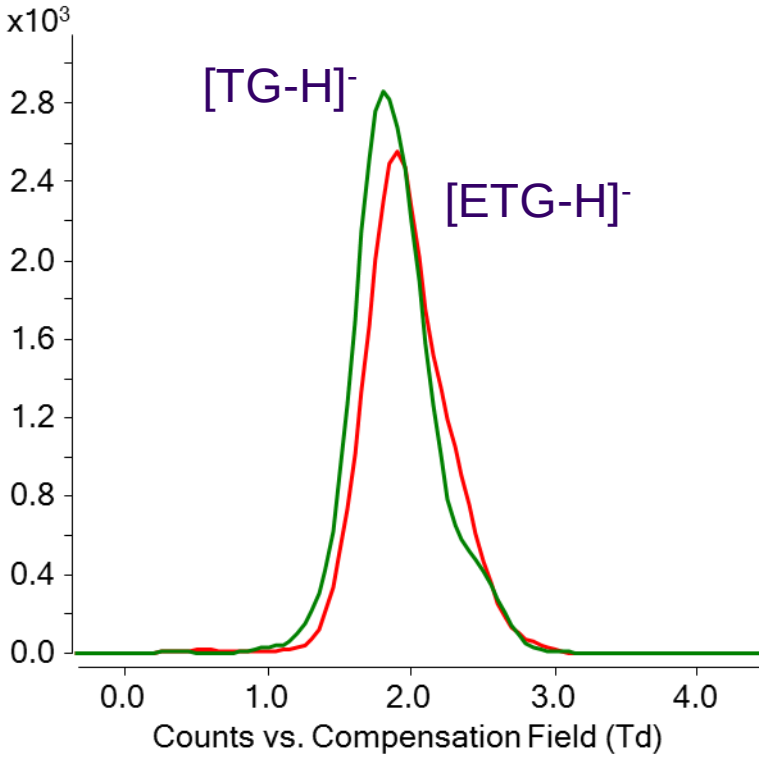
Separation of steroids using MS alone



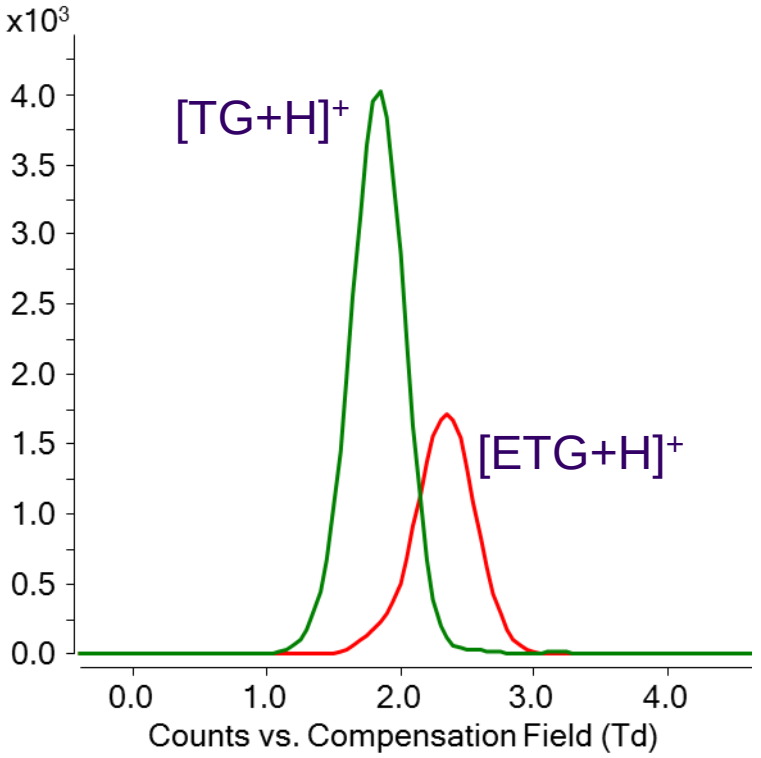
Isobaric pairs/trio unresolved using MS alone

