

# (U)HPLC for Hyphenation

Prepared by  
Gerard Rozing, Karlsruhe, Germany

Delivered by  
Jan Preisler, Masaryk University, Brno, Czech Republic

## About the Author



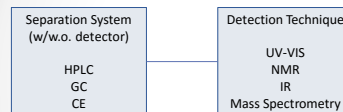
- Undergraduate and graduate studies at University of Amsterdam, 1964-1976. Majors in Organic Chemistry and Chemical Engineering
- Post-doctoral research at State University of Ghent, Belgium, 1977 and post-doctoral training Analytical Chemistry, University of Amsterdam, 1978-1979
- R&D Chemist, group & project Leader, R&D section manager, HPLC column and HPLC system development at Hewlett-Packard, Waldbronn, Germany, 1979-'99
- Since 2000, Agilent Technologies University Relations and External Scientific Collaborations Manager
- Since 2006 Agilent Research Fellow
- Retired September 1, 2012. Since then, working on freelance basis. Visit my website at <http://www.rozing.com> e-mail: [gerard@rozing.com](mailto:gerard@rozing.com)

## Acknowledgements

- Monika Dittmann, Stephan Buckenmaier, Udo Huber, Christian Scholz, Konstantin Choikhet all at Agilent Technologies, Waldbronn, Germany
- Paul Goodley, Chris Miller, Agilent Technologies, Santa Clara, USA
- Oliver Schmitz, University of Duisburg-Essen, Germany
- Achille Cappiello, Univ. Urbino, Italy
- Remco van Soest, Eksigent part of AB Sciex, Dublin, USA

## Hyphenation in Separation Science

- Coupling of a separation method with a spectroscopic detection method resulting in **3 (or more) dimensions of information**



- Detection Technique is coupled "on-line" with the separation system
- Detection Technique is coupled with the Separation System through an interface in case of incompatibility between the phases or systems

## Hyphenation in Separation Science

- Is not Multidimensional Separation Techniques
- In this case the "x" is used  
examples **LCxLC** coupling, **LCxGC** coupling etc.

## Focus on LC-MS

- Important aspects of LC-MS
  - Detector response type
  - Ionization mechanisms and interfaces
- HPLC separation factors influencing ESI and APCI process and mass detection
- HPLC Column Technology, Special Techniques and New Developments

### Concentration Sensitive Detector in HPLC

- Response proportional to concentration (e.g. UV detection)  
 $Abs_{\lambda} = \epsilon_{\lambda} \cdot c_l \cdot L_{cell}$  Lambert Beer's law
- Response independent of flow rate (infusion experiment!)
- Chromatographic peak height does not change with flow rate (e.g. in FIA or neglecting any dispersion)
- Chromatographic peak area is given by:  
 $A_i = \int c_i(v)dv = F \int c_i(t)dt$   
 in case flow rate is constant ( $V_{R,i} = F \cdot t_{R,i}$ ) and inversely proportional with flow rate (peak width decreases in time domain)
- In almost all cases, non-destructive

Methods of Chemical Research 6.12.2013 Slide 7

### Mass Flow Sensitive Detector in HPLC

- Response is proportional to mass/time (or cps)  $R = a \cdot \frac{\partial m_i}{\partial t}$
- Response increases with flow rate (infusion experiment!)
- Chromatographic peak height increases with flow rate (e.g. in FIA neglecting any dispersion) and the peak width decreases
- Chromatographic peak area is given by:  
 $A_i = \int \frac{m_i}{t} dt$   
 and is independent of flow rate (peak width decreases in volume domain)
- In most cases a destructive detection method (FID, MS, ELSD, ICP/MS)

Methods of Chemical Research 6.12.2013 8

### Important Aspects of LC-MS

- Detector response
- Ionization mechanisms and interfaces

Methods of Chemical Research 6.12.2013 9

### Ionization Techniques in LC-MS

- Soft, Atmospheric Pressure (in principle no fragmentation)
  - Electrospray Ionization- ESI
  - Chemical Ionization - APCI
  - Photo Ionization - APLI
  - Laser Ionization - APLI
  - Surface Ionization (MALDI, DART)
- Soft, Vacuum Ionization
  - Matrix assisted laser desorption – MALDI
- Hard, Vacuum Ionization (with fragmentation)
  - Particle Beam
  - Direct Electron Impact
  - Supersonic Molecular Beam

Methods of Chemical Research 6.12.2013 10

### Pneumatically Assisted Electro spray Ionization

Charged solvent droplets formed about 2 μm in diameter

Electrical fields on the cylinder, end plate and capillary charge the droplets

Initially Called Ion Spray

Courtesy of Agilent Technologies Kundensschulung

Methods of Chemical Research 6.12.2013 11

### Pneumatically Assisted Electro spray Ionization

Polar/charged analytes

Solvent evaporation

Rayleigh Limit

From column

Nebulizing Gas

TaylorCone

Solvent Ion Cluster

Ion Emission Model Charge Residue Model

Analyte Ions

An detailed explanation of the electro spray ionization process can be found at:  
<http://www.mcponline.org/content/early/2011/05/19/mcp.R111.009467/suppl/DC1>

Courtesy of Agilent Technologies Kundensschulung

Methods of Chemical Research 6.12.2013 12

### Pneumatically Assisted Electro spray Ionization

From HPLC  
Nebulizing Gas  
Spray needle  
MS Inlet

Courtesy of Agilent Technologies Kundenschoolung  
6. 12. 2013 Slide 13

### Atmospheric Pressure Chemical Ionization

Neutral/Low Polarity Analytes in Aerosol  
Analytes Evaporate!!!  
Gasphase  
Solvent ionization by charged nitrogen molecules  
Proton Transfer  
Analyte ions

Methods of Chemical Research  
6. 12. 2013 Slide 14

### APCI Detailed Mechanism- Gas Phase Ionization

Nebulizer Pressure  
Corona current  
Heater  
Vcap  
Drying gas Temperature and Flow  
Fragmentor

Courtesy of Agilent Technologies Kundenschoolung  
6. 12. 2013 Slide 15

### APCI-Interface

APCI corona needle

Methods of Chemical Research  
6. 12. 2013 Slide 16

### Atmospheric Pressure Photo Ionization

From LC  
Nebulizer Gas  
Heater Block  
UV lamp  
To Mass Analyser  
vacuum

Courtesy of Oliver Schmitz, Univ. Duisburg/Essen, Germany  
6. 12. 2013 Slide 17

### Atmospheric Pressure Photo Ionization

Analyses in aerosol  
Analyses evaporate!!!  
Gasphase  
Photons ionize analyte  
Krypton 10.0 eV, 10.6 eV  
Or a dopant (acetone) added is photo-ionized which acts as reagent gas  
Analyte ions

Methods of Chemical Research  
6. 12. 2013 Slide 18

### APPI-Interface

Lamp Source instead of Discharge Needle

Courtesy of Agilent Technologies KundenSchulung

Methods of Chemical Research 6. 12. 2013 19

### Atmospheric Pressure Laser Ionization

Alternative for High MW substances:  
Combine ESI with APLI!!

