Reversed Phase Method Development

Step 1 Characteristics

Applicational characteristics
- HPLC/UHPLC-method?
- Which detector? LC/MS?
- Isocratic/gradient?

Analyte characteristics
- Hydrophobicity, polarity, ionicity
- Structure/molecular weight
- Stability
- How do the analytes differ?
- Matrix

Step 2 Screening

Typical screening conditions:
- Steep gradient
- Mostly short column e.g. 50 mm
- ID depends on compound, pressure

Columns X **Mobile phase** X

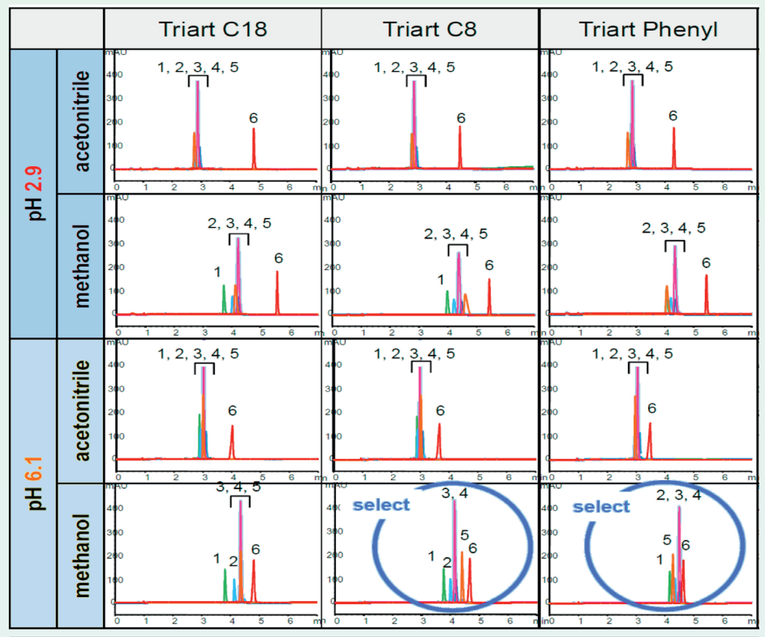
Screening kit
1. YMC-Triart C18
2. YMC-Triart C18 ExRS
3. YMC-Triart C8
4. YMC-Triart Phenyl
5. YMC-Triart PFP

Solvent
❶ Acetonitrile
❷ Methanol

Gradient
5–90% solvent

pH (solution)
I. Acidic
II. Neutral
III. Basic

Choose the most promising conditions



Example 6 pigments on YMC-Triart columns
Column: 50 x 2.0 mm ID
Gradient: 5–90%B(0–5min), 90%B(5–7min), 5%B(7–12min)
Flow rate: 0.2 mL/min
Temperature: 40°C
Detection: UV at 254 nm

1. Acid green 16
2. Acid blue 1
3. Acid red 52
4. Acid blue 3
5. Methyl orange
6. Methyl red

Step 3 Optimisation

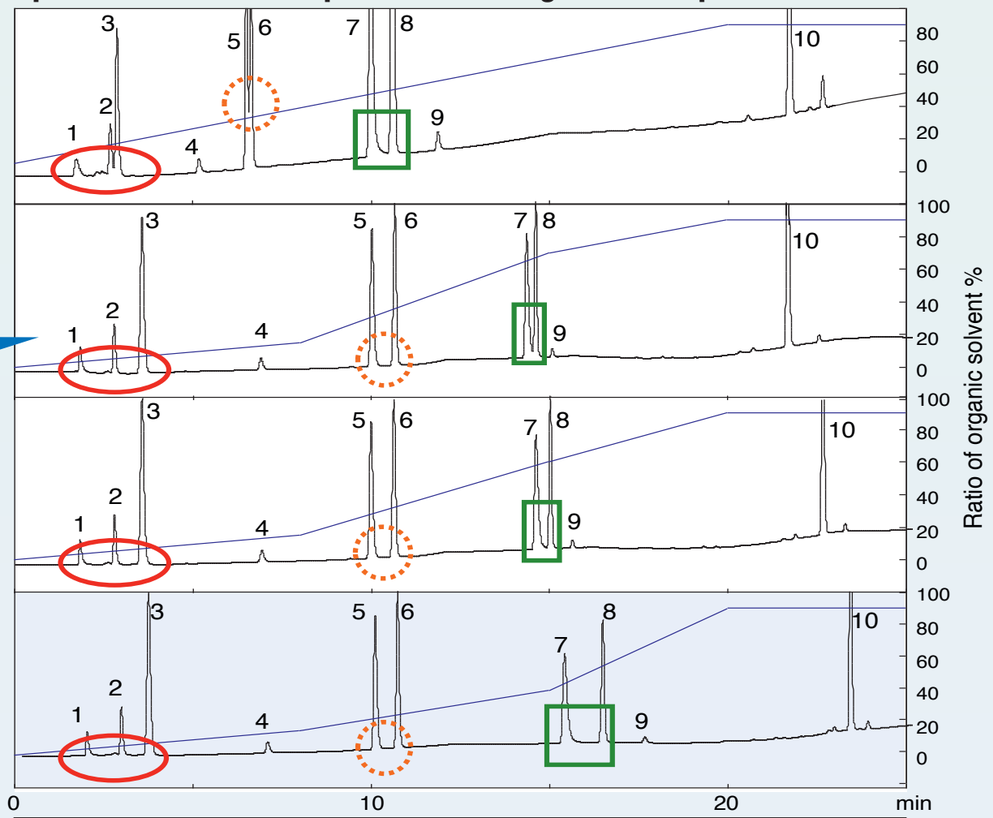
Adjustment options
- Mobile phase ratio
- Gradient slope/isocratic
- Precision tuning of temperature
- Column dimensions
- Particle size
- Add org. modifier
- pH (additives)

