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Overview of preparative HPLC

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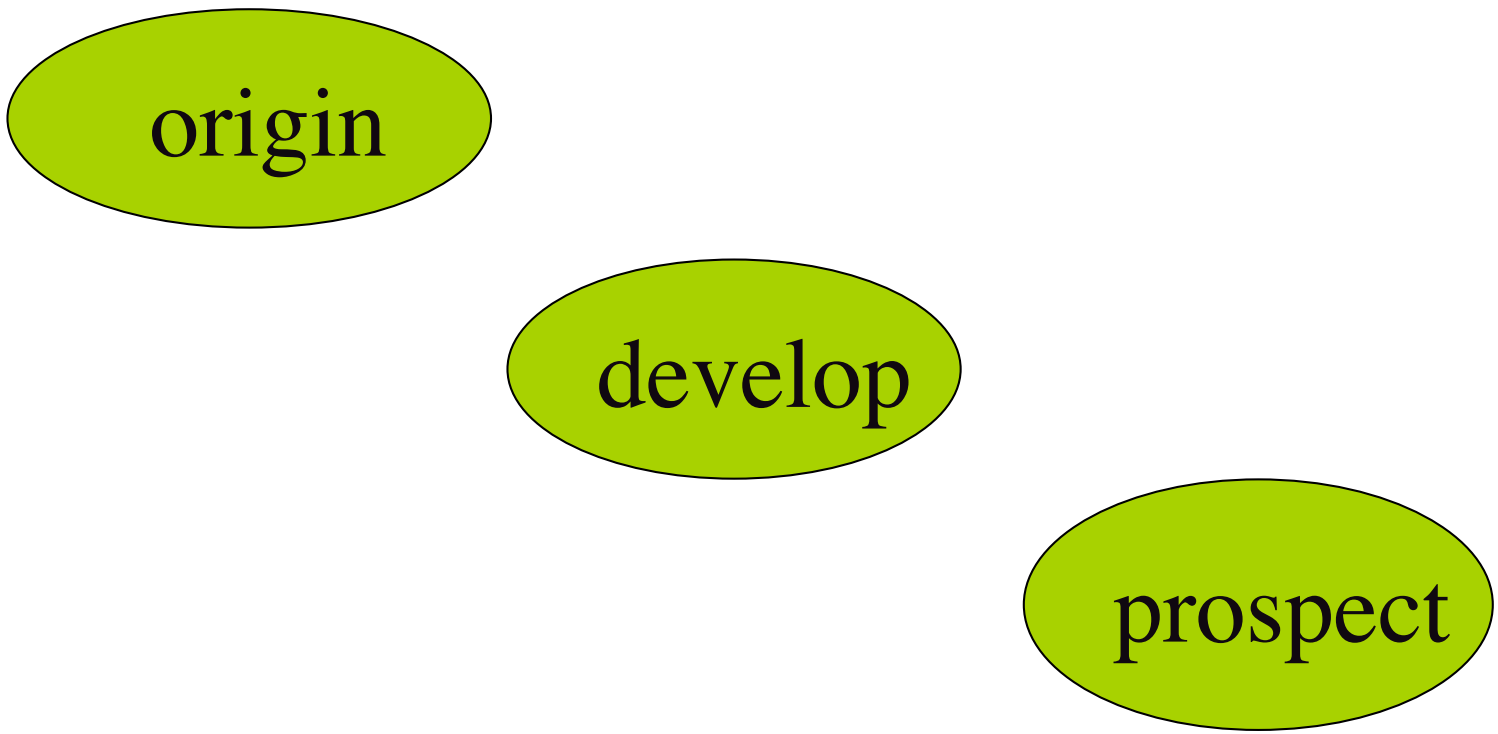
RESUME

