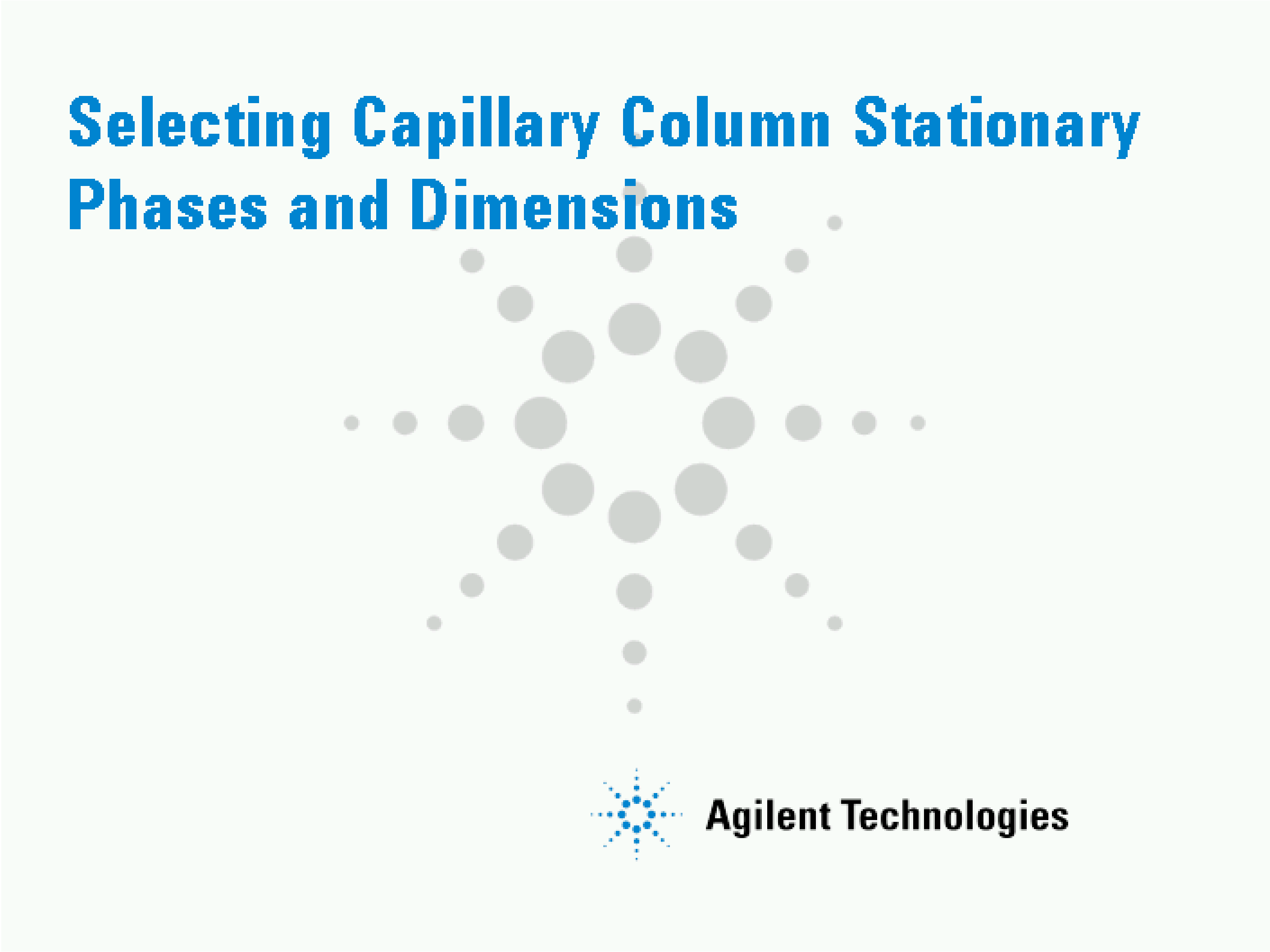
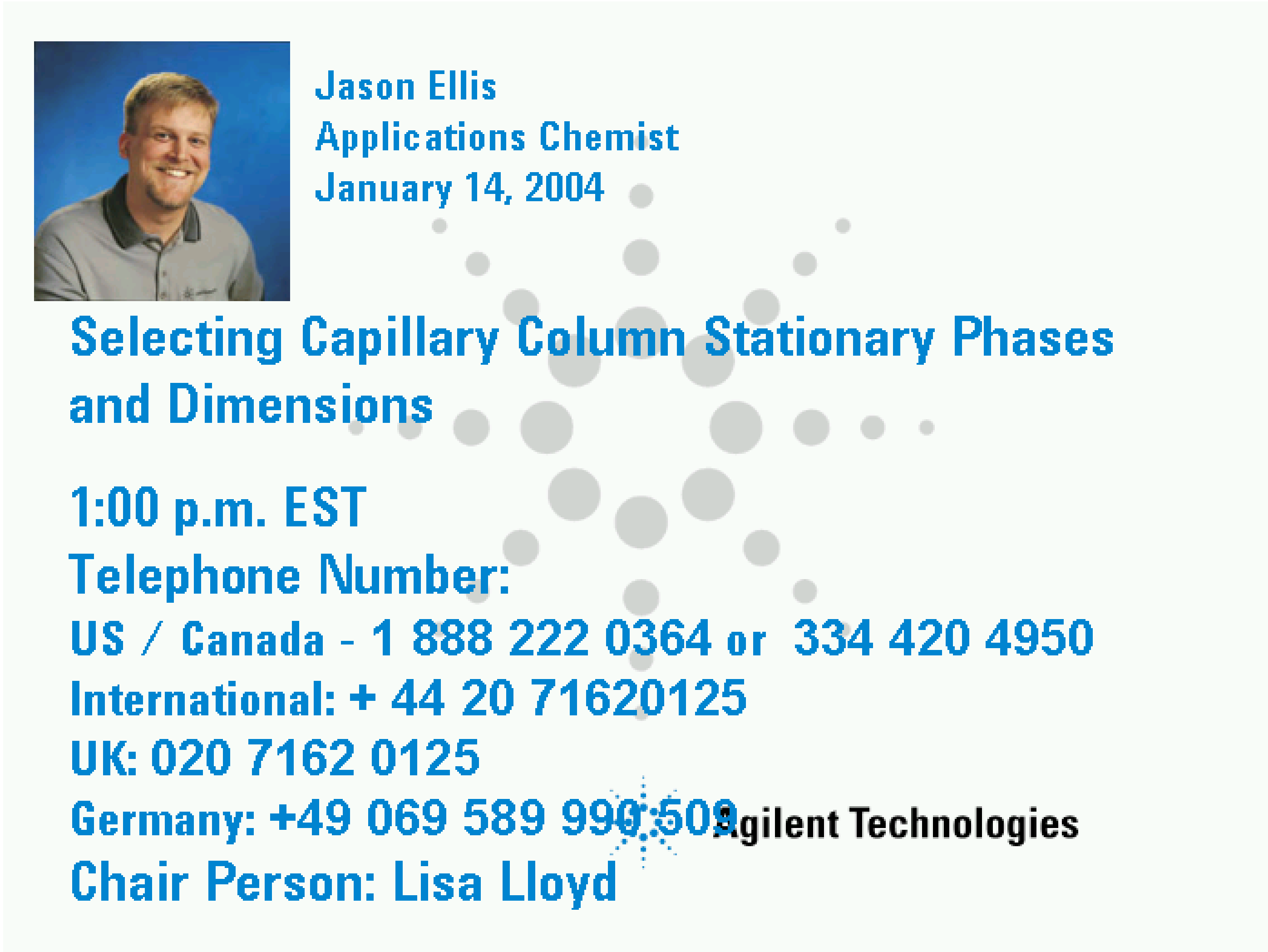
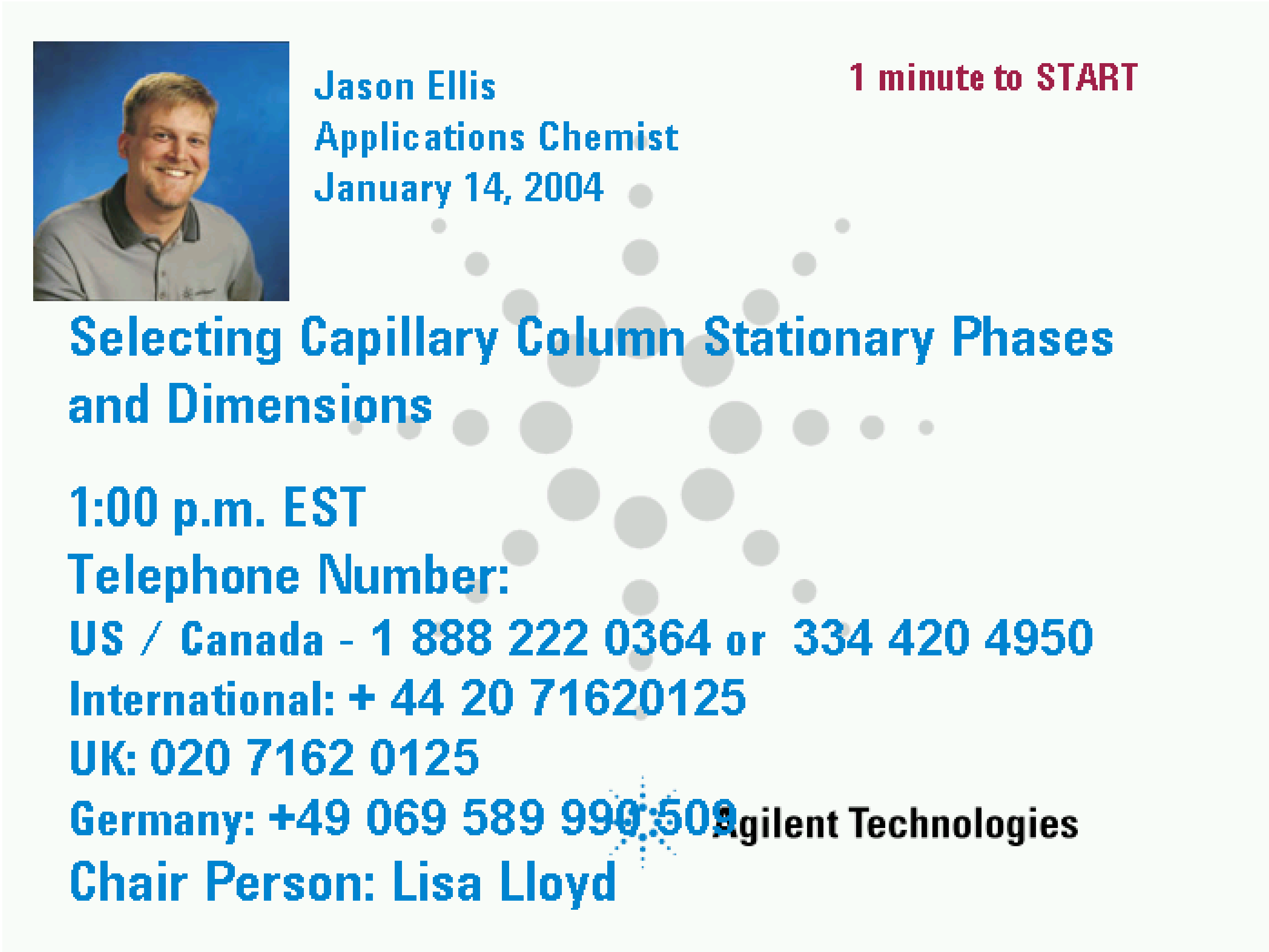

Selecting Capillary Column Stationary
Phases and Dimensions
Agilent Technologies

Slide 1

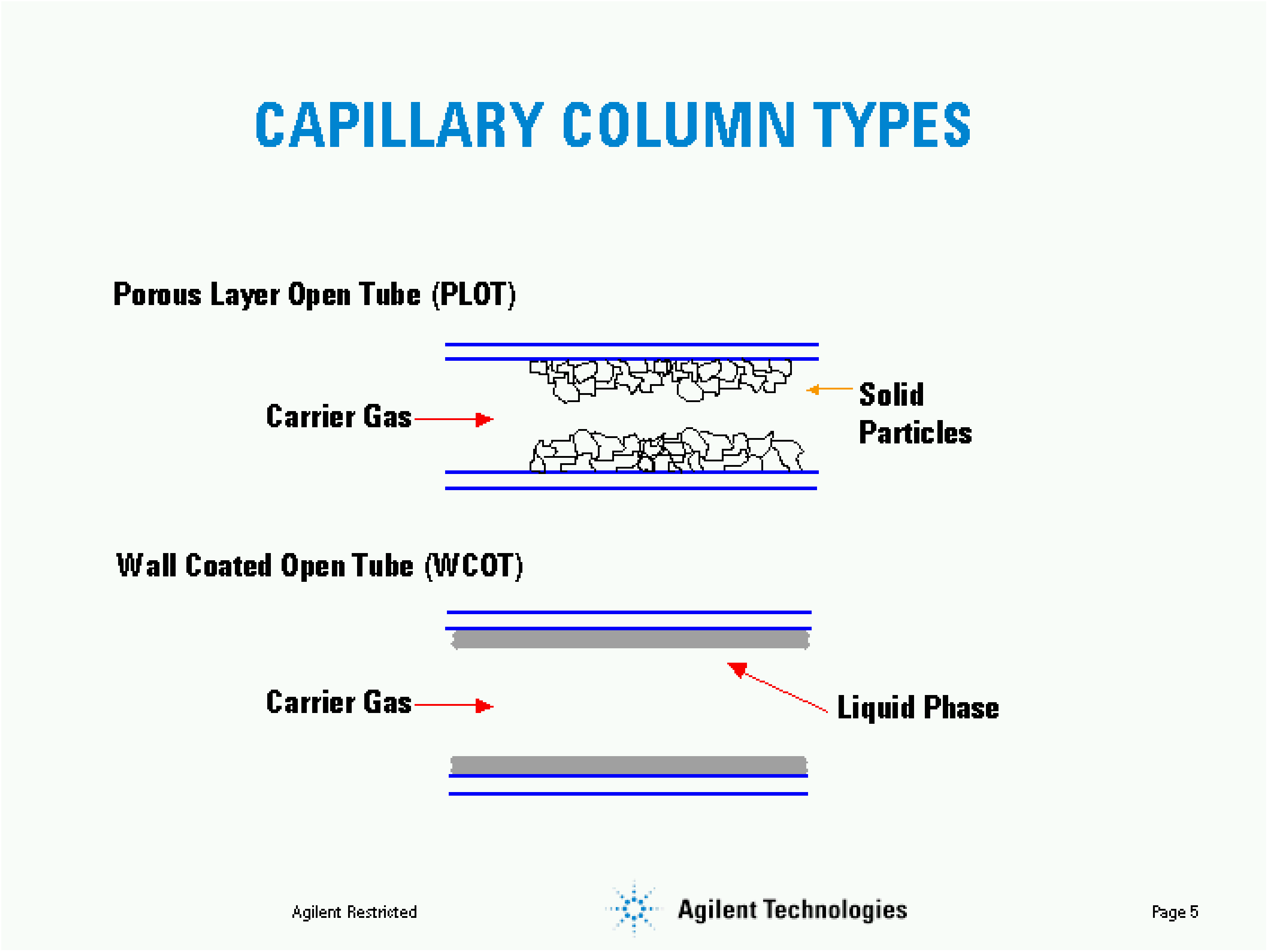


Jason Ellis Applications Chemist January 14, 2004

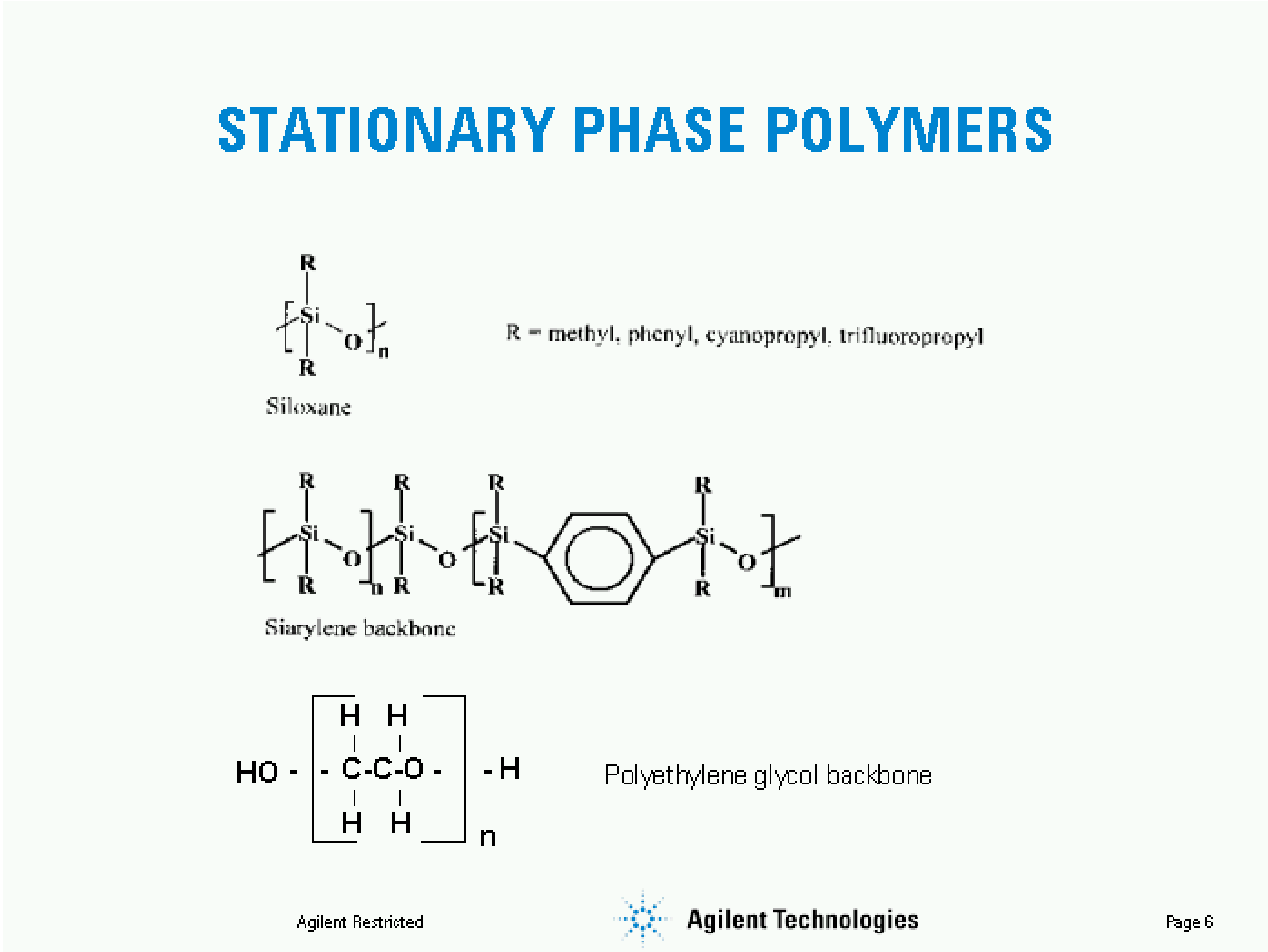
Jason Ellis Applications Chemist January 14, 2004



