Improving Method Performance through Fast LC

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Abstract

The analysis time needed for many separations can be drastically reduced by the use of fast HPLC techniques. Several separations were converted using fast HPLC techniques. Analysis times for some separations that previously took over 35 minutes were reduced to less than 12 minutes with improvement in selectivity between the components. Qualitative TLC techniques can be converted to truly quantitative HPLC. Since columns employed in the fast LC analysis were typically less than 100mm in length, the reduction in analysis time also resulted in increased sensitivity due to reduction of band spreading.

In addition to improving performance through reduction of particle size and column length, performance gains may also be realized by using columns equipped with an appropriately optimized and highly selective stationary phase. These phases allow improved separation of the components without the drastic increase in k that often results when reduction in mobile phase strength is used to improve selectivity.

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

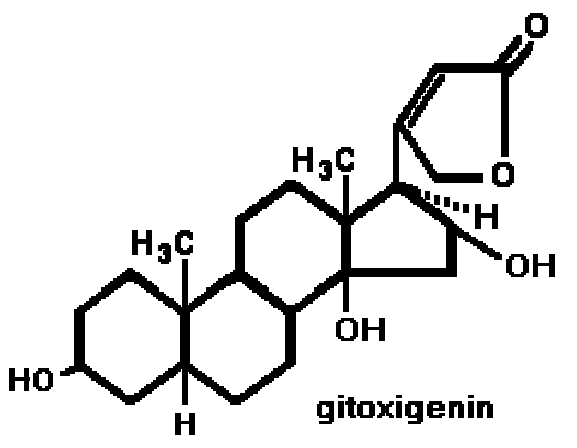
- Fast re-equilibration (when using gradients)
- Sensitivity improvements.
- Older qualitative techniques can be adapted to a highly automated quantitative technique.
- Allows potentially high increases in sample throughput.
- Great technique when performed by LC-MS
- Shorter analysis times reduce solvent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

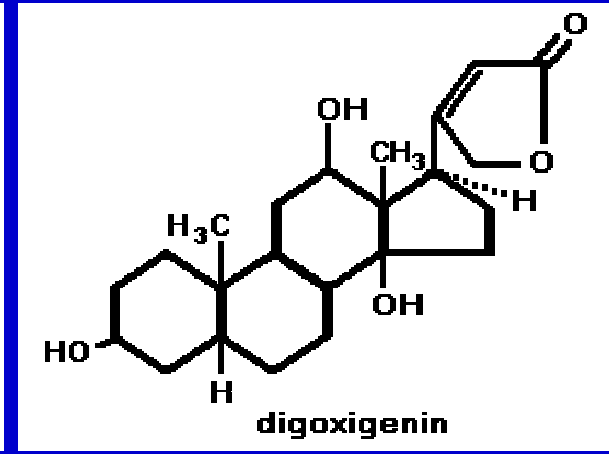
Fast LC Technique – Disadvantages

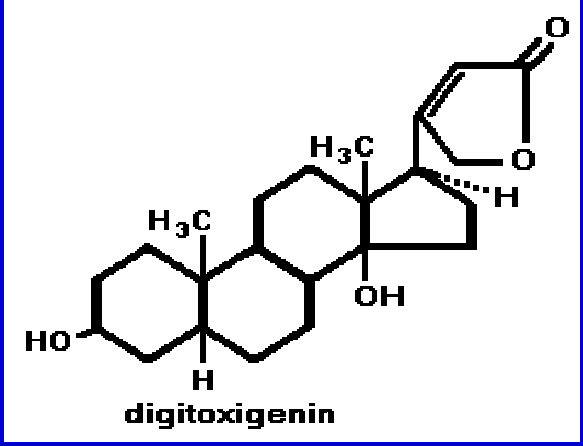
- Critical separations are more sensitive to extra-column volume (as post column reactors).
- Extremely selective stationary phase must be used to maximize selectivity – especially for structural isomers.
- May not be well suited to normal phase or ion pairing separations (with gradients).

