



Purdue-UAB Botanicals Center for Age-Related Diseases

Nanoelectrospray Ionization and High Sensitivity Analysis

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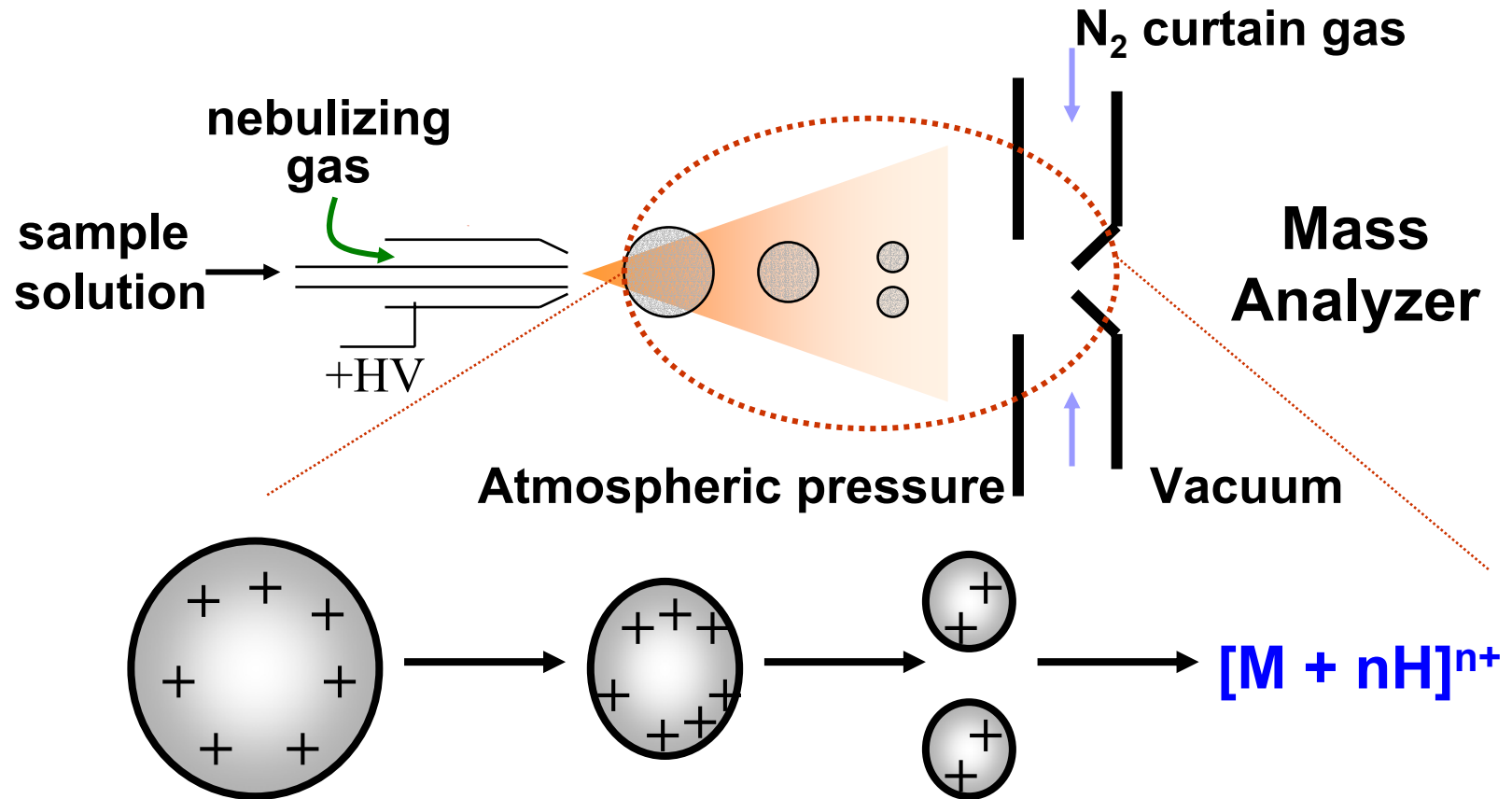
Purdue-UAB Botanicals Center Workshop 2002
Mass Spectrometry Methods in Botanicals Research



Outline

- **Concentration rules in ESI**
- **Micro-nano flow ESI**
- **Development of microESI-MS method**
- **Application to polyphenols**

Electrospray Ionization (ESI)



1. Solvent evaporation
2. Coulombic repulsion



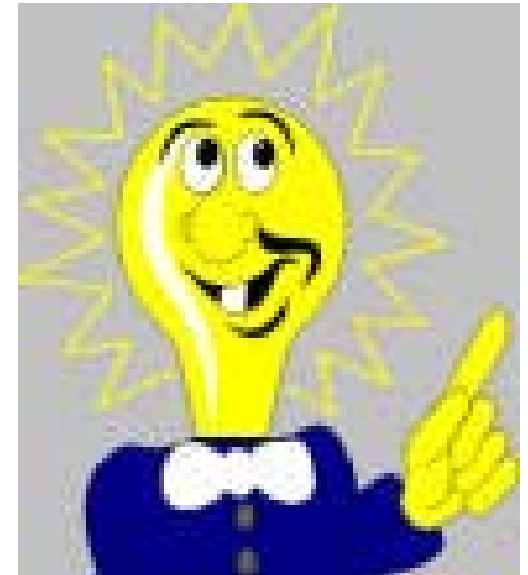
Properties of Electrospray

- **10 ~ 1000 $\mu\text{l}/\text{min}$ flow rate
with pneumatically assisted interface**
- **Concentration-sensitive type detection
- flow rate affects peak width and area,
but intensity**
- **$10^{-5} \sim 10^{-7}\text{M}$**
- **Picomole (10^{-12}mol) detection limit
- 10 ~ 20 μl for LC-MS analysis**



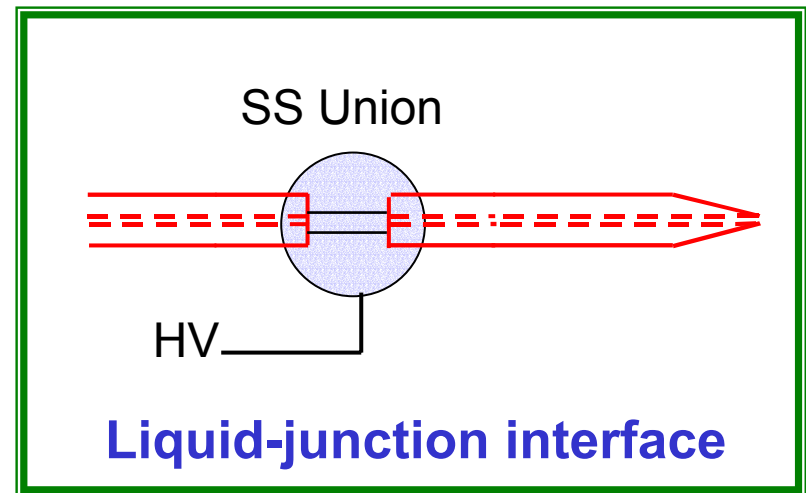
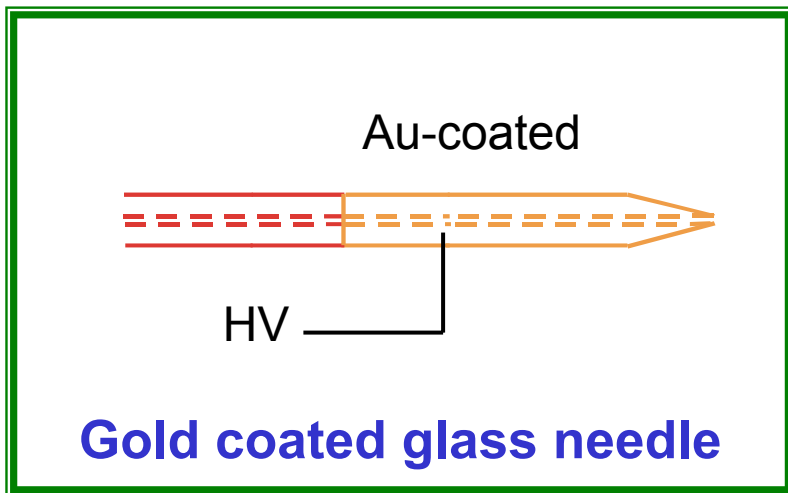
What about small volume and low amount samples ??

