University of Southampton

SOUThAmPtON Chemistry

THE ELEMENTS OF SUCCESS

Ionisation in the absence of high voltage using SFC-MS: a route to greater sensitivity

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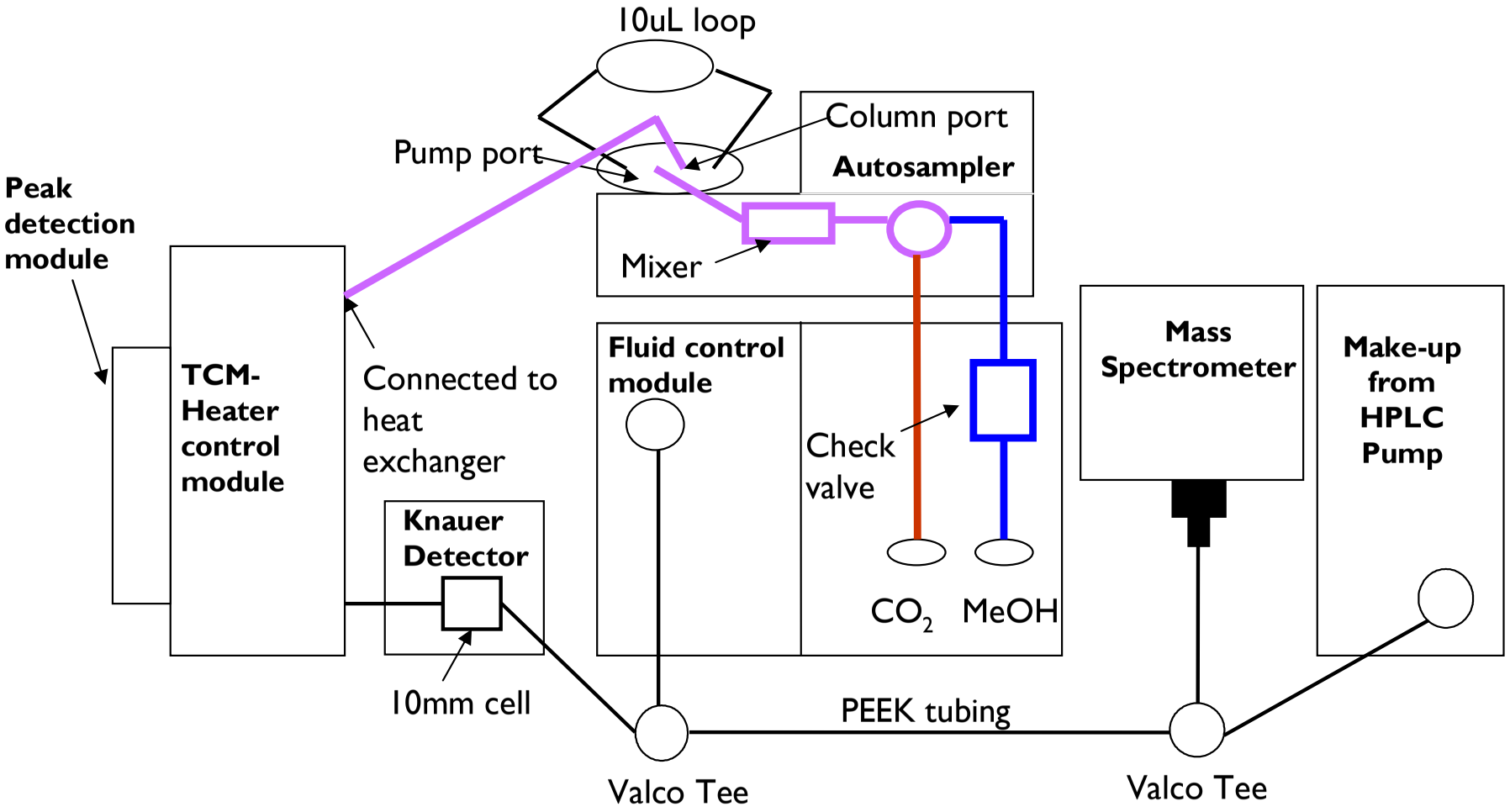
Outline

- What is SFC?
- Coupling SFC-MS
- Experimental Design
- Detection
  - ESI-APCI
  - SFC conditions
    - Pressure
    - Modifier
    - Flow rate

What is SFC?

- Supercritical fluid chromatography
  - Mobile phase in supercritical state
- Mobile phase: non polar carbon dioxide
- Modifier: polar organic solvent to improve chromatography
- Polar stationary phases
  - e.g. silica, amine, 2-EP
- Considered as normal phase technique

SFC-MS Coupling



Ref: M. Garzotti, M. Hamdan, J. Chromatogr B,770 (2002) 53-61.