FAIMS-MS of steroid metabolites

Negative ion mode



Positive ion mode



Dispersion Field 300 Td

Initial FAIMS-MS in MeOH:H_2O 50:50 + 0.1% FA

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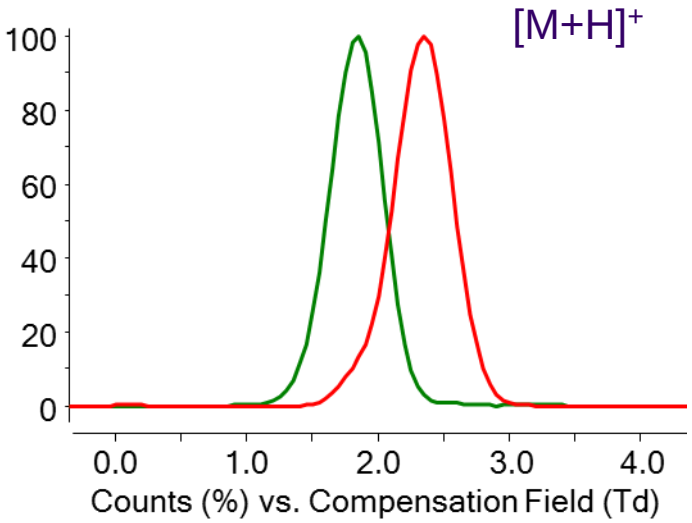
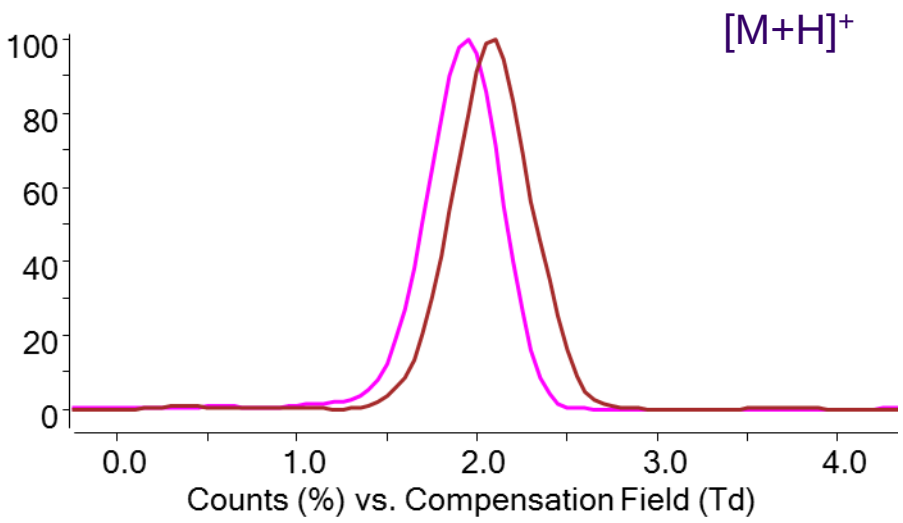
FAIMS-MS of steroid metabolites

Positive ion mode

Dispersion Field 300 Td





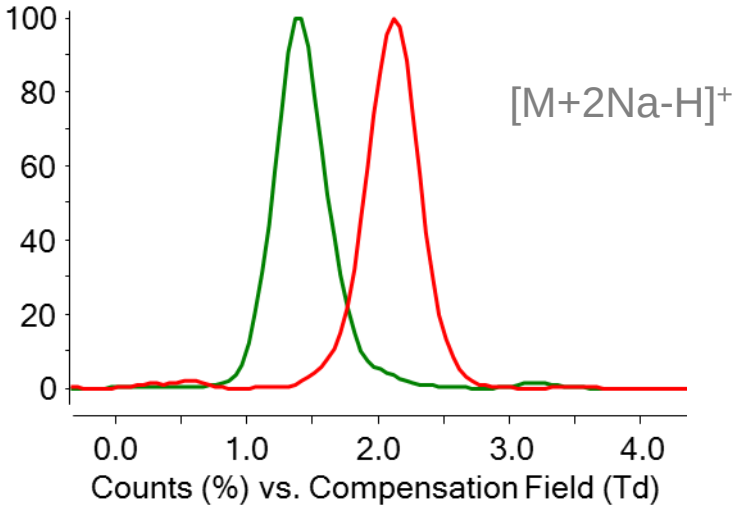
Testosterone glucuronide
Epitestosterone glucuronide

