

Thomas Waeghe, Ph.D.
August 16, 2005

Choosing a Column and Conditions for LC/MS

Toll Free Telephone Number for US/Canada:		888-839-9630	
International Telephone Number:		01-703-871-3881	
Toll Free International Numbers:			
Belgium	0800 72206	Norway	800 15405
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Italy	800 872246	UK	0500 892521
Netherlands	0800 0221179		



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Starts in **Five** Minutes



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Starts in One Minute



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Presenter: Thomas Waeghe, Ph.D.



In November 2001, Tom joined Agilent Technologies as a Technical Marketing Engineer. He currently is an Inside Application Engineer in the Americas Field Organization in the Customer Contact Center at the Little Falls site here in Wilmington, Delaware. His primary responsibility is to provide LC column and application technical support for Agilent's Americas' customers. Tom also supports solid-phase extraction applications, and has secondary responsibilities for GC columns and applications and capillary electrophoresis capillaries and applications. Tom is involved in writing new Frequently Asked Questions for the Agilent web site, and has written articles on several topics for Agilent in Pharmaceutical Analysis and Separation Times magazines.

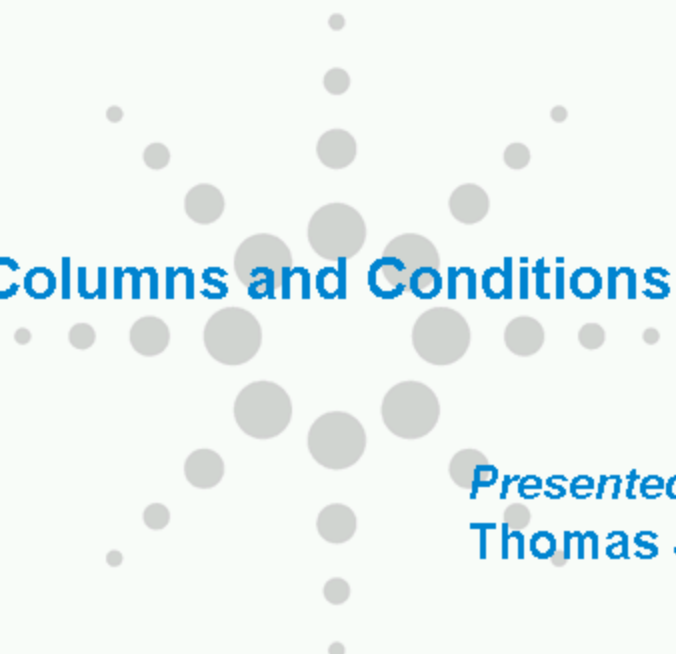
Before joining Agilent Technologies, Dr. Waeghe came to Delaware in 1983 and joined DuPont's Agricultural Products business as an analytical chemist.. Tom supported a variety of different functions within the agrichemical business unit, including formulation development, process development, product registration, and residue analysis.



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Page 4

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Choosing Columns and Conditions for LC/MS

Presented by
Thomas J. Waeghe



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Choosing Columns and Conditions for LC/MS

Atmospheric Pressure Ionization (API) LC/MS Techniques

1. Electrospray (ESI)
2. Atmospheric Pressure Chemical Ionization (APCI)
3. Atmospheric Pressure Photo Ionization (APPI)

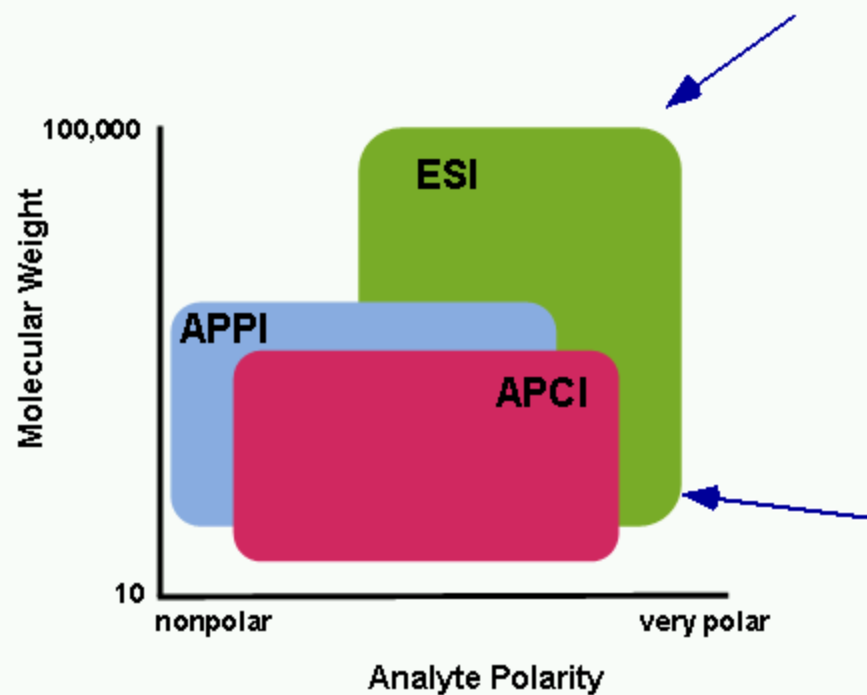


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Page 6

Atmospheric Pressure Ionization (API) LC/MS Techniques

Relative Applicability of Atmospheric Pressure Ionization Techniques



Relative Applicability of Atmospheric Pressure Ionization Techniques

Definition and Application of Electrospray Technique

- **Electrospray can be used for high and low molecular weight ionizable solutes.**
 - **Compounds which are ions in solution**
 - **Samples that carry multiply charges in solution (e.g., peptides, proteins)**
 - **Samples that contain heteroatoms**
 - **Compounds which can accept a charge by induction**
 - **Avoid samples with extremely non-polar analytes, where charge induction is inefficient**
- **Ions are formed from the mobile phase by ejection from shrinking charged droplets. The solvent is dried to shrink the particles and a strong electric field is applied to the charged droplet.**

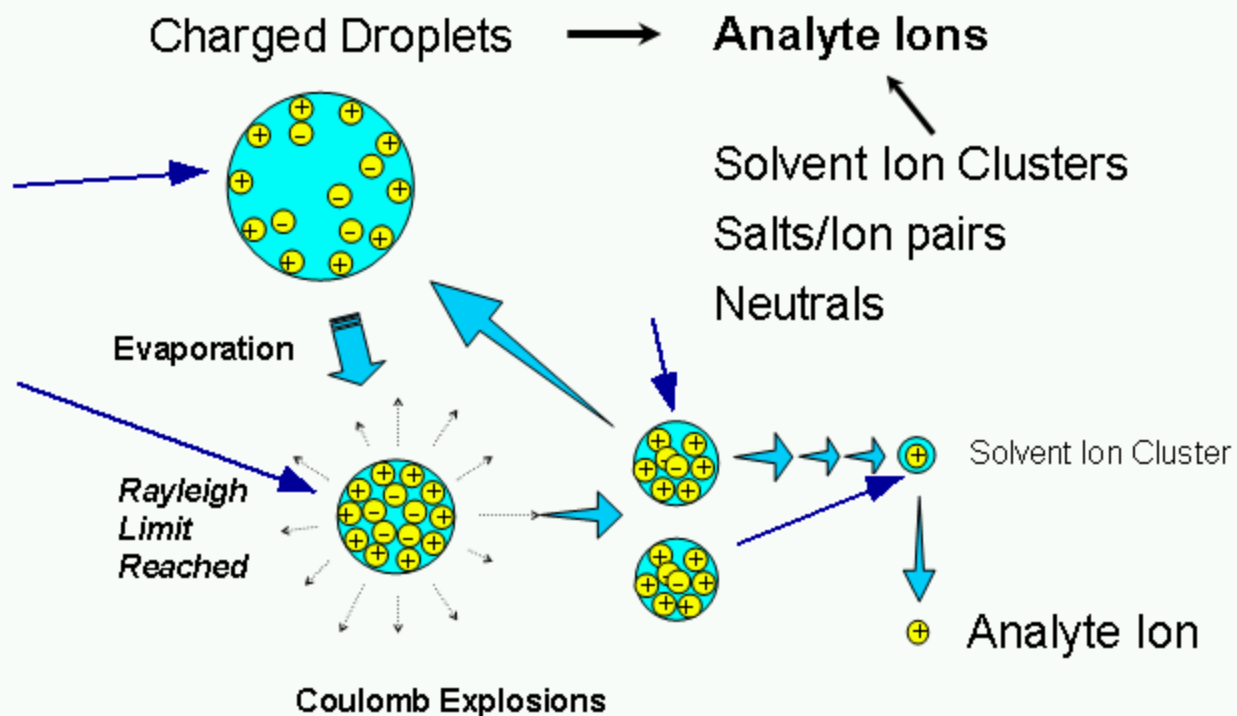


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Page 8

Definition and Application of Electrospray Technique

ESI Process

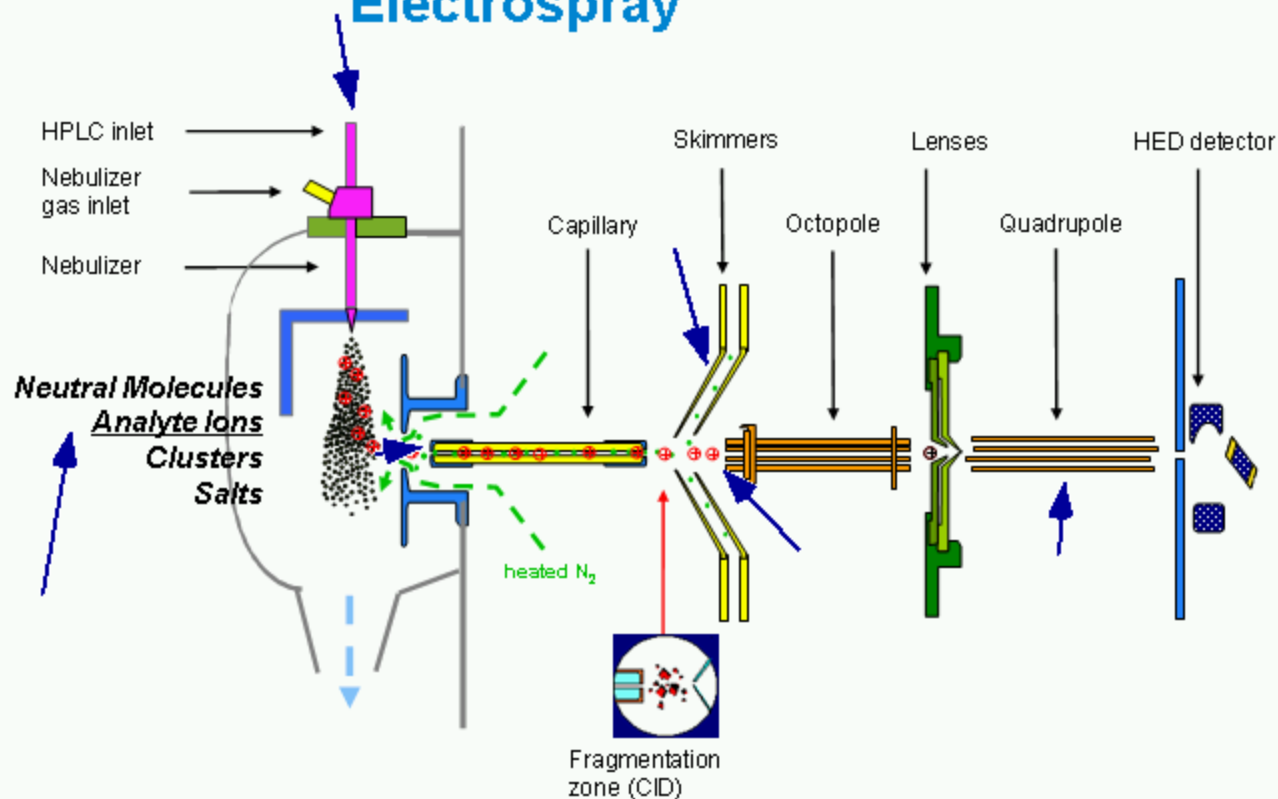


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Page 9

ESI Process

Agilent 1100 LC/MSD VL AP Electrospray



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Page 10

Slide 10

Key Chromatographic Points in Use of Electrospray

- Mobile phase can provide charged analytes
 - Mobile phase pH is critical
 - Know the pK_a values of sample components
- • Analyze acids, bases – anything with a charge
- Operates over a wide flow rate range – 1 μ L/min up to 1 mL/min (with Agilent LC/MS)
- • Accommodates columns from nano/capillary (proteins and peptides) up to analytical 4.6 mm ID with smaller IDs usually preferred for best sensitivity
- • Compatible with reversed-phase solvents
- • Reversed-phase column selection for some charged analytes may be difficult due to limited retention