MS Detection

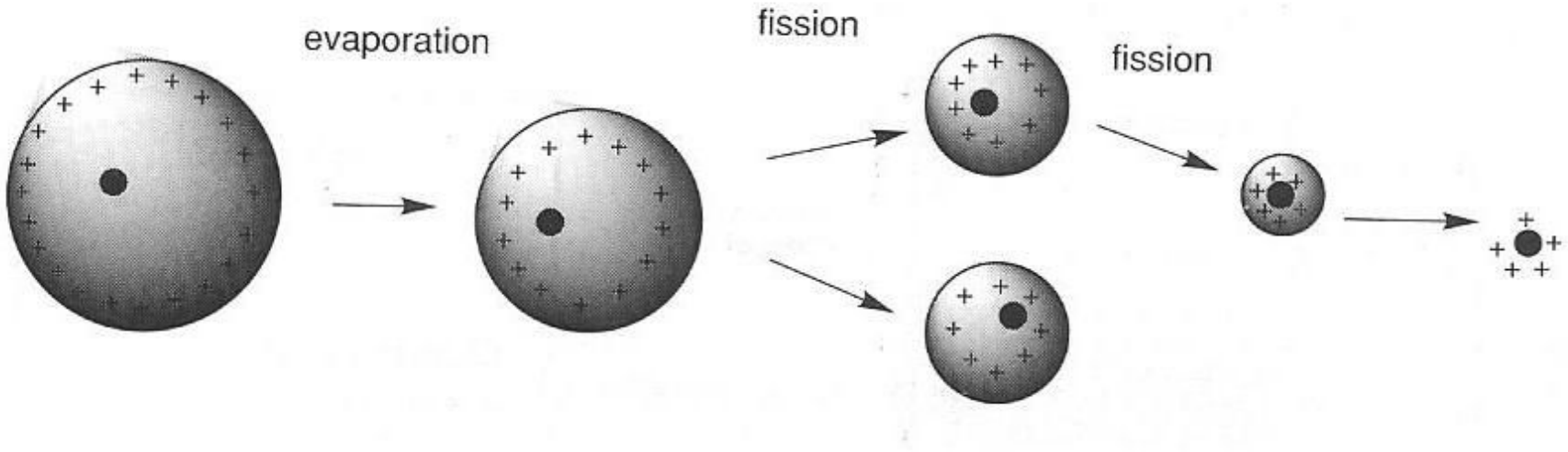
- Initial studies utilised ESI
- Optimisation of API for SFC
- Observation of samples ionising in the absence of high voltages
- Specific test compounds analysed to probe this ionisation mechanism

What is causing ionisation?

- API source configuration dependent?
  - ESI *cf.* APCI
- Chromatographic system specific?
  - HPLC *cf.* SFC

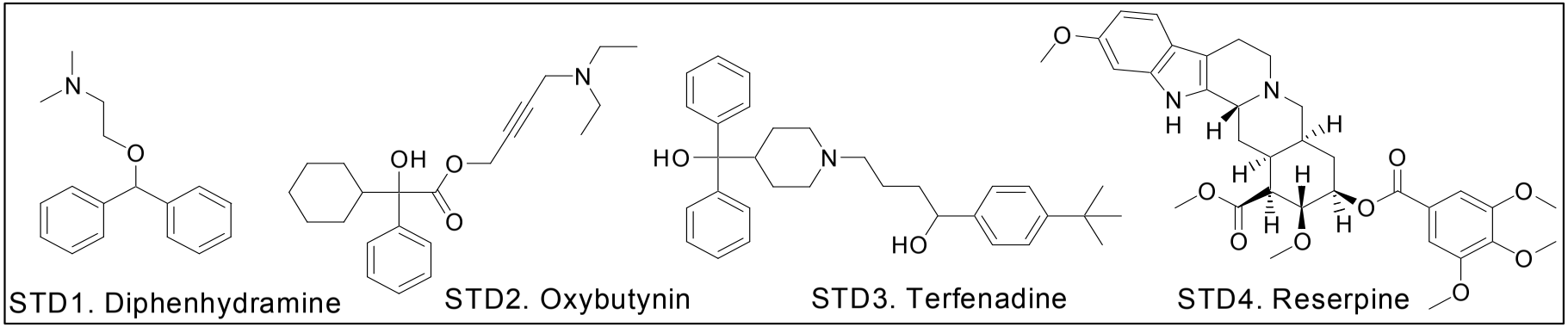
Is ionisation related to?