Scale Down !!

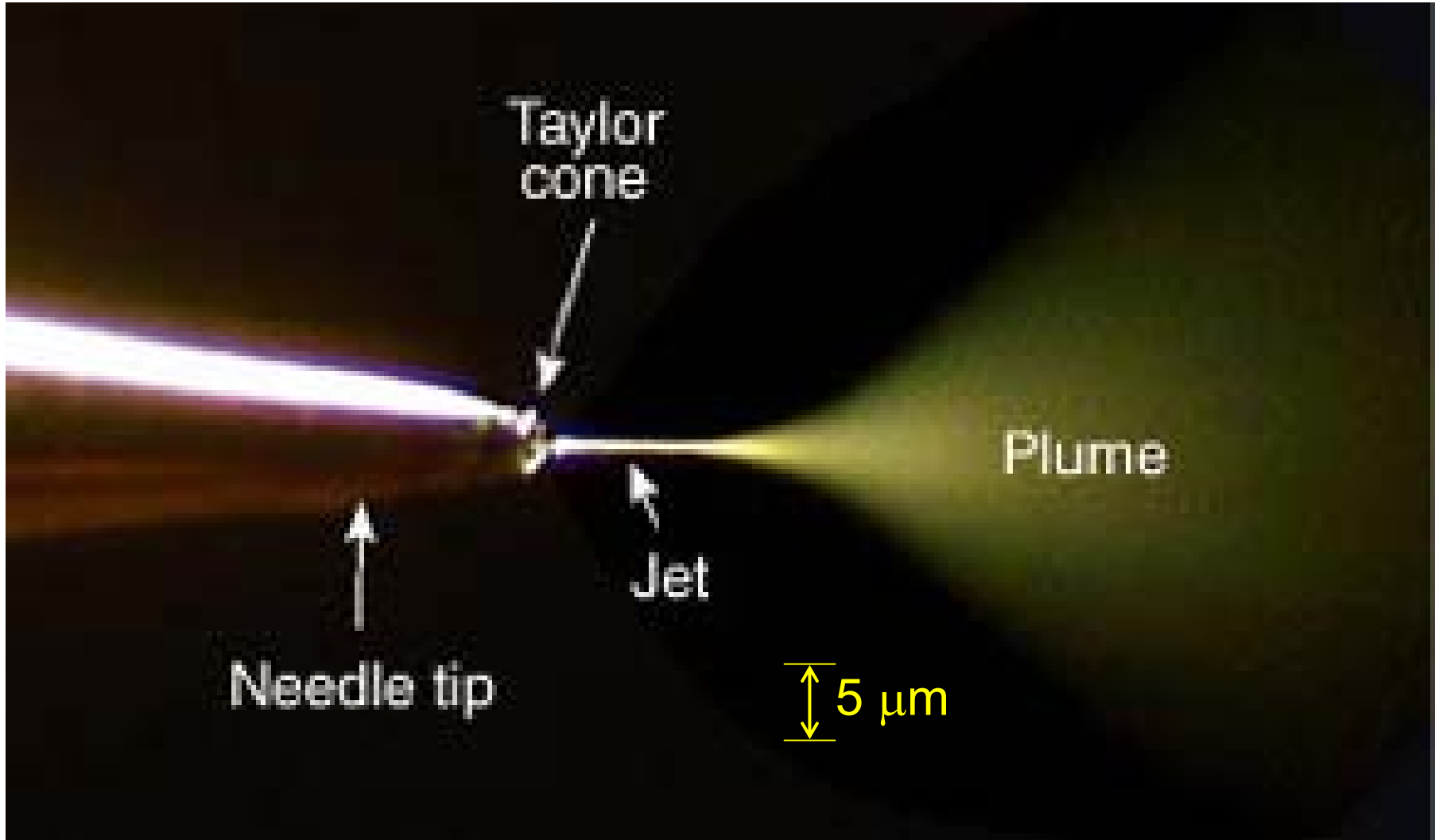


Micro- & Nano- Electrospray

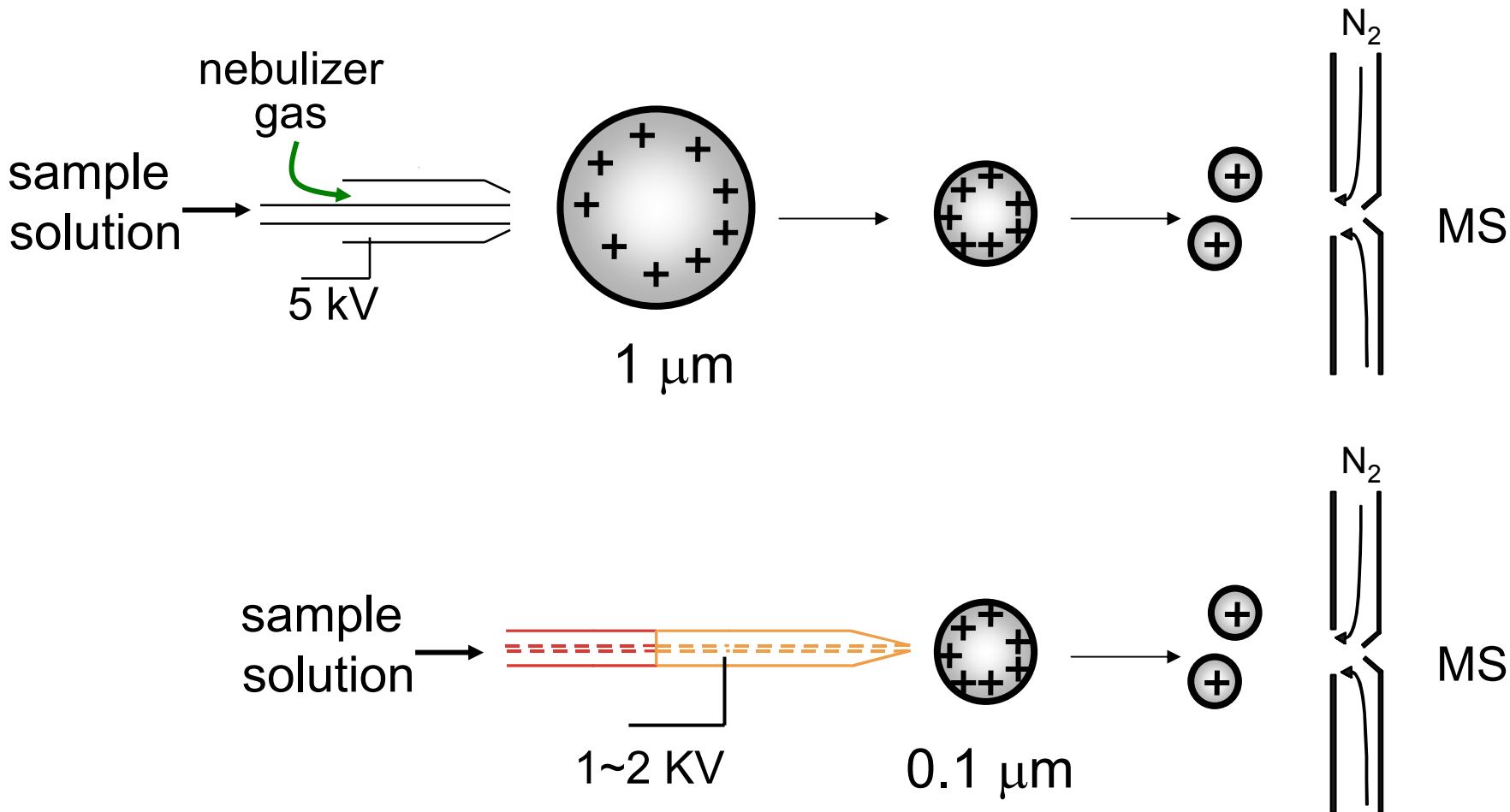
- **Spraying tips (2~50 μm)**
- **Flow Rate: 1000 ~ 10 nL/min**
- **Electrical contact (1~2 kV)**