Fast LC improvement of USP TLC and HPLC Method

Digitalis Extracts and Derivatives



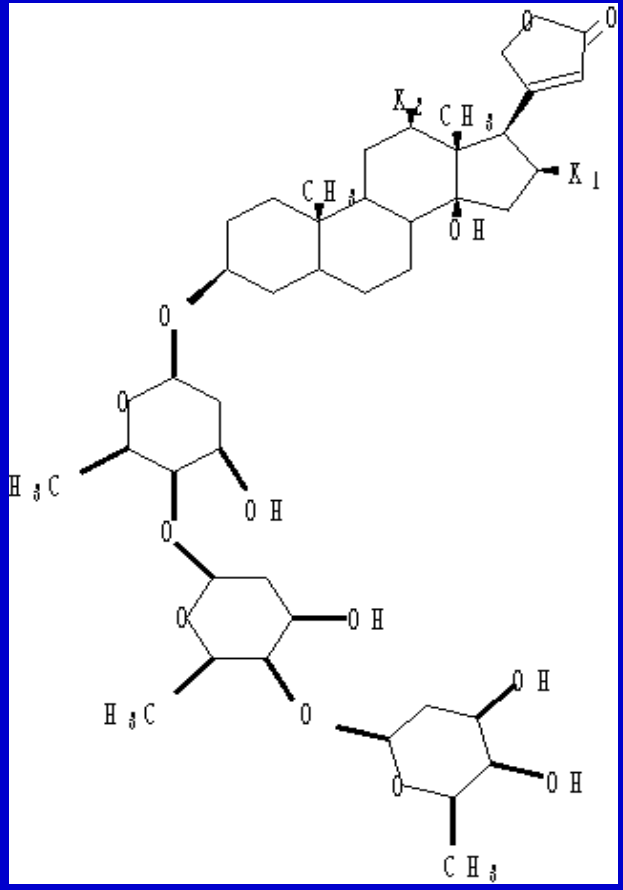




Digitoxin: X_1=X_2=H

Digoxin: X_1=H; X_2=OH

Gitoxin: X_1=OH; X_2=H



Digitoxin Substitutions

Figure 1

Fast LC Separation of Digitalis Derivatives (3 minutes)

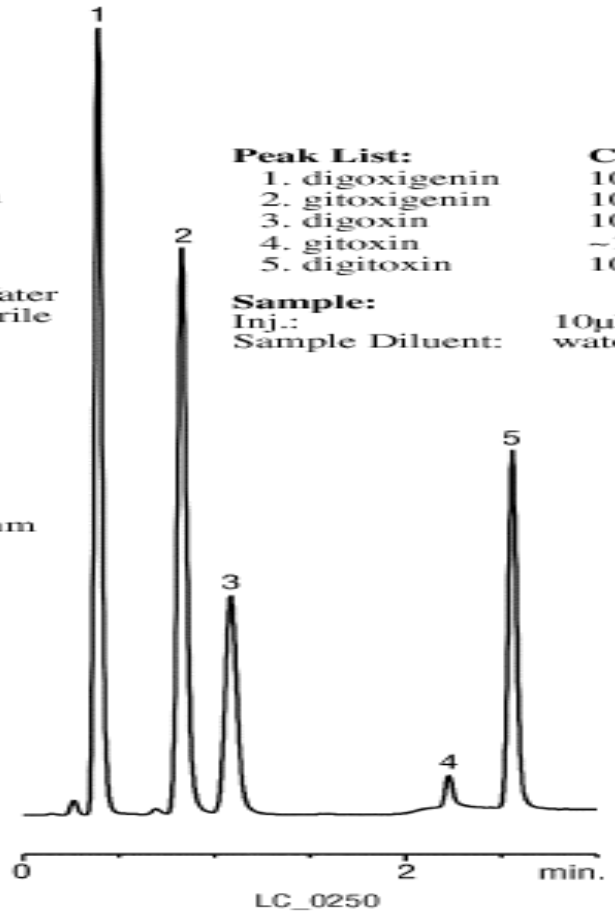
Digitalis Extracts/Derivatives on Ultra Alkaloids Cartridge Column: Fast LC

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 30cm 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 Carbamates

