Improving Method Performance through Fast LC

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Abstract

Fast LC methods can be created by taking advantage of a wide range of separation variables to optimize resolution. Increases in resolution are created through increases in theoretical plates, selectivity, and retention. Alpha, the selectivity variable, is the greatest factor in separation optimization. Sometimes a simple phase change is all that is needed to convert a method to a Fast LC separation. Optimization of alpha through stationary phase selection can change a gradient system to a faster isocratic system, allow creation of a single method in lieu of multiple analysis methods, and reduce analysis times to a fraction of the original. This study examines the variables of the resolution equation and gives significant attention to the optimization of alpha in HPLC separations.

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.
- Significant increases in sample throughput possible.
- 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

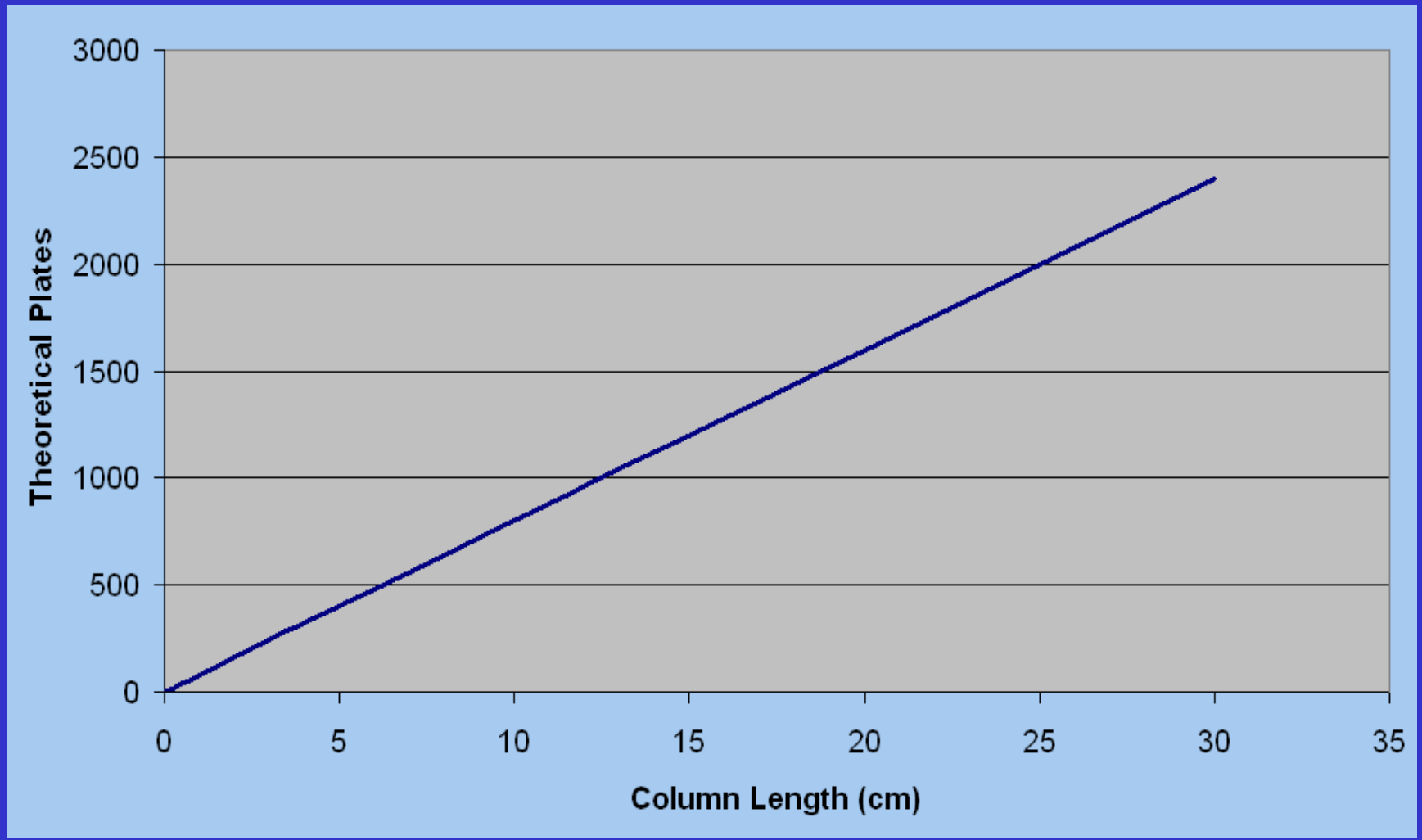
Efficiency

- particle size
- column length

Retention

- chain length
- mobile phase strength

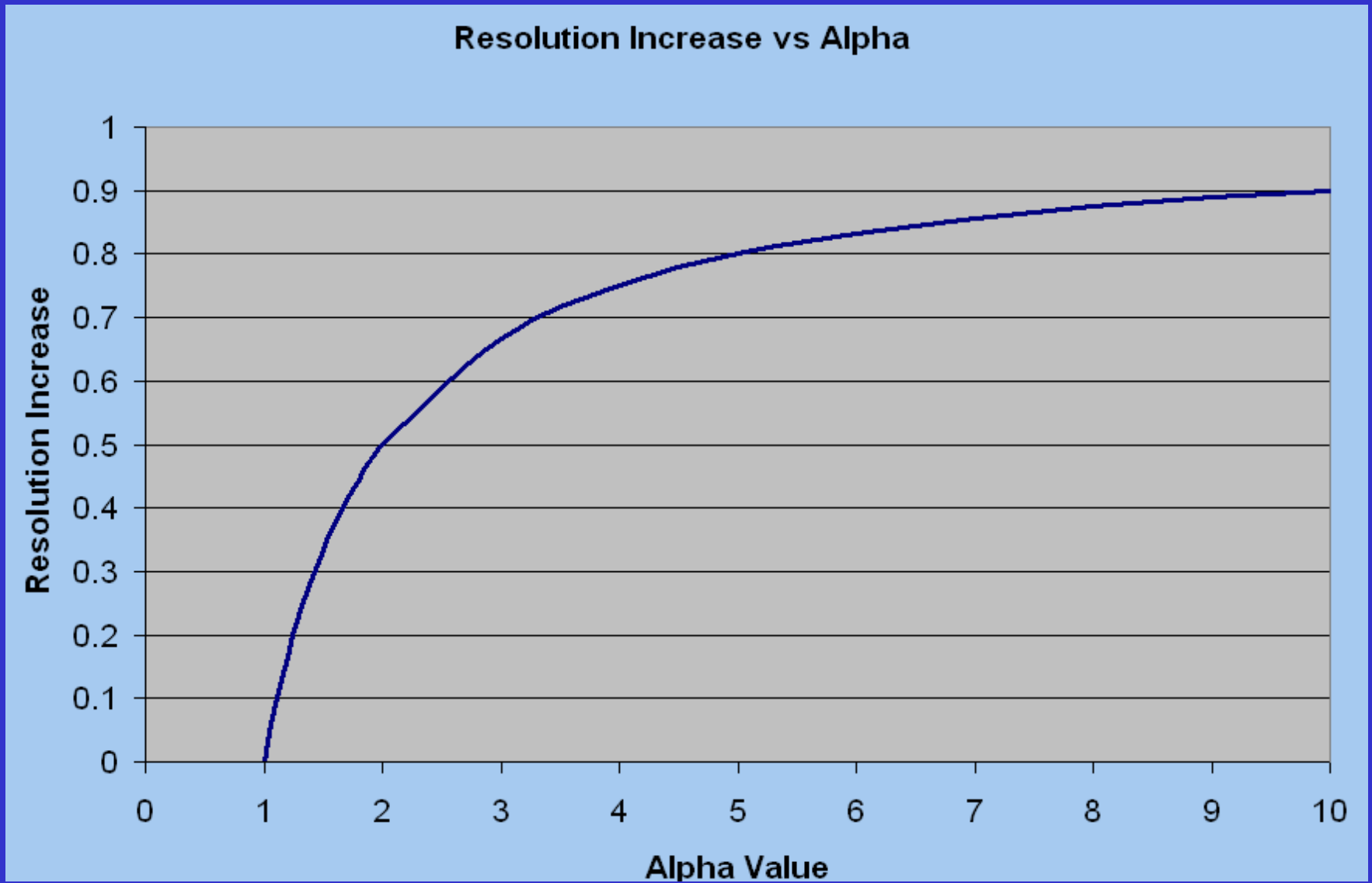
Column Length vs Theoretical Plates



Theoretical Plates and Fast LC