Testosterone sulfate
Epitestosterone sulfate

FAIMS-MS of steroid metabolites

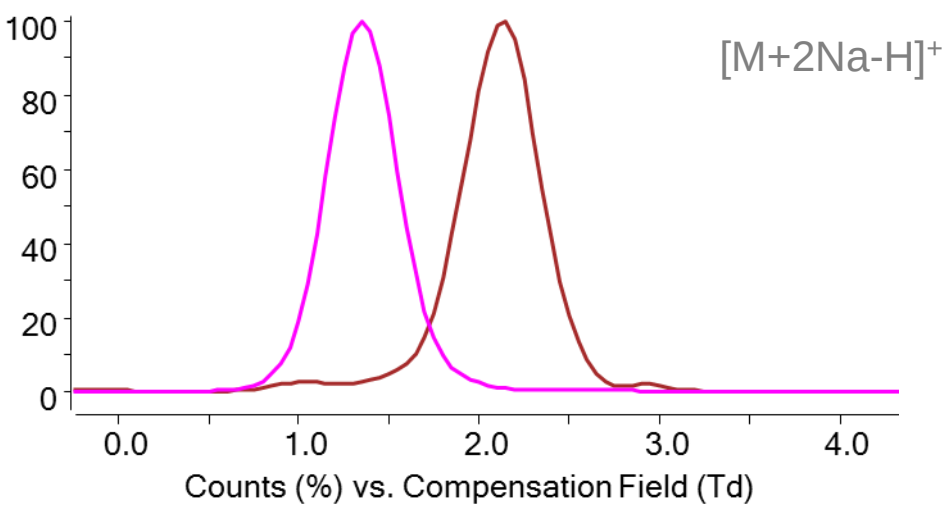
Positive ion mode

Dispersion Field 300 Td



Testosterone glucuronide

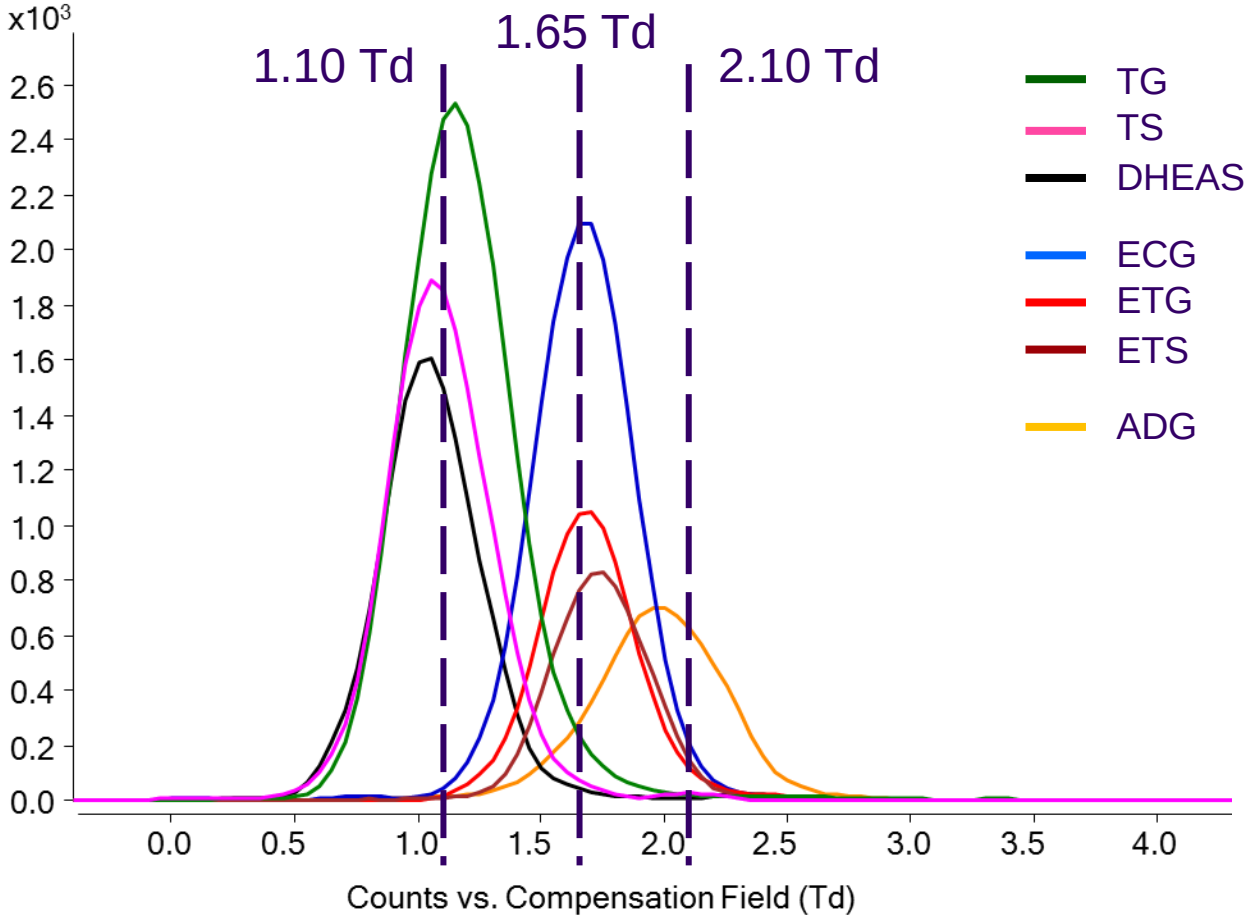
Epitestosterone glucuronide



Testosterone sulfate

Epitestosterone sulfate

FAIMS-MS of steroid metabolites



Dispersion Field 280 Td

FAIMS-MS in ACN:H_2O (LC mobile phase)