NanoElectrospray



Comparison of ESI and Nano-ESI - Ionization Efficiency -

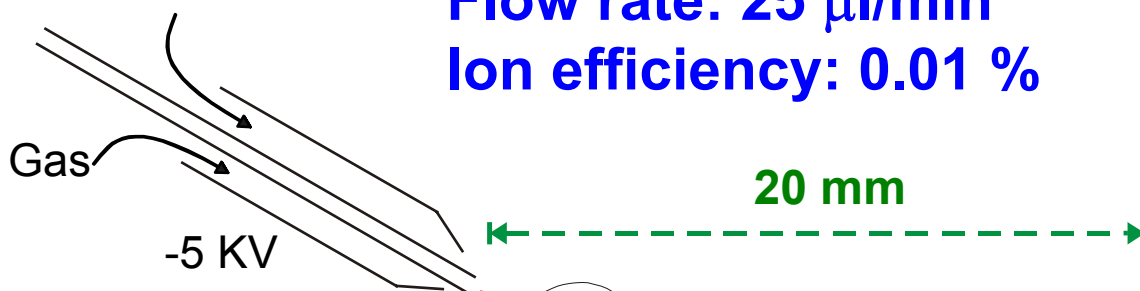


Comparison of ESI and Nano-ESI - Ion Transfer Efficiency -

Electrospray

Flow rate: 25 $\mu\text{l}/\text{min}$

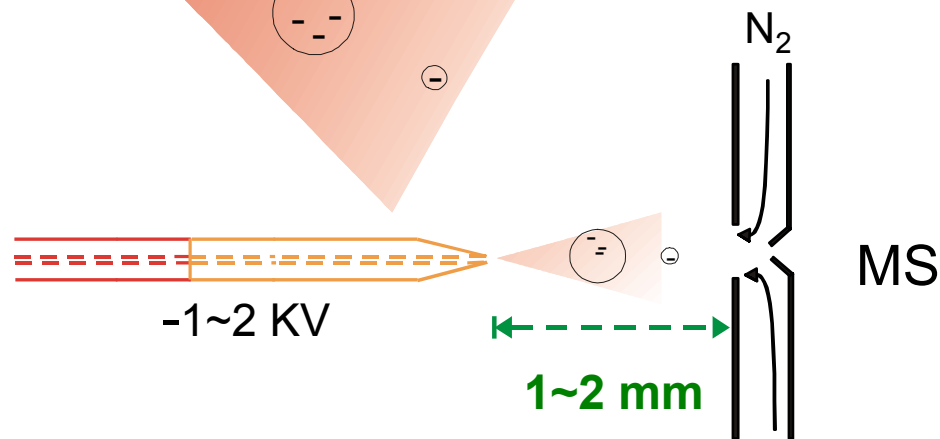
Ion efficiency: 0.01 %



Nano-Electrospray

Flow rate: < 500 nl/min

Ion efficiency: 50~60%

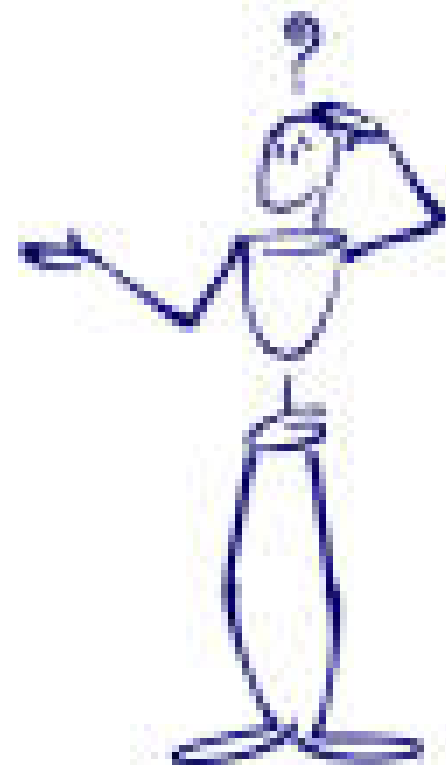




Advantages of NanoElectrospray

- **sensitivity (femto - attomole level)**
- **tolerance toward buffer composition**
-- **aq. and salt concentration (10 mM NaCl)**
- **longer analysis time (for MS-MS)**
- **more compatible with high efficiency separation techniques (nano LC & CE)**

- **How about biological samples with 150 mM salt ?**
- **Many, many of them ??**

