Improving Method Performance through Fast LC

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Fast LC Methods

- Use of columns that can operate at high flow rates with reduced pressures (increased k´)
- Use of a reduced particle size substrate (<3µm) to force higher theoretical plate counts (increased N)
- Use of shortened standard packed columns with optimized and possibly unique stationary phases (increased alpha)

Fast LC Technique

- Highly selective stationary phase is desired to maximize alpha values.
- Elution of components is typically accomplished through the use of gradients to reduce retention of highly retained components.
- Simple resolution of methylene substitutions /additions may be accomplished isocratically.
- Good screening technique for unknowns.

Fast LC Technique – Advantages

- Faster re-equilibration (when using gradients)
- Sensitivity improvements.
- Older qualitative techniques can be adapted to a highly automated quantitative technique.
- Allows potentially high increases in sample throughput.
- Great technique when performed by LC-MS
- Shorter analysis times reduce solvent consumption and waste.

Fast LC Technique – Disadvantages

- Critical separations are more sensitive to extra-column volume (as post column reactors.)
- Extremely selective stationary phase must be used to maximize selectivity – especially for structural isomers.
- May not be well suited to normal phase or ion pairing separations (with gradients).

Principles and Theory of HPLC

General Resolution Equation

$$R = 1/4 \left(\frac{a-1}{a} \right) \sqrt{N} \left(\frac{k'}{k'+1} \right)$$

Selectivity

- stationary phase
- mobile phase composition
- additives

Efficiency

- particle size
- column length

Retention

- chain length
- mobile phase strength

Column Length vs Theoretical Plates

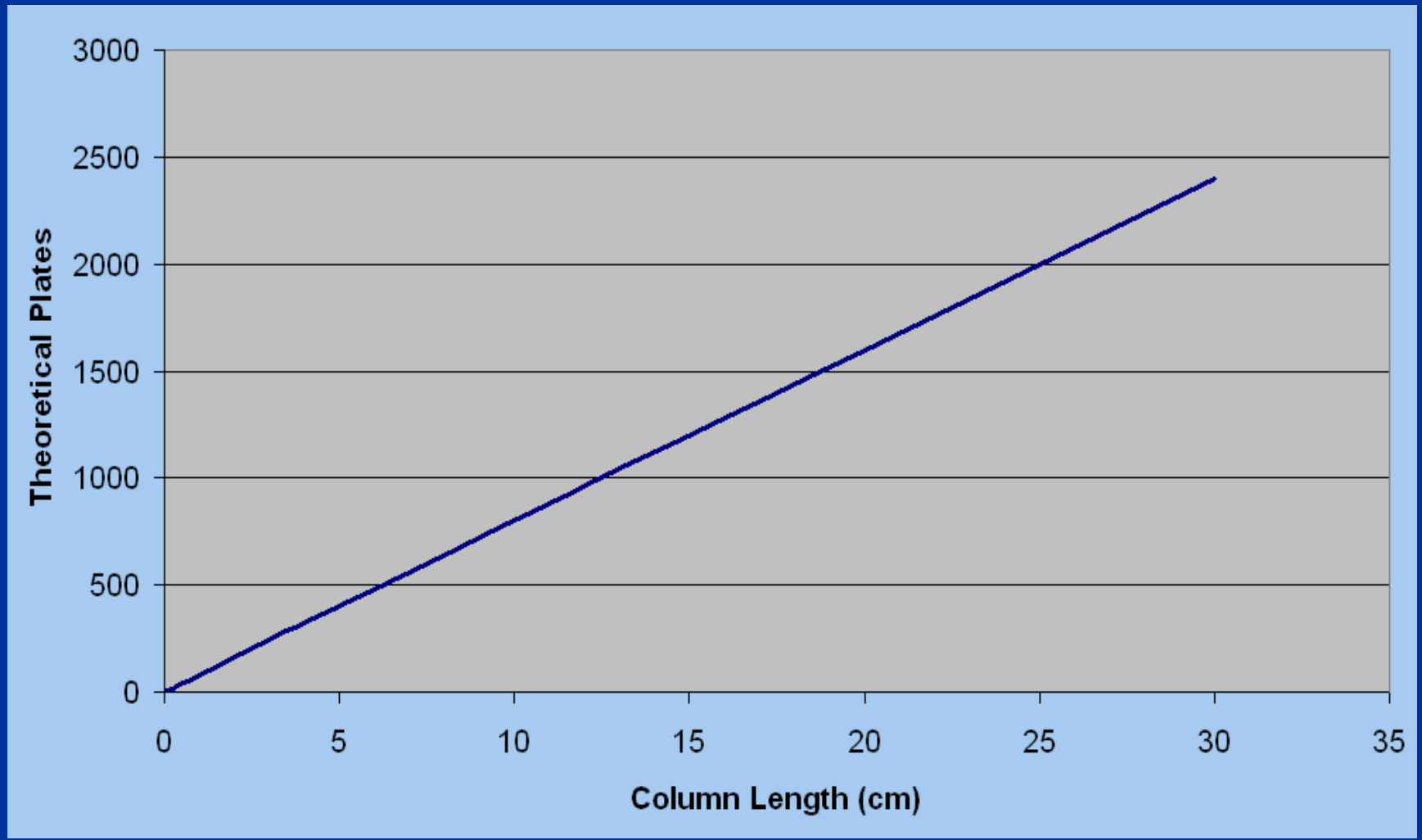
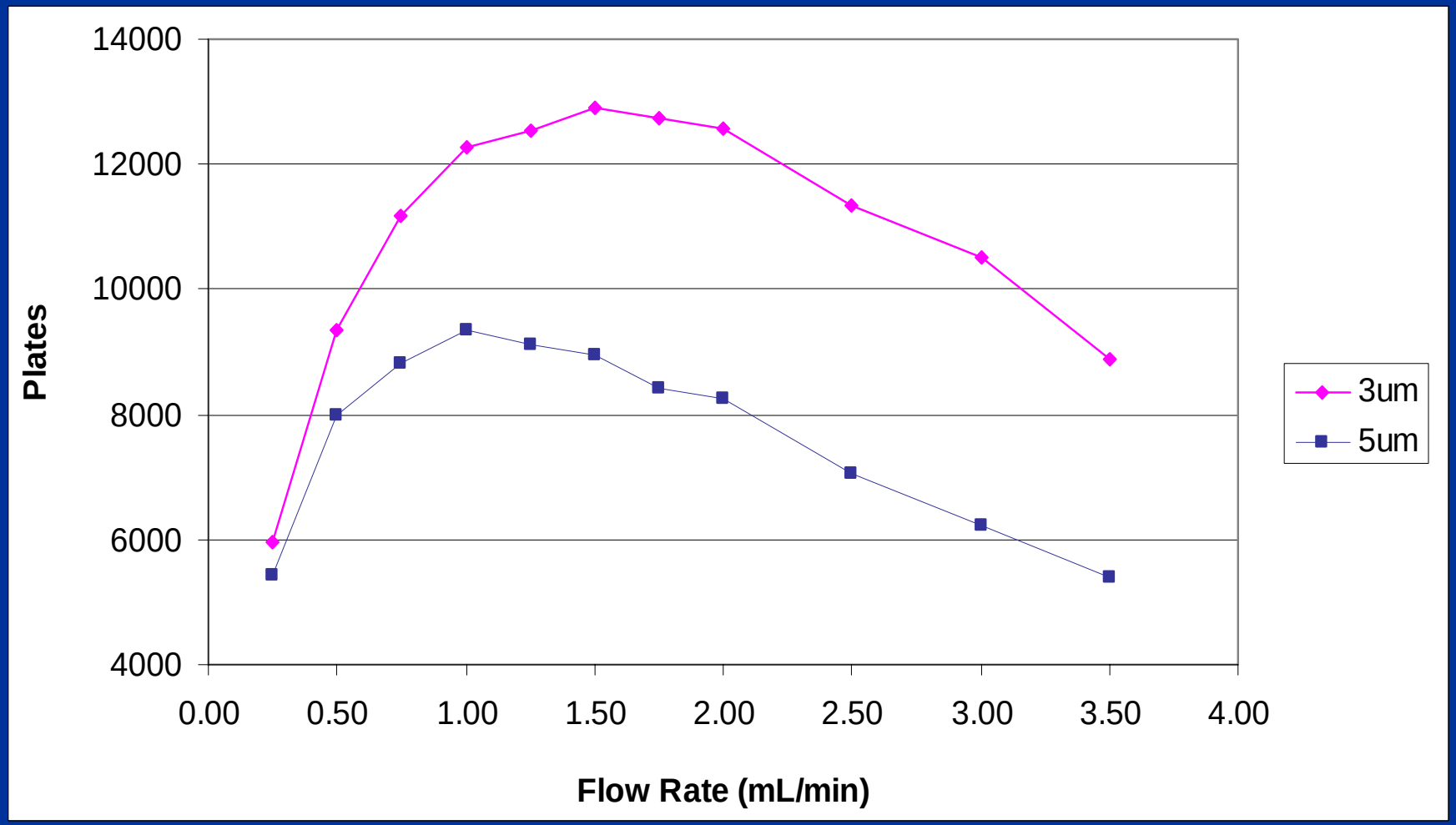