Courtesy of Oliver Schmitz, Univ. Duisburg/Essen, Germany

Methods of Chemical Research 6. 12. 2013 Slide 20

### Atmospheric Pressure Laser Ionization

Courtesy of Oliver Schmitz, Univ. Duisburg/Essen, Germany

Methods of Chemical Research 6. 12. 2013 Slide 21

### Summary

Methods of Chemical Research 6. 12. 2013 Slide 22

### Quoted from Marja-Liisa Riekkola, Helsinki, Finland\*

*"Many important advances in column materials and technology have contributed to improve the resolution of analytes in liquid chromatography. As is well known, liquid chromatographic separations critically depend on column type, choice of stationary phase, and type and composition of the eluent employed as mobile phase. The selectivity of separations can be enhanced by adjusting the stationary or mobile phase. The best separations are achieved through careful optimization of conditions.*

*Liquid chromatography-mass spectrometry (LC-MS) has become increasingly popular in recent years. Although three atmospheric pressure ionization (API) techniques (electrospray ionization, atmospheric pressure chemical ionization and atmospheric pressure photoionization) are available to facilitate the coupling of LC to MS, the MS detection is not always compatible with the solvents and additives required in the preceding LC separation. Compromises must be accepted between the best LC separation conditions, especially eluent composition, and the best ionization conditions if highest selectivity and sensitivity are to be achieved."*

\*J. Chromatography, 1216, 684 (2009)

Methods of Chemical Research 6. 12. 2013 Slide 24

### HPLC Separation Factors Influencing ESI and APCI Process and Mass Detection

- Interface Parameters (voltage(s), gases used)
- Eluent Solvent Properties
  - Flow rate
  - Composition, volatility, viscosity, conductivity
  - Mobile phase additives, pH
  - Ion Suppression/Matrix effects
- Practice of LC-MS
  - Use of inorganic buffers
  - Common background ions & contaminants

System variables	Compound variables	Method variables
Electric field	Surface activity	Flow rate
ES-capillary diameter	Proton affinity	Electrolyte concentration
ES-capillary voltage	pKa	pH
Distance to counter electrode	Solvation energy	Solvent properties (boiling point, surface tension, etc.)
Heat capacity of ambient gas		
Solvent saturation level of ambient gas		

R. King et al., J. Am. Soc. Mass Spectrom., 2000, 11, 942-950

Methods of Chemical Research 6. 12. 2013 Slide 25

# Eluent Solvent Properties

...

ROZING.COM

Methods of Chemical Research 6.12.2013 26

## Electrospray Ionization – Influence of Flow Rate

- Pneumatically assisted electrospray

Initially electrospray was not pneumatically assisted – no direct countercurrent drying gas, no nebulizer gas

Only working with very low LC flow rates.

Bruins and Henion introduce pneumatically assisted electrospray (ion spray) \*

\*A.P. Bruins, Th. R. Covey, J. D. Henion, Anal. Chem., 1987, 59 (21), pp 2642-2646  
Picture taken from G. Hopfgartner et al., J. Chrom. A, 647, 51 (1993)

ROZING.COM

Methods of Chemical Research 6.12.2013 27

## Influence of Flow Rate on Response in LC-MS\*

Pneumatically Assisted ESI (Ion Spray)

Solv.: MeOH/Water 50/50, 0.1% AcOH  
Source: Analytica of Branford  
MS: SQ HP89A, 100-1000 m/z p.s.

This ESI works as an Concentration Sensitivity Detector

Fig. 1. Ion signal from the direct infusion of a 10 pmol/µl solution of methionine enkephalin as a function of sample flow-rate. F. Banks Jr., J. Chrom. A, 743, 99, 1996

ROZING.COM

Methods of Chemical Research 6.12.2013 Slide 28

## Influence of Flow Rate on Response in LC-MS\*

Pneumatically Assisted ESI (Ion Spray)

Injection of equal amounts (50 pmol) of methionine enkephaline on columns with different i.d.

Signal height increase is 163x short of 339x by column diameter ratio<sup>2</sup>  
Attributed to poor packing of the microbore column

Fig. 2. TICs from methionine enkephalin injections (50 pmol each) on columns with different diameters. F. Banks Jr., J. Chrom. A, 743, 99, 1996

ROZING.COM

Methods of Chemical Research 6.12.2013 Slide 29

## Influence of Flow Rate on Response in LC-MS\*

Pneumatically Assisted ESI vs. APCI

### ESI

### APCI

Fig. 3. 314–318–325–328 signal response of seven particles depending on the flow-rate of eluent (methanol-water 97:3, v/v); standard peptide substance: c = 100 µg/ml. Top: peak area plotted versus flow-rate. Bottom: peak height plotted versus flow-rate.

Not all analyte ions are captured with the same efficiency

\*M. Engewald et al., Journal of Chromatography A, 937 (2001) 65–72

ROZING.COM

Methods of Chemical Research 6.12.2013 Slide 30

## Nano-electrospray Ionization

Developed by Matthias Mann & Matthias Wilm\*

- Smaller droplets → generation of more ions
- No orthogonal design – sprayer is 1 – 2 mm from MS entrance
- Higher sampling rate of ions into MS

**Result:** dramatically higher sensitivity than standard ESI

\*M. Wilm & M. Mann, International Journal of Mass Spectrometry and Ion Processes, 136, 167 (1994)

ROZING.COM

Methods of Chemical Research 6.12.2013 Page 31

### Influence of Flow Rate on Response in NanoESI

Emitter tip orifice diameter proportionally reduced

**Figure 8.** Relationship between nanoESI-MS response and the mobile-phase flow (or inner diameters) for individual species in the yeast soluble protein digest. Conditions: the flow rates were measured.

**Table 1. NanoLC Packed Capillary Parameters Operated at 10 000 Psi\***

column (mm i.d. x cm)	$d_p$ (nm)	$R_p$ (Å)	$u$ (cm/s)	$F$ (nl/min)	$\gamma$	$\epsilon$
74.5 x 87.0	3.6	300	0.19	380	20.7	0.78
47.1 x 87.0	3.6	300	0.19	155	13.1	0.79
20.7 x 87.0	3.6	300	0.22	76	8.3	0.81
19.8 x 87.0	3.6	300	0.22	33	5.1	0.81
14.8 x 87.0	3.6	300	0.23	20	4.1	0.83

R.D. Smith et al., Anal. Chem. 74, 4235 (2002)

Methods of Chemical Research 6. 12. 2013 Slide 32

### Preliminary Conclusions – Flow Dependence ESI

- The pneumatically assisted ESI interface (IF) tolerates maximally 1 mL/min
- Pneumatically assisted ESI IF behaves largely like a concentration sensitive detector
- Reduction of column i.d. demands very low extra column dispersion and well packed columns in order to exploit sensitivity gain with concentration sensitive detection
- Nano-ESI response increases dramatically at very low flow rates (<20 nL/min)
- At low flow rate nor mass flow sensitive or concentration sensitive detector because more ions reach the MS inlet
- Ion suppression is much reduced (vide infra)

Methods of Chemical Research 6. 12. 2013 Slide 33

### Influence of Gradient Elution on Response in LC-MS\*

Nano-electrospray Ionization

Column 75  $\mu$ m i.d. Solvent 0.2% AcOH 0.1% TFA in water vs. 0.1% TFA in ACN, 0-100%