- The longer the column, the more theoretical plates the column is capable of providing.
- Shortening columns will reduce runtime at the expense of resolution.
- The loss in resolution can sometimes be compensated by the use of smaller particles.
- To achieve the separation with a shorter column, the important factor is to maintain the number of theoretical plates necessary for the resolution.

Resolution Increase vs Alpha



Alpha (Selectivity) in Fast LC

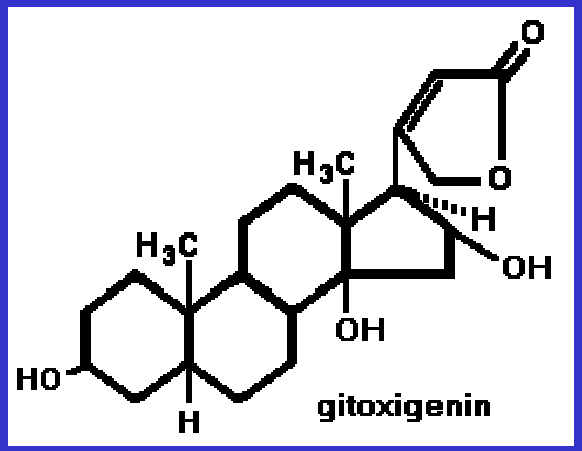
- Alpha has the greatest influence over the separation efficiency.
- Small changes in Alpha can lead to dramatic increase in resolution.
- Small changes in Alpha from 1-2 have the greatest influence on resolution.
- Additional increases in Alpha can be realized by taking advantage of other chemical and physical properties of the analyte, mobile phase composition, and the stationary phase.

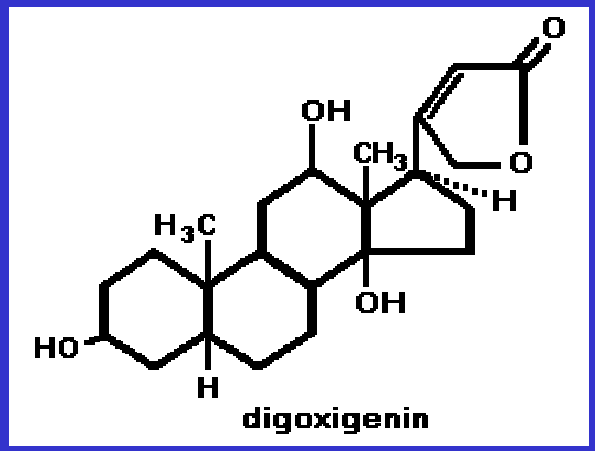
Alpha (Selectivity) in Fast LC

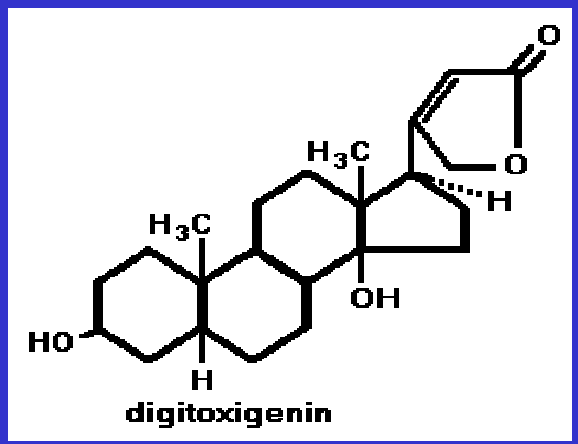
- Taking advantage of mixed mode interactions, size exclusion, shape selectivity, and other properties will lead to further increases in Alpha.
- High increases in Selectivity mean shorter HPLC columns can be used to achieve the desired resolution, while reducing analysis time.

