Method scouting by using Triart columns

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(R151013AE_W)

Common problem in method development

Have you experienced anything like shown below?

- Suited column cannot be selected.
- A way of analytic condition optimization is not known.
- Satisfactory retention, resolution and peak shape cannot be obtained.
- Condition optimization needs time.

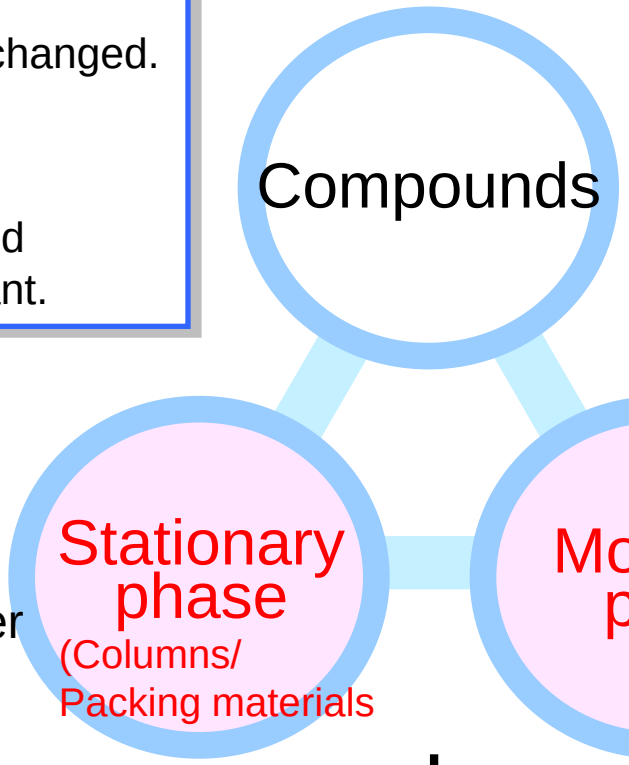
Approach for efficient method development is proposed.

1. Reversed-phase chromatography

- 3 factors by which separation is strongly affected -

In method development,
compounds cannot be changed.

Selection of **columns** and
mobile phase is important.