R.D. Smith et al., J. Amer. Soc. Mass Spectrometry, 20, 682 (2009)

Methods of Chemical Research 6. 12. 2013 34

### Influence of Gradient Elution on Response in LC-MS\*

Nano-electrospray Ionization

C = cone jet  
P = pulsed cone jet  
M = multi jet  
D = dripping  
S = spindle

G. Valaskovic, J. Am. Soc. Mass Spectrom., 2004, 15, 1201-1215

Methods of Chemical Research 6. 12. 2013 Slide 35

### HPLC Separation Factors Influencing ESI and APCI Process and Mass Detection

- Interface Parameters (voltage(s), gases used)
- Eluent Solvent Properties
  - Flow rate
  - Composition, volatility, viscosity, conductivity
  - Mobile phase additives, pH
  - Ion Suppression/Matrix effects
- Practice of LC-MS
  - Use of inorganic buffers
  - Common background ions & contaminants

Methods of Chemical Research 6. 12. 2013 Slide 36

### Frequently Used Mobile Phase Additives in ESI/MS

Positive Mode	Negative Mode
Ammonium Acetate	Ammonium Acetate
Ammonium Formate	Ammonium Formate
Acetic Acid (pH 3-4)	Ammonia/Ammonium Hydroxide (pH>7)
Formic Acid (pH 2-3)	Triethylamine (pH >7)
Trifluoro-acetic Acid (pH 1-2)	N-Methylmorpholin

- As additive or by post-column addition in case the solvent pH for optimal separation differs from the pH for optimal ionization.
- Additives will cause a high background signal (TFA (m/z 113) in negative mode, TEA (m/z 102) in positive mode), increase conductivity of the solvent and may cause ion suppression

Courtesy of Agilent Technologies Kundensschulung

Methods of Chemical Research 6. 12. 2013 37

### Solution Chemistry is Important

ESI mandates the formation of analyte ions in the eluent solution

**Positive Mode**

$$\begin{matrix} R_1 \\ | \\ :N - R_2 + HA \rightleftharpoons [HN - R_2 + A^- \\ | \\ R_3 \end{matrix}$$

Base      Acid      Analyte Cation

**Negative Mode**

$$\begin{matrix} O \\ || \\ R - C - OH + :B \rightleftharpoons R - C - O^- + H:B^+ \end{matrix}$$

Acid      Base      Analyte Anion

Methods of Chemical Research      6.12.2013      38

### Influence of Additive Concentration on Response

Pneumatically Assisted ESI

CN1C=NC2=C1C(=O)N(C(=O)N2C)C  
**Caffeine**

CN1C=NC2=C1C(=O)N(C(=O)N2C)C  
**Reserpine**

ESI

APCI

Courtesy of Agilent Technologies Kundensschulung

Methods of Chemical Research      6.12.2013      39

### Influence of Additive Concentration on Response

Pneumatically Assisted ESI

Taken from: HPLC Analysis of Biomolecules, Technical Guide Thermo Electron Corporation

Methods of Chemical Research      6.12.2013      40

### TFA Containing Solvents for Tryptic Peptides LC-MS

- Ideally suited for RP LC since trypsin cleaves at lysine or arginine leaving a basic peptide. With TFA is ion-pair separation on RP column possible.
- TFA neutralizes "hot" sites on the silica surface
- TFA forms a strong ion pair with basic peptides
- But spray instability due to high conductivity and high surface tension of the solution has been reported
- Strong signal reduction observed

Methods of Chemical Research      6.12.2013      41

### FIA with 1% AcOH/0.25% TFA\*

Pneumatically Assisted ESI

**1.0% HOAc**

**0.2% TFA**

\*A. Apffel et al., J. Chrom., 712 177 (1995)

Methods of Chemical Research      6.12.2013      Slide 42

### Remedy in Practice

- Post-column addition of the "TFA-fix"
  - No compromise on chromatography
  - Additional hardware required (cost, reliability, mixing efficiency)
- New stationary phases that have low silanophilic interactions allowing good peptide separations without compromising chromatograph by using formic acid etc.
  - Dionex Acclaim Pepmap
  - Waters CSH130 C<sup>18</sup>
  - Thermo BioBasic columns
  - Agilent AdvanceBio Peptide Mapping columns

Methods of Chemical Research      6.12.2013      43

## Pneumatically Assisted API

### ESI

- In principle concentration sensitive
- In many cases more sensitive
- Wide flow rate range → nanobore – normal bore columns
- Solvent composition (gradient) OK
- Mobile phase additives compromise response
- Tolerates low concentration of inorganic buffers
- High matrix effect

### APCI

- In principle mass flow sensitive
- More selective, non-polar substances
- No advantage at low flow rates
- Organic solvent may have a large influence on response
- Chlorinated solvents will assist ionization
- Tolerate up to 100 mmol inorganic buffers
- Low matrix effect

Methods of Chemical Research

6. 12. 2013

44

## HPLC Separation Factors Influencing ESI and APCI Process and Mass Detection

- Interface Parameters (voltage(s), gases used)
- Eluent Solvent Properties
  - Flow rate
  - Composition, volatility, viscosity, conductivity
  - Mobile phase additives, pH
  - Ion Suppression/Matrix effects in ESI/MS
- Practice of LC-MS
  - Use of inorganic buffers
  - Common background ions & contaminants

Methods of Chemical Research

6. 12. 2013

Slide 45

## What is Matrix Effect/Ion Suppression in LC-ESI/MS\*

- Ionization efficiency of ESI depends
  - Solvent properties – mostly constant but for gradient elution
  - Source parameters
  - Compounds co-eluting with analyte
- Standards are clean solutions
- Different response for the same analyte concentration in sample solution than in standard solution
- Matrix effect depends on analyte concentration

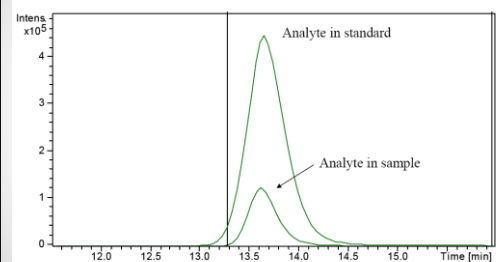
\*Annelie Kruve, Univ. of Tartu, Estonia

Methods of Chemical Research

6. 12. 2013

46

## Example of Matrix Effect in LC-ESI/MS\*



\*Annelie Kruve, Univ. of Tartu, Estonia

Methods of Chemical Research

6. 12. 2013

47

## Matrix Effect - Causes

- Competition for available charges  
(Keep in mind that a very low fraction from the analytes actually make it into the MS)
- Interfering substances may cause increase of viscosity and surface tension therewith hampering the formation of droplet
- Formation of solid particles including the analyte
- As with TFA ion pair formation renders the analyte neutral.