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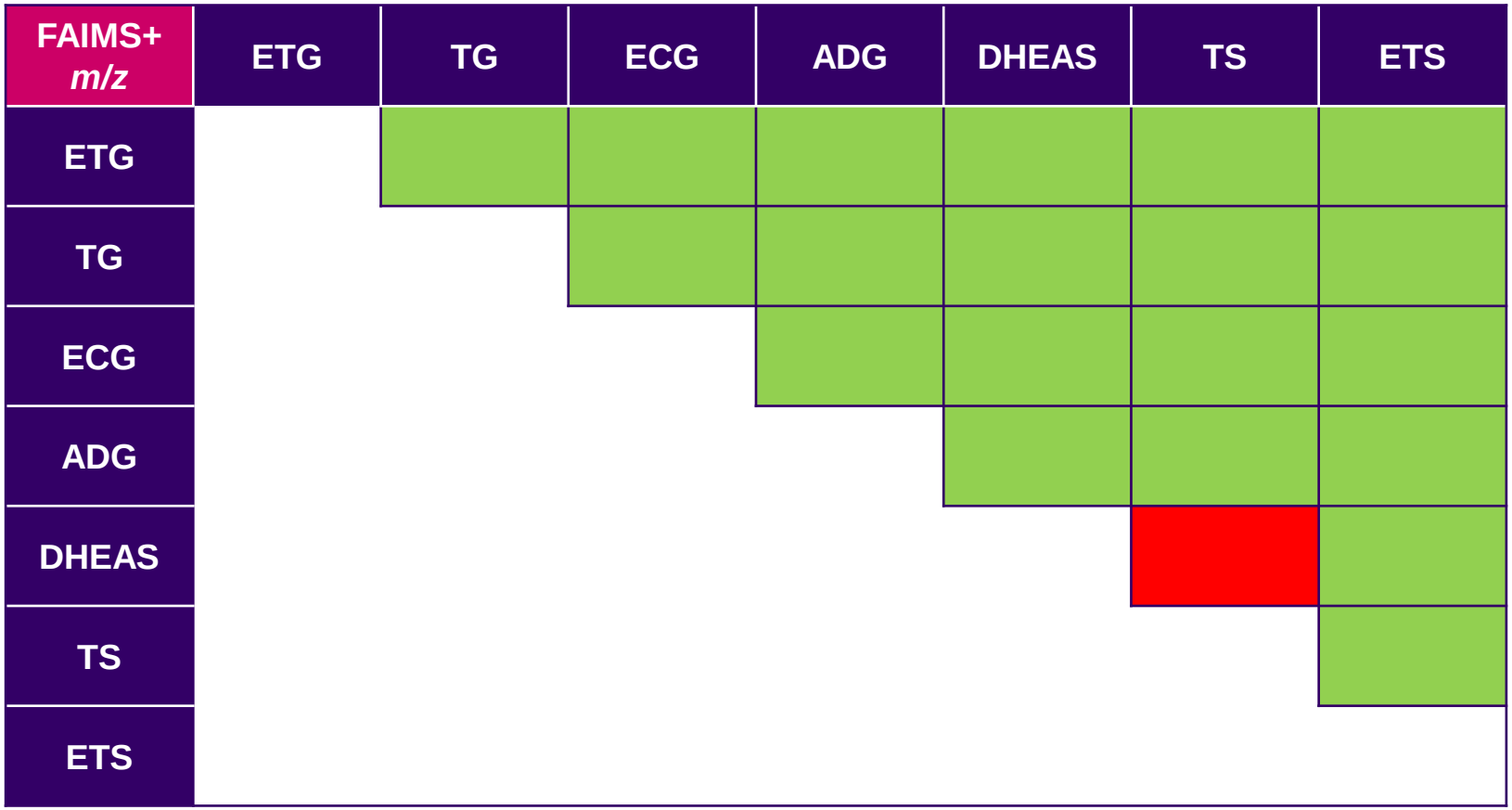
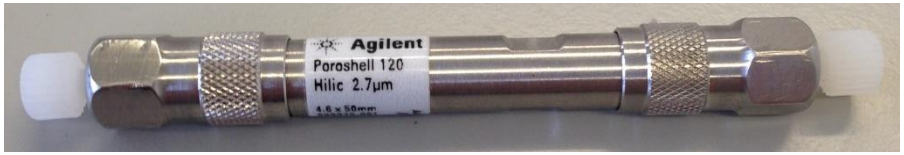
Separation of steroids using FAIMS-MS