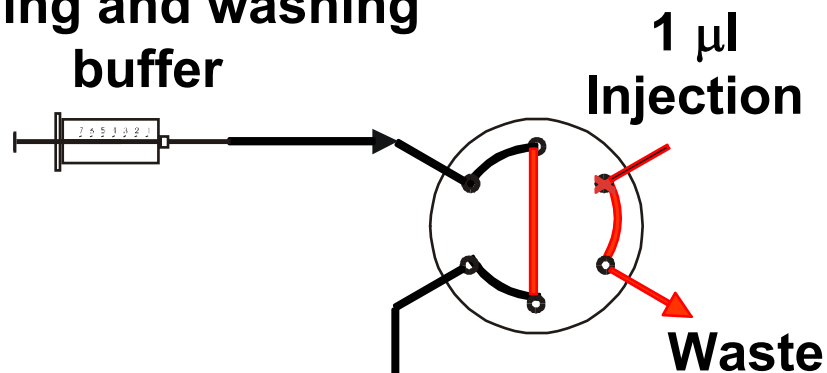


Microscale method

- **on-line sample cleanup**
- **microESI-MS !!**

Microscale System

Loading and washing
buffer



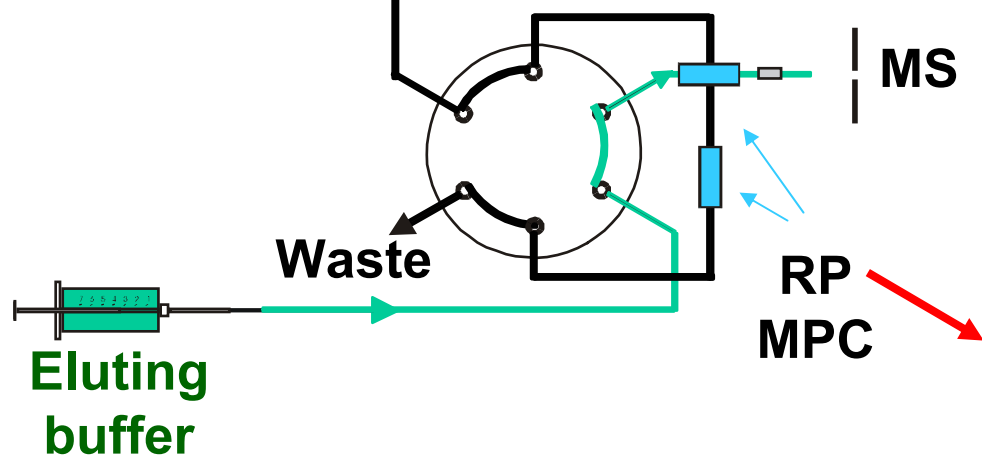
< 1000 nl/min



N₂

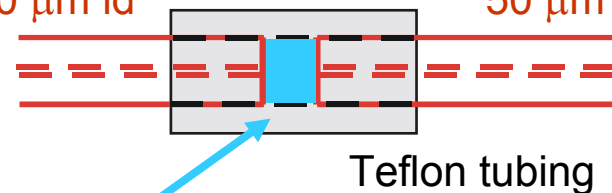
MS

MicroES Interface



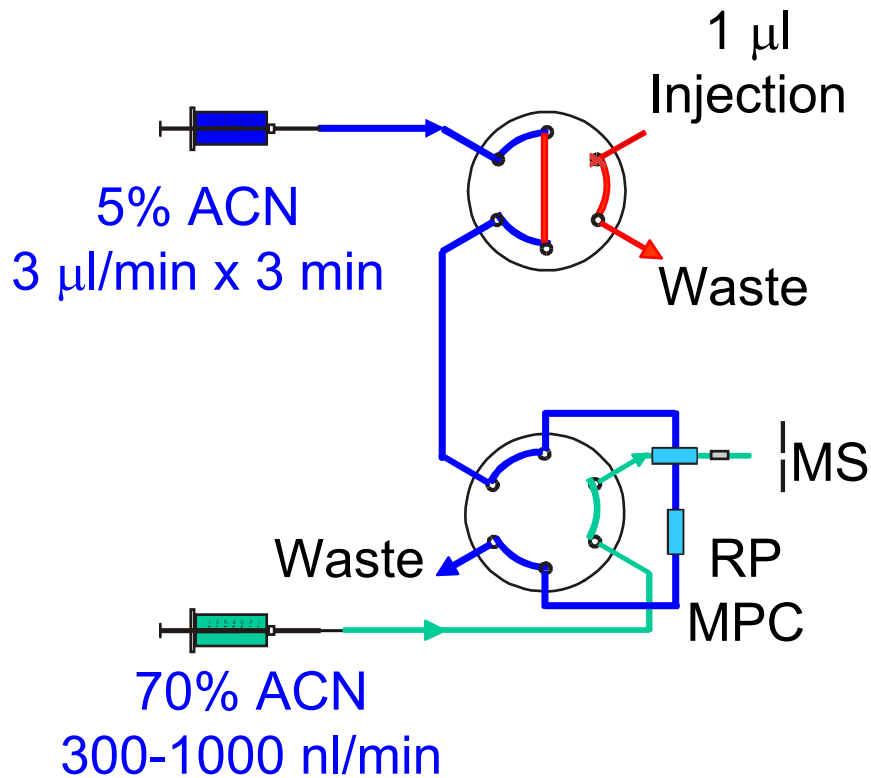
FS Capillary
50 μ m id

FS Capillary
50 μ m id

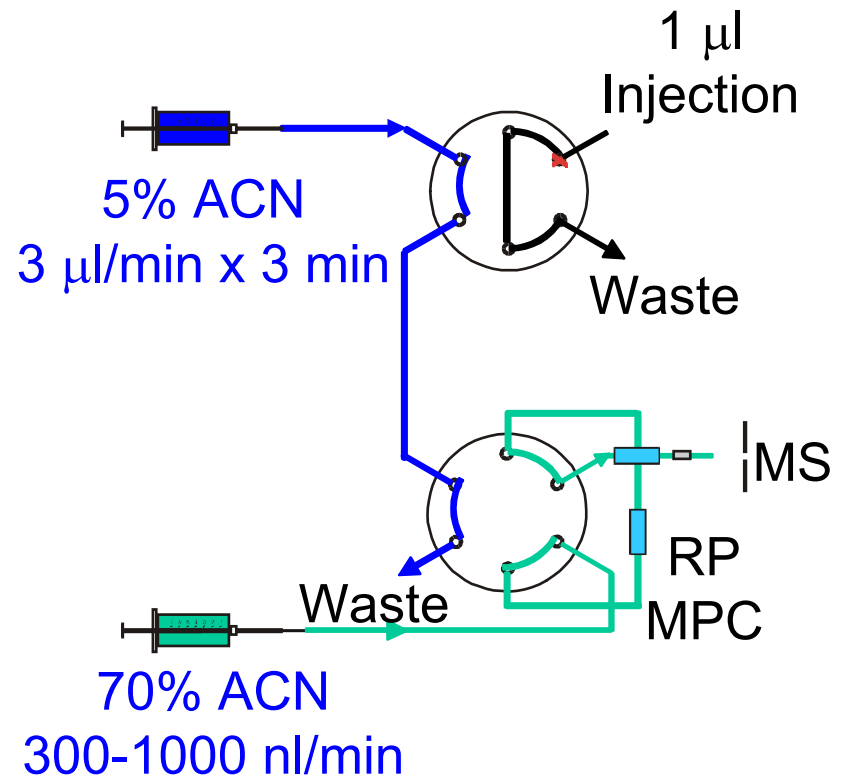


Micro Preconcentrator

Operation of Microscale System



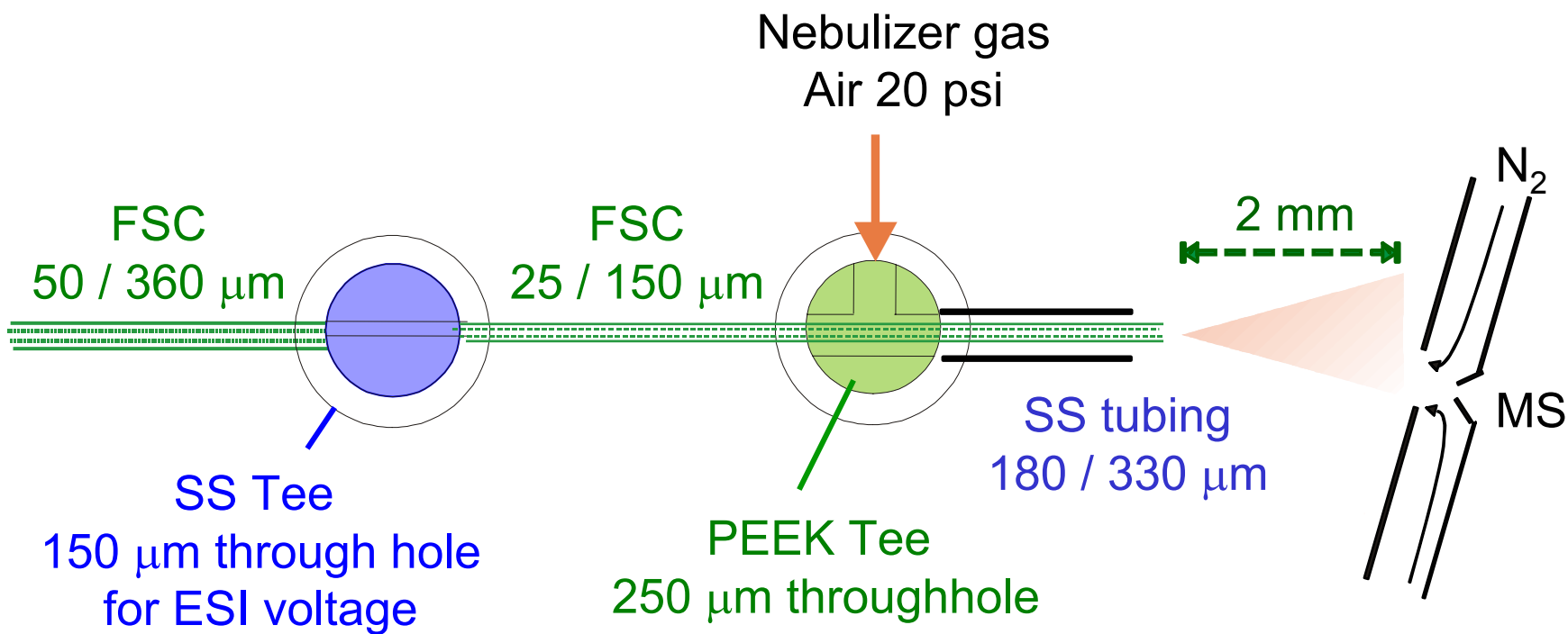
**Sample
Loading & Washing**



Sample Elution



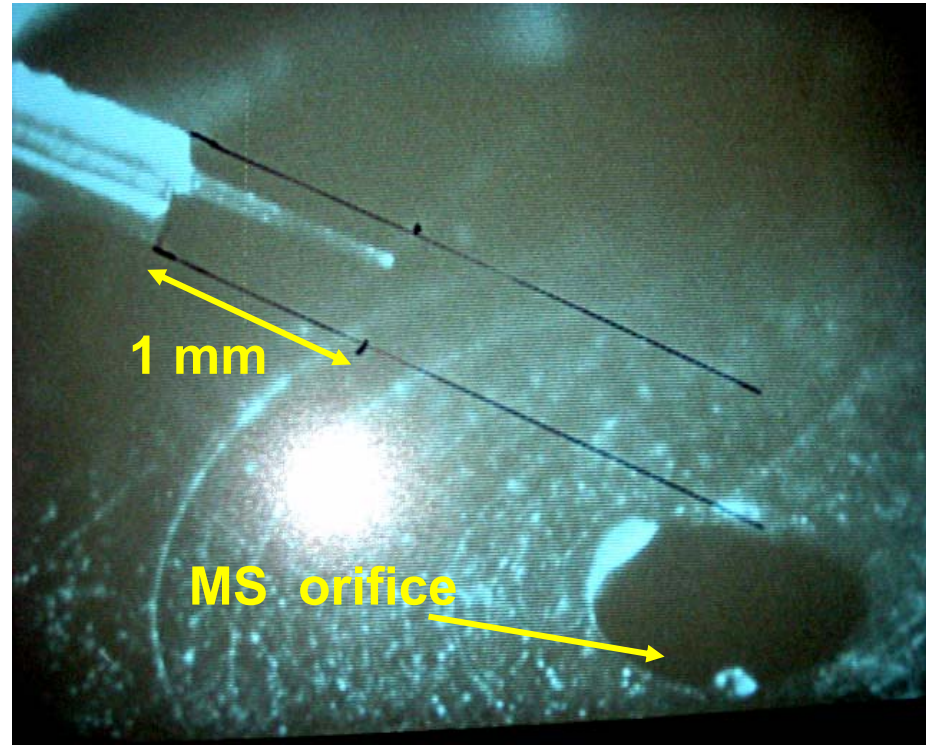
MicroESI - Nebulizer Interface





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MicroESI - Nebulizer Interface