- Charge residual model?
  - Additives for ionisation
  - % modifier changed
  - Nebuliser gas



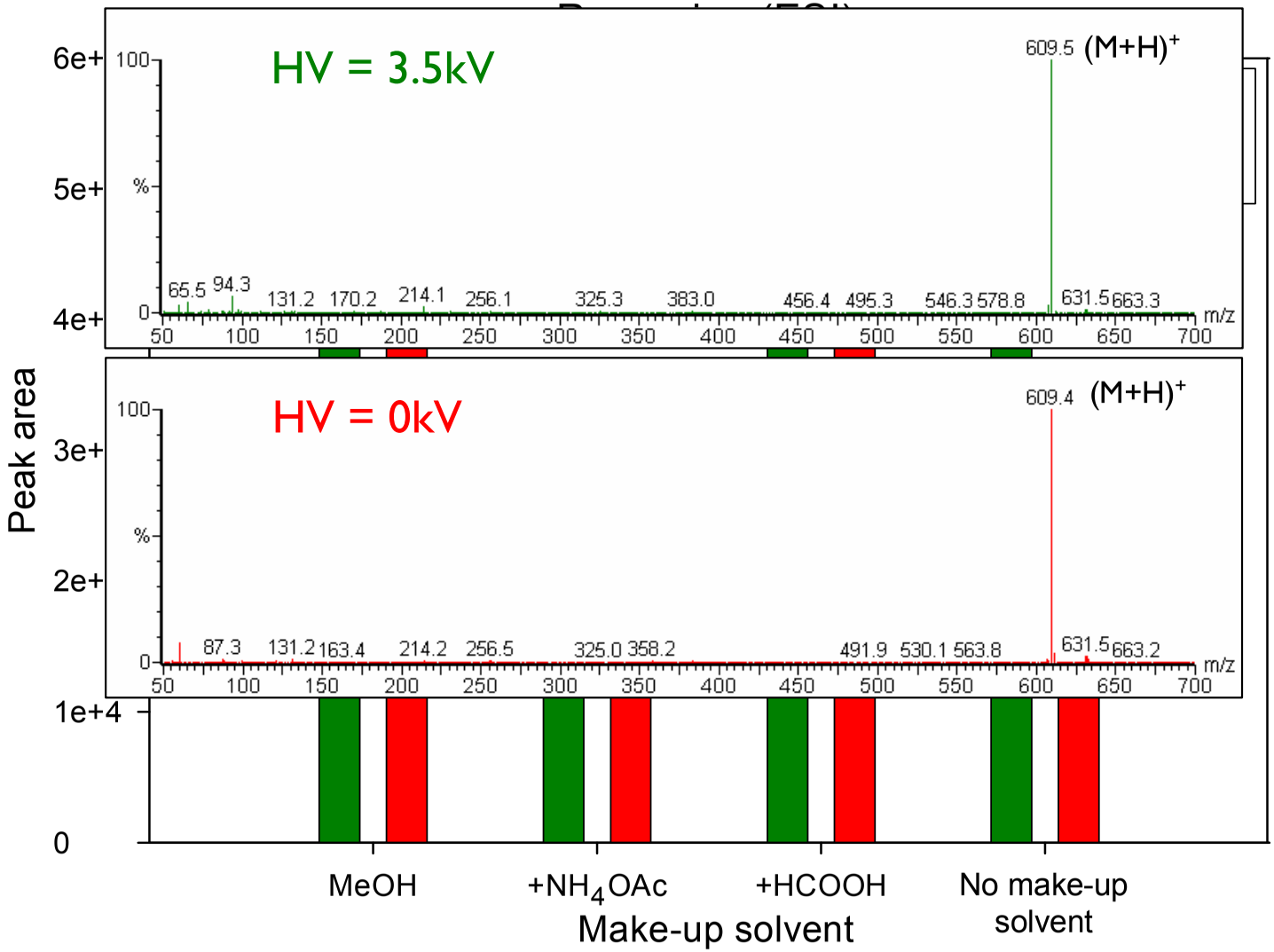
Ref: C. Dass, Principles and Practice of Biological Mass Spectrometry, John Wiley & Sons, Inc., New York, 2001.