Jason Ellis Applications Chemist January 14, 2004


CAPILLARY COLUMN TYPES
Porous Layer Open Tube (PLOT)
Carrier Gas
Solid Particles
Wall Coated Open Tube (WCOT)
Carrier Gas
Liquid Phase
Agilent Restricted
Agilent Technologies
Page 5

Slide 5


STATIONARY PHASE POLYMERS
R = methyl, phenyl, cyanopropyl, trifluoropropyl
Siloxane
Siarylene backbone
Polyethylene glycol backbone
Agilent Restricted
Agilent Technologies
Page 6

Slide 6

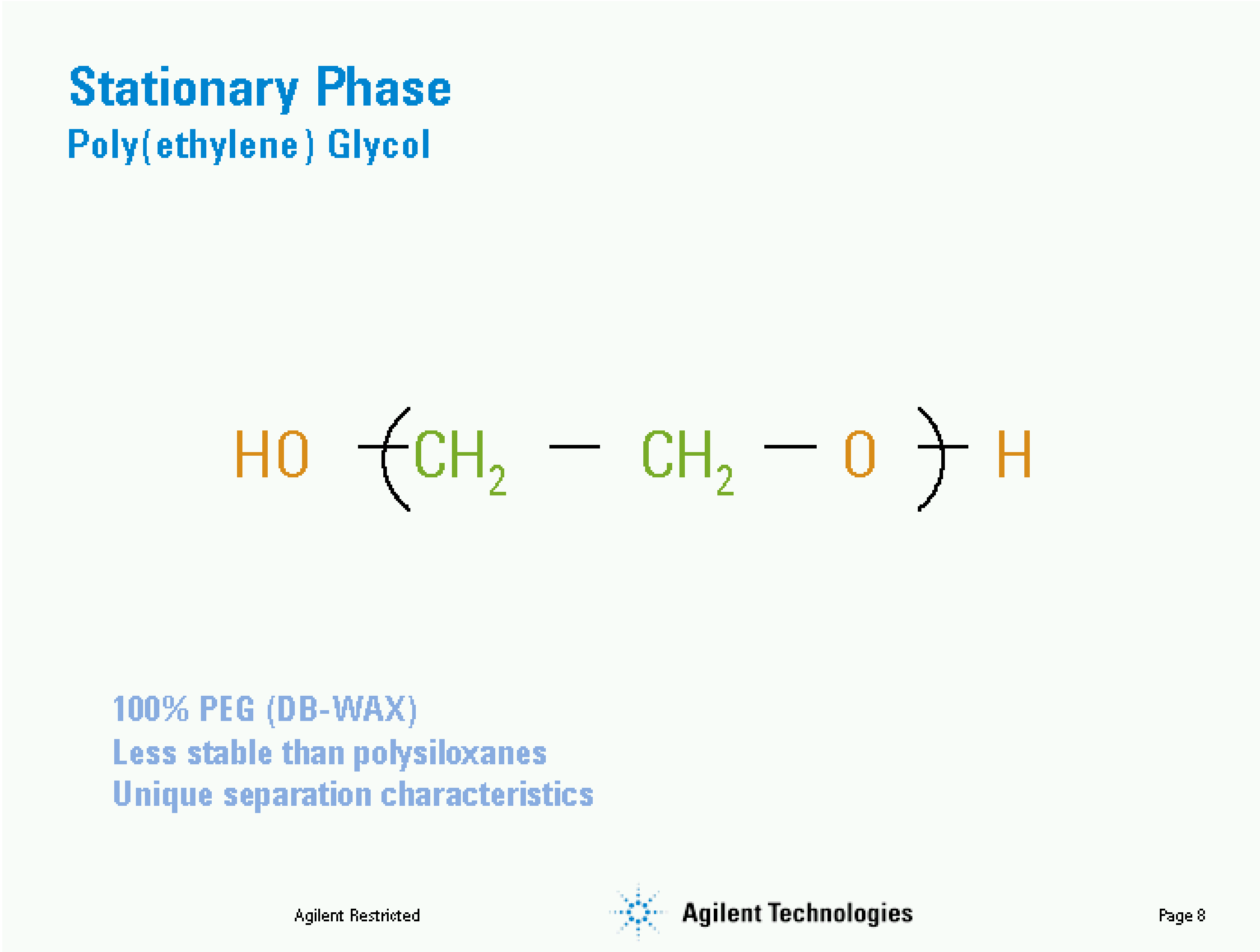
Stationary Phase
% Substitution -- polysiloxanes

% = # of sites on silicon atoms occupied

Balance is methyl

Agilent Restricted — Agilent Technologies — Page 7

Stationary Phase % Substitution -- polysiloxanes



Stationary Phase Poly(ethylene) Glycol

Poly(Ethylene) Glycol
Modified

Base deactivated (CAM)

Acid Modified (DB-FFAP)

Agilent Restricted Agilent Technologies Page 9

Poly(Ethylene) Glycol Modified

Specialty Phases

Columns developed for particular applications

Examples: DB-VRX, DB-MTBE, DB-TPH, DB-ALC1, DB-ALC2, DB-HTSimDis, DB-Dioxin

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Specialty Phases

Three Types Of Low Bleed Phases

Phases tailored to "mimic" currently existing polymers

- -Examples: DB-5ms, DB-35ms, DB-17ms

New phases unrelated to any previously existing polymers

- -Examples: DB-XLB

Optimized manufacturing processes

- -DB-1ms, HP-1ms, HP-5ms

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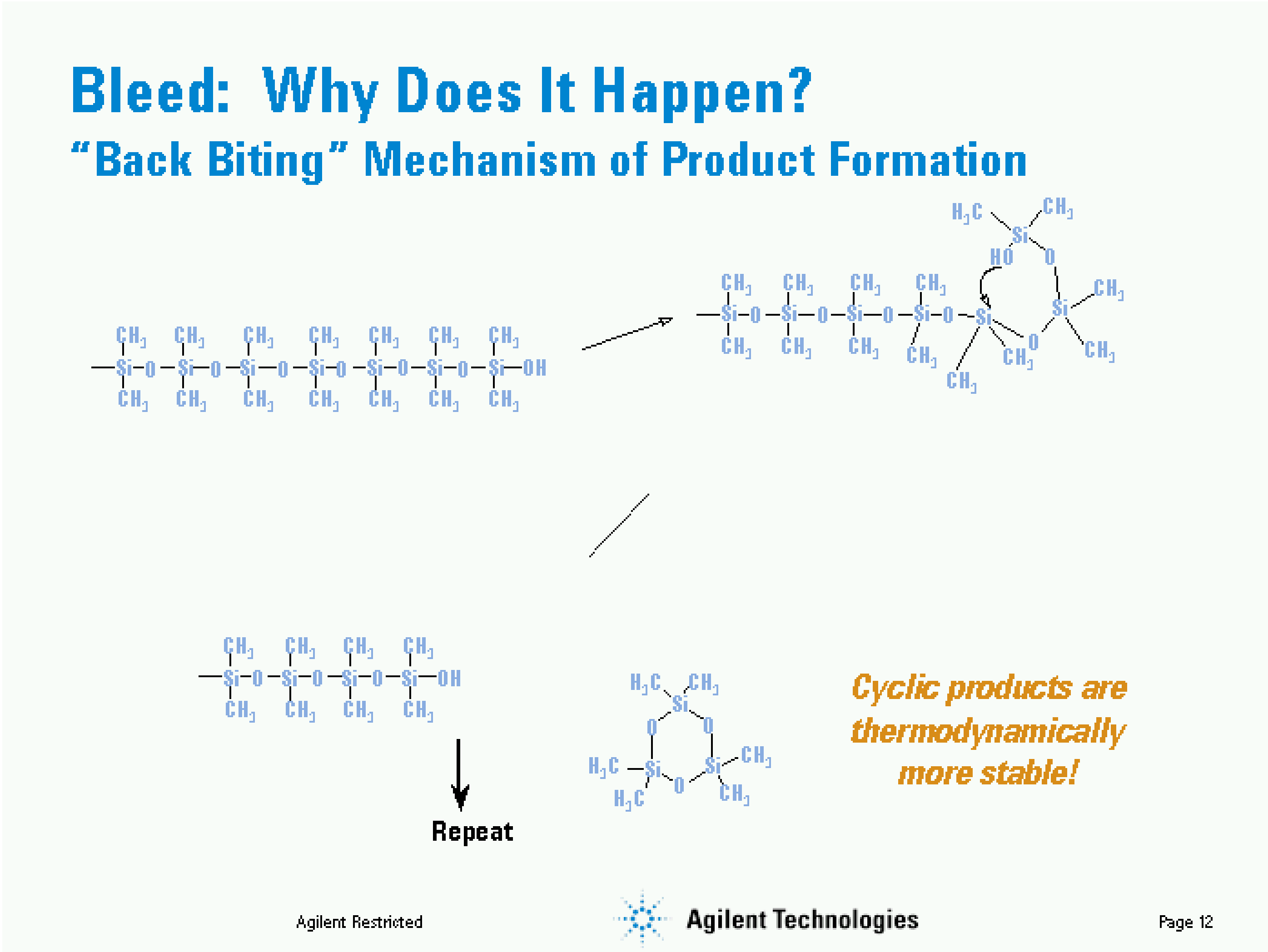
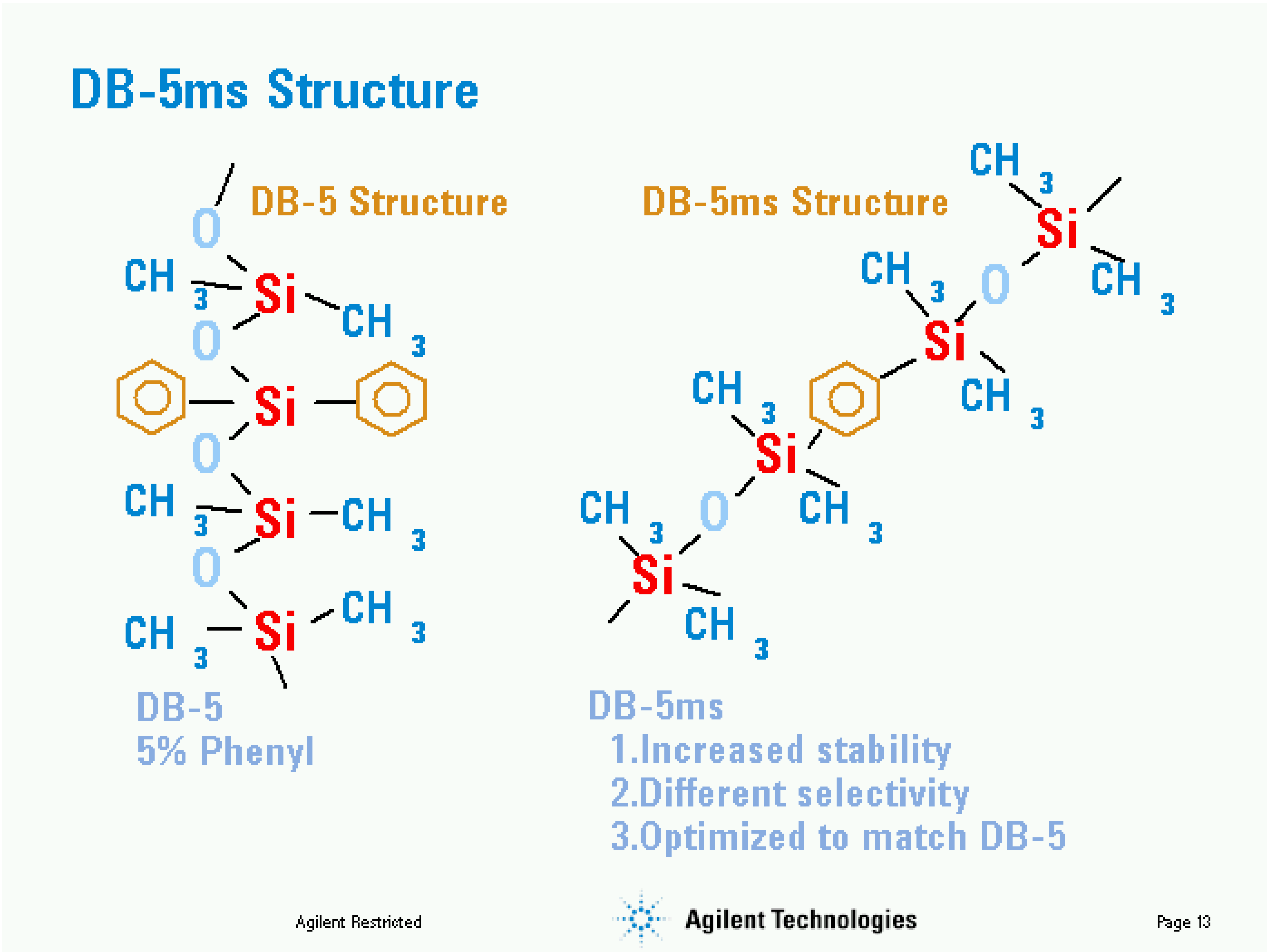
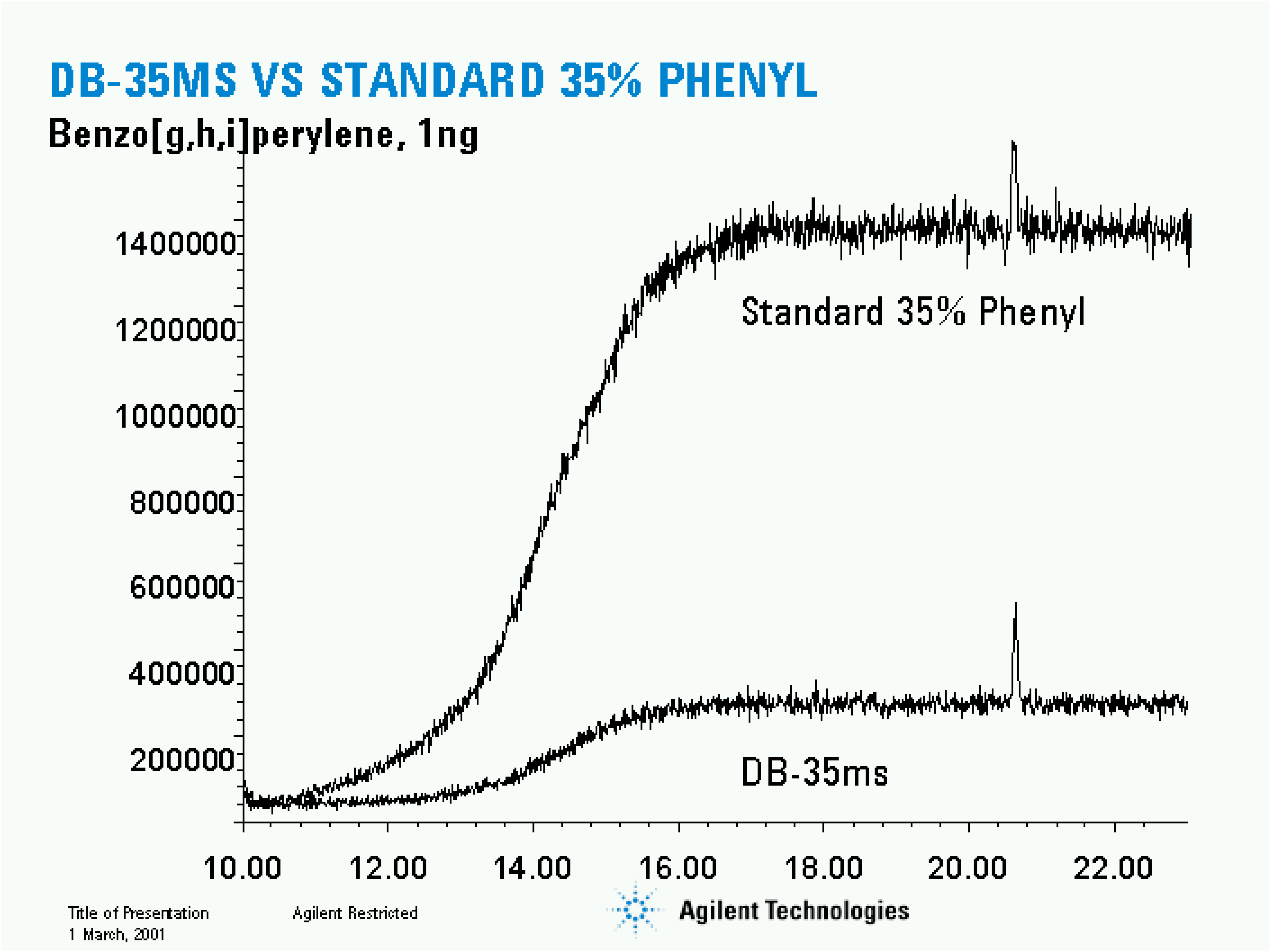
Three Types Of Low Bleed Phases



Bleed: Why Does It Happen? "Back Biting" Mechanism of Product Formation



DB-5ms Structure



DB-35MS VS STANDARD 35% PHENYL Benzo[g,h,i]perylene, 1ng

Why is stationary phase type important?

Influence of α

$$\alpha = \frac{k_2}{k_1}$$

k_2 = partition ratio of 2nd peak
k_1 = partition ratio of 1st peak

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Why is stationary phase type important?

Selectivity

Relative spacing of the chromatographic peaks.

The result of all non-polar, polarizable and polar interactions that cause a stationary phase to be more or less retentive to one analyte than another.