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Page 11

Key Chromatographic Points in Use of Electrospray

Definition and Application of APCI Technique

- In APCI, electrons discharged from a corona needle ionize the vaporized mobile phase, which subsequently undergoes a gas phase chemical reaction in which charged solvent molecules transfer that charge to the gas phase analyte
- Ions formed in the spray by charge transfer from solvent molecules (>> No preformed ions needed for APCI)
- Good for small molecules (< 1500), polar to non-polar and semi-volatile.
- Not good for biomolecules because they are rarely volatile
- Avoid samples that are typically charged in solution because ESI will usually be more sensitive.
- Avoid samples that are extremely thermally unstable or photosensitive because they will tend to fragment completely in APCI. For the latter, try APPI instead.

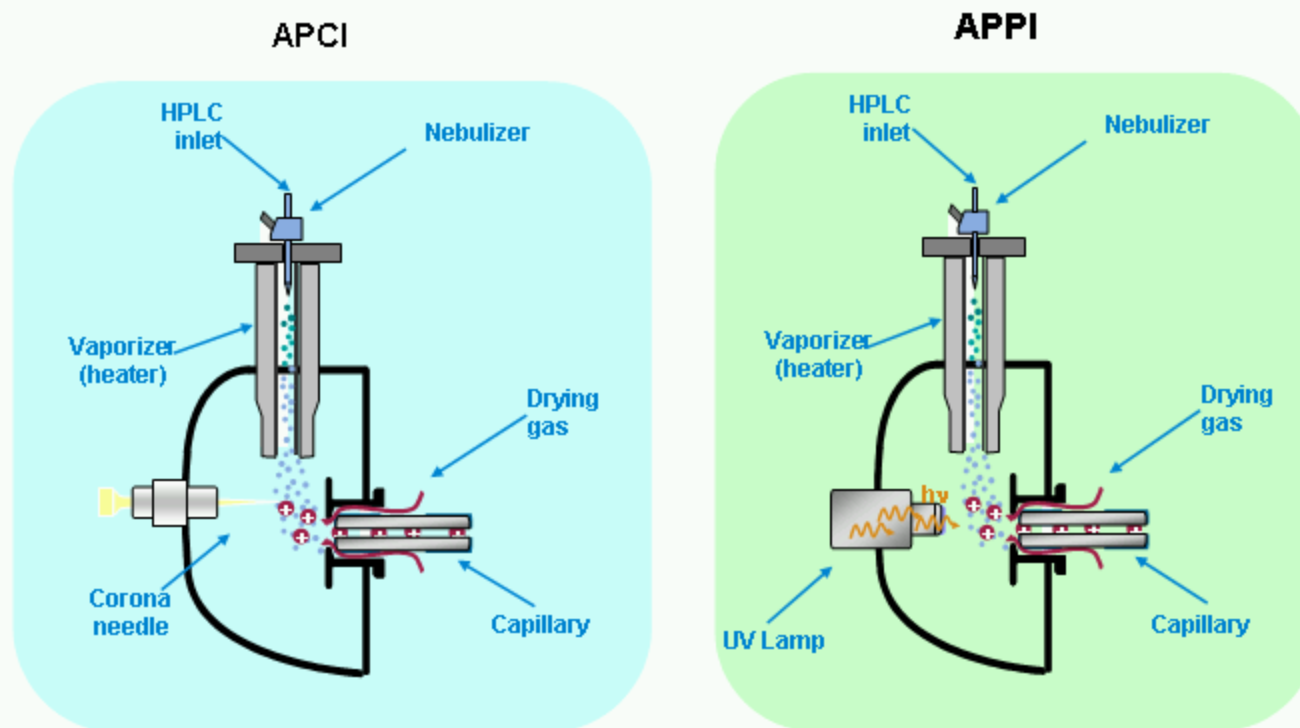


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Page 12

Definition and Application of APCI Technique

APCI and APPI Sources for the LC/MSD



• **Corona needle in APCI vs. UV lamp in APPI**

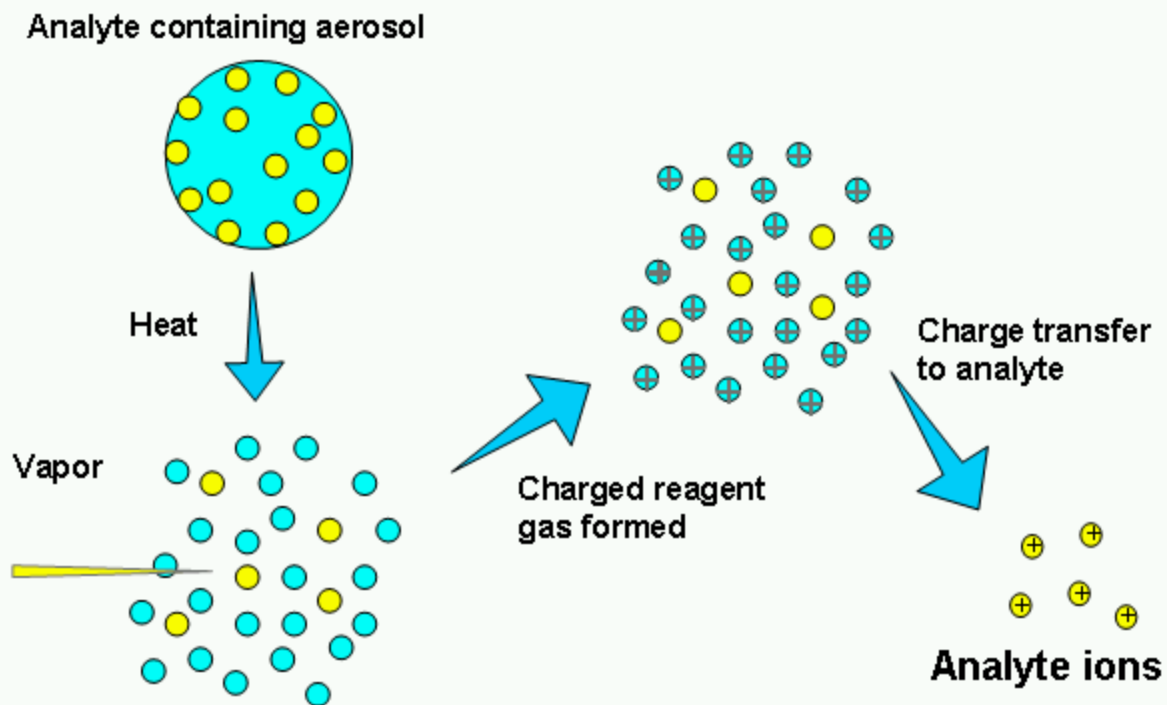


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Page 13

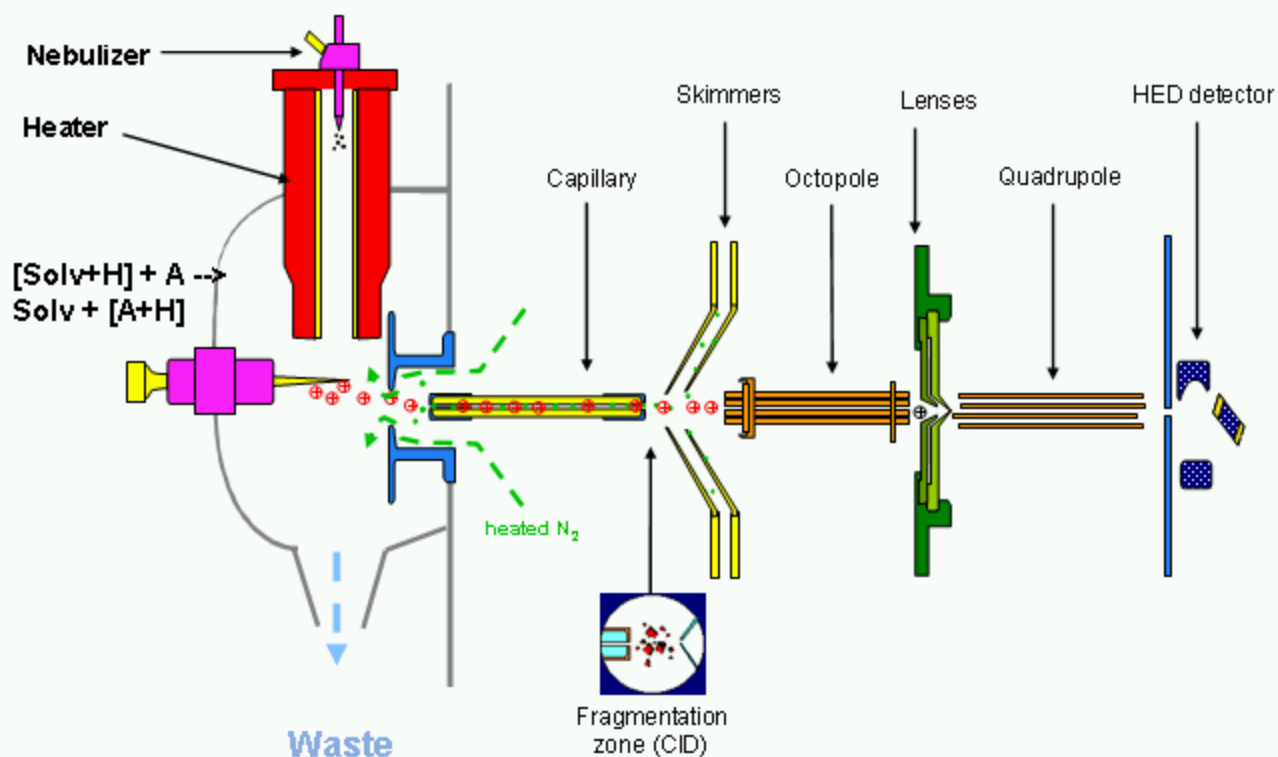
APCI and APPI Sources for the LC/MSD

AP Chemical Ionization (APCI) Process



Agilent 1100 LC/MSD - APCI

Vaporize in gas phase and ionize the gas with a discharge



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Page 15

Slide 15

Key Chromatographic Points in Use of APCI

- Operates over a wide flow rate range – up to 1.5 mL/min
- Therefore, can use with 2.1 – 4.6 mm id columns, with 3.0 mm id popular for APCI and ESI with same instrument
- Use with reversed-phase buffered mobile phases up to 100 mM
- Use with selected normal phase solvents to accommodate nonpolar analytes.

