A Streamlined Approach for Reversed-Phase Method Development using a Combination of Column Screening and Software Modelling

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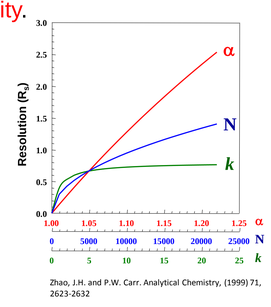
1. Introduction

- Method development can be a lengthy and expensive process defined by the separation goals and sample complexity.
- Efficient method development requires logical exploration of key chromatographic parameters affecting selectivity.
- Rationally designed method development strategies assess key parameters and allow well informed decision making leading to robust methods.



Zhao, J.H. and P.W. Carr. Analytical Chemistry, (1999) 71, 2623-2632

Isocratic Separations		Gradient Separations
- Column stationary phase - pH (ionisable analytes only) - Organic modifier type - % Organic modifier - Buffer selection - Column temperature - Buffer concentration	MOST Influential ⇑ LEAST Influential	All parameters for isocratic separations **PLUS:** - Gradient steepness - Dwell volume - Column dimensions

1 Adapted from 'Introduction to Modern Liquid Chromatography", 3rd Edition, Snyder, Kirkland, Dolan, 2010, p.29, Wiley & sons

- Stationary phase and organic modifier are the most powerful parameters to assess in method development.

2. Screening Strategy with ACE Columns

- A screening strategy based on stationary phase and organic modifier has the most potential to streamline method development:

- Select mobile phase pH depending on analyte properties. (For unknown samples, start at low pH).
- Screen six ACE reversed-phase columns with different properties:

Bonded phase	Separation Mechanism and Relative Strength[1]				
	Hydrophobic Binding	π-π Interaction	Dipole-Dipole	Hydrogen Bonding	Shape Selectivity
ACE C18	****	-	-	*	**
ACE C18-AR	****	*** (donor)	*	**	***
ACE C18-PFP	****	*** (acceptor)	****	***	****
ACE SuperC18	****	-	-	-	**
ACE C18-Amide	****	-	**	****	**/***
ACE CN-ES	***	*	***	**	*

[1]Approximate value – determined by semi-quantitative mechanism weightings and/or by reference to other ACE phases using >100 characterising analytes.

- The ACE range consists of phases specifically designed to offer maximum selectivity differences – ideal for method development.

3. Method Optimisation with ChromSword 2.0

- Once a suitable stationary phase/organic modifier is selected, the method needs optimising (gradient slope, % organic, temp etc.).
- ChromSword 2.0 computer assisted method development software can help to streamline the optimisation step.

ChromSword

- From as few as 2 runs, ChromSword can model the separation.
- Thousands of potential separations can be automatically simulated to find a final method.
- Manual editing of conditions and simulation of associated chromatograms is also possible.
- In Reversed-Phase, ChromSword 2 can be used to optimise %organic, gradient time, pH, temperature.
- Also compatible with Normal Phase and Ion Exchange Chromatography.

4. Example: Triple API Column Screen

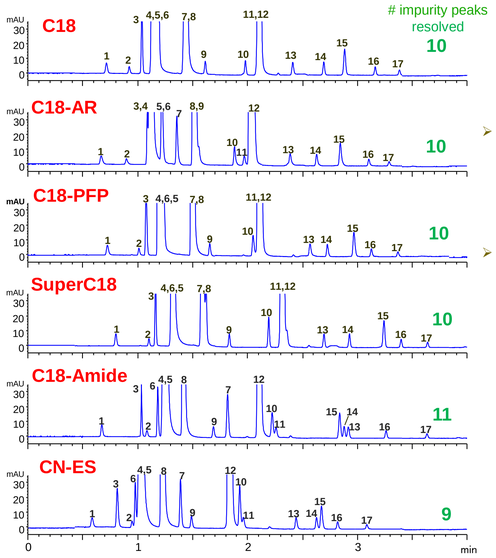
- A triple API pharmaceutical sample containing 14 impurities was screened on the six ACE phases using both MeCN and MeOH as the organic modifier

- **Columns:** 6 x ACE Excel 2 µm, 100 x 3.0 mm
- **System:** VWR Hitachi ChromasterUltra Rs UHPLC with 6-column switching valve

- **A1:** 10 mM ammonium formate pH 3.0
- **B1:** 10 mM ammonium formate pH 3.0 in MeCN:H_2O 9:1 v/v
- **B2:** 10 mM ammonium formate pH 3.0 in MeOH:H_2O 9:1 v/v

- **Gradient:** 5-95% B in 5 minutes
- **Temperature:** 40 °C
- **Flow rate:** 1.2 mL/min
- **Detection:** UV, 270 nm
- **Sample:** Aspirin: 5 mg/mL
  Paracetamol: 3.3 mg/mL
  Caffeine: 0.75 mg/mL
  Impurities spiked at 0.1% (wrt aspirin)