Plate Count vs Particle Size and Flow Rate



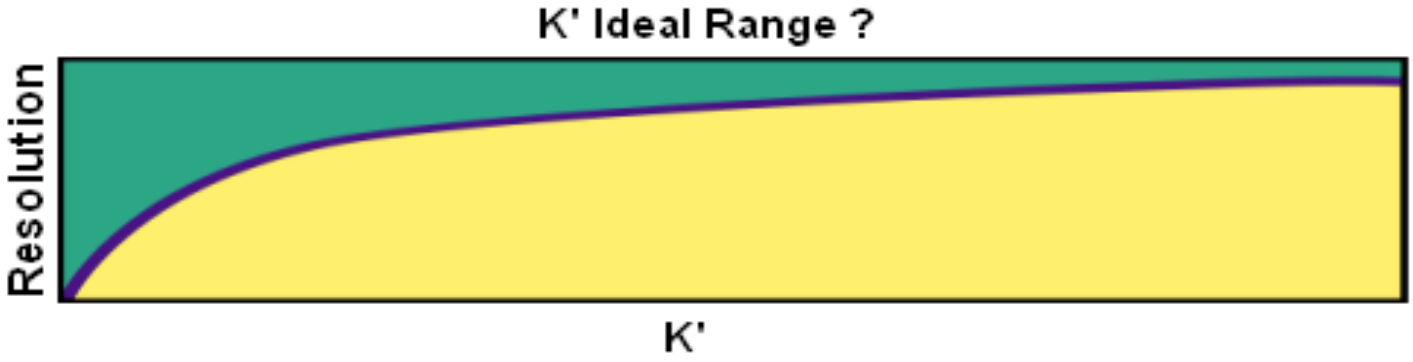
4.6 mm Column

Principles and Theory of HPLC

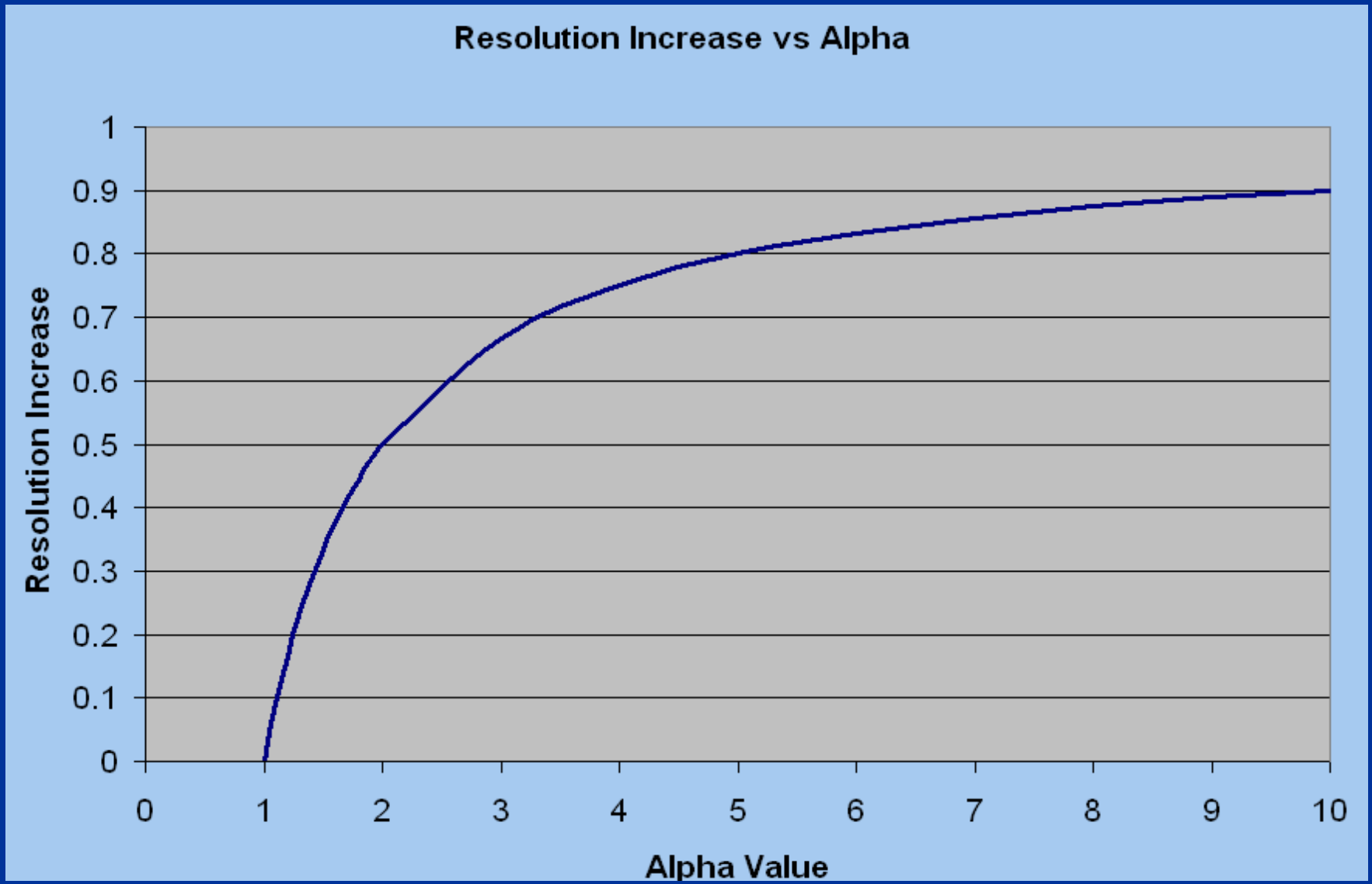
$$\frac{K'}{1+K'}$$

Resolution Depends on k'

K' Value	K' Term	K' Resolution ?
0	0	0
1	1/2	.50
2	2/3	.67
3	3/4	.75
10	10/11	.91
20	20/21	.95