Definition and Application of APPI Atmospheric Photoionization

- **APPI** – atmospheric pressure photoionization is a new source for LC/MS
- Good for many non-polar compounds, e.g., PAHs, Vitamin E
- Two modes – with dopant and without dopant
- Dopant - a solvent that ionizes easily at the wavelength of the photoionization lamp and then transfers a charge to the analytes
- No dopant – the analyte is ionized directly by a photon
- APPI is a complimentary API technique relative to APCI and ESI
- Simple method development – no corona discharge current optimization – lamp is either on or off



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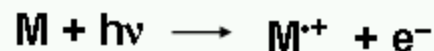
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Page 17

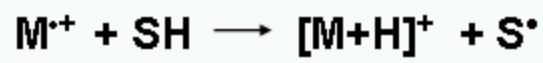
Definition and Application of APPI Atmospheric Photoionization

APPI Mechanisms

Direct APPI



Analyte molecule M is ionized to molecular ion $M^{+\bullet}$
Analyte ionization potential must be below
photon energy

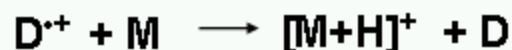


Molecular ion $M^{+\bullet}$ may abstract a hydrogen
to form $[M+H]^{+}$

Dopant APPI



Photoionizable dopant D is in excess and yields
many $D^{+\bullet}$ ions

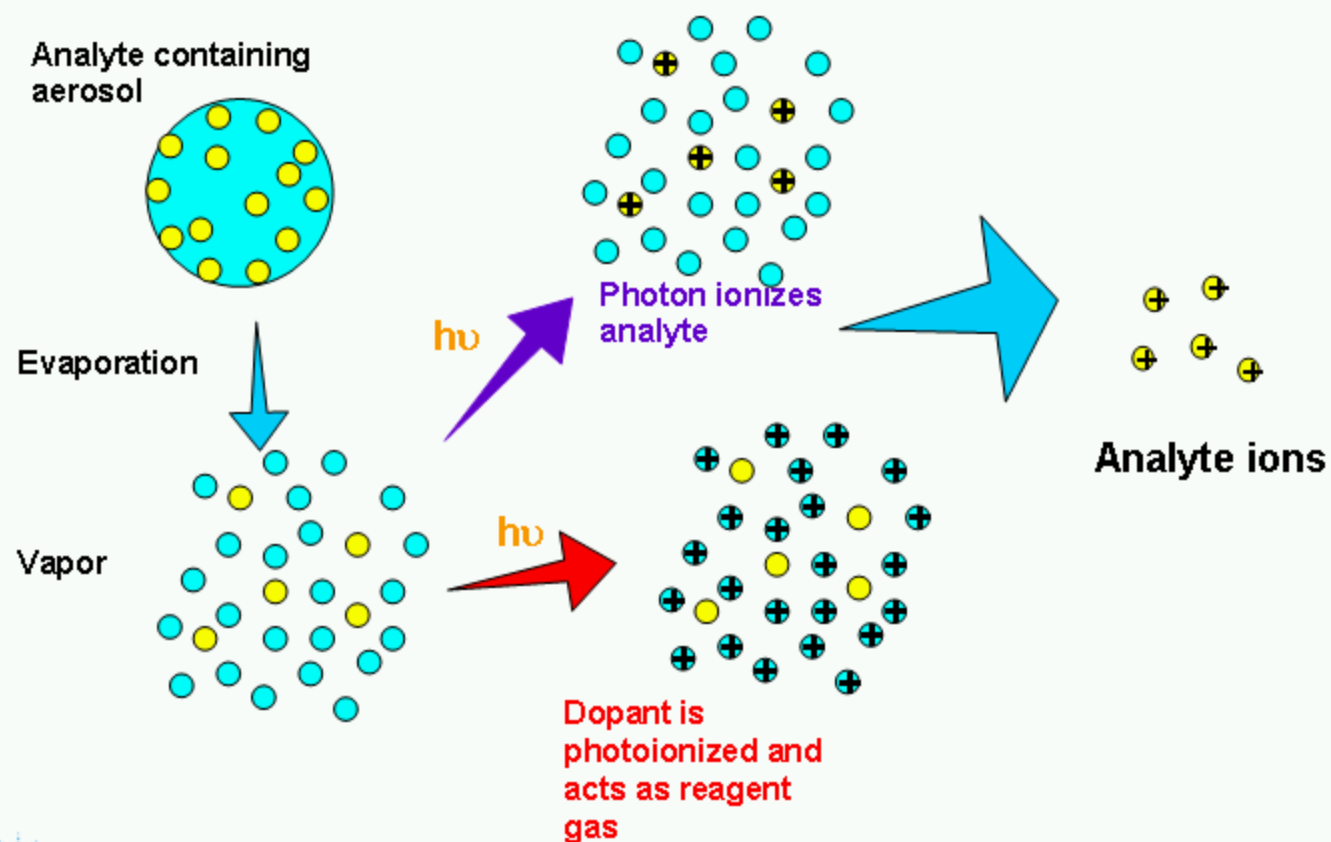


Analyte M ionizes by proton transfer from
dopant or solvent



$D^{+\bullet}$ ionizes analyte M by electron transfer

APPI Process



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Page 19

APPI Process

Atmospheric Pressure Ionization Techniques

ESI

- Volatility not required
- Preferred technique for thermally sensitive analytes
- Ions formed in solution
- Can form multiply charged ions
- Upper limit for molecular weight at 100,000 Da – ionization technique for small molecules and biomolecules

APCI/APPI

- Some volatility required
- Analyte must be thermally stable
- Ions formed in gas phase—produces singly charged ions
- Upper limit for molecular weight at 1000 Da – ionization technique for small molecules



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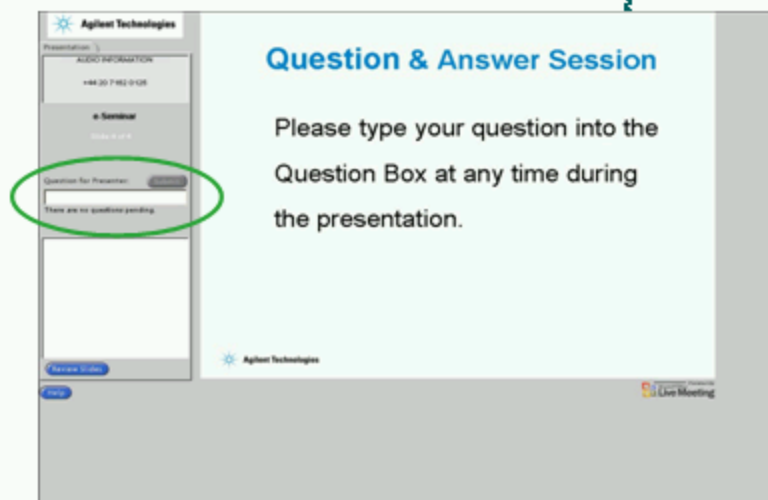
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Atmospheric Pressure Ionization Techniques

Break Number 1



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Page 21

Slide 21

Selecting Columns and Conditions for LC/MS

- **Column Configuration**
 - Column length – chromatographic resolution
 - Column particle size – chromatographic resolution
 - Column internal diameter – flow rate, available sample
- **Mobile Phase Selection**
 - Enhance ionization of solutes – based on AP technique used – ESI, APCI, APPI
 - Improve sensitivity
 - Volatile additives
- **Bonded phase**
 - Choose for optimum lifetime and low bleed at pH of mobile phase



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Page 22

Selecting Columns and Conditions for LC/MS

Agilent LC/MS Column Configurations

Column Type	Column I.D.	Column Lengths	Particle Sizes	Flow Rate Range
Nano	0.075, 0.10 mm	50, 150 mm	3.5 μ m	100 – 600 nL/min
Capillary	0.3, 0.5 mm	35, 150, 250 mm	3.5, 5 μ m	1 – 10 μ L/min
MicroBore	1.0 mm	30, 50, 150 mm	3.5, 5 μ m	30 – 60 μ L/min
Narrow Bore	2.1 mm	15 – 250 mm	1.8, 3.5, 5 μ m	0.1 – 0.3 mL/min
Solvent Saver	3.0 mm	50 – 250 mm	3.5, 5 μ m	0.3 – 1 mL/min
Analytical	4.6 mm	15 – 250 mm	1.8, 3.5, 5 μ m	1 – 1.5 mL/min