Fast LC improvement of USP TLC and HPLC Method

Digitalis Extracts and Derivatives



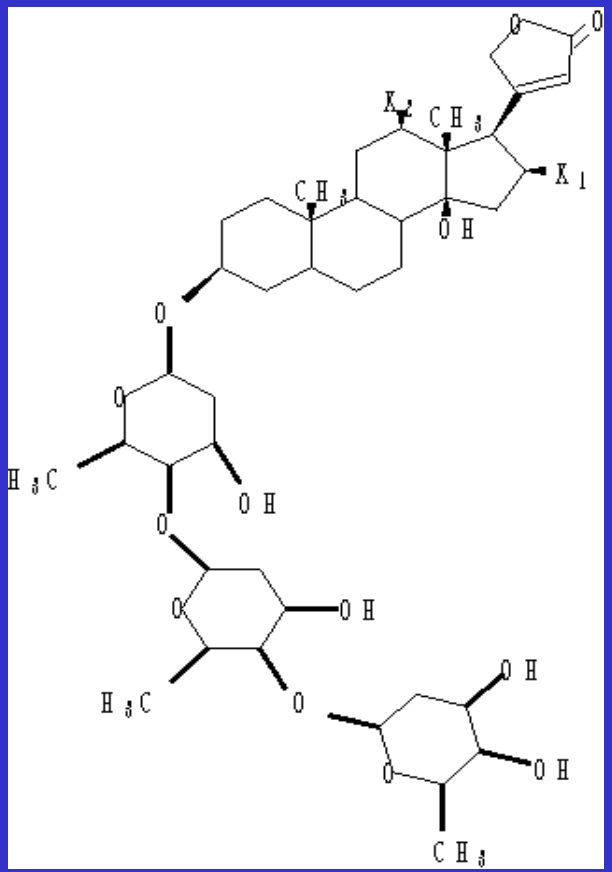




Digitoxin: X_1=X_2=H

Digoxin: X_1=H; X_2=OH

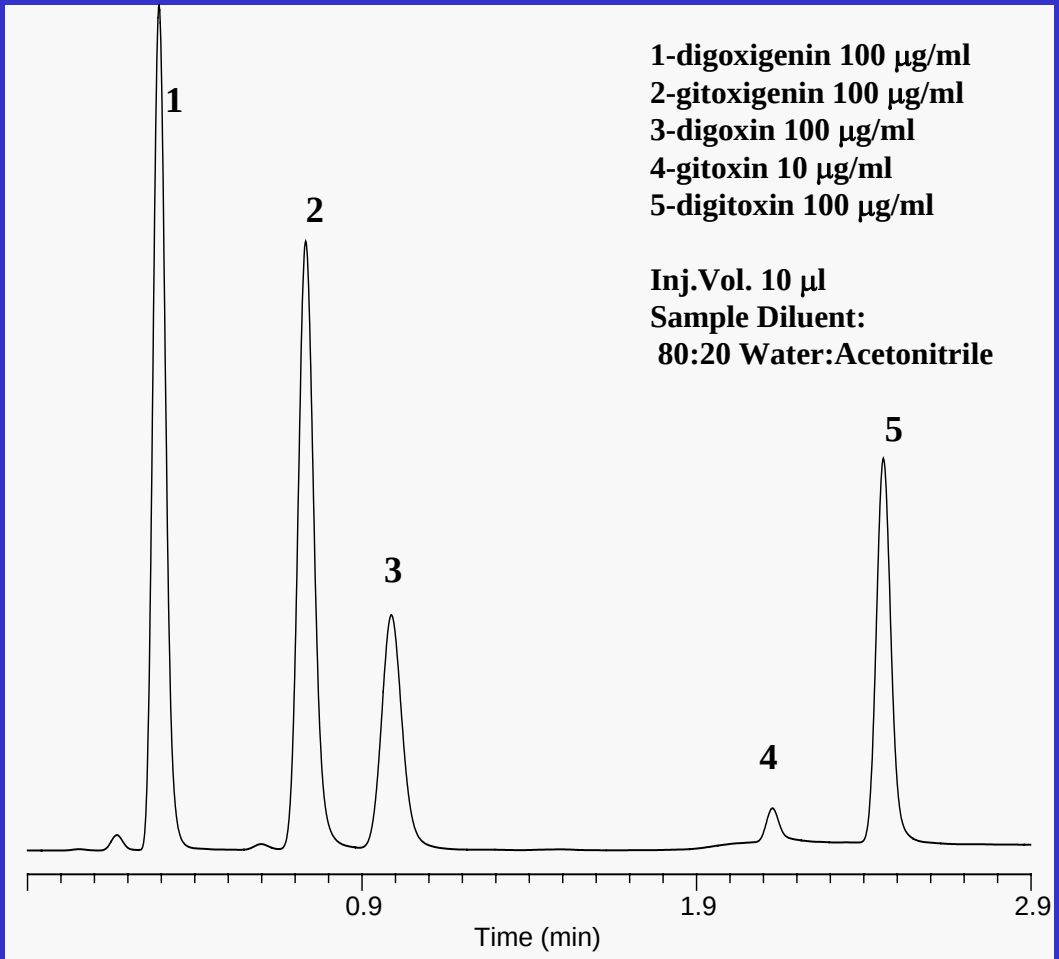
Gitoxin: X_1=OH; X_2=H



Digitoxin Substitutions

Figure 1

Fast LC Separation of Digitalis Derivatives on Ultra PFP Propyl (3 minutes)



1-digoxigenin 100 µg/ml
2-gitoxigenin 100 µg/ml
3-digoxin 100 µg/ml
4-gitoxin 10 µg/ml
5-digitoxin 100 µg/ml

Inj.Vol. 10 µl
Sample Diluent:
80:20 Water:Acetonitrile

Part number: 5179335

Particle Size: 3µm

Pore Size: 100 Å

Dimensions: 30mm x 4.0 mm

Flow Rate: 2.0 ml/min

Temp: 27° C

Detection: UV @ 230 nm

Mobile Phase:

A: 100% Water

B:Acetonitrile

Time (min):	%B
0.0	20
1.5	20
1.51	35
3.0	35
3.01	20

LC_250

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

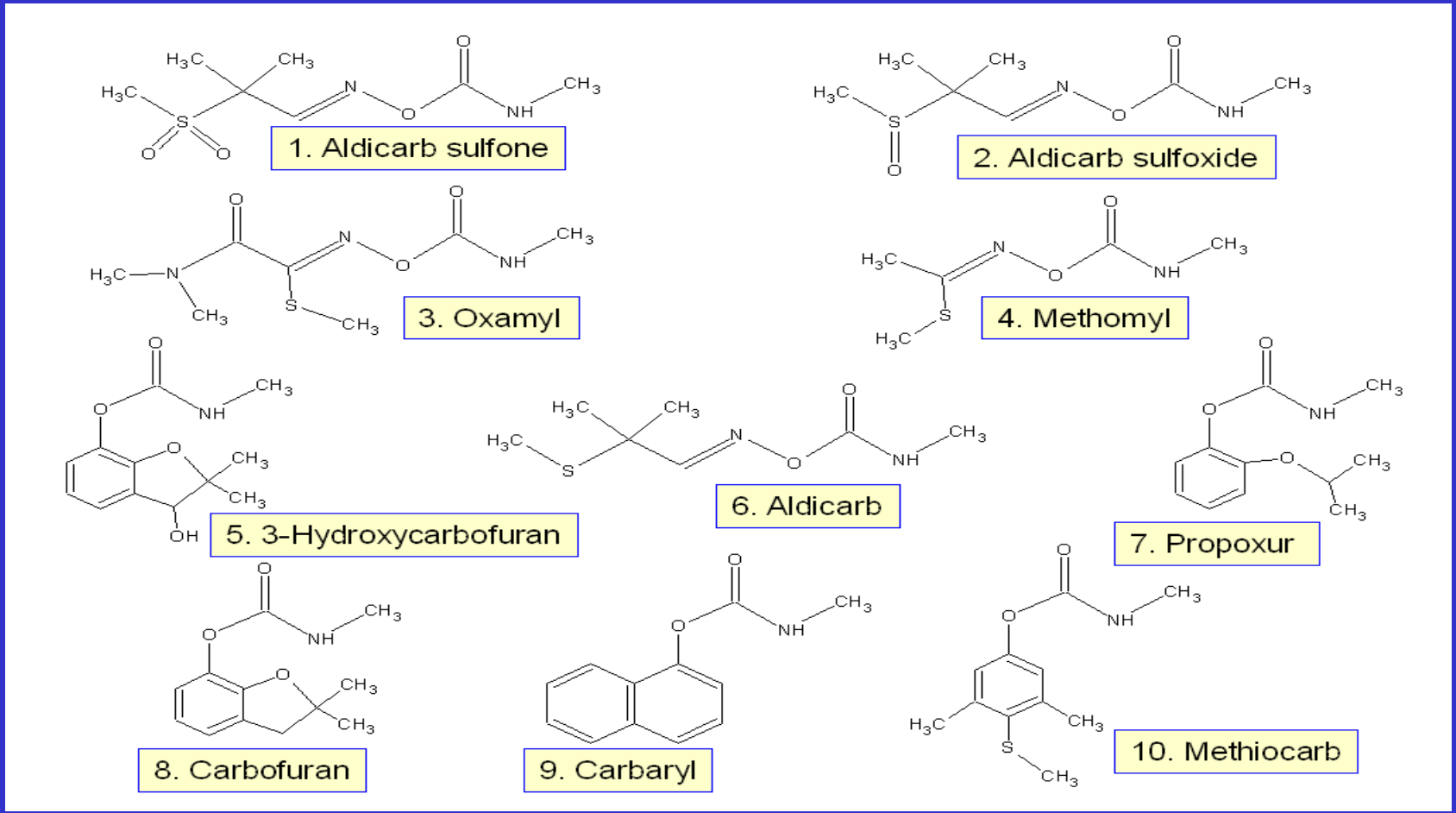


Figure 3-Structures of Commonly Analyzed Carbamates

Carbamate Analysis using Standard HPLC Methodology (About 40 minutes)

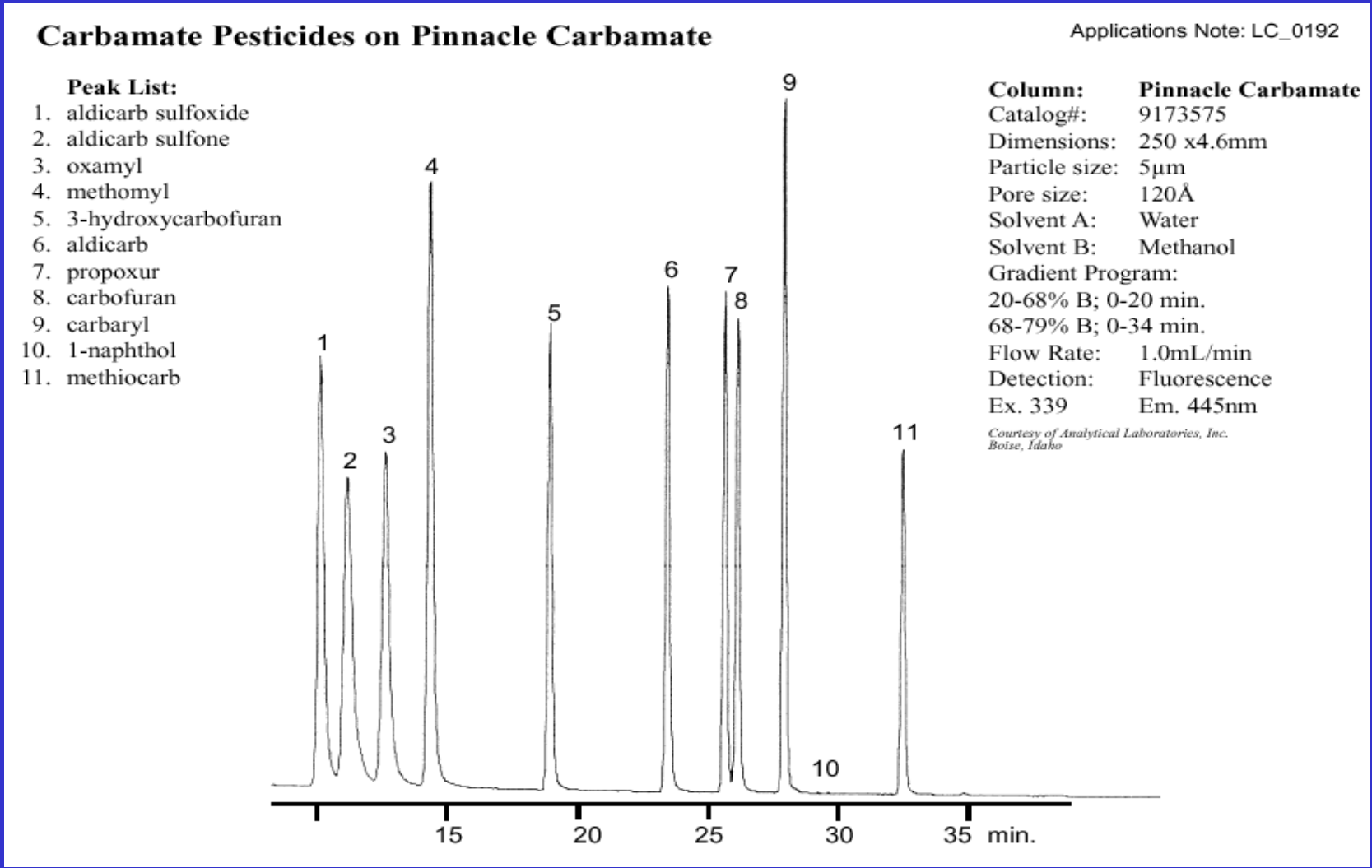


Figure 4

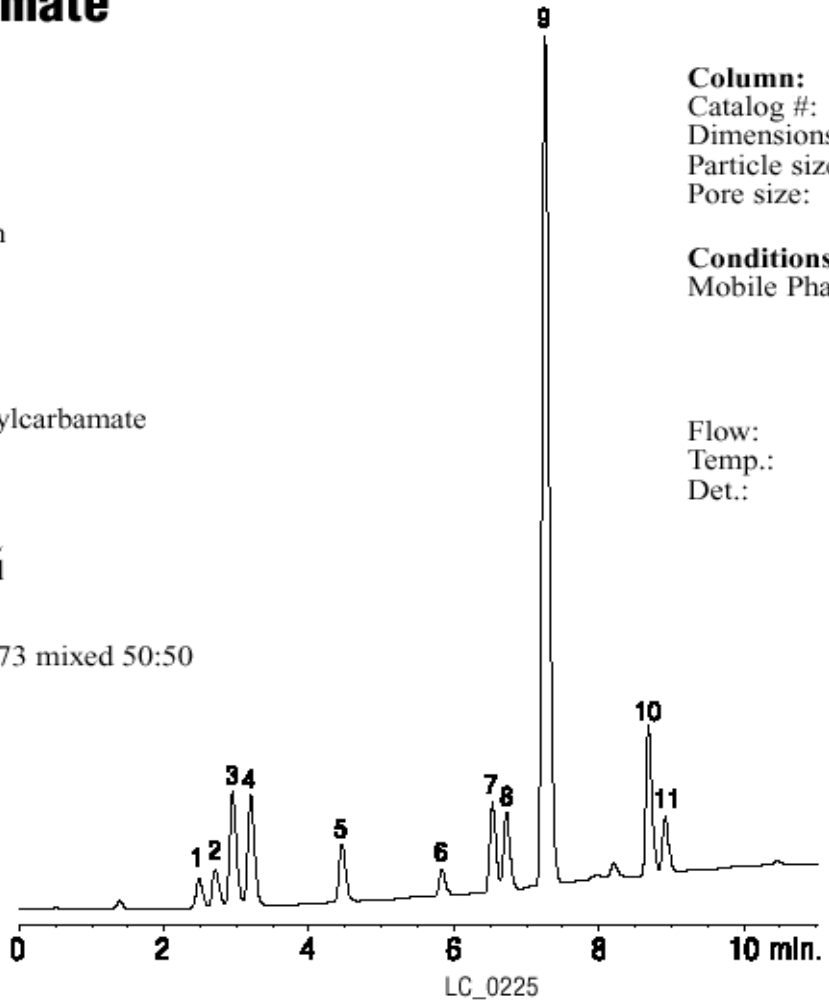
Fast LC Separation of Carbamates (About 13 minutes)