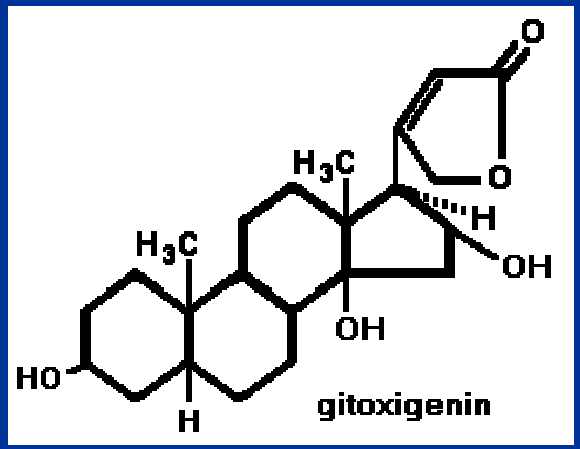


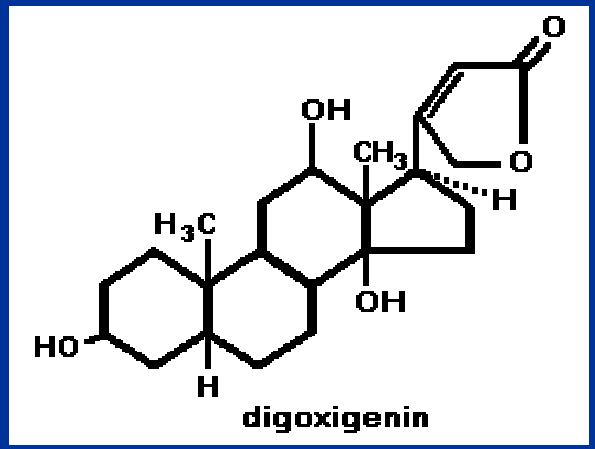
Resolution Increase vs Alpha

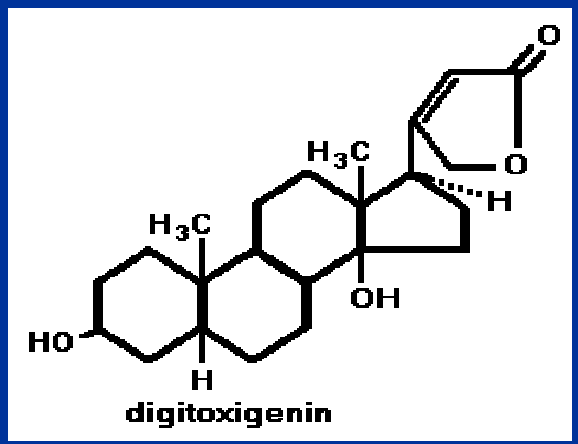


Fast LC improvement of USP TLC and HPLC Method

Digitalis Extracts and Derivatives



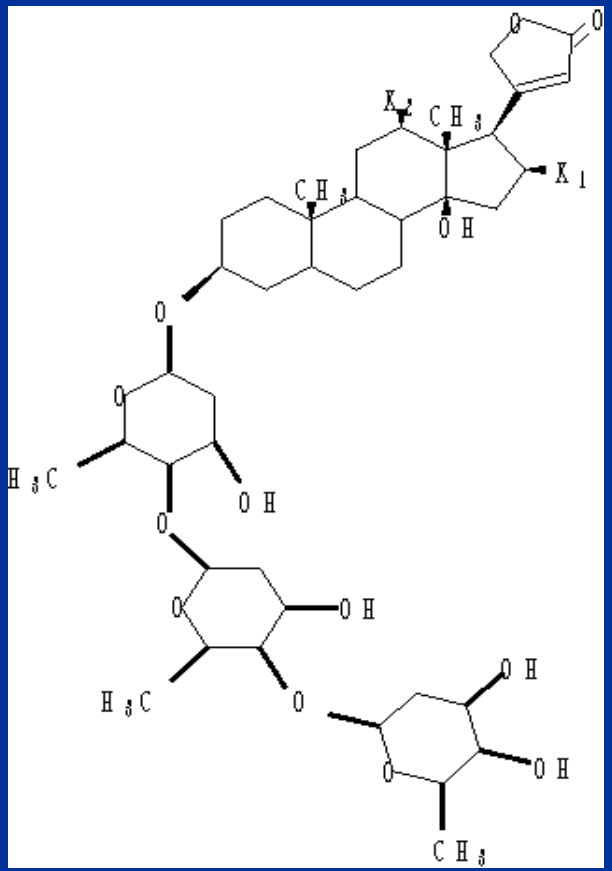




Digitoxin: $X_1=X_2=H$

Digoxin: $X_1=H; X_2=OH$

Gitoxin: $X_1=OH; X_2=H$



Digitoxin Substitutions

Figure 1

Fast LC Separation of Digitalis Derivatives Experimental Fluorophase (3 minutes)

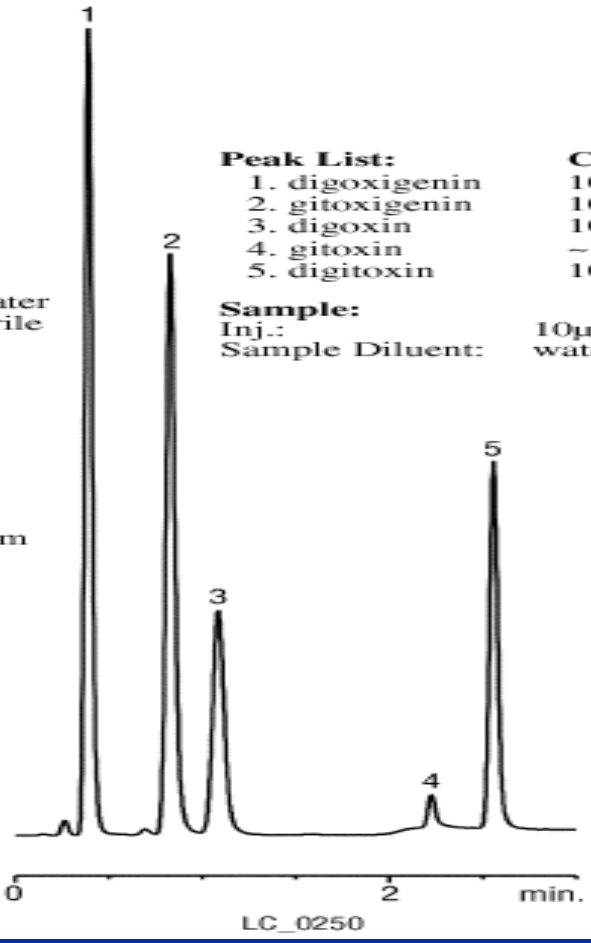
Digitalis Extracts/Derivatives on Ultra Alkaloids Cartridge Column: Fast LC

Column:
Catalog #:
Dimensions: 30 x 4.0mm
Particle Size: 3µm
Pore Size: 100Å

Conditions:
Mobile Phase: A: 100% Water
B: Acetonitrile

Time (min.)	%B
0.0	20
1.5	20
1.51	35
3.0	35
3.1	20

Flow: 2.0mL/min
Temp.: 27°C
Det.: UV @ 230nm



Peak List:	Conc.	Ret. Time (min.)
1. digoxigenin	100µg/mL	0.40
2. gitoxigenin	100µg/mL	0.80
3. digoxin	100µg/mL	1.10
4. gitoxin	~10µg/mL	2.20
5. digitoxin	100µg/mL	2.60

Sample:
Inj.: 10µL
Sample Diluent: water:acetonitrile (80:20 v/v)

Figure 2

Advantages of Digitalis Fast LC over Current Methods

- Improved automation and analysis throughput.
- Reduction of analysis time – previously a 30 cm length C18 column was required for resolution.
- Ability to analyze all materials by HPLC.
- Ability to precisely quantitate materials vs TLC.
- Highly selective stationary phase.
- Technique can also be applied to purification and analysis of digoxin labeled materials for biological activity.
- Perfect technique for use in high speed cleaning validations.