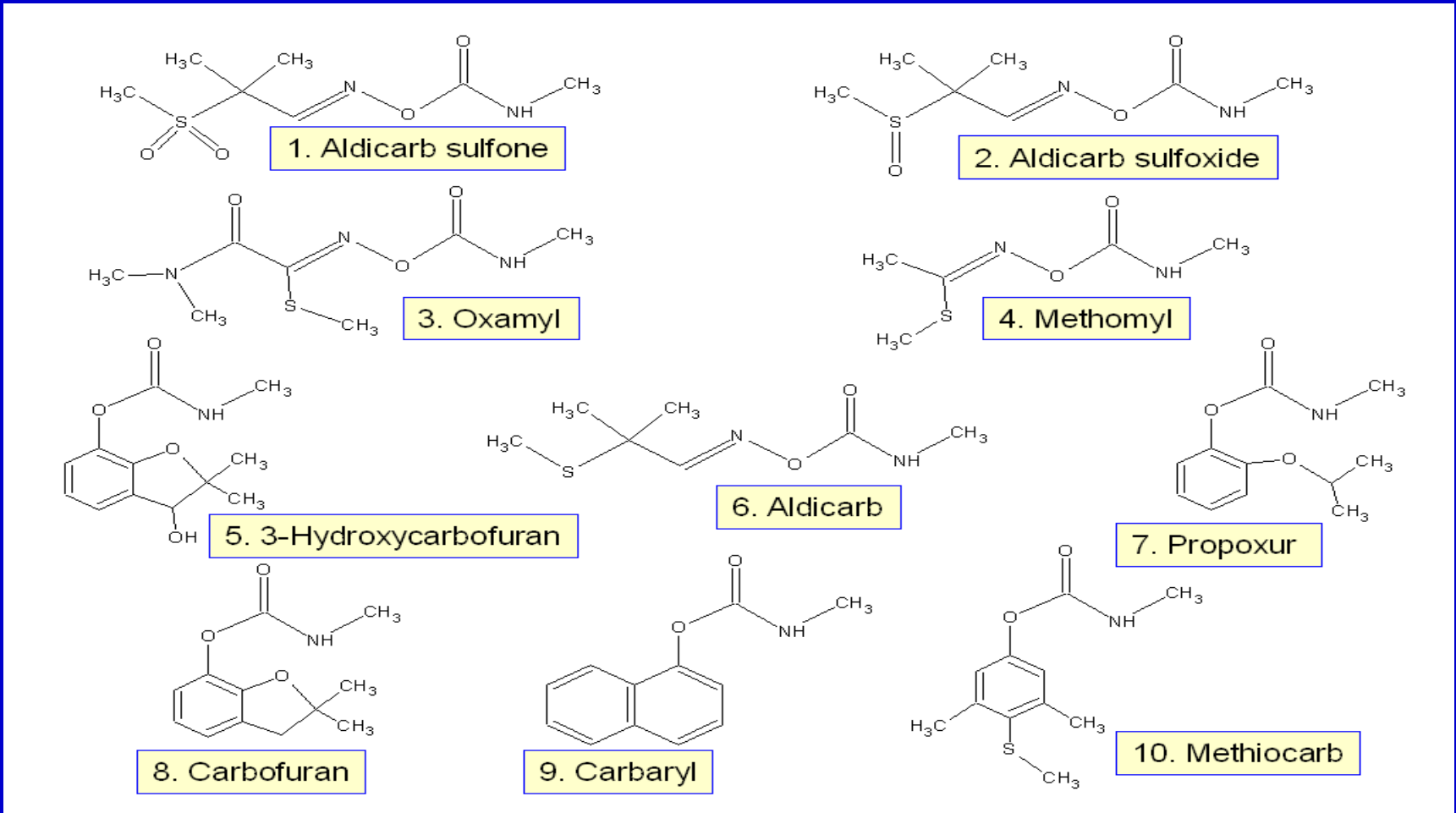


Figure 3-Structures of Commonly Analyzed Carbamates

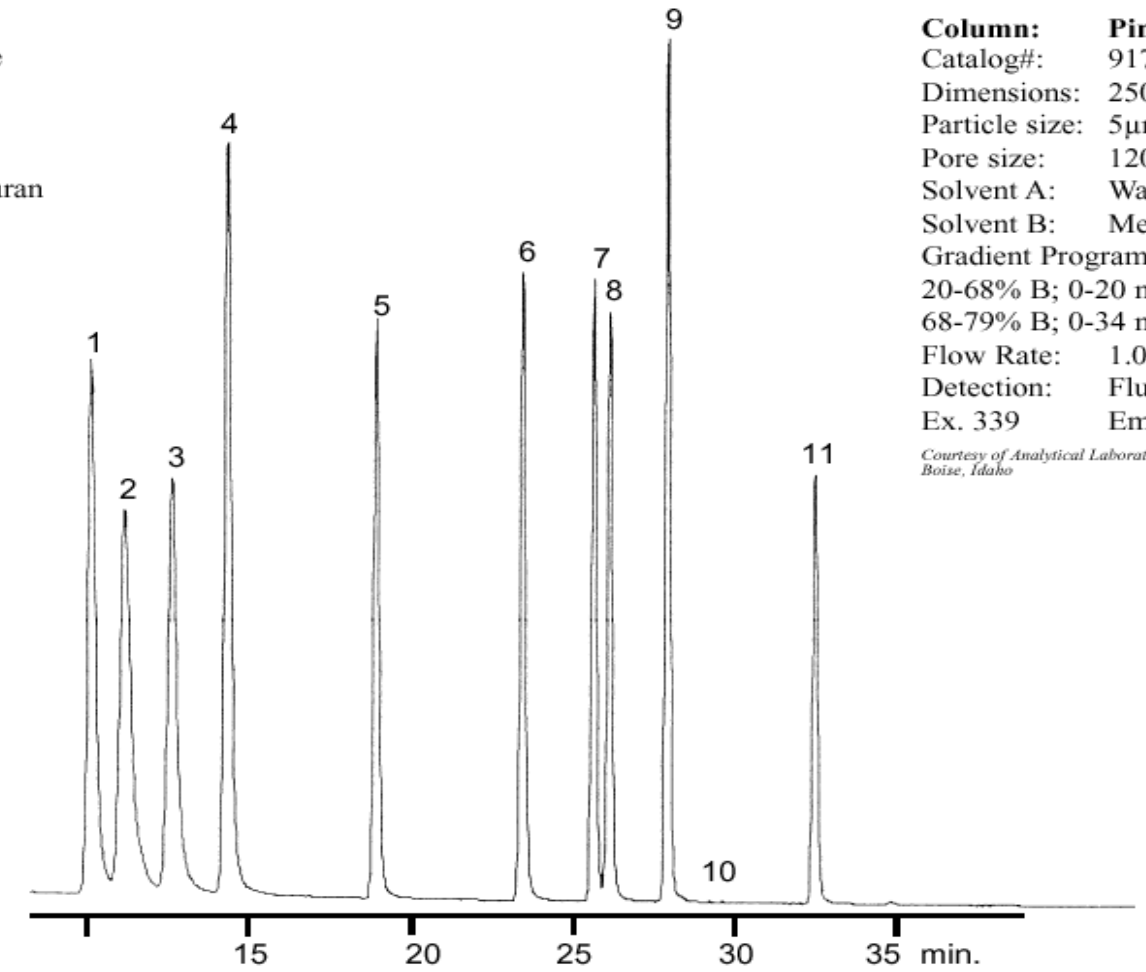
Carbamate Analysis using Standard HPLC Methodology (About 40 minutes)

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
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:

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

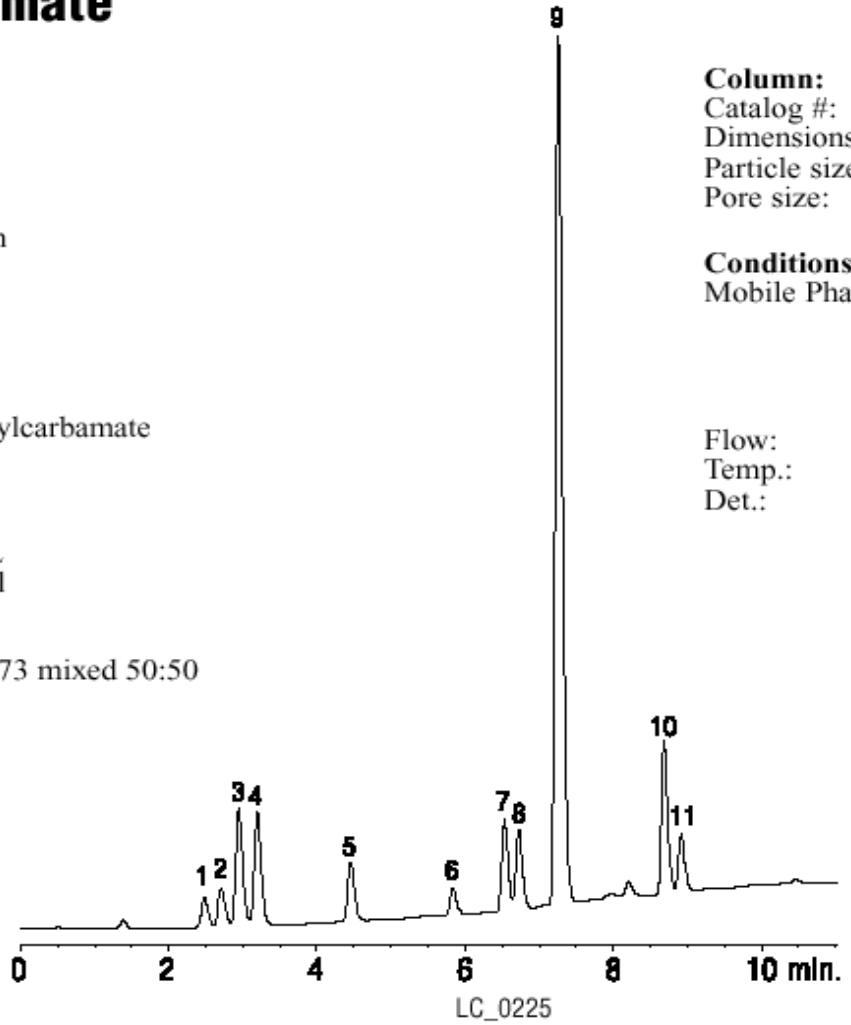


Figure 5

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)

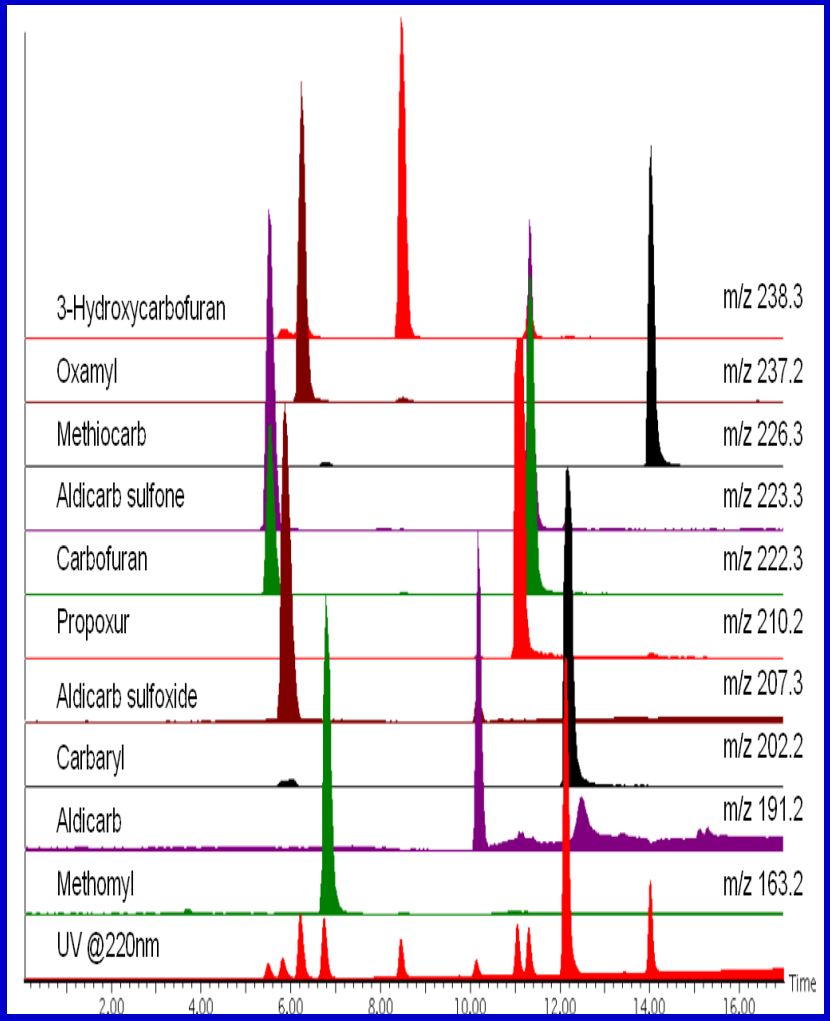


Figure 6

Fast LC Analysis – Carbamate Separation Loss

- Post Column Volumes produced by external reactors can be detrimental to critical separations – especially to smaller bore columns.