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)

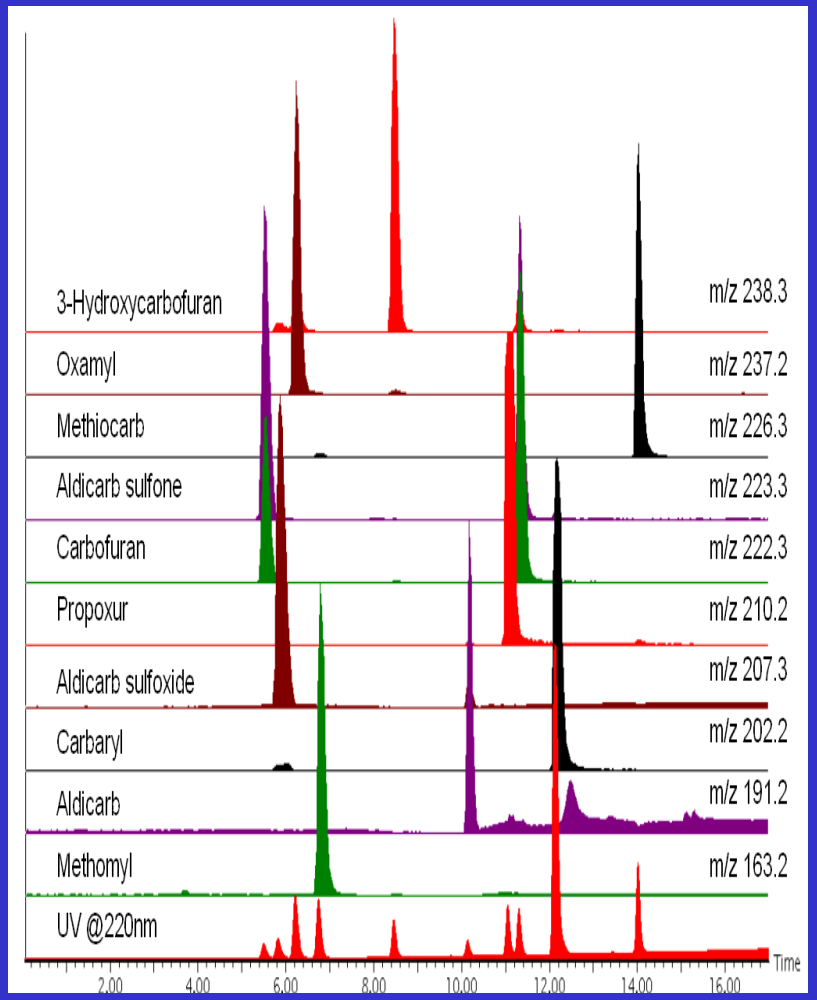


Figure 6

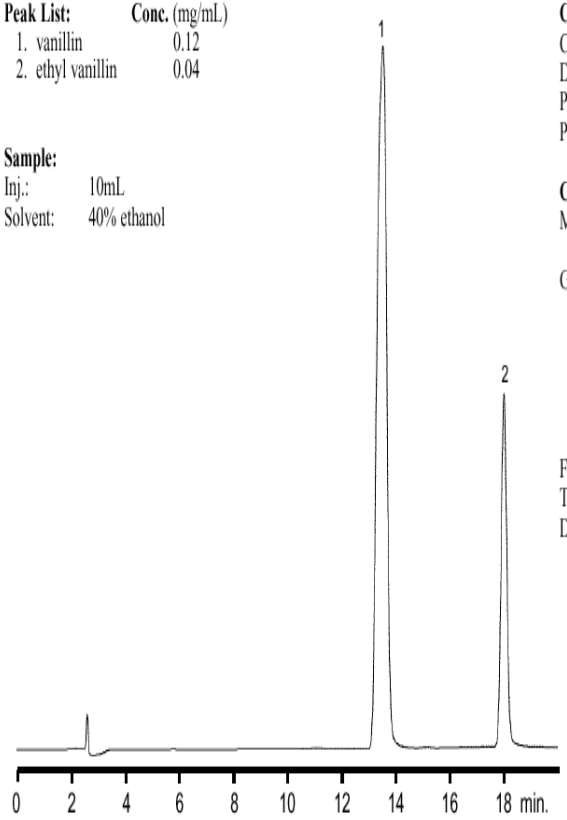
Conventional Vanilla Flavoring Analysis (About 20 minutes with gradient)

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

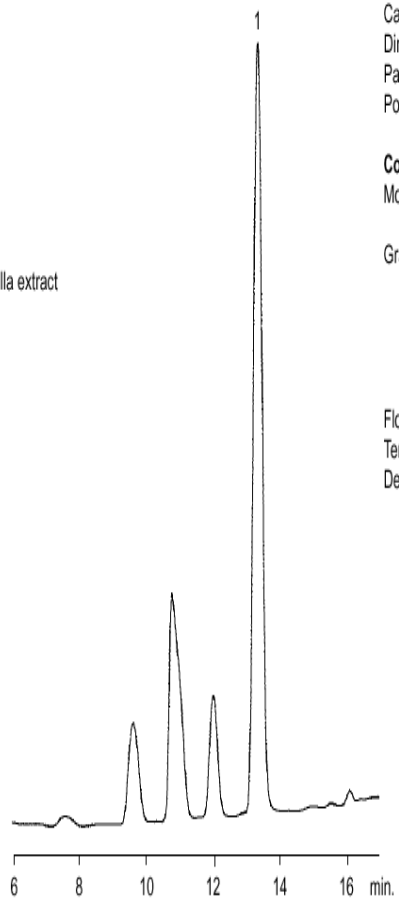
Flow: 1.0mL/min.
Temp.: 28°C
Det.: UV @ 254nm