Slide 16

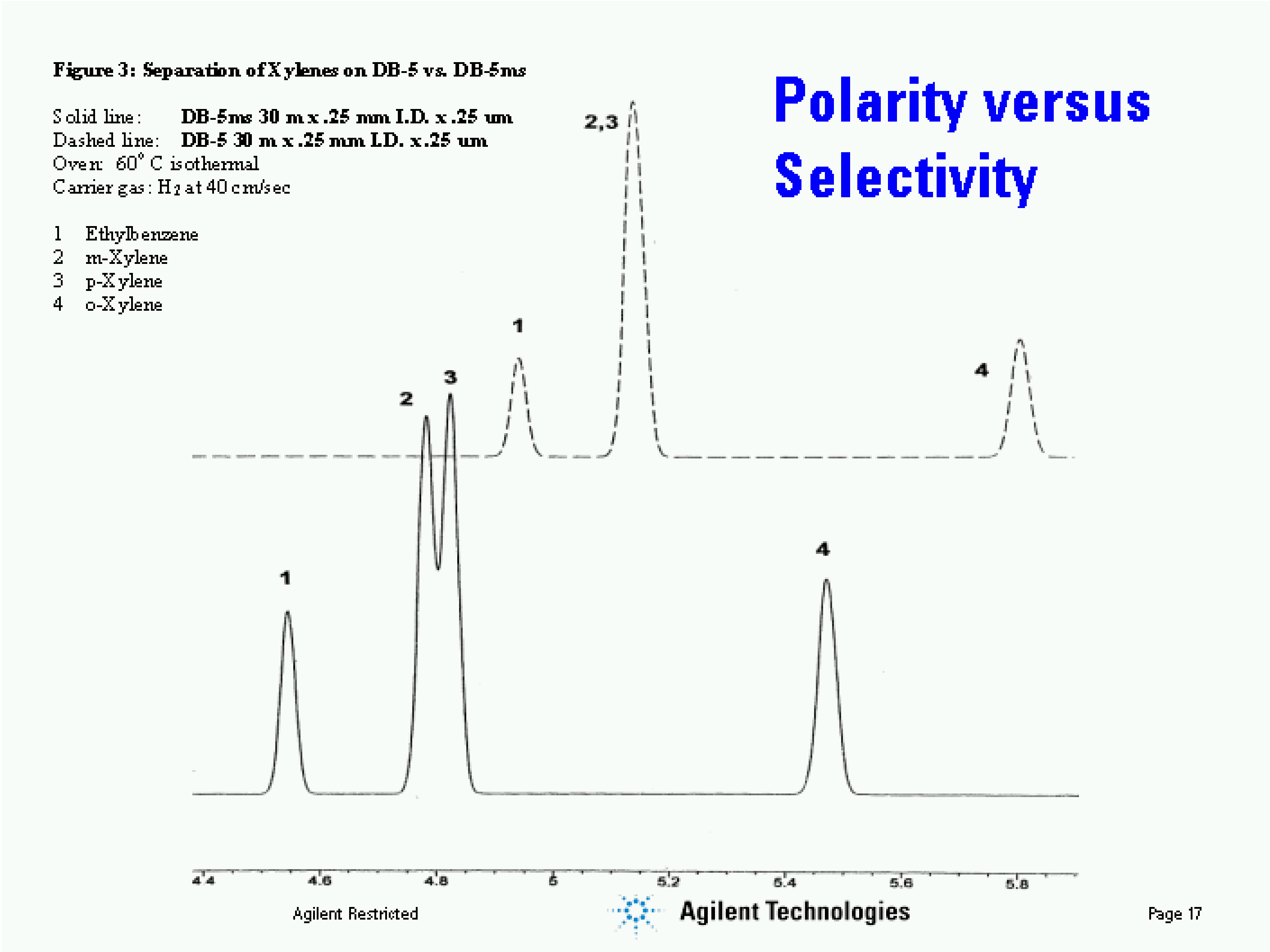
Figure 3: Separation of Xylenes on DB-5 vs. DB-5ms
Solid line: DB-5ms 30 m x .25 mm I.D. x .25 um
Dashed line: DB-5 30 m x .25 mm I.D. x .25 um
Oven: 60° C isothermal
Carrier gas: H2 at 40 cm/sec
1 Ethylbenzene
2 m-Xylene
3 p-Xylene
4 o-Xylene
Polarity versus Selectivity
2,3
1
4
2
3
1
4
4.4
4.6
4.8
5
5.2
5.4
5.6
5.8
Agilent Restricted
Agilent Technologies
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Slide 17

Optimizing Selectivity (α)

- Match analyte polarity to stationary phase polarity ("like dissolves like")
  - *maybe, but not always the best approach for method development*

- Take advantage of unique interactions between analyte and stationary phase functional groups to enhance separation of key solutes.

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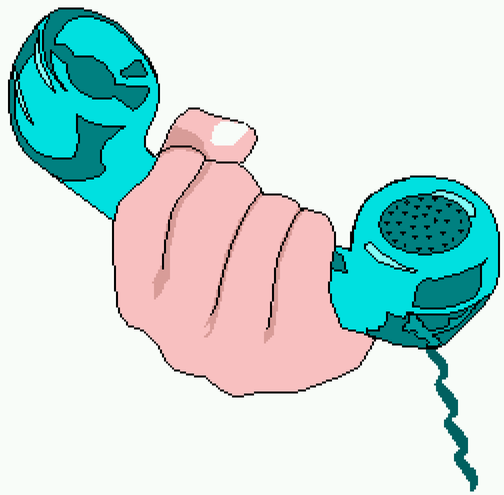
Optimizing Selectivity (?)

Break Number 1



- For Questions and Answers
- Press *1 on Your Phone to
- Ask a Question

Break Number 1

Analyte Polarity

Nonpolar Molecules - generally composed of only carbon and hydrogen and exhibit no dipole moment (Straight-chained hydrocarbons (n-alkanes))

Polar Molecules - primarily composed of carbon and hydrogen but also contain atoms of nitrogen, oxygen, phosphorus, sulfur, or a halogen (Alcohols, amines, thiols, ketones, nitriles, organo-halides, etc. Includes dipole-dipole interactions and H-bonding)

Polarizable Molecules - primarily composed of carbon and hydrogen, but also contain unsaturated bonds (Alkenes, alkynes and aromatic compounds)

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Selectivity Interactions

Dispersion

Dipole

Hydrogen bonding

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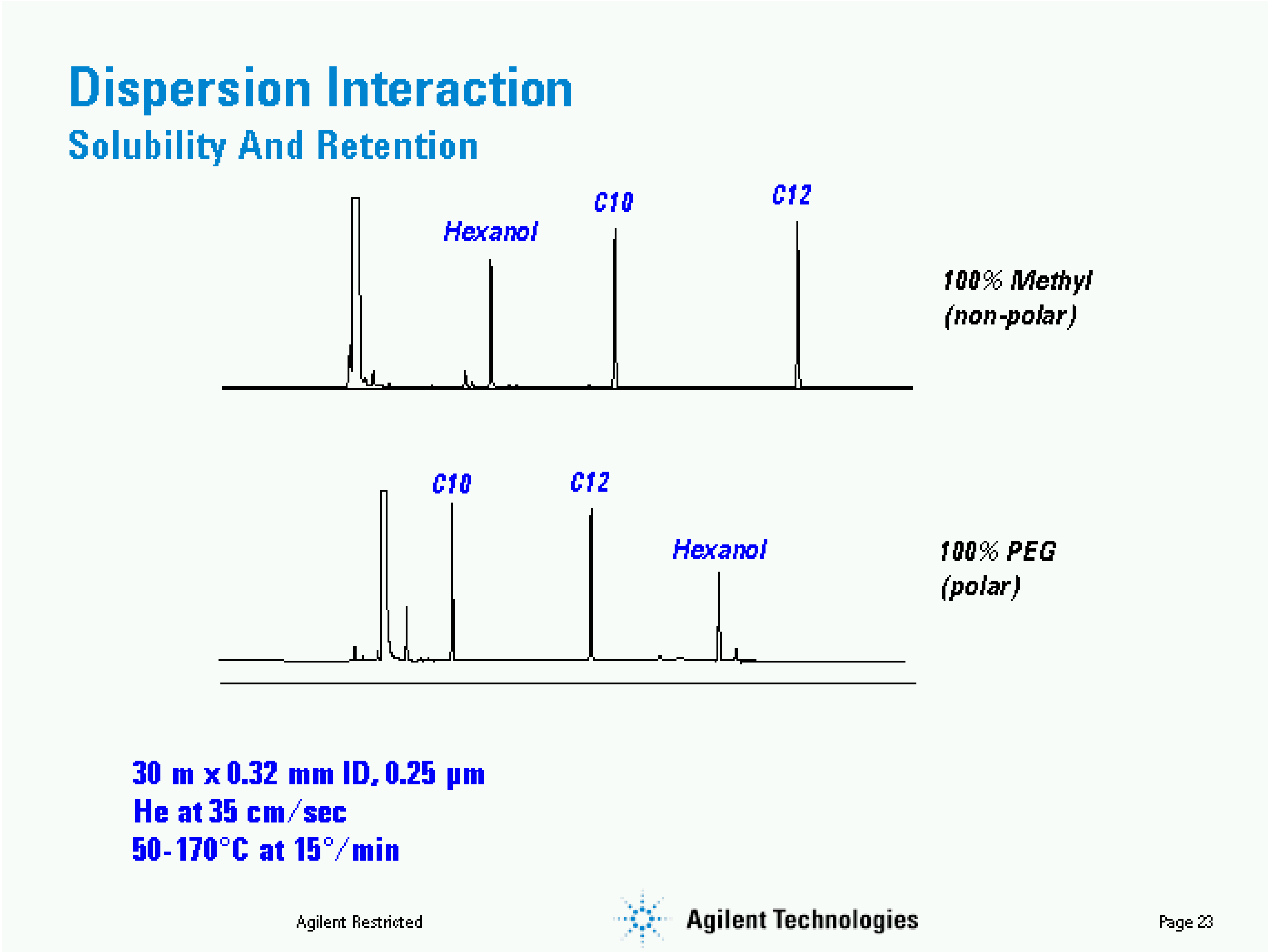
Selectivity Interactions

Dispersion Interaction
ΔH_{vap}

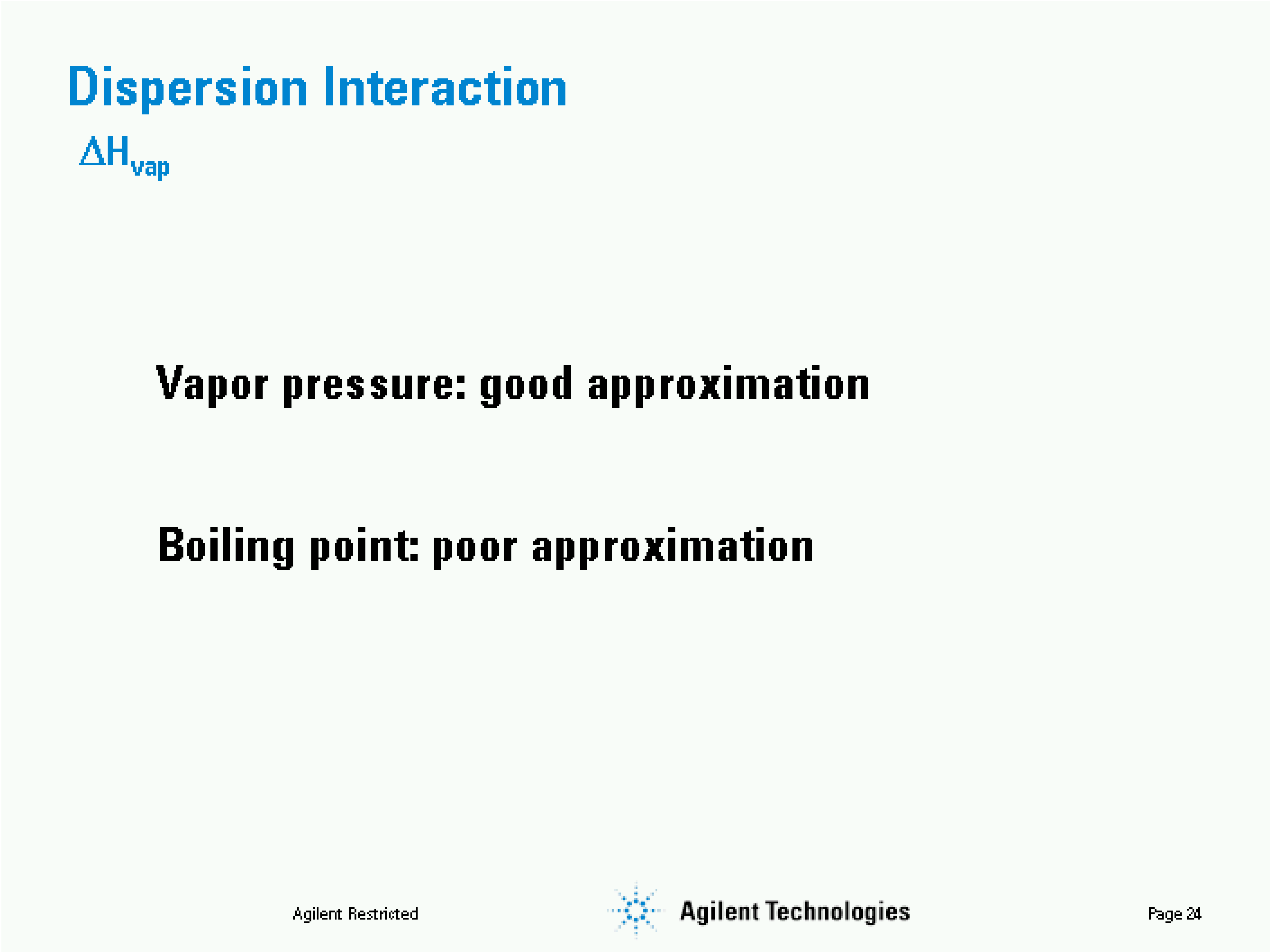
Separation by differences in solute heat of vaporizations (ΔH_{vap})

Heat necessary to convert a liquid into a gas (at the same temperature)

Dispersion Interaction ?Hvap



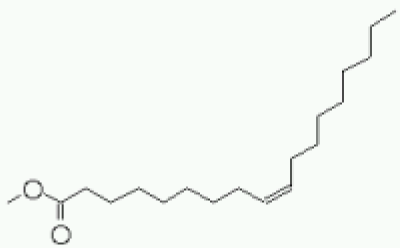
Dispersion Interaction Solubility And Retention



Dispersion Interaction ?Hvap

Dipole Interaction

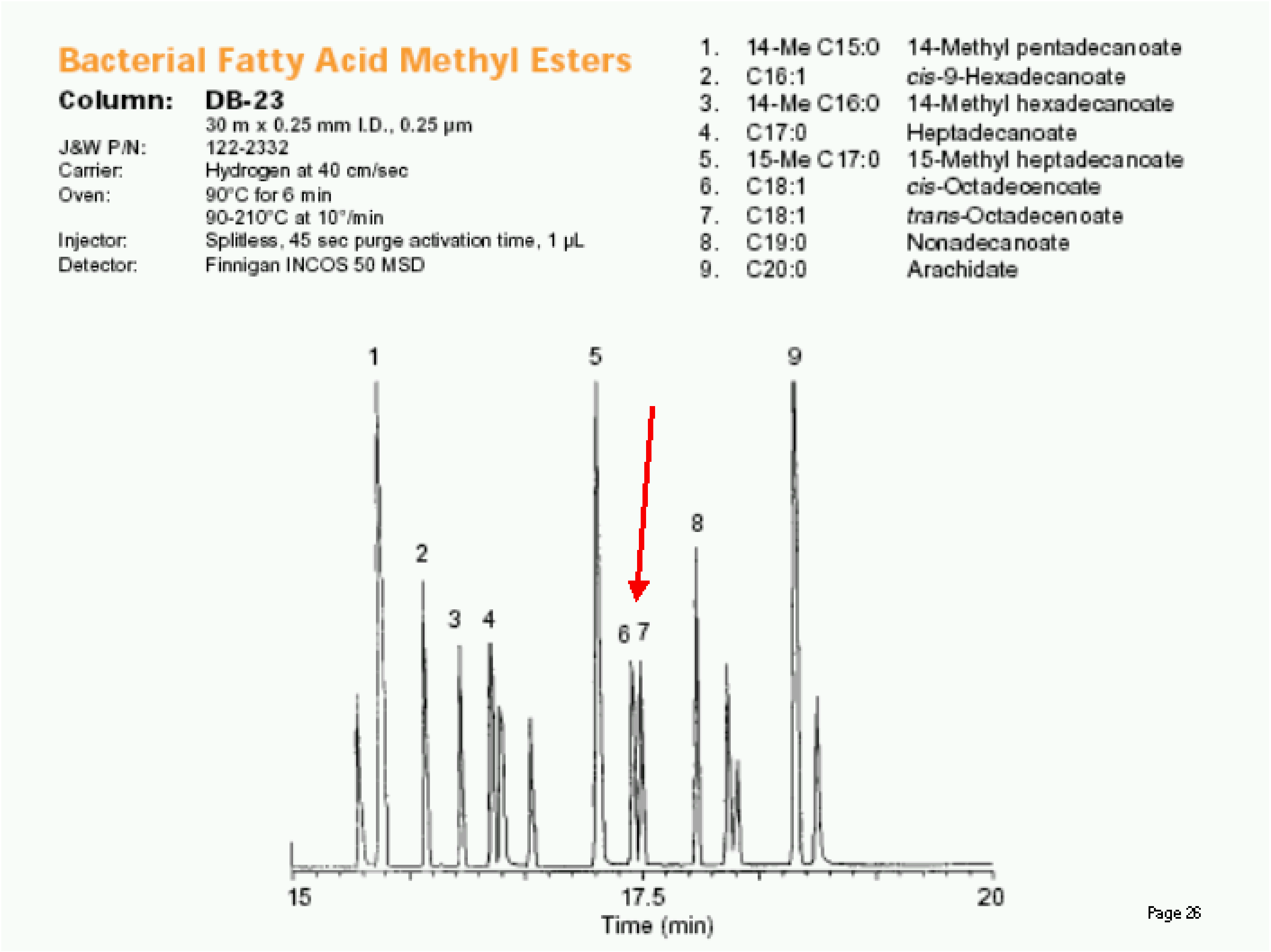
C18:1 (Methyl *trans*-9-octadecenoate)



C18:1 (Methyl *cis*-9-octadecenoate)