- Hydrophobicity, polarity, ionity
- Structure, molecular weight

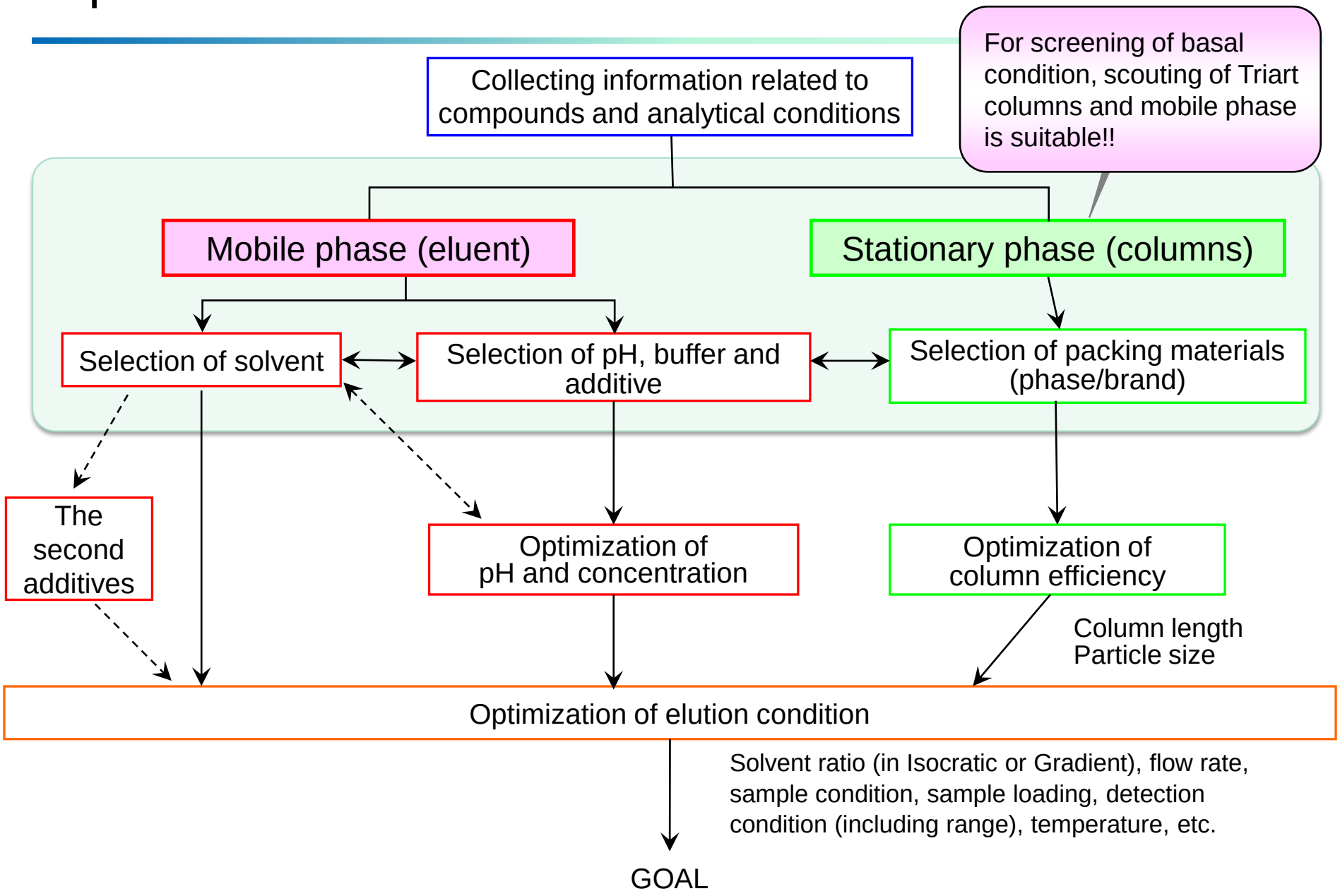
- Type of phases
- Separation character

- Type of organic solvent
- Buffer, pH

+

Temperature, flow rate, sample condition, etc.

Optimization workflow of HPLC methods



Method scouting for reversed-phase chromatography

＜Columns＞

1st choice

1. Triart C18
2. Triart C18 ExRS
3. Triart C8
4. Triart Phenyl
5. Triart PFP

×

＜Mobile phase＞

Organic solvent

❶ acetonitrile

❷ methanol

Gradient elution
5-90% organic solvent

×

pH (Solution)

Ⅰ. Acidity

Ⅱ. Neutral

Ⅲ. Alkalinity

↓

Selection of basal conditions
(**Columns** and **Mobile phase**)

↓

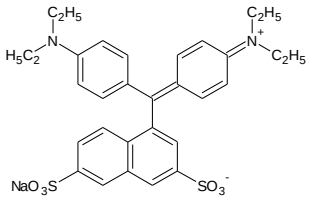
Optimizing separation (Establishing a final method)

Method scouting by short-time separation

Compound mixture with similar structure (6 elements of pigment)

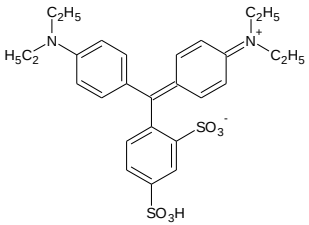
Column	: 50 X 2.0 mmI.D.	Flow rate	: 0.2 mL/min
Gradient	: 5-90%B (0-5 min), 90%B (5-7 min)	Temperature	: 40℃
	5%B (7-12 min) (Equilibration for next analysis)	Detection	: UV at 254 nm

1



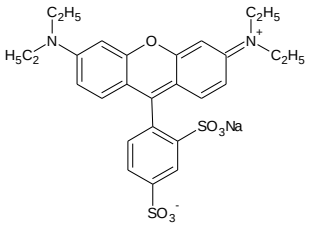
Acid green 16

2



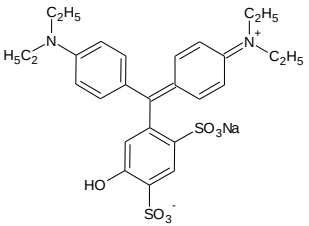
Acid brue 1

3



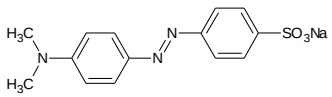
Acid red 52

4



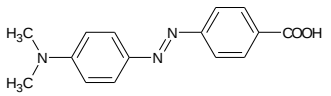
Acid blue 3

5



Methyl orange

6



Methyl red

Sample

Mixture
（6 elements）

1

×

Columns

1. Triart C18
2. Triart C18 ExRS
3. Triart C8
4. Triart Phenyl
5. Triart PFP

5

×

Organic solvent

❶ acetonitrile

❷ methanol

2

×

pH (Solution)

Ⅰ. Acidity (pH 2.9)

Ⅱ. Neutral (pH 6.1)