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Applications to Polyphenols

- **Qualitative analysis : MS and MS-MS**
- **Quantitative analysis : MRM-MS**

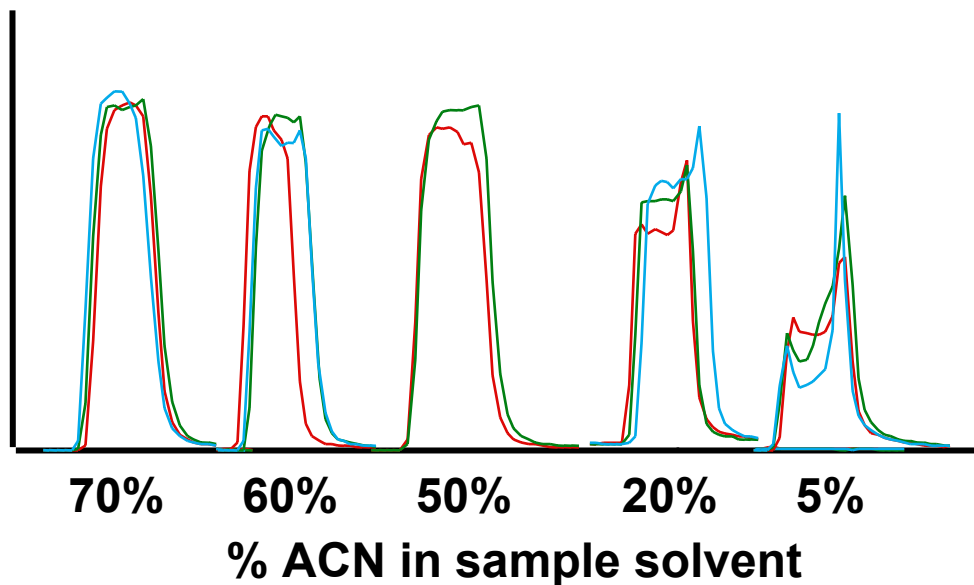
Characterization of MicroESI Interface

Solvent Composition

Methods: flow injection

Carrier: 70% ACN, 10 mM NH₄OAc
1000 nl/min

Sample: isoflavone standards
in various solvent composition

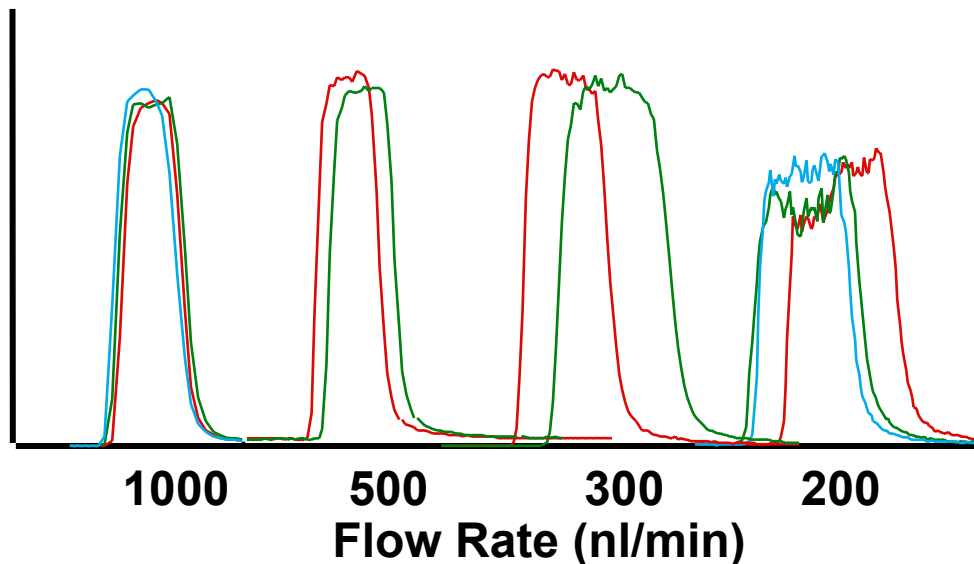


Flow Rate

Methods: flow injection