- **Smaller differences require a stronger dipole phase**

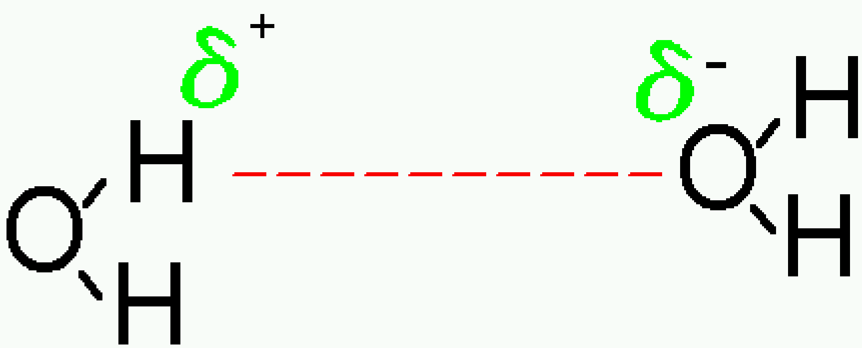
Dipole Interaction


Bacterial Fatty Acid Methyl Esters
Column: DB-23
30 m x 0.25 mm I.D., 0.25 µm
J&W P/N: 122-2332
Carrier: Hydrogen at 40 cm/sec
Oven: 90°C for 6 min
90-210°C at 10°/min
Injector: Splitless, 45 sec purge activation time, 1 µL
Detector: Finnigan INCOS 50 MSD
1. 14-Me C15:0 14-Methyl pentadecanoate
2. C16:1 cis-9-Hexadecanoate
3. 14-Me C16:0 14-Methyl hexadecanoate
4. C17:0 Heptadecanoate
5. 15-Me C17:0 15-Methyl heptadecanoate
6. C18:1 cis-Octadecenoate
7. C18:1 trans-Octadecenoate
8. C19:0 Nonadecanoate
9. C20:0 Arachidate
1
2
3
4
5
6
7
8
9
15
17.5
20
Time (min)
Page 26

Slide 26

Hydrogen Bonding Interaction

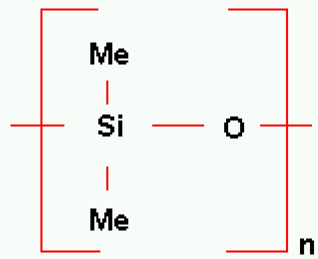
- Dipole-Dipole interaction with H bound to O or N interacting with an O or N



Hydrogen Bonding Interaction

NONPOLAR PHASES

Typified by 100% polydimethylsiloxanes such as HP-1, DB-1, DB-1ms, HP-1ms



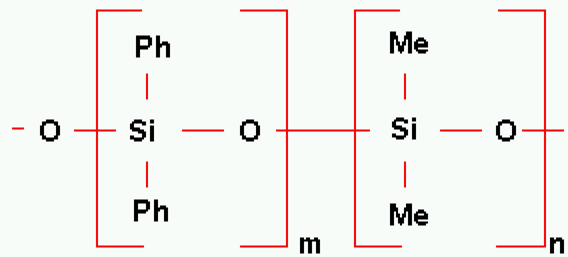
Separation Mechanisms:

- Dispersion only

Slide 28

POLARIZABLE PHASES

Typified by phenyl substituted siloxanes, substituted at 5-50% (HP-5, HP-5ms, DB-35, DB-35ms, DB-17, DB-17ms)



5%--weakly polar, rest--mid polar

Separation Mechanisms:

- Dispersion
- Inducible dipole at phenyl groups

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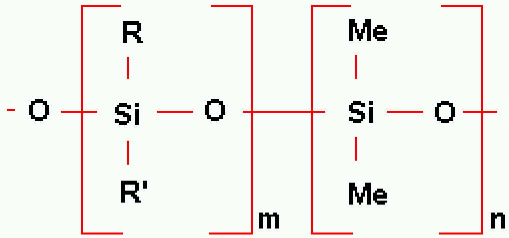
STRONG DIPOLE PHASES

Typified by cyanopropyl or trifluoropropyl substituted siloxanes, substituted 6-50% (DB-1701, DB-1301, DB-200, DB-23, DB-225)



R = cyanopropyl or trifluoropropyl
R′ = phenyl or methyl

Separation Mechanisms:

- Dispersion
- Inducible dipole at phenyl groups
- Strong permanent dipole
- Hydrogen bonding

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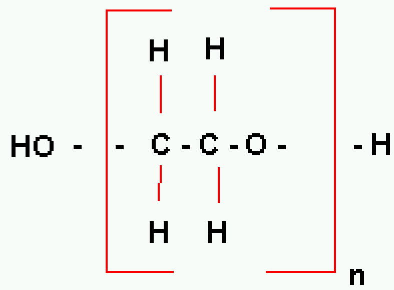
HYDROGEN BONDING PHASES

Typified by polyethylene glycol polymers (Carbowax, HP-INNOWax, DB-WAX, DB-FFAP, CAM)



Separation Mechanisms:

- Dispersion
- Strong permanent dipole
- Hydrogen bonding

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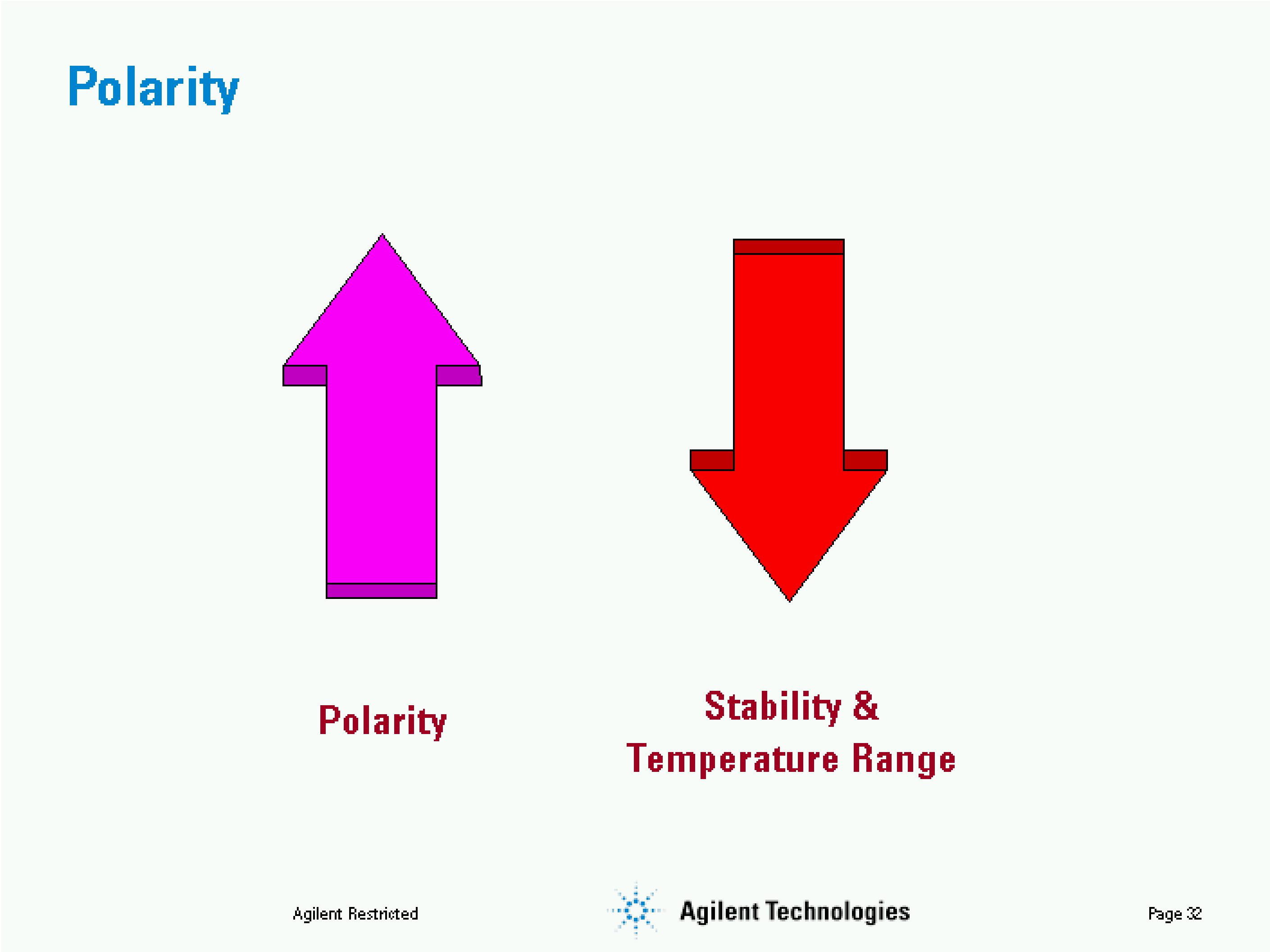
Polarity
Polarity
Stability &
Temperature Range
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Polarity

Selectivity
Interaction Strengths

Phase	Dispersion	Dipole	H Bonding
Methyl	Strong	None	None
Phenyl	Strong	None	Weak
Cyanopropyl	Strong	Strong	Moderate
Trifluoropropyl	Strong	Moderate	Weak
PEG	Strong	Strong	Moderate

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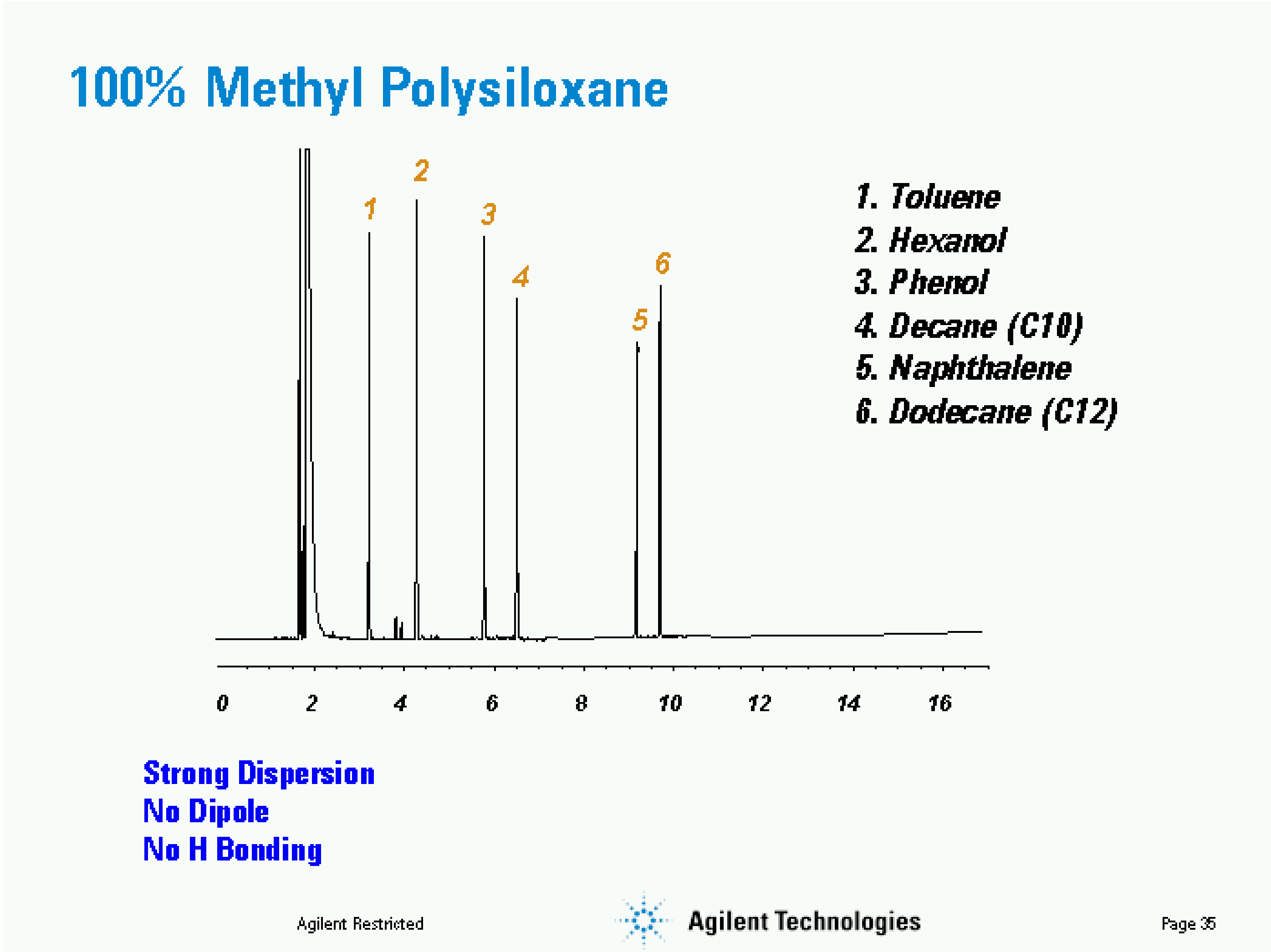
Selectivity Interaction Strengths

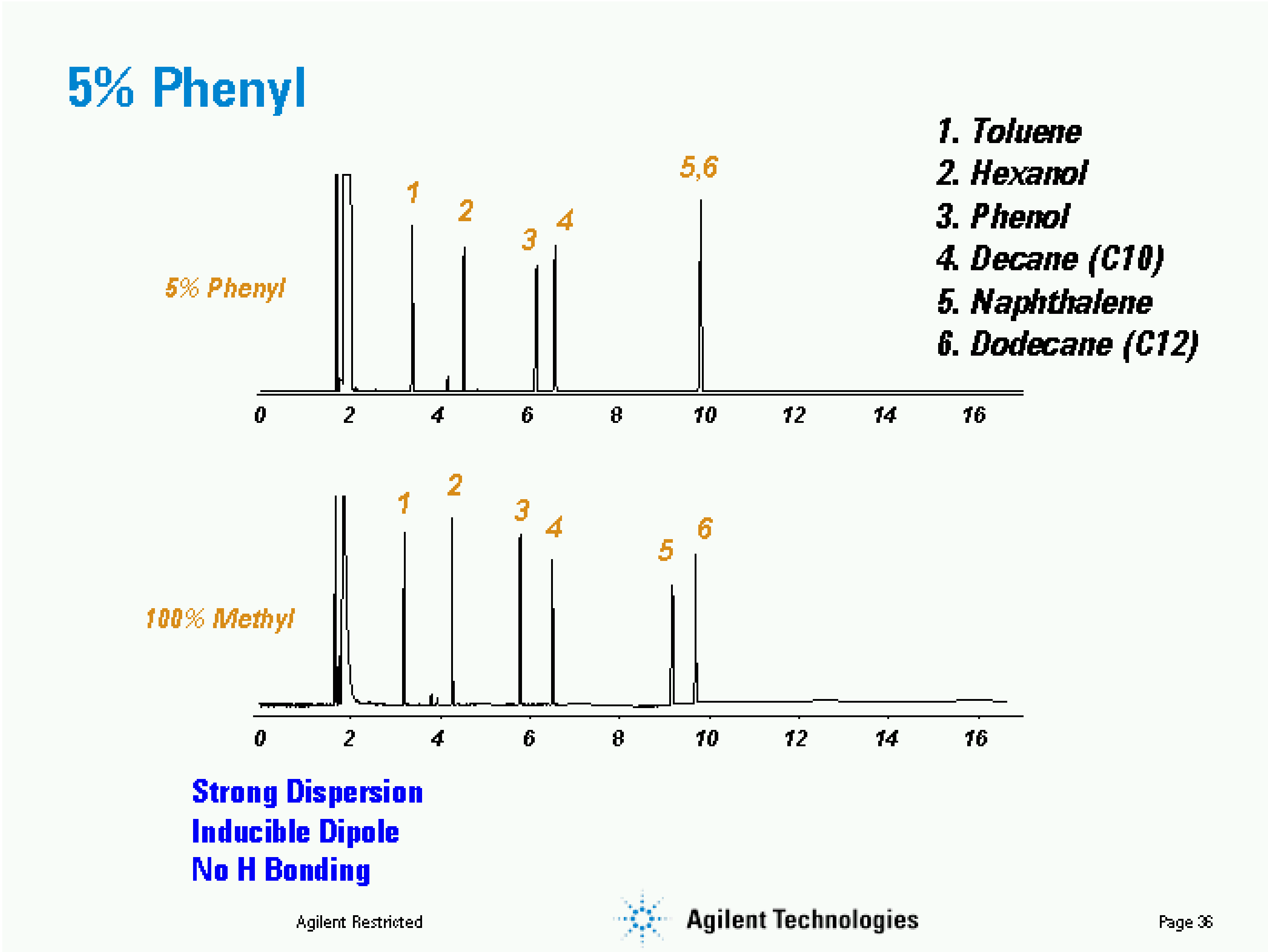
Compounds Properties

Compounds	Polar	Aromatic	Hydrogen Bonding	Dipole
Toluene	no	yes	no	induced
Hexanol	yes	no	yes	yes
Phenol	yes	yes	yes	yes
Decane	no	no	no	no
Naphthalene	no	yes	no	induced
Dodecane	no	no	no	no

Compounds Properties



100% Methyl Polysiloxane


5% Phenyl
1. Toluene
2. Hexanol
3. Phenol
4. Decane (C10)
5. Naphthalene
6. Dodecane (C12)
5% Phenyl
1
2
3
4
5,6
0 2 4 6 8 10 12 14 16
100% Methyl
1
2
3
4
5
6
0 2 4 6 8 10 12 14 16
Strong Dispersion
Inducible Dipole
No H Bonding
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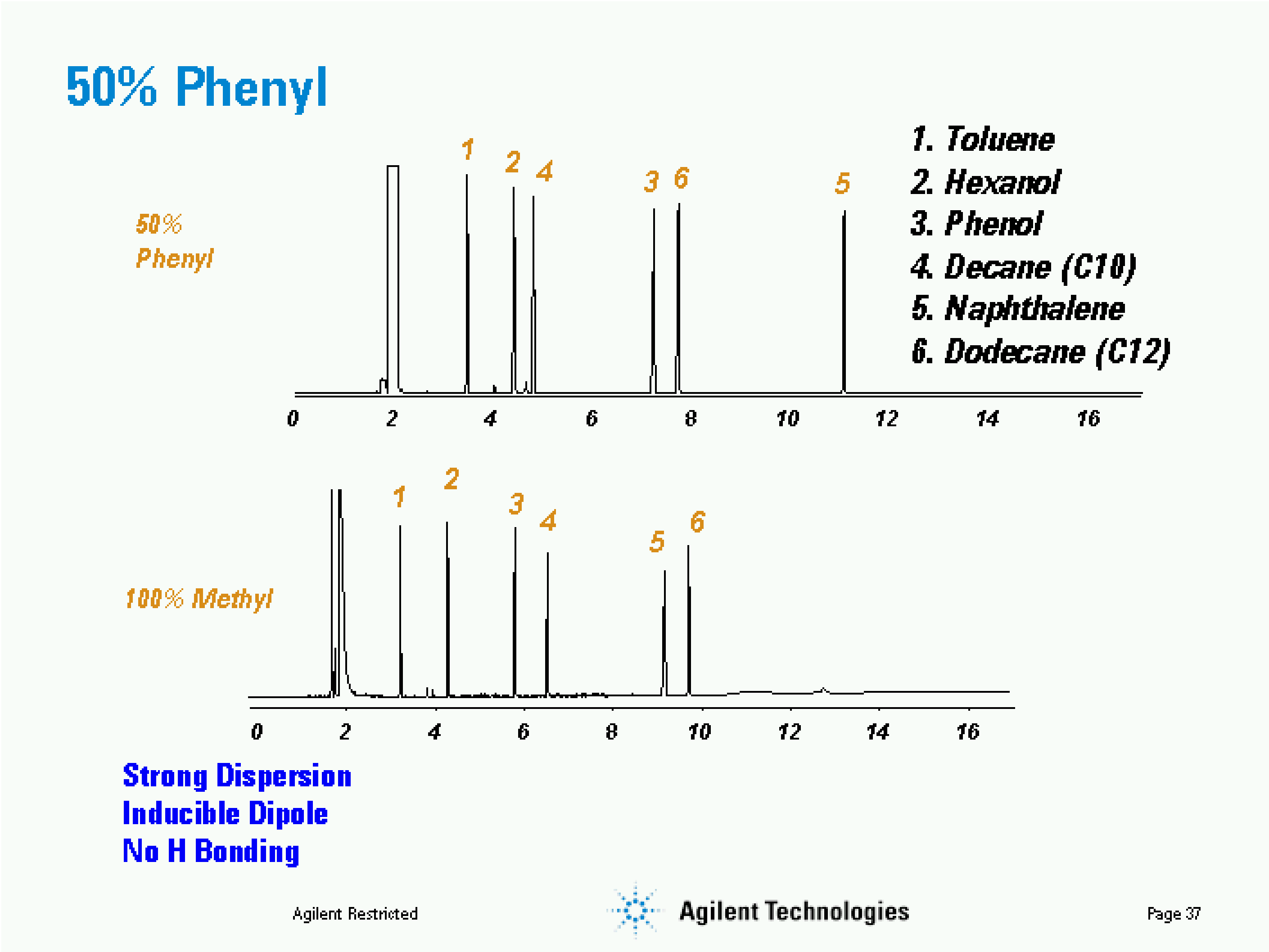
5% Phenyl


