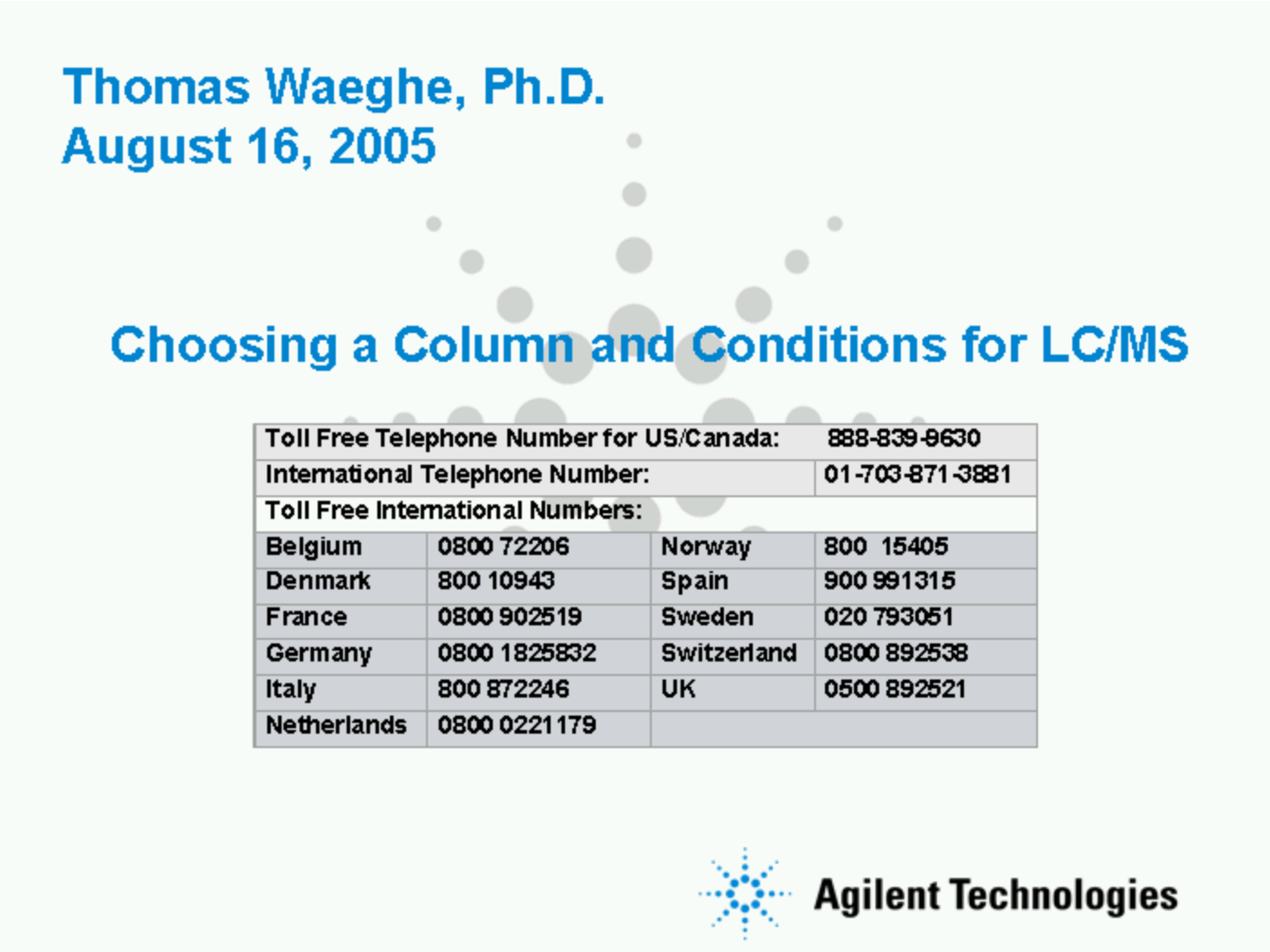
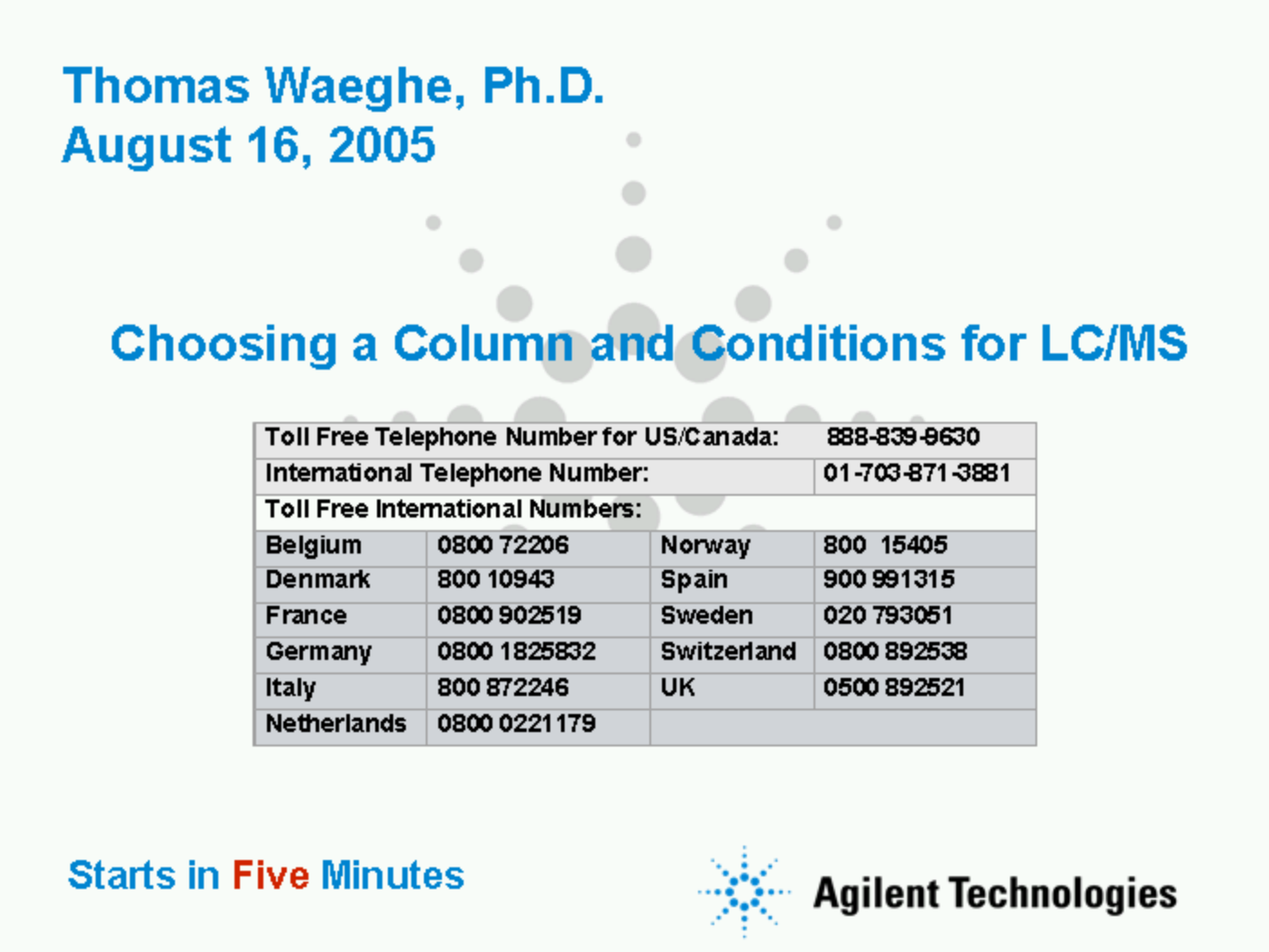
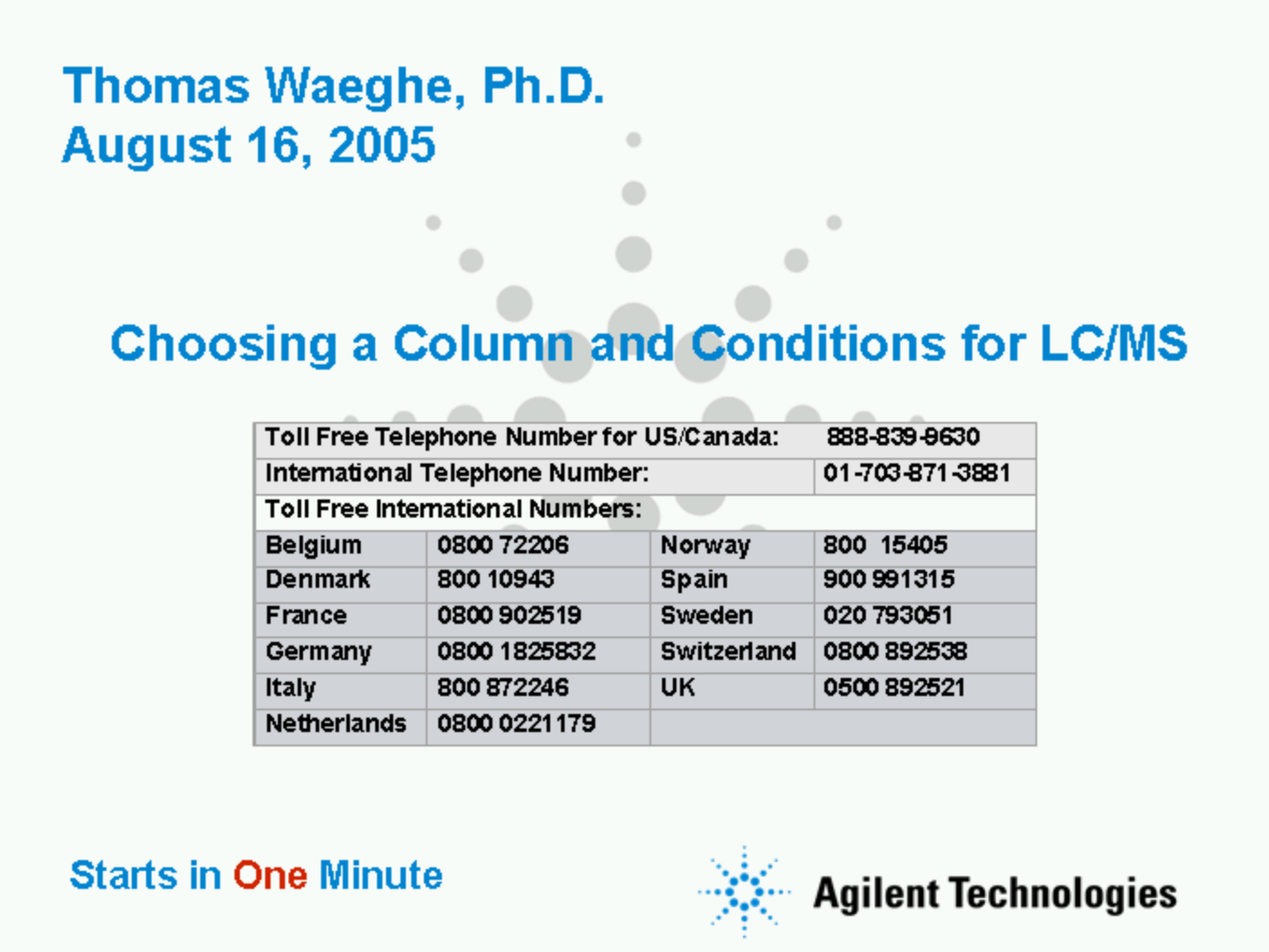


Thomas Waeghe, Ph.D. August 16, 2005



Thomas Waeghe, Ph.D. August 16, 2005



Thomas Waeghe, Ph.D. August 16, 2005

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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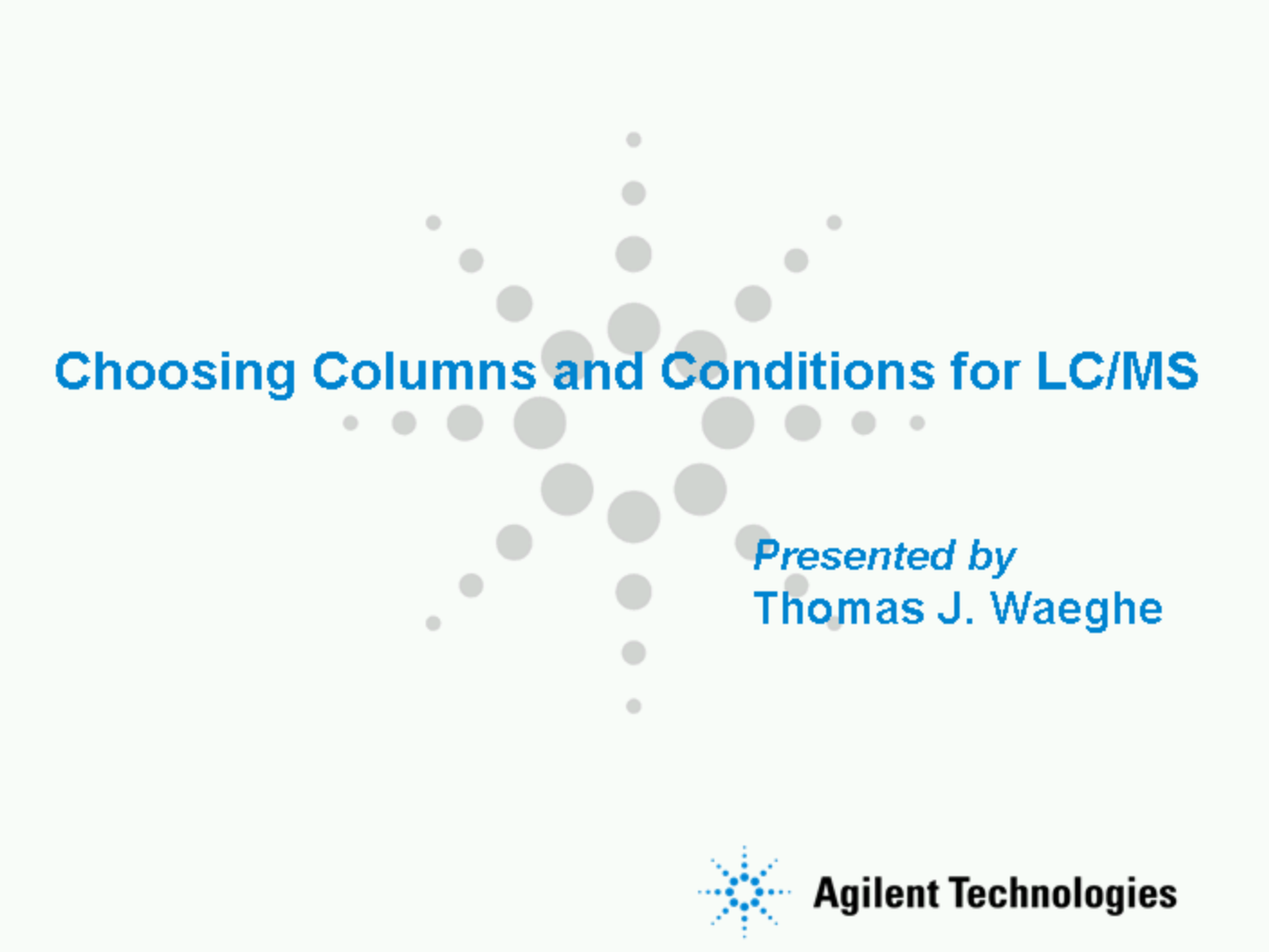
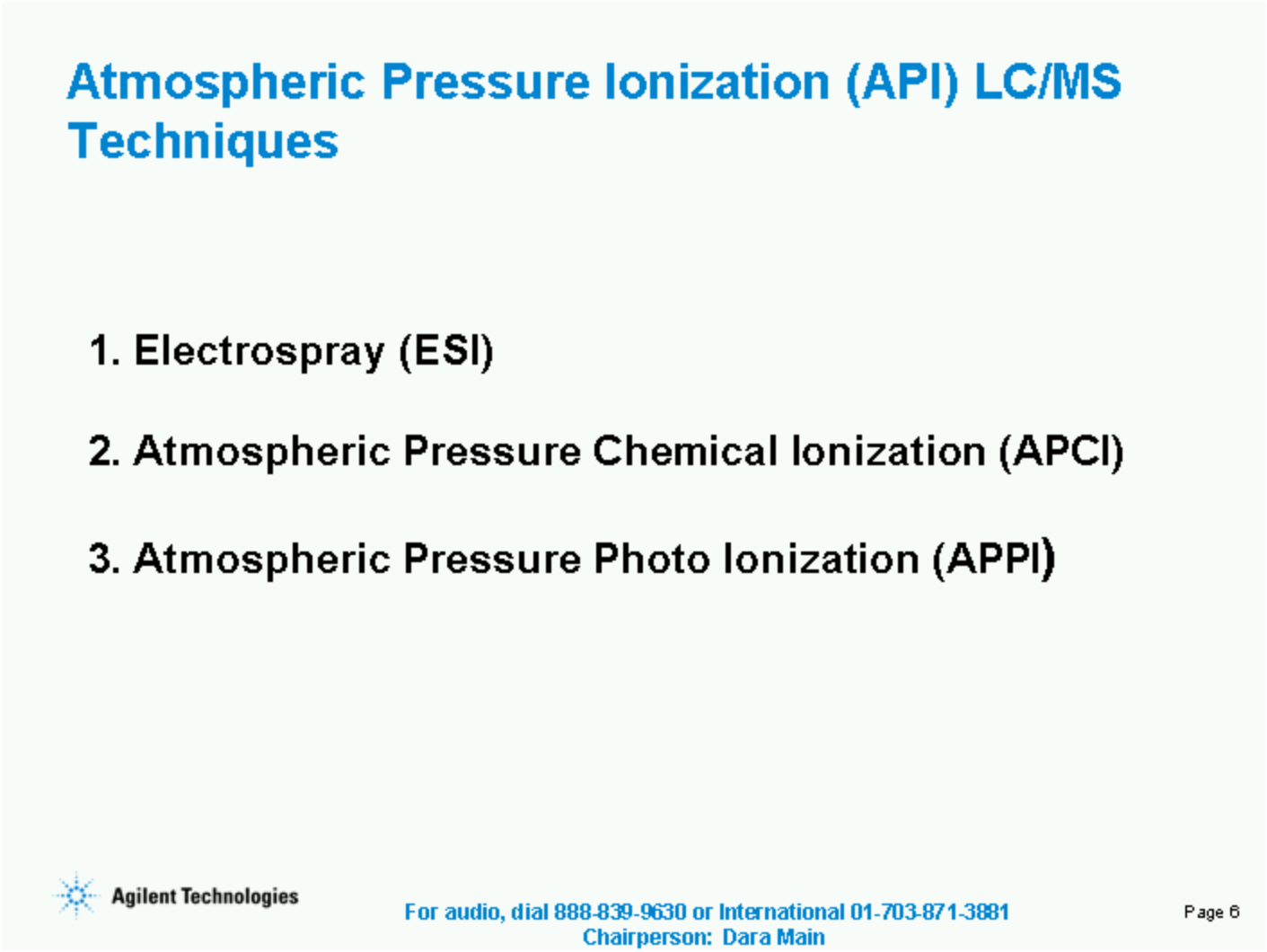
Page 4

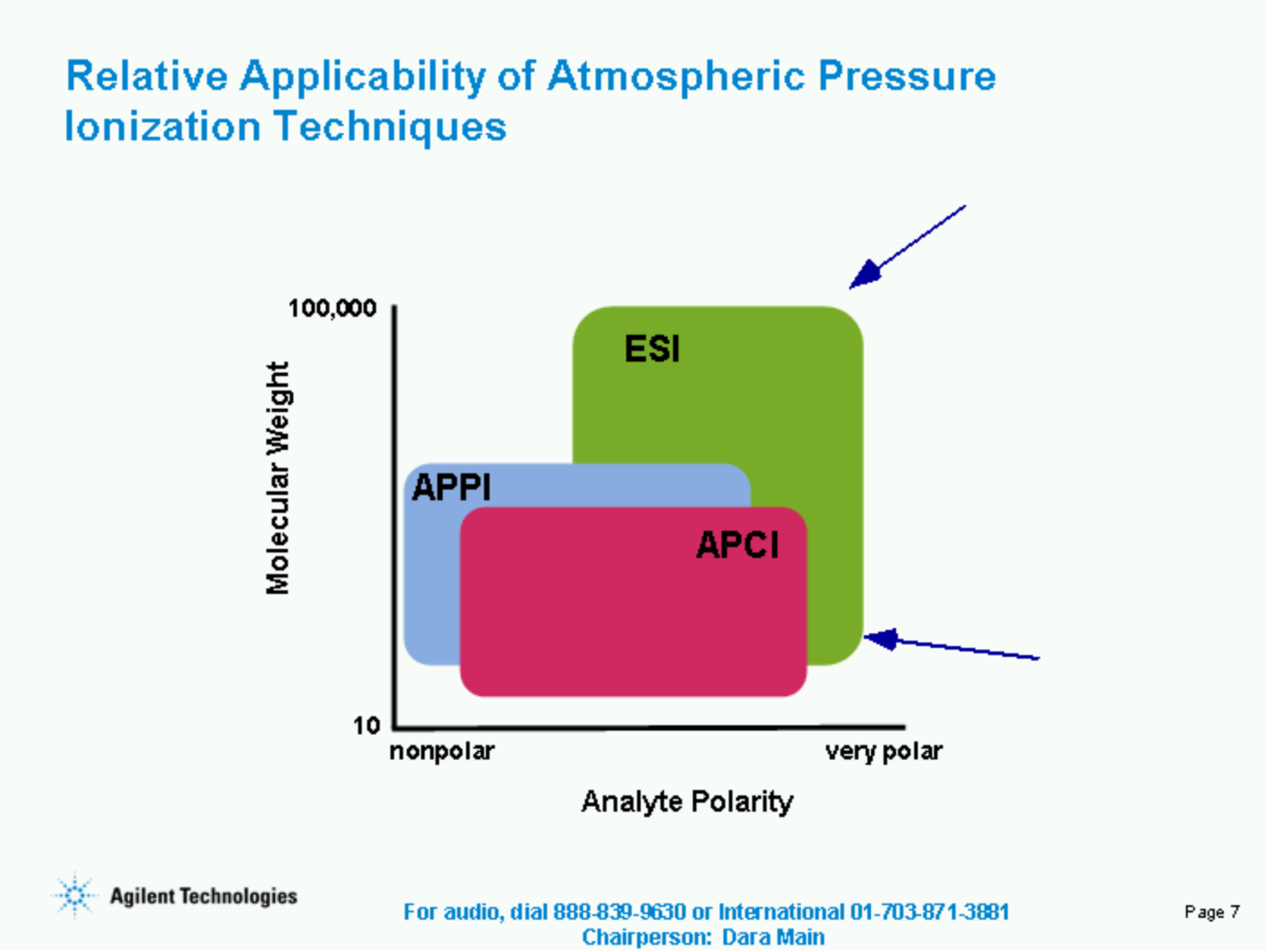
Presenter: Thomas Waeghe, Ph.D.