Fast LC Analysis of Carbamate Insecticides

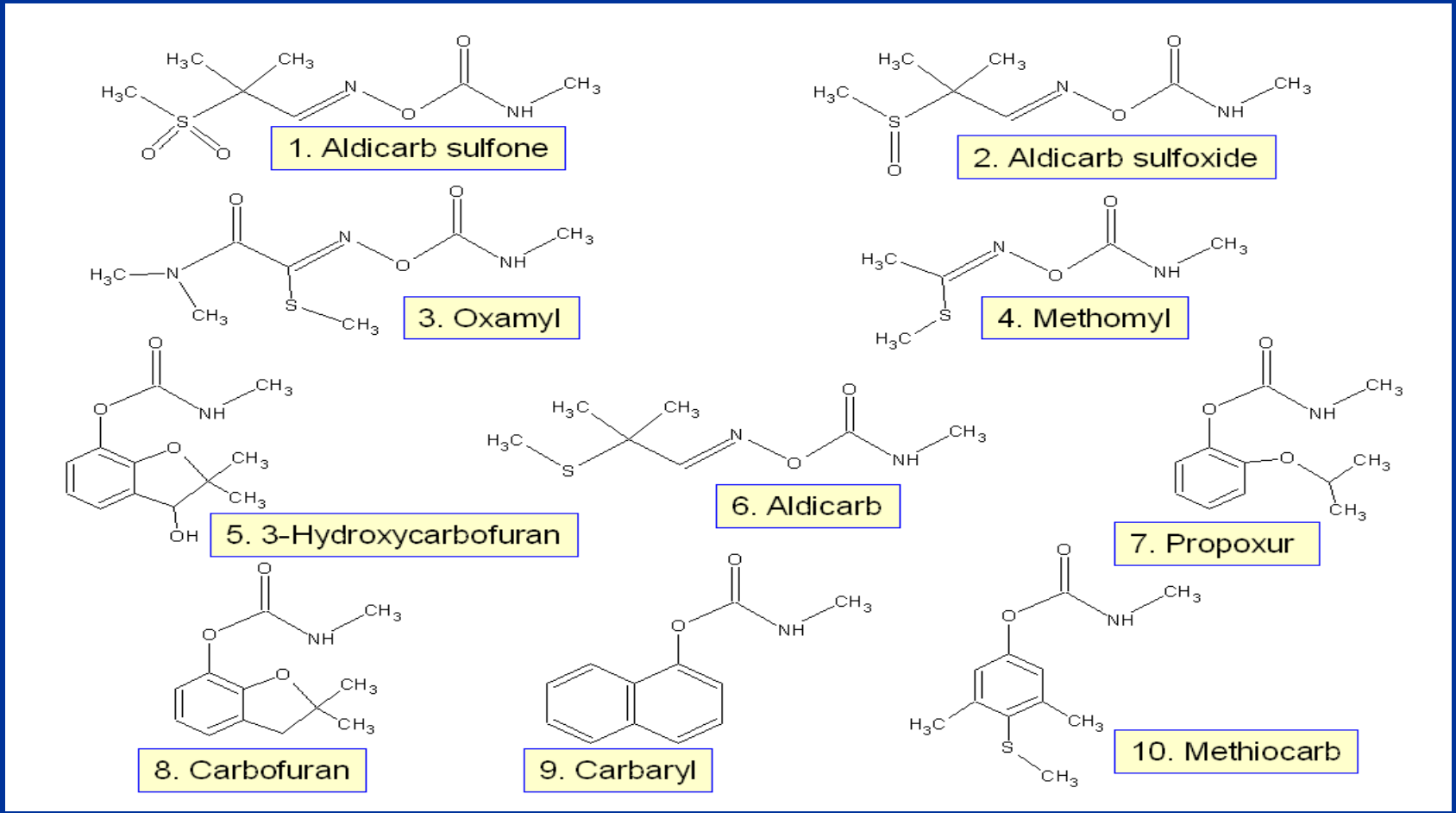


Figure 3-Structures of Commonly Analyzed Carbamates

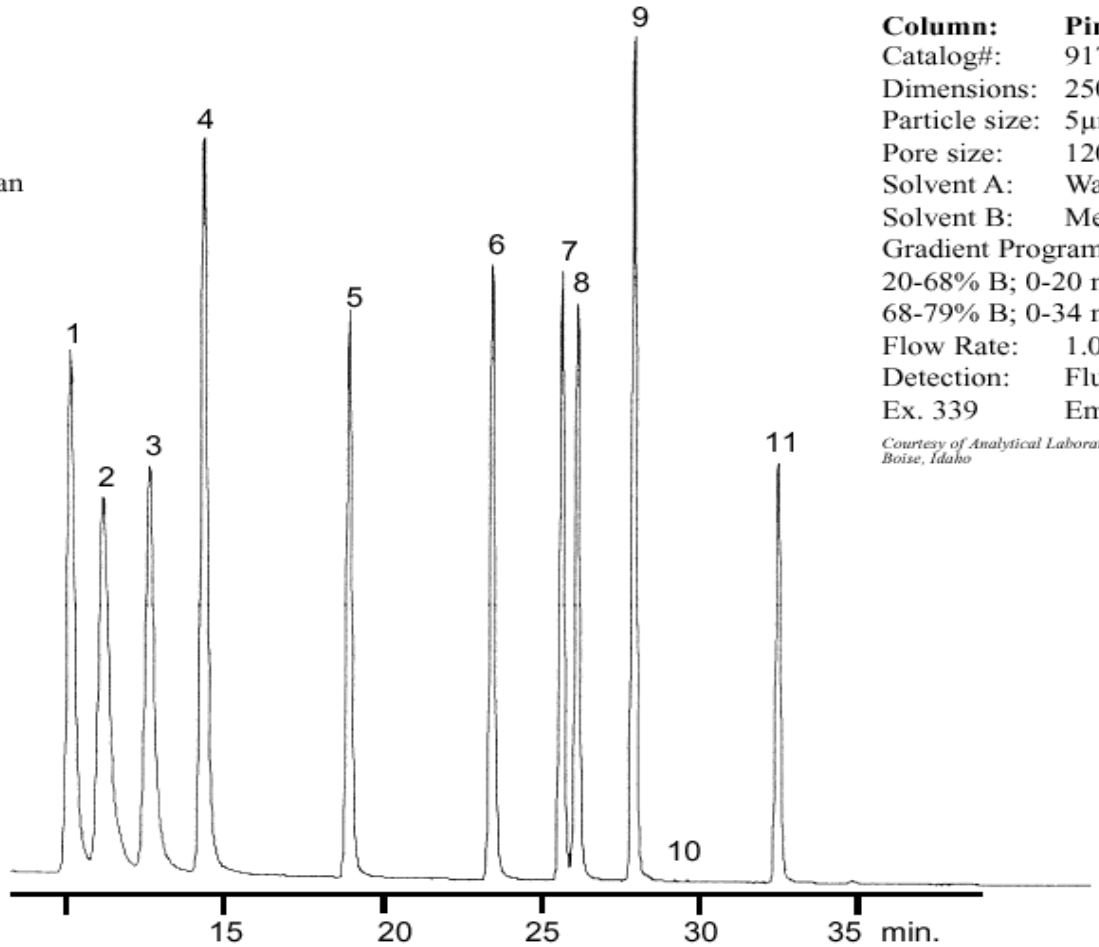
Carbamate Analysis using Standard HPLC Methodology (About 40 minutes)

Carbamate Pesticides on Pinnacle Carbamate

Applications Note: LC_0192

Peak List:

1. aldicarb sulfoxide
2. aldicarb sulfone
3. oxamyl
4. methomyl
5. 3-hydroxycarbofuran
6. aldicarb
7. propoxur
8. carbofuran
9. carbaryl
10. 1-naphthol
11. methiocarb



Column:	**Pinnacle Carbamate**
Catalog#:	9173575
Dimensions:	250 x4.6mm
Particle size:	5µm
Pore size:	120Å
Solvent A:	Water
Solvent B:	Methanol

Gradient Program:
20-68% B; 0-20 min.
68-79% B; 0-34 min.

Flow Rate:	1.0mL/min
Detection:	Fluorescence
Ex. 339	Em. 445nm

Courtesy of Analytical Laboratories, Inc. Boise, Idaho

Figure 4

Fast LC Separation of Carbamates (About 13 minutes)

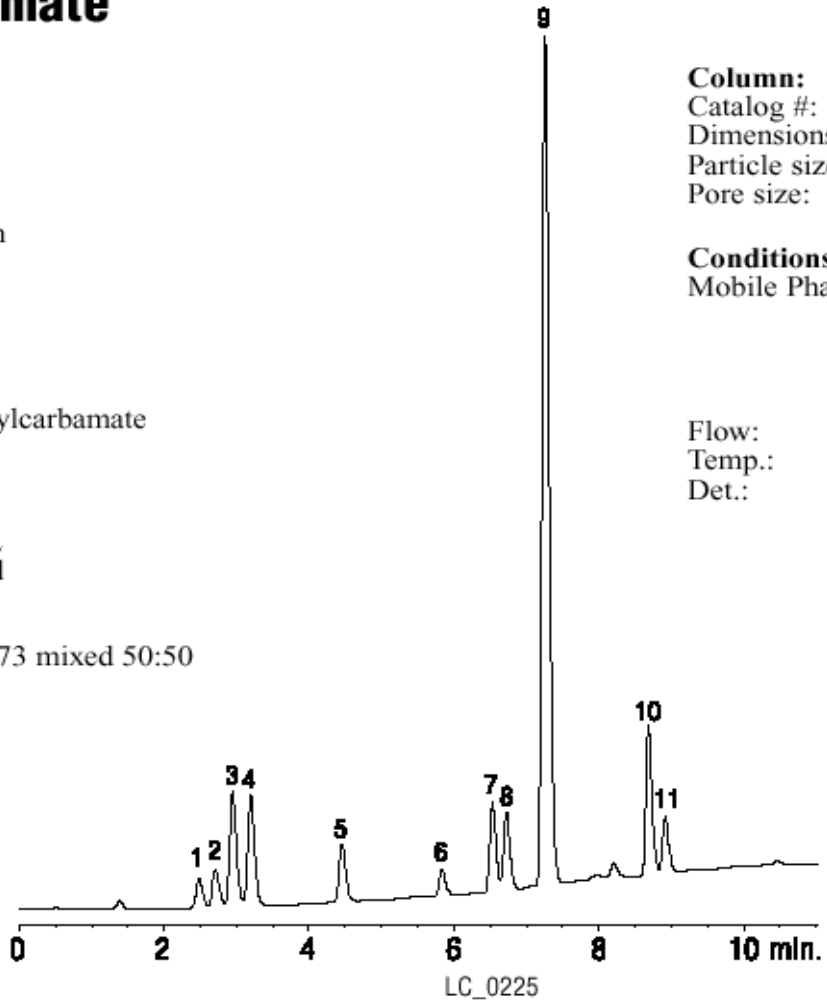
Fast LC Separation of 11 Carbamates on Ultra Carbamate