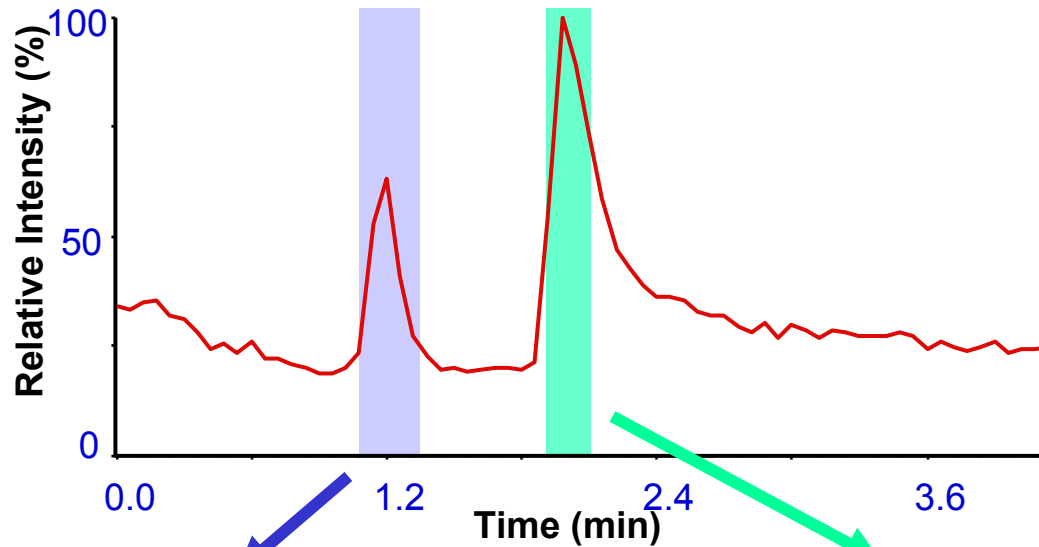
Sample: isoflavone standards in
70% ACN, 10mM NH₄OAc

Carrier: 70% ACN, 10 mM NH₄OAc
at various flow rate

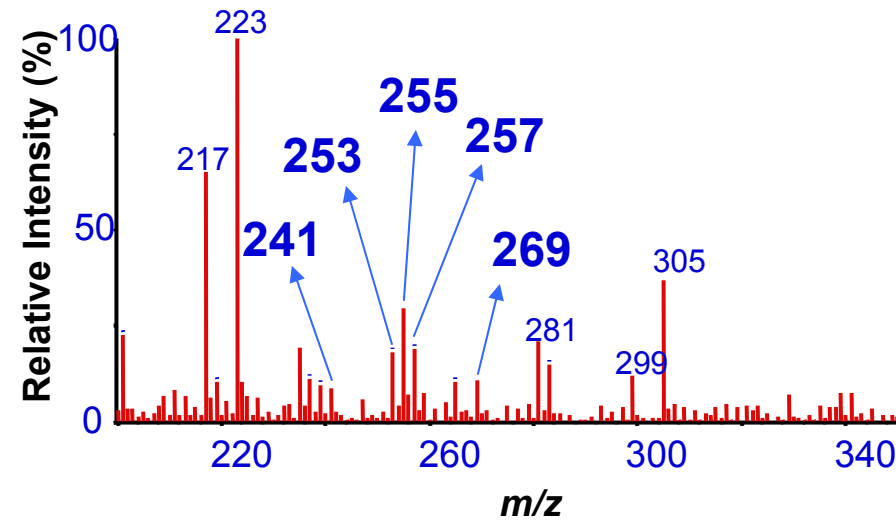
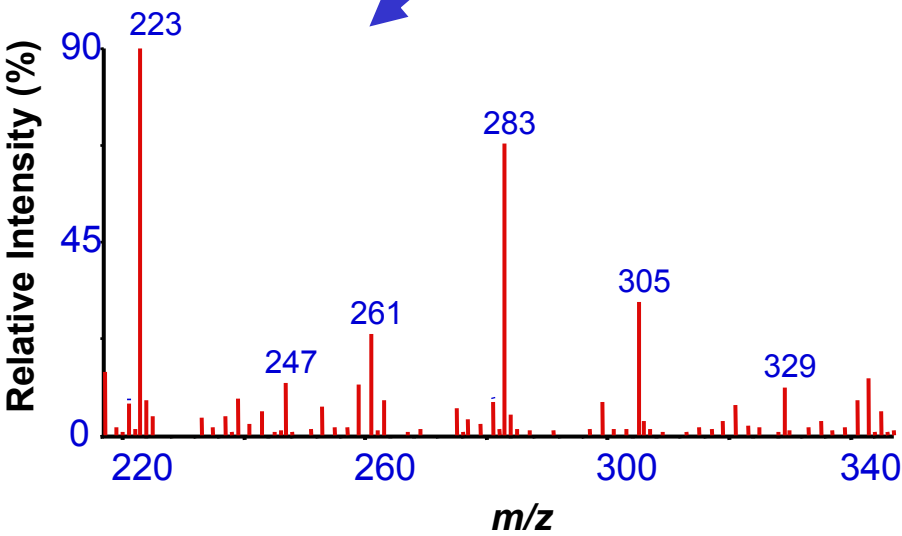


Microscale System for Qualitative MS Analysis of Isoflavones in PBS

Sample: 1 μ l, 100 nM isoflavones in PBS (100 fmol)

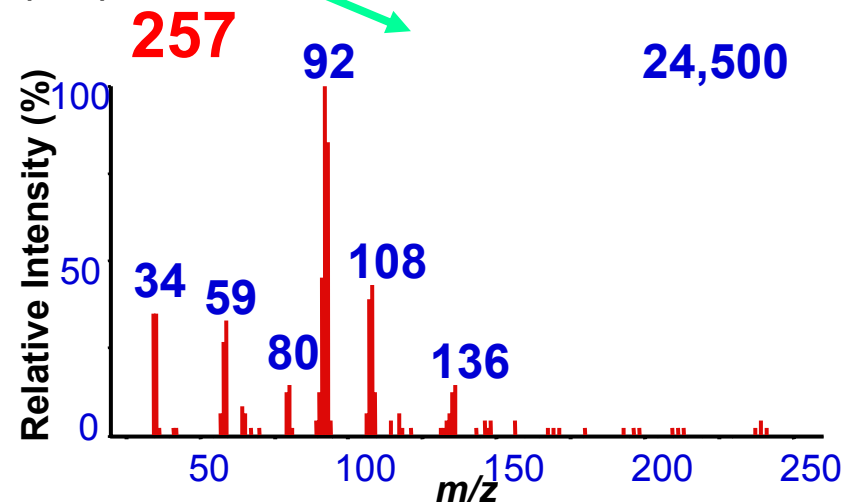
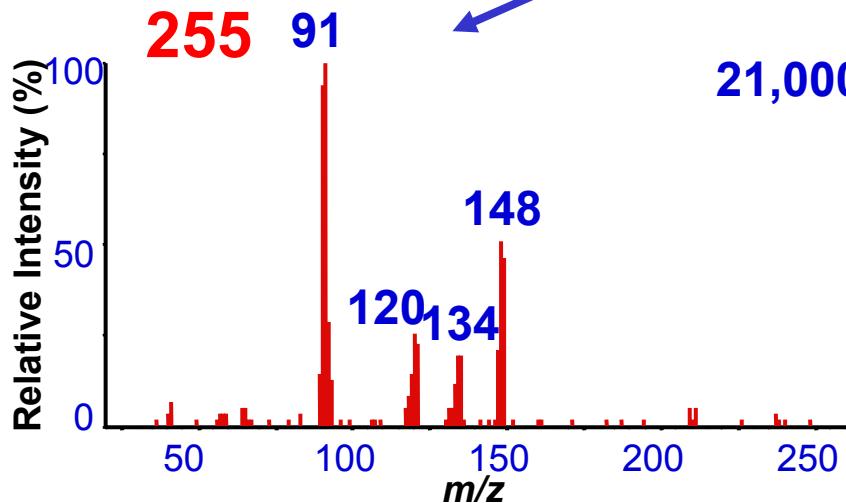
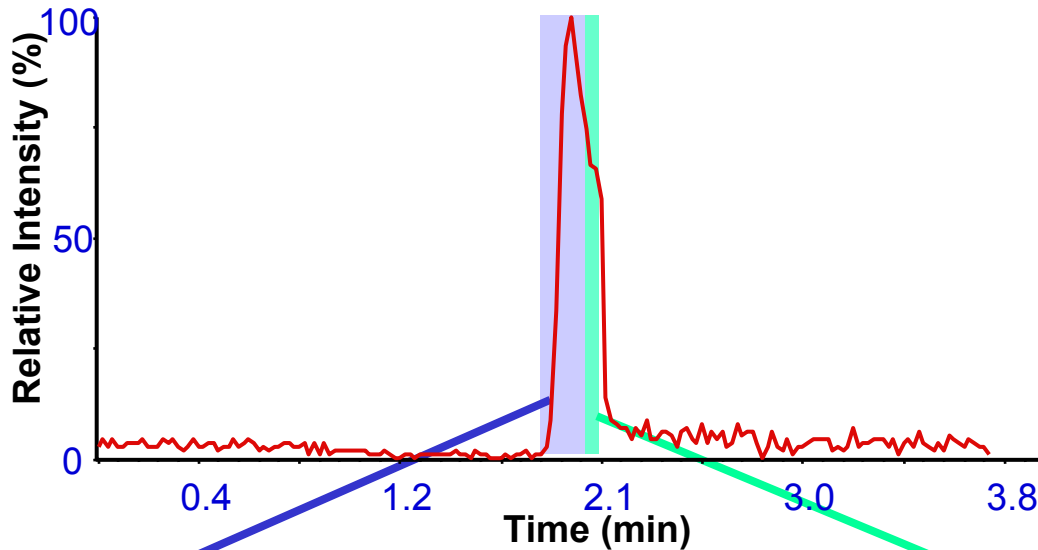