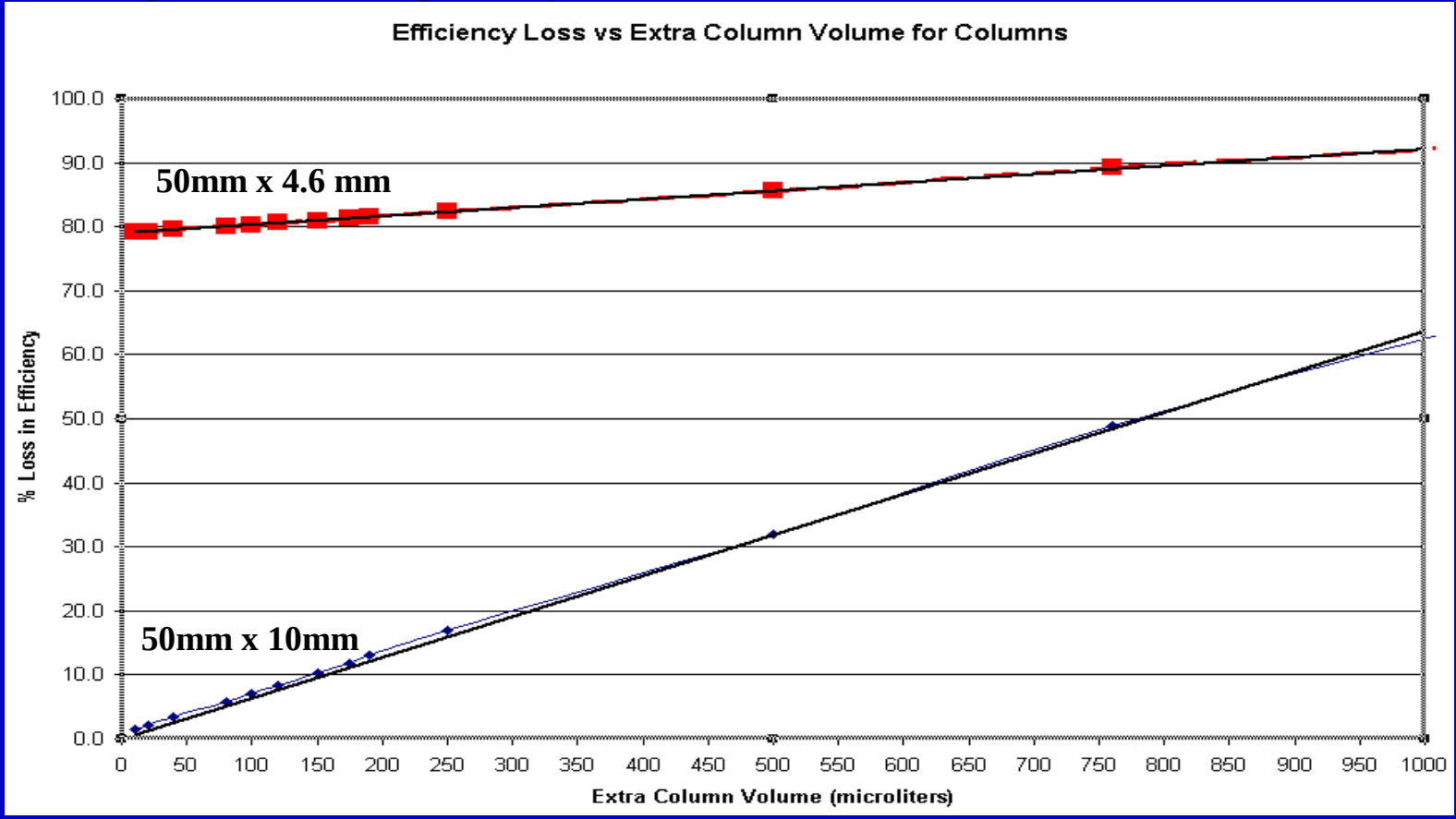


Figure 7

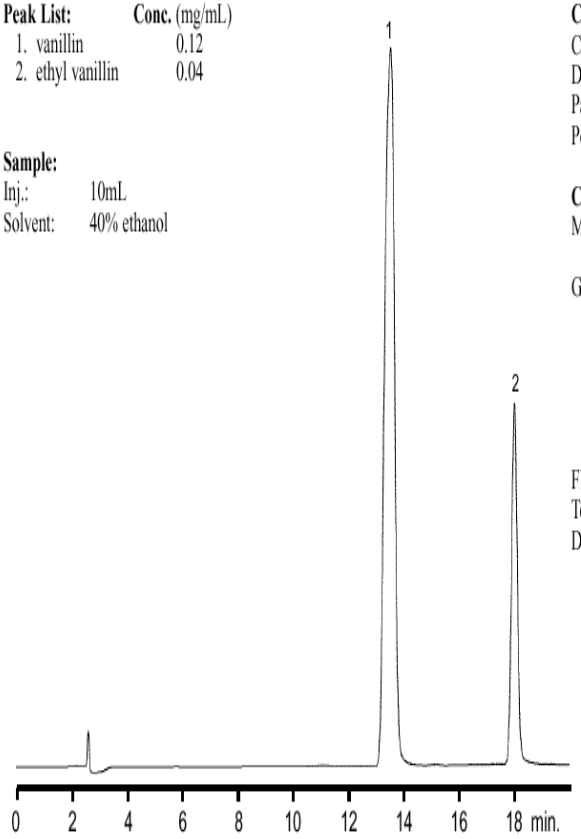
Conventional Vanilla Flavoring Analysis (About 20 minutes with gradient)

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

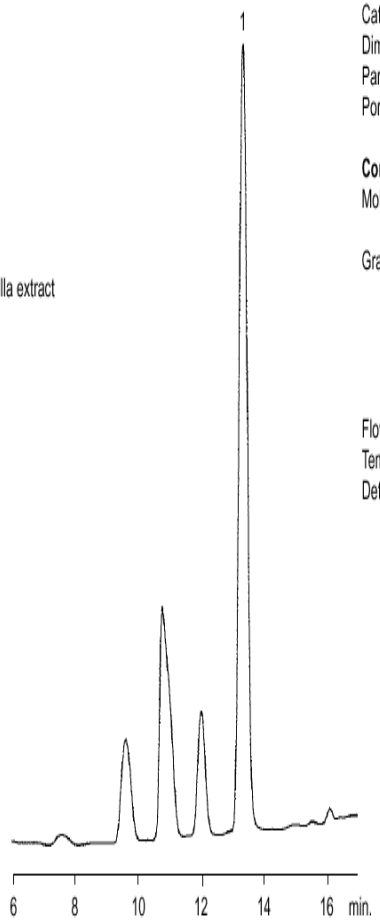
Flow: 1.0mL/min.
Temp.: 28°C
Det.: UV @ 254nm

Vanillin on Ultra C8