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

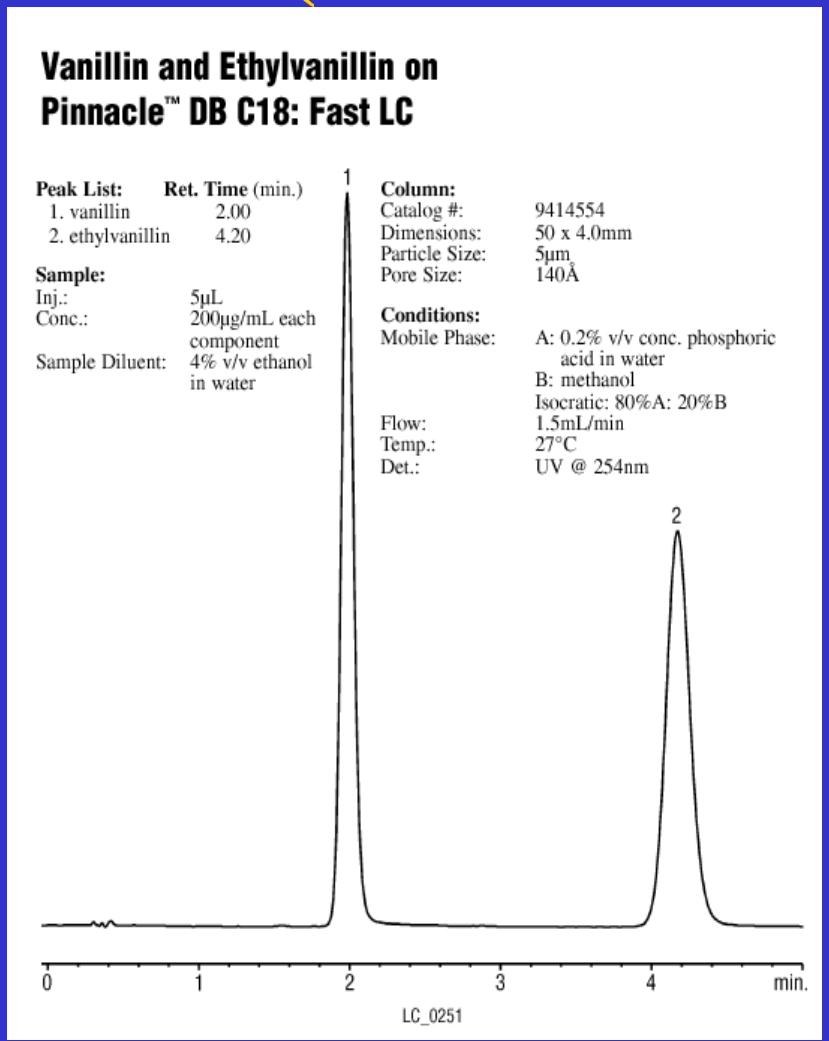
Conditions:
Mobile phases: A: 1.2% acetic acid
B: methanol

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)



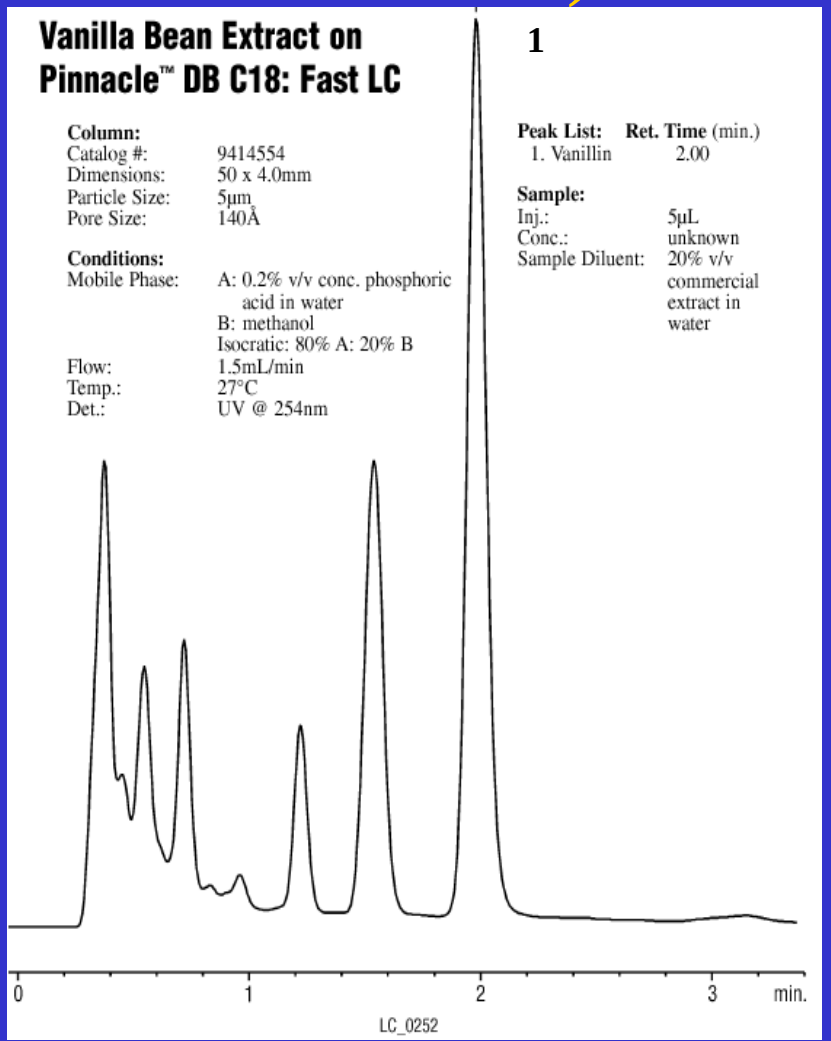
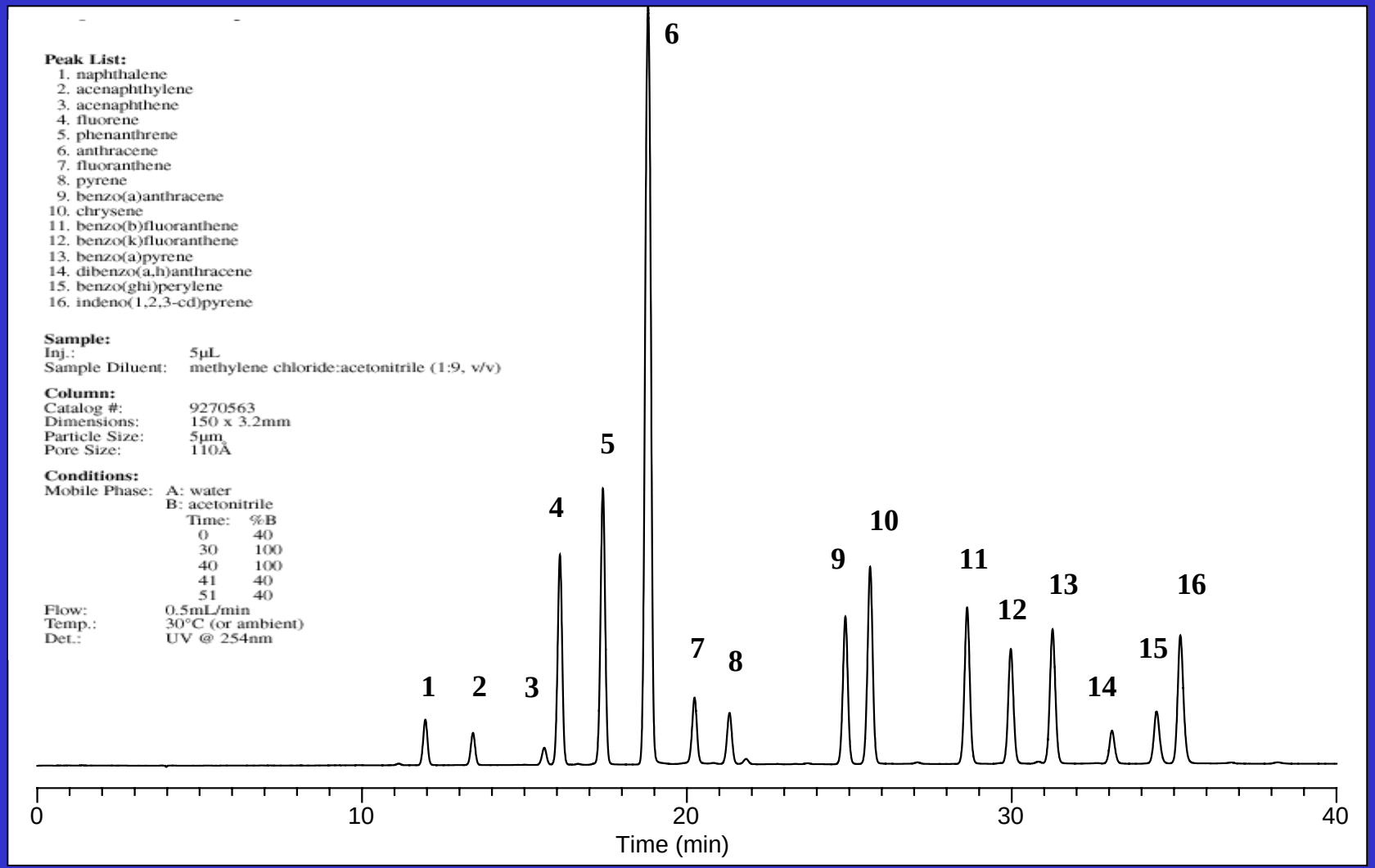