50% Phenyl
50% Phenyl
100% Methyl
1 2 4 3 6 5
1 2 3 4 5 6
0 2 4 6 8 10 12 14 16
1. Toluene
2. Hexanol
3. Phenol
4. Decane (C10)
5. Naphthalene
6. Dodecane (C12)
Strong Dispersion
Inducible Dipole
No H Bonding
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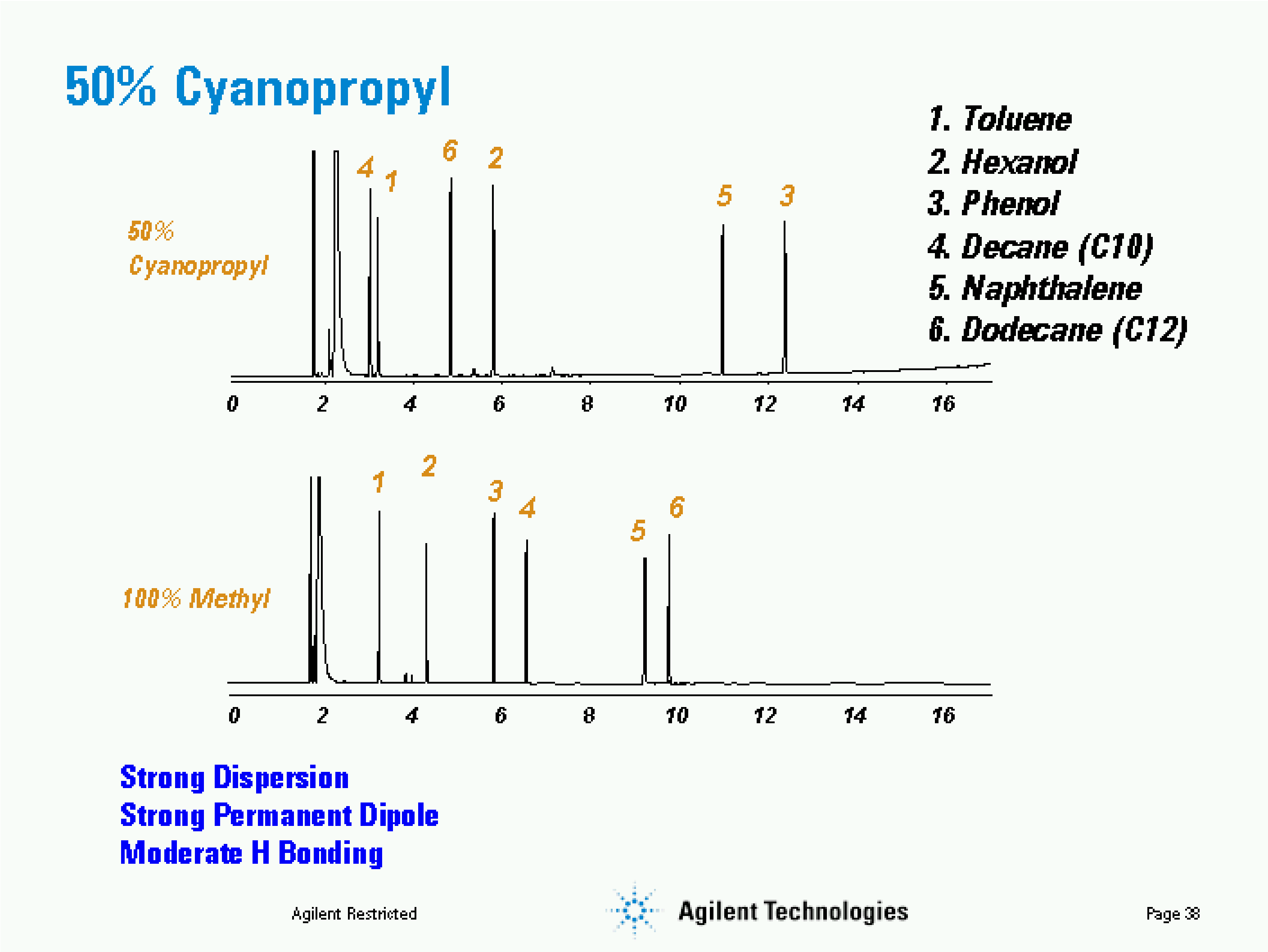
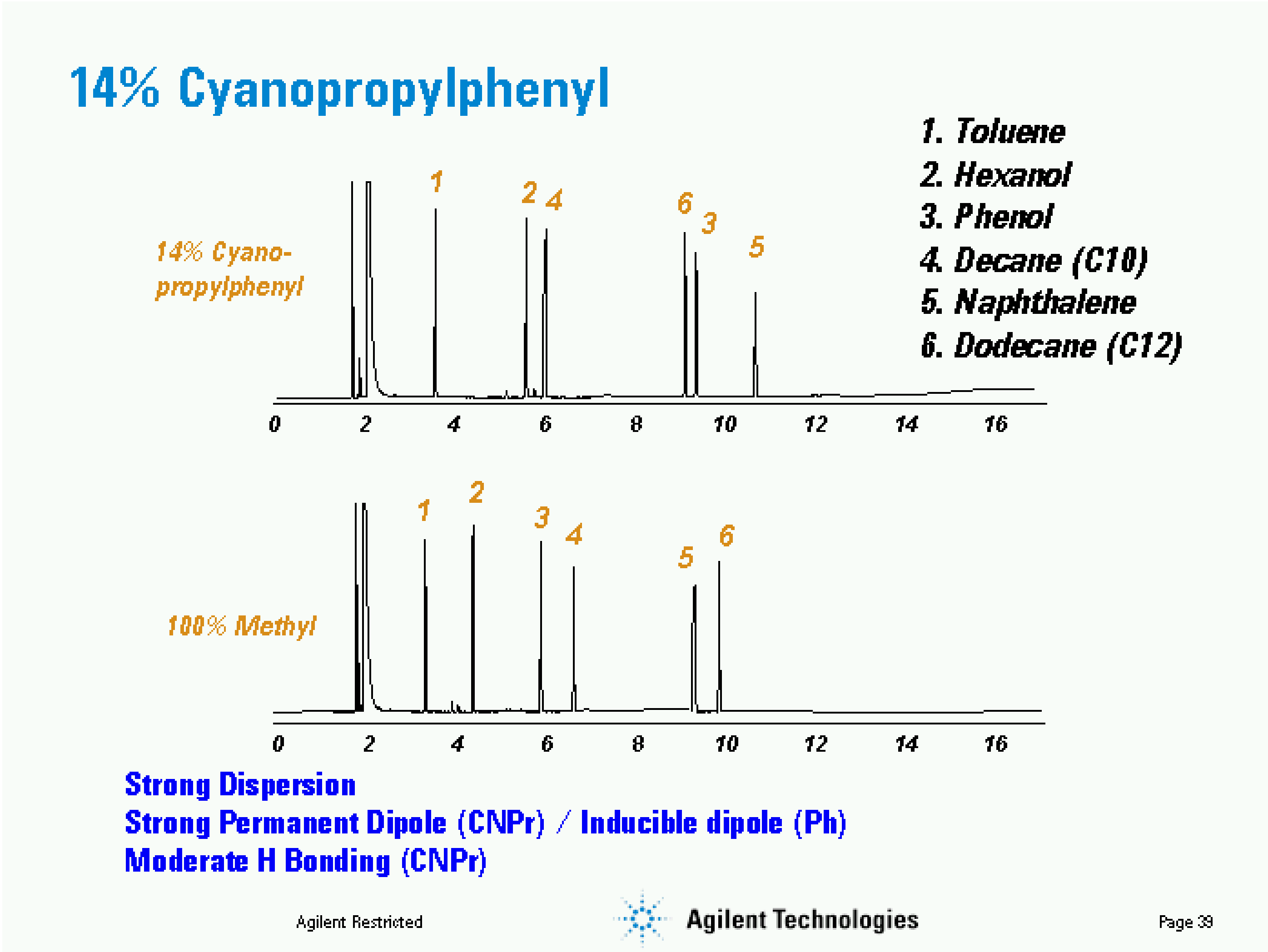
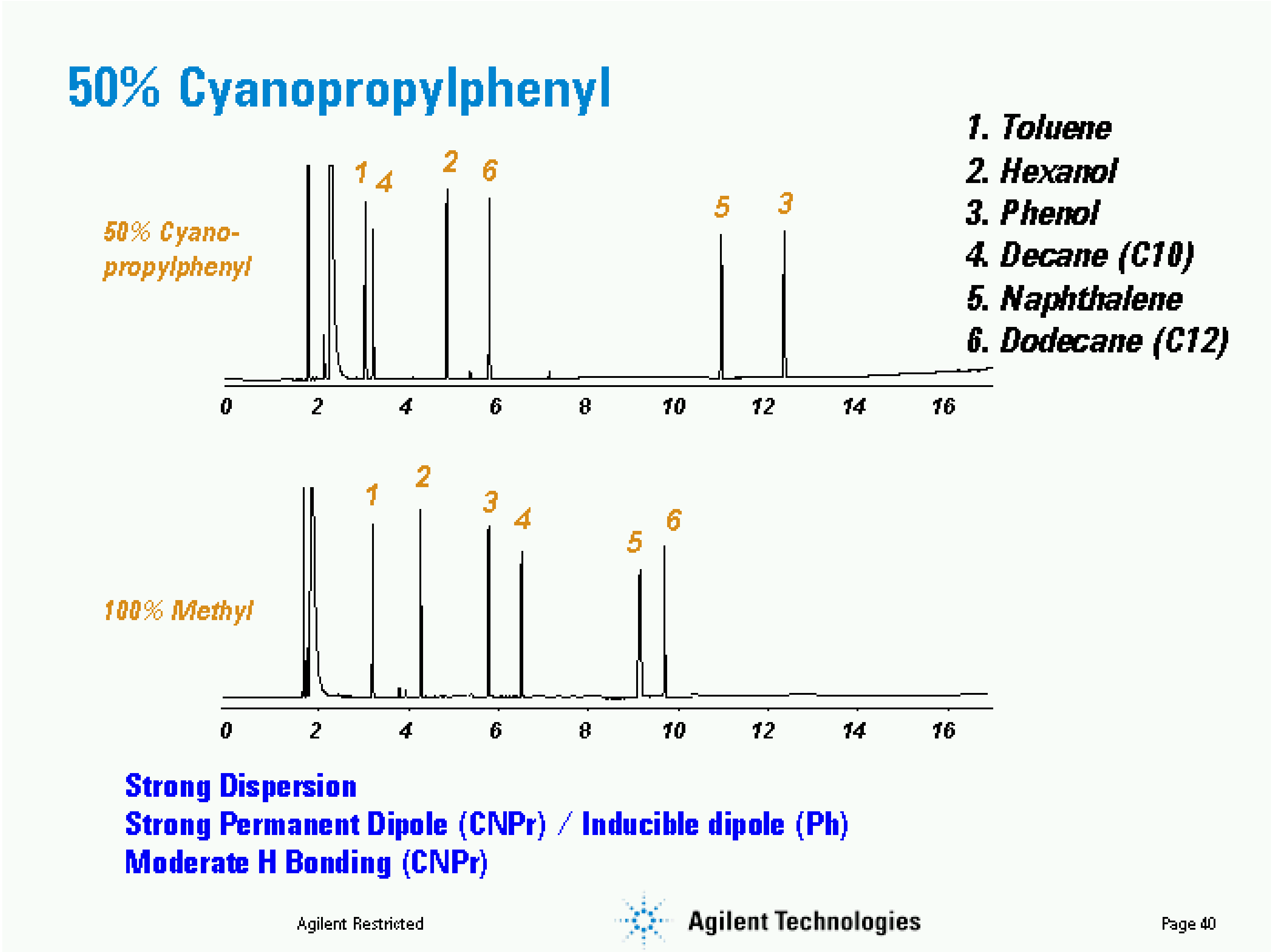
50% Phenyl



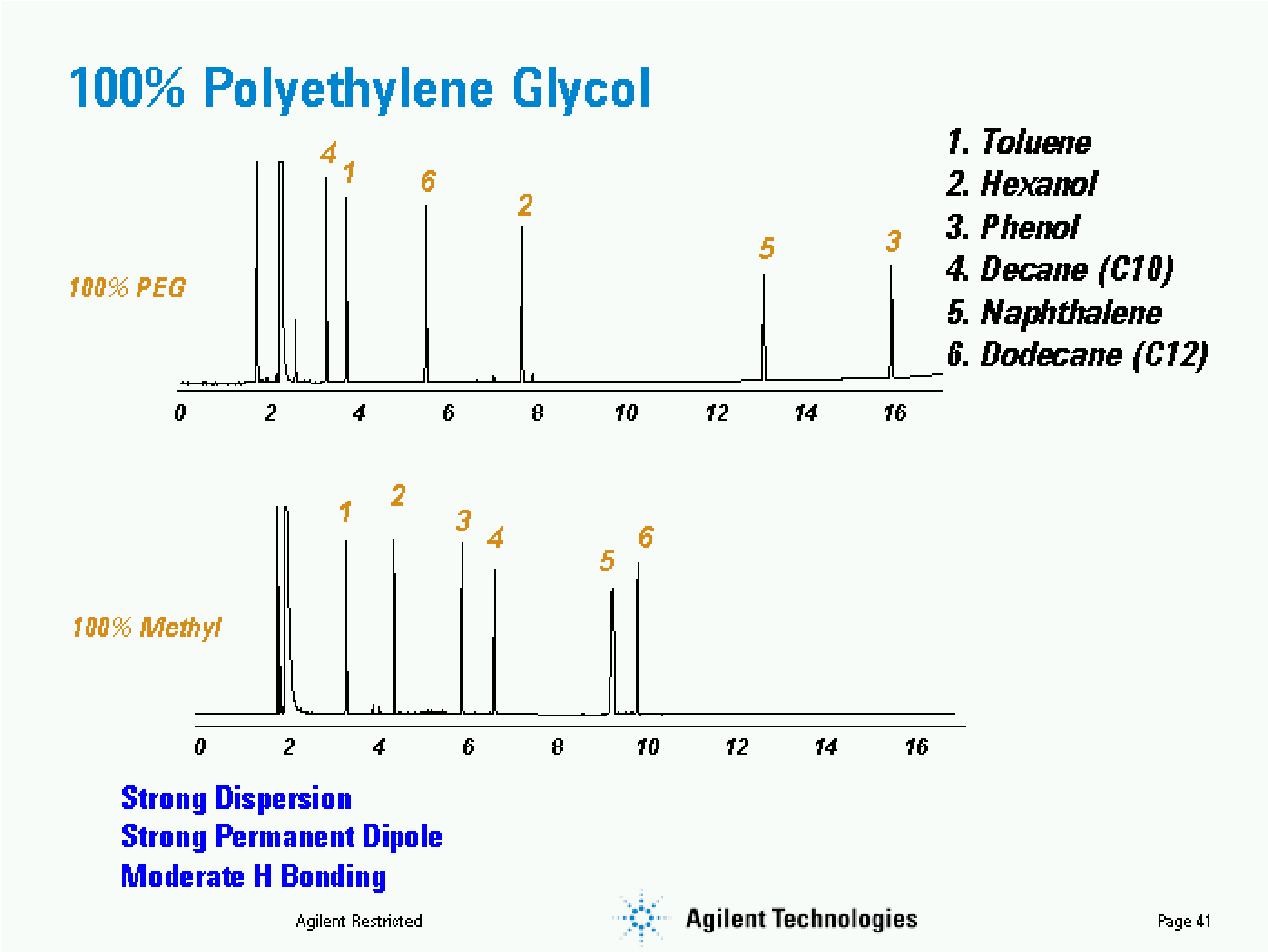
50% Cyanopropyl



14% Cyanopropylphenyl



50% Cyanopropylphenyl



100% Polyethylene Glycol

Stationary Phase Selection
Part 1

- Existing information
- Selectivity
- Polarity
- Critical separations
- Temperature limits