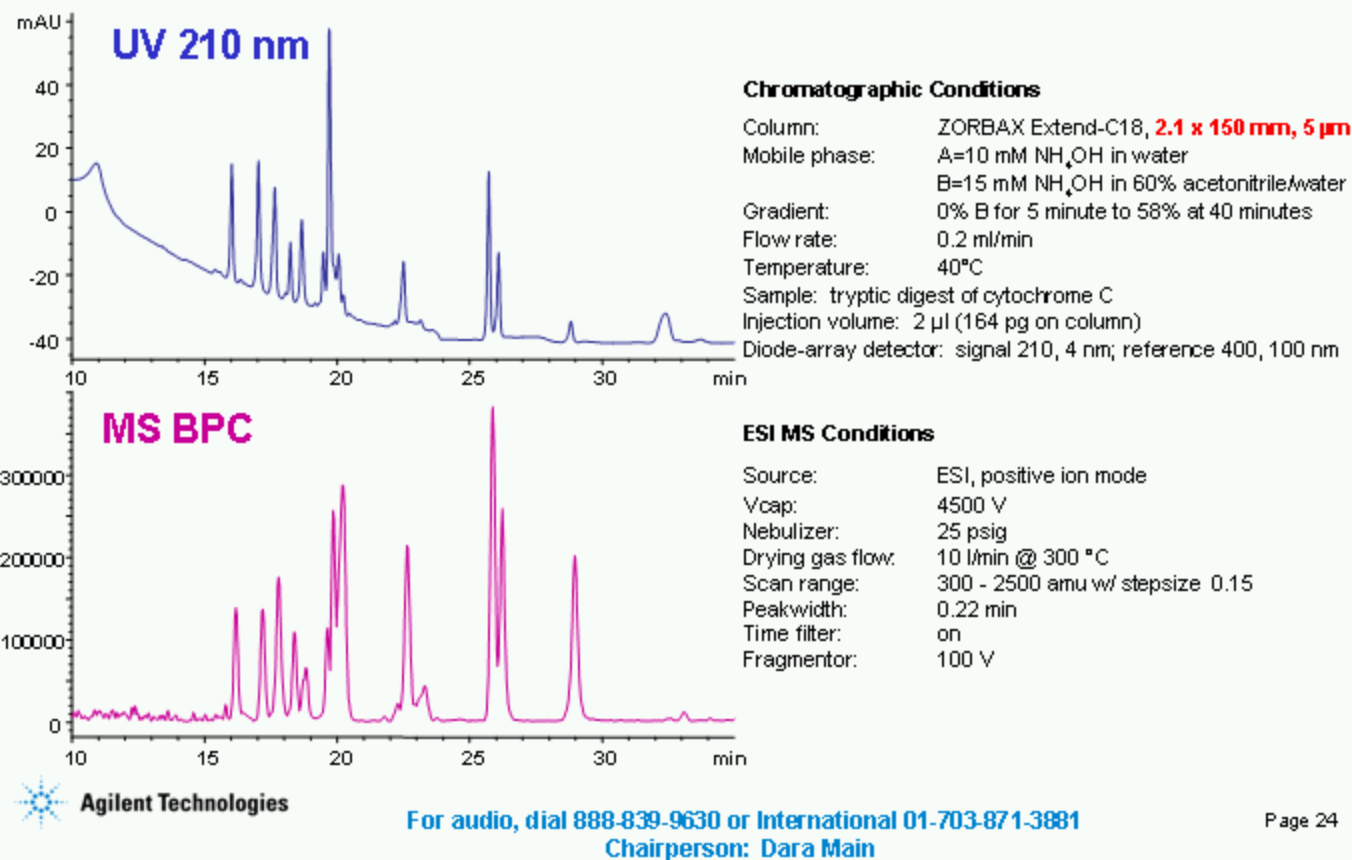


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Page 23

Agilent LC/MS Column Configurations

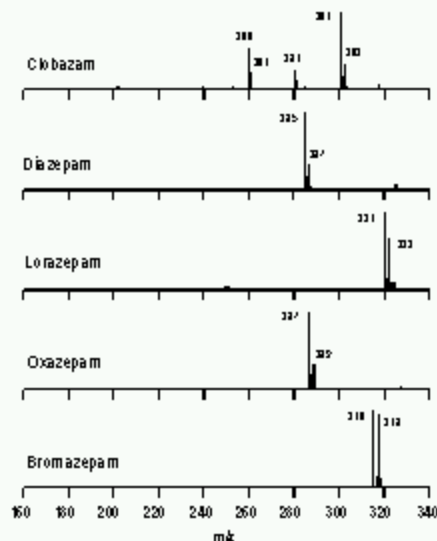
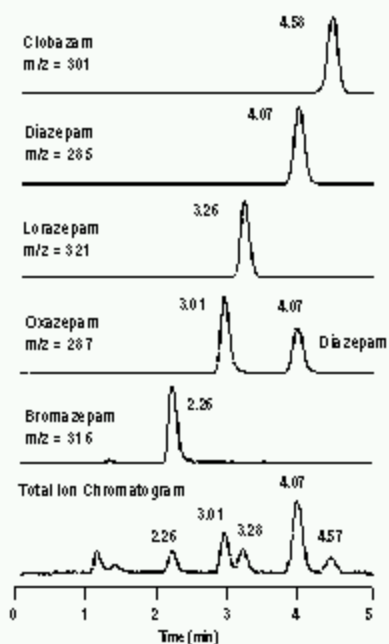
Choose Longer Columns for Complex Samples



Choose Longer Columns for Complex Samples

Choose Longer Columns for Maximum Chromatographic Resolution

2.1 x 150 mm Column



Column: ZORBAX SB-CN
2.1 x 150 mm, 5 μ m
Mobile Phase: 60% water + 0.2% formic acid
40% ACN
Flow Rate: 0.25 mL/min
Detection: Electrospray Positive Ion
Sample: Benzo diazepines
1. Bromazepam
2. Oxazepam
3. Lorazepam
4. Diazepam
5. Clobazam

• Longer columns provide chromatographic resolution, while the MS identifies the components.



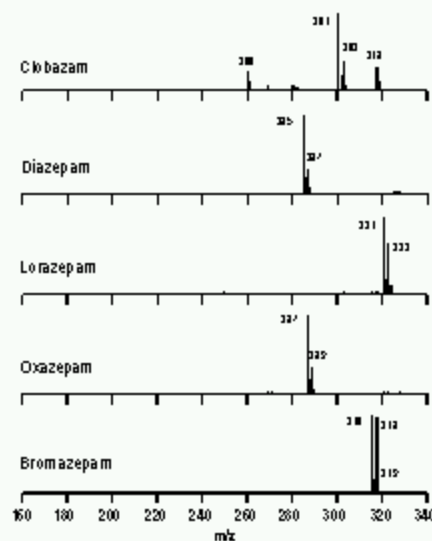
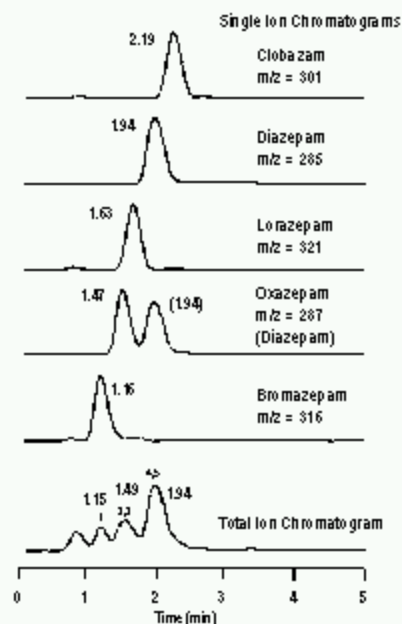
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Page 25

Choose Longer Columns for Maximum Chromatographic Resolution 2.1 x 150 mm Column

Choose Shorter Columns for Fast Analysis with MS Identification

2.1 x 50 mm Column



Column: ZORBAX SB-CN
2.1 x 50 mm, 5 μ m
Mobile Phase: 60% water + 0.2% formic acid
40% ACN
Flow Rate: 0.20 mL/min
Detection: Electrospray Positive Ion
Sample: Benzodiazepines
1. Bromazepam
2. Oxazepam
3. Lorazepam
4. Diazepam
5. Clobazam

- A shorter column provides faster analysis and enough separation for accurate identification by MS.



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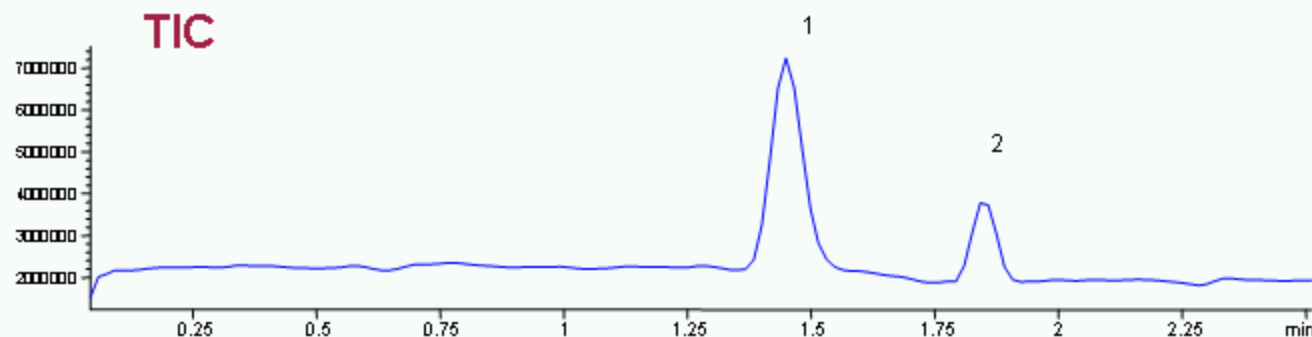
Page 26

Choose Shorter Columns for Fast Analysis with MS Identification 2.1 x 50 mm Column

Choose Short Columns with Sub Two-Micron Particles for Rapid LC/MS

APCI Analysis of Clindamycin and Lincomycin

Column: ZORBAX Rapid Resolution HT SB-C18 2.1 x 30 mm, 1.8 μ m Mobile Phase: Gradient: 15 – 50% B in 1 min, hold for 1.5 min, A: 0.2% formic acid pH, 2.8 B: ACN + 0.2% formic acid Post time: 1.5 min Flow Rate: 0.5 mL/min Injection Volume: 1 μ L Temperature: Ambient
HPLC: Agilent 1100 with WPS and ADVR on
Detection: APCI, Positive ion MS Conditions: Peakwidth: 0.10 min Scan: 150 – 600 Da, step 0.1 Fragmentor: 70 Gas Temp: 350° C Vaporizer: 350° C
Drying gas: 12 l/min, Nebulizer pres. 50 psi, Vcap +3000V, Corona: 4.0 mA
Sample: 1. Lincomycin 2. Clindamycin



- High throughput gradient LC/MS is readily achieved with the Rapid Resolution HT columns.
- A narrow bore (2.1 mm id) column and formic acid were chosen for LC/MS compatibility.



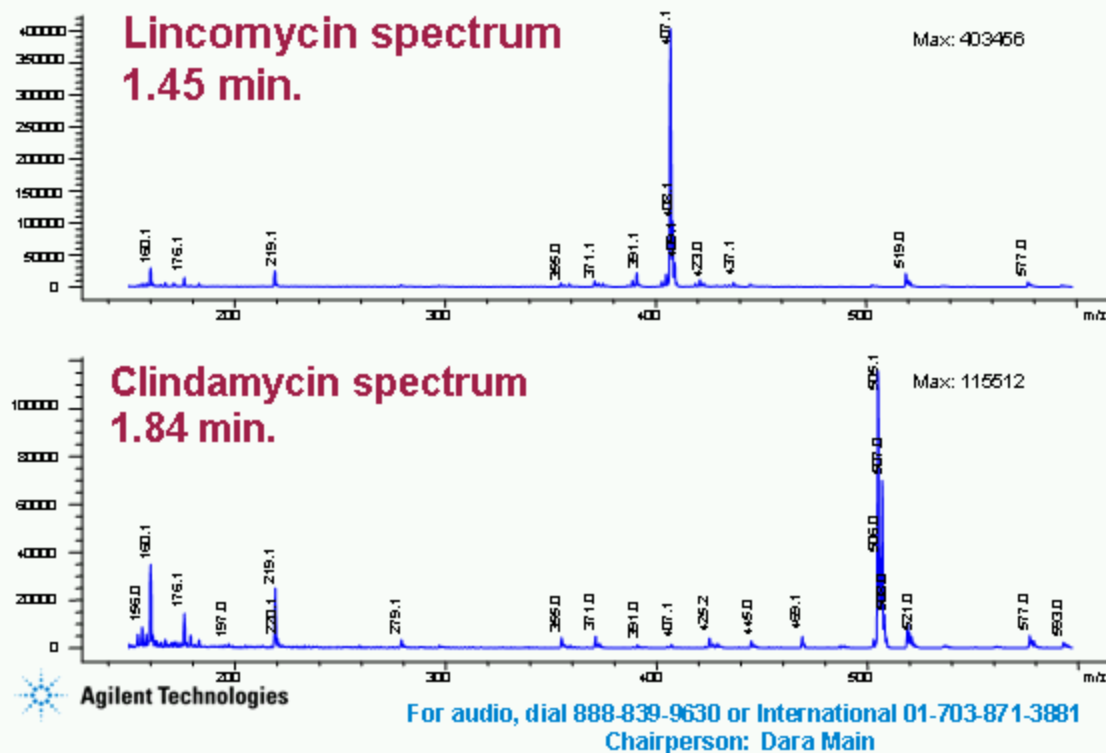
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Page 27

Choose Short Columns with Sub Two-Micron Particles for Rapid LC/MS APCI Analysis of
Clindamycin and...