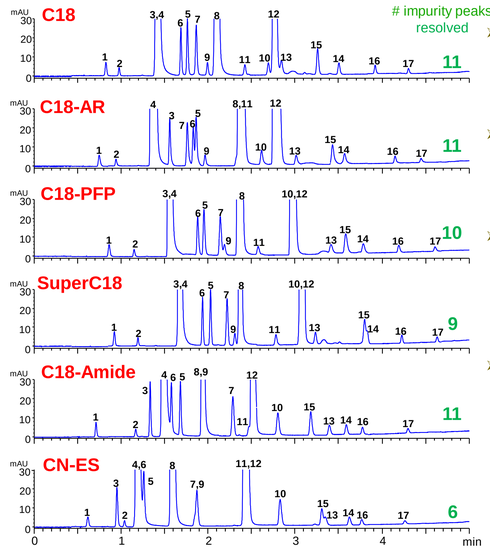
5. Example: Triple API Column Screen - MeCN



- The sample was initially screened using acetonitrile as the organic modifier
- The ACE C18-Amide was found to resolve 11 of the 14 impurity peaks.

1. 2-Aminophenol, 2. Hydroquinone, 3. Theobromine, 4. Paracetamol, 5. Theophylline, 6. Paraxanthine, 7. 4-Hydroxybenzoic acid, 8. Caffeine, 9. 2-Acetamidophenol, 10. 2-Hydroxybenzoic acid, 11. Phenol, 12. Aspirin, 13. 4-Nitrophenol, 14. 4-Chloroacetanilide, 15. 2-Nitrophenol, 16. Acetylsalicylsalicylic acid, 17. Salsalate

6. Example: Triple API Column Screen - MeOH

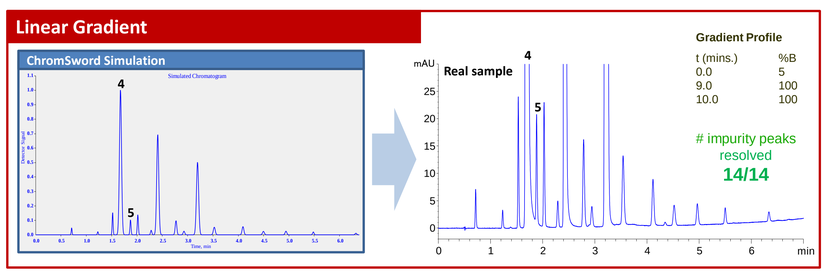


- The sample was then screened using methanol as the organic modifier.
- Better retention and selectivity for the more hydrophilic analytes.
- The screening approach provides multiple options to pursue for obtaining a full separation.
- The ACE C18, C18-AR and C18-Amide all resolve 11 of the 14 impurity peaks.

1. 2-Aminophenol, 2. Hydroquinone, 3. Theobromine, 4. Paracetamol, 5. Theophylline, 6. Paraxanthine, 7. 4-Hydroxybenzoic acid, 8. Caffeine, 9. 2-Acetamidophenol, 10. 2-Hydroxybenzoic acid, 11. Phenol, 12. Aspirin, 13. 4-Nitrophenol, 14. 4-Chloroacetanilide, 15. 2-Nitrophenol, 16. Acetylsalicylsalicylic acid, 17. Salsalate

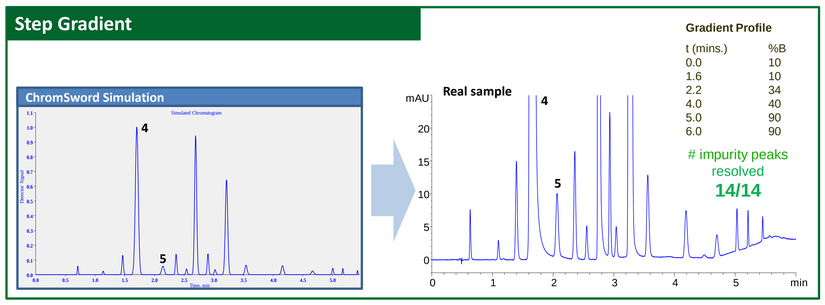
7. Optimisation with ChromSword: Linear Gradient

- The ACE Excel 2 C18-Amide, with methanol as the organic modifier, was selected as the best candidate for optimisation.
- The 5 minute screening gradient run was input into ChromSword.
- 10 and 15 minute gradients were also run and input to generate the model.
- The separation was optimised and the optimum linear gradient was predicted.



8. Optimisation with ChromSword: Step Gradient

- The separation was further improved by using step gradients.
- ChromSword automatically generates a set of proposed step gradients.
- The resolution of peaks 4 and 5 was improved over the linear gradient method by giving this peak-pair priority in the optimisation algorithm.
- An additional gradient step was manually added to the end of the gradient table to reduce run time.
- Only the original 3 input runs were required to optimise the separation.



9. Summary and Conclusions

- Screening a new sample on the six ACE phases with two different organic modifiers is a powerful way to explore selectivity in method development.
- Once the optimum stationary phase and organic modifier have been selected, the final method needs to be optimised.
- ChromSword 2.0 can accelerate method development by streamlining the optimisation stage.
- Method optimisation can be achieved using data from as little as two experimental runs.
- The ACE ChromSword 2 Method Development Kit combines the six unique ACE phases with the ChromSword 2 software in one package.