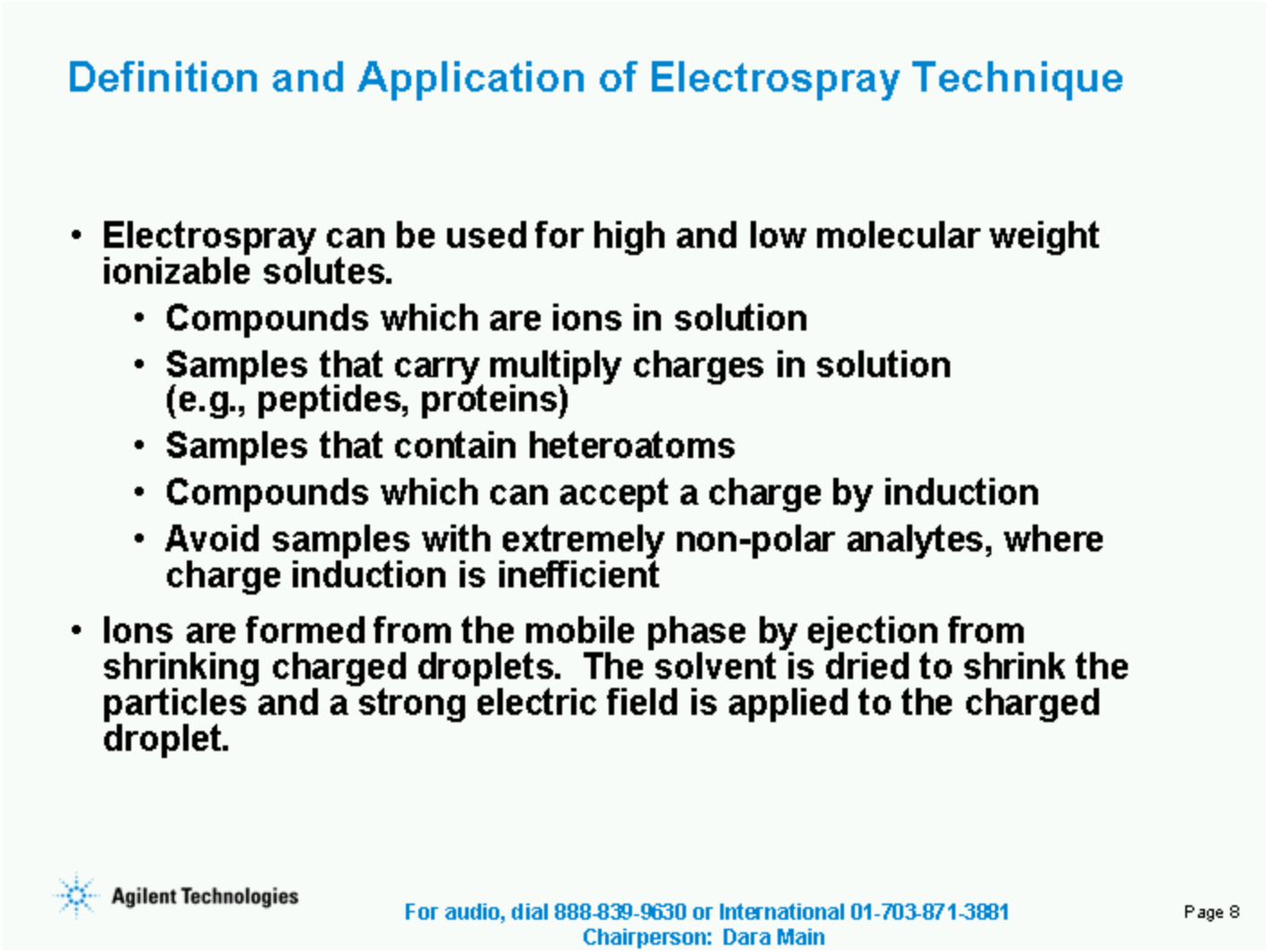
Choosing Columns and Conditions for LC/MS



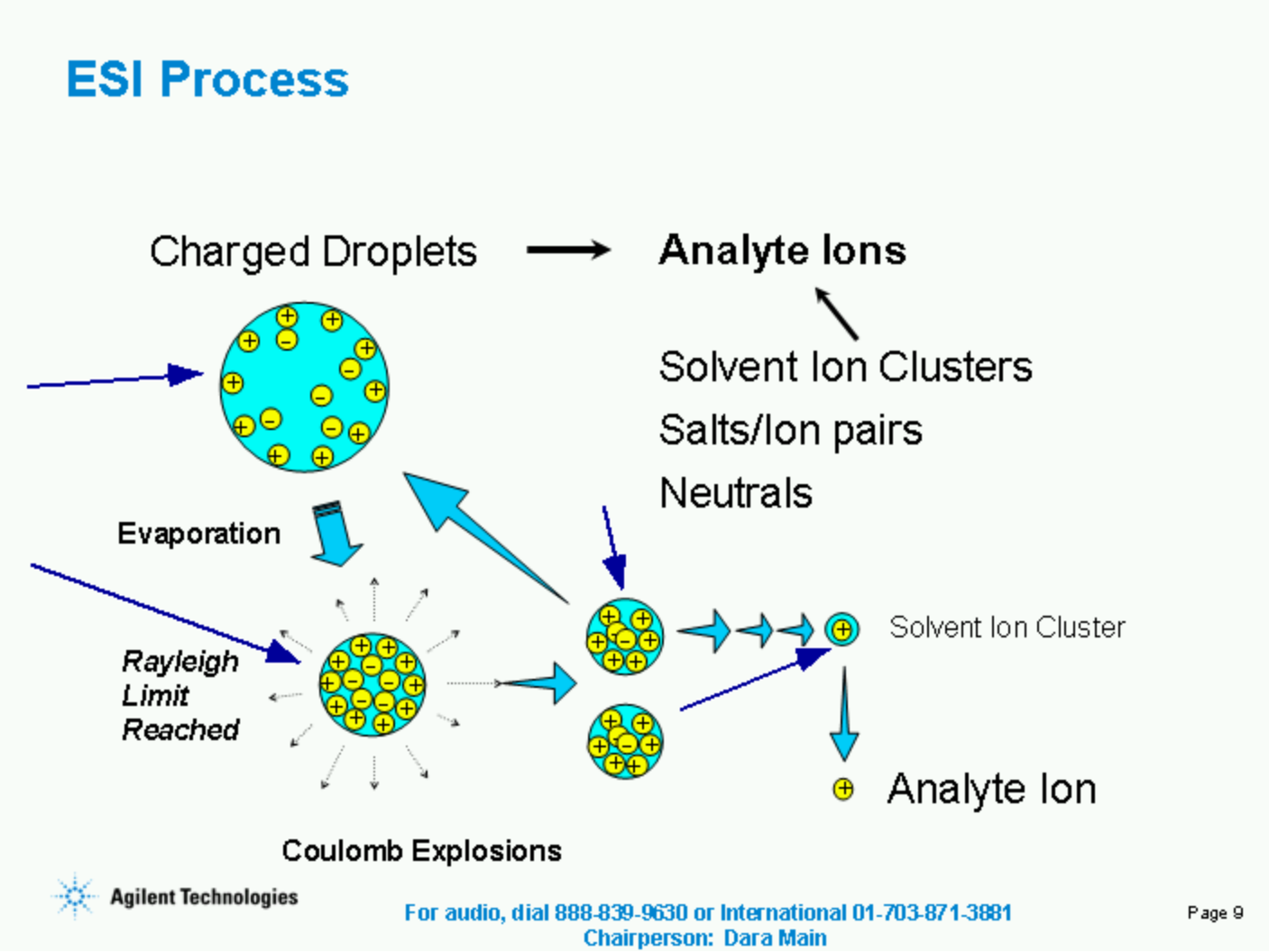
Atmospheric Pressure Ionization (API) LC/MS Techniques



Relative Applicability of Atmospheric Pressure Ionization Techniques



Definition and Application of Electrospray Technique


ESI Process
Charged Droplets
Analyte Ions
Solvent Ion Clusters
Salts/Ion pairs
Neutrals
Evaporation
Rayleigh Limit Reached
Solvent Ion Cluster
Analyte Ion
Coulomb Explosions
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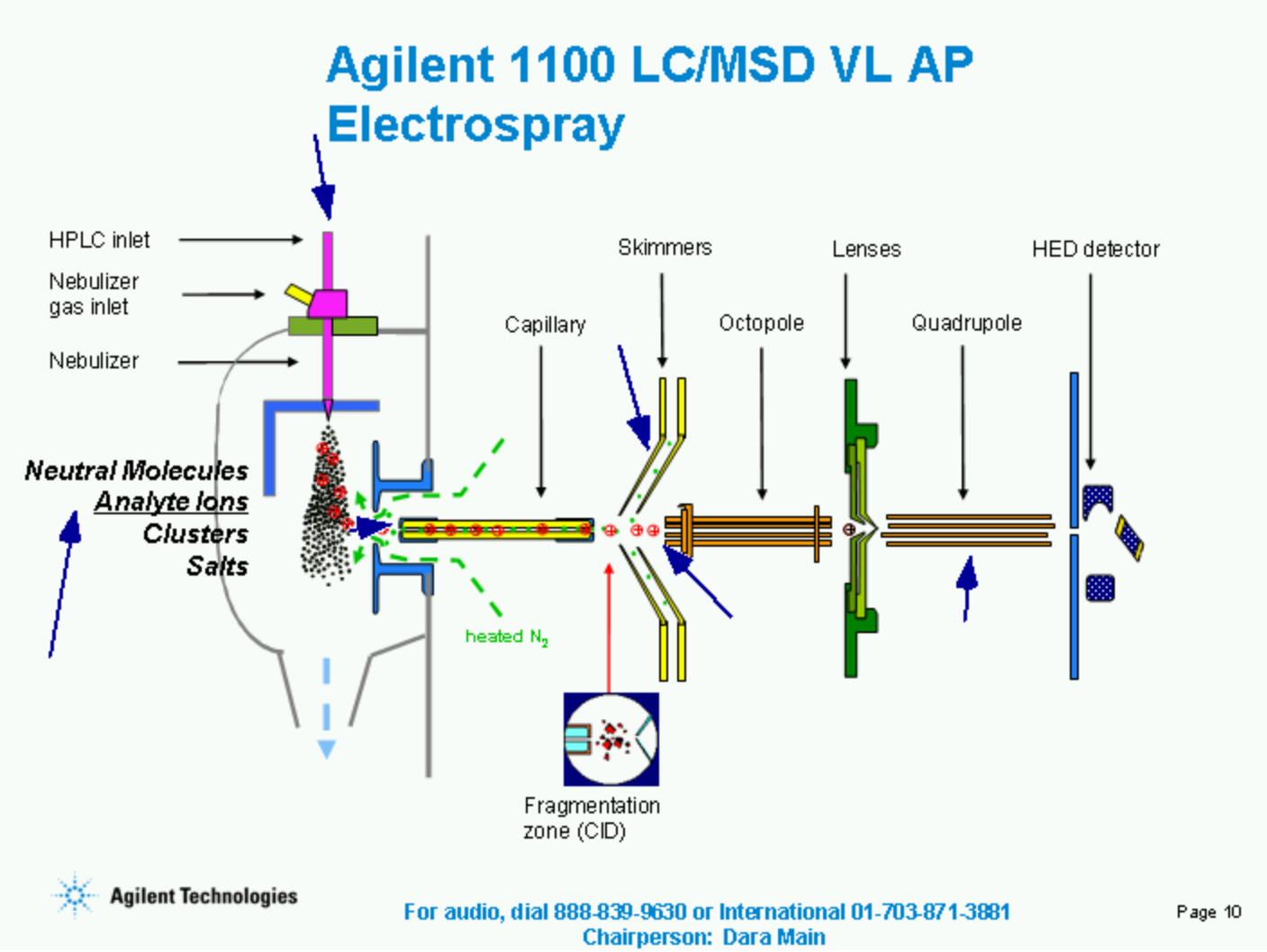
Page 9

ESI Process


Agilent 1100 LC/MSD VL AP Electrospray
HPLC inlet
Nebulizer gas inlet
Nebulizer
Neutral Molecules
Analyte Ions
Clusters
Salts
Capillary
Skimmers
Octopole
Lenses
Quadrupole
HED detector
heated N_2
Fragmentation zone (CID)
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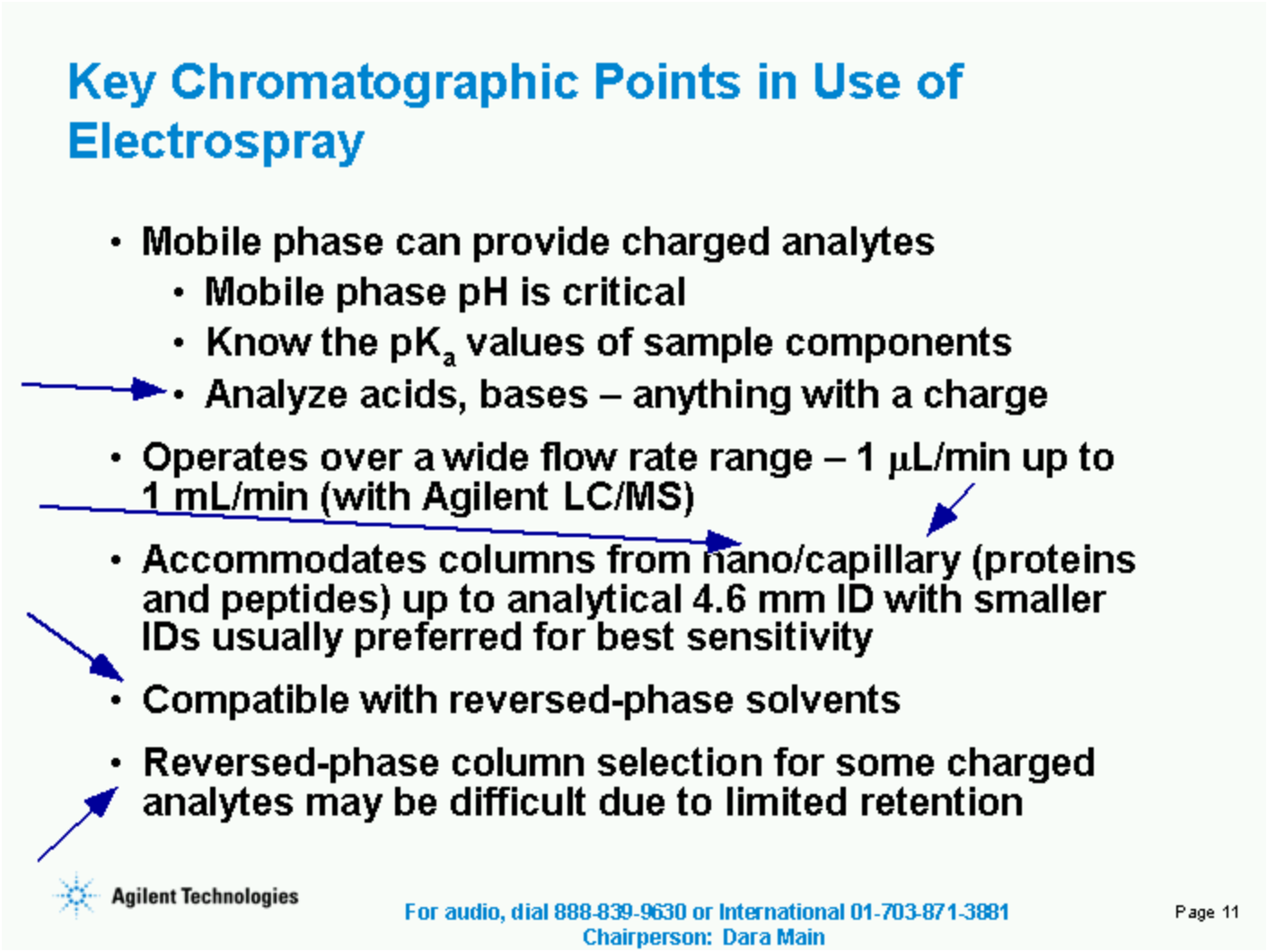
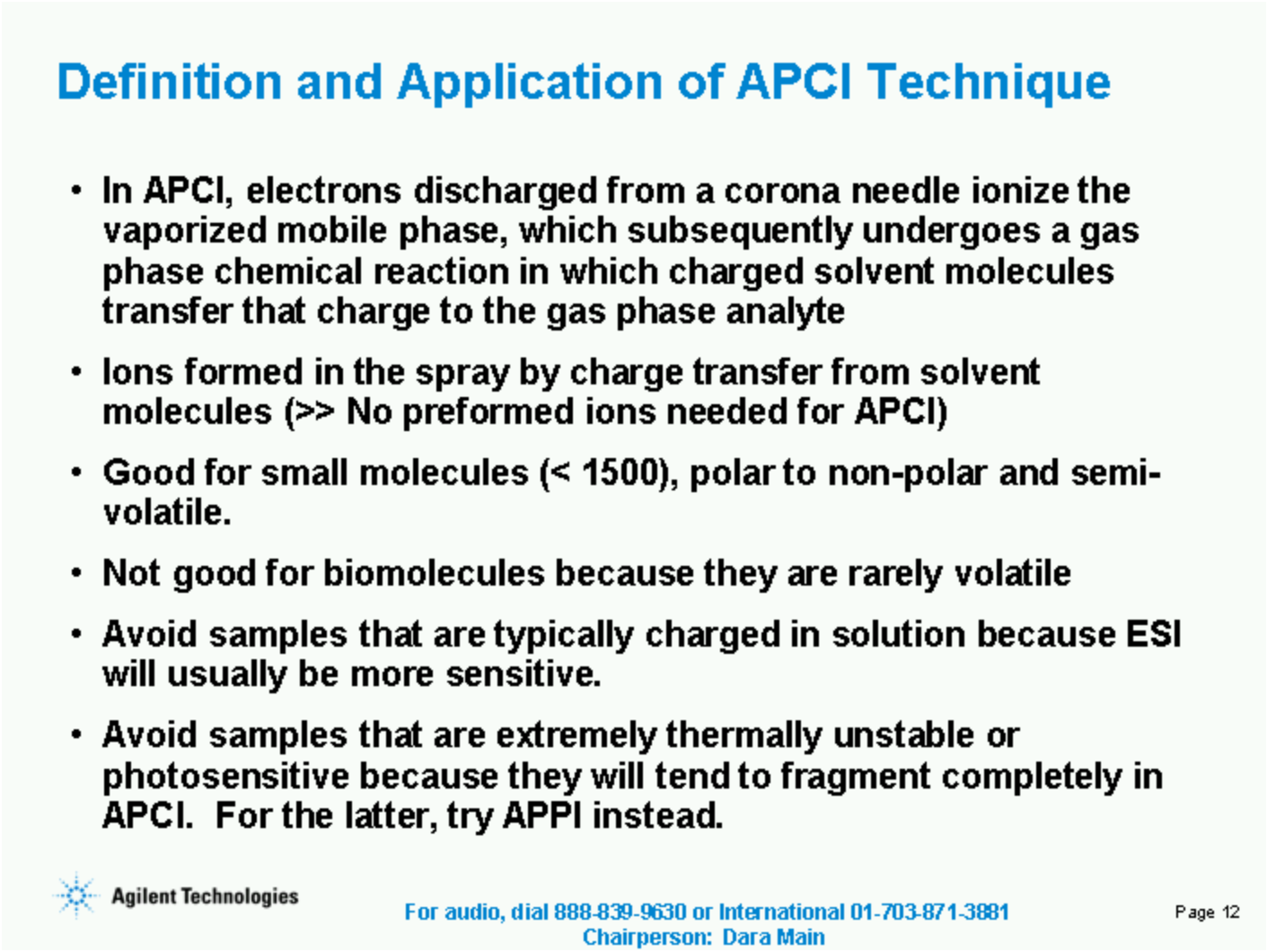
Page 10

