

Scouting for Columns

New Strategy for UPLC Methods Development Employing a Column Manager for ACQUITY UPLC

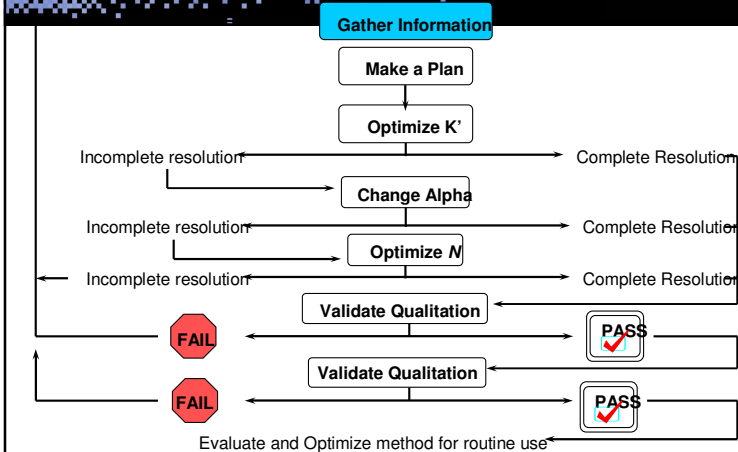
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Seven Basic Considerations in Choosing HPLC Operating Parameters

- 1) **Solubility** - Hexane, Chloroform, Methanol, Water (buffer pH), other?
- 2) **Molecular Weight** - Would GPC be useful in either the analysis or sample prep?
- 3) **Functional Groups** - Any ionizable groups? Acidic, Basic, or Neutral?
- 4) **Sample Matrix** - What amounts are expected in matrix for either analytical or preparative isolation?
- 5) **Levels in Matrix** - What amounts are expected in matrix for either analytical or preparative isolation?
- 6) **Detectability** - Any chromophores or fluorophores? Consider Redox or derivatization. Together with point #5, an appropriate detector is chosen.
- 7) **How Do Species Differ** - An important clue to manipulate selectivity in the separation, especially if compounds are similar in their structure.

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Methods Development Strategy



- Step by step method development strategy -

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ACQUITY UPLC Column Manager and Column Heater/Cooler

- Column Manager
 - Thermostatted from 10 – 90 °C
 - Automated switching among four columns and a bypass channel
 - eCord Information Management for each column
 - Accepts column sizes from 2.1 x 30 mm to 4.6 x 150 mm
 - Passive pre-column solvent heating and post-column cooling
- Column Heater/Cooler
 - Same as Column Manager but with no switching valves



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Scouting for Columns

UPLC Systematic Screening

- Four ACQUITY UPLC Chemistries 2.1 x 50 mm, 1.7 μ m
 - ACQUITY UPLC BEH C₁₈
 - ACQUITY UPLC BEH Shield RP₁₈
 - ACQUITY UPLC BEH C₈
 - ACQUITY UPLC BEH Phenyl
- Solvents:
 - Acetonitrile
 - Methanol
- Buffers:
 - pH 3 ammonium formate
 - pH 10 ammonium bicarbonate

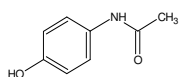
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The Task

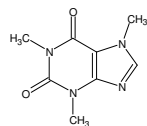
- Develop a method to assay a common over the counter (OTC) analgesic:
 - Excedrin Extra-Strength Tablets



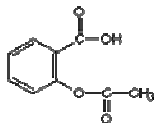
Target Compounds



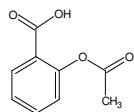
acetaminophen
pKa : 9.71
MW: 151.16



caffeine
pKa : 0.6; 14.0
MW: 194.19



acetylsalicylic acid
pKa : 3.5
MW: 180.16



salicylic acid
pKa : 3.5

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UPLC Systematic Screening for Analgesics: Experimental Details