Stationary Phase Selection Part 1

Stationary Phase Selection
Part 2

- Capacity
- Analysis time
- Bleed
- Versatility
- Selective detectors

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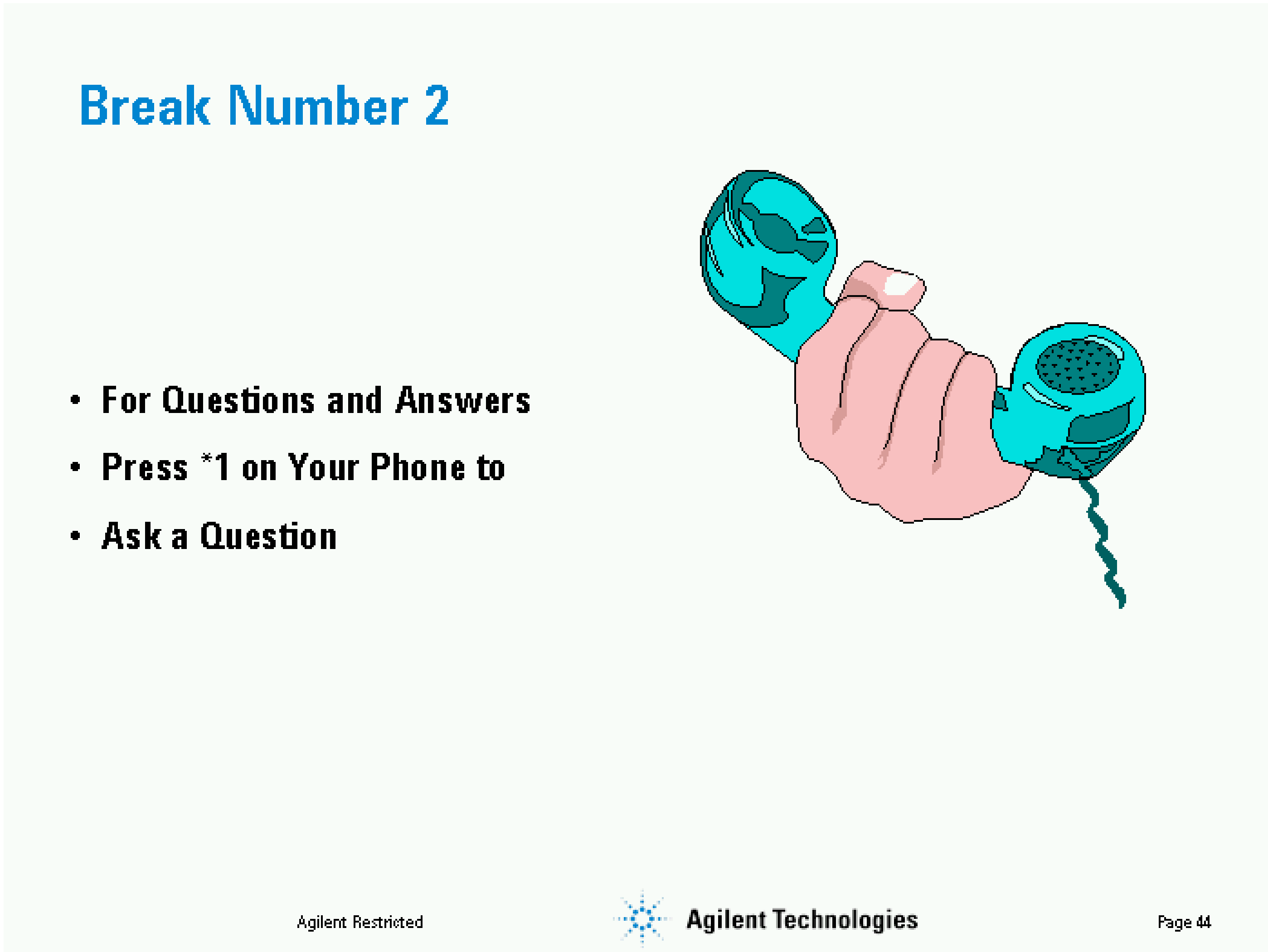
Stationary Phase Selection Part 2



Break Number 2

Column Dimensions

Diameter

Length

Film Thickness

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Column Dimensions

Column Diameter

Capillary Columns

I.D. (mm)	Common Name
0.53	Megabore
0.45	High speed Megabore
0.32	Wide
0.20-0.25	Narrow
0.18	Minibore

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Column Diameter Capillary Columns

Column Diameter
Theoretical Efficiency

I.D. (mm)	N/m
0.10	11905
0.18	6666
0.20	5941
0.25	4762
0.32	3717
0.53	2242

k = 5

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Column Diameter Theoretical Efficiency

Efficiency and Resolution

Relationship

$$\sqrt{N} \;\alpha\; R_s$$

Efficiency X 4 = Resolution X 2

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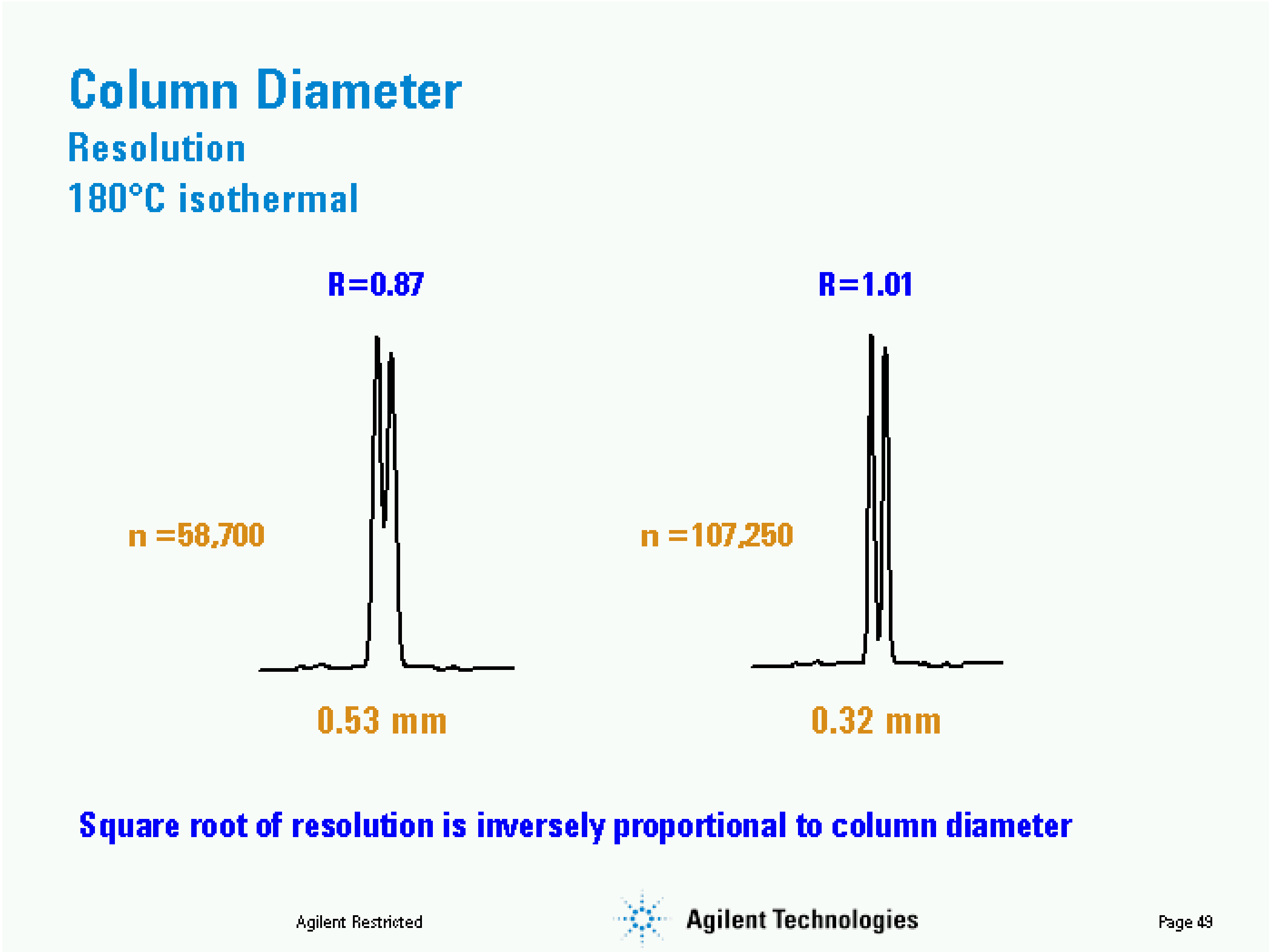
Efficiency and Resolution Relationship



Column Diameter Resolution 180°C isothermal

Column Diameter
Inlet Head Pressures
Helium

I.D (mm)	Pressure (psig)
0.10	225-250
0.20	25-35
0.25	15-25
0.32	10-20
0.53	2-4

30 meters
Hydrogen pressures x 1/2

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Column Diameter Inlet Head Pressures Helium

Column Diameter

Capacity

Like Polarity Phase/Solute

I.D. (mm)	Capacity (ng)
0.20	50-100
0.25	75-150
0.32	125-250
0.53	200-400

0.25 µm film thickness

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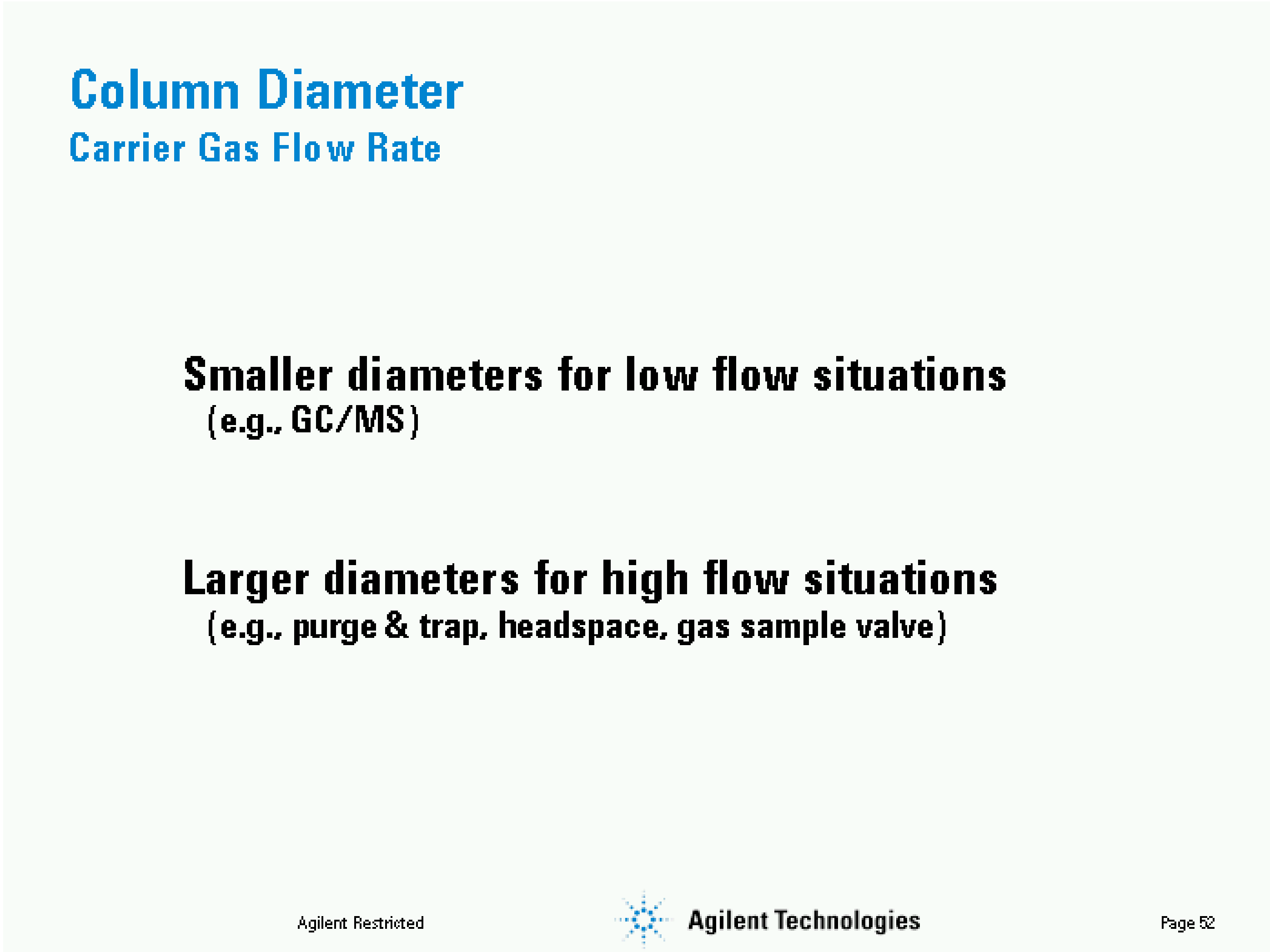
Column Diameter Capacity Like Polarity Phase/Solute



Column Diameter Carrier Gas Flow Rate

Column Length

Most common: 15-60 meters

Available: 5-150 meters

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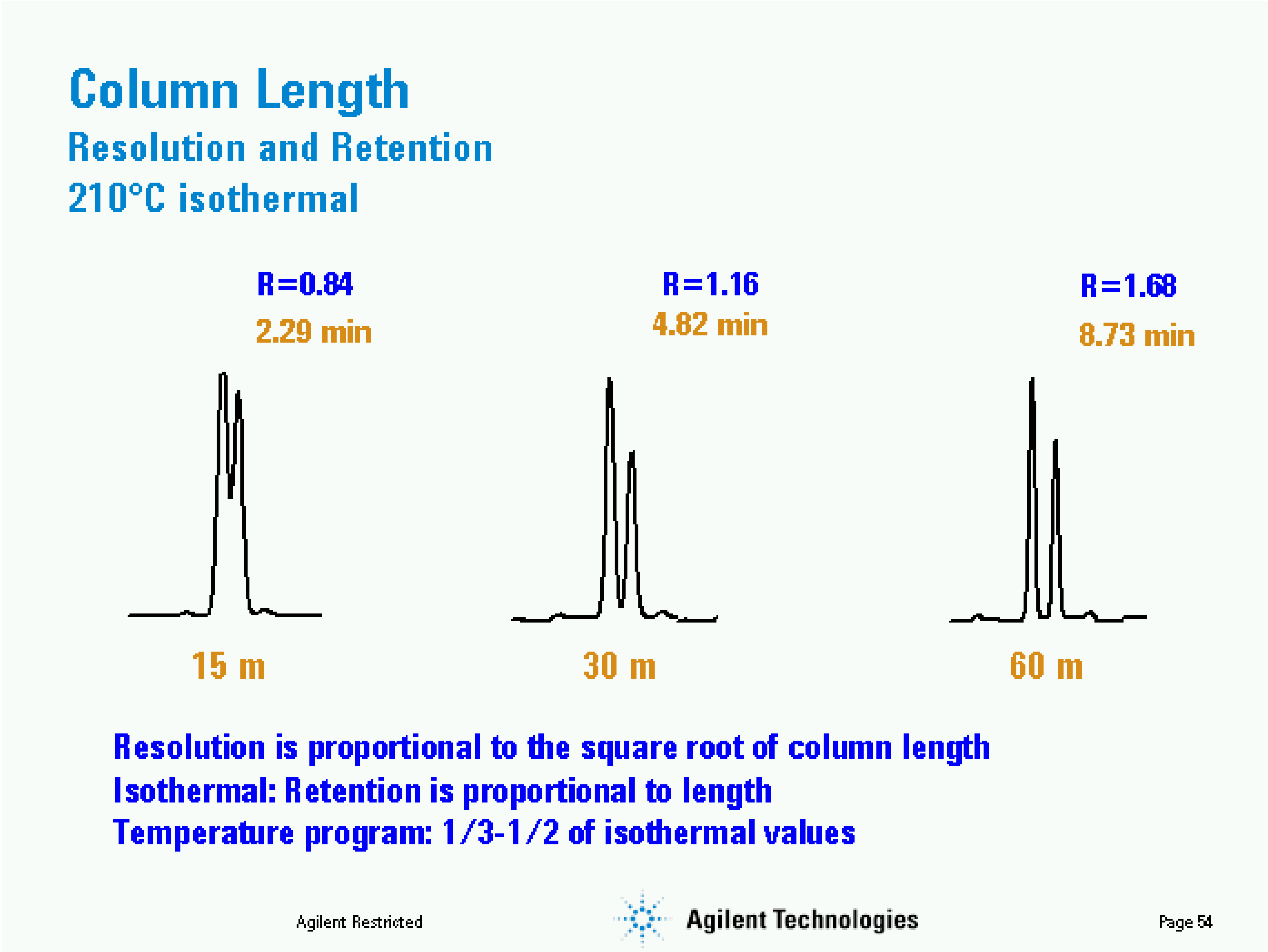
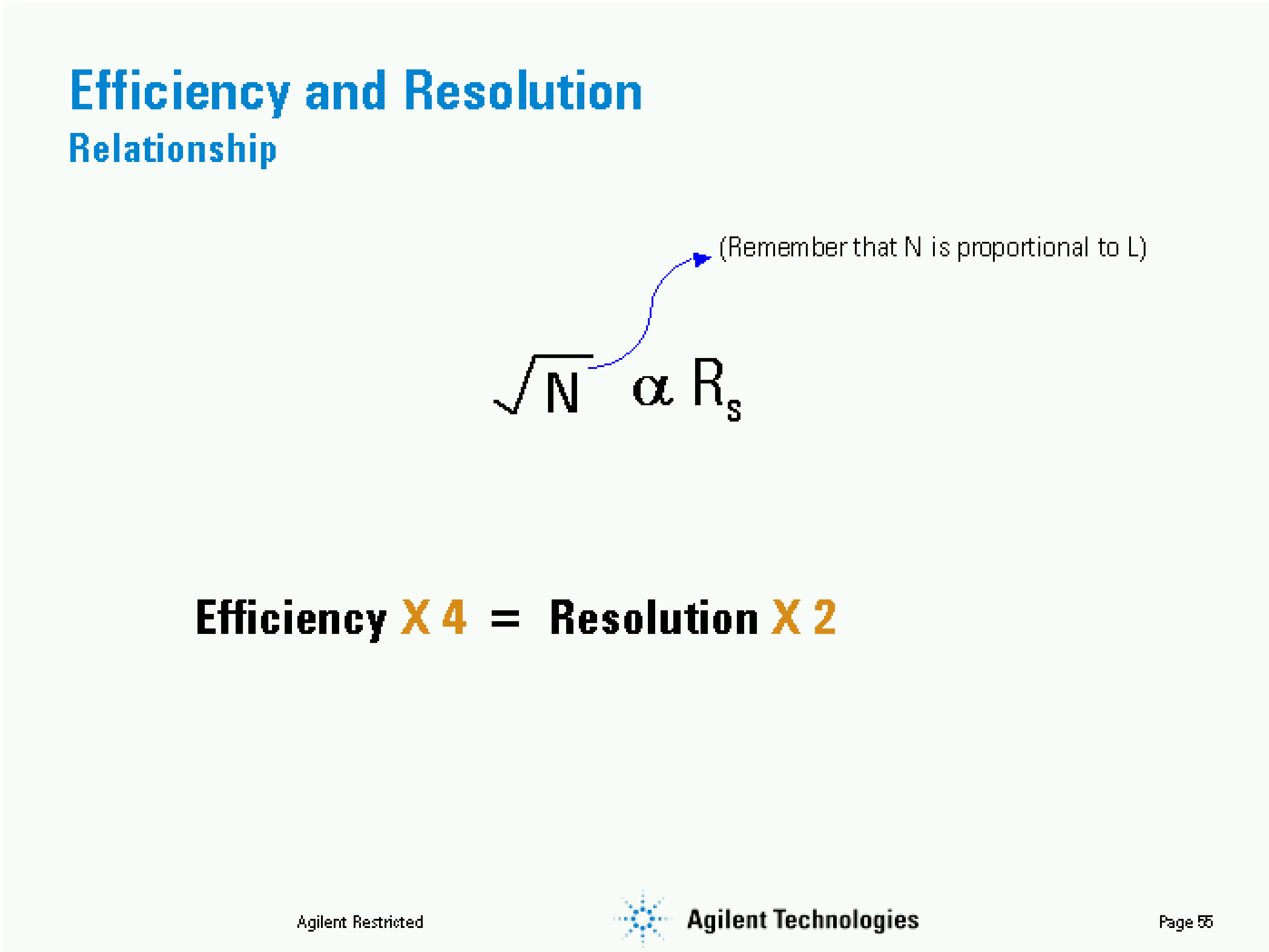
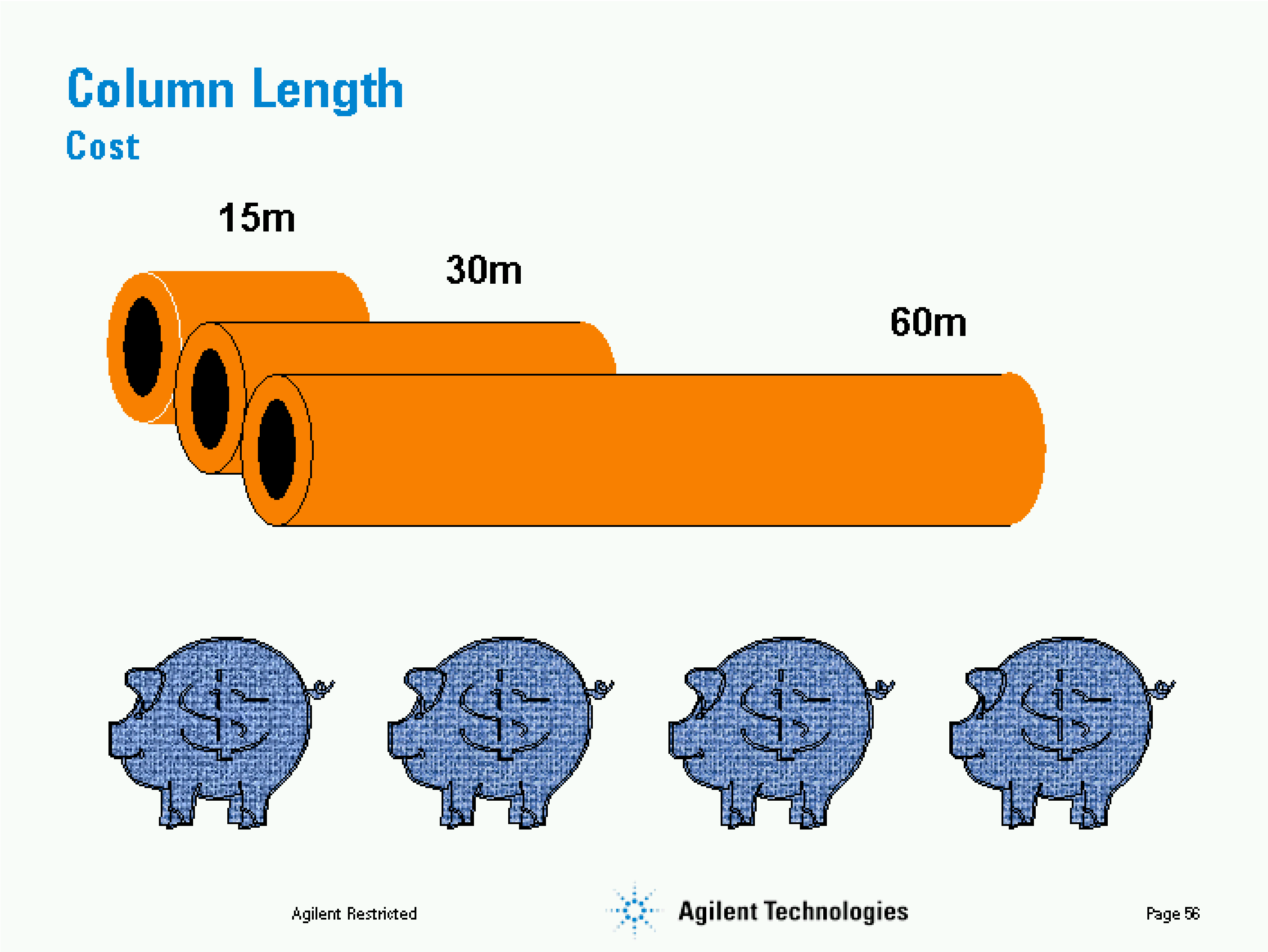
Column Length