Mass Spectra of Clindamycin and Lincomycin High Throughput LC/MS with Rapid Resolution HT Column

Conditions: see previous slide

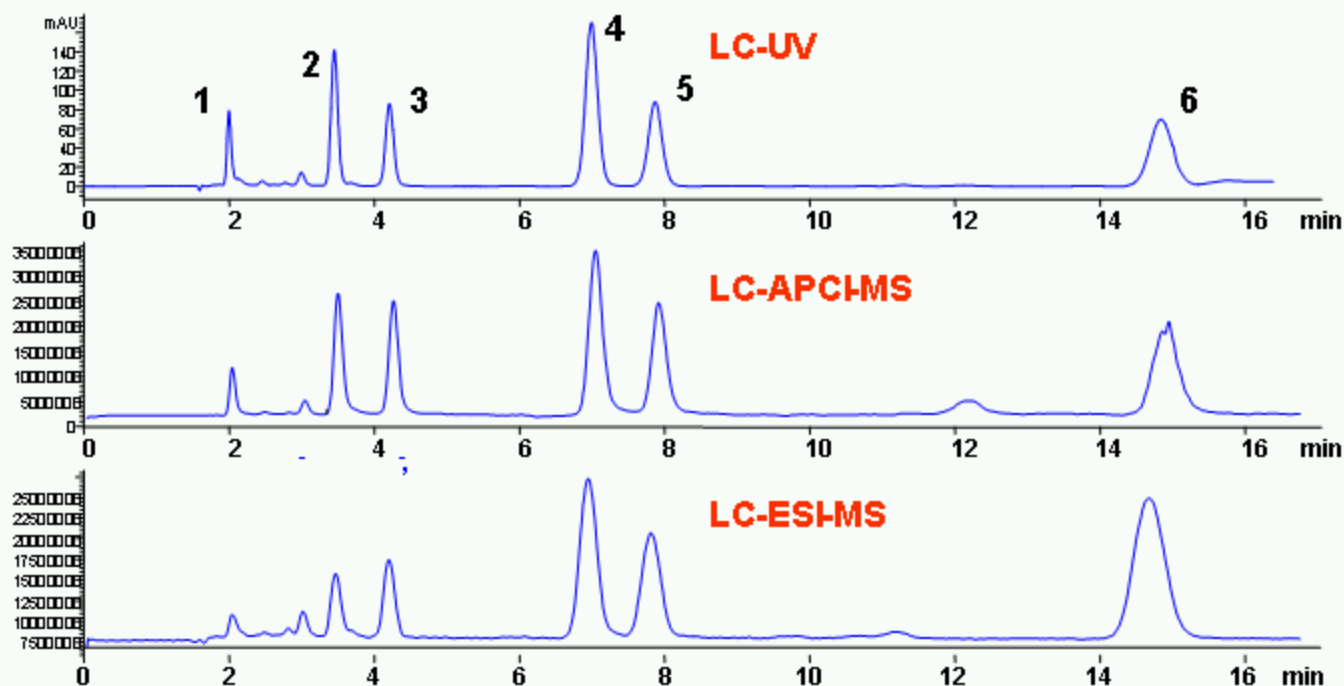


Page 28

Mass Spectra of Clindamycin and Lincomycin High Throughput LC/MS with Rapid Resolution HT Column

Solvent Saver 3.0 mm I.D. is Ideal for Converting from LC to LC/MS

Conditions: Column: ZORBAX SB-C18, Solvent Saver Plus, 3.0 x 100 mm, 3.5 μ m, Mobile Phase: 70% Methanol + 0.4% formic acid: 30% water + 0.4% formic acid Flow Rate: 0.425 mL/min Detection A: UV 254 nm B: Positive ion APCI C: Positive Ion Electrospray
Sample: Steroids 1. Triamcinolone 2. Hydrocortisone 3. Cortisone acetate 4. Deoxycorticosterone 5. Hydroxyprogesterone 6. Progesterone



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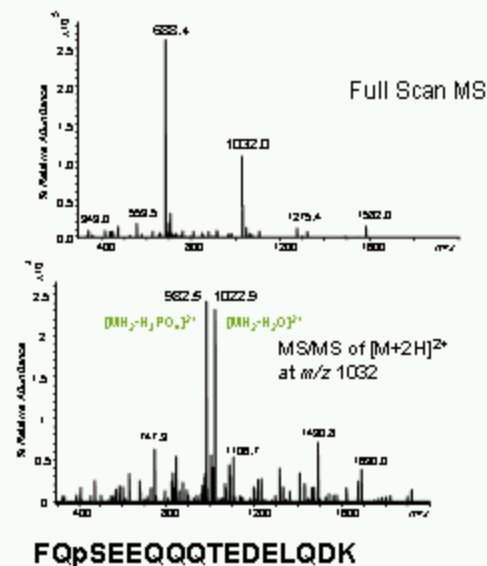
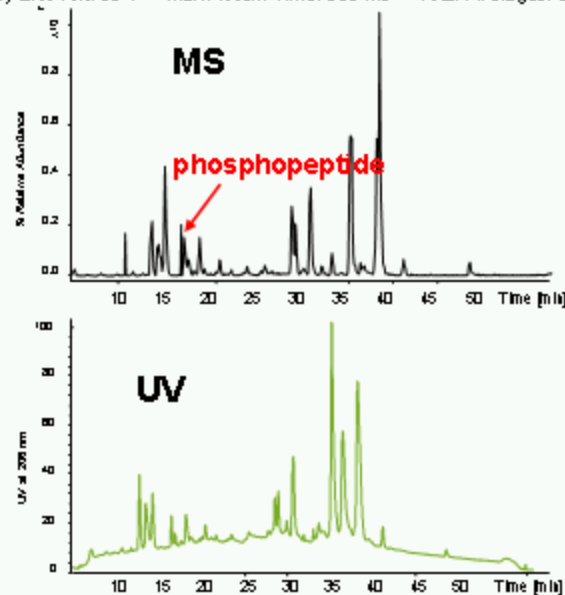
Page 29

Solvent Saver 3.0 mm I.D. is Ideal for Converting from LC to LC/MS

Choose Capillary Columns for LC/MS of Proteins and Peptides

Identify Peptide Phosphorylation Sites by LC/MS

Column: ZORBAX 300SB-C18, 0.3 x 150 mm, 5 μ m Mobile phase gradient: 5–55% B in 50 min, to 85% B from 55–57 min. A: 0.1% formic acid in water B: 0.1% formic acid in ACN, Flow Rate: 5.5 μ L/min Detection: UV 206 nm Sample: Beta casein digest (4 pmol) Injection Volume: 100 nL LC/MS: Pos. Ion ESI with LC/MSD trap –, Vcap 4000 V, Drying gas flow: 7 l/min Drying gas temperature: 250°C Nebulizer: 15 psi, Capillary Exit Volt: 50 V Max Accum Time: 300 ms Total Averages: 3 Isolation Width: 3 m/z Frag Amplitude: 1.0 V



• LC/MS/MS identifies the phosphorylated peptide site in the digest sample.

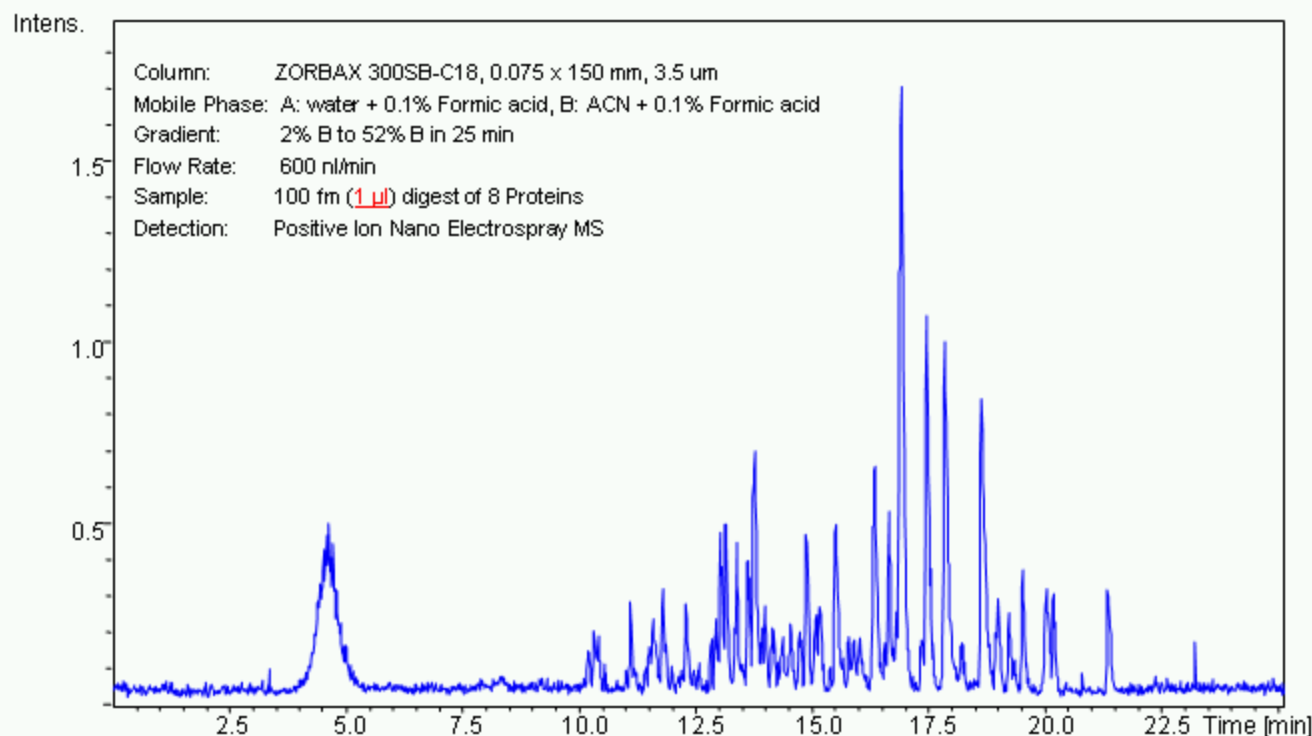
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Page 30

Choose Capillary Columns for LC/MS of Proteins and Peptides Identify Peptide Phosphorylation Site...