Methods of Chemical Research

6. 12. 2013

Slide 48

## Remedies for the Matrix Effect

- Assess the scope of the effect by the post-column addition method\*
- If possible prepare standard in sample matrix (e.g. serum) and run it through the sample prep procedure
- Address in the whole procedures the probable mechanism of the matrix effect
- Smaller droplets → nanoelectrospray!
- Use another ionization method e.g. APCI or Direct Election Impact LC-MS interface (vide infra)

\*Matuszewski et al., *Anal. Chem.* 2003, 75, 3019-3030)

Methods of Chemical Research

6. 12. 2013

49



### HPLC Separation Factors Influencing ESI and APCI Process and Mass Detection

- Interface Parameters (voltage(s), gases used)
- Eluent Solvent Properties
  - Flow rate
  - Composition, volatility, viscosity, conductivity
  - Mobile phase additives, pH
  - Ion Suppression/Matrix effects
- Practice of LC-MS
  - Use of inorganic buffers
  - Common background ions & contaminants

Methods of Chemical Research 6.12.2013 Slide 50

### Using Non-volatile Buffers in the Mobile Phase

**LC Conditions:**  
 Mobile phase: 8% methanol in one of the following:  
 A: water  
 B: 0.2% acetic acid in water  
 C: 50 mM ammonium phosphate, pH 7  
 D: 50 mM sodium phosphate, pH 7  
 Flow rate: ESI - 0.3 ml/min; APCI - 0.7 ml/min  
 Injection: 1 µl of a mixture containing 10 ng/µl each of lincomycin, caffeine and sulfachloropyradizine  
 Column: Zorbax Eclipse XDB C8 2.1 mm x 50 mm @ 30 °C

**MS Conditions:**  
 SIM Ions:  
 Positive ion mode: 195, 285 and 407 amu  
 Negative ion mode: 193, 283 and 405 amu  
 Fragmentor: Ramped 70 V for 193/195; 50 V for 283/285; 80 V for 405/407  
 Vcap: ESI - 4000 V, APCI - 3000 V  
 Drying gas: ESI - 350°C, 10 l/min; APCI - 350 °C, 5 l/min  
 Nebulizer: ESI - 25 psig; APCI - 60 psig

Methods of Chemical Research 6.12.2013 51

### Using Non-volatile Buffers in the Mobile Phase

Influence on Response

Positive Ion Mode

Mobile Phase Conditions:  
 (B) 0.2% acetic acid;  
 (C) 50 ammonium phosphate;  
 (D) 50 mM sodium phosphate

Methods of Chemical Research 6.12.2013 52

### API-Spray Chamber after using a 25 mM Phosphate Buffer

No comment needed

Methods of Chemical Research 6.12.2013 53

### HPLC Separation Factors Influencing ESI and APCI Process and Mass Detection

- Interface Parameters (voltage(s), gases used)
- Eluent Solvent Properties
  - Flow rate
  - Composition, volatility, viscosity, conductivity
  - Mobile phase additives, pH
  - Ion Suppression/Matrix effects
- Practice of LC-MS
  - Use of inorganic buffers
  - Common background ions & contaminants

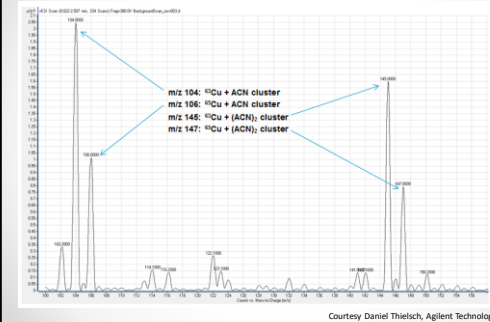
Methods of Chemical Research 6.12.2013 Slide 54