Application Note: LC_0149

Peak List:
1. vanillin

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



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Mobile phases: A: 1.2% acetic acid
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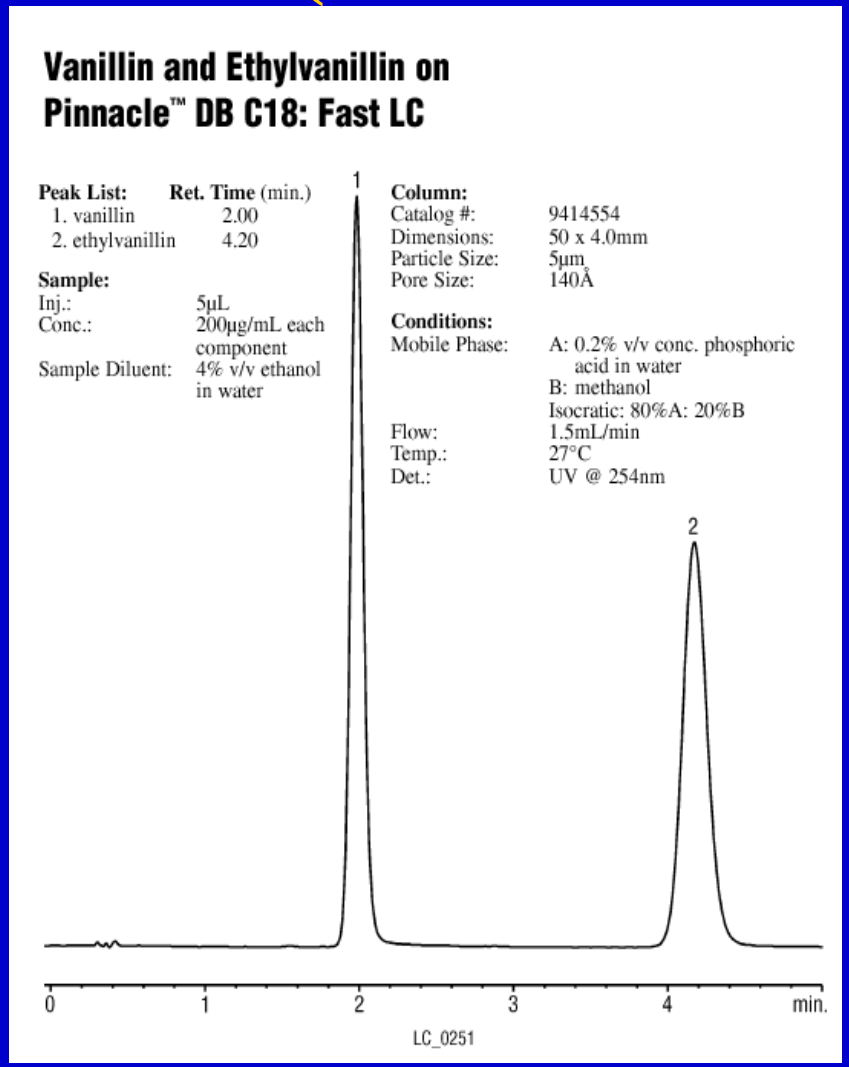
	0.0	20.0
	5.0	20.0
	15.0	40.0
	20.0	40.0
	25.0	20.0