Figure 9

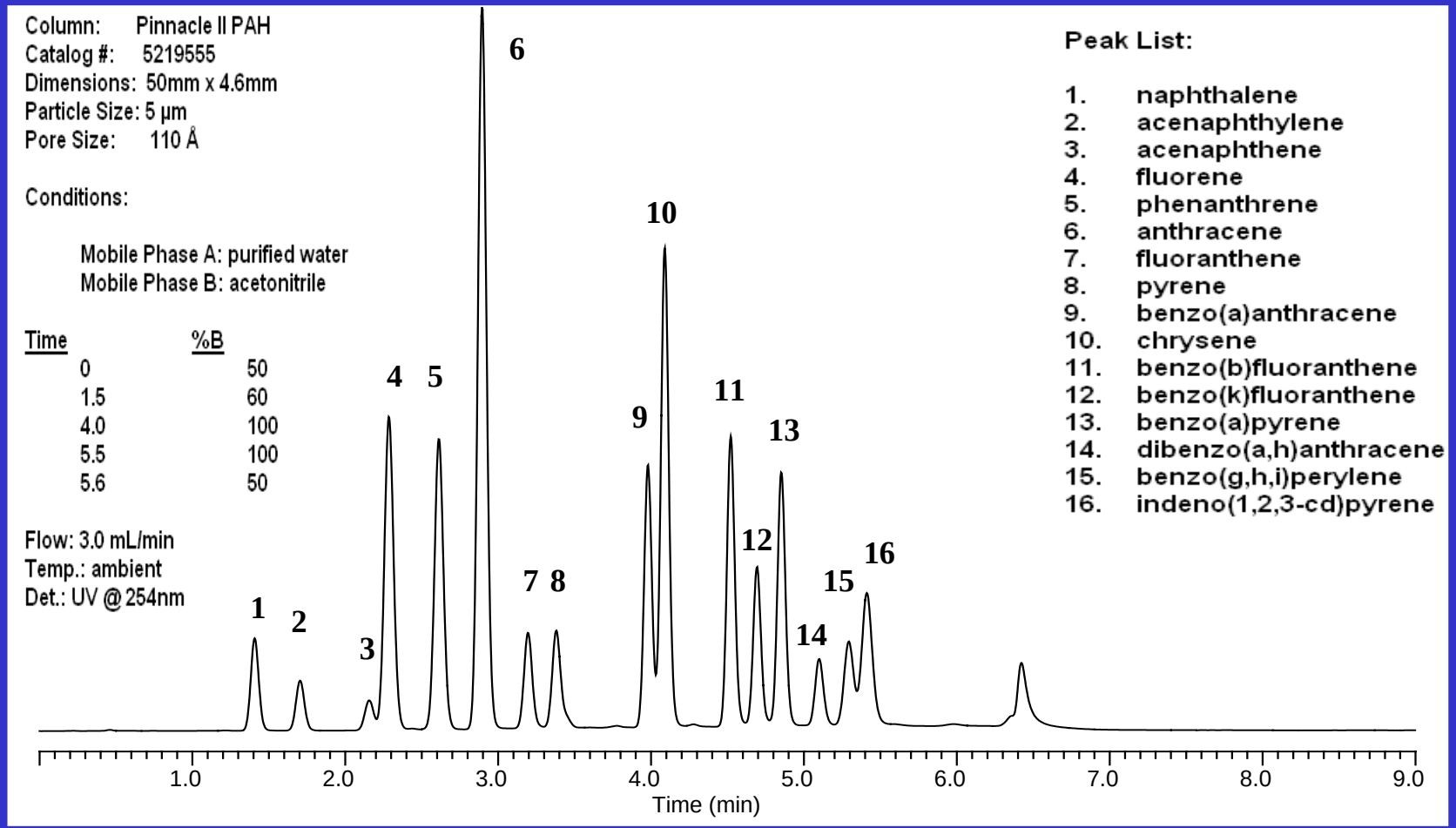
Vanillin Fast LC Analysis Advantages

- 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 20-25 minute methods requiring re-equilibration.
- 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.