Experimental conditions

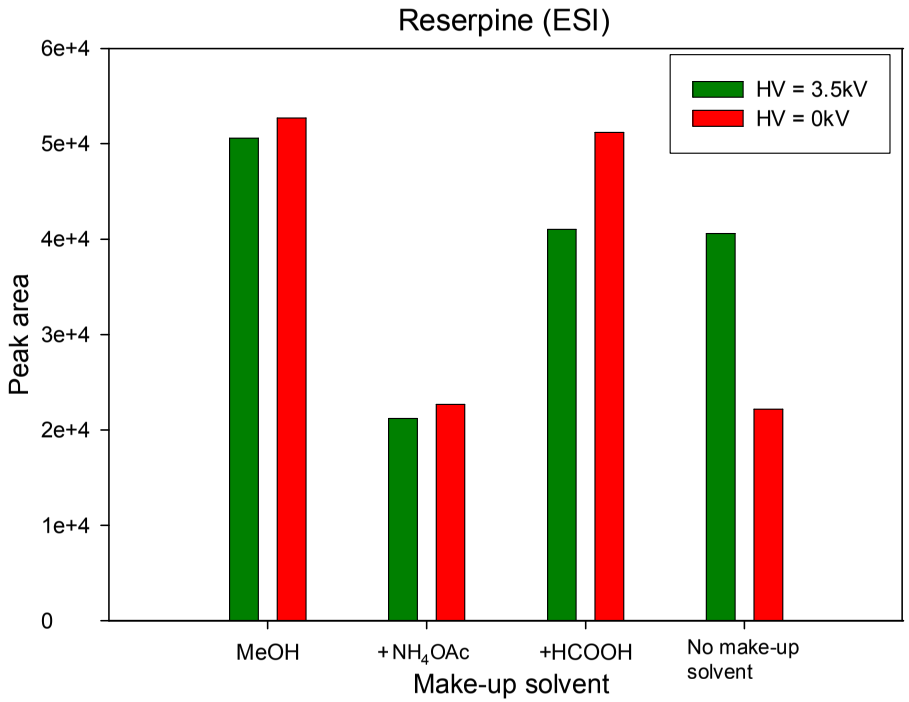


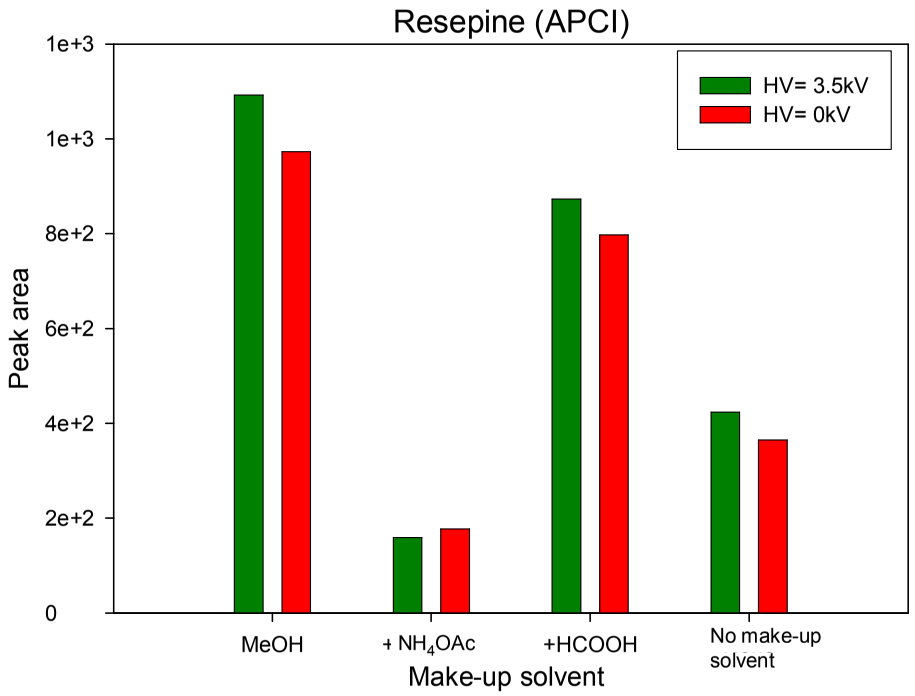
	1	2	3	4
Pure methanol	HV: 3.5kV CV: 20V	HV: 3.5kV CV: 0V	HV: 0kV CV: 20V	HV: 0kV CV: 0V
Methanol + 0.1% NH_4OAc	HV: 3.5kV CV: 20V	HV: 3.5kV CV: 0V	HV: 0kV CV: 20V	HV: 0kV CV: 0V
Methanol + 0.1% HCOOH	HV: 3.5kV CV: 20V	HV: 3.5kV CV: 0V	HV: 0kV CV: 20V	HV: 0kV CV: 0V
No make-up	HV: 3.5kV CV: 20V	HV: 3.5kV CV: 0V	HV: 0kV CV: 20V	HV: 0kV CV: 0V