Equol	$m/z = 241$
Daidzein	$m/z = 253$
DHD	$m/z = 255$
O-DMA	$m/z = 257$
Genistein	$m/z = 269$



Microscale System for Qualitative MS-MS Analysis of Isoflavones in PBS

Sample: 1 μ l, 100 nM isoflavone in PBS (100 fmol)



LC-MRM-MS Analysis of Isoflavones

Column: RP C₈ column
4.6 mm id x 10 cm

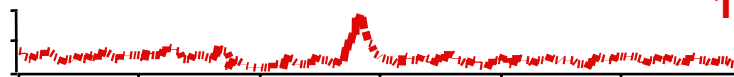
Flow rate : 1 ml/min
25 μ l/min to MS

Sample : isoflavones
50 nM in 80% MeOH

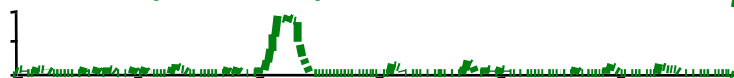
Injection volume : 20 μ l

Sample consumed : 1 pmol

Equol (241/119) 199



Daidzein (253/223) 76



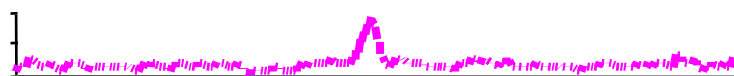
Genistein (269/133) 194



DHD (255/149) 210



O-DMA (257/108) 246



Apigenin (269/149) 6348
(5 μ M)



0.00 1.00 2.00 3.00 4.00 5.00 6.00
Time (min)

Application of Microscale System for MRM Analysis of Isoflavones in PBS

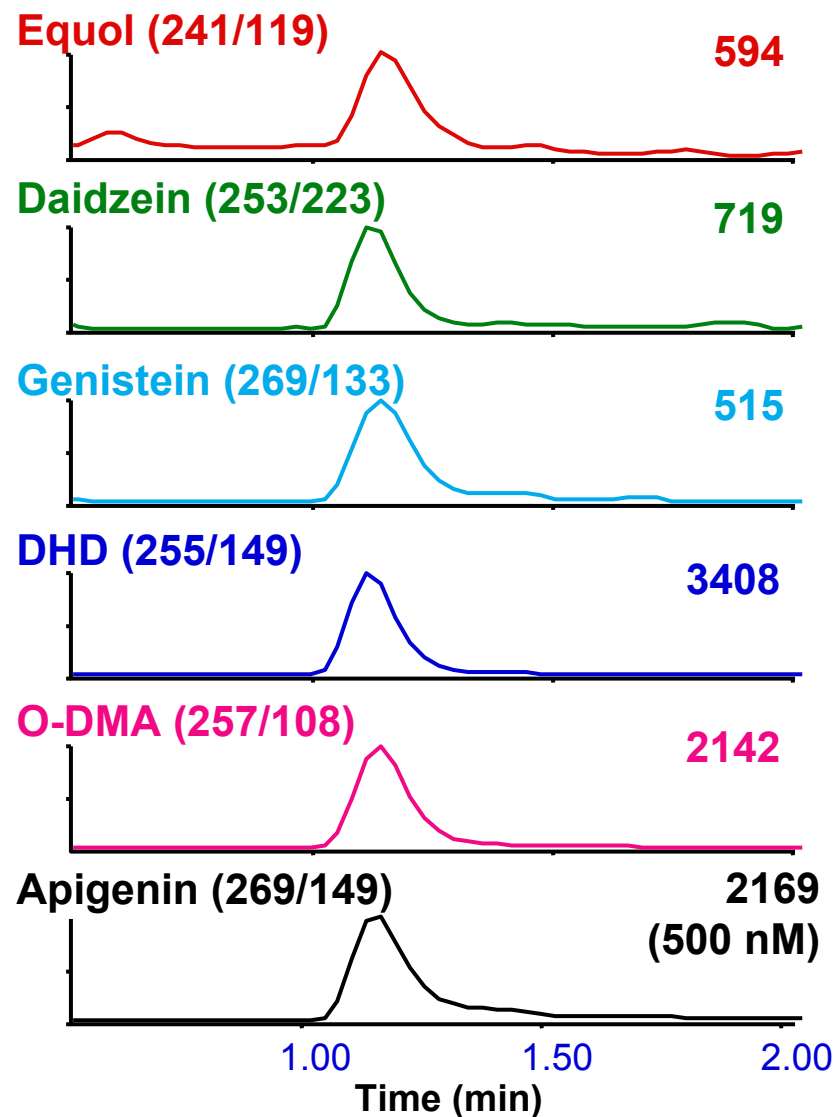
Column: 3M SDB-XC polymer
packed bed: 85 nl