Peak List:
1. aldicarb sulfone
2. aldicarb sulfoxide
3. oxamyl
4. methomyl
5. 3-hydroxycarbofuran
6. aldicarb
7. propoxur
8. carbofuran
9. carbaryl
10. methiocarb
11. 4-bromo-3,5-dimethylcarbamate

Sample:

Inj.:	5µL
Conc.:	50µg/mL
Solvent:	methanol

Restek standards:
Catalog# 32274 and 32273 mixed 50:50



Column: **Ultra Carbamate**

Catalog #:	9177355
Dimensions:	50 x 4.6mm
Particle size:	3µm
Pore size:	100Å

Conditions:

Mobile Phase:	A: 90:10 water:methanol
	B: 90:10 methanol:acetonitrile

Time (min):	%B
0	10
10	90

Flow:	1.5mL/min
Temp.:	27°C
Det.:	UV @ 220nm

Figure 5

Fast LC Analysis of Carbamates with MS Detection

Table II. Experimental conditions for the LC/MS analysis of carbamate compounds.

HPLC Conditions	
Column:	Ultra Carbamate, 100 mm x 4.6 mm, 3 µm
Mobile Phase A:	90% water:10% methanol with 10mM ammonium formate
Mobile Phase B:	10% acetonitrile:90% methanol with 10mM ammonium formate
Gradient:	90%A:10%B to 10%A:90%B from 0-15 minutes
Inj. Volume:	10 µL
Flow Rate:	0.75 mL/min to UV detector, 0.25 mL/min to MSD

MSD Conditions		**Compound**	**Ion**	**Cone V**
Detector:	Micromass ZMD	1	223.3	25V
Mode:	ESI+	2	207.3	18V
Capillary V:	3.50	3	237.2*	10V
Extractor:	4.0	4	163.2	15V
Ion Energy:	0.4	5	238.3	15V
Multiplier:	650	6	191.2	8V
Source Temp:	100°C	7	210.2	18V
Desolv. Temp:	250°C	8	222.3	22V
Gas Flow:	490 L/hr.	9	202.2	18V
		10	226.3	19V

*Ammonium adduct (all other are [M+H]+ ions)

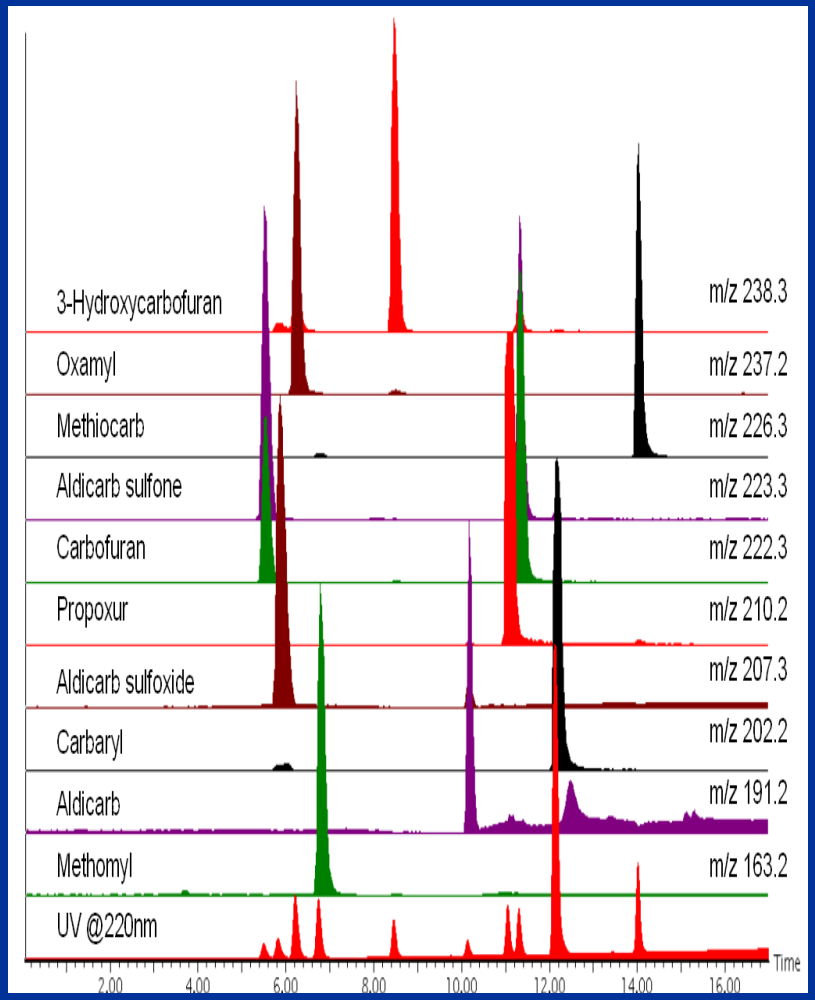


Figure 6

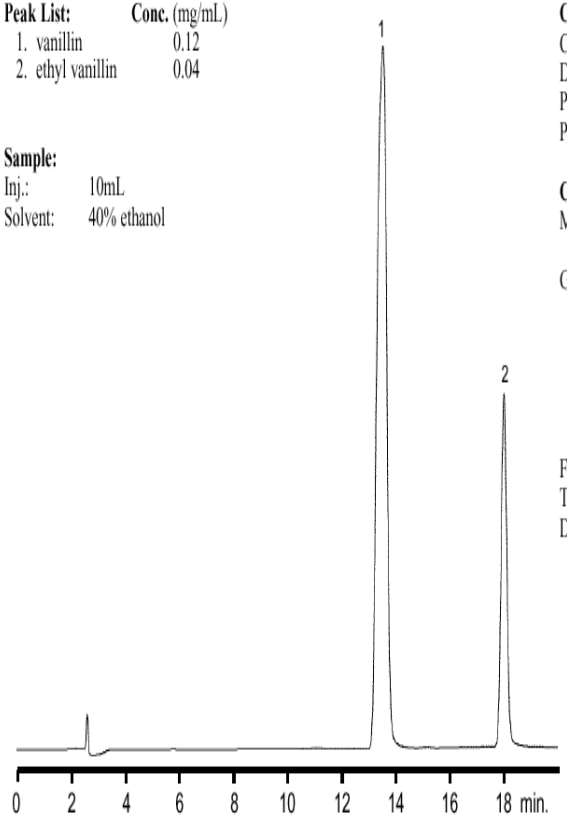
Conventional Vanilla Flavoring Analysis (About 20 minutes with gradient)

Vanillin and Ethyl Vanillin on Ultra C8

Applications Note: LC_0148

Peak List: **Conc.** (mg/mL)
1. vanillin 0.12
2. ethyl vanillin 0.04

Sample:
Inj.: 10mL
Solvent: 40% ethanol



Column: **Ultra C8**
Catalog#: 9103565
Dimensions: 150x4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phases: A: 1.2% acetic acid
B: methanol