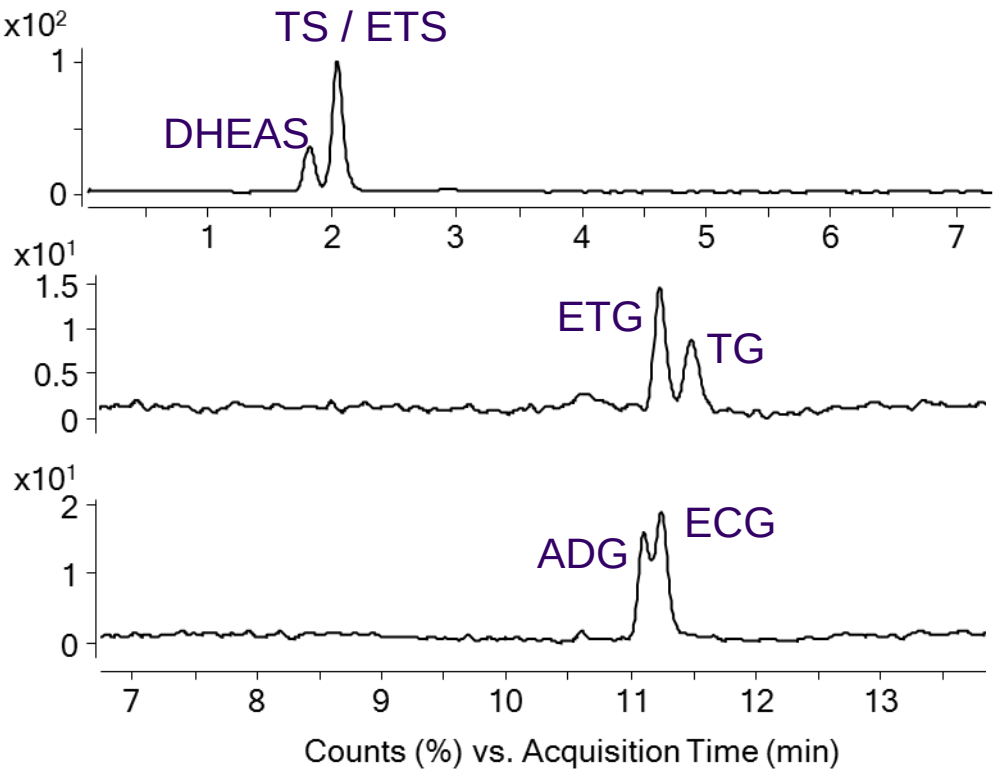
HILIC of steroid metabolites

- DHEAS is not separated from TS by FAIMS or MS, so must be separated chromatographically using LC
- LC method should be a quick and reliable method for the determination of all of the compounds of interest in a urine matrix
- Hydrophilic interaction liquid chromatography (HILIC) was chosen as separation between DHEAS / TS could be achieved on a fast timescale