ESI reserpine HV on/off



ESI and APCI

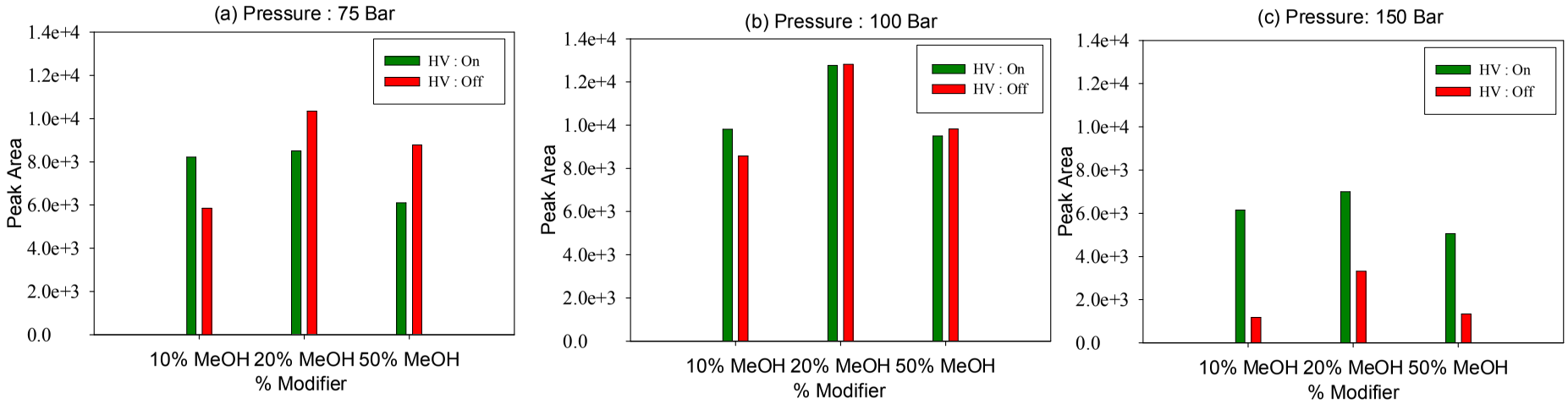

Reserpine (ESI)
6e+4
5e+4
4e+4
3e+4
2e+4
1e+4
0
Peak area
HV = 3.5kV
HV = 0kV
MeOH
+NH4OAc
+HCOOH
No make-up solvent
Make-up solvent


Resepine (APCI)
1e+3
1e+3
8e+2
6e+2
4e+2
2e+2
0
Peak area
HV= 3.5kV
HV= 0kV
MeOH
+ NH4OAc
+HCOOH
No make-up solvent
Make-up solvent

Summary of ESI and APCI

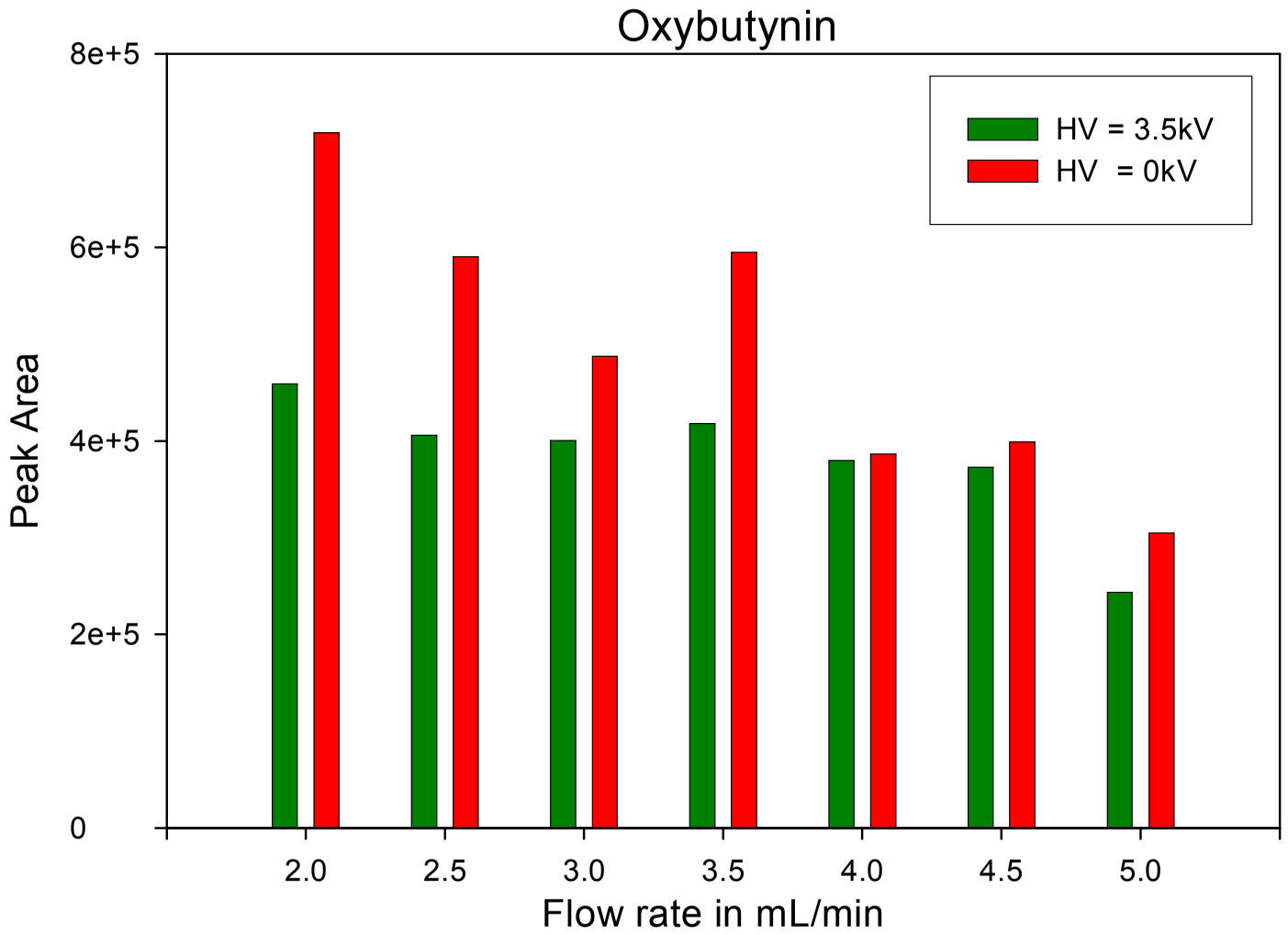
- Comparable/Improved ionisation in absence of high voltage
- Ionisation suppressed with addition of ammonium acetate
- Formic acid does not enhance ionisation
- Data for APCI source comparable with ESI source
- Ionisation independent of capillary type