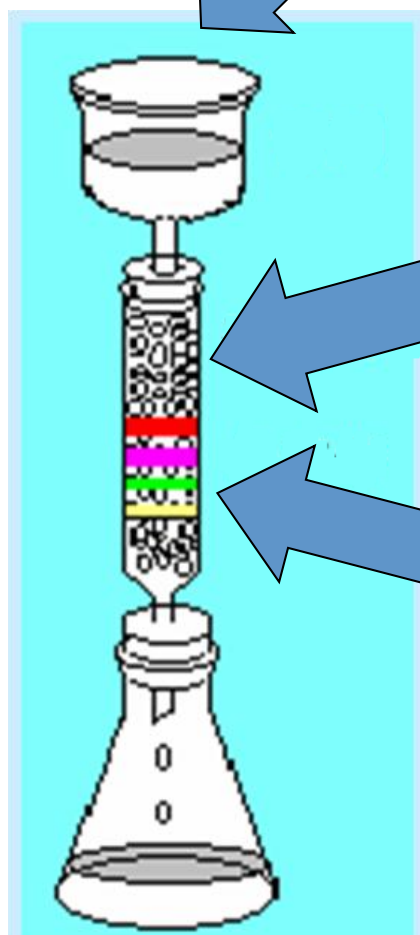
- Review of liquid phase chromatography
- ATL preparative HPLC system
- Industry purify HPLC system
- The case of application

1.Review of liquid phase chromatography



Tewett 1903

origin



Mobile phase

Stationary phase

Separation result

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Liquid-stationary chromatographic theory

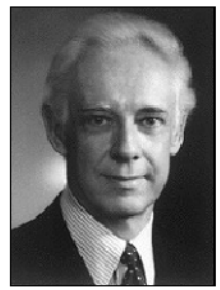
Paper chromatography and plate theory

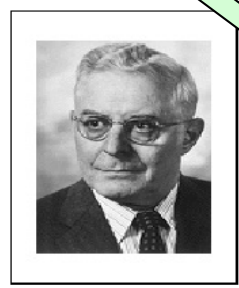
Kuhn

AA analyzer

Martin & Synge

Develop





Stanford Moore & William Howard Stein

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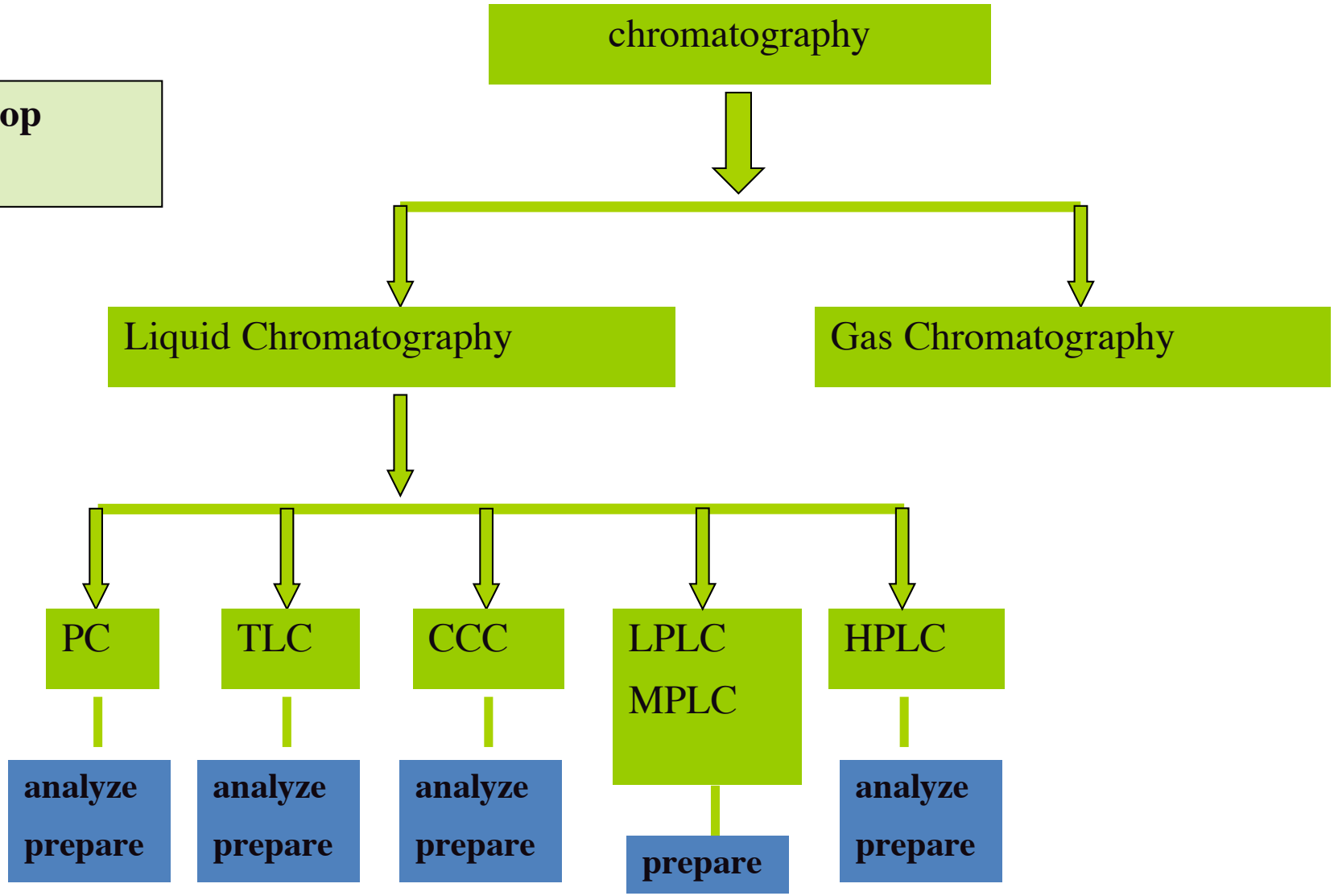
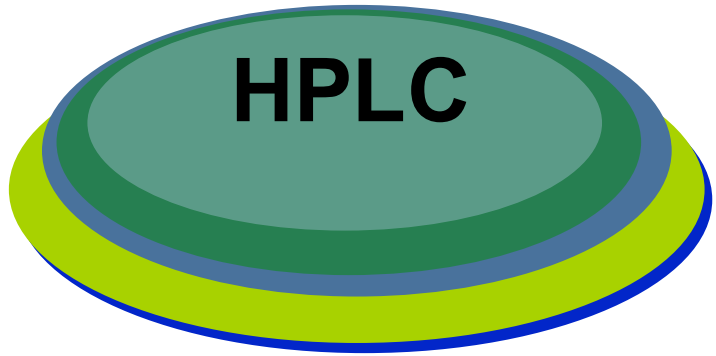
Chromatography develops chronology