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

Figure 8

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



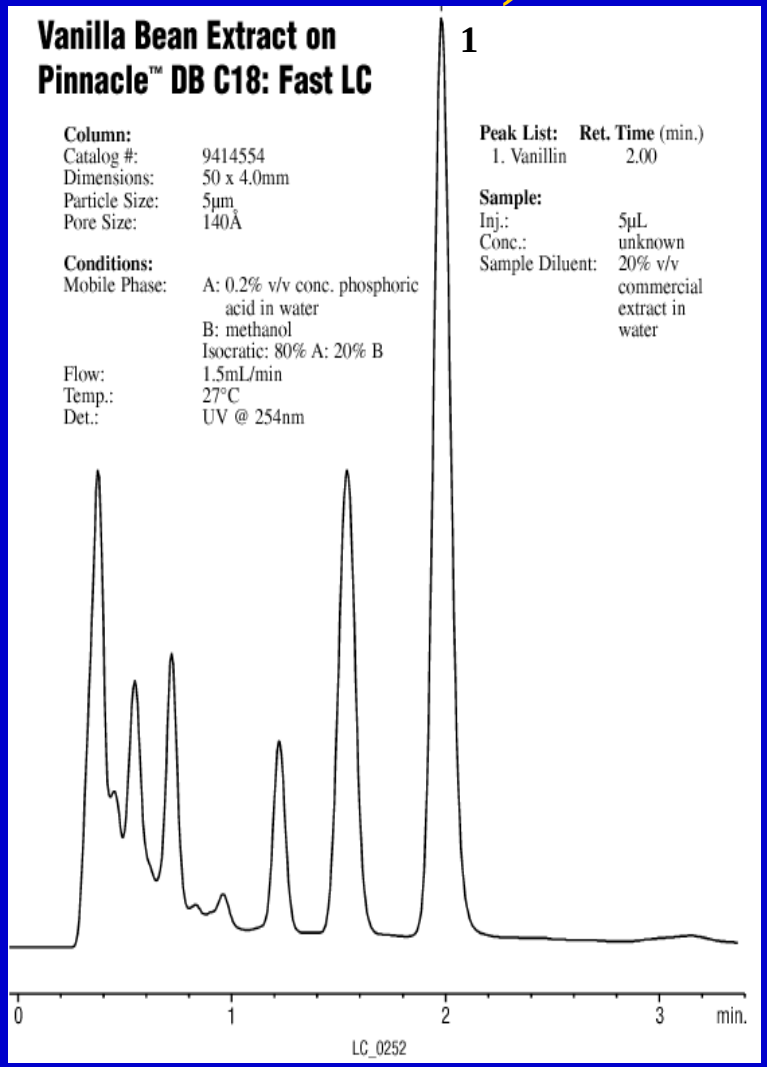


Figure 9

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.

Conclusion

- Fast LC techniques applied upon highly selective stationary phases create a viable, precise quantitative alternative for analyses previously performed by Thin Layer Chromatography. In addition, these techniques can be used to improve method sensitivity, reduce solvent waste, and enhance laboratory throughput. Simplification of methods from gradient elution to isocratic elution can also occur when the proper stationary phase is used with a drastic reduction in analysis time, however, extraneous column volume caused by items as post column reactors can have a greater adverse effect when using fast columns.

Acknowledgements

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