### Common Contaminant & Background Ions

m/z	Ion	Compound
43	[M+H] <sup>+</sup>	Acetic acid
55	[M+H] <sup>+</sup>	Formic acid
67	[M+H] <sup>+</sup>	Propionic acid
77	[M+H] <sup>+</sup>	Acetic acid
83	[M+H] <sup>+</sup>	Formic acid
95	[M+H] <sup>+</sup>	Propionic acid
105	[M+H] <sup>+</sup>	Acetic acid
117	[M+H] <sup>+</sup>	Formic acid
129	[M+H] <sup>+</sup>	Propionic acid
137	[M+H] <sup>+</sup>	Acetic acid
151	[M+H] <sup>+</sup>	Formic acid
165	[M+H] <sup>+</sup>	Propionic acid
173	[M+H] <sup>+</sup>	Acetic acid
187	[M+H] <sup>+</sup>	Formic acid
201	[M+H] <sup>+</sup>	Propionic acid
211	[M+H] <sup>+</sup>	Acetic acid
225	[M+H] <sup>+</sup>	Formic acid
239	[M+H] <sup>+</sup>	Propionic acid
249	[M+H] <sup>+</sup>	Acetic acid
263	[M+H] <sup>+</sup>	Formic acid
277	[M+H] <sup>+</sup>	Propionic acid
285	[M+H] <sup>+</sup>	Acetic acid
300	[M+H] <sup>+</sup>	Formic acid
315	[M+H] <sup>+</sup>	Propionic acid
323	[M+H] <sup>+</sup>	Acetic acid
337	[M+H] <sup>+</sup>	Formic acid
351	[M+H] <sup>+</sup>	Propionic acid
361	[M+H] <sup>+</sup>	Acetic acid
375	[M+H] <sup>+</sup>	Formic acid
389	[M+H] <sup>+</sup>	Propionic acid
397	[M+H] <sup>+</sup>	Acetic acid
411	[M+H] <sup>+</sup>	Formic acid
425	[M+H] <sup>+</sup>	Propionic acid
435	[M+H] <sup>+</sup>	Acetic acid
449	[M+H] <sup>+</sup>	Formic acid
463	[M+H] <sup>+</sup>	Propionic acid
473	[M+H] <sup>+</sup>	Acetic acid
487	[M+H] <sup>+</sup>	Formic acid
501	[M+H] <sup>+</sup>	Propionic acid
511	[M+H] <sup>+</sup>	Acetic acid
525	[M+H] <sup>+</sup>	Formic acid
539	[M+H] <sup>+</sup>	Propionic acid
549	[M+H] <sup>+</sup>	Acetic acid
563	[M+H] <sup>+</sup>	Formic acid
577	[M+H] <sup>+</sup>	Propionic acid
587	[M+H] <sup>+</sup>	Acetic acid
601	[M+H] <sup>+</sup>	Formic acid
615	[M+H] <sup>+</sup>	Propionic acid
625	[M+H] <sup>+</sup>	Acetic acid
639	[M+H] <sup>+</sup>	Formic acid
653	[M+H] <sup>+</sup>	Propionic acid
663	[M+H] <sup>+</sup>	Acetic acid
677	[M+H] <sup>+</sup>	Formic acid
691	[M+H] <sup>+</sup>	Propionic acid
701	[M+H] <sup>+</sup>	Acetic acid
715	[M+H] <sup>+</sup>	Formic acid
729	[M+H] <sup>+</sup>	Propionic acid
739	[M+H] <sup>+</sup>	Acetic acid
753	[M+H] <sup>+</sup>	Formic acid
767	[M+H] <sup>+</sup>	Propionic acid
777	[M+H] <sup>+</sup>	Acetic acid
791	[M+H] <sup>+</sup>	Formic acid
805	[M+H] <sup>+</sup>	Propionic acid
815	[M+H] <sup>+</sup>	Acetic acid
829	[M+H] <sup>+</sup>	Formic acid
843	[M+H] <sup>+</sup>	Propionic acid
853	[M+H] <sup>+</sup>	Acetic acid
867	[M+H] <sup>+</sup>	Formic acid
881	[M+H] <sup>+</sup>	Propionic acid
891	[M+H] <sup>+</sup>	Acetic acid
905	[M+H] <sup>+</sup>	Formic acid
919	[M+H] <sup>+</sup>	Propionic acid
929	[M+H] <sup>+</sup>	Acetic acid
943	[M+H] <sup>+</sup>	Formic acid
957	[M+H] <sup>+</sup>	Propionic acid
967	[M+H] <sup>+</sup>	Acetic acid
981	[M+H] <sup>+</sup>	Formic acid
995	[M+H] <sup>+</sup>	Propionic acid
1005	[M+H] <sup>+</sup>	Acetic acid
1019	[M+H] <sup>+</sup>	Formic acid
1033	[M+H] <sup>+</sup>	Propionic acid
1043	[M+H] <sup>+</sup>	Acetic acid
1057	[M+H] <sup>+</sup>	Formic acid
1071	[M+H] <sup>+</sup>	Propionic acid
1081	[M+H] <sup>+</sup>	Acetic acid
1095	[M+H] <sup>+</sup>	Formic acid
1109	[M+H] <sup>+</sup>	Propionic acid
1119	[M+H] <sup>+</sup>	Acetic acid
1133	[M+H] <sup>+</sup>	Formic acid
1147	[M+H] <sup>+</sup>	Propionic acid
1157	[M+H] <sup>+</sup>	Acetic acid
1171	[M+H] <sup>+</sup>	Formic acid
1185	[M+H] <sup>+</sup>	Propionic acid
1195	[M+H] <sup>+</sup>	Acetic acid
1209	[M+H] <sup>+</sup>	Formic acid
1223	[M+H] <sup>+</sup>	Propionic acid
1233	[M+H] <sup>+</sup>	Acetic acid
1247	[M+H] <sup>+</sup>	Formic acid
1261	[M+H] <sup>+</sup>	Propionic acid
1271	[M+H] <sup>+</sup>	Acetic acid
1285	[M+H] <sup>+</sup>	Formic acid
1299	[M+H] <sup>+</sup>	Propionic acid
1309	[M+H] <sup>+</sup>	Acetic acid
1323	[M+H] <sup>+</sup>	Formic acid
1337	[M+H] <sup>+</sup>	Propionic acid
1347	[M+H] <sup>+</sup>	Acetic acid
1361	[M+H] <sup>+</sup>	Formic acid
1375	[M+H] <sup>+</sup>	Propionic acid
1385	[M+H] <sup>+</sup>	Acetic acid
1399	[M+H] <sup>+</sup>	Formic acid
1413	[M+H] <sup>+</sup>	Propionic acid
1423	[M+H] <sup>+</sup>	Acetic acid
1437	[M+H] <sup>+</sup>	Formic acid
1451	[M+H] <sup>+</sup>	Propionic acid
1461	[M+H] <sup>+</sup>	Acetic acid
1475	[M+H] <sup>+</sup>	Formic acid
1489	[M+H] <sup>+</sup>	Propionic acid
1499	[M+H] <sup>+</sup>	Acetic acid
1513	[M+H] <sup>+</sup>	Formic acid
1527	[M+H] <sup>+</sup>	Propionic acid
1537	[M+H] <sup>+</sup>	Acetic acid
1551	[M+H] <sup>+</sup>	Formic acid
1565	[M+H] <sup>+</sup>	Propionic acid
1575	[M+H] <sup>+</sup>	Acetic acid
1589	[M+H] <sup>+</sup>	Formic acid
1603	[M+H] <sup>+</sup>	Propionic acid
1613	[M+H] <sup>+</sup>	Acetic acid
1627	[M+H] <sup>+</sup>	Formic acid
1641	[M+H] <sup>+</sup>	Propionic acid
1651	[M+H] <sup>+</sup>	Acetic acid
1665	[M+H] <sup>+</sup>	Formic acid
1679	[M+H] <sup>+</sup>	Propionic acid
1689	[M+H] <sup>+</sup>	Acetic acid
1703	[M+H] <sup>+</sup>	Formic acid
1717	[M+H] <sup>+</sup>	Propionic acid
1727	[M+H] <sup>+</sup>	Acetic acid
1741	[M+H] <sup>+</sup>	Formic acid
1755	[M+H] <sup>+</sup>	Propionic acid
1765	[M+H] <sup>+</sup>	Acetic acid
1779	[M+H] <sup>+</sup>	Formic acid
1793	[M+H] <sup>+</sup>	Propionic acid
1803	[M+H] <sup>+</sup>	Acetic acid
1817	[M+H] <sup>+</sup>	Formic acid
1831	[M+H] <sup>+</sup>	Propionic acid
1841	[M+H] <sup>+</sup>	Acetic acid
1855	[M+H] <sup>+</sup>	Formic acid
1869	[M+H] <sup>+</sup>	Propionic acid
1879	[M+H] <sup>+</sup>	Acetic acid
1893	[M+H] <sup>+</sup>	Formic acid
1907	[M+H] <sup>+</sup>	Propionic acid
1917	[M+H] <sup>+</sup>	Acetic acid
1931	[M+H] <sup>+</sup>	Formic acid
1945	[M+H] <sup>+</sup>	Propionic acid
1955	[M+H] <sup>+</sup>	Acetic acid
1969	[M+H] <sup>+</sup>	Formic acid
1983	[M+H] <sup>+</sup>	Propionic acid
1993	[M+H] <sup>+</sup>	Acetic acid
2007	[M+H] <sup>+</sup>	Formic acid
2021	[M+H] <sup>+</sup>	Propionic acid