Gradient:	Minutes	%B
	0.0	20.0
	5.0	20.0
	15.0	40.0
	20.0	40.0
	25.0	20.0

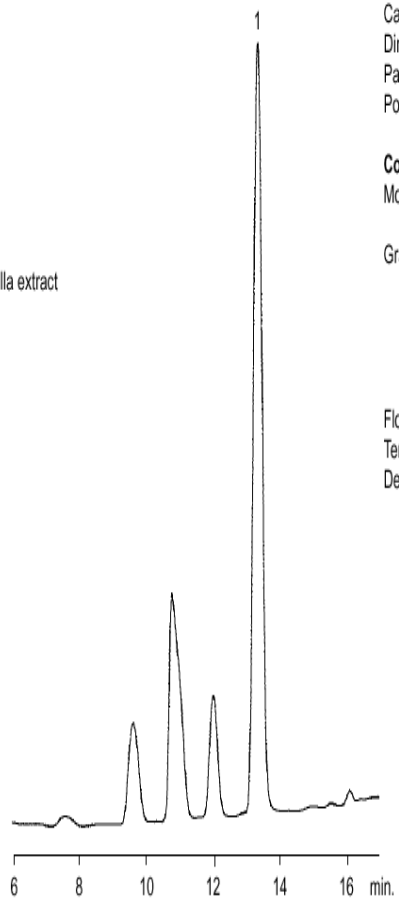
Flow: 1.0mL/min.
Temp.: 28°C
Det.: UV @ 254nm

Vanillin on Ultra C8

Application Note: LC_0149

Peak List:
1. vanillin

Sample:
Inj.: 10mL
Conc.: 5% solution of vanilla extract
Solvent: 40% ethanol



Column: **Ultra C8**
Catalog#: 9103565
Dimensions: 150x4.6mm
Particle size: 5µm
Pore size: 100Å

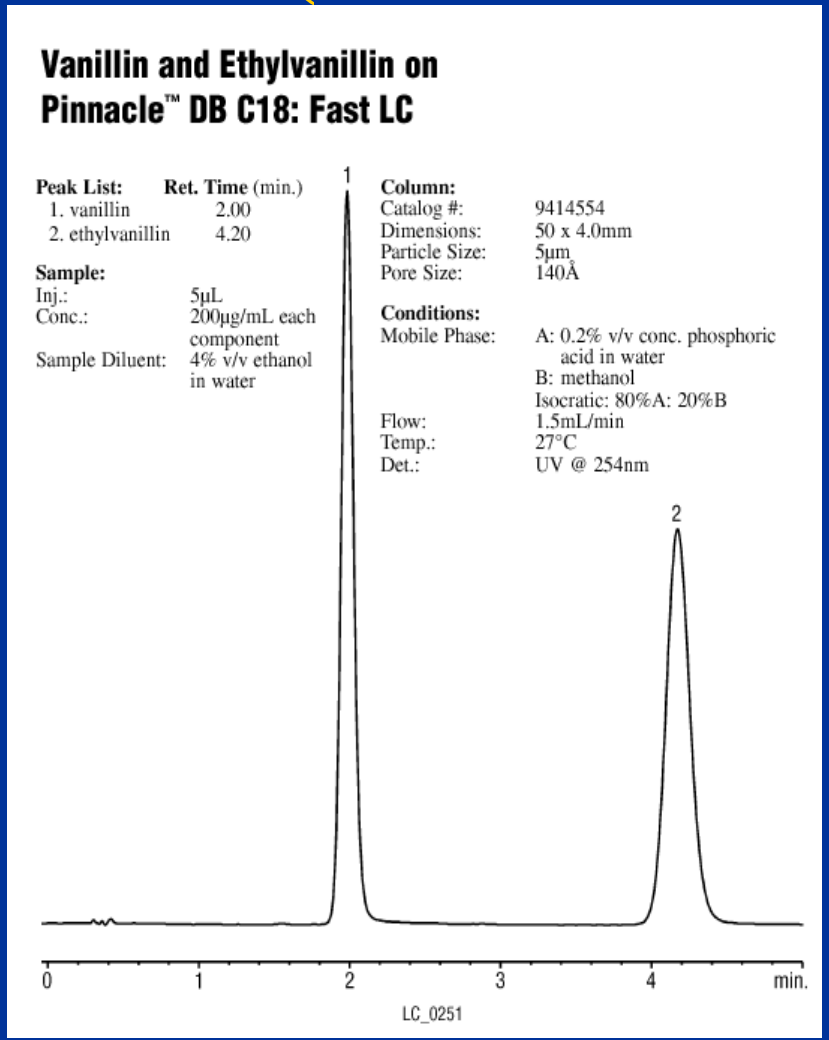
Conditions:
Mobile phases: A: 1.2% acetic acid
B: methanol

Gradient:	Minutes	%B
	0.0	20.0
	5.0	20.0
	15.0	40.0
	20.0	40.0
	25.0	20.0

Flow: 1.0mL/min.
Temp.: 28°C
Det.: UV @ 254nm

Figure 8

Highly Selective Fast LC Vanillin Analysis (Less than 5 minutes and isocratic)



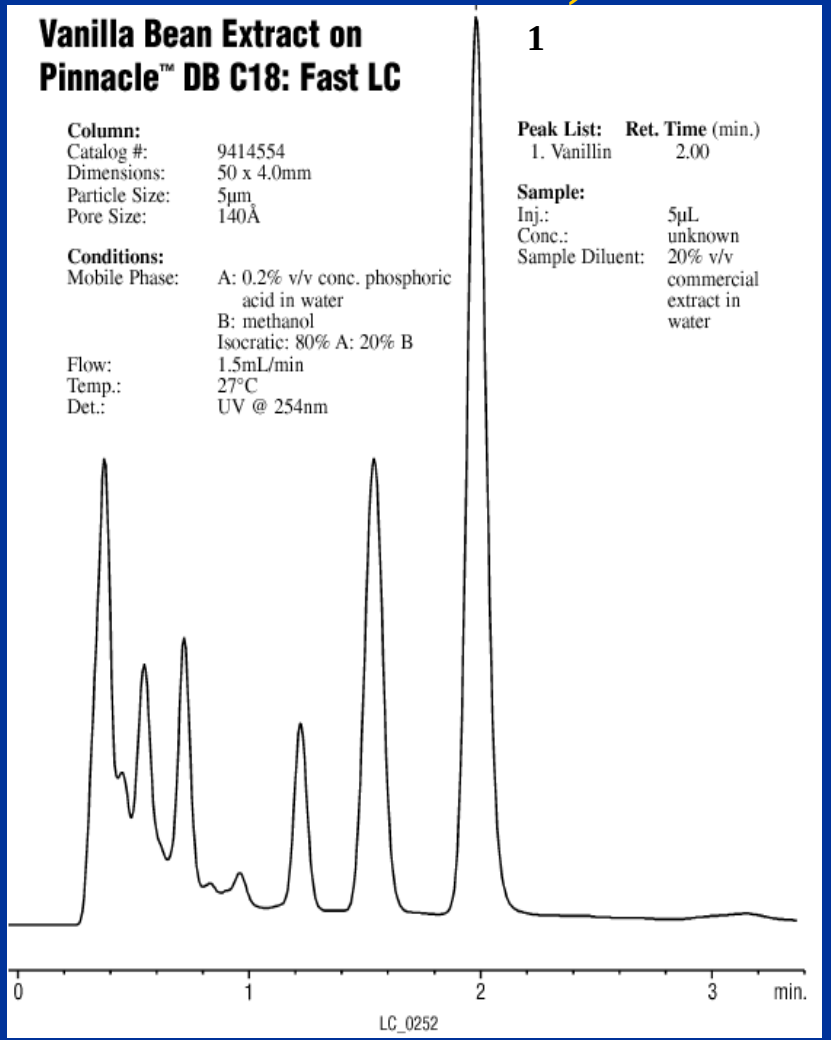
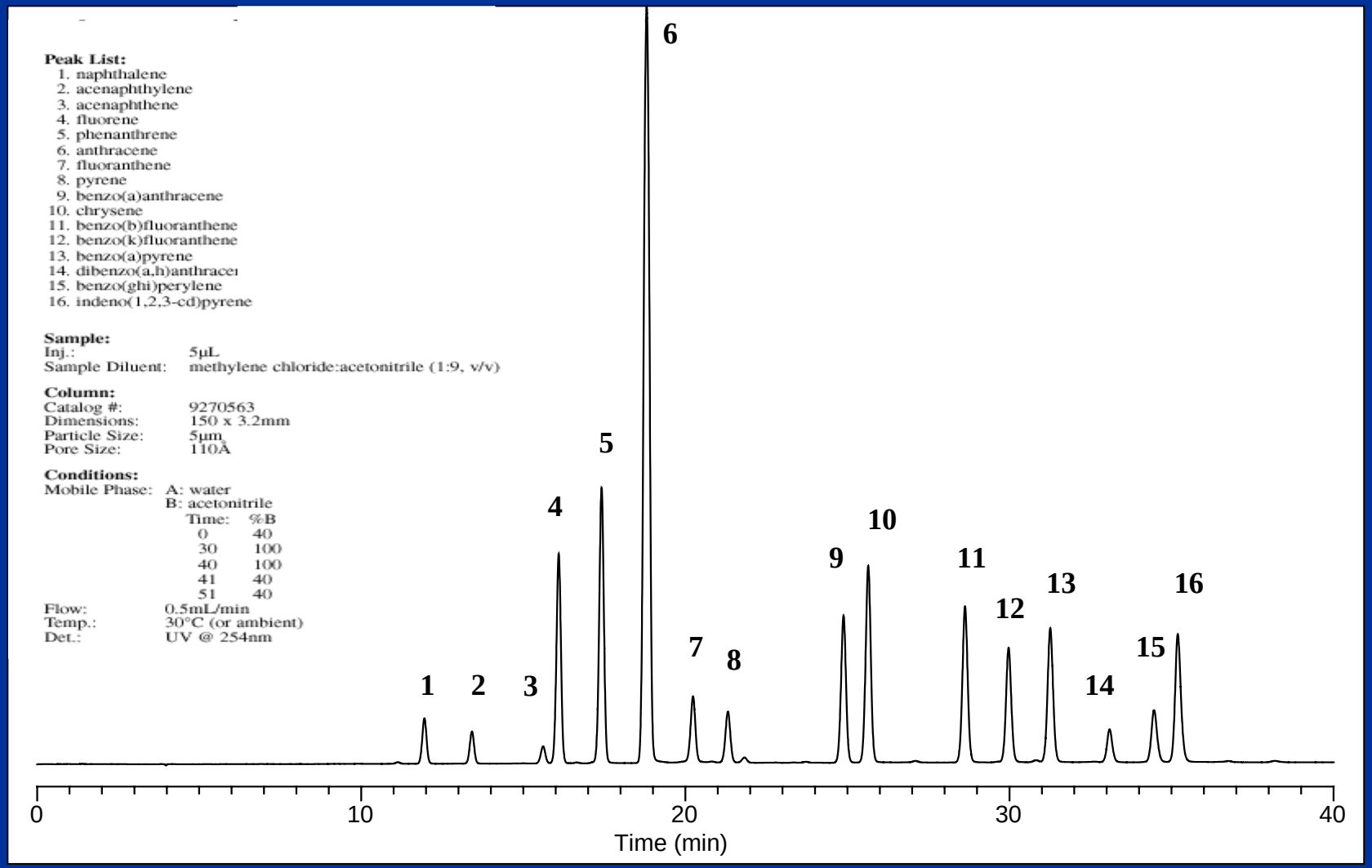