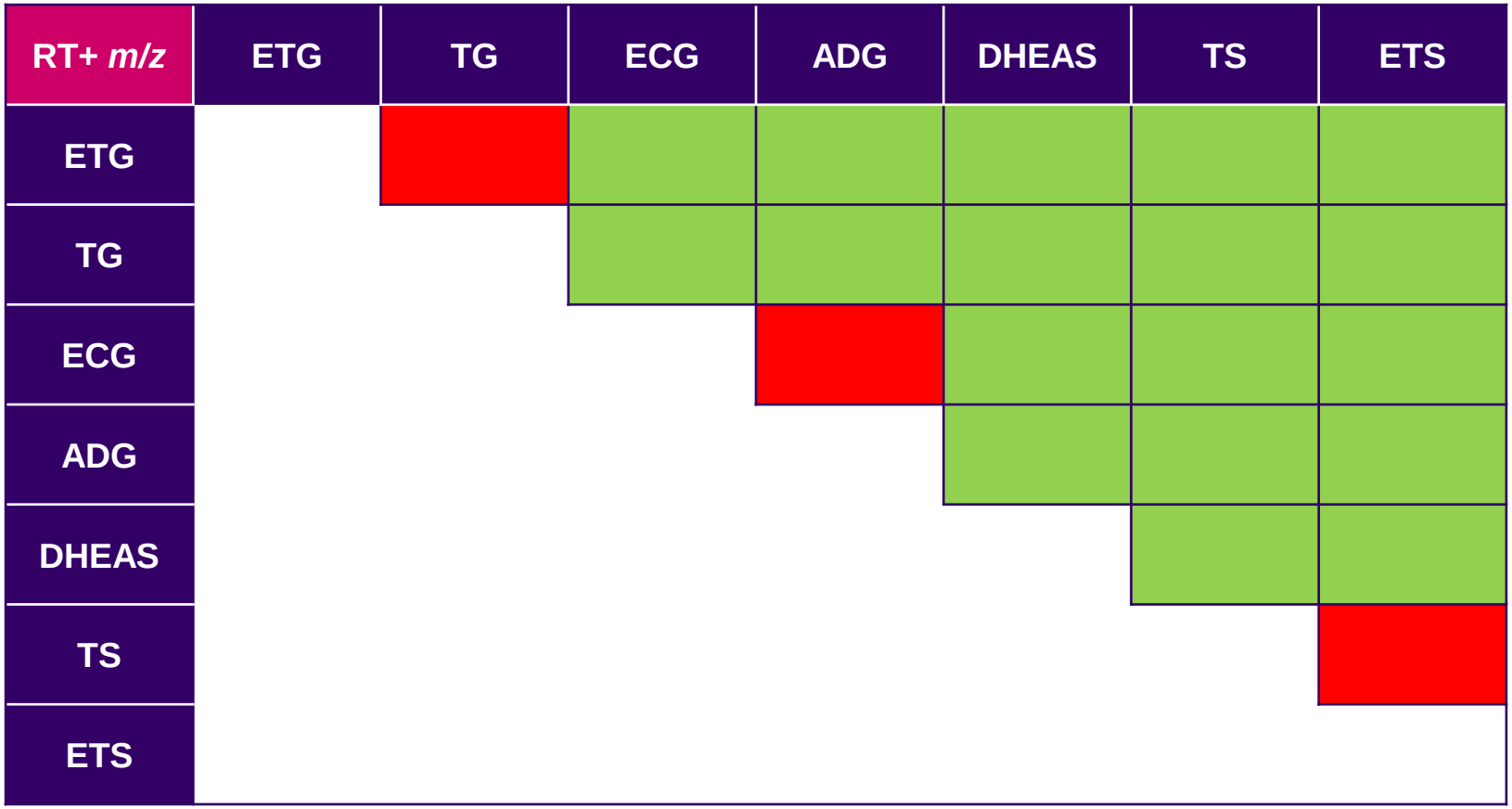


LC-MS of steroids

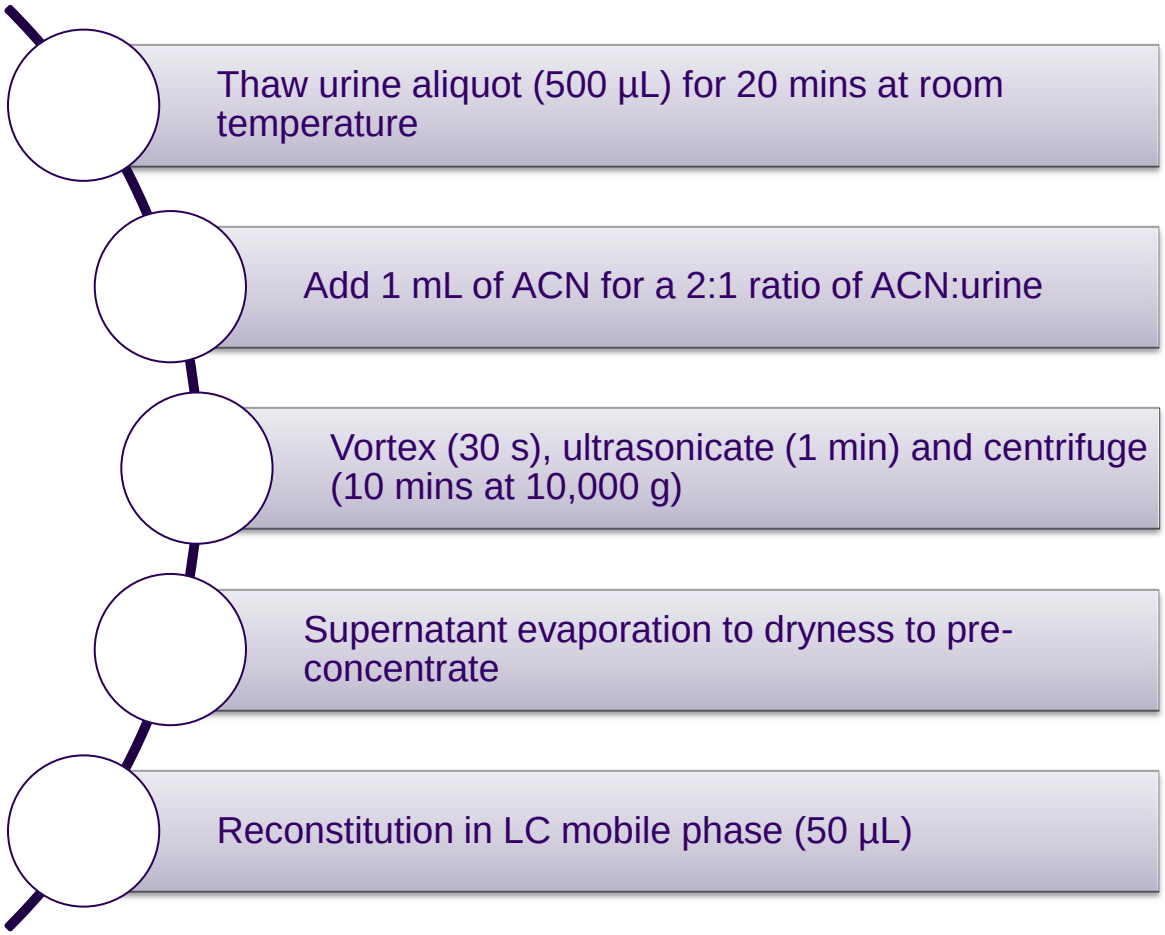
- Selected ion responses for *m/z*:
  - 413.14
  - 509.21
  - 511.23
- No chromatographic separation between TS / ETS
- ETG / TG are not baseline resolved
- Only partial separation between ADG / ECG