1903's propose chromatographic method
1931's liquid- stationary absorption chromatography
1940's paper chromatography
1950's thin-layer chromatography
50-60's fast develop period of GC
70's HPLC
1975's ion-exchange chromatography
80's rip period of HPLC
80's development of capillary electrophoresis
2003's UPLC

develop

develop


chromatography
Liquid Chromatography
Gas Chromatography
PC
TLC
CCC
LPLC
MPLC
HPLC
analyze
prepare
analyze
prepare
analyze
prepare
prepare
analyze
prepare



develop

Analyze
qualitatively
quantitatively
analyze the
consist of sample

prepare
extract
separate
purify
the valuable
compound of row
material

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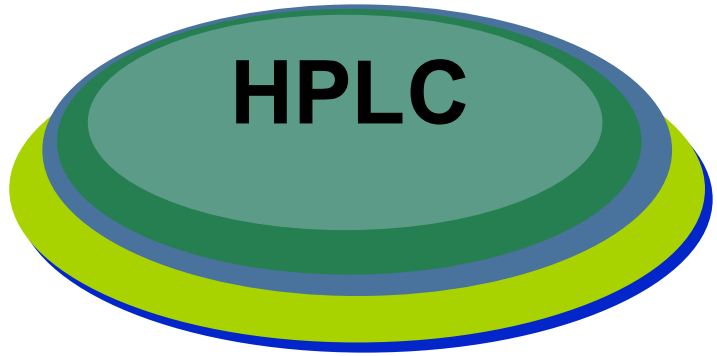

prospect

analyze

prepare

High /Ultra Performance Liquid Chromatography (H/UPLC)

Dynamic Axial compression (DAC)

Simulated Moving Bed (SMB)

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2.ATL preparative HPLC system

From analysis to preparation

Correlation technique of preparation

ATL instrument system

From A To P

Preparative high pressure chromatography

has no essential difference with traditionally classical column chromatography. It has higher efficiency and achieves automatic operation. About the instrument, a high pressure pump is adopted to deliver mobile phase. At the same time, a detector is connected to the outlet of the column which can detect the fraction on-line constantly.

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From A To P

The preparative high pressure chromatography has several features as following:

1.shortter column length;

2. larger column ID;

3. higher flow rate.