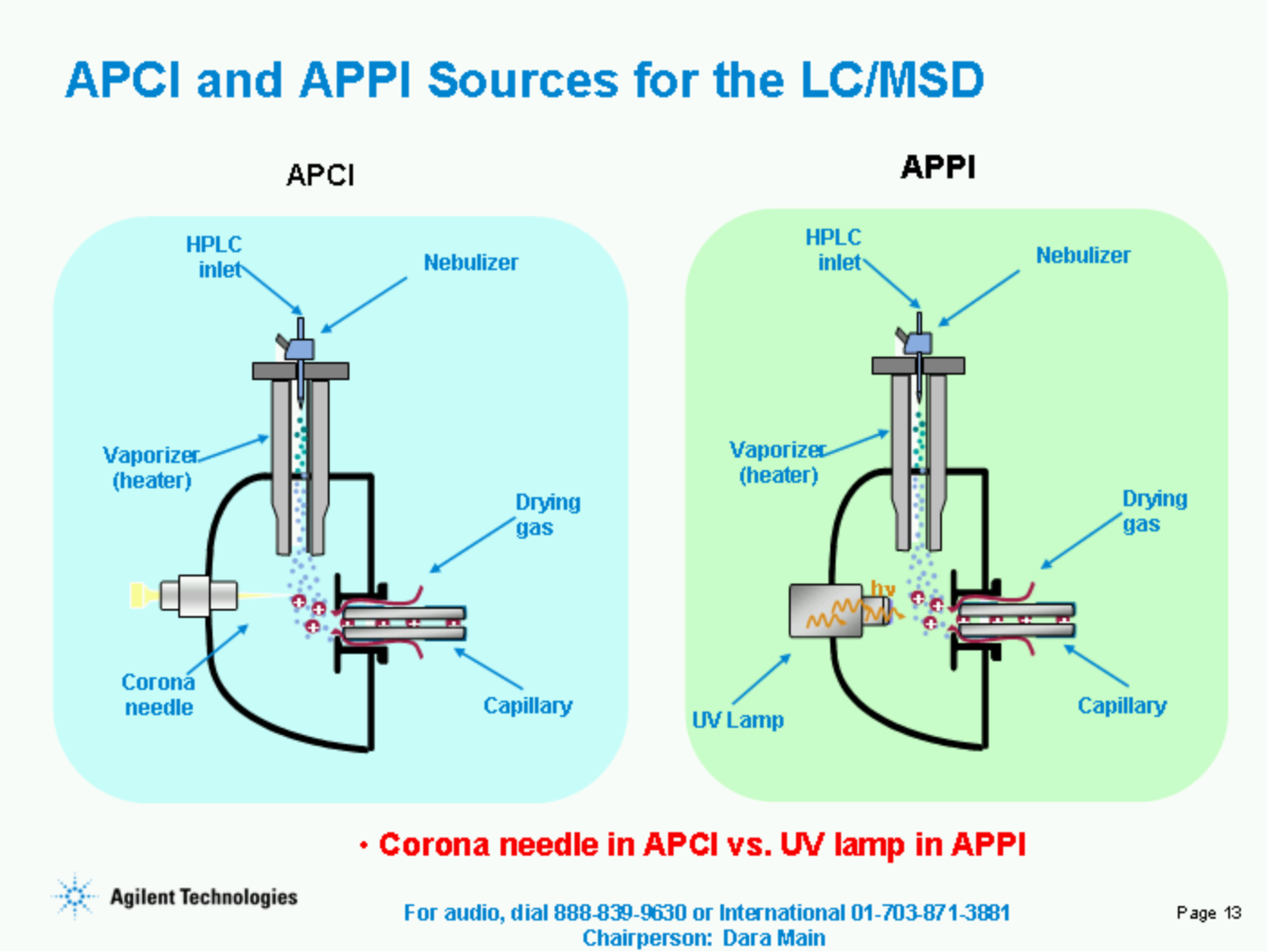
Slide 10



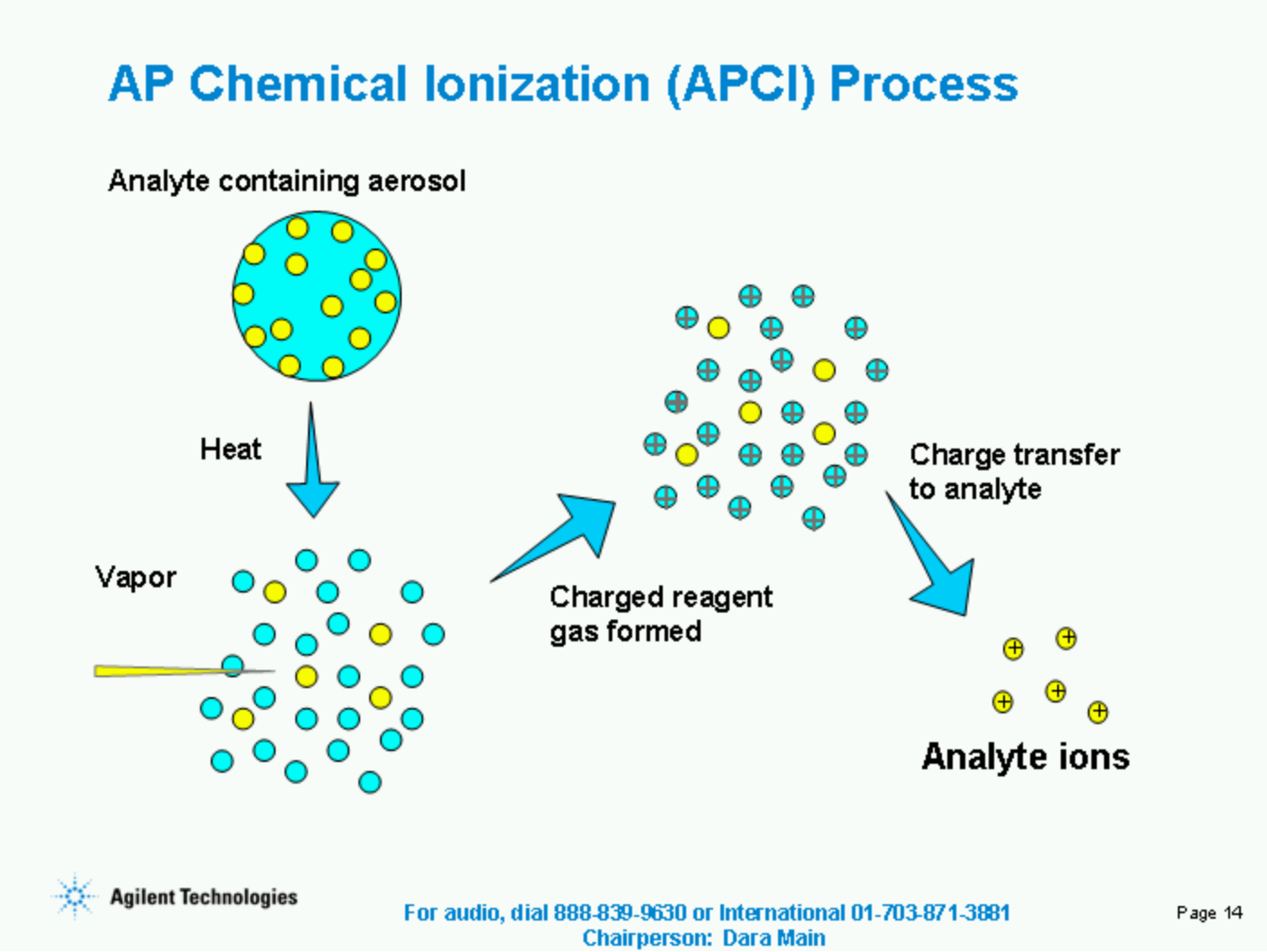
Key Chromatographic Points in Use of Electrospray



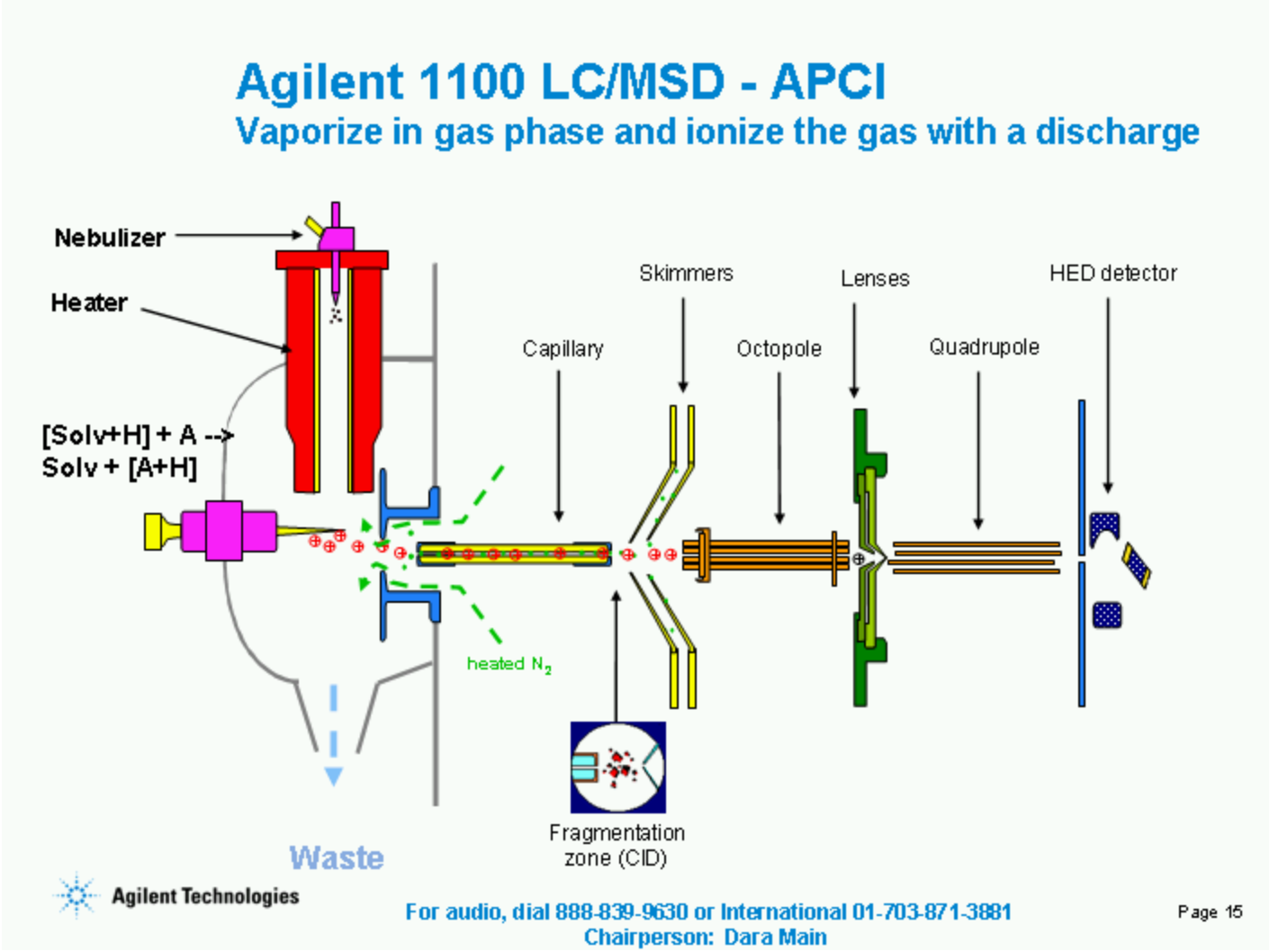
Definition and Application of APCI Technique



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
Nebulizer
Heater
[Solv+H] + A -->
Solv + [A+H]
Capillary
Skimmers
Octopole
Lenses
Quadrupole
HED detector
heated N2
Waste
Fragmentation
zone (CID)
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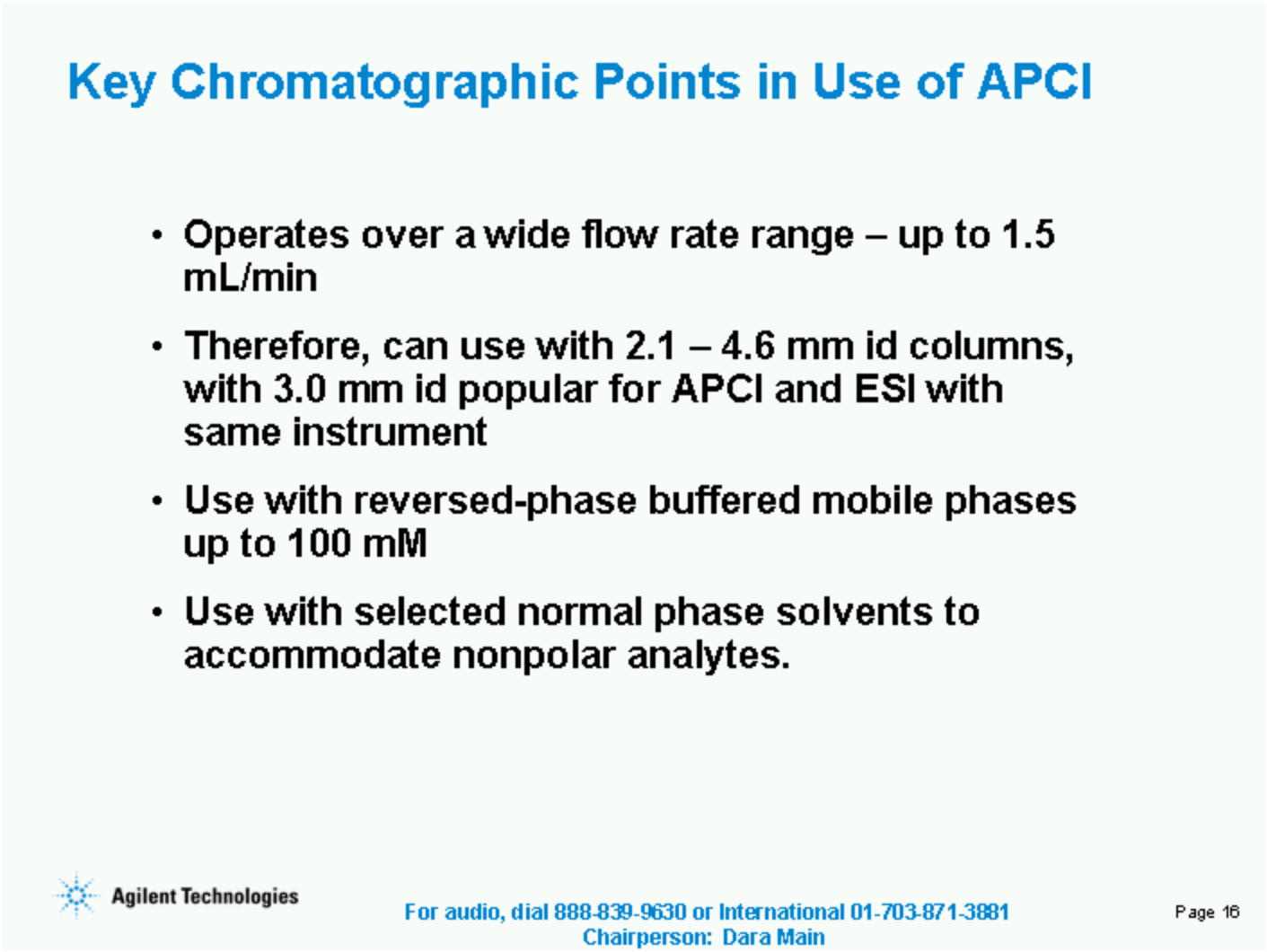
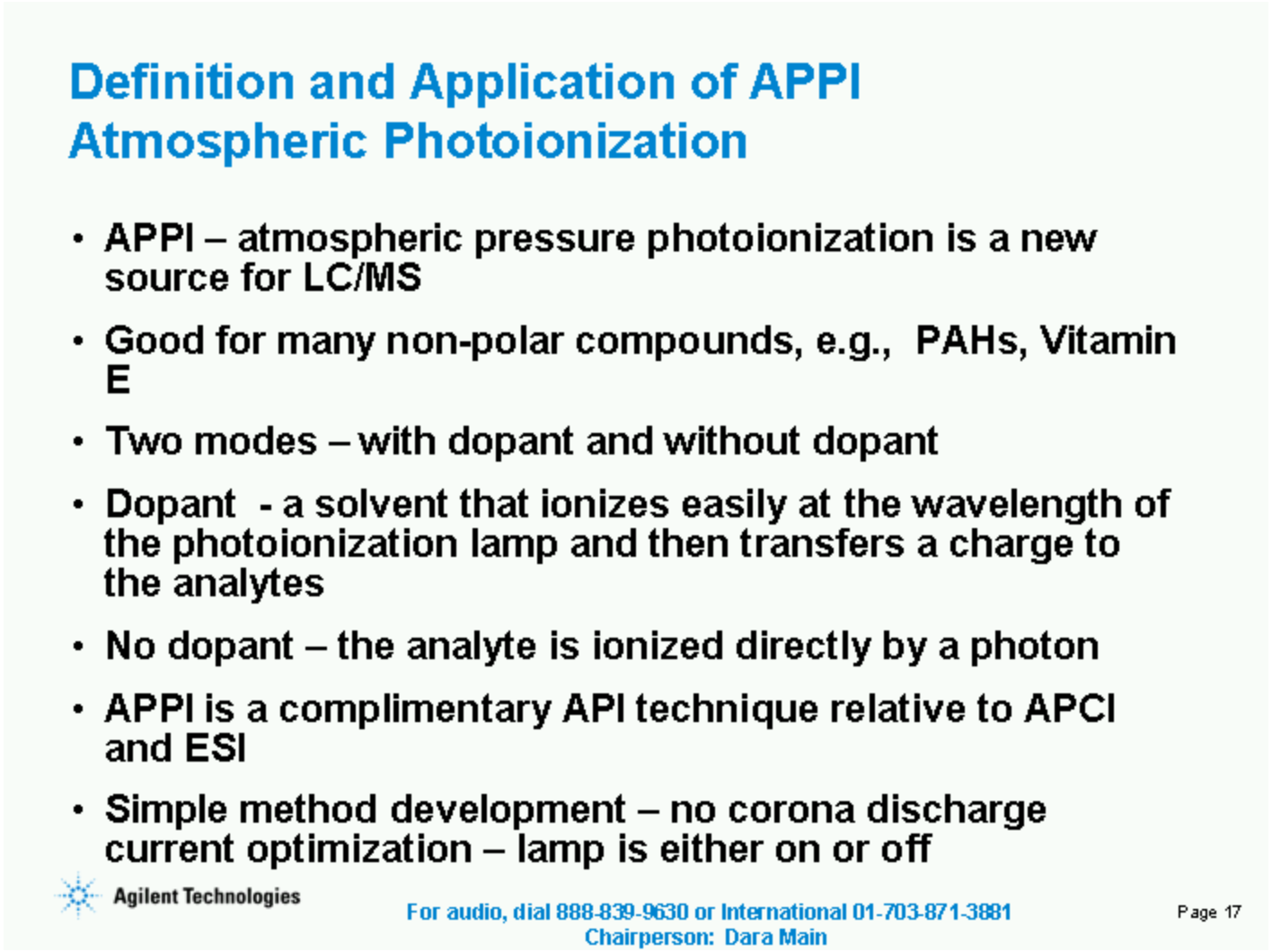
Page 15

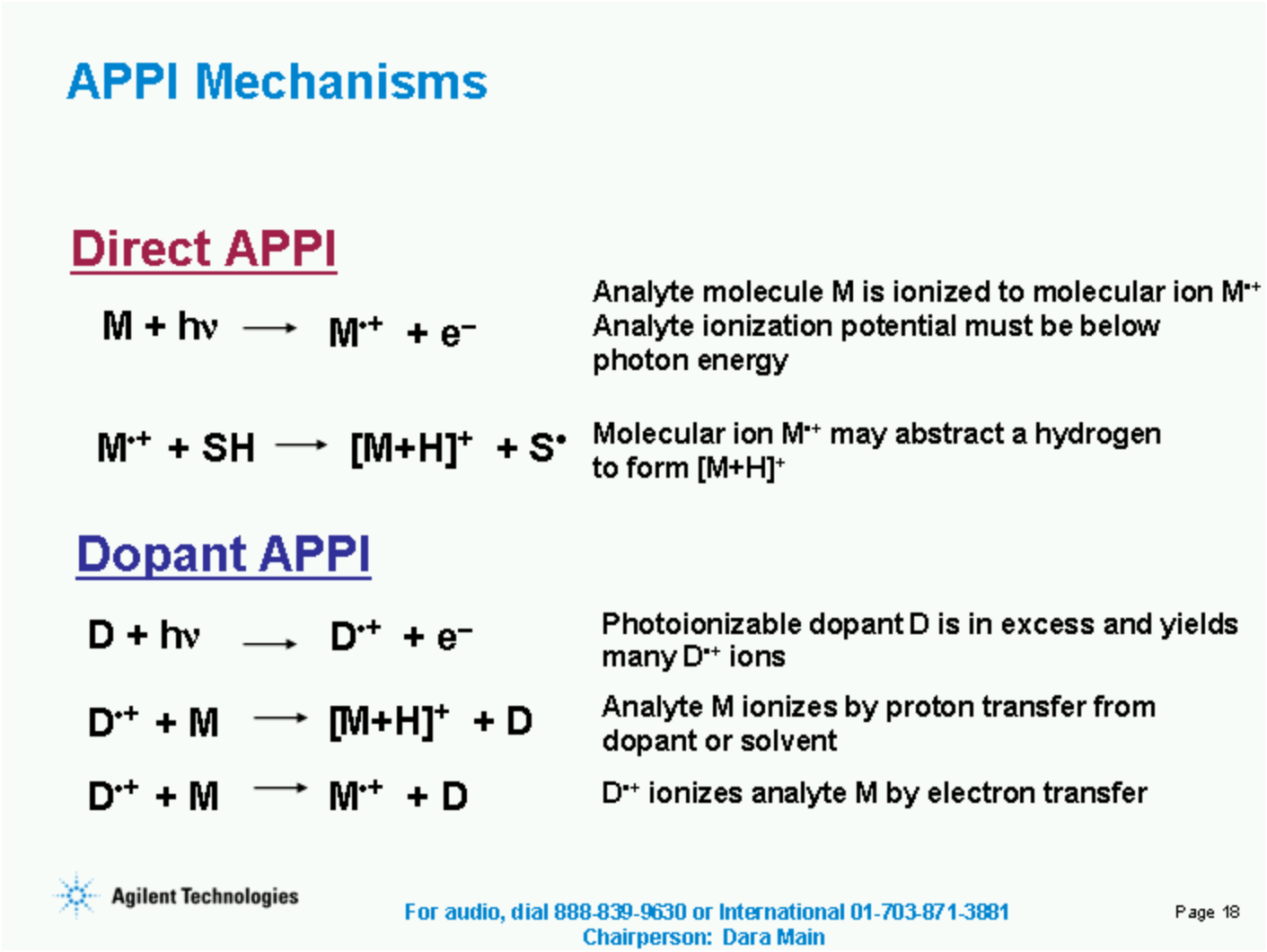
Slide 15



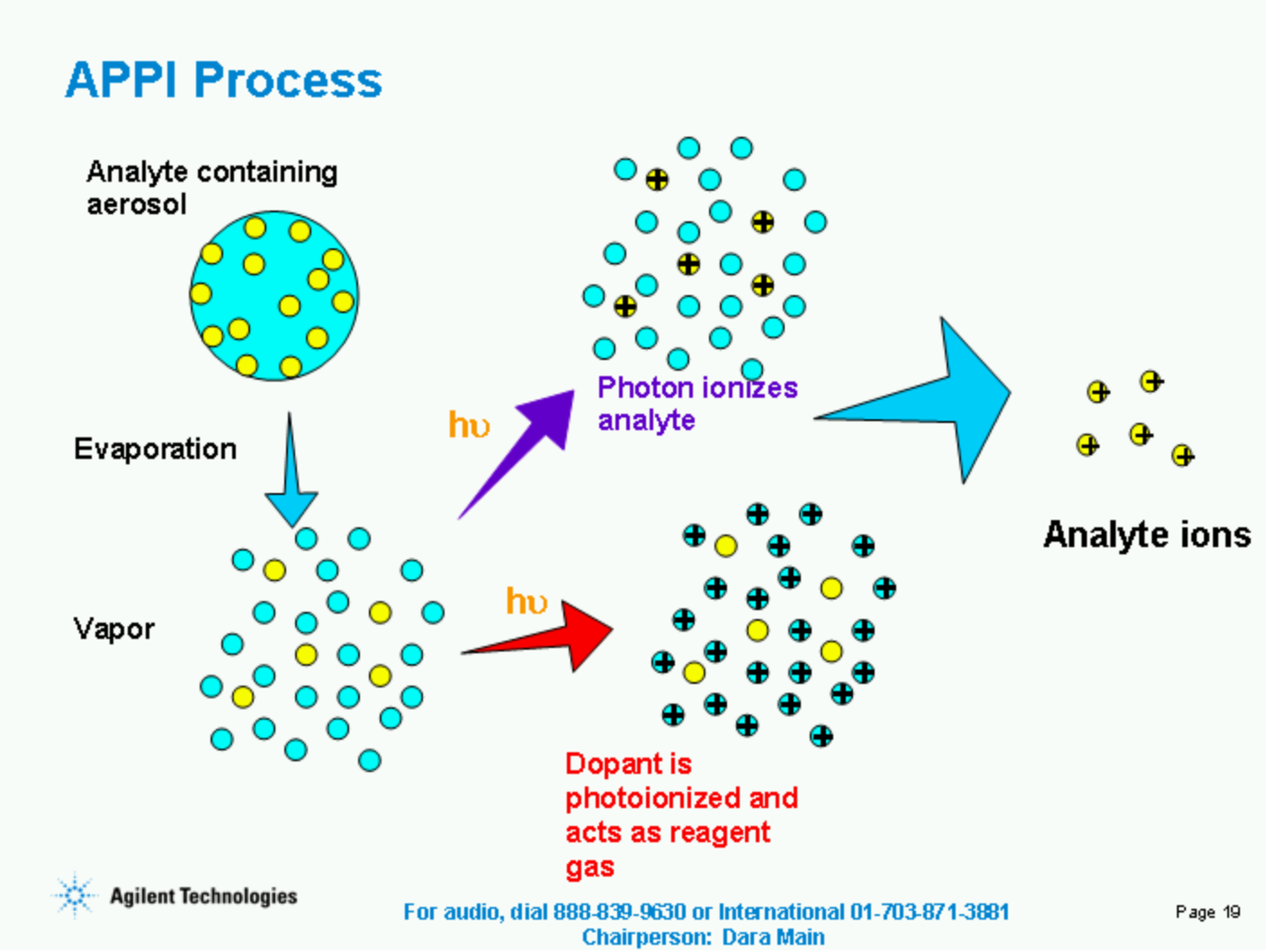
Key Chromatographic Points in Use of APCI