Column Length Resolution and Retention 210°C isothermal



Efficiency and Resolution Relationship



Column Length Cost

Film Thickness

Most common: 0.1-3.0 µm

Available: 0.1-10.0 µm

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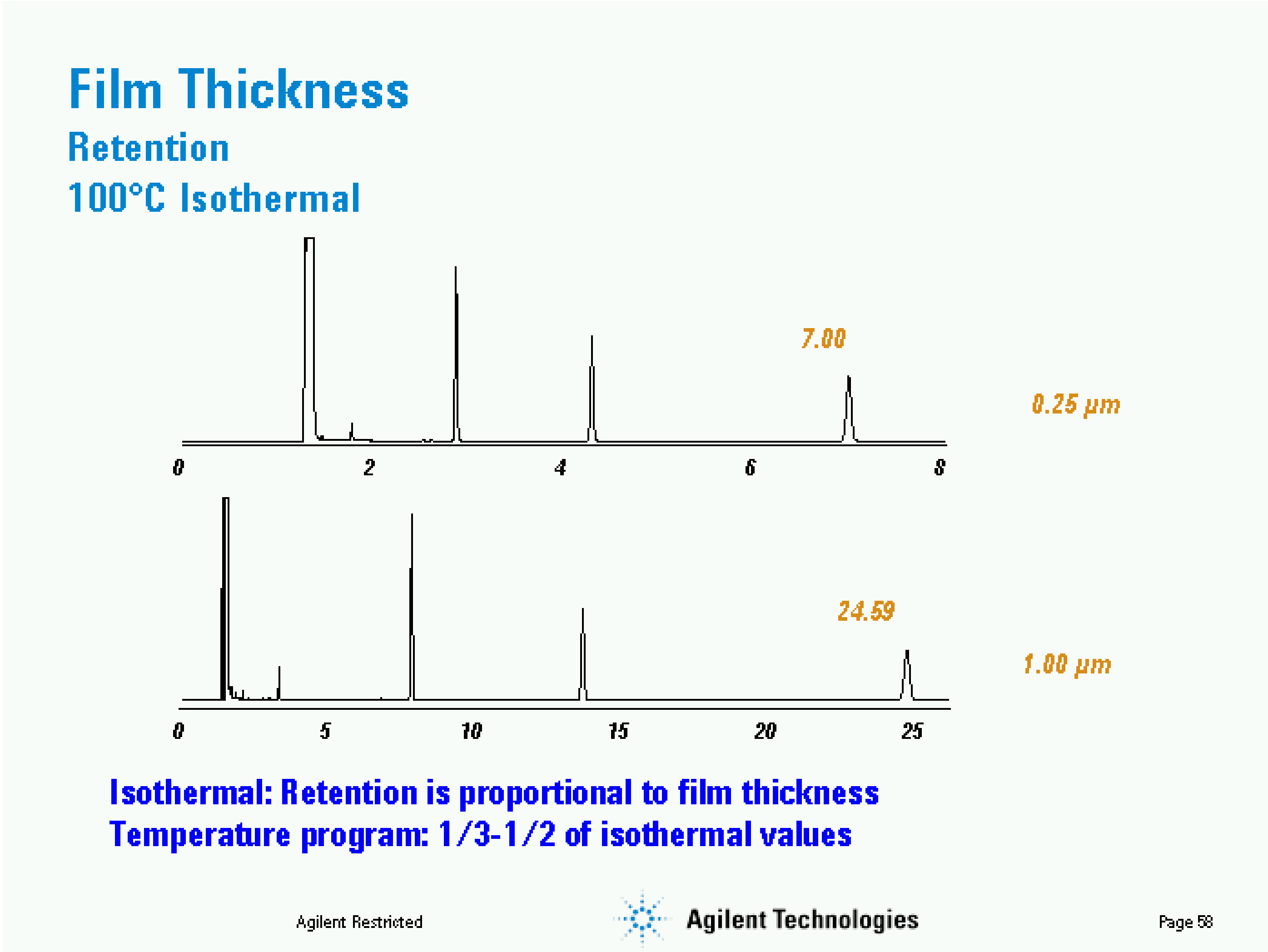
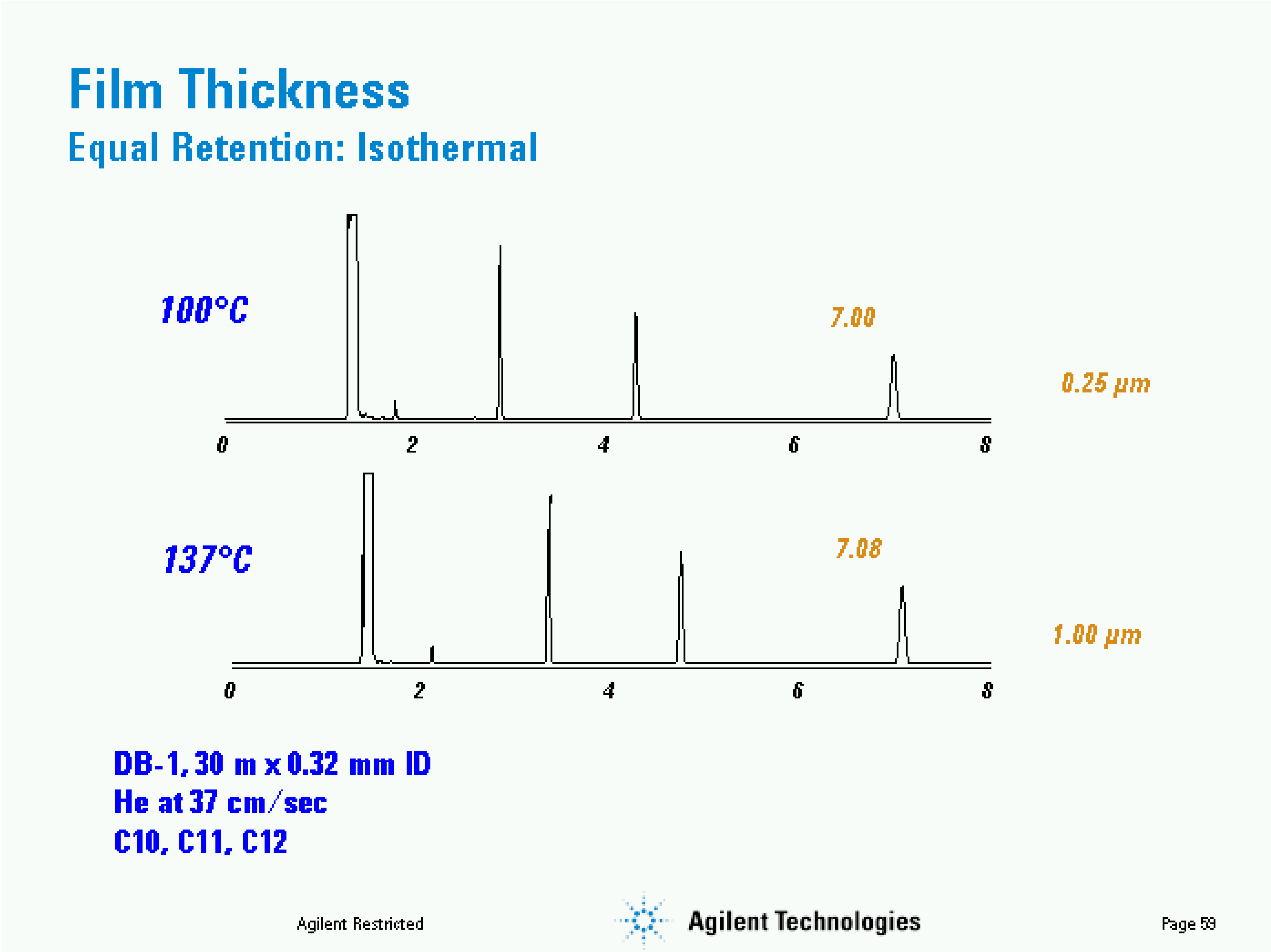
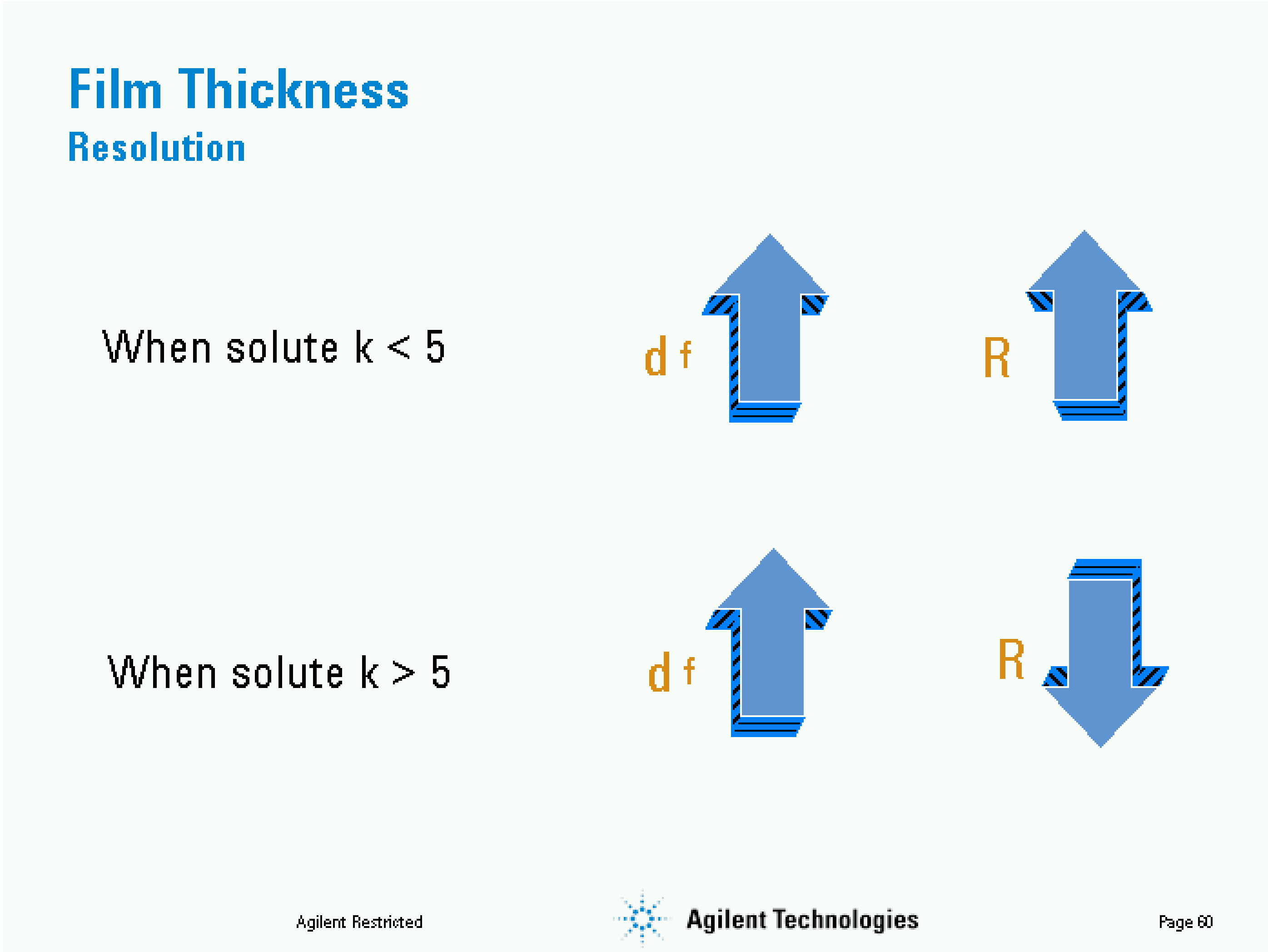
Film Thickness