Figure 9

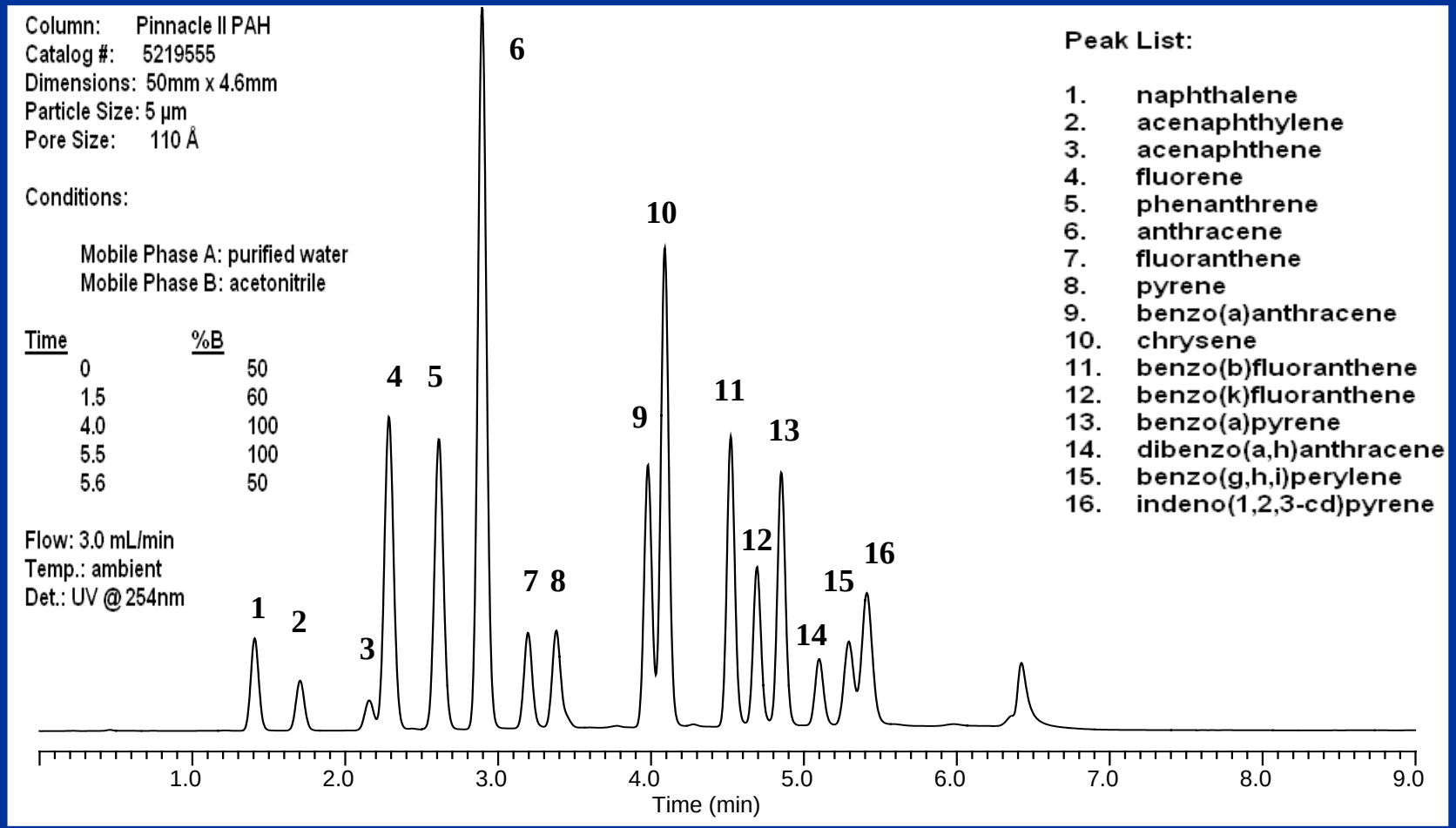
Vanillin Fast LC Analysis Advantages

- Takes advantage of improved methylene selectivity by the use of C18 phase versus a C8
- Analysis can be conducted in 3 to 5 minutes versus older 15 minute methods.
- High but not not excessive selectivity of C18 phases toward simple methylene substitutions allow the use of an isocratic mobile phase.
- Reduction of column length also reduces the time needed for more hydrophobic analytes to elute from system.