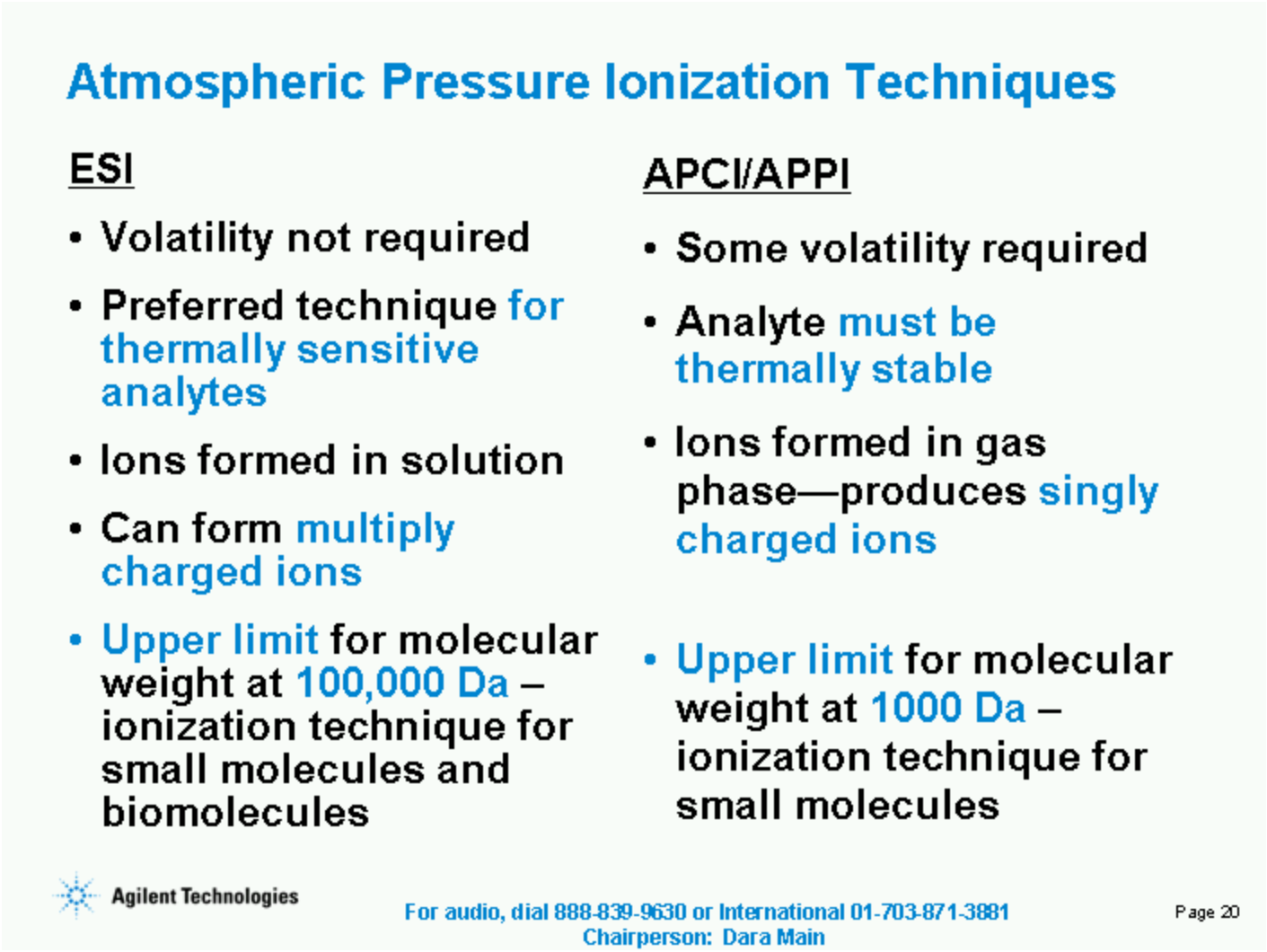
Definition and Application of APPI Atmospheric Photoionization



APPI Mechanisms



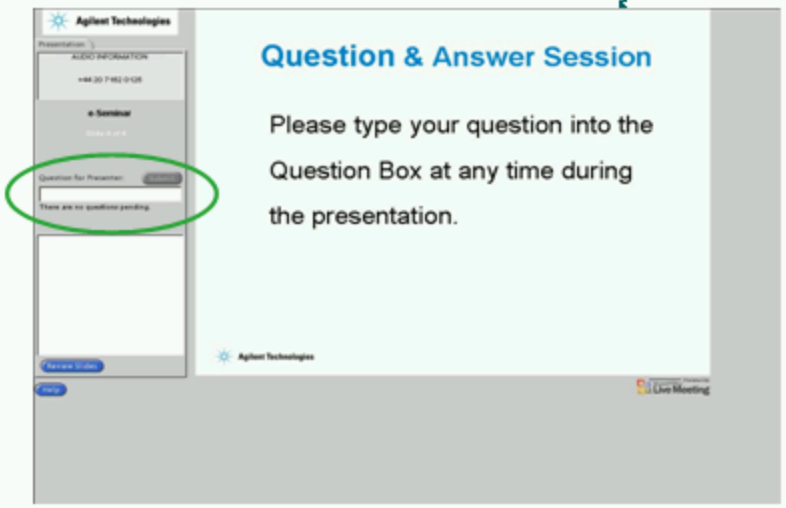
APPI Process



Atmospheric Pressure Ionization Techniques

Break Number 1

For questions, at break please dial 1 on your phone, or type in the Question Box at any time during the presentation.



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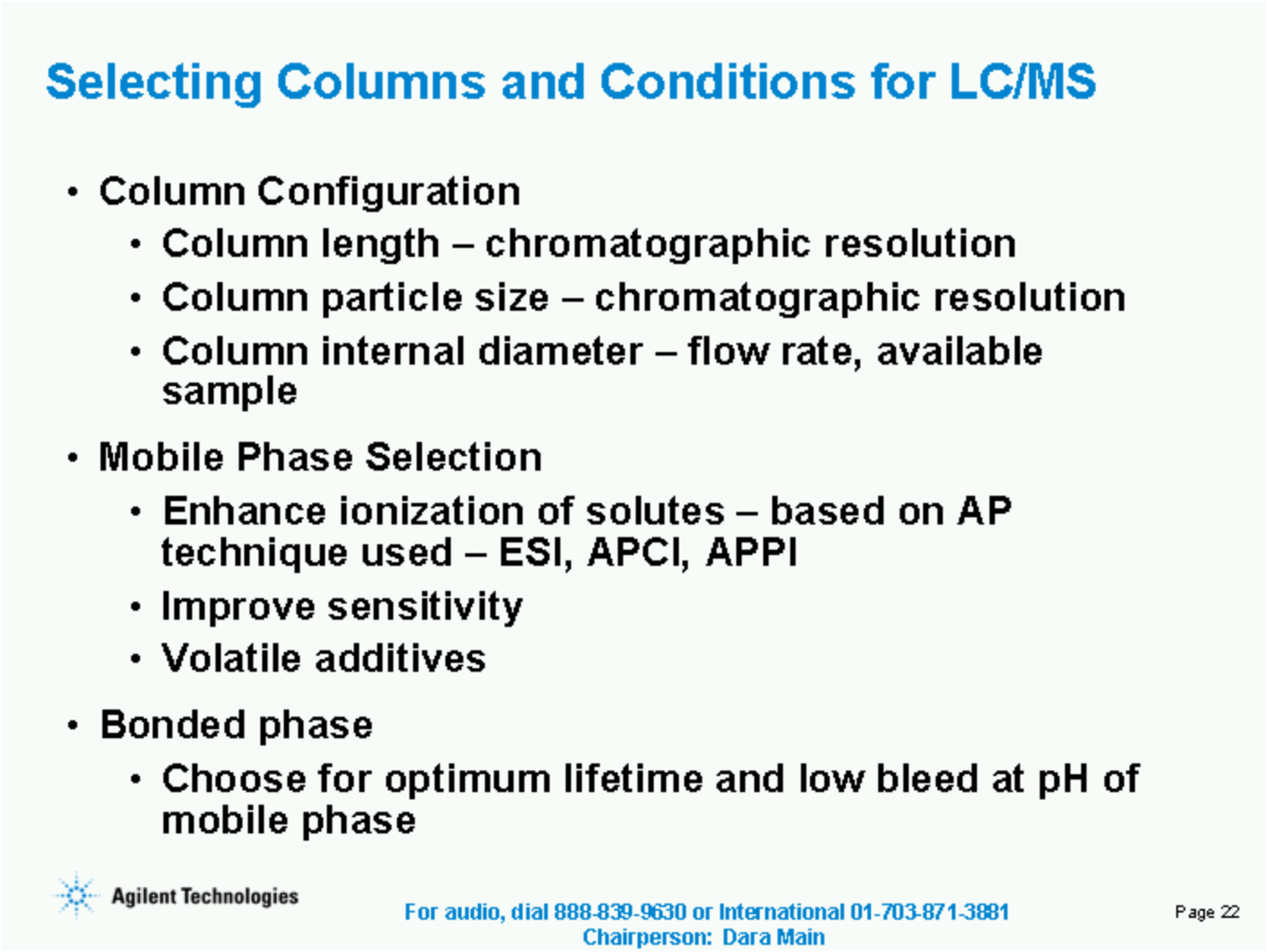
Page 21

Slide 21



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 um	100 – 600 nL/min
Capillary	0.3, 0.5 mm	35, 150, 250 mm	3.5, 5 um	1 – 10 uL/min
MicroBore	1.0 mm	30, 50, 150 mm	3.5, 5 um	30 – 60 ul/min
Narrow Bore	2.1 mm	15 – 250 mm	1.8, 3.5, 5 um	0.1 – 0.3 mL/min
Solvent Saver	3.0 mm	50 – 250 mm	3.5, 5 um	0.3 – 1 mL/min
Analytical	4.6 mm	15 – 250 mm	1.8, 3.5, 5um	1 – 1.5 mL/min

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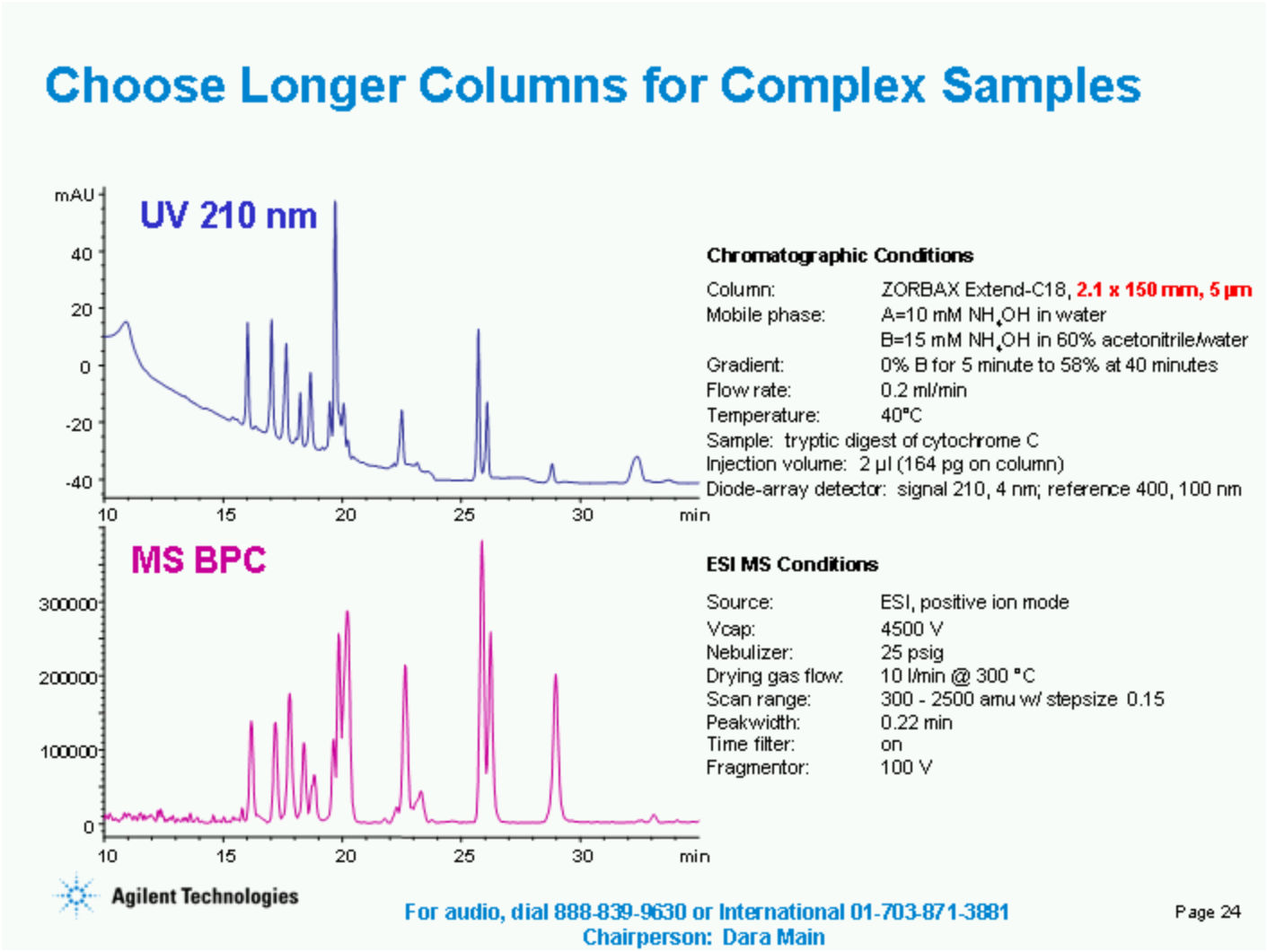
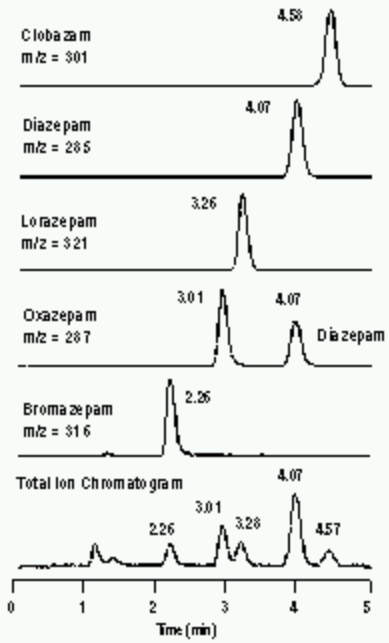
Page 23

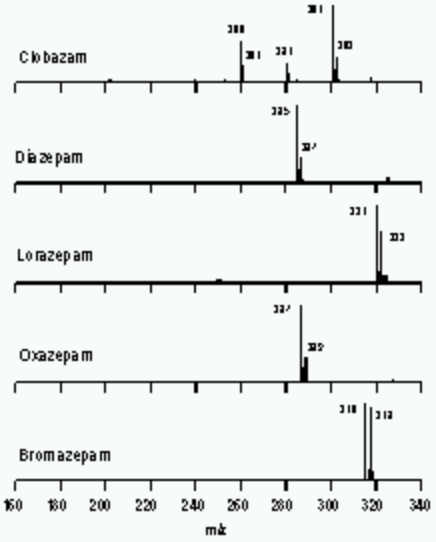
Agilent LC/MS Column Configurations



Choose Longer Columns for Complex Samples

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





Column: ZORBAX SB-CN
2.1 x 150 mm, 5 um
Mobile Phase: 60% water + 0.2% formic acid
40% ACN
Flow Rate: 0.25 mL/min
Detection: Electrospray Positive Ion
Sample: Benzodiazepines
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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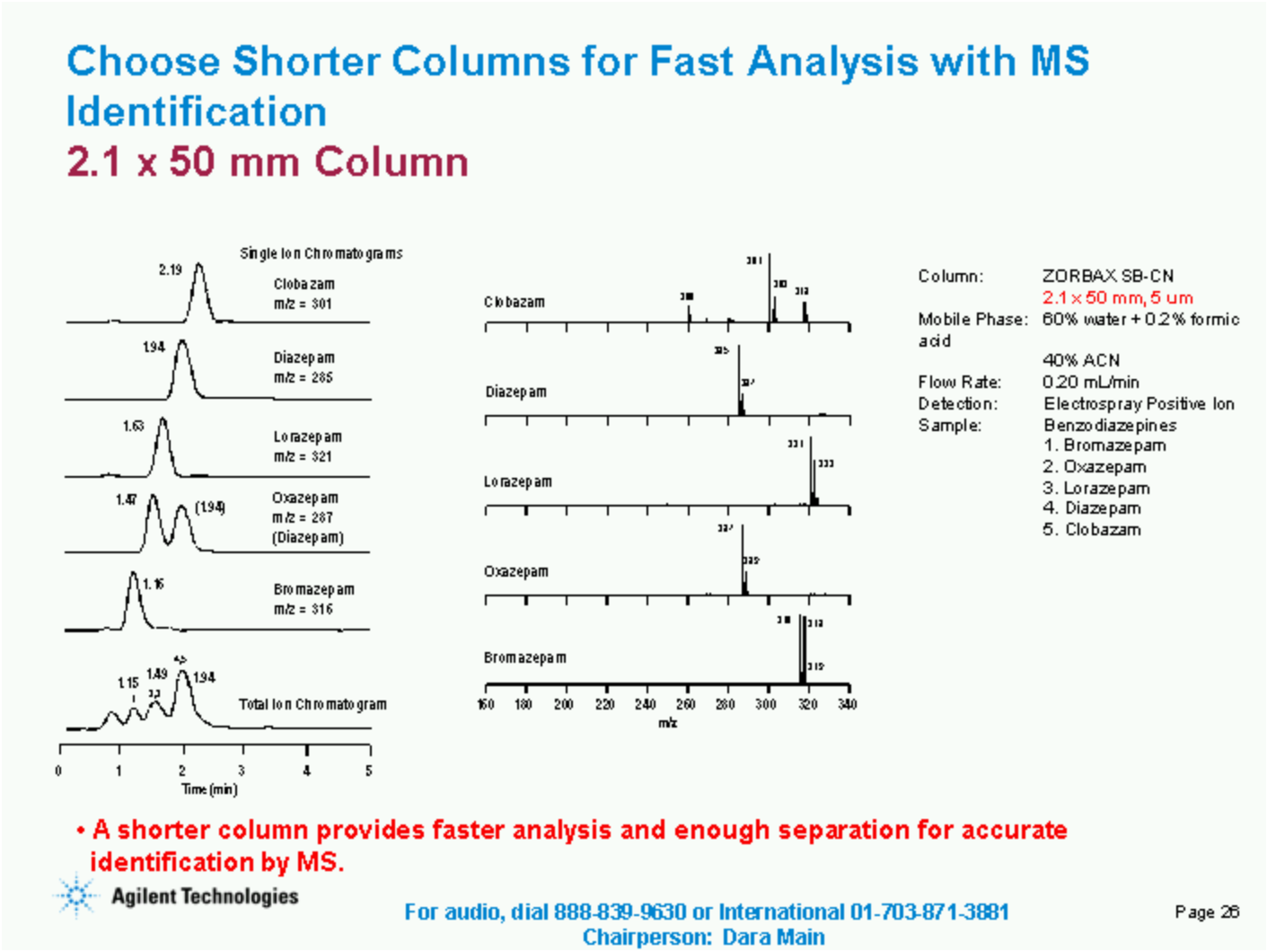
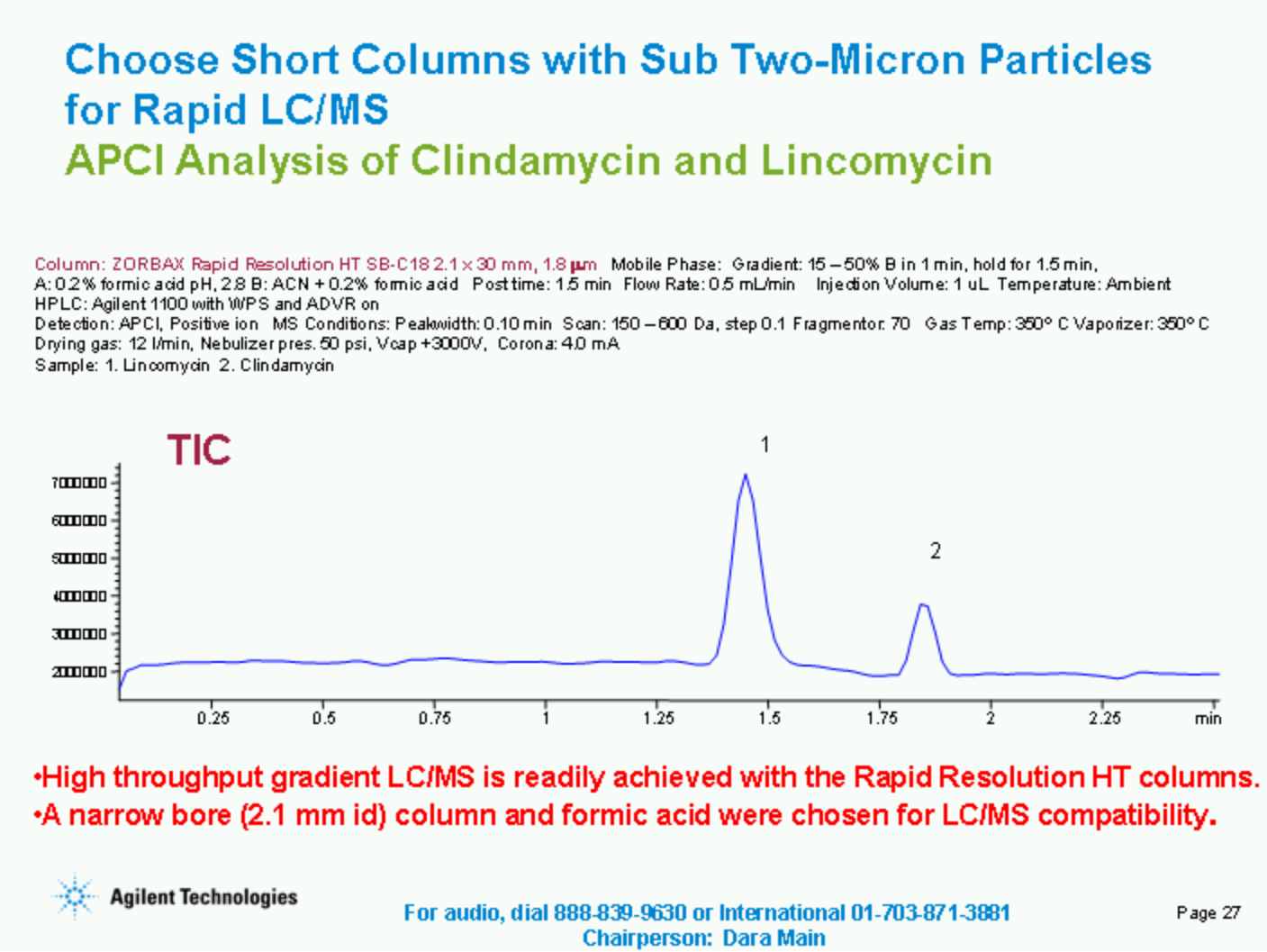
Page 25