Protein Separation on a NanoHPLC Column with Nano-electrospray MS Detection



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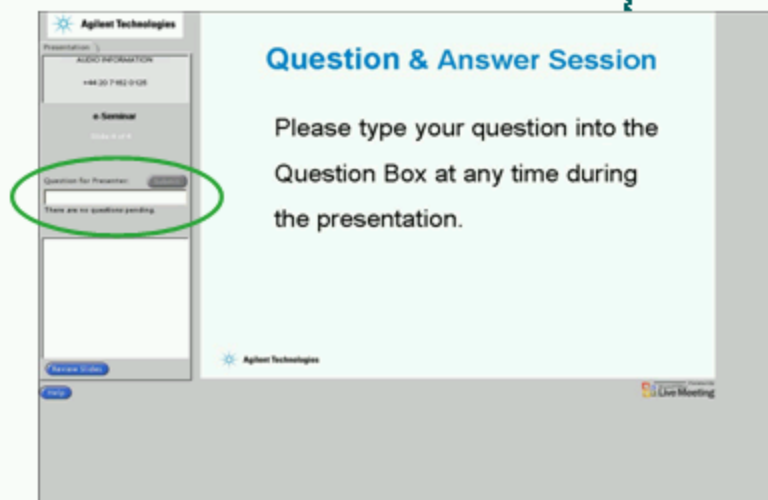
Page 31

Protein Separation on a NanoHPLC Column with Nano-electrospray MS Detection

Break Number 2



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Page 32

Break Number 2

LC/MS Mobile Phase Selection - ESI

- **Select more volatile buffers to reduce the buildup of salts in the MS**
 - TFA, formate, acetate, ammonium hydroxide
- **Adjust mobile phase pH to ionize analyte ions – and possibly control polarity of ions**
 - Based on sample pKa
- **Use solvents that enhance ion desorption (low surface tension, low heat of vaporization)**
- **Avoid solvents and additives that suppress ionization**
 - Salts suppresses the ionization process
 - Detergents interfere with the evaporation process
- **Consider post column addition of solvents if no mobile phase choice produces good chromatography – “TFA Fix”**



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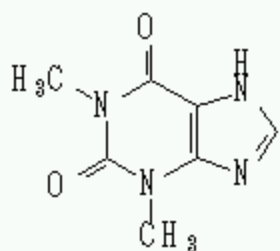
Page 33

LC/MS Mobile Phase Selection - ESI

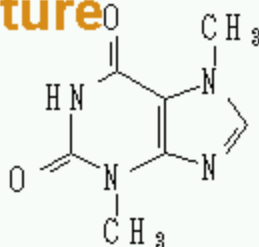
Choosing a Volatile Mobile Phase

Comparison of Results with Volatile and Non-volatile Mobile Phase Additives

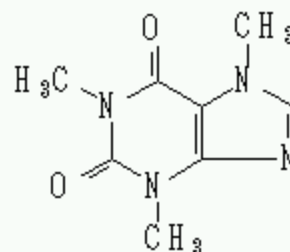
Chemical Structure



Theophylline (TP)
M.W=180.17
pKa <1, 8.6



Theobromine (TB)
M.W=180.17
pKa <1, 10.0



Caffeine (CF)
M.W=194.19
pKa = 14

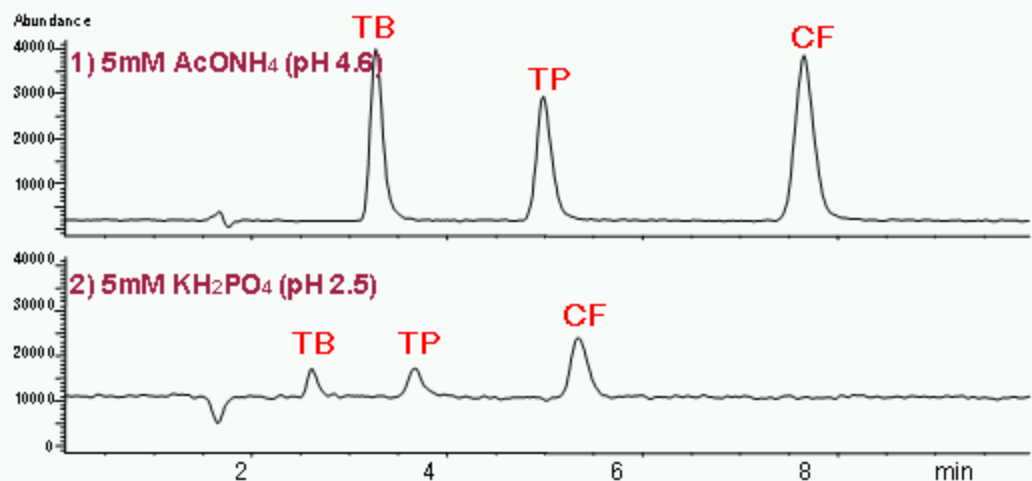


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Page 34

Choosing a Volatile Mobile Phase Comparison of Results with Volatile and Non-volatile Mobile Phase Additives

Comparison Between Volatile Buffer and Non Volatile Phosphate Buffer (pH 2.5) (10 ppm injection:scan mode TIC)

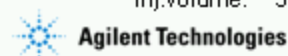


LC conditions

Column: ZORBAX Eclipse XDB-C18
2.1 x 150 mm, 5µm
Mobile Phase: 1) 5mM AcONH₄ (pH 4.6)/MeOH=80:20
2) 5mM KH₂PO₄ (pH 2.5)/MeOH=80:20
Flow rate: 0.2mL/min
Temp: 40°C
Inj. volume: 5µL

MS conditions

Ionization: ESI
Mode: Positive
Mass range: m/z 100~200
Capillary volt.: 3.5kV
Fragmentor volt : 100V
Drying gas: N₂ (12.0L/min , 350°C)
Nebulizer gas: N₂ (50psi)



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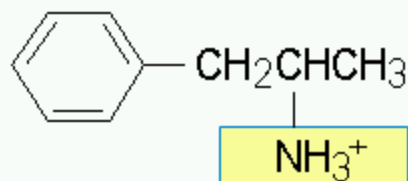
Page 35

Comparison Between Volatile Buffer and Non Volatile Phosphate Buffer (pH 2.5) (10 ppm injection:sc...

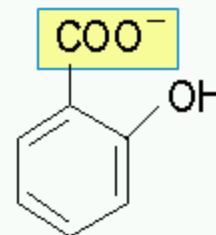
Adjust Mobile Phase pH to Ionize Molecules in Solution

ESI - LC/MS of Bases and Acids

Amphetamine



Salicylic Acid



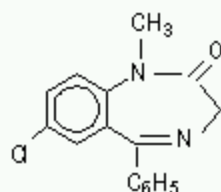
- Choose buffers that create charged analytes.
- Ionize sample based on analyte pK_a .

Adjust Mobile Phase pH to Ionize Molecules in Solution ESI - LC/MS of Bases and Acids

Basic Compounds - Benzodiazepines

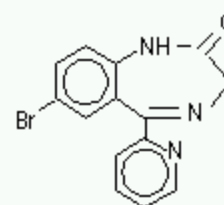
Diazepam

pK_a 3.3

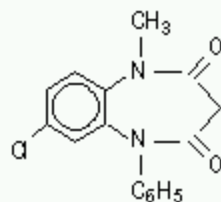


Bromazepam

pK_a 2.9, 11.0

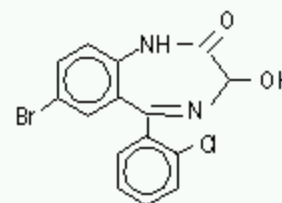


Clobazam



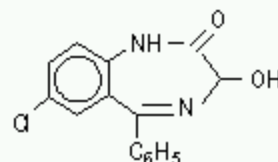
Lorazepam

pK_a 1.3, 11.5



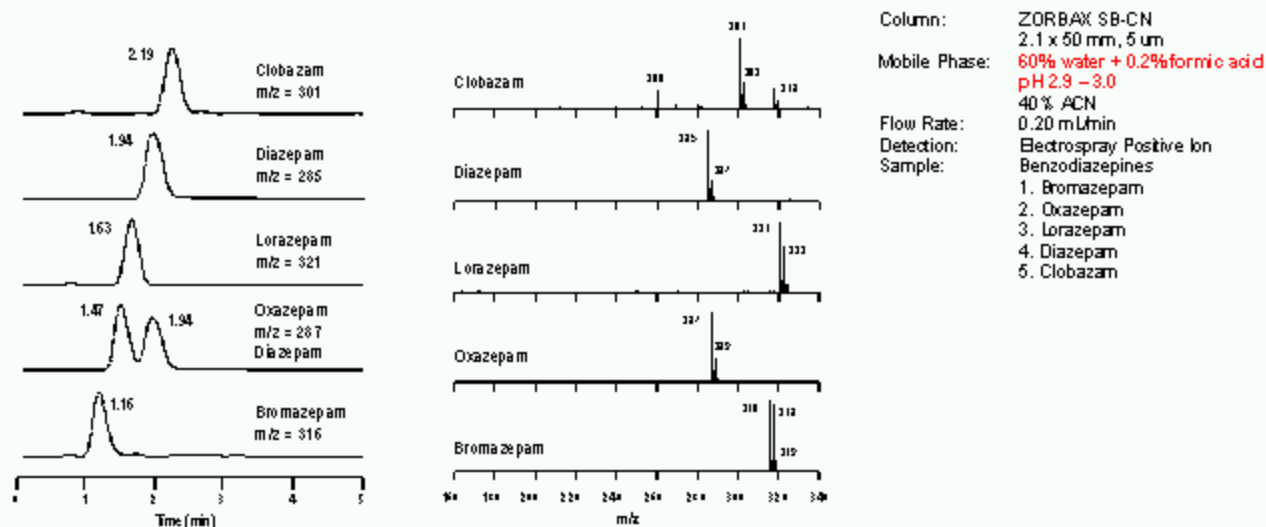
Oxazepam

pK_a 1.7, 11.6



Choose Low pH Mobile Phase for Ionized Basic Compounds

Positive Ion ESI LC/MS of Benzodiazepines



• Formic acid (pH 2.8 – 3.8) will keep basic compounds positively charged for positive ion ESI.



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Page 38

Choose Low pH Mobile Phase for Ionized Basic Compounds Positive Ion ESI LC/MS of Benzodiazepines

Mobile Phase Buffers for ESI

- **Positive ion detection of basic analytes**
- **Buffer choices (10 mM or less)**
 - Acetate pKa 4.8
 - Propionic acid pKa 4.8
 - Formate pKa 3.8
 - TFA highly acidic
- **Typical analytes – amines, amides, antibiotics**

- **Negative ion detection of acidic analytes**
- **Buffer choices (10 mM or less)**
 - Ammonia pKa 9.2
 - Diethylamine pKa 10.5
 - Triethylamine pKa 10.7
 - Piperidine pKa 11.1
- **Typical analytes – acids, hydroxyls, phosphates, sulfates**

Keep pH 1 – 2 pH units above or below pKa of analytes.