2

×

Time

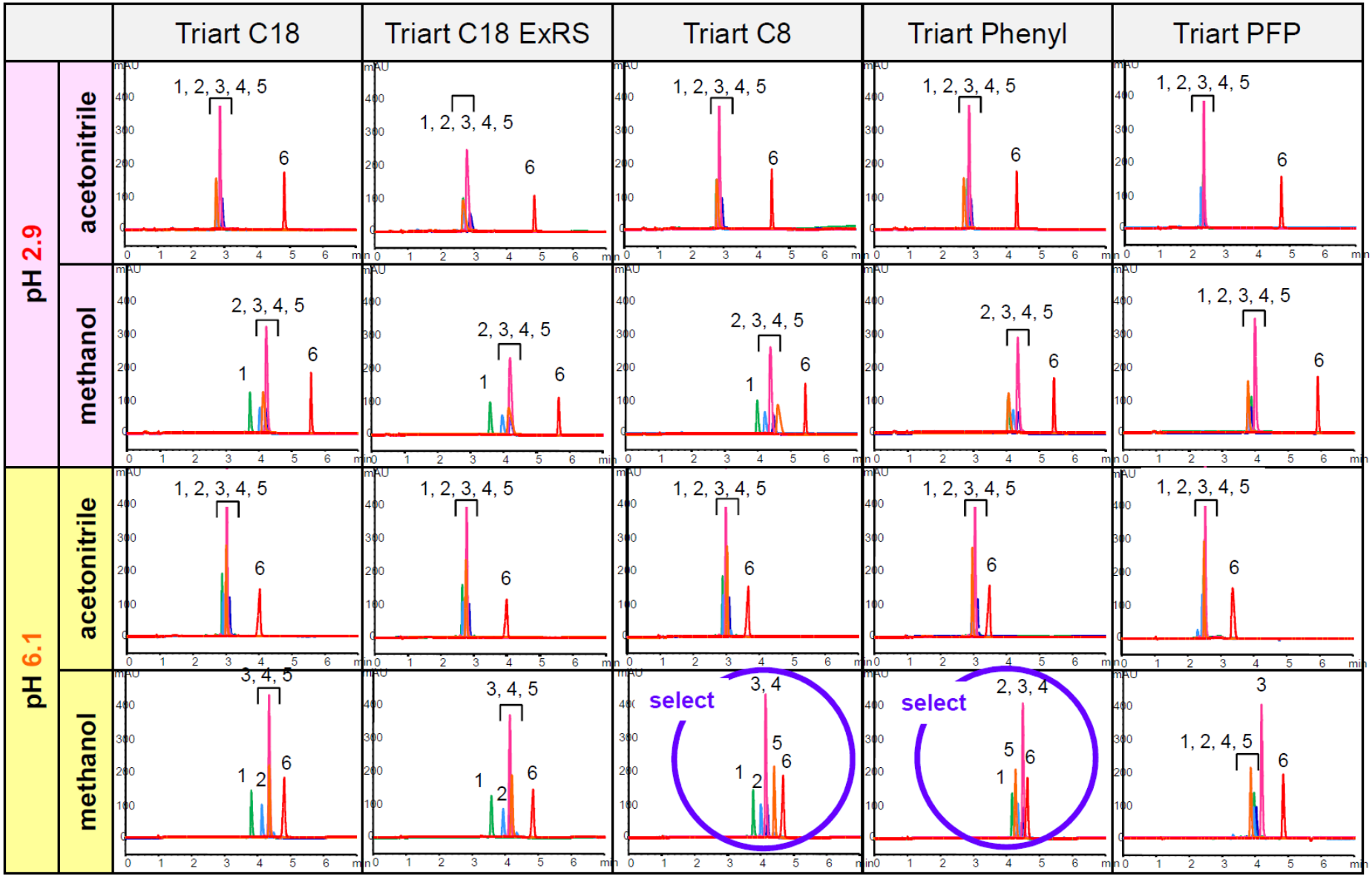
12 min / run

n=2
(n: The number of triala)

2

1 x 5 x 2 x 2 x 2 x 12 min = 480 min (8 hr)

Method scouting by short-time separation (Using 6 elements of pigment mixture)



Method scouting by short-time separation (Using 6 elements of pigment mixture)

Selection of basal conditions (columns and mobile phase) ⇒ Optimized

Selected column
&
Gradient elution with selected mobile phase

Transfer from gradien elution to isocratic eluton

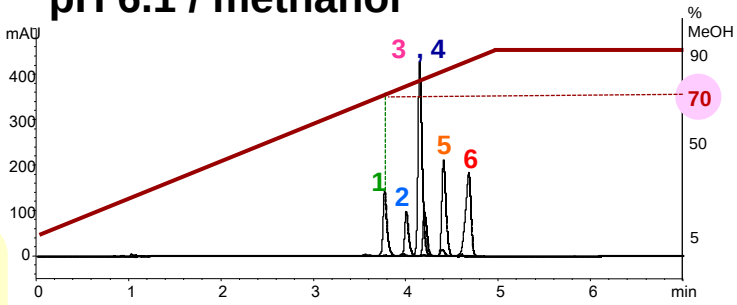
point Beginning to test with concentration (%) of solvent in 20% lower than a concentration at which samples were eluted in gradient elution.