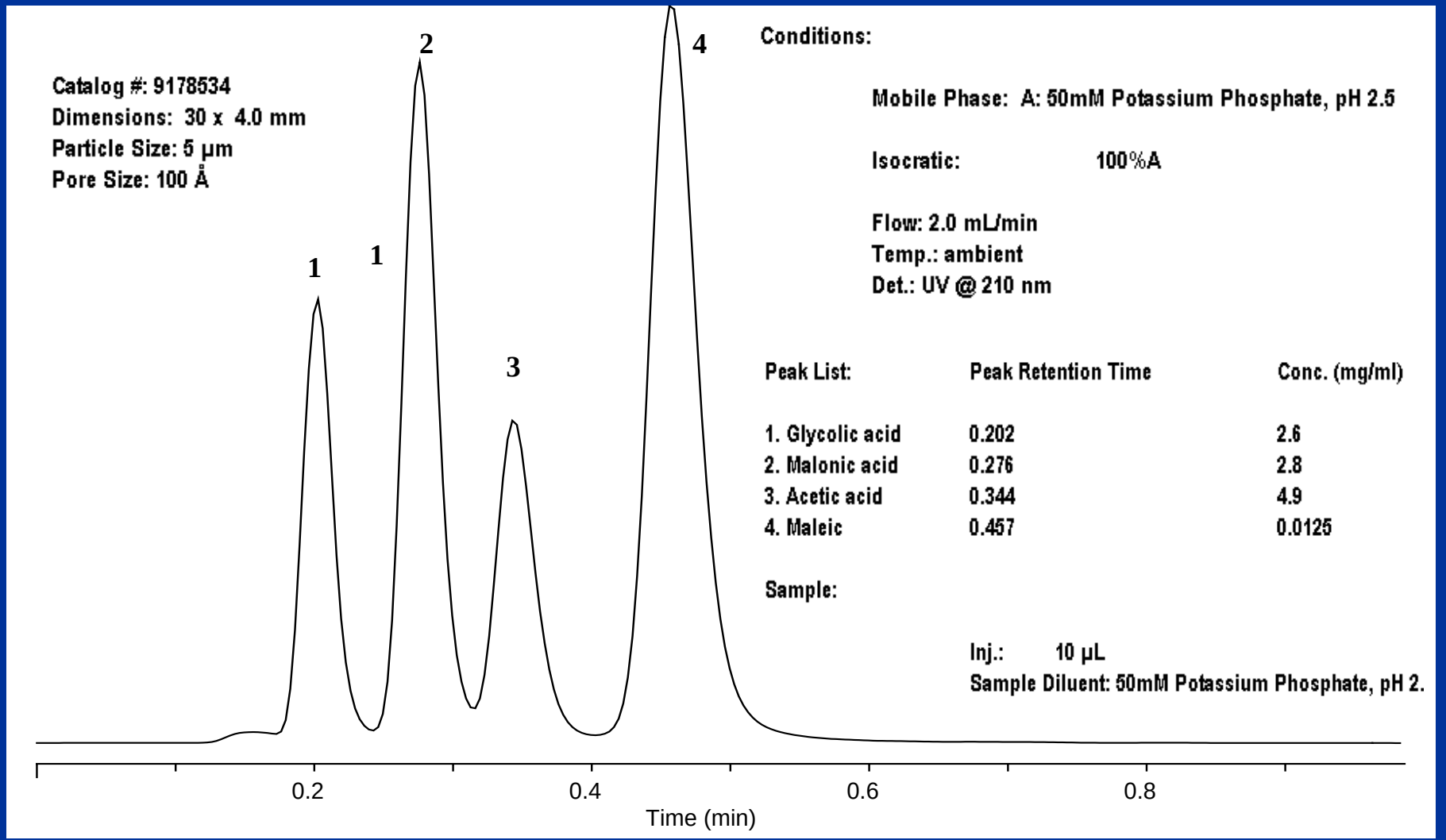
EPA Method 610 – PAHs on Pinnacle II PAH



PAHs on Pinnacle II PAH- Fast LC



Fast LC Analysis of Carboxylic Acids on Ultra Aqueous C18



Fast LC Analysis of Aromatic Amino Acids on Ultra Aqueous C18

Column:

Catalog #: 9178535
Dimensions: 30 mm x 4.0 mm
Particle Size: 5 µm
Pore Size: 100 Å

Conditions:

Mobile Phase: A: 50mM Potassium Phosphate, pH 2.5
B: Acetonitrile

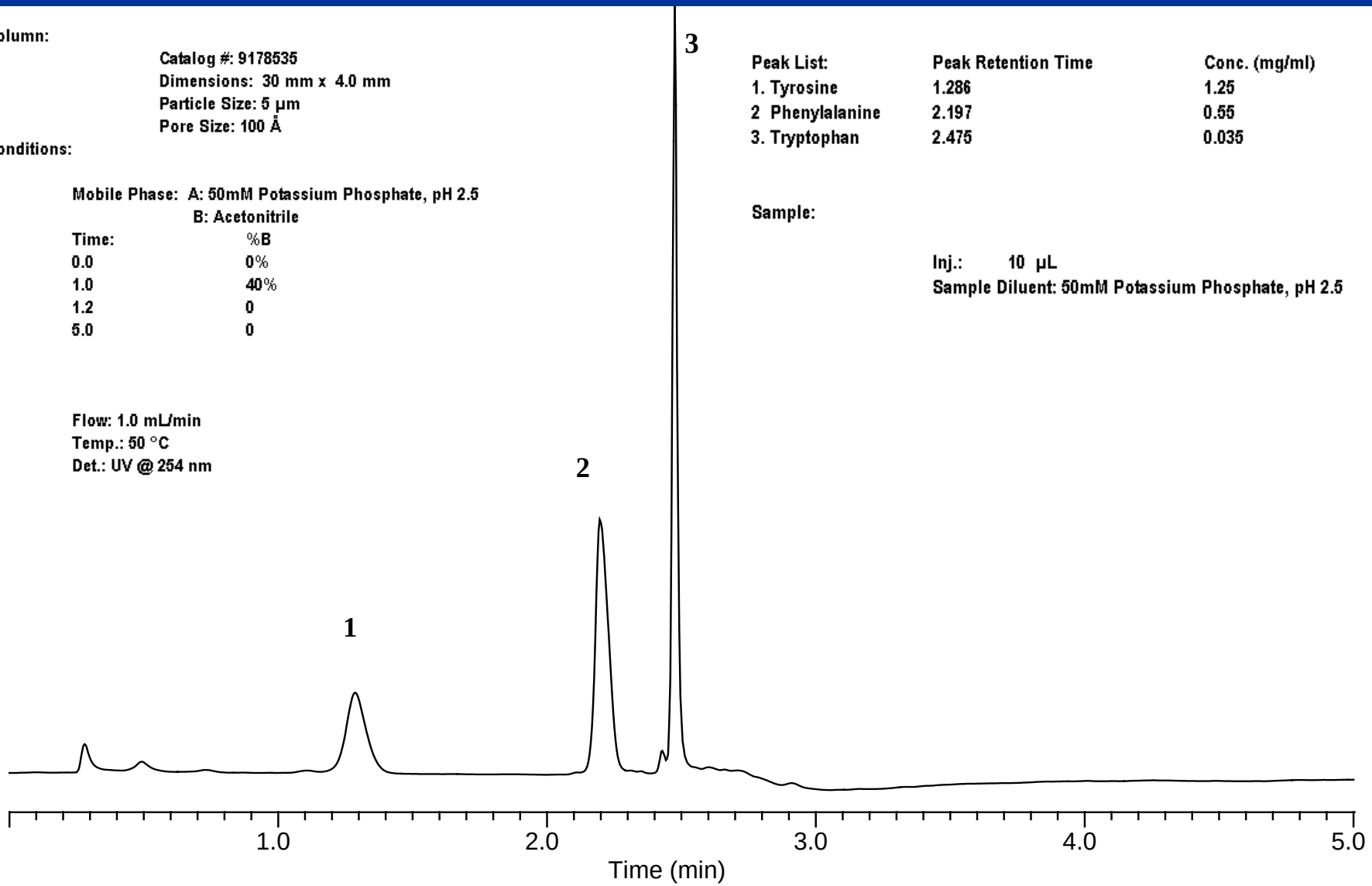
Time:	%B
0.0	0%
1.0	40%
1.2	0
5.0	0

Flow: 1.0 mL/min
Temp.: 50 °C
Det.: UV @ 254 nm

Peak List:	Peak Retention Time	Conc. (mg/ml)
1. Tyrosine	1.286	1.25
2 Phenylalanine	2.197	0.55
3. Tryptophan	2.475	0.035

Sample:

Inj.: 10 µL
Sample Diluent: 50mM Potassium Phosphate, pH 2.5



Conclusions

- Highly selective and sometimes unique stationary phases make Fast LC a reality using conventional hardware and techniques.
- Fast LC is viable, precise quantitative alternative for analysis previously performed by Thin Layer Chromatography.
- Fast LC can improve method sensitivity, reduce solvent waste, and enhance laboratory throughput.

Conclusions

- The proper stationary phase selection can change a gradient system to a faster isocratic system.
- Sometimes only a simple phase change is needed to convert a method to a Fast LC separation.
- Selectivity is still the greatest factor in separation optimization.

Acknowledgements

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