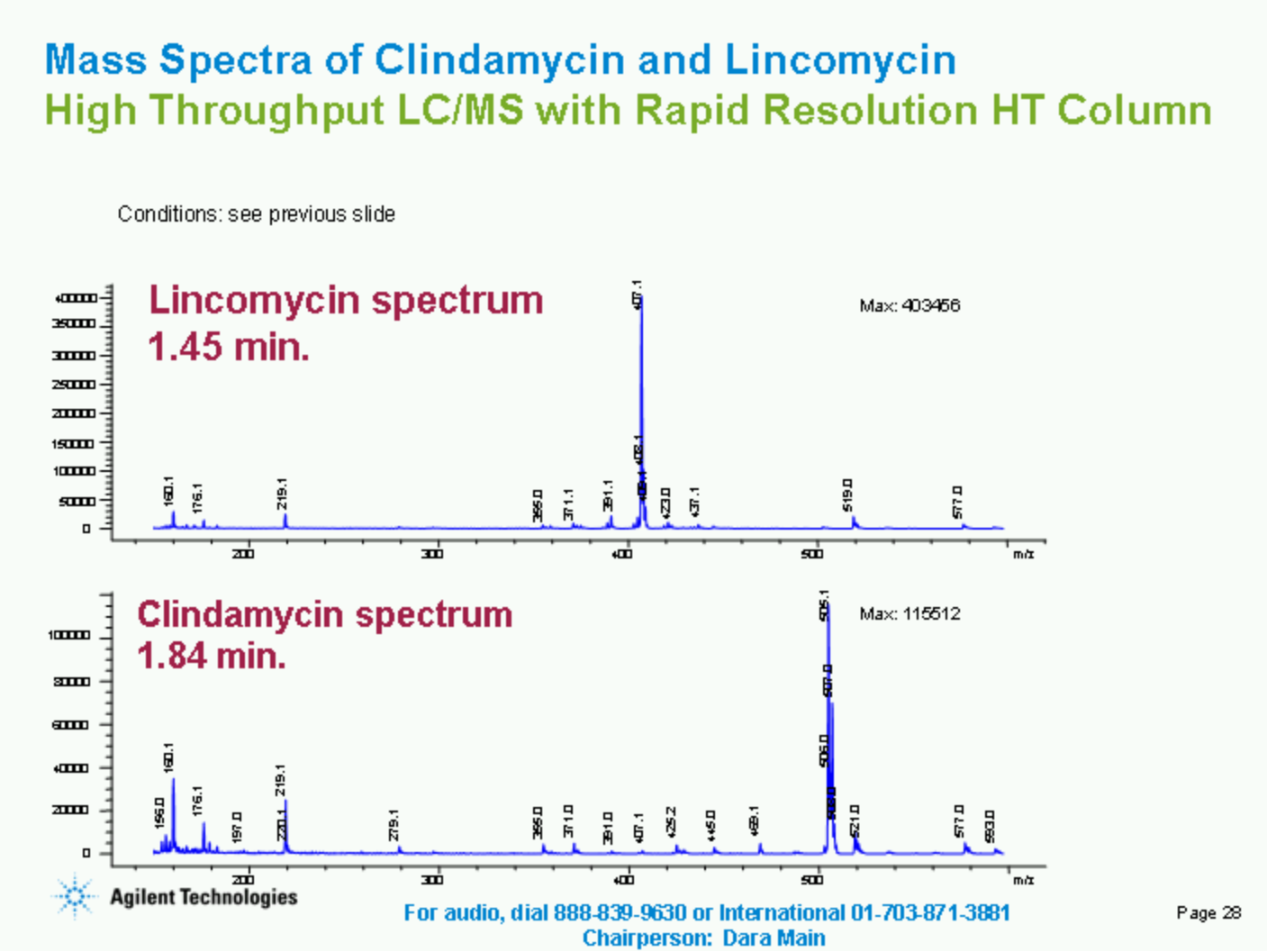
Choose Longer Columns for Maximum Chromatographic Resolution 2.1 x 150 mm Column



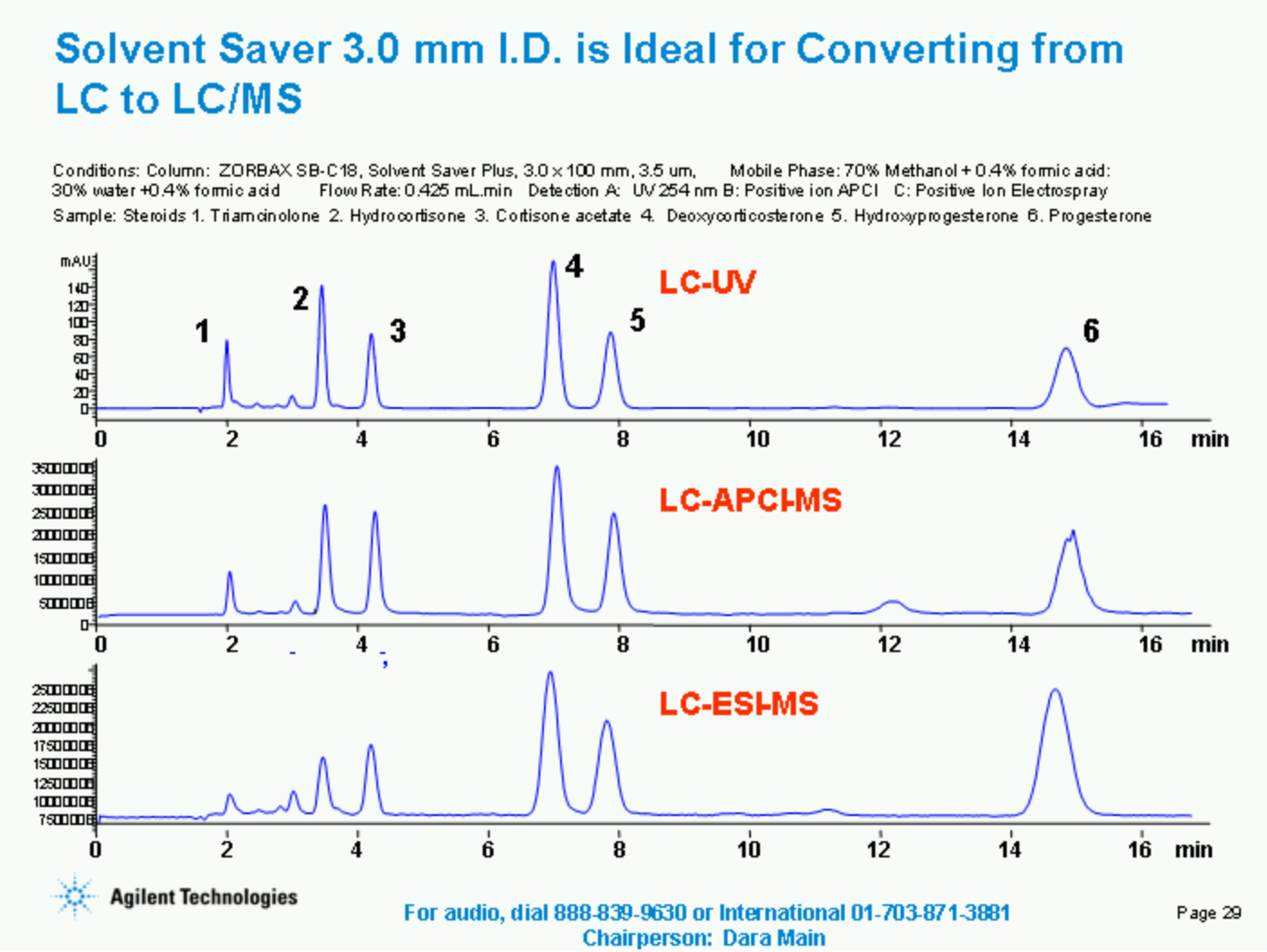
Choose Shorter Columns for Fast Analysis with MS Identification 2.1 x 50 mm Column



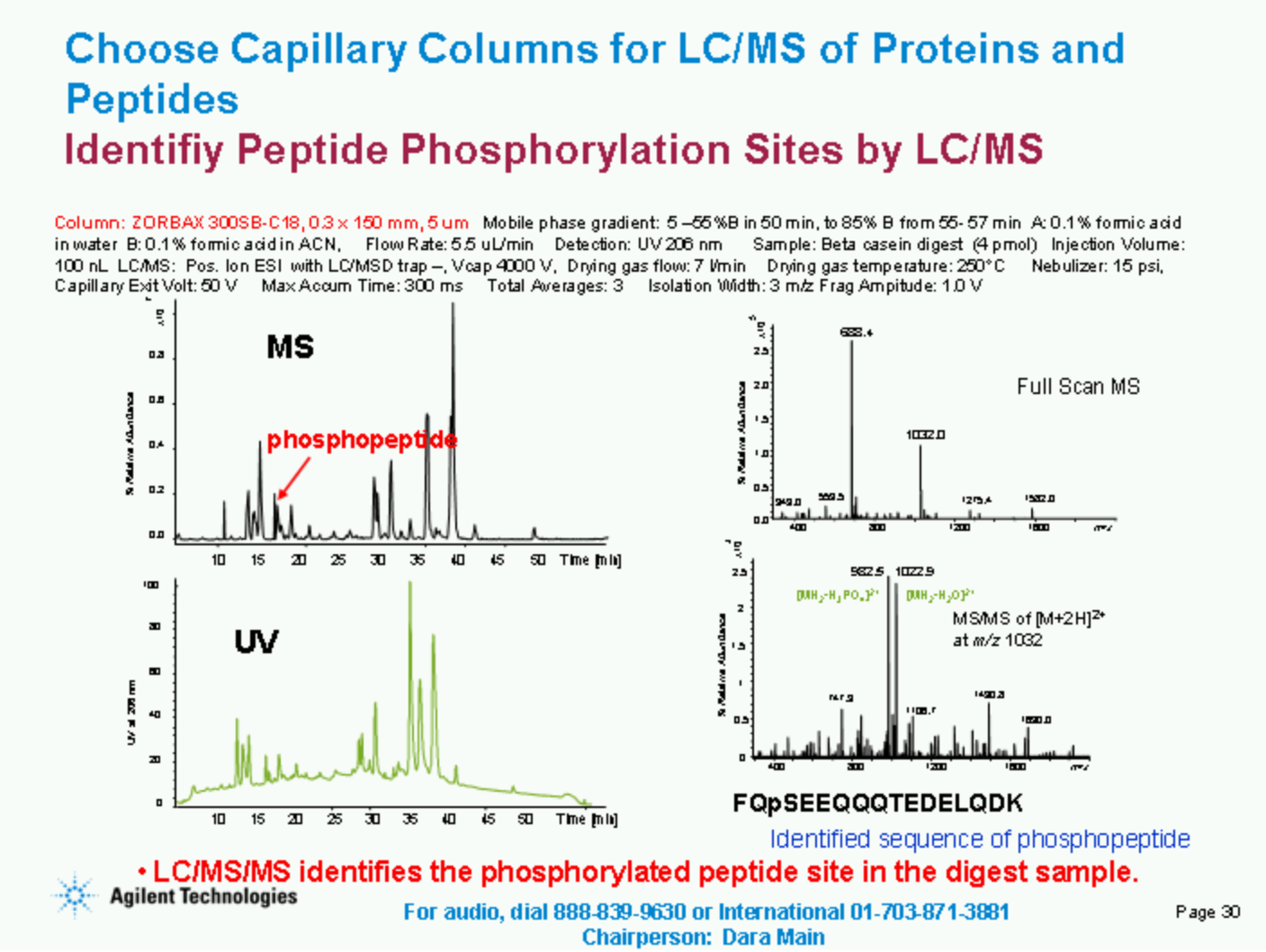
Choose Short Columns with Sub Two-Micron Particles for Rapid LC/MS APCI Analysis of Clindamycin and...



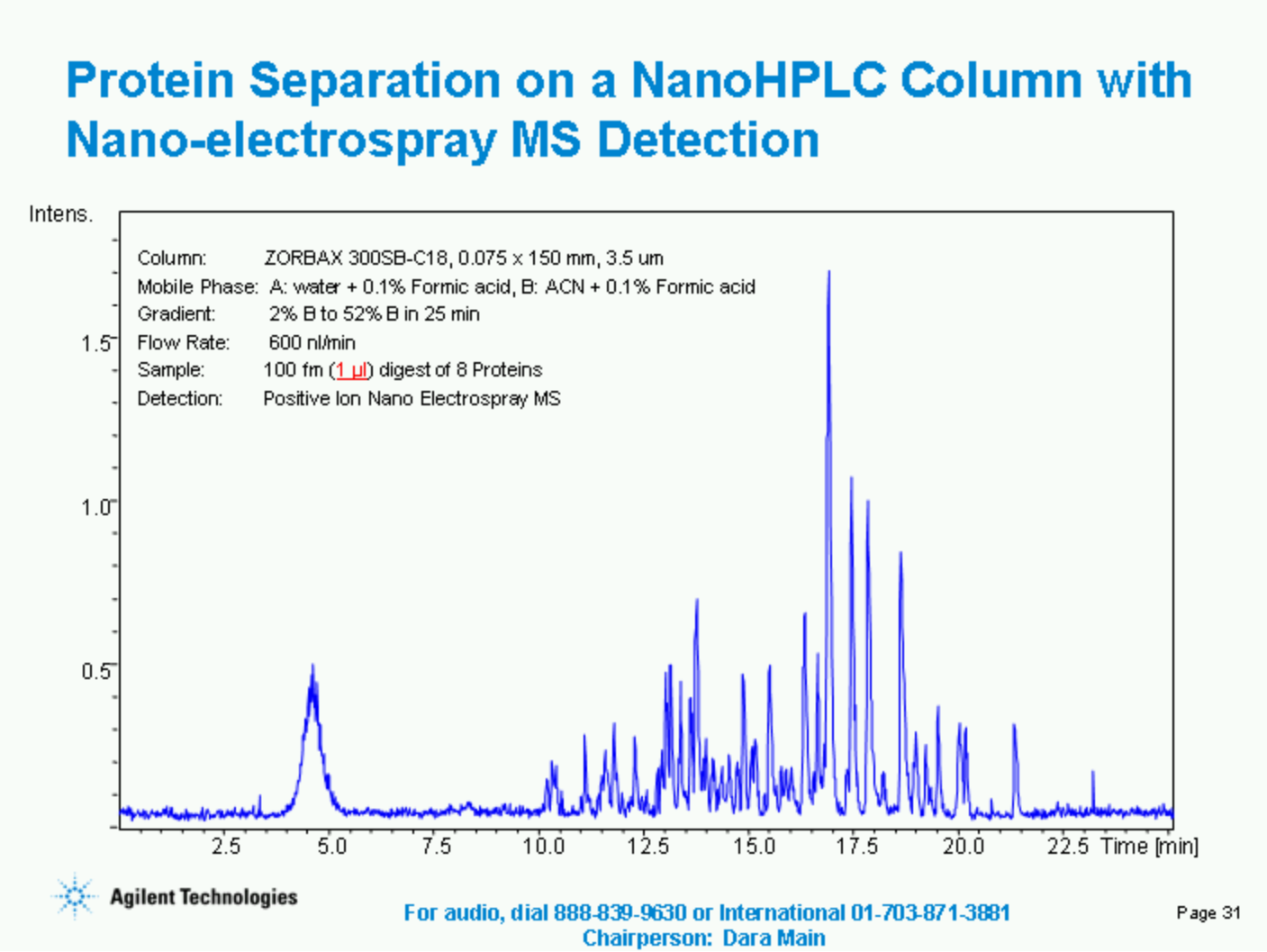
Mass Spectra of Clindamycin and Lincomycin High Throughput LC/MS with Rapid Resolution HT Column



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



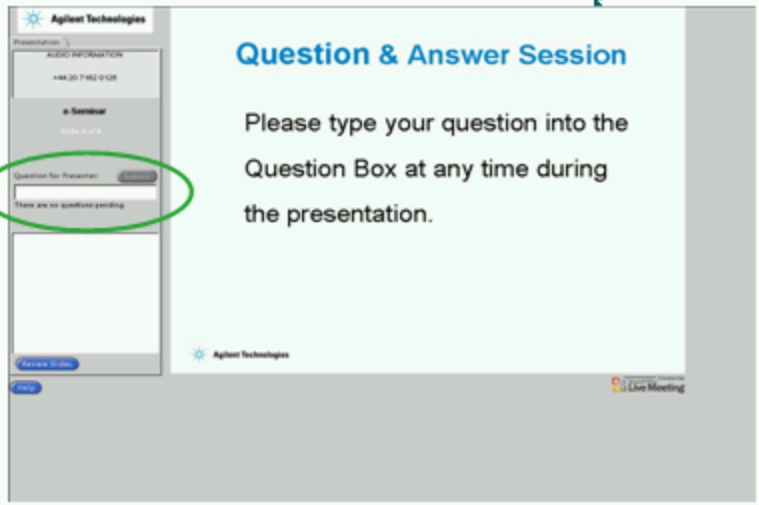
Choose Capillary Columns for LC/MS of Proteins and Peptides Identifiy Peptide Phosphorylation Site...



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

Break Number 2

For questions, at break please dial 1 on your phone, or type in the Question Box at any time during the presentation.