Pressure and modifier

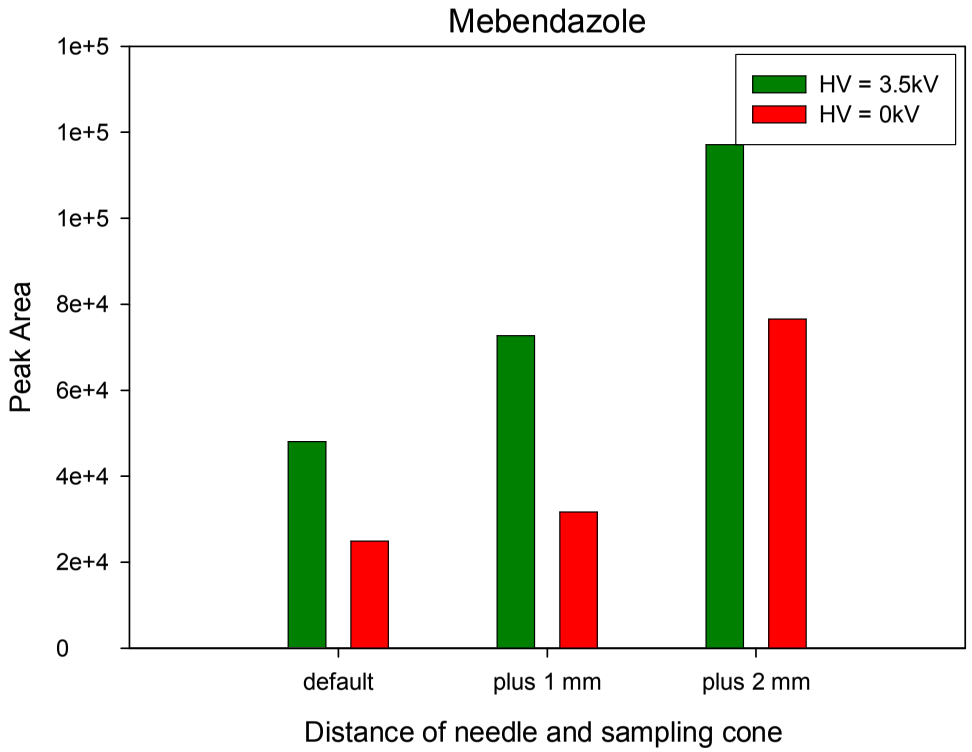


- Mixture of terfenadine, oxybutynin and erythromycin analysed at varying pressure and modifier percentage
- Optimum conditions for maximum sensitivity in presence or absence of high voltage:
  Pressure 100bar and modifier 30% MeOH