5–90%B(0–20 min)

Separated by decreasing in gradient slope

0–15%B(0–8 min)
15–40%B(8–15 min)
40–90%B(15–20 min)

Optimisation of mobile phase ratio and gradient slope



Example cold medicine on Hydrosphere C18
Column: Hydrosphere C18 (5 µm, 120 Å) 150 x 4.6 mm ID
Eluent: A) 20 mM phosphate buffer (pH 2.5)
B) methanol
Flow rate: 1.0 mL/min
Temperature: 37 °C
Detection: UV at 210 nm (0–15 min), 235 nm (15–25 min)

1. Thiamine hydrochloride
2. Unknown
3. L-Ascorbic acid
4. Maleic acid
5. dl-Methylephedrine hydrochloride
6. Dihydrocodeine phosphate
7. Saccharin sodium
8. Caffeine
9. Chlorpheniramine
10. Ibuprofen

Buffer Selection
Buffers should be choosen as following: **pH 2 values under pKa** of the analyte for acidic compounds and for **basic** compounds **2 values above the pKa**. For LC/MS methods it is recommended to use buffer concentrations <15 mM.

Buffer	pKa	Buffer range [pH]	Standard Concentration	LC/MS compatibility
Trifluoroacetic acid (TFA)	<1.0	–	0.01~0.1%	✓
Phosphoric acid	2.1		0.01~0.1%	✘
Ammonium dihydrogen phosphate (Na^+, K^+ salt)	2.1	1.1 ~3.1	5~50 mM (<20 mM recommended)	✘
Formic acid	3.7	–	0.1~1.0%	✓
Ammonium formate (Na^+, K^+ salt)*	3.7	2.7~4.7	5~50 mM	NH_4^+ salt: ✓ [Na^+, K^+ salt: ✘]
Acetic acid	4.8	–	0.5~5.0%	✓
Ammonium acetate (Na^+, K^+ salt)*	4.8	3.8~5.8	5~50 mM	NH_4^+ salt: ✓ [Na^+, K^+ salt: ✘]
Ammonium hydrogen phosphate (Na^+, K^+ salt)	7.2	6.2~8.2	5~50 mM (<20 mM recommended)	✘
Triethylamine acetic acid (TEAA)	–	4.6~6, 10~11	<20 mM	✓
Ammonium formate, ammonium acetate**	9.2	8.2~10.2	<20 mM	✓
Sodium phosphate, potassium phosphate	12.3	11.3~13.3	<10 mM	✘
Ammonium bicarbonate**	–	8.5~10.5	<10 mM	✓

* adjusted with acid **adjusted with ammonia

YMC-Triart Phase Specifications

	C18	C18 ExRS	Bio C18	C8	Bio C4	Phenyl	PFP
Base	organic/inorganic silica						
Stationary phase	C18 (USP L1)	C18 (USP L1)	C18 (USP L1)	C8 (USP L7)	C4 (USP L26)	Phenyl (USP L11)	Penta-fluorophe-nyl (USP L43)
Particle size	1.9, 3 and 5 µm						
Pore size	12 nm	8 nm	30 nm	12 nm	30 nm	12 nm	12 nm
Carbon content	20%	25%	—	17%	—	17%	15%
Endcapping	multi-stage	multi-stage	multi-stage	multi-stage	multi-stage	multi-stage	none
pH range	1 ~ 12	1 ~ 12	1 ~ 12	1 ~ 12	1 ~ 10	1 ~ 10	1 ~ 8
Temperature range	pH<7: 90°C pH>7: 50°C	pH<7: 90°C pH>7: 50°C	pH<7: 90 °C pH>7: 50 °C	pH<7: 90°C pH>7: 50°C	pH<7: 90°C pH>7: 50°C	50°C	50°C
100% aqueous eluents	✓	✘	✓	✘	✓	✓	✓

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