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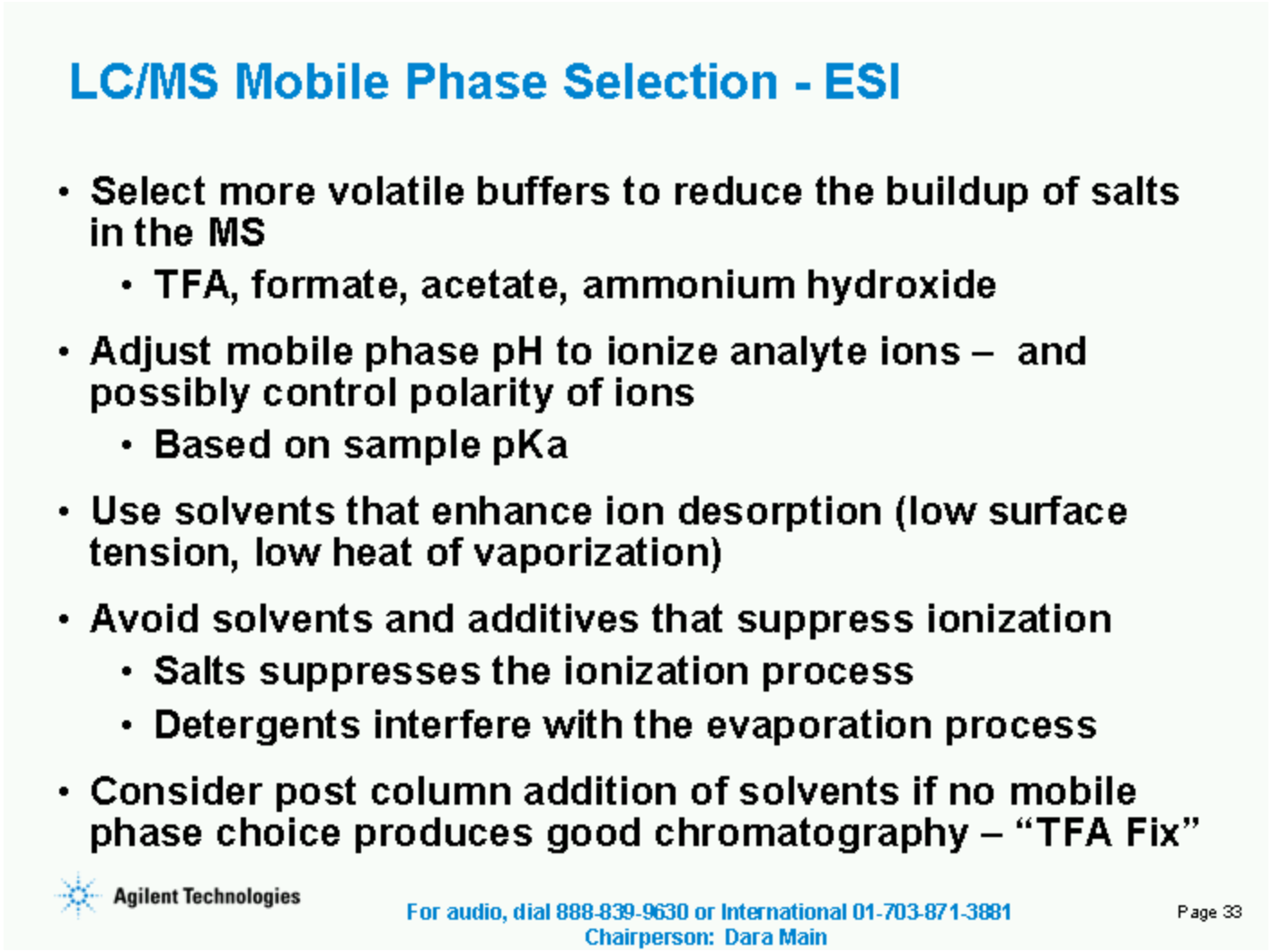
Page 32

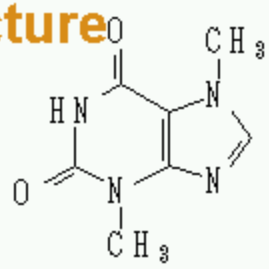
Break Number 2



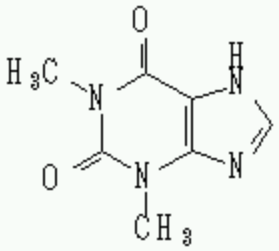
LC/MS Mobile Phase Selection - ESI

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

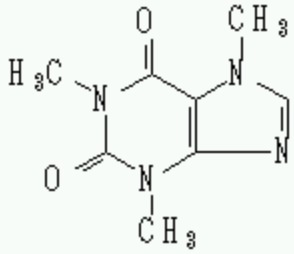
Chemical Structure



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



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



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

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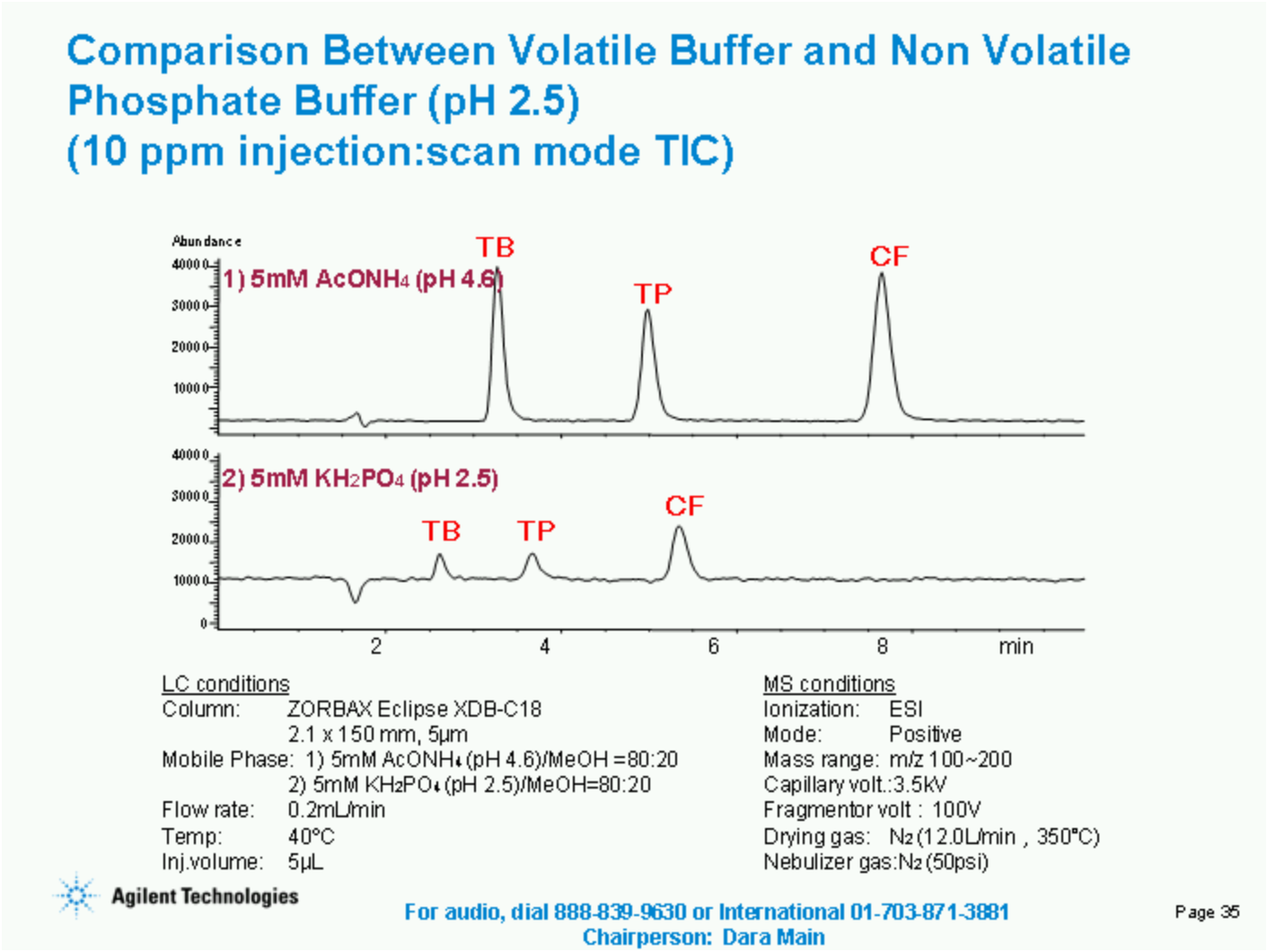
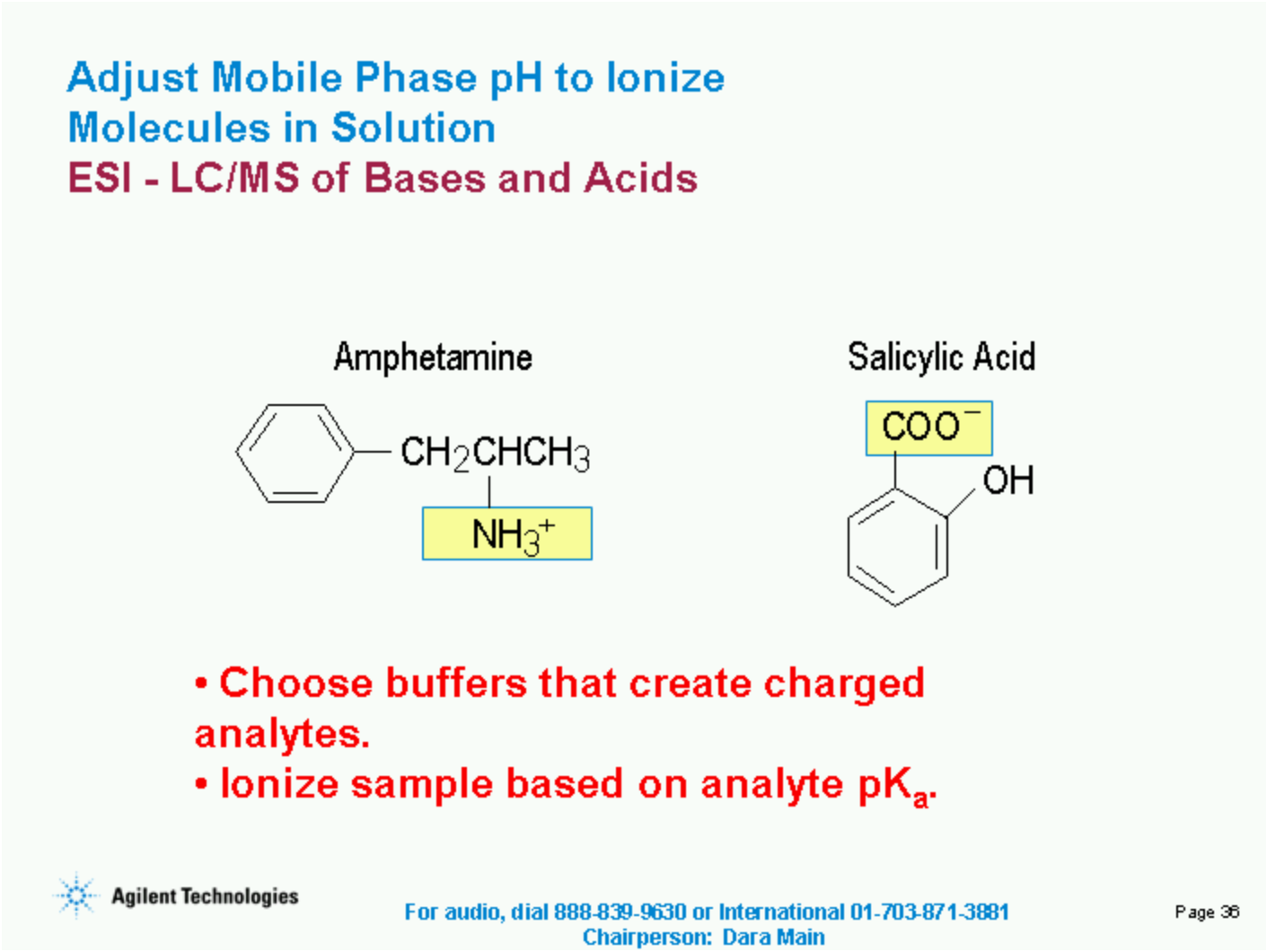
Page 34

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



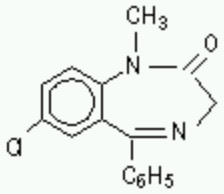
Comparison Between Volatile Buffer and Non Volatile Phosphate Buffer (pH 2.5) (10 ppm injection:sc...



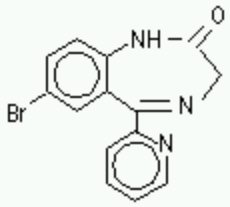
Adjust Mobile Phase pH to Ionize Molecules in Solution ESI - LC/MS of Bases and Acids

Basic Compounds - Benzodiazepines

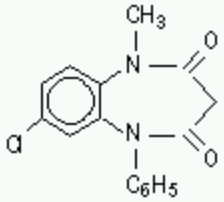
Diazepam
pK a 3.3



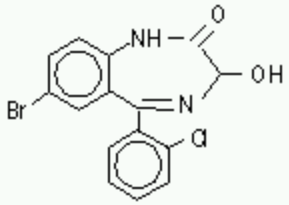
Bromazepam
pK a 2.9, 11.0



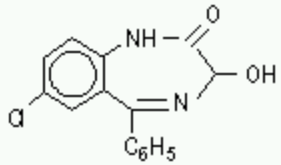
Clobazam



Lorazepam
pK a 1.3, 11.5



Oxazepam
pK a 1.7, 11.6



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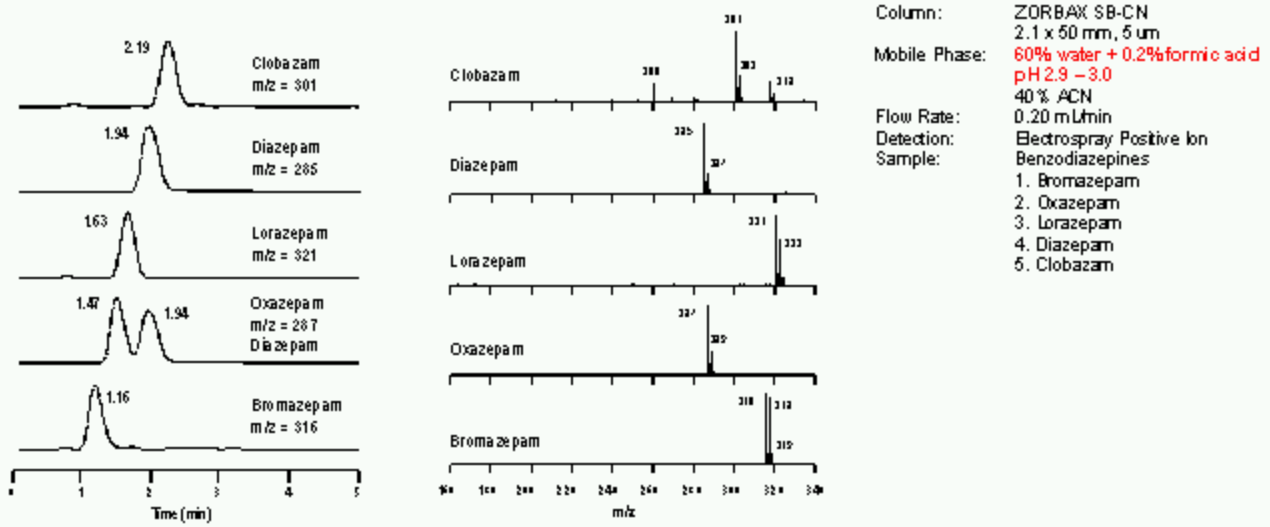
Page 37

Basic Compounds - Benzodiazepines

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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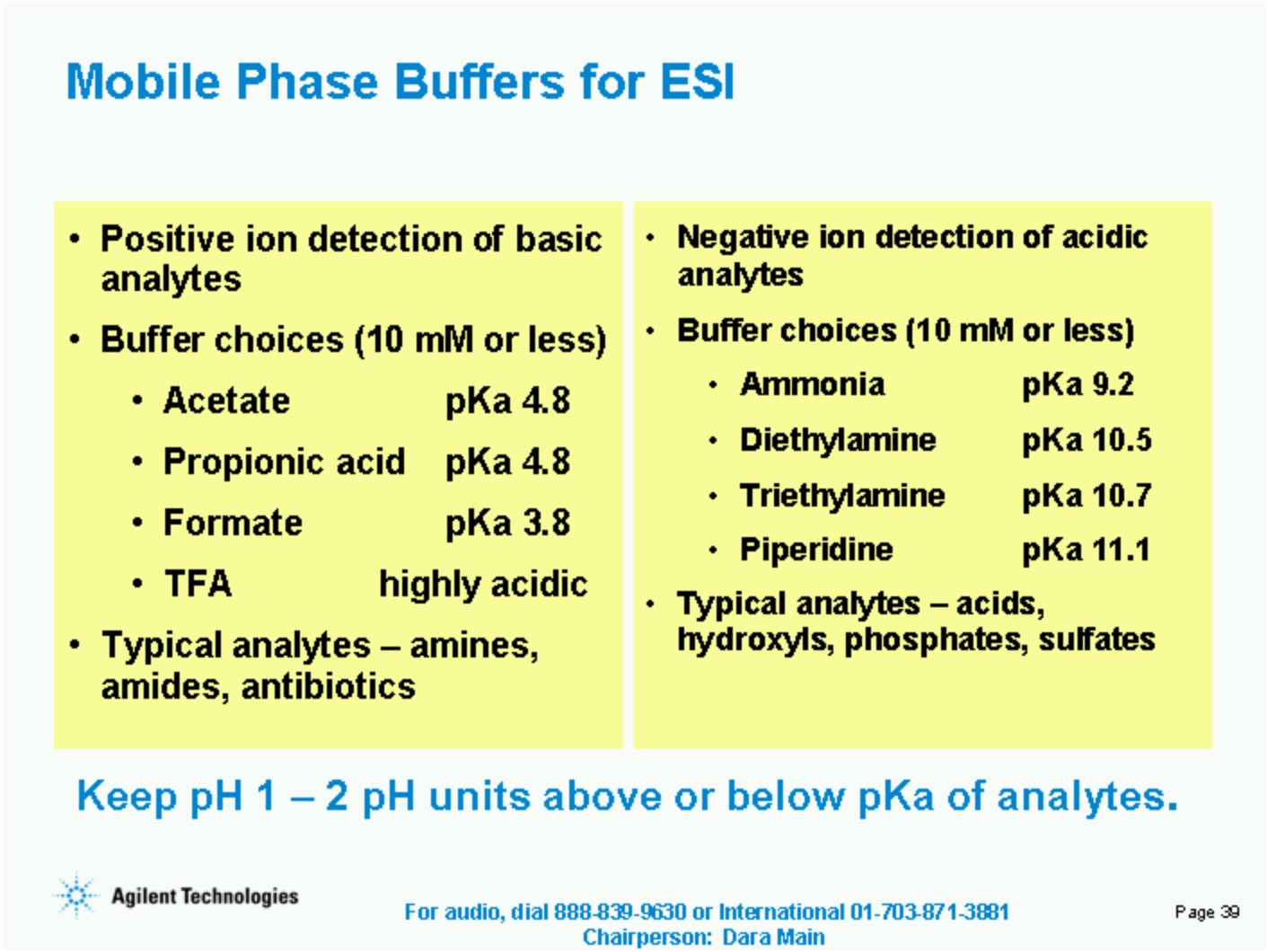
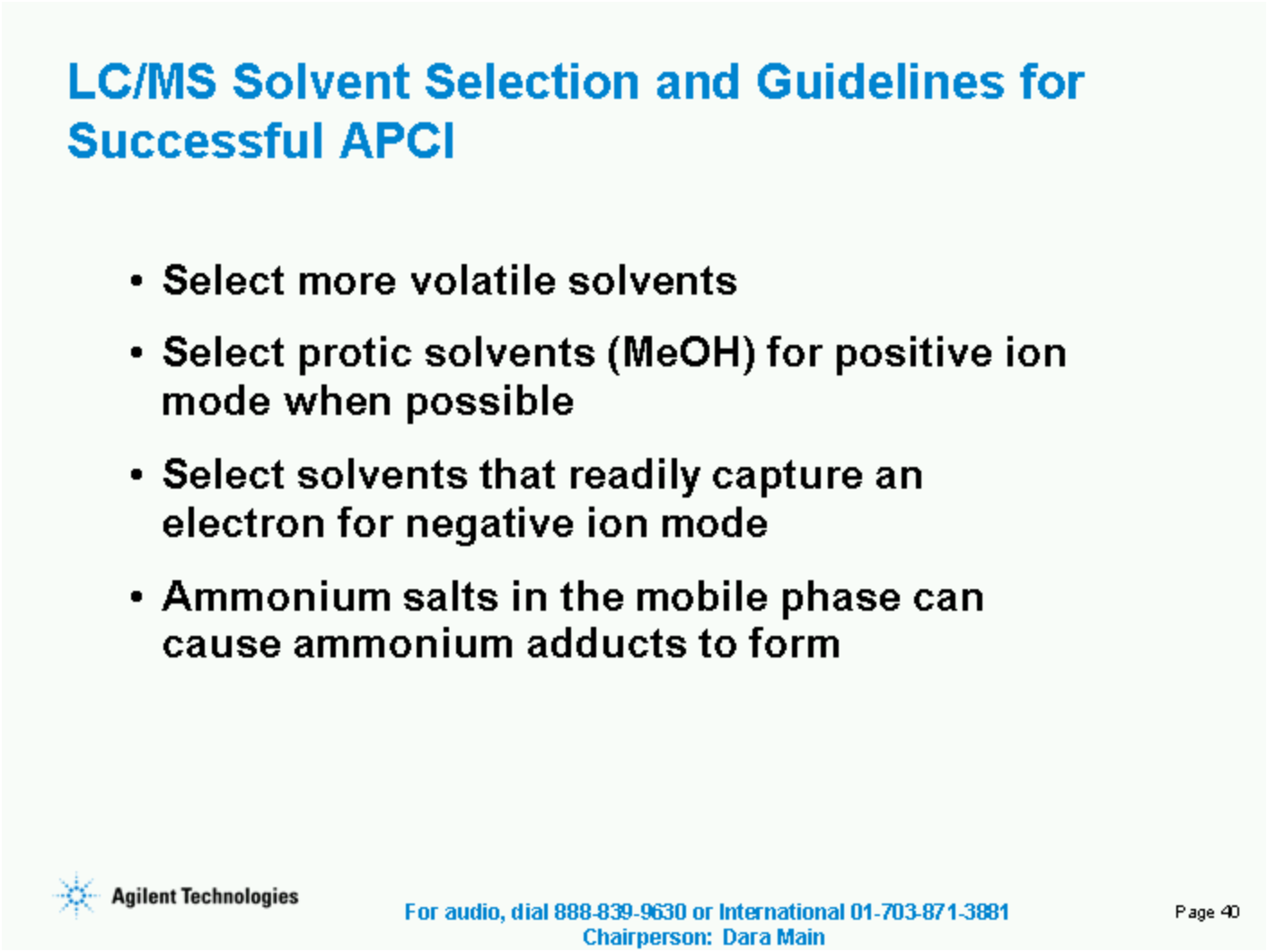
Page 38

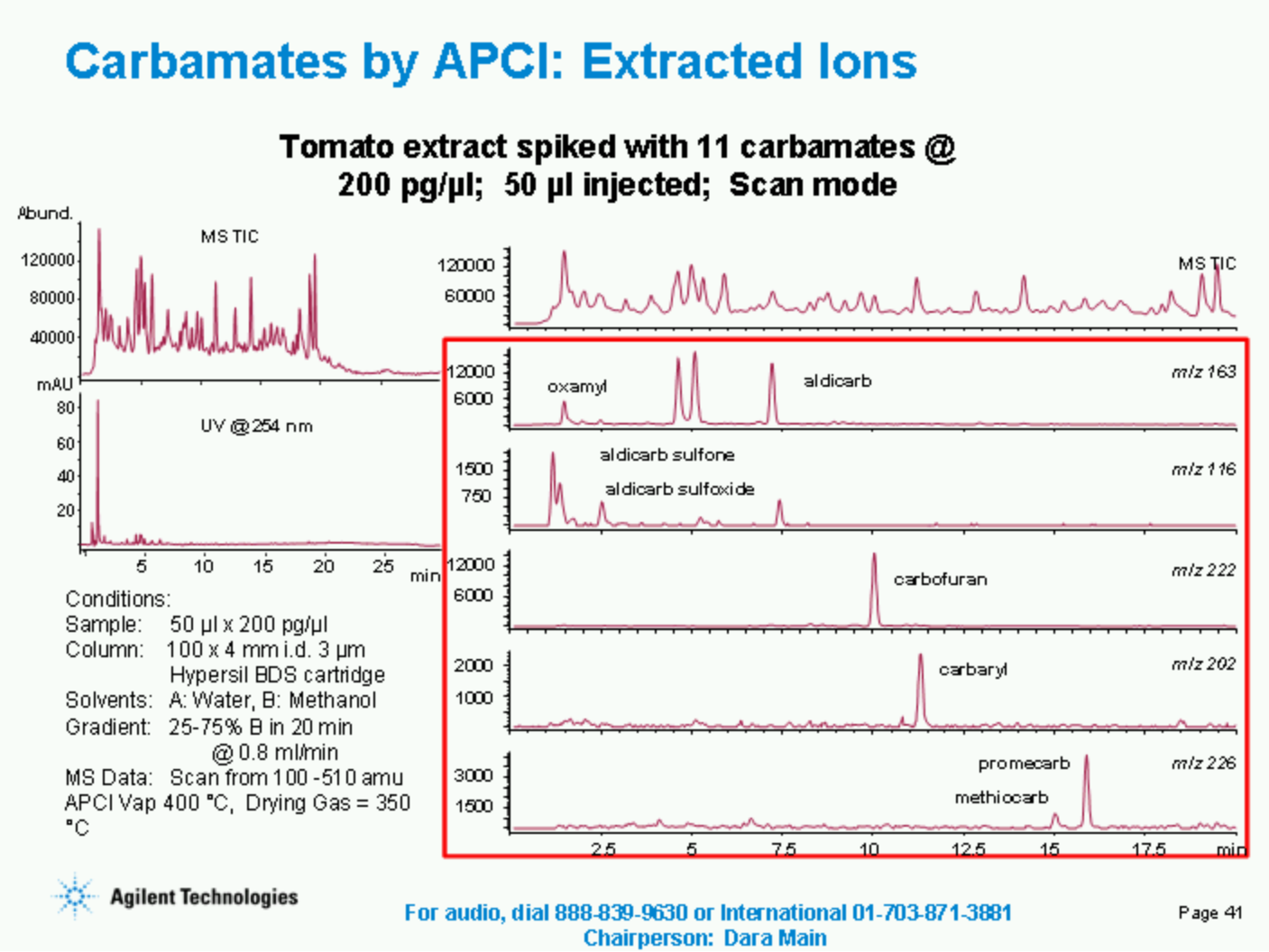
Choose Low pH Mobile Phase for Ionized Basic Compounds Positive Ion ESI LC/MS of Benzodiazepines