Flow rate

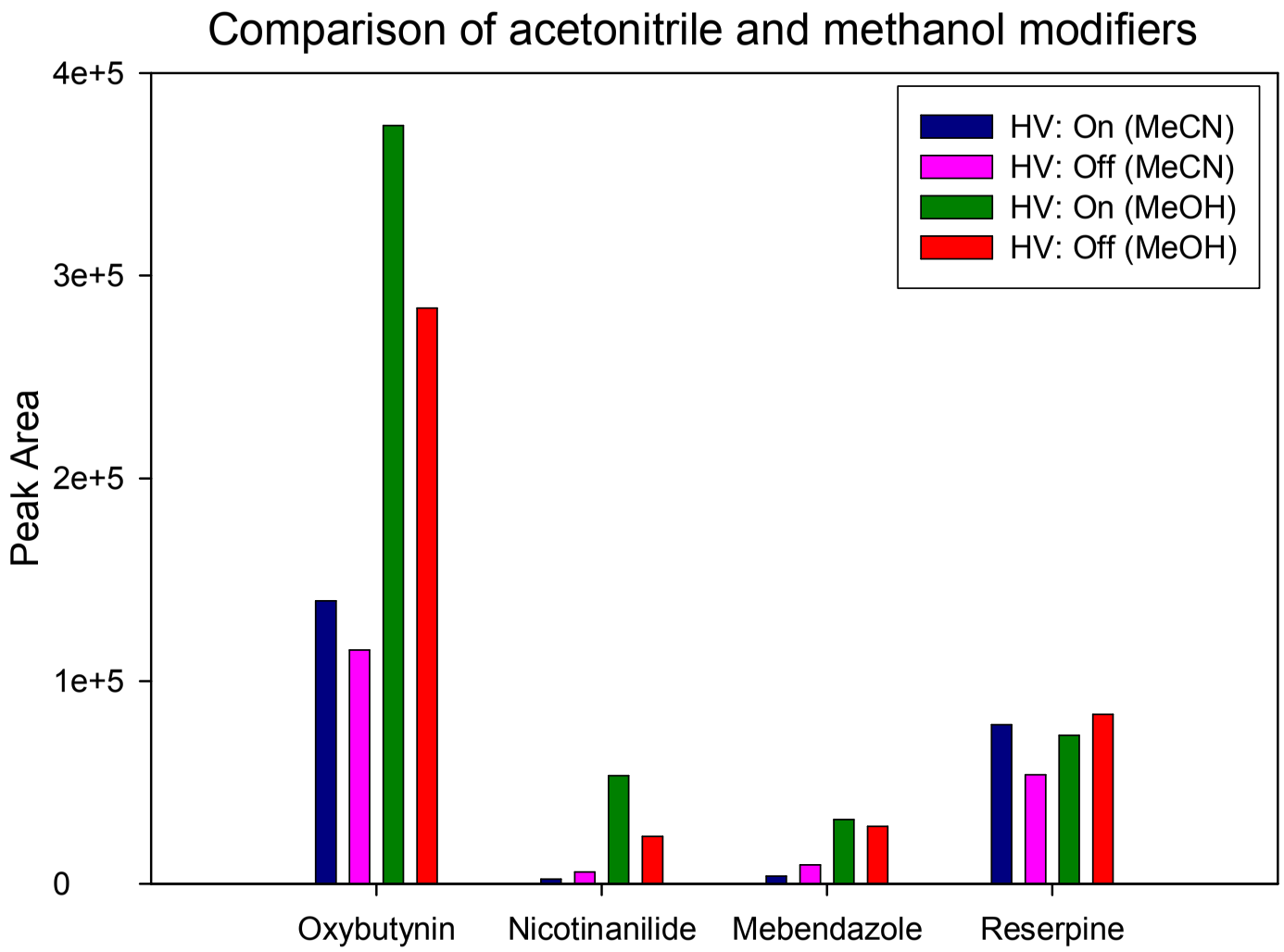

Oxybutynin
HV = 3.5kV
HV = 0kV
Peak Area
8e+5
6e+5
4e+5
2e+5
0
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Flow rate in mL/min

High flow rate (5mL/min)

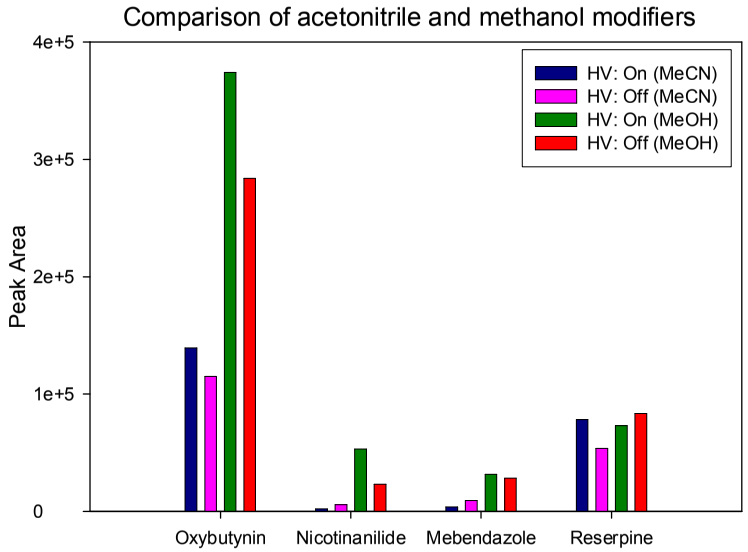


- Improvement in sensitivity upon increasing the distance between the ESI needle tip and the sample cone