2031	[M+H] <sup>+</sup>	Acetic acid
2045	[M+H] <sup>+</sup>	Formic acid
2059	[M+H] <sup>+</sup>	Propionic acid
2069	[M+H] <sup>+</sup>	Acetic acid
2083	[M+H] <sup>+</sup>	Formic acid
2097	[M+H] <sup>+</sup>	Propionic acid
2107	[M+H] <sup>+</sup>	Acetic acid
2121	[M+H] <sup>+</sup>	Formic acid
2135	[M+H] <sup>+</sup>	Propionic acid
2145	[M+H] <sup>+</sup>	Acetic acid
2159	[M+H] <sup>+</sup>	Formic acid
2173	[M+H] <sup>+</sup>	Propionic acid
2183	[M+H] <sup>+</sup>	Acetic acid
2197	[M+H] <sup>+</sup>	Formic acid
2211	[M+H] <sup>+</sup>	Propionic acid
2221	[M+H] <sup>+</sup>	Acetic acid
2235	[M+H] <sup>+</sup>	Formic acid
2249	[M+H] <sup>+</sup>	Propionic acid
2259	[M+H] <sup>+</sup>	Acetic acid
2273	[M+H] <sup>+</sup>	Formic acid
2287	[M+H] <sup>+</sup>	Propionic acid
2297	[M+H] <sup>+</sup>	Acetic acid
2311	[M+H] <sup>+</sup>	Formic acid
2325	[M+H] <sup>+</sup>	Propionic acid
2335	[M+H] <sup>+</sup>	Acetic acid
2349	[M+H] <sup>+</sup>	Formic acid
2363	[M+H] <sup>+</sup>	Propionic acid
2373	[M+H] <sup>+</sup>	Acetic acid
2387	[M+H] <sup>+</sup>	Formic acid
2401	[M+H] <sup>+</sup>	Propionic acid
2411	[M+H] <sup>+</sup>	Acetic acid
2425	[M+H] <sup>+</sup>	Formic acid
2439	[M+H] <sup>+</sup>	Propionic acid
2449	[M+H] <sup>+</sup>	Acetic acid
2463	[M+H] <sup>+</sup>	Formic acid
2477	[M+H] <sup>+</sup>	Propionic acid
2487	[M+H] <sup>+</sup>	Acetic acid
2501	[M+H] <sup>+</sup>	Formic acid
2515	[M+H] <sup>+</sup>	Propionic acid
2525	[M+H] <sup>+</sup>	Acetic acid
2539	[M+H] <sup>+</sup>	Formic acid
2553	[M+H] <sup>+</sup>	Propionic acid
2563	[M+H] <sup>+</sup>	Acetic acid
2577	[M+H] <sup>+</sup>	Formic acid
2591	[M+H] <sup>+</sup>	Propionic acid
2601	[M+H] <sup>+</sup>	Acetic acid
2615	[M+H] <sup>+</sup>	Formic acid
2629	[M+H] <sup>+</sup>	Propionic acid
2639	[M+H] <sup>+</sup>	Acetic acid
2653	[M+H] <sup>+</sup>	Formic acid
2667	[M+H] <sup>+</sup>	Propionic acid
2677	[M+H] <sup>+</sup>	Acetic acid
2691	[M+H] <sup>+</sup>	Formic acid
2705	[M+H] <sup>+</sup>	Propionic acid
2715	[M+H] <sup>+</sup>	Acetic acid
2729	[M+H] <sup>+</sup>	Formic acid
2743	[M+H] <sup>+</sup>	Propionic acid
2753	[M+H] <sup>+</sup>	Acetic acid
2767	[M+H] <sup>+</sup>	Formic acid
2781	[M+H] <sup>+</sup>	Propionic acid
2791	[M+H] <sup>+</sup>	Acetic acid
2805	[M+H] <sup>+</sup>	Formic acid
2819	[M+H] <sup>+</sup>	Propionic acid
2829	[M+H] <sup>+</sup>	Acetic acid
2843	[M+H] <sup>+</sup>	Formic acid
2857	[M+H] <sup>+</sup>	Propionic acid
2867	[M+H] <sup>+</sup>	Acetic acid
2881	[M+H] <sup>+</sup>	Formic acid
2895	[M+H] <sup>+</sup>	Propionic acid
2905	[M+H] <sup>+</sup>	Acetic acid
2919	[M+H] <sup>+</sup>	Formic acid
2933	[M+H] <sup>+</sup>	Propionic acid
2943	[M+H] <sup>+</sup>	Acetic acid
2957	[M+H] <sup>+</sup>	Formic acid
2971	[M+H] <sup>+</sup>	Propionic acid
2981	[M+H] <sup>+</sup>	Acetic acid
2995	[M+H] <sup>+</sup>	Formic acid
3009	[M+H] <sup>+</sup>	Propionic acid
3019	[M+H] <sup>+</sup>	Acetic acid
3033	[M+H] <sup>+</sup>	Formic acid
3047	[M+H] <sup>+</sup>	Propionic acid
3057	[M+H] <sup>+</sup>	Acetic acid
3071	[M+H] <sup>+</sup>	Formic acid
3085	[M+H] <sup>+</sup>	Propionic acid
3095	[M+H] <sup>+</sup>	Acetic acid
3109	[M+H] <sup>+</sup>	Formic acid
3123	[M+H] <sup>+</sup>	Propionic acid
3133	[M+H] <sup>+</sup>	Acetic acid
3147	[M+H] <sup>+</sup>	Formic acid
3161	[M+H] <sup>+</sup>	Propionic acid
3171	[M+H] <sup>+</sup>	Acetic acid
3185	[M+H] <sup>+</sup>	Formic acid
3199	[M+H] <sup>+</sup>	Propionic acid
3209	[M+H] <sup>+</sup>	Acetic acid
3223	[M+H] <sup>+</sup>	Formic acid
3237	[M+H] <sup>+</sup>	Propionic acid
3247	[M+H] <sup>+</sup>	Acetic acid
3261	[M+H] <sup>+</sup>	Formic acid
3275	[M+H] <sup>+</sup>	Propionic acid
3285	[M+H] <sup>+</sup>	Acetic acid
3299	[M+H] <sup>+</sup>	Formic acid
3313	[M+H] <sup>+</sup>	Propionic acid
3323	[M+H] <sup>+</sup>	Acetic acid
3337	[M+H] <sup>+</sup>	Formic acid
3351	[M+H] <sup>+</sup>	Propionic acid
3361	[M+H] <sup>+</sup>	Acetic acid
3375	[M+H] <sup>+</sup>	Formic acid
3389	[M+H] <sup>+</sup>	Propionic acid
3399	[M+H] <sup>+</sup>	Acetic acid
3413	[M+H] <sup>+</sup>	Formic acid
3427	[M+H] <sup>+</sup>	Propionic acid
3437	[M+H] <sup>+</sup>	Acetic acid
3451	[M+H] <sup>+</sup>	Formic acid
3465	[M+H] <sup>+</sup>	Propionic acid
3475	[M+H] <sup>+</sup>	Acetic acid
3489	[M+H] <sup>+</sup>	Formic acid
3503	[M+H] <sup>+</sup>	Propionic acid
3513	[M+H] <sup>+</sup>	Acetic acid
3527	[M+H] <sup>+</sup>	Formic acid
3541	[M+H] <sup>+</sup>	Propionic acid
3551	[M+H] <sup>+</sup>	Acetic acid
3565	[M+H] <sup>+</sup>	Formic acid
3579	[M+H] <sup>+</sup>	Propionic acid
3589	[M+H] <sup>+</sup>	Acetic acid
3603	[M+H] <sup>+</sup>	Formic acid
3617	[M+H] <sup>+</sup>	Propionic acid
3627	[M+H] <sup>+</sup>	Acetic acid
3641	[M+H] <sup>+</sup>	Formic acid
3655	[M+H] <sup>+</sup>	Propionic acid
3665	[M+H] <sup>+</sup>	Acetic acid
3679	[M+H] <sup>+</sup>	Formic acid
3693	[M+H] <sup>+</sup>	Propionic acid
3703	[M+H] <sup>+</sup>	Acetic acid
3717	[M+H] <sup>+</sup>	Formic acid
3731	[M+H] <sup>+</sup>	Propionic acid
3741	[M+H] <sup>+</sup>	Acetic acid
3755	[M+H] <sup>+</sup>	Formic acid
3769	[M+H] <sup>+</sup>	Propionic acid
3779	[M+H] <sup>+</sup>	Acetic acid
3793	[M+H] <sup>+</sup>	Formic acid
3807	[M+H] <sup>+</sup>	Propionic acid
3817	[M+H] <sup>+</sup>	Acetic acid
3831	[M+H] <sup>+</sup>	Formic acid
3845	[M+H] <sup>+</sup>	Propionic acid
3855	[M+H] <sup>+</sup>	Acetic acid
3869	[M+H] <sup>+</sup>	Formic acid
3883	[M+H] <sup>+</sup>	Propionic acid
3893	[M+H] <sup>+</sup>	Acetic acid
3907	[M+H] <sup>+</sup>	Formic acid
3921	[M+H] <sup>+</sup>	Propionic acid
3931	[M+H] <sup>+</sup>	Acetic acid
3945	[M+H] <sup>+</sup>	Formic acid
3959	[M+H] <sup>+</sup>	Propionic acid
3969	[M+H] <sup>+</sup>	Acetic acid
3983	[M+H] <sup>+</sup>	Formic acid
3997	[M+H] <sup>+</sup>	Propionic acid
4007	[M+H] <sup>+</sup>	Acetic acid
4021	[M+H] <sup>+</sup>	Formic acid
4035</		