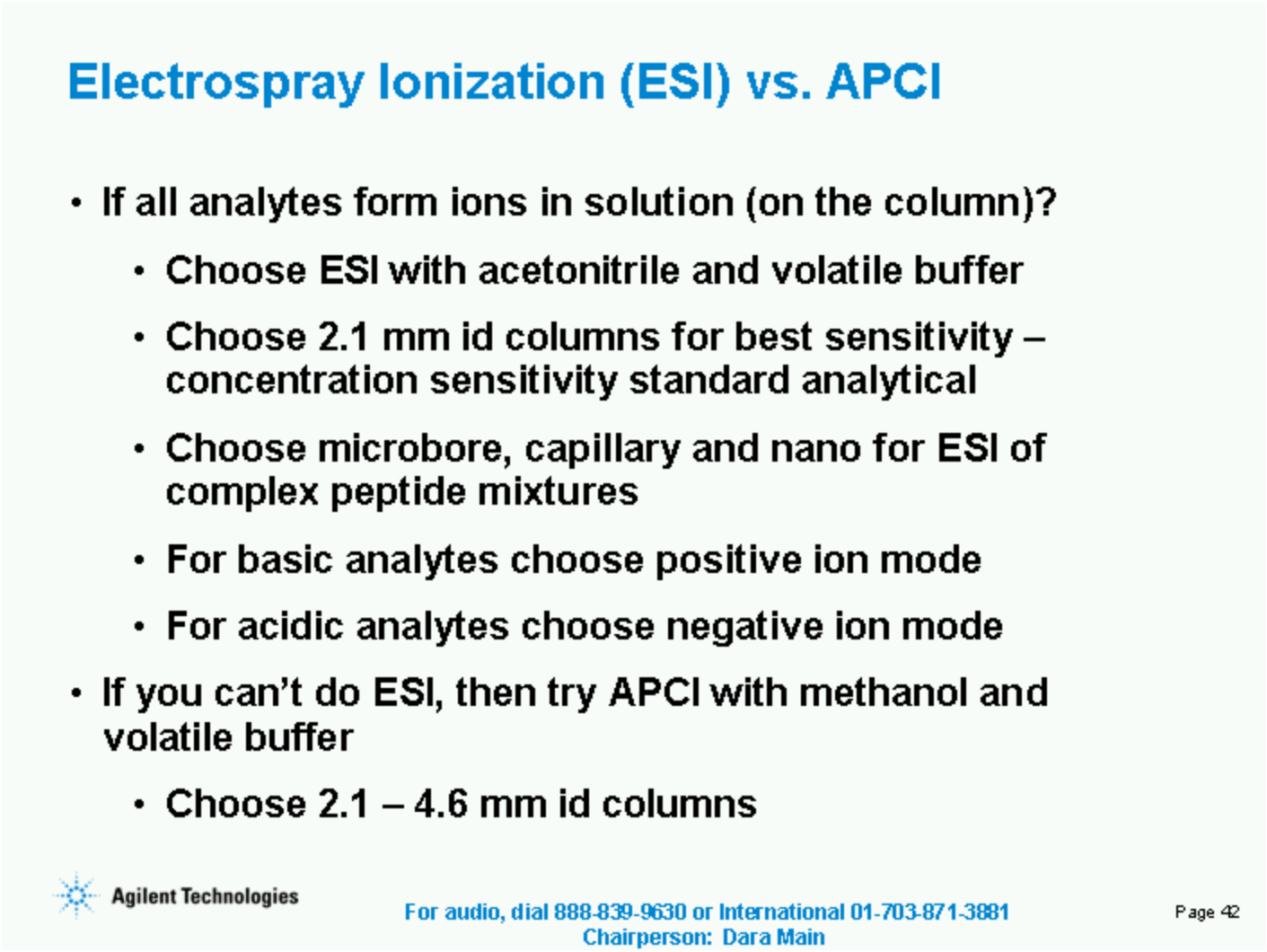
Mobile Phase Buffers for ESI



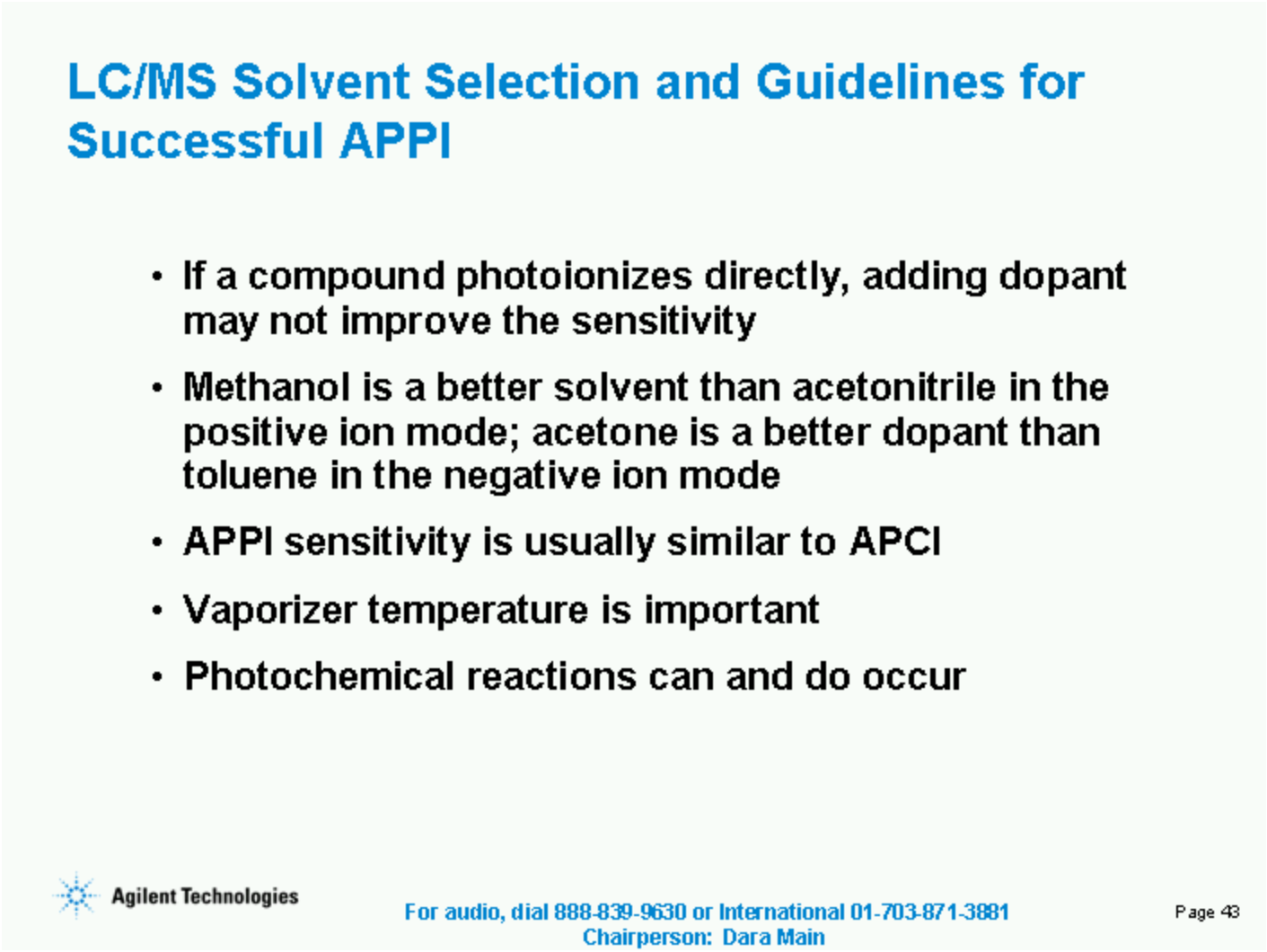
LC/MS Solvent Selection and Guidelines for Successful APCI



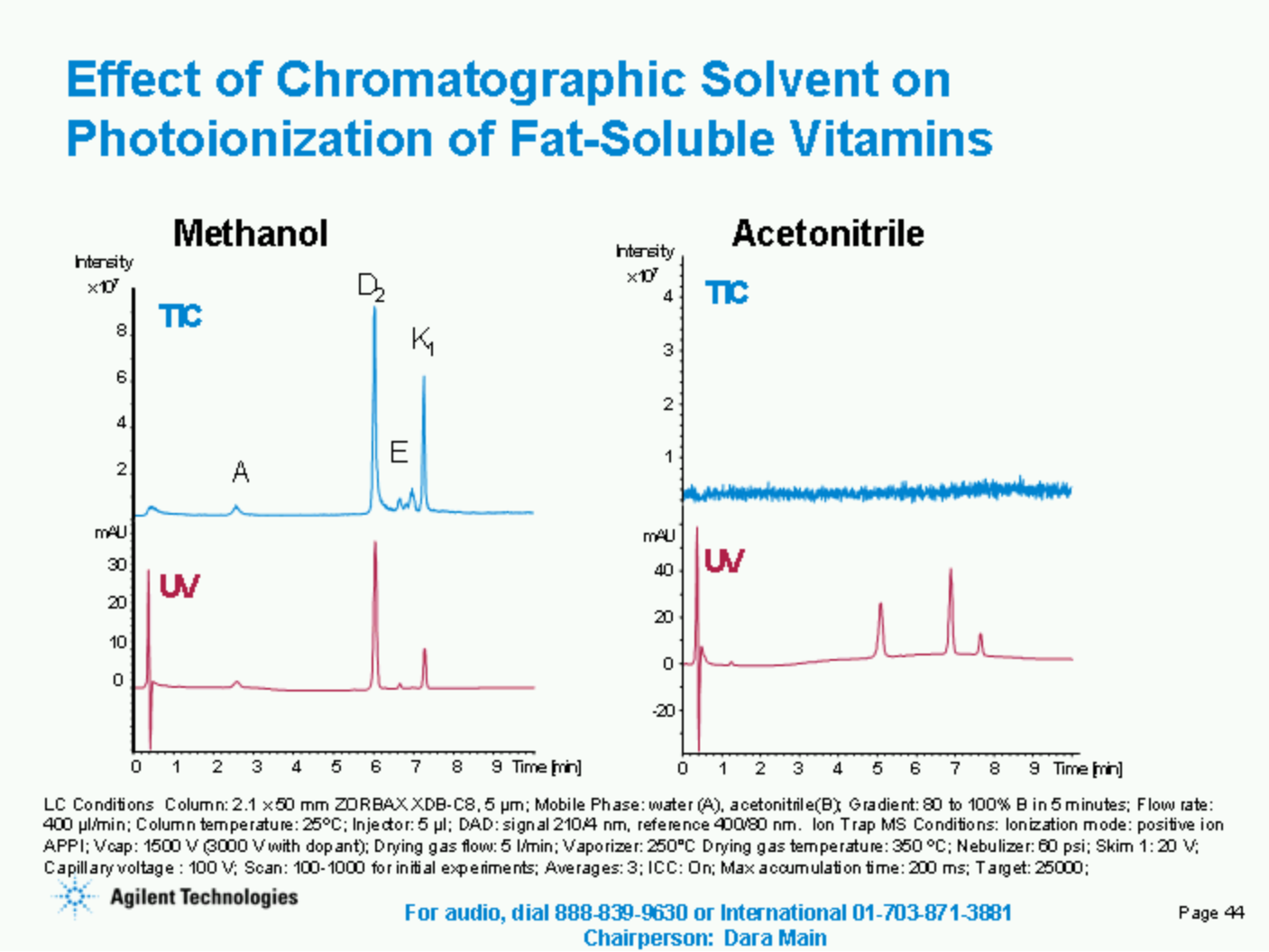
Carbamates by APCI: Extracted Ions



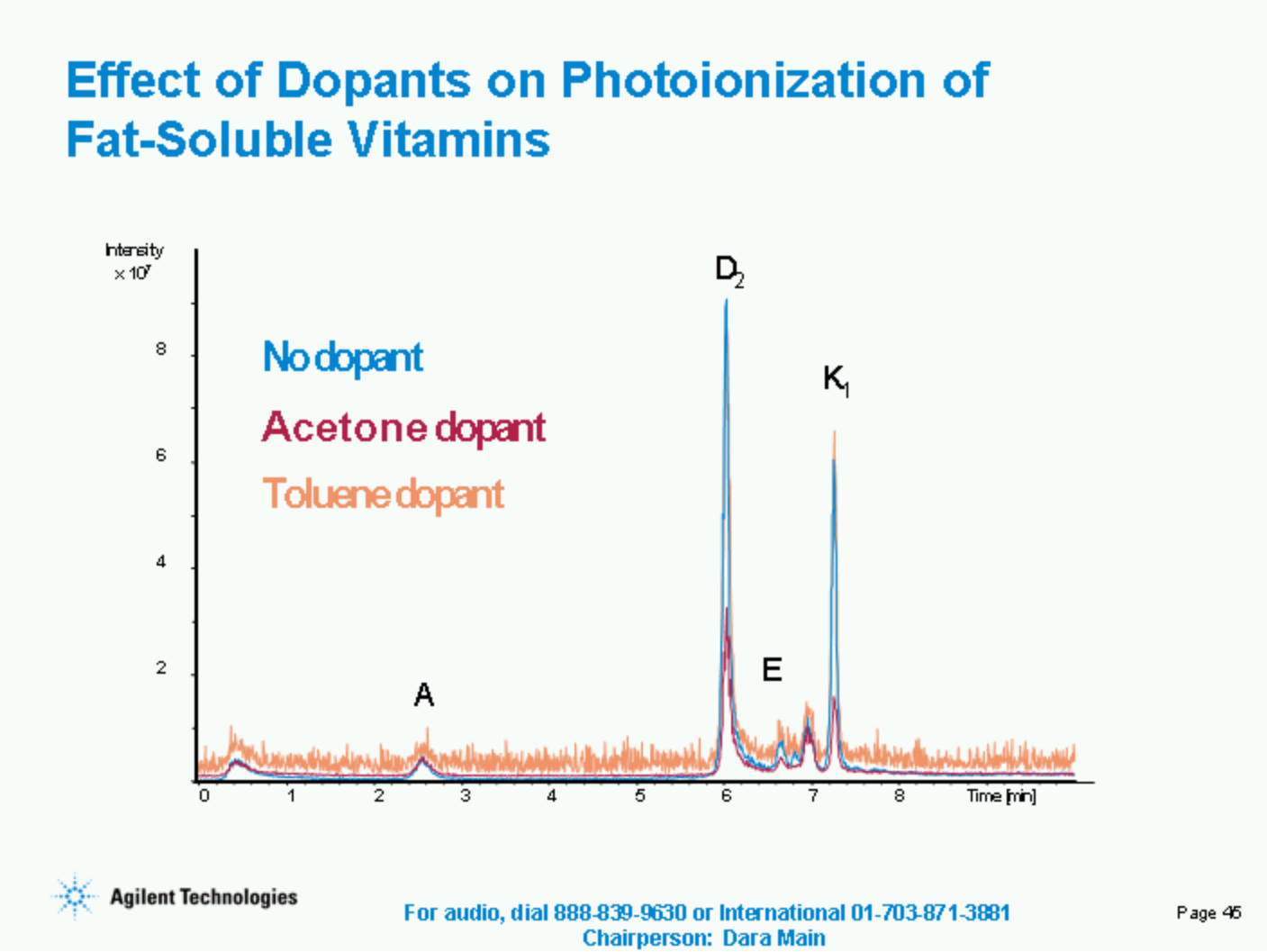
Electrospray Ionization (ESI) vs. APCI



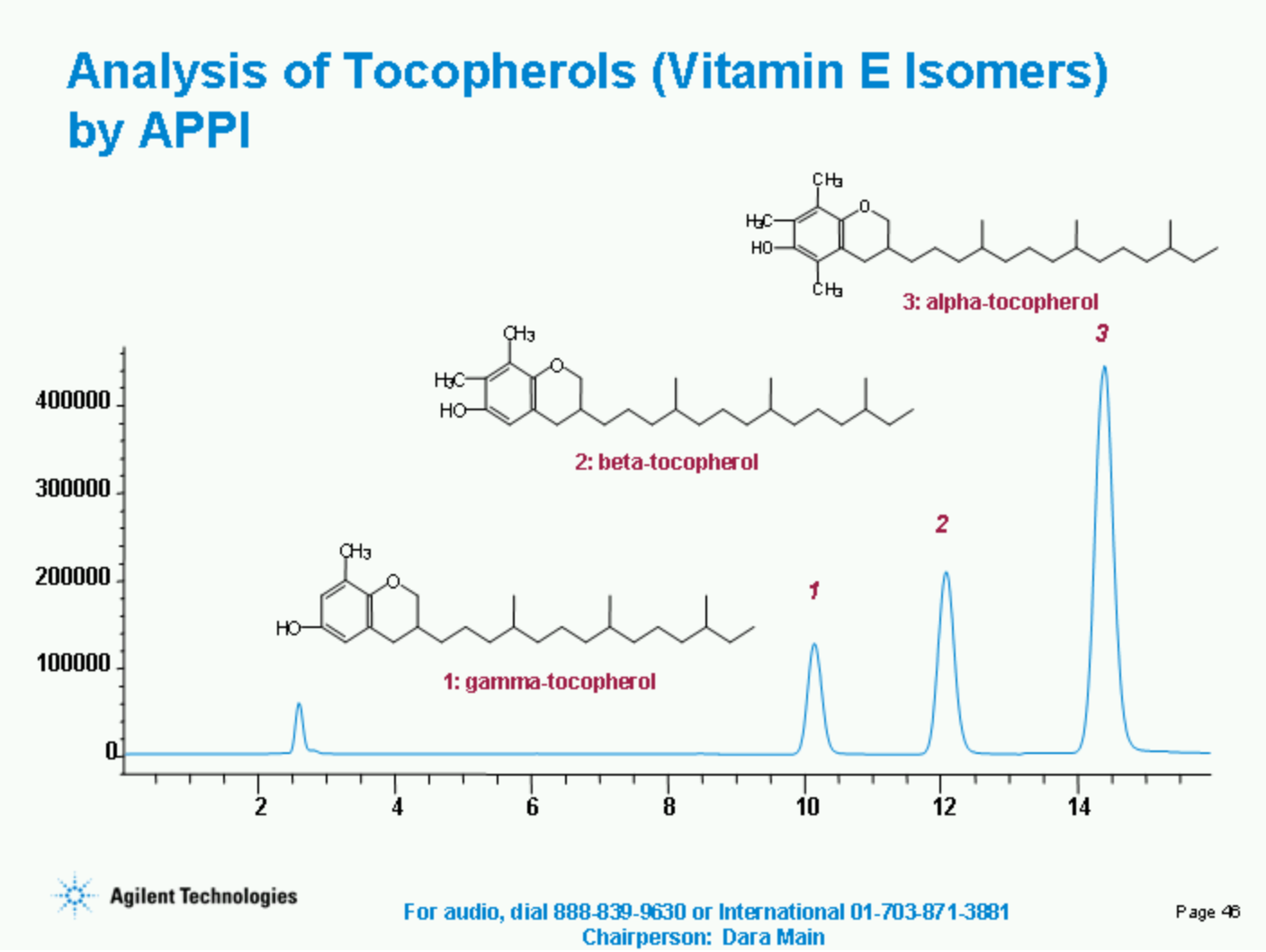
LC/MS Solvent Selection and Guidelines for Successful APPI



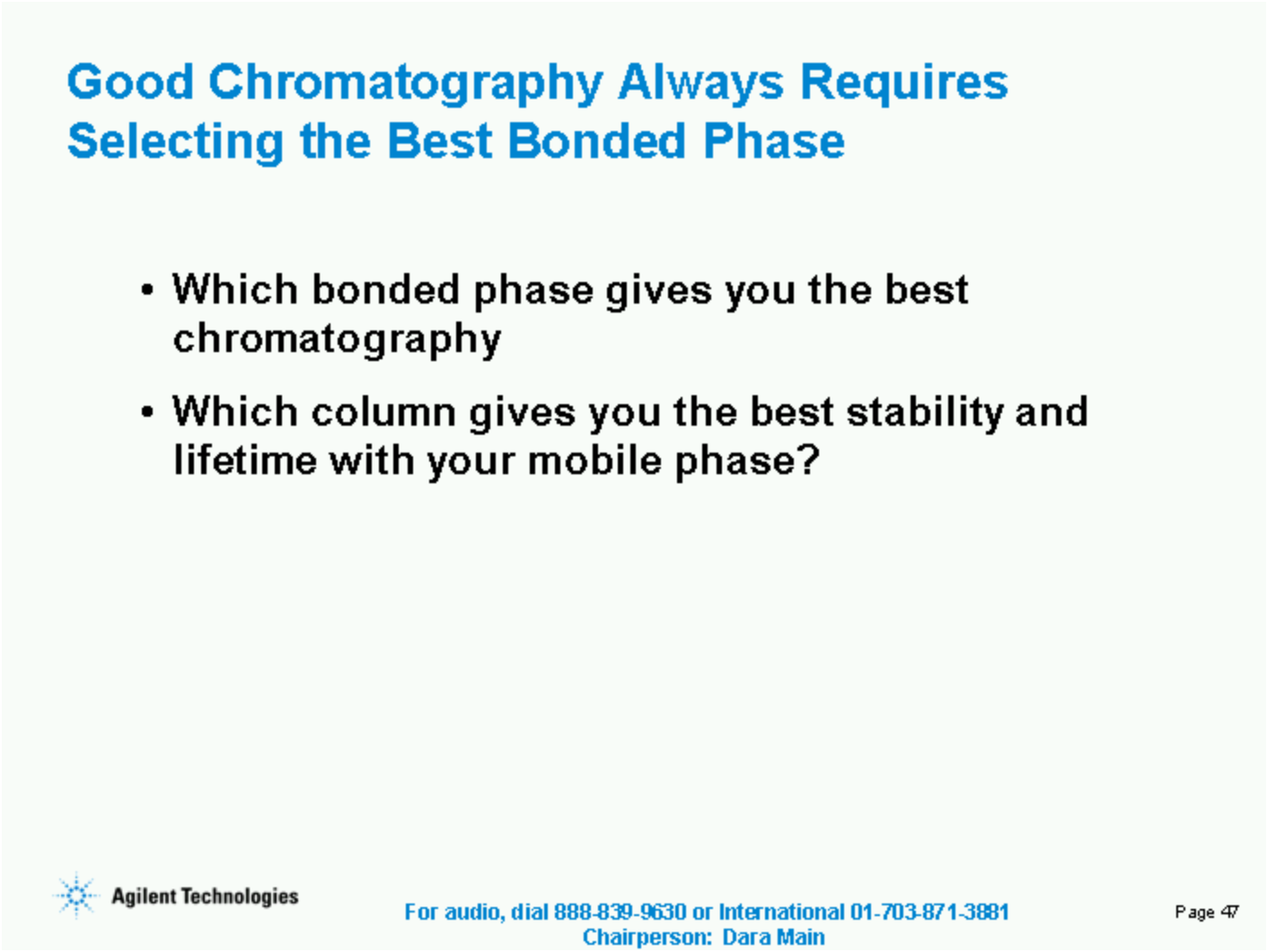
Effect of Chromatographic Solvent on Photoionization of Fat-Soluble Vitamins