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Page 39

Mobile Phase Buffers for ESI

LC/MS Solvent Selection and Guidelines for Successful APCI

- **Select more volatile solvents**
- **Select protic solvents (MeOH) for positive ion mode when possible**
- **Select solvents that readily capture an electron for negative ion mode**
- **Ammonium salts in the mobile phase can cause ammonium adducts to form**



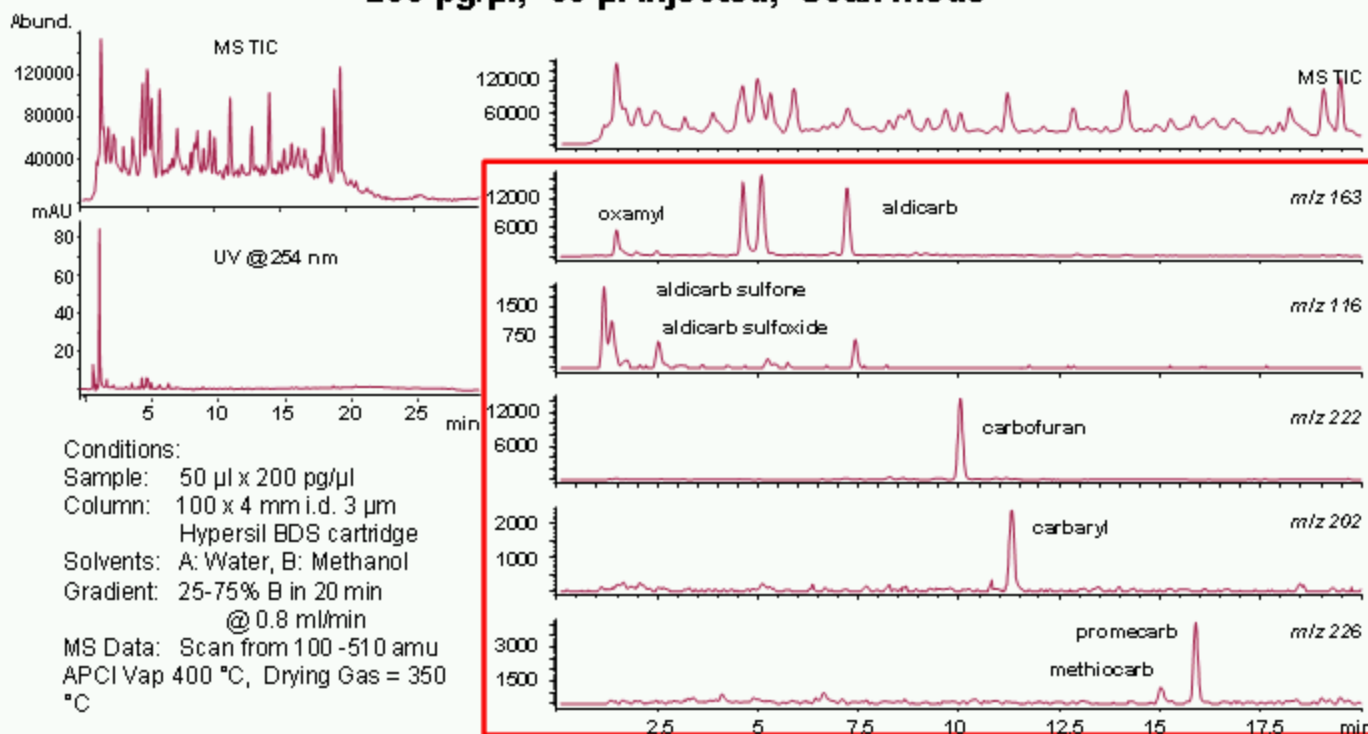
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Page 40

LC/MS Solvent Selection and Guidelines for Successful APCI

Carbamates by APCI: Extracted Ions

**Tomato extract spiked with 11 carbamates @
200 pg/ μ l; 50 μ l injected; Scan mode**



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Page 41

Carbamates by APCI: Extracted Ions

Electrospray Ionization (ESI) vs. APCI

- If all analytes form ions in solution (on the column)?
 - Choose ESI with acetonitrile and volatile buffer
 - Choose 2.1 mm id columns for best sensitivity – concentration sensitivity standard analytical
 - Choose microbore, capillary and nano for ESI of complex peptide mixtures
 - For basic analytes choose positive ion mode
 - For acidic analytes choose negative ion mode
- If you can't do ESI, then try APCI with methanol and volatile buffer
 - Choose 2.1 – 4.6 mm id columns



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Page 42

Electrospray Ionization (ESI) vs. APCI

LC/MS Solvent Selection and Guidelines for Successful APPI

- If a compound photoionizes directly, adding dopant may not improve the sensitivity
- Methanol is a better solvent than acetonitrile in the positive ion mode; acetone is a better dopant than toluene in the negative ion mode
- APPI sensitivity is usually similar to APCI
- Vaporizer temperature is important
- Photochemical reactions can and do occur

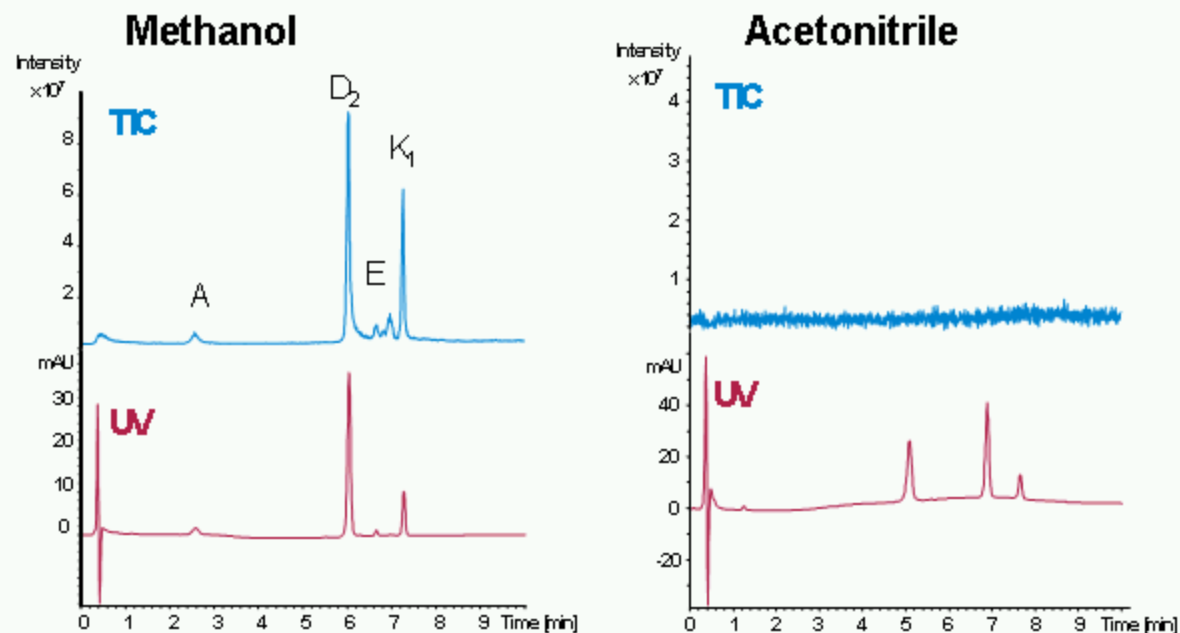


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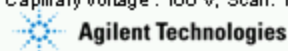
Page 43

LC/MS Solvent Selection and Guidelines for Successful APPI

Effect of Chromatographic Solvent on Photoionization of Fat-Soluble Vitamins



LC Conditions: Column: 2.1 x 50 mm ZORBAX XDB-C8, 5 μ m; Mobile Phase: water (A), acetonitrile (B); Gradient: 80 to 100% B in 5 minutes; Flow rate: 400 μ l/min; Column temperature: 25°C; Injector: 5 μ l; DAD: signal 210/4 nm, reference 400/80 nm. Ion Trap MS Conditions: Ionization mode: positive ion APPI; Vcap: 1500 V (3000 V with dopant); Drying gas flow: 5 l/min; Vaporizer: 250°C Drying gas temperature: 350 °C; Nebulizer: 60 psi; Skim 1: 20 V; Capillary voltage: 100 V; Scan: 100-1000 for initial experiments; Averages: 3; ICC: On; Max accumulation time: 200 ms; Target: 25000;

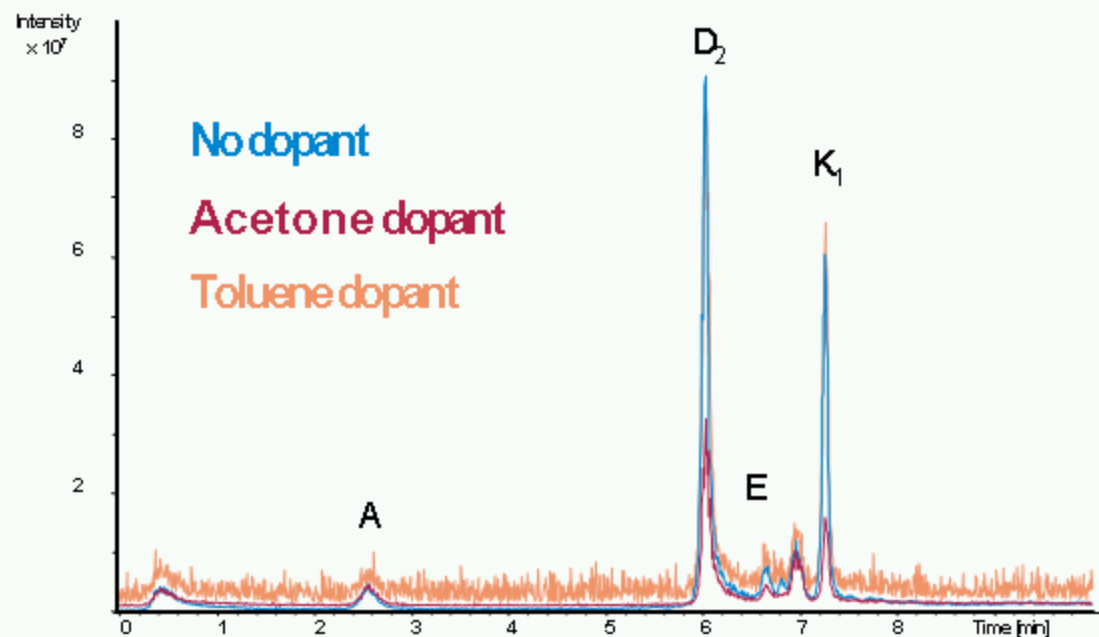


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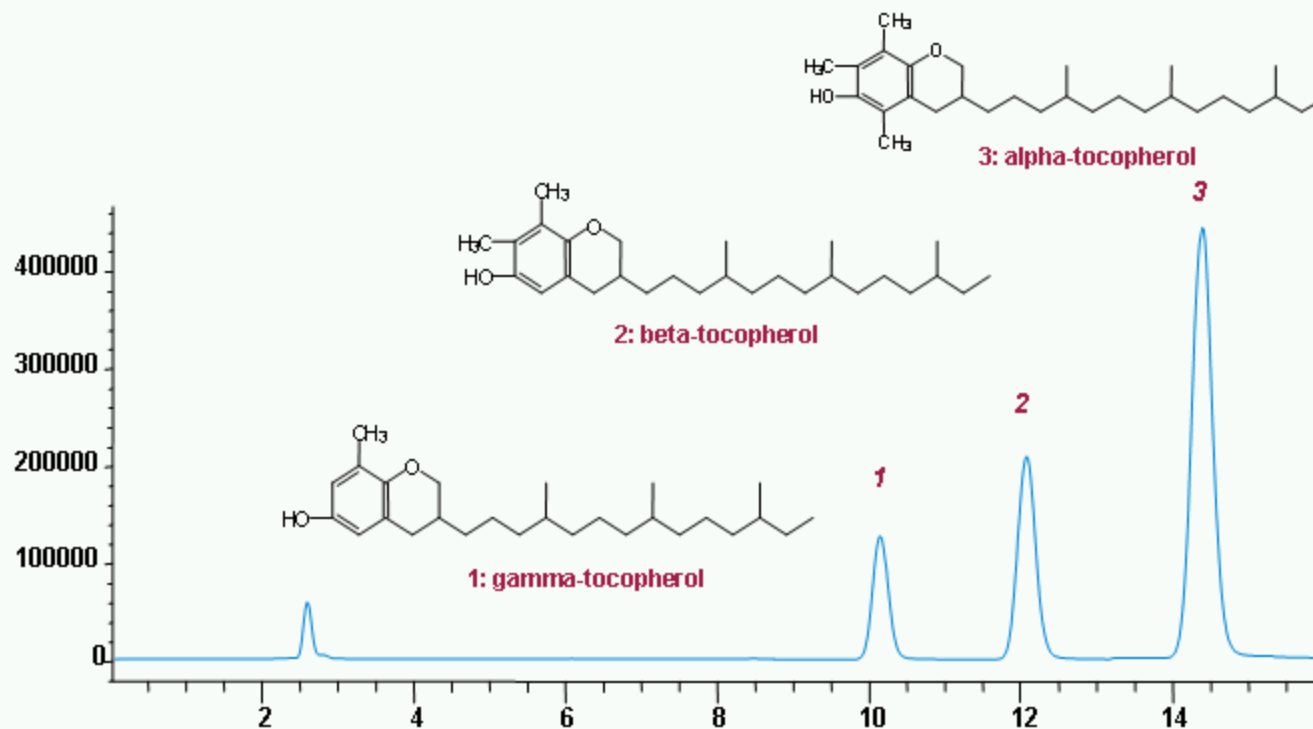
Page 44

Effect of Chromatographic Solvent on Photoionization of Fat-Soluble Vitamins

Effect of Dopants on Photoionization of Fat-Soluble Vitamins



Analysis of Tocopherols (Vitamin E Isomers) by APPI



Good Chromatography Always Requires Selecting the Best Bonded Phase

- Which bonded phase gives you the best chromatography
- Which column gives you the best stability and lifetime with your mobile phase?