## Background Ions in LC-MS

Copper/Acetonitrile Adducts



Courtesy Daniel Thielsch, Agilent Technologies

Methods of Chemical Research

6. 12. 2013

Slide 56

## Avoid/Eliminate Contamination

- Utmost cleanliness of lab articles, solvents etc.
  - Unlike UV-VIS, remember a MS “sees” everything!
- Run solvent only – no HPLC column
  - Step gradient – monitor and identify background ions
  - Locate source of contamination
  - Replace parts, modules or clean system (see next page)
- Run with HPLC column
  - Step gradient – monitor and identify background ions
  - Inject a blank sample
- Use a sample divert valve to avoid sample salts and early eluting sample components enter the MS

Methods of Chemical Research

6. 12. 2013

Slide 57

## Clean-up your HPLC System

- Flush with water (no column, bypass UV-detection cell, outlet to waste) e.g. at 3 mL/min for 15-20 minutes to remove salts
- Flush with i-propanol as above or at low flow rate overnight. Do blank sample injections with i-propanol to clean injection path
- Flush with organics cleaning solution as above  
(e.g. from Agilent (50:25:15:10 acetonitrile/isopropanol/cyclohexane/dichloromethane)  
Do blank sample injections with cleaning solution
- Change back to isopropanol and flush. Do blank injections with i-propanol to clean injection path
- Flush with 100% methanol HPLC grade
- Install column and flush with 100% methanol at elevated temperature
- Switch to mobile phase. In case of gradient analysis do a reverse gradient.
- After pumping down MS connect LC
- As an alternative, one may use a solution of a few % formic acid in acetonitrile
- Formal passivation with strong acid only after checking manufacturer literature

Methods of Chemical Research

6. 12. 2013

58

## Focus on LC-MS

- Important aspects of LC-MS
- Factors Influencing ESI Process and Mass Detection
- HPLC Column Technology, Special Techniques and New Developments
  - Is separation prior to MS needed?
  - HPLC instrumental factors
  - What column diameter to use
  - HPLC Chip column technologies for LC-MS
  - Direct EI LC-MS

Methods of Chemical Research

6. 12. 2013

59

## HPLC Column Technology, Special Techniques and New Developments

- Is separation prior to MS needed?
- HPLC instrumental factors
- What column diameter to use
- HPLC Chip column technologies for LC-MS
- Direct EI LC-MS

Methods of Chemical Research

6. 12. 2013

60

“Chromatographic separation is not required when using MS. Extract individual m/z values, do SIM or choose precursor ions for MS/MS.”

...

Is separation prior to MS needed?

Methods of Chemical Research

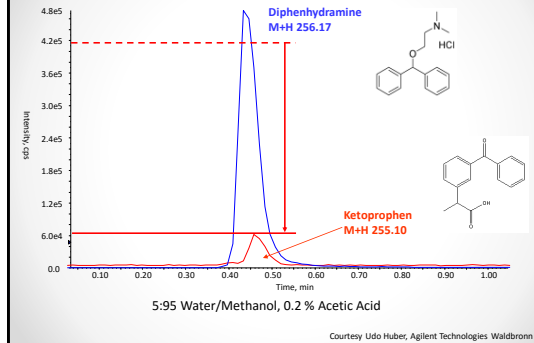
6. 12. 2013

61

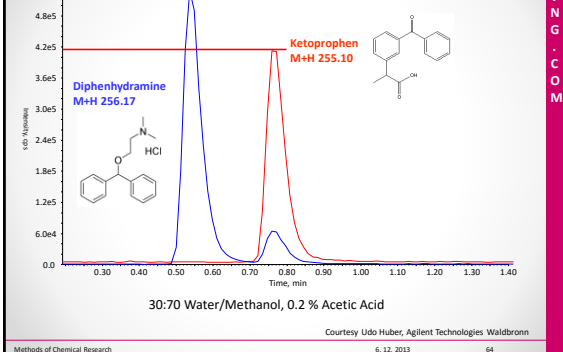
### Why is Separation Needed?

- MS will not/barely differentiate isomeric substances (same MW but different structure of stereoisomers)
- MS will not/barely differentiate isobaric substances (same molecular formula but different molecules)
- May mitigate matrix effect

### No Separation before MS



### Separation before MS

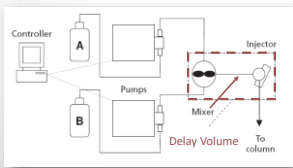


### HPLC Column Technology, Special Techniques and New Developments

- Is separation prior to MS needed?
- HPLC instrumental factors
- What column diameter to use
- HPLC Chip column technologies for LC-MS
- Direct EI LC-MS

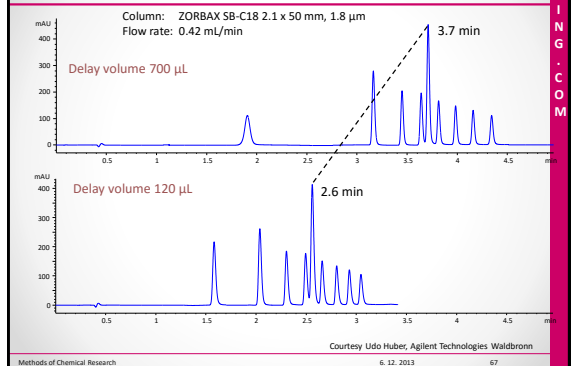
### HPLC Instrumental Factors – System Dwell Volume\*

- Volume from the point of mobile phase mixing to the column head
- Delays the arrival of eluent composition change (gradient)



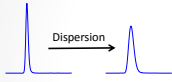
\*J.W. Dolan LCGC 2006, Vol 24, 458-466

### Influence of Dwell Volume



## HPLC Instrumental Factors : Extra Column Dispersion

- "Dispersion is the sample bandspreading or dilution which occurs in connecting tubing, sample valves, flow cells and in column end-fittings."