Effect of Dopants on Photoionization of Fat-Soluble Vitamins

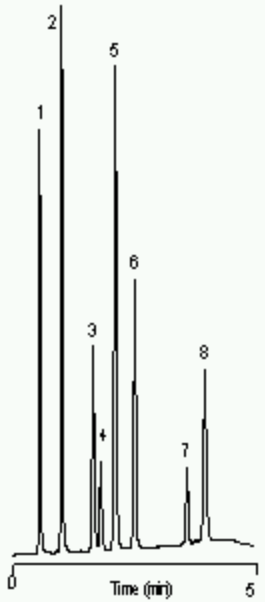


Analysis of Tocopherols (Vitamin E Isomers) by APPI



Good Chromatography Always Requires Selecting the Best Bonded Phase

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	5. Vanillic
2. Protocatechuric	6. Syringic
3. Hydrocaffeic	7. Sinapinic
4. Gentisic	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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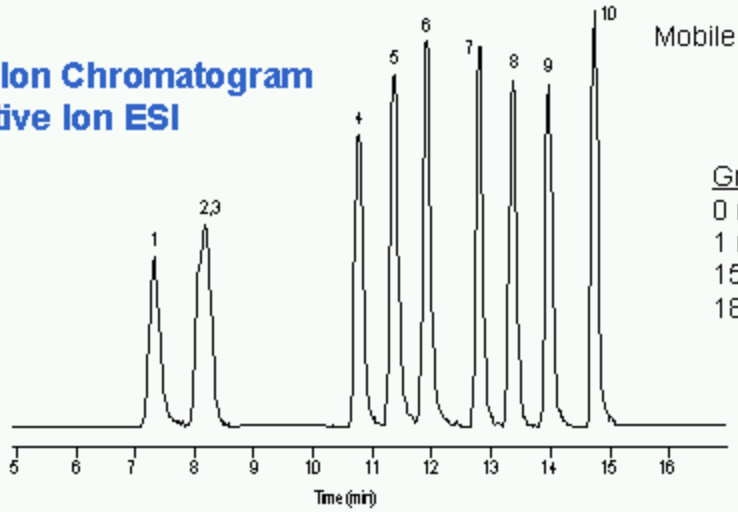
Page 48

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 um Flow Rate: 0.3 mL/min Temperature: RT Sample:
1. Naproxen 2. Tolmetin 3. Ketoprofen 4. Phenylbutazone 5. Fenoprofen 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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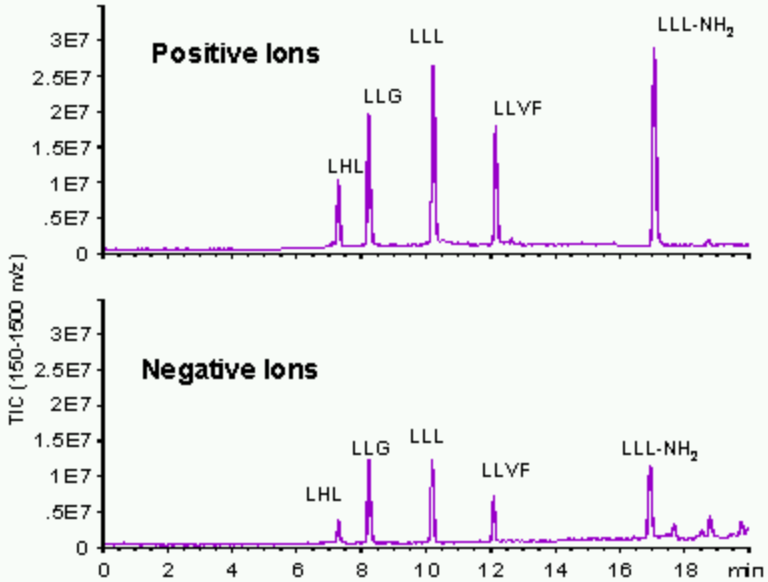
Page 49

LC/MS Bonded-Phase Selection at Mid pH

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



Column: **ZORBAX Extend C18 2.1 x 150 mm, 5 um**
Flow rate: 0.25mL/min
Temp: 25°C
Gradient: 5-60% B in 20 min
LC/MS: Pos. Ion ESI- Vf 70V, Vcap 4.5 kV, N2- 35 psi, 12L/min, 300°C
Sample: 4µL (50 ng each peptide)

- 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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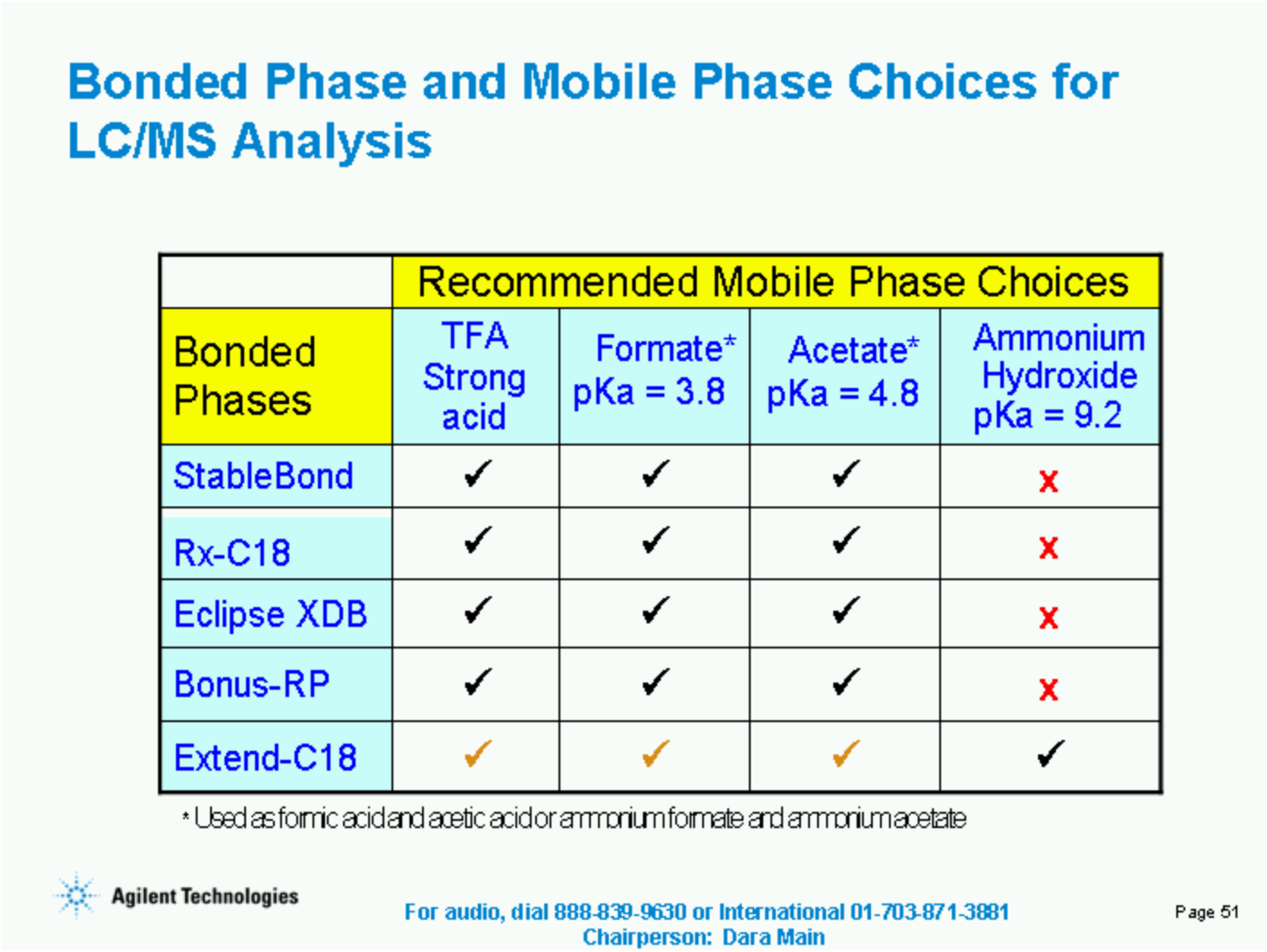
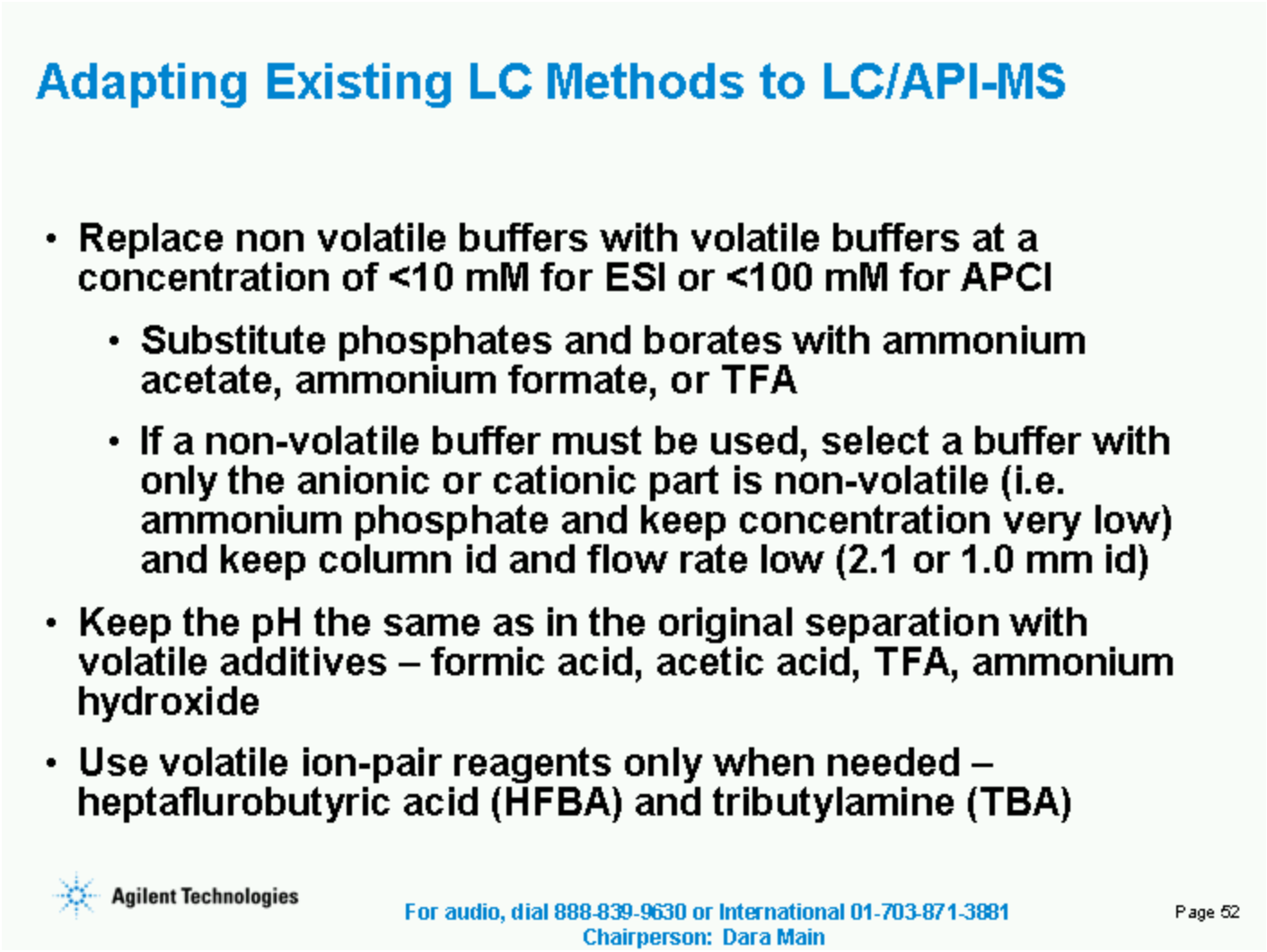
Page 50

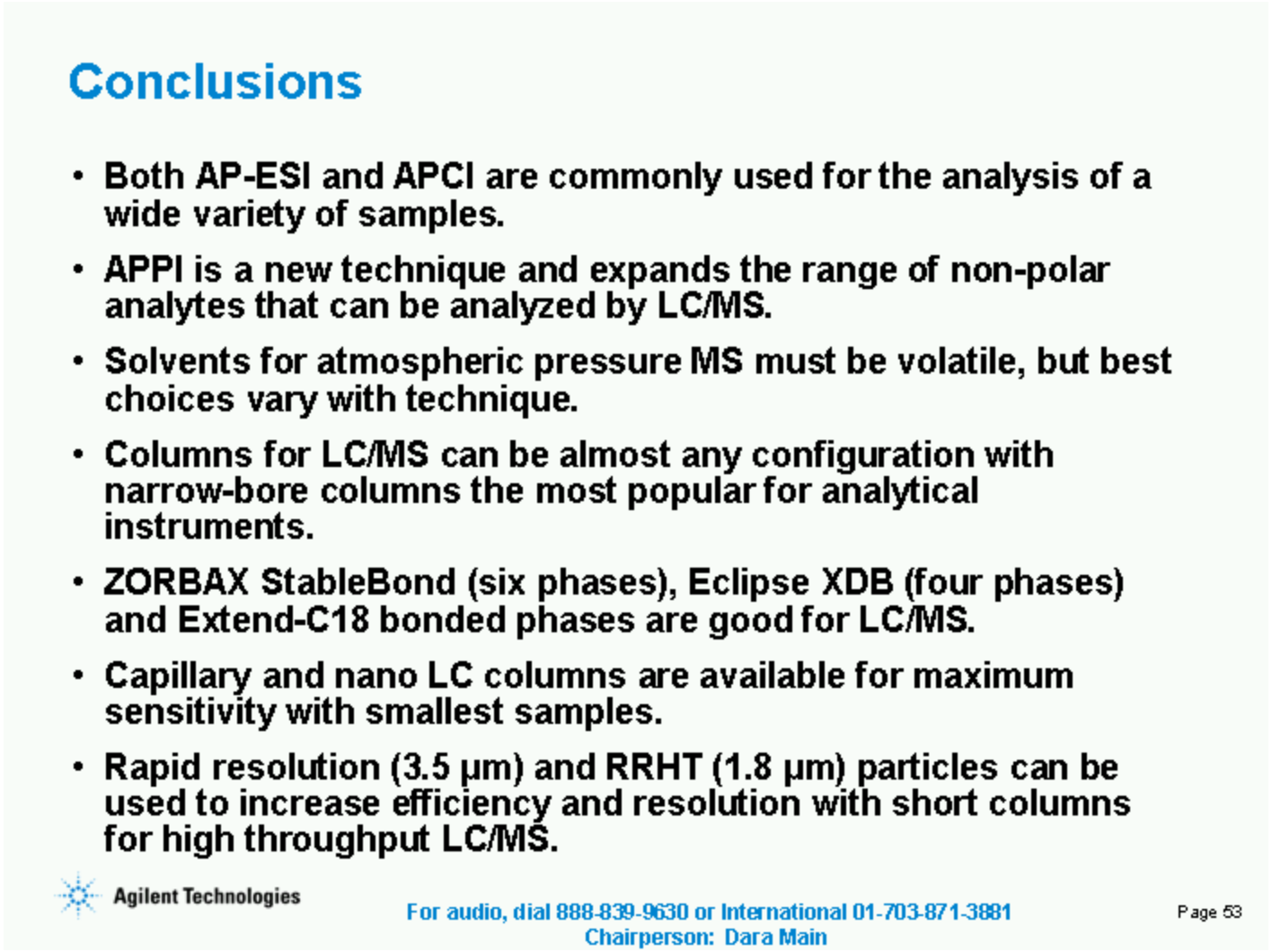
LC/MS Bonded Phase Selection at High pH – Extend-C18



Bonded Phase and Mobile Phase Choices for LC/MS Analysis



Adapting Existing LC Methods to LC/API-MS



Conclusions

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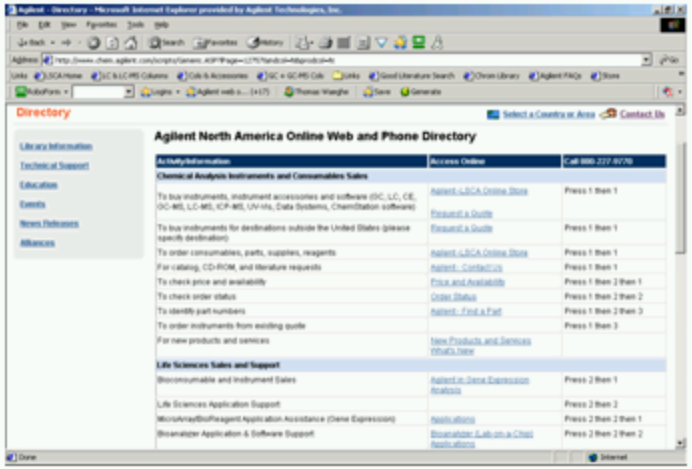

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Page 54

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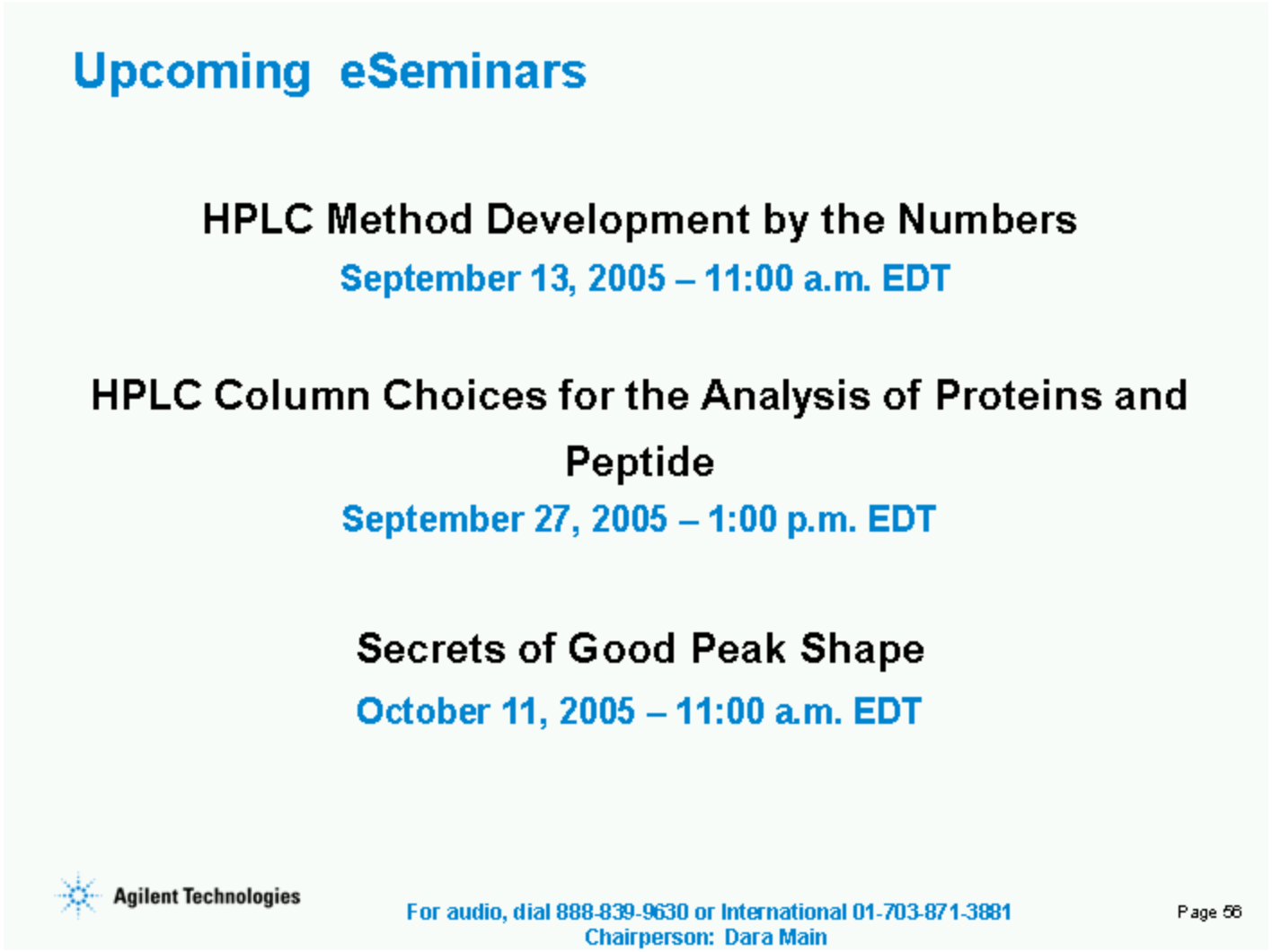
Page 55

Slide 55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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 56

Slide 56