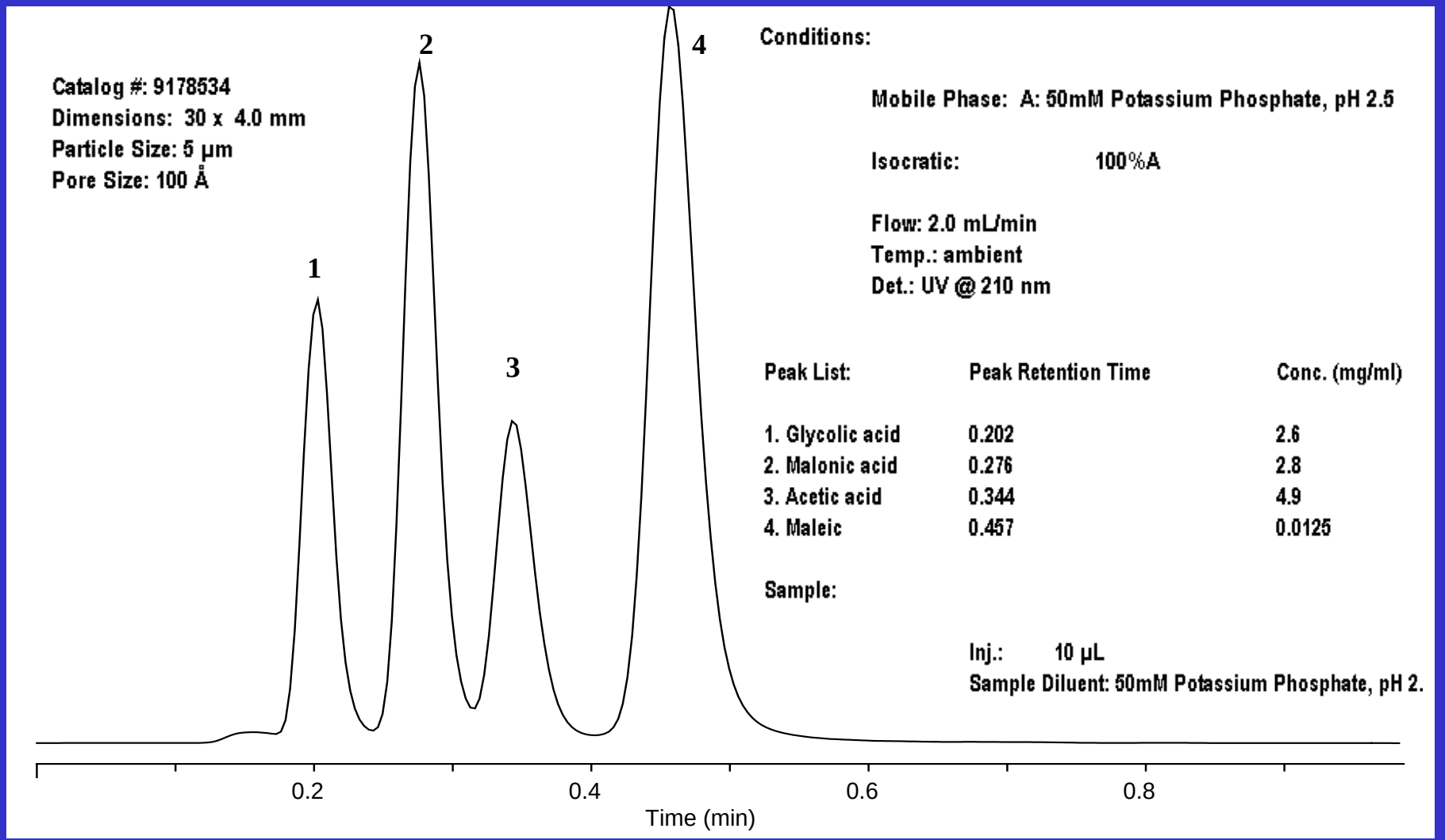
EPA Method 610 – PAHs on Pinnacle II PAH



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

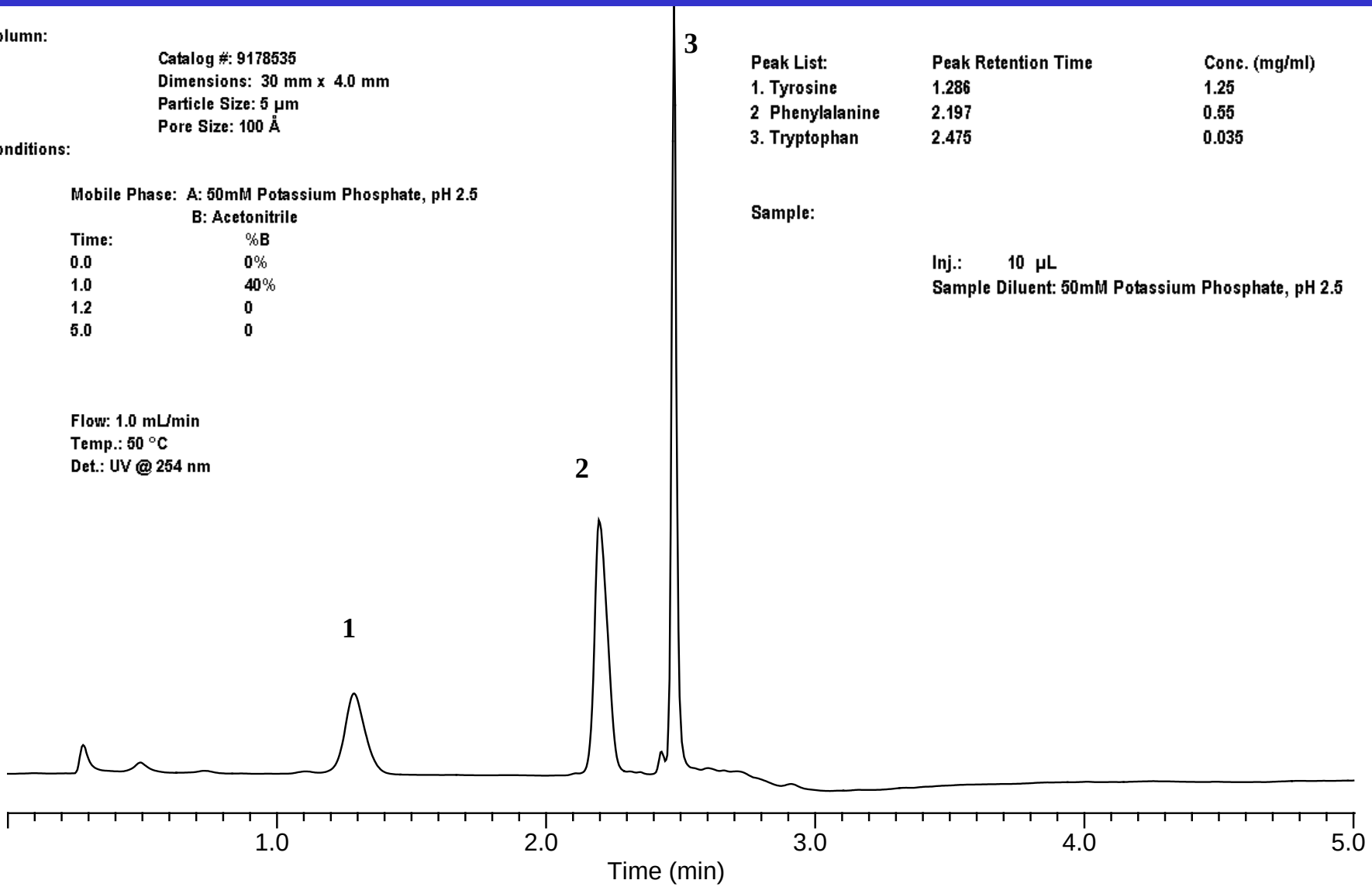
Time:	%B
0.0	0%
1.0	40%
1.2	0
5.0	0

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 analyses 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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