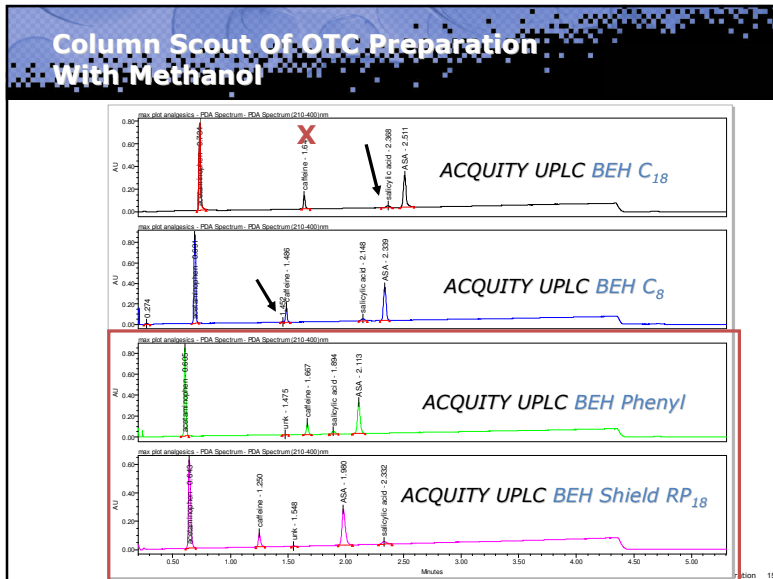
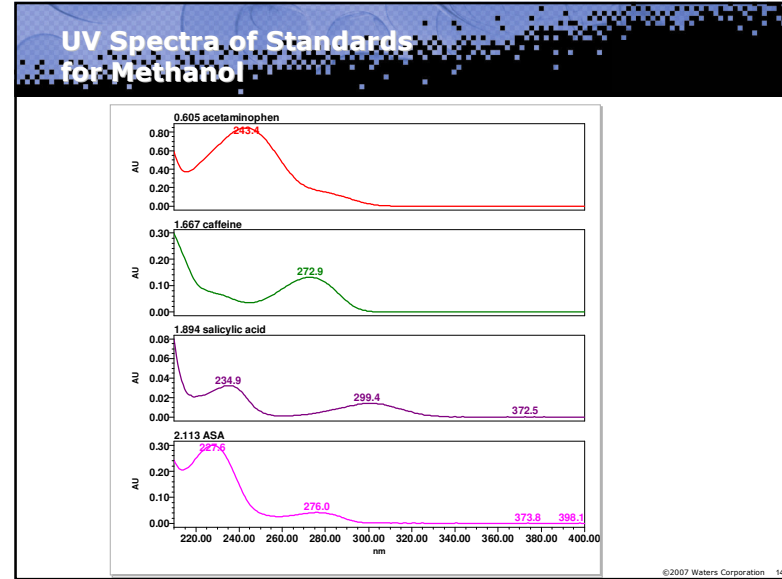
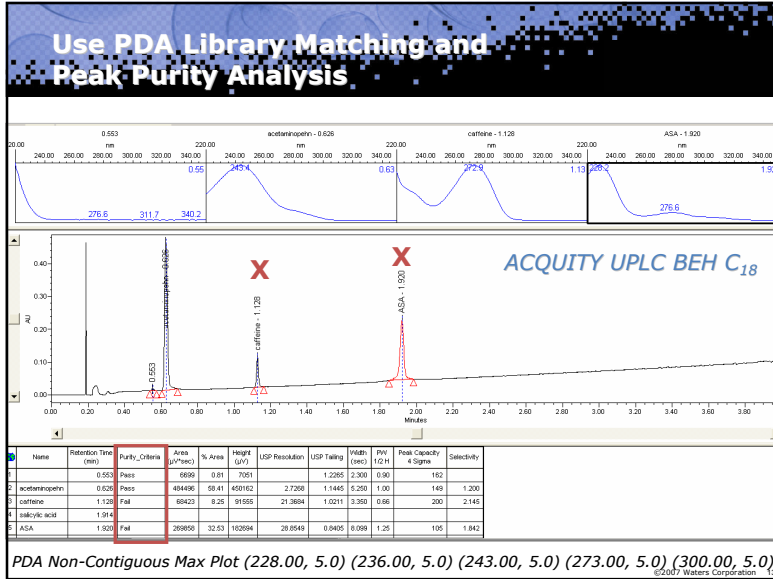
System Configuration:

Hardware:
ACQUITY UPLC Binary Solvent Manager with standard 50 μ L Mixer
ACQUITY Sample Manager with 2 μ L loop
ACQUITY Column Manager
ACQUITY PDA detector with 10 mm 500 nL flow cell

