Nanoelectrospray Ionization and High Sensitivity Analysis

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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 µl/min flow rate with pneumatically assisted interface
- Concentration-sensitive type detection
  - flow rate affects peak width and area, but intensity
- 10⁻⁵ ~ 10⁻⁷M
- Picomole (10⁻¹²mol) 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)

Gold coated glass needle

Liquid-junction interface
NanoElectrospray

Taylor cone

Jet

Needle tip

Plume

\[ \frac{5 \mu m}{\text{Vertical}} \]
Comparison of ESI and Nano-ESI - Ionization Efficiency -

sample solution → nebulizer gas → 5 kV → 1 µm → MS → N₂

sample solution → 1~2 KV → 0.1 µm → MS → N₂
Comparison of ESI and Nano-ESI - Ion Transfer Efficiency -

**Electrospray**
- Flow rate: 25 µl/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

1 μl Injection

< 1000 nl/min

Waste

MicroES Interface

Micro Preconcentrator

Eluting buffer

Loading and washing buffer

Waste

RP MPC

RP polymer

Teflon tubing

FS Capillary 50 μm id

FS Capillary 50 μm id

N₂

HV

MS
Operation of Microscale System

Sample Loading & Washing

- 5% ACN
- 3 µl/min x 3 min

Sample Elution

- 70% ACN
- 300-1000 nl/min
MicroESI - Nebulizer Interface

Nebulizer gas
Air 20 psi

FSC
50 / 360 µm

SS Tee
150 µm through hole for ESI voltage

FSC
25 / 150 µm

PEEK Tee
250 µm throughhole

SS tubing
180 / 330 µm

2 mm

N2

MS
MicroESI - Nebulizer Interface

1 mm

MS orifice
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, 10 mM NH₄OAc
Carrier: 70% ACN, 10 mM NH₄OAc at various flow rate
Microscale System for Qualitative MS Analysis of Isoflavones in PBS

Sample: 1 µl, 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 μl, 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 µl/min to MS

Sample: isoflavones
50 nM in 80% MeOH

Injection volume: 20 µl

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 µM)
Application of Microscale System for MRM Analysis of Isoflavones in PBS

Column: 3M SDB-XC polymer packed bed: 85 nl
Flow rate: 1 \( \mu l \)/min
Sample: isoflavones 12.5 nM in PBS
Injection volume: 1 \( \mu l \)
Sample consumed: 12.5 fmol

Equol (241/119) 594
Daidzein (253/223) 719
Genistein (269/133) 515
DHD (255/149) 3408
O-DMA (257/108) 2142
Apigenin (269/149) 2169 (500 nM)

Time (min)
Application of Microscale System for MRM Analysis of Isoflavones in PBS

Load: 1 µl, Wash: 5% ACN 3 µl x 3 min
Elute: 10 mM NH₄OAc in 70% ACN, 1000 nl/min

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

Equol (241/119) 151
Daidzein (253/223) 107
Genistein (269/133) 245
DHD (255/149) 598
O-DMA (257/108) 311
Apigenin (269/149) 1733

Time (min)
Application of Microscale System for MRM Analysis of Isoflavones in PBS

Conc. Range: 2.5 ~ 125 nM

- R² = 0.999 DHD
- R² = 0.999 O-DMA
- R² = 0.999 Daidzein
- R² = 0.994 Equol
- R² = 0.998 Genistein
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 µl 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 !!