Peakheight: Reduced sensitivity  
Peakwidth: Resolution loss

- Connection capillaries (I.D. Length)

$$\sigma_v^2 = \frac{\pi \cdot d^4 \cdot F \cdot L}{96 \cdot D_m}$$

Aris-Taylor Gleichung

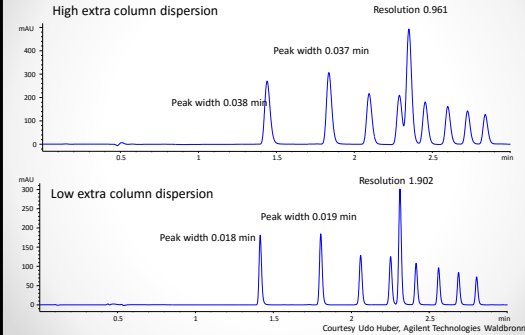
Courtesy Udo Huber, Agilent Technologies Waldbronn

Methods of Chemical Research

6. 12. 2013

68

## HPLC Instrumental Factors : Extra Column Dispersion

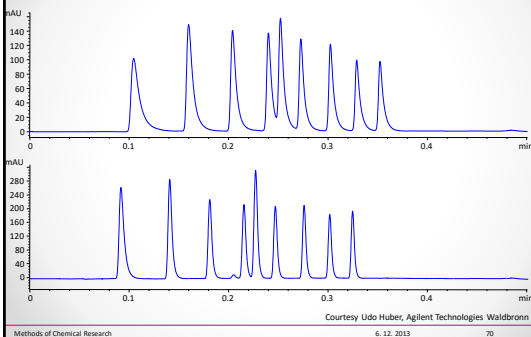


Methods of Chemical Research

6. 12. 2013

69

## Influence of Poor Connections



Methods of Chemical Research

6. 12. 2013

70

## Recommendations for Sample Preparation

### Positive ion ESI

- Dissolve samples in acid
- Basic sites (N and O) bind to proton to give the molecule a positive charge
- Other cat ions (Na+, or K+) may also be used to form positive ions
- Anions (Cl-) may be removed from a molecule to form a positive ions

- ESI works best when the samples are free of salt
- Samples that contain salt can be desalted in many ways (divert valve)

### Negative ion ESI

- Dissolve samples in base
- Acidic sites (acids) give up a proton to form a negative ion.

Methods of Chemical Research

6. 12. 2013

71

## HPLC Column Technology, Special Techniques and New Developments

- Is separation prior to MS needed?
- HPLC instrumental factors
- What column diameter to use
- HPLC Chip column technologies for LC-MS
- Direct EI LC-MS

Methods of Chemical Research

6. 12. 2013

72

## What Column Diameter to Use?

For a chromatographic separation

$$c_{l,max} = \frac{m_{inj,i}}{\sqrt{2\pi} \cdot \sigma_{v,vol}} = \frac{c_{i,0} \cdot V_{inj}}{\sqrt{2\pi} \cdot \sigma_{v,vol}} \quad c_{l,max} \propto \frac{m_i}{d_c^2}$$

When the column diameter is reduced:

For a concentration sensitive detector, response increases with the square root of the diameter ratio in case the same amount of analyte is injected.

Methods of Chemical Research

6. 12. 2013

Slide 73

### Influence of Column Diameter – UV response

Mobile phase: Water/CAN, 0.1% FA gradients from 5 – 90 %B in 15' Inj. vol. 0.5 µl

Peak height increase

Courtesy Stephan Buckenmaier, Agilent Technologies, Waldbronn

Methods of Chemical Research 6. 12. 2013 Slide 74

### Jetstream IF, Ion Funnel

1. Nebulizer w/ 50 µm needle
2. Nebulizing gas (35 psi)
3. Sampling capillary (Vcap 4 kV)
4. Drying gas (200°C, 13 L/min)
5. Sheath gas (400°C, 12 L/min)
6. Nozzle voltage (1500 V)
7. Thermal gradient focussing region

Higher sampling from ion spray

Courtesy Stephan Buckenmaier, Agilent Technologies, Waldbronn

Methods of Chemical Research 6. 12. 2013 Slide 75

### Influence of Column Diameter – MS response

with Jetstream IF and Ion Funnel

Cpd	H-ratio (0.3/2.1)	A-ratio (0.3/2.1)
#3	0.6	1.0
#4	0.4	0.8
#6	1.5	2.7
#9	0.6	1.0
#13	0.4	0.7

Advances in ionization and sampling efficiency peak abundances are maintained, independent of column-ID, flow rates, and sample concentration.

Courtesy Stephan Buckenmaier, Agilent Technologies, Waldbronn

Methods of Chemical Research 6. 12. 2013 Slide 76

### Columns for NanoESI/MS

Nano-electrospray MS mandates flow rates 100 – 1000 nL/min. For (U)HPLC to work properly the solvent has to move with a velocity of 1-10 mm/s

$$d_c = \sqrt{\frac{4 \cdot F}{\epsilon \cdot \pi \cdot u}}$$

Column I.D. must be between 0.05 and 0.15 mm

Sensitivity of NanoESI/MS increases dramatically at flow rates <50 nL/min

Proteomics research  
 → Ultra small samples  
 → Ultra high sensitivity mandated

Methods of Chemical Research 6. 12. 2013 Slide 77

### Example – NanoESI Interface Agilent

Methods of Chemical Research 6. 12. 2013 Slide 78

### Typical Set-up of Nanoflow HPLC Electro-Spray Ionization MS System

- Sensitivity
  - 75µm ID analytical column
- Challenging to Set-up & Maintain
  - Multiple Parts
  - Possible leaks, misalignments
- Robustness & Ease-of-use
  - Clogging of spray needle
  - After part replacement system can take hours to stabilize
- Chromatographic Fidelity
  - Rel. large extra column volume leads to band broadening
  - Limited to peptide separation

Methods of Chemical Research 6. 12. 2013 Slide 79

### Agilent HPLC-Chip Technology

Height 50  $\mu\text{m}$   
 Width 75  $\mu\text{m}$   
 Length 43 mm  
 Particle size 5  $\mu\text{m}$

Methods of Chemical Research 6. 12. 2013 Slide 80

### HPLC-Chip MS

Essential Components for Nanoflow HPLC are chip-integrated

- No extra column volume
- Chromatographic performance is conserved
- Sensitivity for ESI LC-MS easily obtained
- Avoids leaks and misalignments

Stator Rotor Sixport valve Autosampler Trap Column Waste Column 43x0.075x0.05 mm Nanoflow HPLC pump Nanospray tip

Methods of Chemical Research 6. 12. 2013 Slide 81

### HPLC-Chip - Phosphopeptide Enrichment Chip Design

Waste P6 P4 P3 P2 P1 Loading Pump RP-TiO<sub>2</sub>-RP Analytical Pump Analytical Column Spray Tip

(c)

RP RP TiO<sub>2</sub> P1 P4

Methods of Chemical Research 6. 12. 2013 Slide 82

### Thanks for your attention

...

PDF Copy can be found at <http://www.rozing.com>  
 (registration required)

Methods of Chemical Research 6. 12. 2013 83