Software:
Empower 2 Software for system control, data collection and processing

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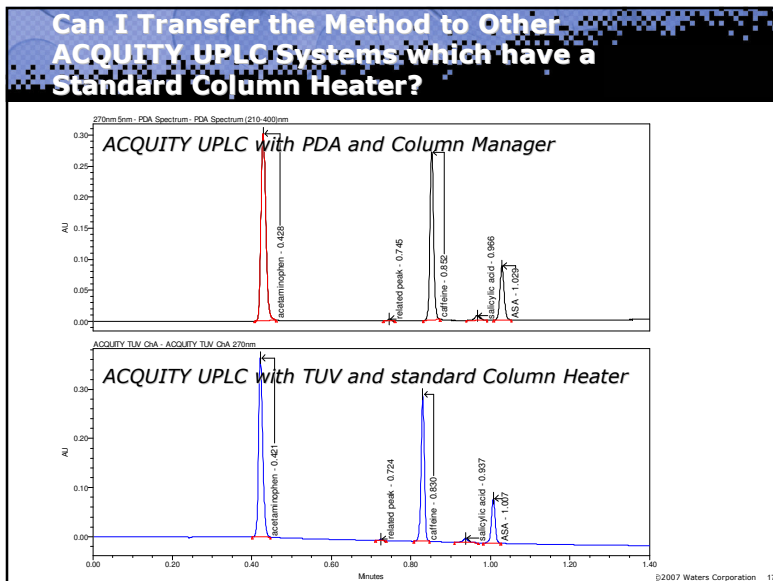
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Compare Phenyl to Shield RP₁₈

	Name	Retention Time (min.)	USP Rs	USP Tailing	PW 1/2 H seconds
Phenyl	acetaminophen	0.6047		1.14	0.96
Phenyl	unk	1.4746	28.4	1.10	1.10
Phenyl	caffeine	1.6670	6.0	1.02	1.09
Phenyl	salicylic acid	1.8936	5.6	1.12	1.78
Phenyl	ASA	2.1130	4.6	1.05	1.55
	Average			1.09	1.30
Shield RP ₁₈	acetaminophen	0.6430		1.36	1.26
Shield RP ₁₈	caffeine	1.2504	17.1	1.30	1.22
Shield RP ₁₈	unk	1.5484	7.4	1.36	1.39
Shield RP ₁₈	ASA	1.9801	8.9	1.25	1.80
Shield RP ₁₈	salicylic acid	2.3318	5.8	1.21	2.42
	Average			1.30	1.62

Scouting for Columns



Develop Methods Faster with UPLC: Time Savings

UPLC Method Development Protocol		EQUIV HPLC Methods Development Protocol	
2.1 x 50 mm, 1.7 μm		4.6 x 150 mm, 5 μm	
pH 3/ acetonitrile	Time	pH 3/ acetonitrile	Time
Flow ramp	5 min	Flow ramp	5 min
Column conditioning (2 blank gradients)	11 min	Column conditioning (2 blank gradients)	80 min
Sample injection (2 replicates)	11 min	Sample injection (2 replicates)	80 min
pH 3/ methanol		pH 3/ methanol	
Flow ramp	5 min	Flow ramp	5 min
Column conditioning (2 blank gradients)	11 min	Column conditioning (2 blank gradients)	80 min
Sample injection (2 replicates)	11 min	Sample injection (2 replicates)	80 min
Column purge	6 min	Column purge	35 min
pH 10/ acetonitrile		pH 10/ acetonitrile	
Flow ramp	5 min	Flow ramp	5 min
Column conditioning (2 blank gradients)	11 min	Column conditioning (2 blank gradients)	80 min
Sample injection (2 replicates)	12 min	Sample injection (2 replicates)	80 min
pH 10/ methanol		pH 10/ methanol	
Flow ramp	5 min	Flow ramp	5 min
Column conditioning (2 blank gradients)	11 min	Column conditioning (2 blank gradients)	80 min
Sample injection (2 replicates)	11 min	Sample injection (2 replicates)	80 min
Column purge	6 min	Column purge	35 min
	120 min		730 min
SCREENING TIME	2 Hours/column x 4 columns	SCREENING TIME	12.2 Hours/column x 4 columns
TOTAL SCREENING TIME	8 HOURS	TOTAL SCREENING TIME	48.8 HOURS

Grumbach et.al. Waters Chemistry Operations 2005

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