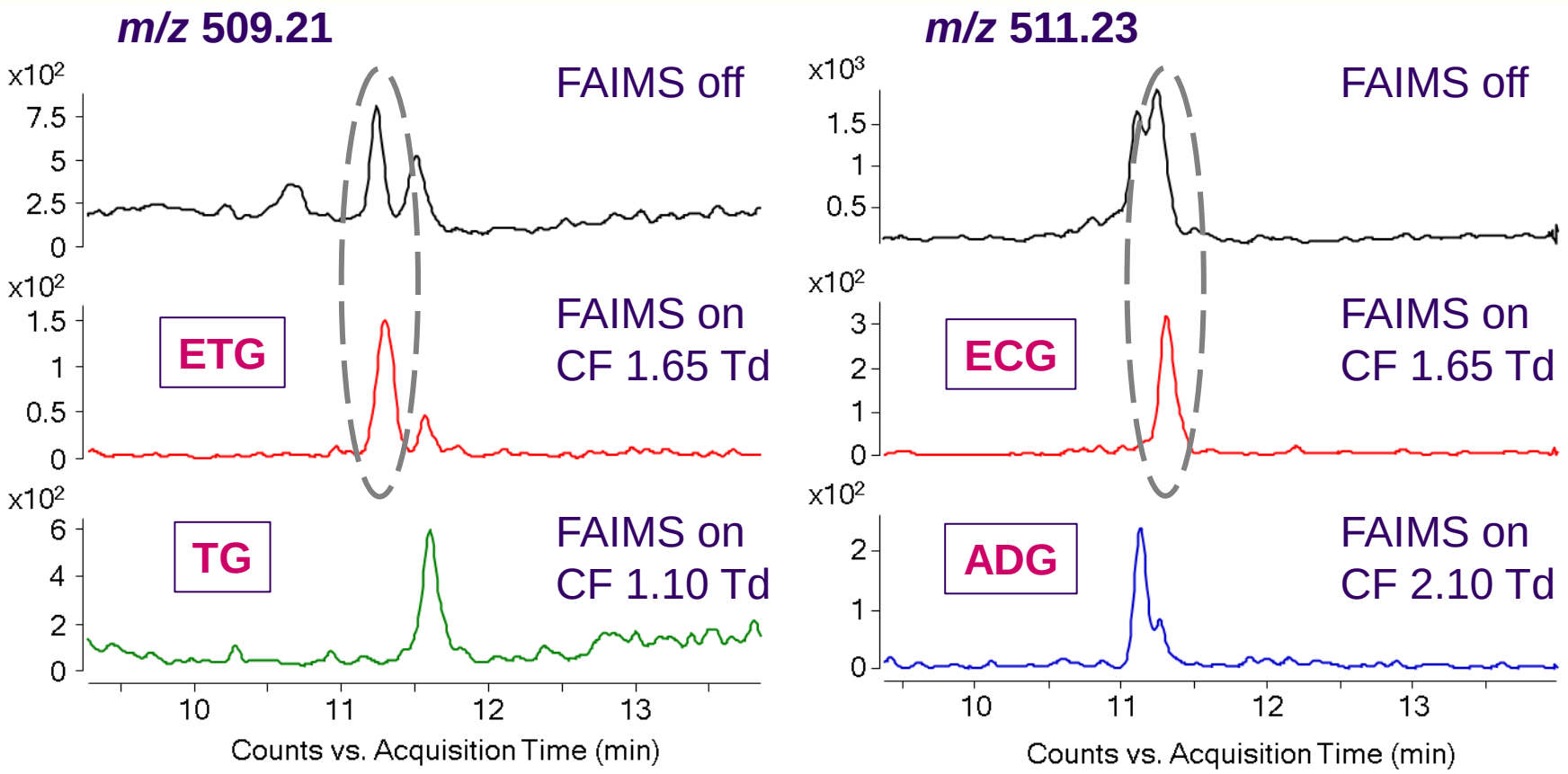
Separation of steroids using LC-MS



Urine sample preparation



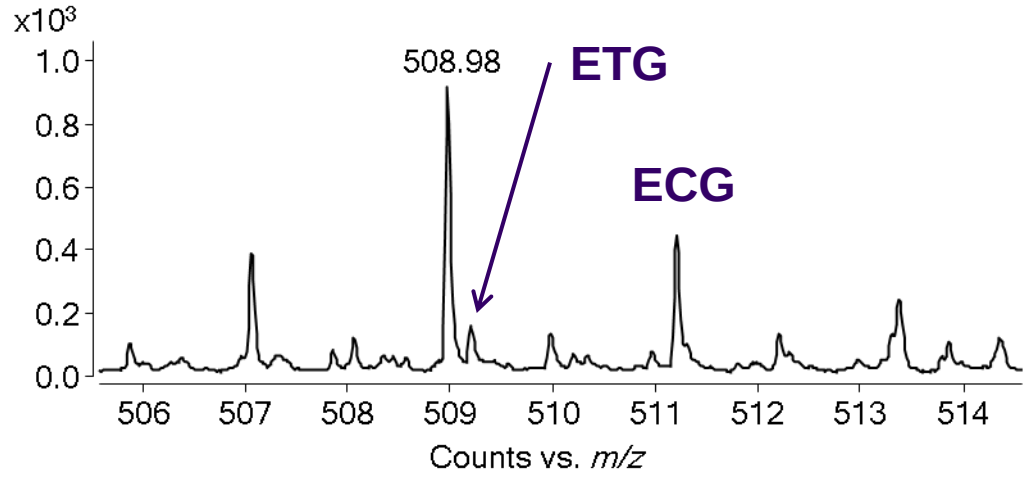
LC-FAIMS-MS of steroids in urine



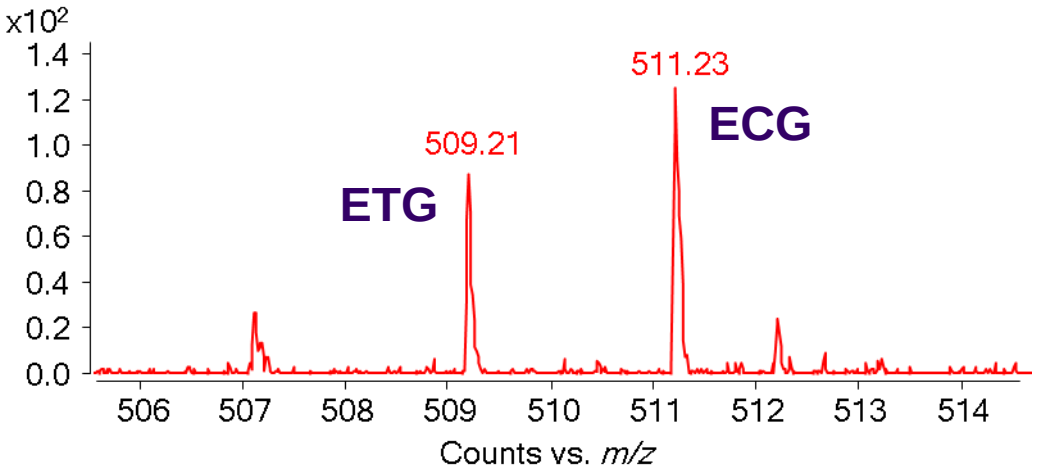
FAIMS on – Dispersion Field 280 Td

LC-FAIMS-MS of steroids in urine

Mass spectra at RT 11.3 mins



FAIMS off



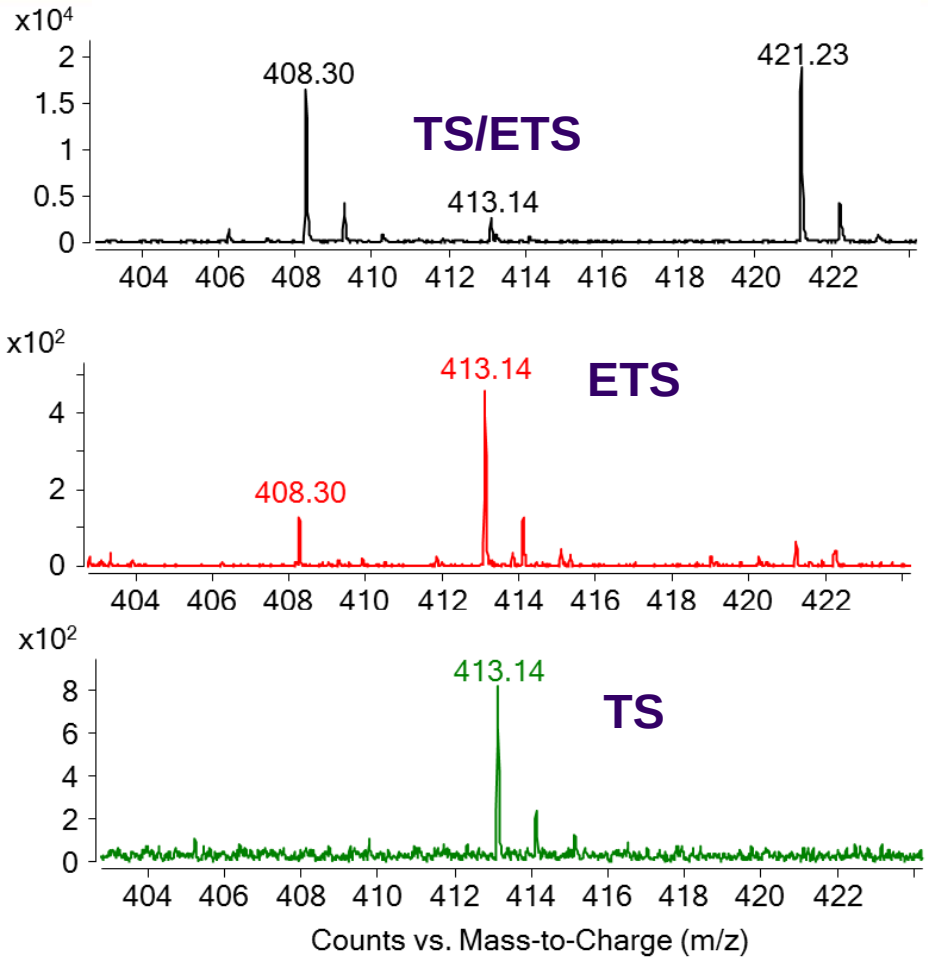
FAIMS on CF 1.65 Td

Dispersion Field 280 Td

LC-FAIMS-MS of steroids in urine

Mass spectra at RT 2.2 mins

Dispersion Field 280 Td



FAIMS off

FAIMS on CF 1.65 Td

FAIMS on CF 1.1 Td

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Separation of steroids using LC-FAIMS-MS

RT+ FAIMS + *m/z*	ETG	TG	ECG	ADG	DHEAS	TS	ETS
ETG							
TG							
ECG							
ADG							
DHEAS							
TS							
ETS							

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Conclusion

- FAIMS-MS separation of steroid sulfates and glucuronides in positive ionisation mode
- Improved FAIMS separation of steroids using sodiated adducts
- Potential to improve quantitative determination of steroids in biological matrices using LC-FAIMS-MS

Further work

- Further optimisation of method parameters
- Develop and optimise urine sample preparation with a pre-concentration step for maximum sensitivity
- Validation of the LC-FAIMS-MS method for urine analysis

Acknowledgements

- **Loughborough University:**
  - Colin Creaser
  - James Reynolds
  - Matthew Turner
  - Richard Gyasie-Hayford

- **Owlstone:**
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  - Lauren Brown
  - Robert Smith
- **Drug Control Centre (KCL):**
  - David Cowan
  - Andrew Kicman
  - Alan Brailsford
- **Staff and researchers at the Centre for Analytical Science**

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