

Quickly How to Develop HPLC Methods

A Five Step Strategy from Mourne Training Services

www.mournetrainingservices.co.uk

Step 1

What are your goals?

Define your requirements:

- What is the purpose of the method?
- What is the analyte and are there more than one?
- What is sample matrix for analysis?
- Is quantification required?
- What level of effort is appropriate and what resources are available?
- What are the desired method criteria, e.g. minimum resolution, maximum runtime, accuracy & precision?



Step 4

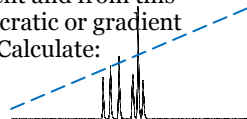
What conditions will you use for the method?

From a standard set of initial conditions modify as necessary based on previous steps, e.g. the pH of the buffer.

Run a full range gradient and from this determine whether isocratic or gradient analysis is preferable. Calculate:

Isocratic - %B.

Gradient - starting %B.



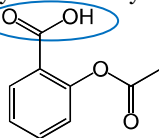
For complex separations - screen different conditions and choose the most promising.

Step 2

What do you already know?

Assess the structure of the analyte(s):

- Molecular weight & polarity - Suitability for RP-HPLC conditions.
- pK_a - Is a buffer required and if so at what pH?
- Solubility - Solvents for sample preparation?
- Chromophores - UV detection possible?



Assess interferences in the sample matrix.

Assess previous work performed on the analyte(s).

Step 3

What sample(s) will you use to develop the method?

Identify the required materials for developing the method.

Source the required materials:

- Can they be purchased?
- Can analyte free sample matrix be obtained?
- Are stress studies required to generate degradation products?

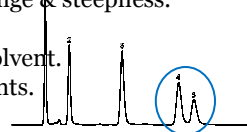
Prepare test sample(s) to be used for method development.

Step 5

What method parameters will you use?

Optimise the conditions from step 4 to achieve the desired separation. Adjust conditions to achieve acceptable resolution for the critical pair:

- %B or gradient range & steepness.
- Temperature.
- Change organic solvent.
- Mix organic solvents.
- Adjust buffer pH.



Define all method parameters.