Flow rate : 1 μ l/min

Sample : **isoflavones**
12.5 nM in PBS

Injection volume : 1 μ l

Sample consumed : 12.5 fmol

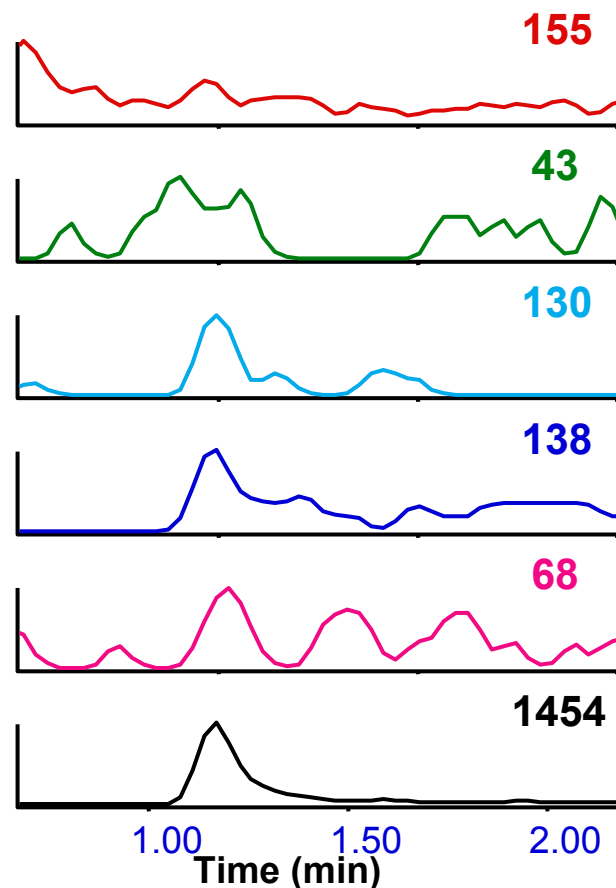
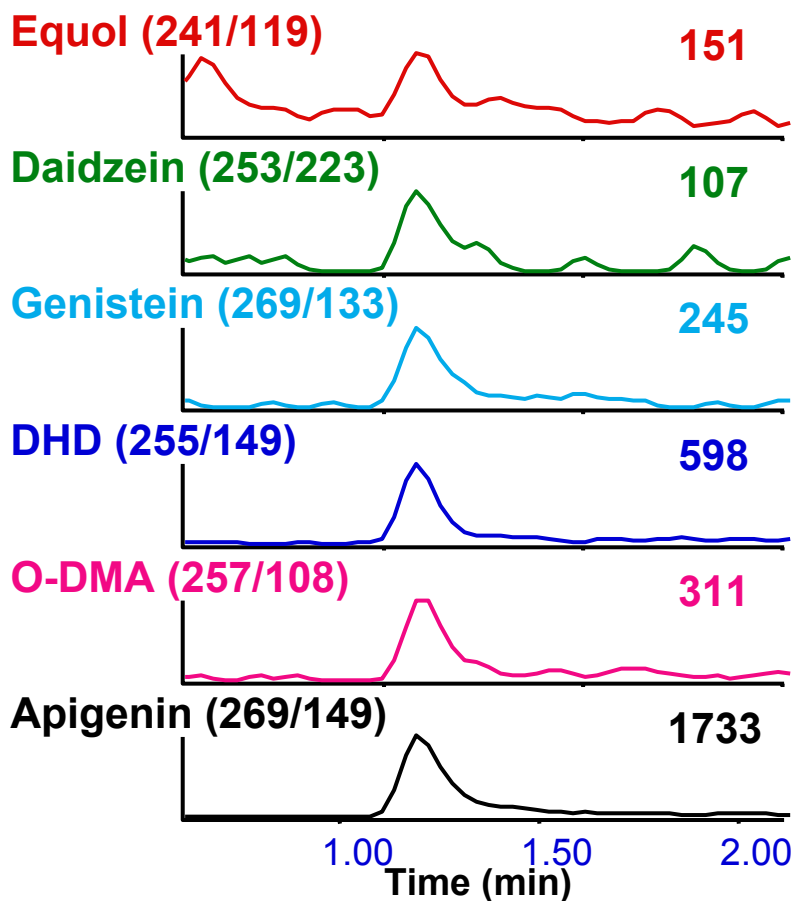


Application of Microscale System for MRM Analysis of Isoflavones in PBS

Load : 1 μ l , Wash: 5% ACN 3 μ l x 3 min

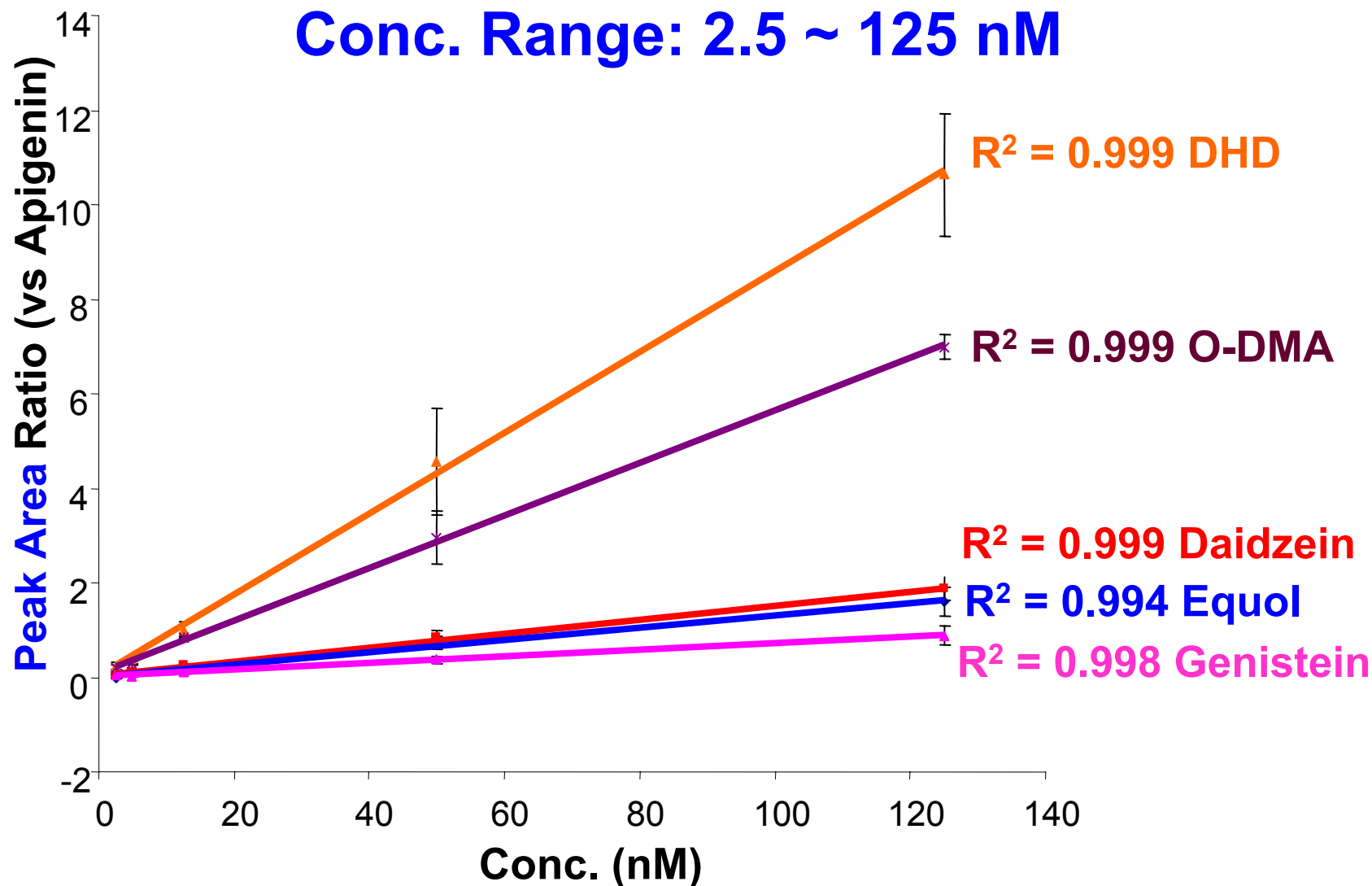
Elute : 10 mM NH₄OAc in 70% ACN, 1000 nl/min

2.5 nM isoflavones in PBS PBS blank w\ internal std.



Application of Microscale System for MRM Analysis of Isoflavones in PBS

Conc. Range: 2.5 ~ 125 nM





Summary

- **Micro- to nano- flow ESI provides many advantages, especially sensitivity, over conventional ESI, due to better ion formation and transfer efficiency.**
- **The microscale system, combining on-line sample cleanup (desalt) and concentration with MicroESI-MRM-MS, provides detection limits at low femtomole levels in 1 μ l of physiological samples.**
- **The microscale system will be ideal for analysis of polyphenols from minimal physiological samples, such as blood extracts of small animals and microdialysates.**

Demonstration

- **Making of micropreconcentrator (hand-on)**
- **Analysis of isoflavones in saline (mimic microdialysis samples)**
 - **desalt or not desalt !!**