Modifier effect

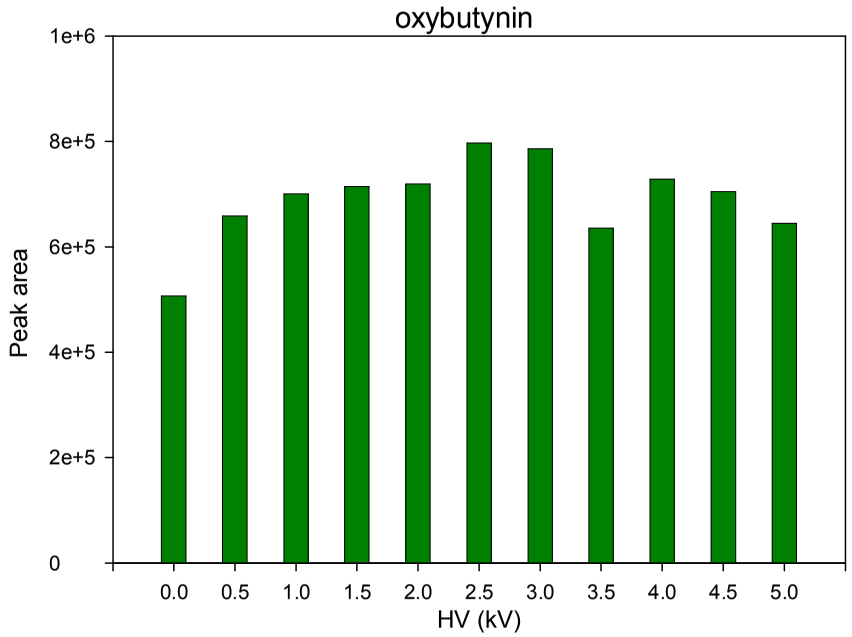


Modifier effect



- Increased ionisation with MeOH modifier compared to MeCN
- Is the ionisation efficiency related to preformed ions in solution?

High voltage profile



- Not electrospray
- CRM - Ions formed in solution or during spray process
- CRM plus other processes, sonic spray

Conclusions

- Compounds ionise in the absence of high voltage
  ∴ not electrospray ionisation

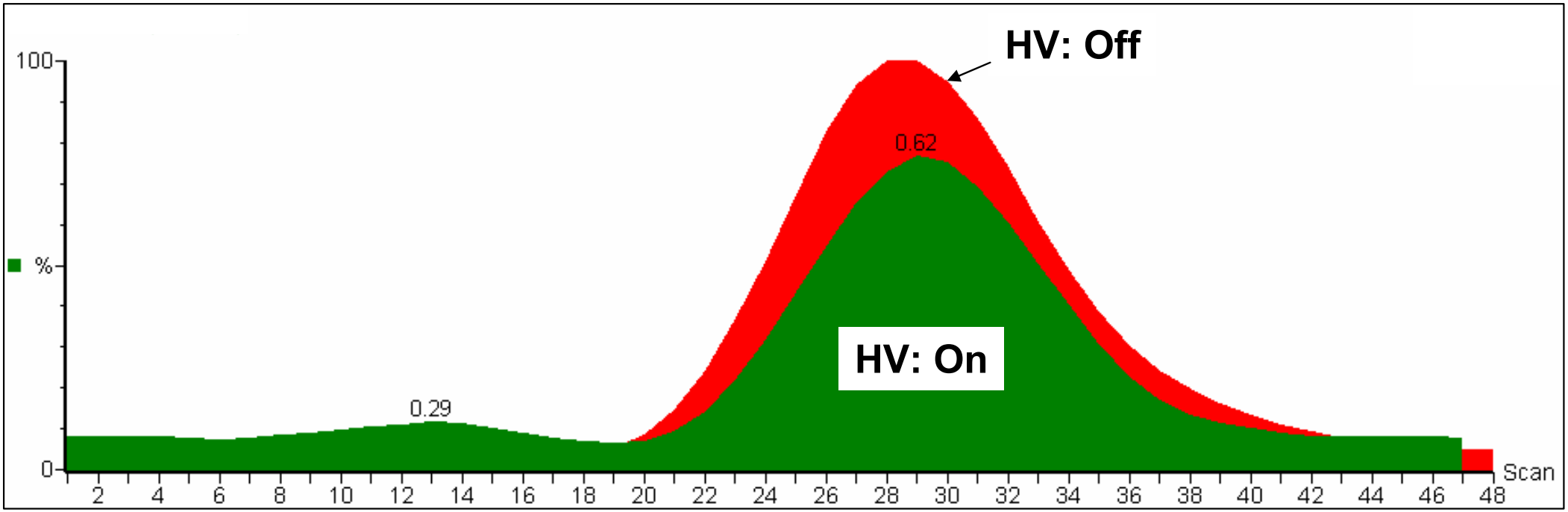
- Addition of ammonium acetate does not aid ionisation ∴ not thermospray ionisation

- Suggests ions are formed in solution or during the supersonic jet expansion

Conclusions

- MeCN as make-up solvent or as modifier does not assist in ionisation, unlike MeOH ∴ supporting CRM?
- Ionisation on FIA-ESI-MS with HV turned off but not as good as SFC-MS
- API-MS response dependent on modifier concentration; this should be taken into account for gradient elution studies

Conclusions



For small molecule analysis if sensitivity is an issue : Turning off the electrospray high voltage could improve LOD - e.g. oxybutynin, reserpine data

Acknowledgements

G. John Langley
Julie Herniman
Amaury Cazenave Gassiot
School of Chemistry, University of Southampton, SO17 1BJ, UK

Laure Hitzel
Frank Pullen
Pfizer Global Research and Development, Sandwich, CT13 9NJ, UK

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Thank You