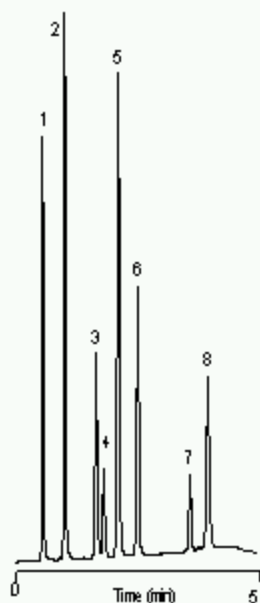


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Page 47

Good Chromatography Always Requires Selecting the Best Bonded Phase

LC/MS Bonded Phase Selection at Low pH



Column: ZORBAX Rapid Resolution SB-C18,
2.1 x 50 mm, 3.5 mm

Mobile Phase: Gradient: 10 – 40% B in 4 min.

A: Water with 0.4% formic acid

B: Methanol with 0.4% formic acid

Flow Rate: 0.5 mL/min

Temperature: 35°C

Detection: UV 254 nm

Sample: Organic acids

1. Gallic

2. Protocatechuric

3. Hydrocaffeic

4. Gentisic

5. Vanillic

6. Syringic

7. Sinapinic

8. Salicylic

- **StableBond is the best choice for low pH MS method development due to long lifetime and no bonded-phase leaching.**



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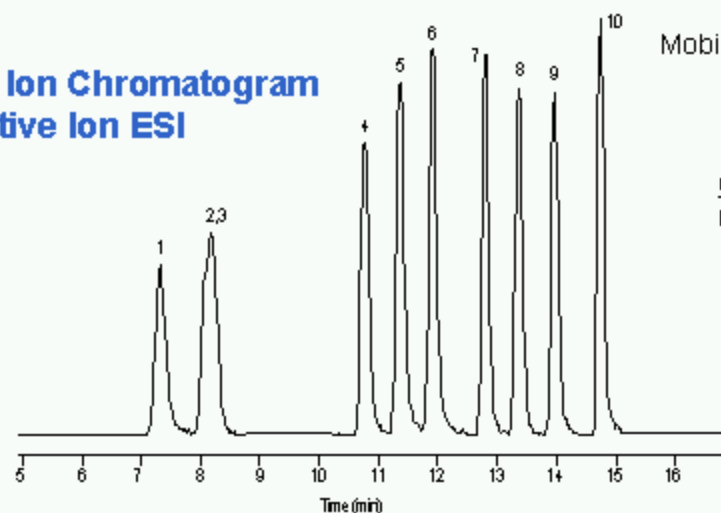
Page 48

LC/MS Bonded Phase Selection at Low pH

LC/MS Bonded-Phase Selection at Mid pH

Column: ZORBAX Eclipse XDB-C8, 2.1 x 150 mm, 5 μ m **Flow Rate:** 0.3 mL/min **Temperature:** RT **Sample:**
1. Naproxen 2. Tolmetin 3. Ketoprofen 4. Phenylbutazone 5. Fenopropfen 6. Flurbiprofen 7. Niflumic acid 8. Ibuprofen
9. Mefenamic acid 10. Flufenamic acid

Total Ion Chromatogram Negative Ion ESI



Mobile Phase: A: Water
B: MeOH
C: 30 mM TEA
pH 7.5 with acetic acid

Gradient:	%A	%B	%C
0 min:	40	40	20
1 min:	40	40	20
15 min:	0	80	20
18 min:	0	80	20

- A narrow-bore Eclipse XDB column is ideal for mid pH separations with MS detection.
- MS identifies all peaks, even the partially resolved pair, 2 and 3.

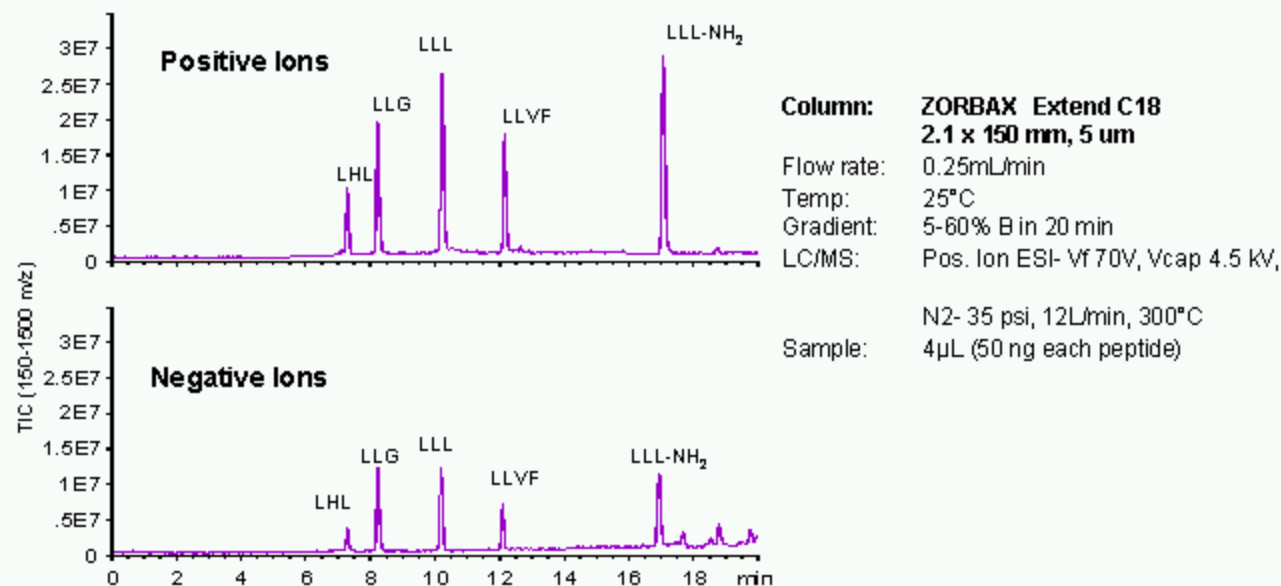


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Page 49

LC/MS Bonded-Phase Selection at Mid pH

LC/MS Bonded Phase Selection at High pH – Extend-C18



- The Extend-C18 column is ideal for LC/MS of proteins and peptides at high pH
- Both positive and negative ion MS are possible with NH_4OH mobile phase.



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Page 50

LC/MS Bonded Phase Selection at High pH – Extend-C18

Bonded Phase and Mobile Phase Choices for LC/MS Analysis

	Recommended Mobile Phase Choices			
Bonded Phases	TFA Strong acid	Formate* pKa = 3.8	Acetate* pKa = 4.8	Ammonium Hydroxide pKa = 9.2
StableBond	✓	✓	✓	✗
Rx-C18	✓	✓	✓	✗
Eclipse XDB	✓	✓	✓	✗
Bonus-RP	✓	✓	✓	✗
Extend-C18	✓	✓	✓	✓

* Used as formic acid and acetic acid or ammonium formate and ammonium acetate

Bonded Phase and Mobile Phase Choices for LC/MS Analysis

Adapting Existing LC Methods to LC/API-MS

- **Replace non volatile buffers with volatile buffers at a concentration of <10 mM for ESI or <100 mM for APCI**
 - **Substitute phosphates and borates with ammonium acetate, ammonium formate, or TFA**
 - **If a non-volatile buffer must be used, select a buffer with only the anionic or cationic part is non-volatile (i.e. ammonium phosphate and keep concentration very low) and keep column id and flow rate low (2.1 or 1.0 mm id)**
- **Keep the pH the same as in the original separation with volatile additives – formic acid, acetic acid, TFA, ammonium hydroxide**
- **Use volatile ion-pair reagents only when needed – heptafluorobutyric acid (HFBA) and tributylamine (TBA)**



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Page 52

Adapting Existing LC Methods to LC/API-MS

Conclusions

- **Both AP-ESI and APCI are commonly used for the analysis of a wide variety of samples.**
- **APPI is a new technique and expands the range of non-polar analytes that can be analyzed by LC/MS.**
- **Solvents for atmospheric pressure MS must be volatile, but best choices vary with technique.**
- **Columns for LC/MS can be almost any configuration with narrow-bore columns the most popular for analytical instruments.**
- **ZORBAX StableBond (six phases), Eclipse XDB (four phases) and Extend-C18 bonded phases are good for LC/MS.**
- **Capillary and nano LC columns are available for maximum sensitivity with smallest samples.**
- **Rapid resolution (3.5 μm) and RRHT (1.8 μm) particles can be used to increase efficiency and resolution with short columns for high throughput LC/MS.**



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Page 53

Conclusions

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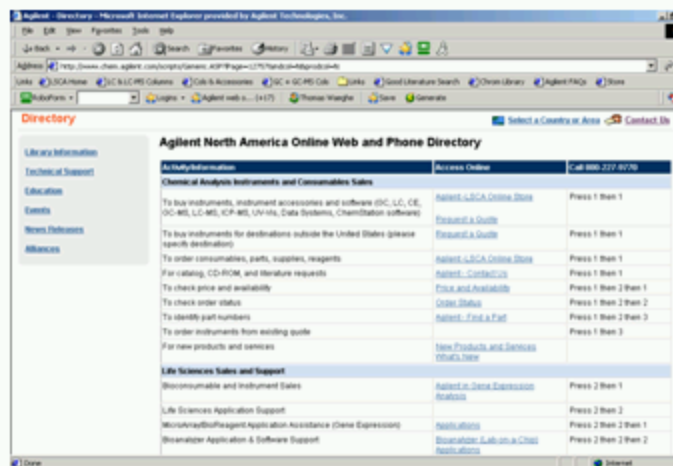
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Page 54

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Page 55

Slide 55

Upcoming eSeminars

HPLC Method Development by the Numbers

September 13, 2005 – 11:00 a.m. EDT

HPLC Column Choices for the Analysis of Proteins and Peptide

September 27, 2005 – 1:00 p.m. EDT

Secrets of Good Peak Shape

October 11, 2005 – 11:00 a.m. EDT



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Page 55

Slide 56