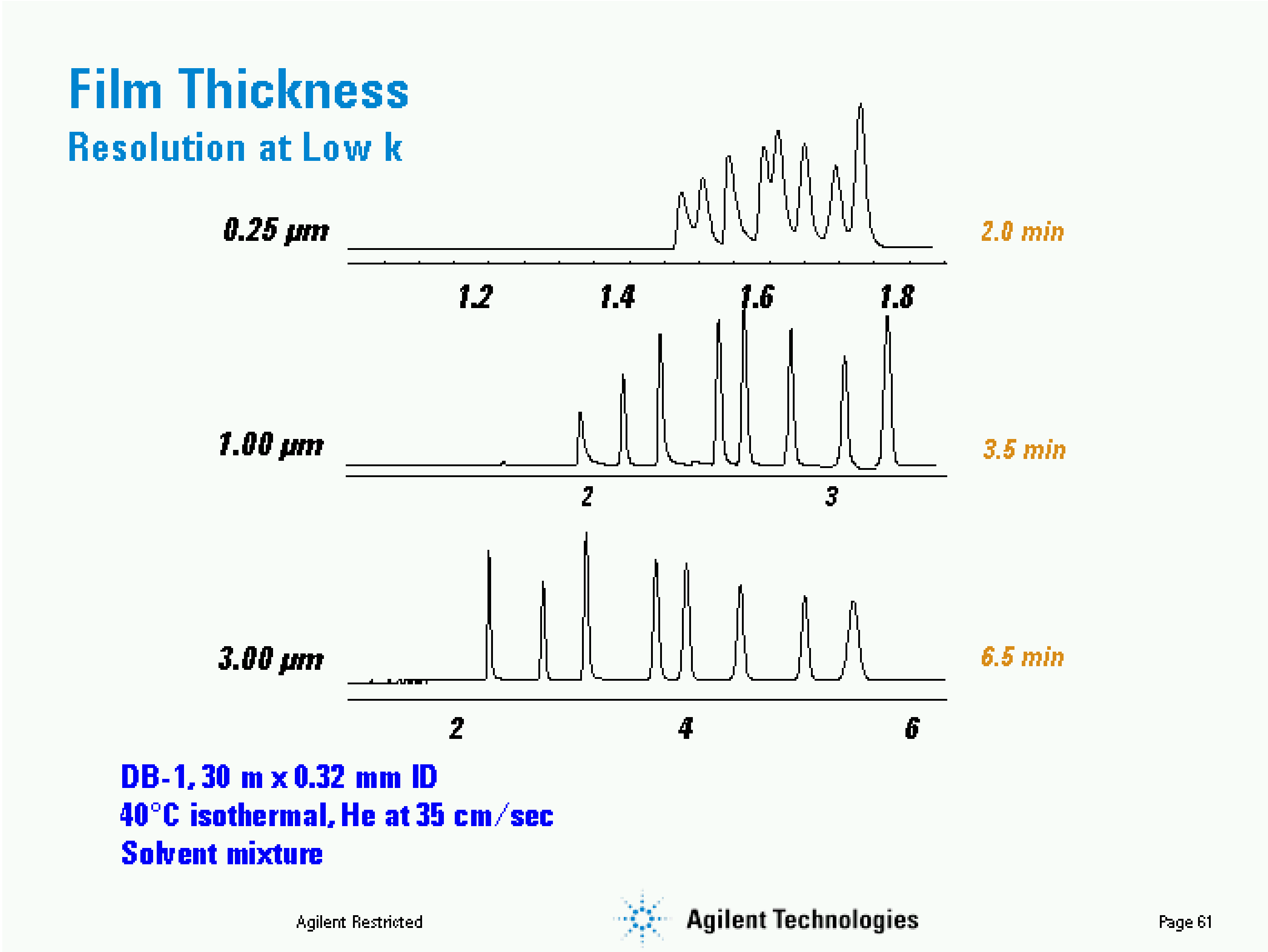
Film Thickness Retention 100°C Isothermal



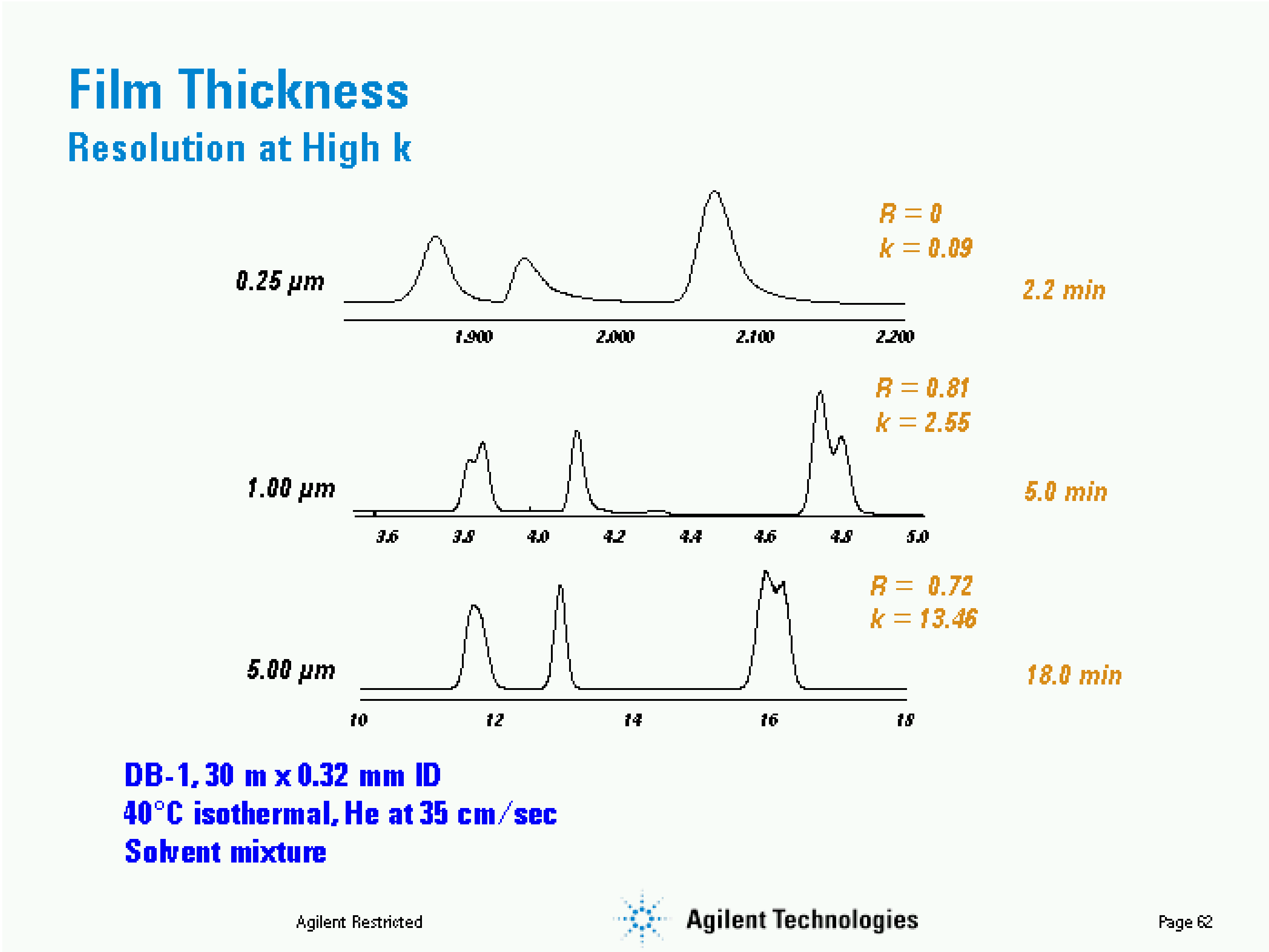
Film Thickness Equal Retention: Isothermal



Film Thickness Resolution



Film Thickness Resolution at Low k



Film Thickness Resolution at High k

Film Thickness

Capacity

Like Polarity Phase/Solute

Thickness (um)	Capacity (ng)
0.10	50-100
0.25	125-250
1.0	500-1000
3.0	1500-3000
5.0	2500-5000

0.32 mm I.D.

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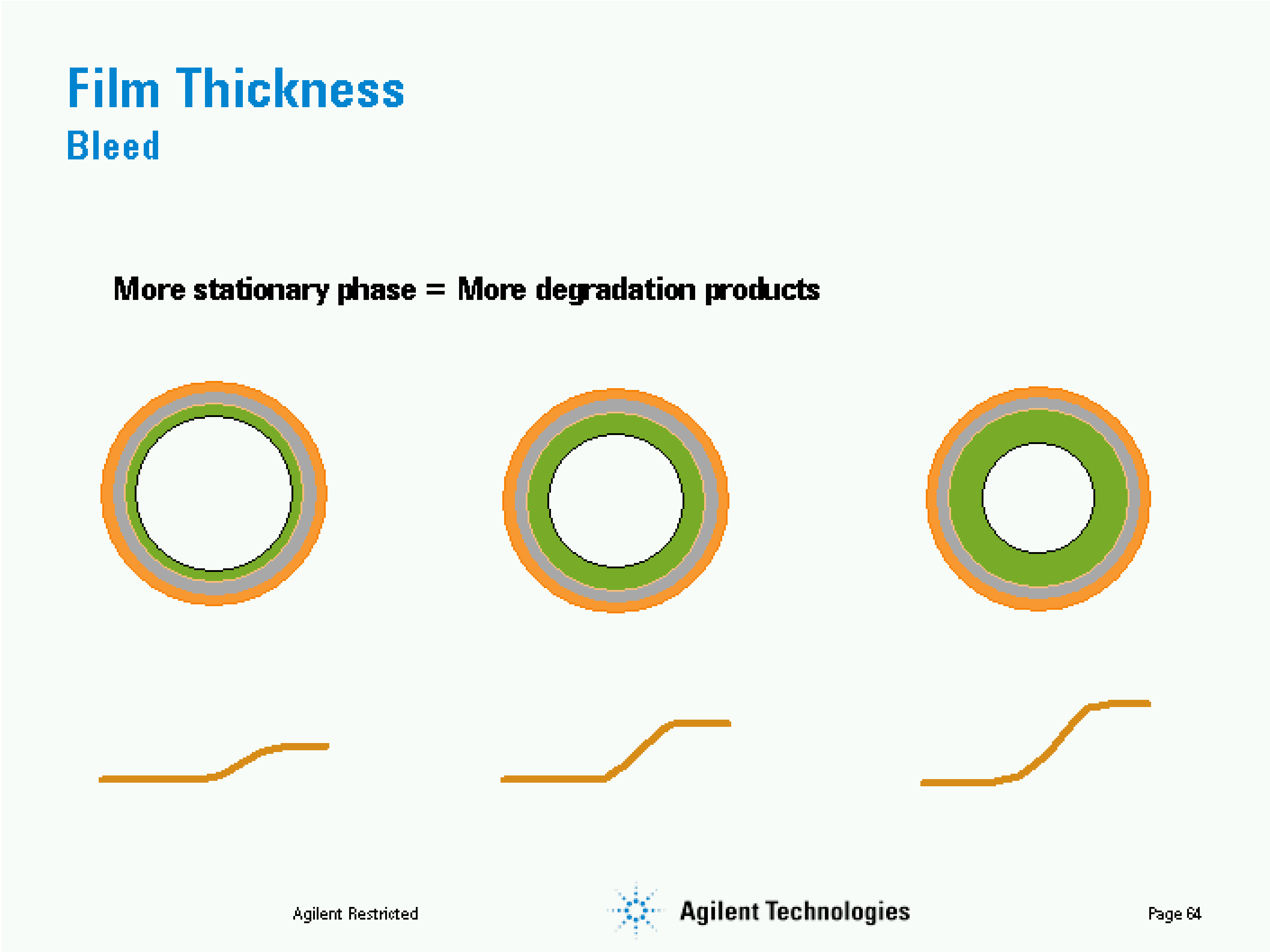
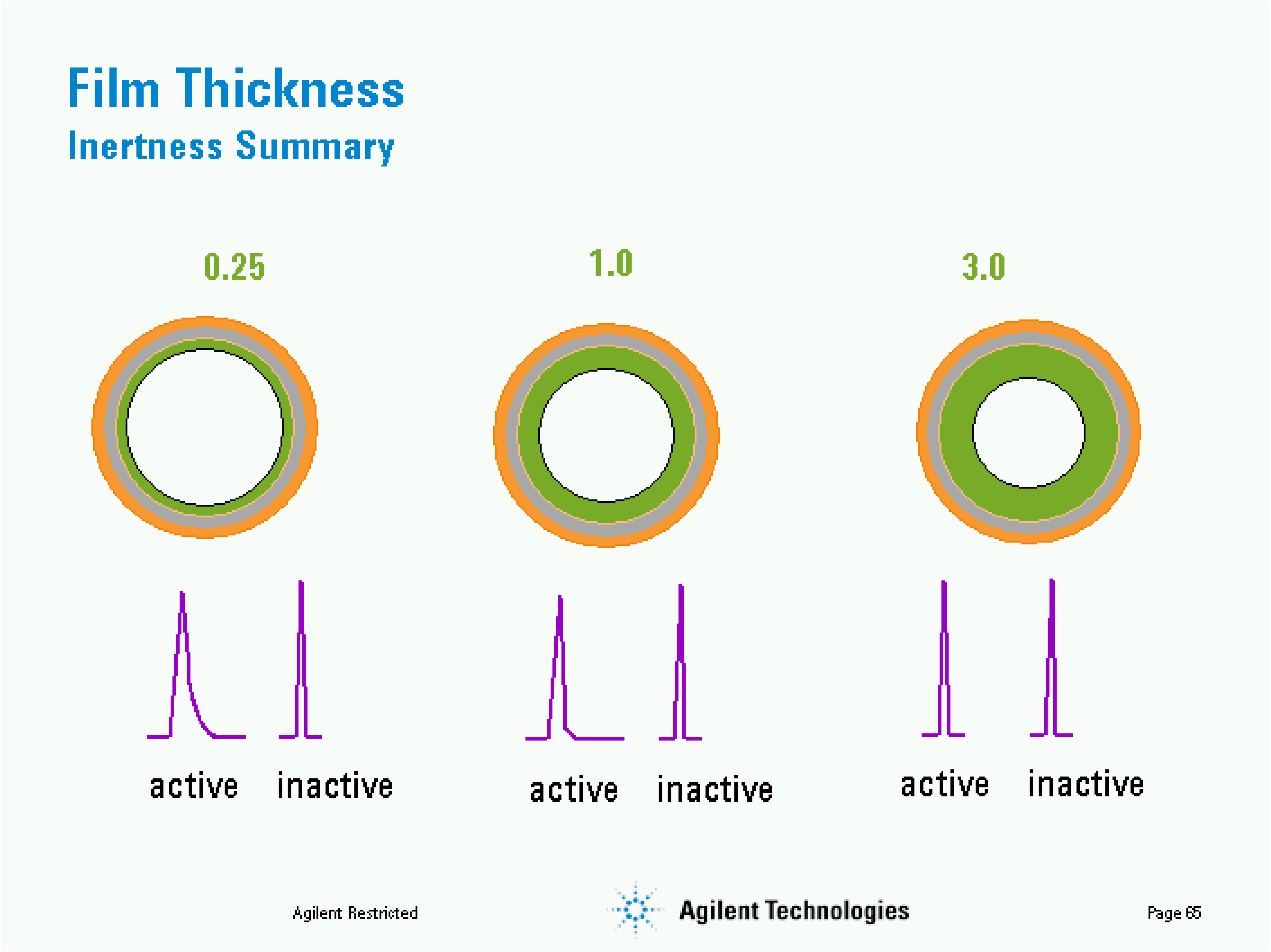
Film Thickness Capacity Like Polarity Phase/Solute



Film Thickness Bleed



Film Thickness Inertness Summary

Column Dimensions
Diameter Summary

To Increase	Make Diameter
Resolution	Smaller
Retention	Smaller
Pressure	Smaller
Flow rate	Larger
Capacity	Larger

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Column Dimensions Diameter Summary

Column Dimensions
Length Summary

To Increase	Make Length
Resolution	Longer
Retention	Longer
Pressure	Longer
Cost	Longer

Column Dimensions Length Summary

Column Dimensions
Film Thickness Summary

To Increase	Make Film
Retention	Thicker
Resolution (k<5)	Thicker
Resolution (k>5)	Thinner
Capacity	Thicker
Inertness	Thicker
Bleed	Thicker

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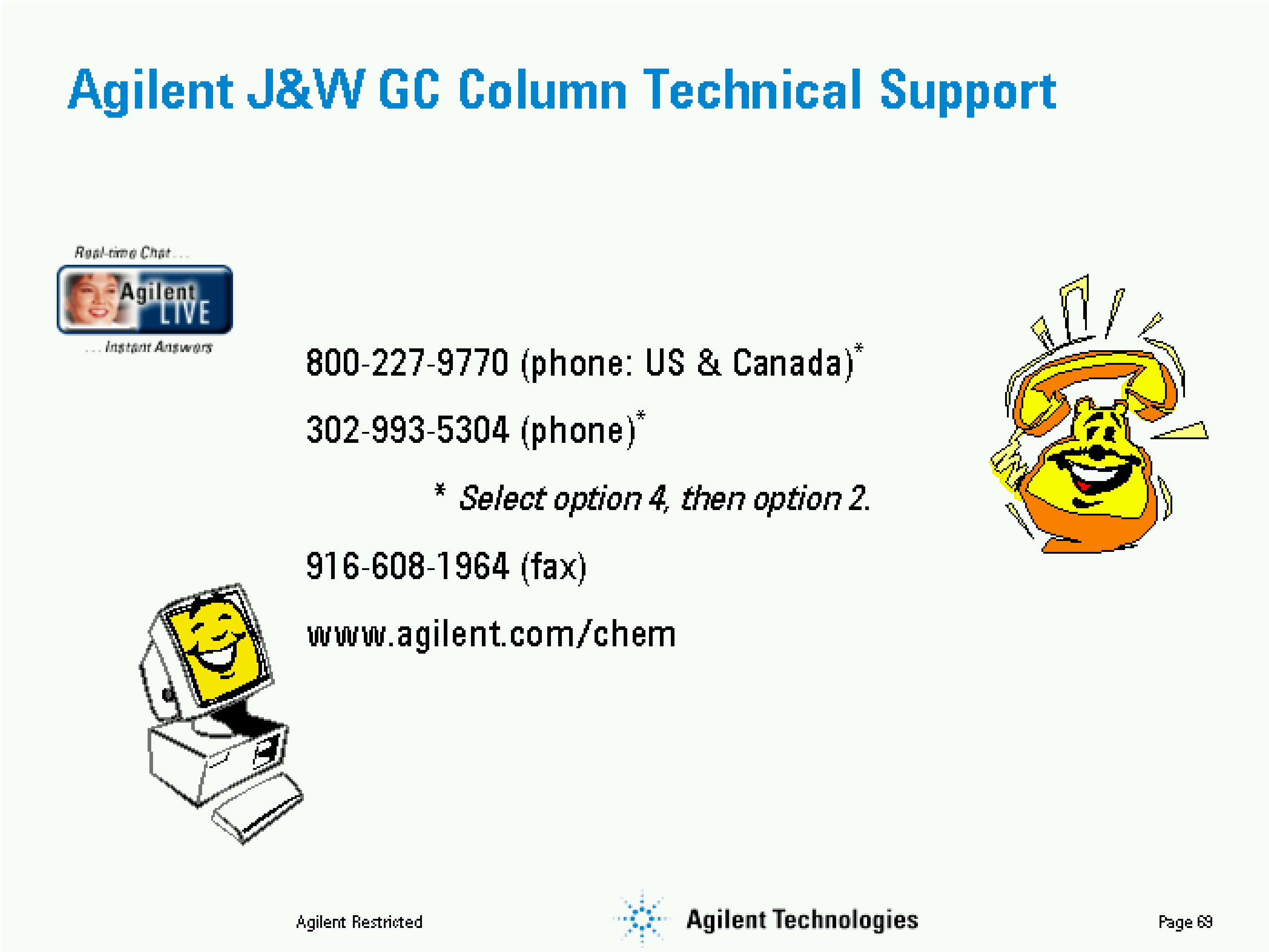
Column Dimensions Film Thickness Summary



Agilent J&W GC Column Technical Support