Isocratic elution
(n=2)
（2 x 2 x 7 min）
= 28 min

Optimized!!
(n=2)
（2 x 7 min）
= 14 min

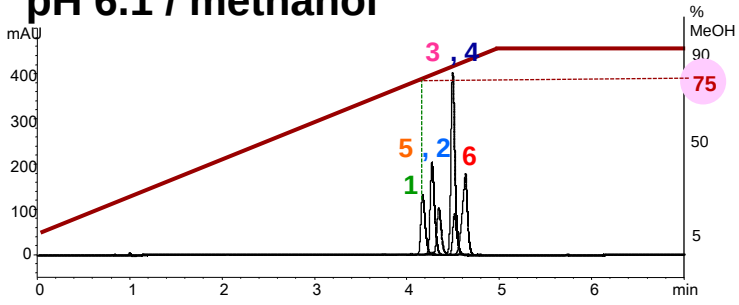
Triart C8

pH 6.1 / methanol

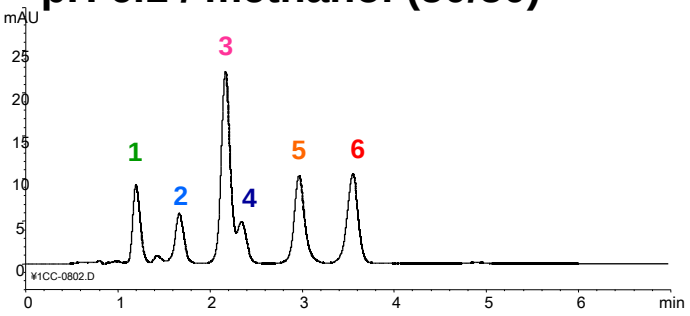


Triart Phenyl

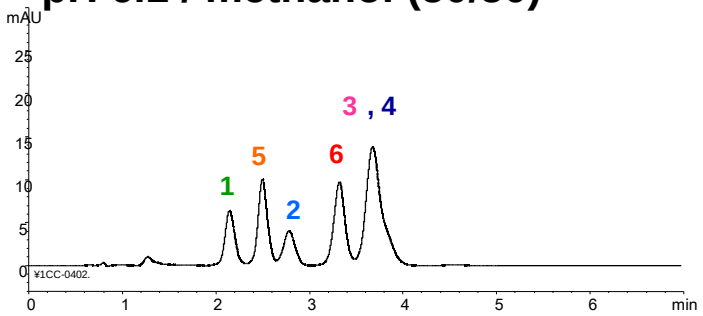
pH 6.1 / methanol



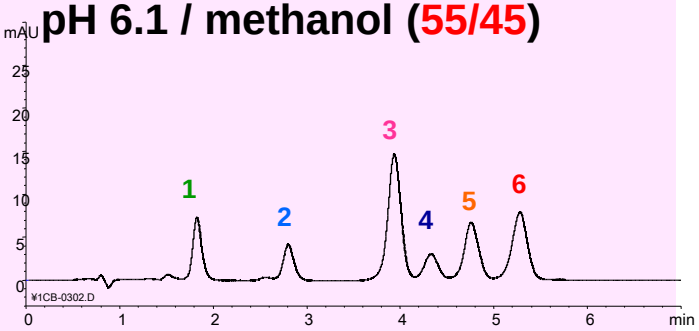
pH 6.1 / methanol (50/50)



pH 6.1 / methanol (50/50)



pH 6.1 / methanol (55/45)



Only method scouting; 8 hr
Including optimization;

total about 9 hr !!

Summary of method scouting for reversed-phase chromatography

- Triart C18 with enough performance required as first choice columns and Triart C18 ExRS by which retention and resolution are affected in a positive way are capable of wide application.

- C18 columns and the other columns with unique selectivity, which are available as our products, can be used complementary each other.

- Initial separation condition has been rapidly (within 9 hours) and successfully developed by building a way of method scouting with combination with Triart, pH of mobile phase and type of organic solvent.