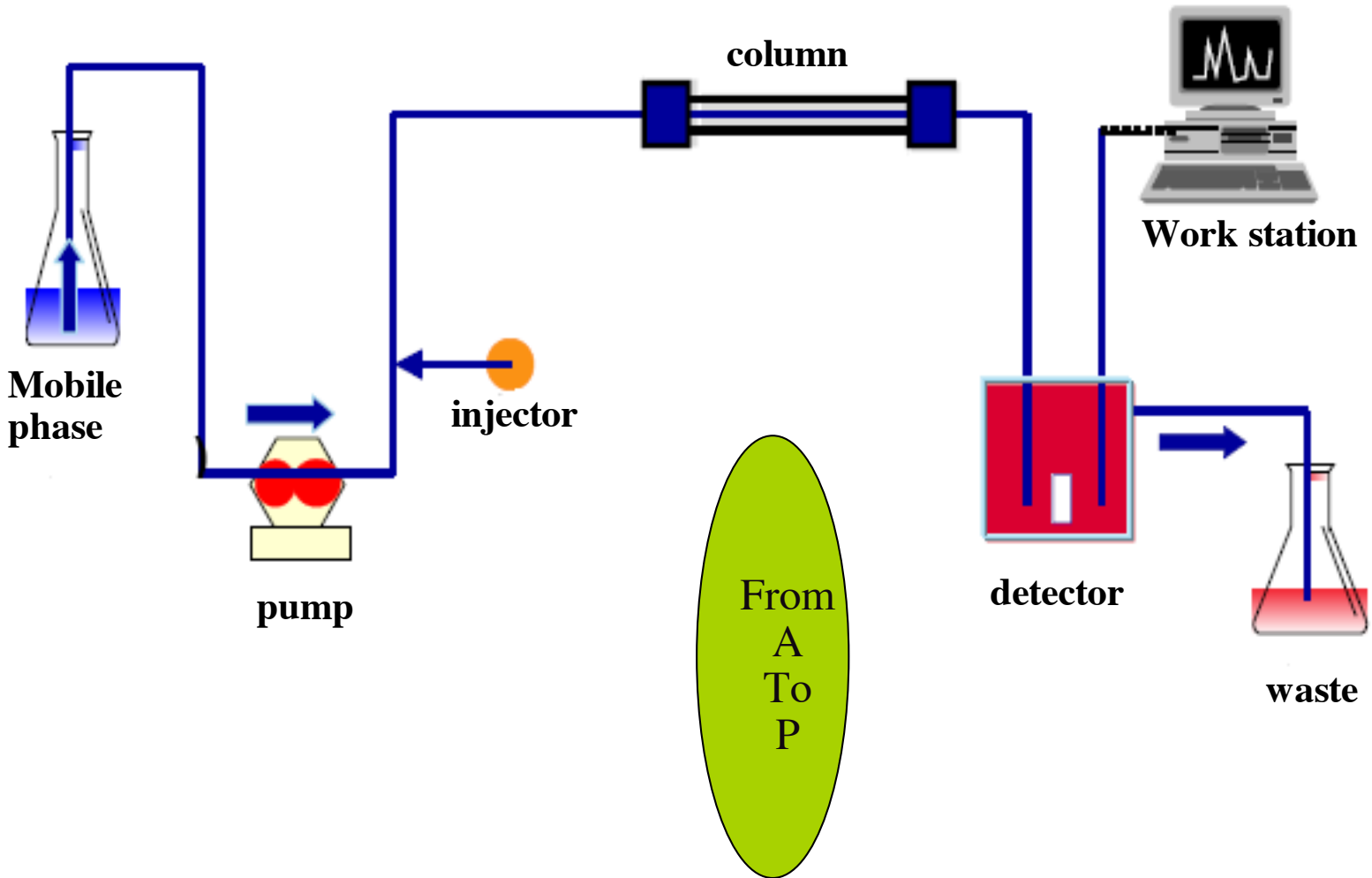
Sometimes it is also called High performance preparative liquid chromatography

The difference and relation of analysis and preparative chromatography

	analysis	preparative
Applied area	qualitatively analyze: sensitivity quantitatively analyze: resolution, repeatability	Extract, separate, purify compounds from mixture of row material Obtain specific fraction with high purification from the sample; pilot preparation and low-cost preparation
Particle size	≤5um	≥10um
Flow rate	0.001-9.999ml/min	100~80000ml/min
Sample size	0.01-2mg	1-500000mg
Capillary ID	1/16”	1/8”~1/4”
Injection method	Inject valve	Inject valve and inject pump
Mobile phase	No recovery	recovery
Separate way	linear	Non-linear

From A To P

Typical analysis HPLC system



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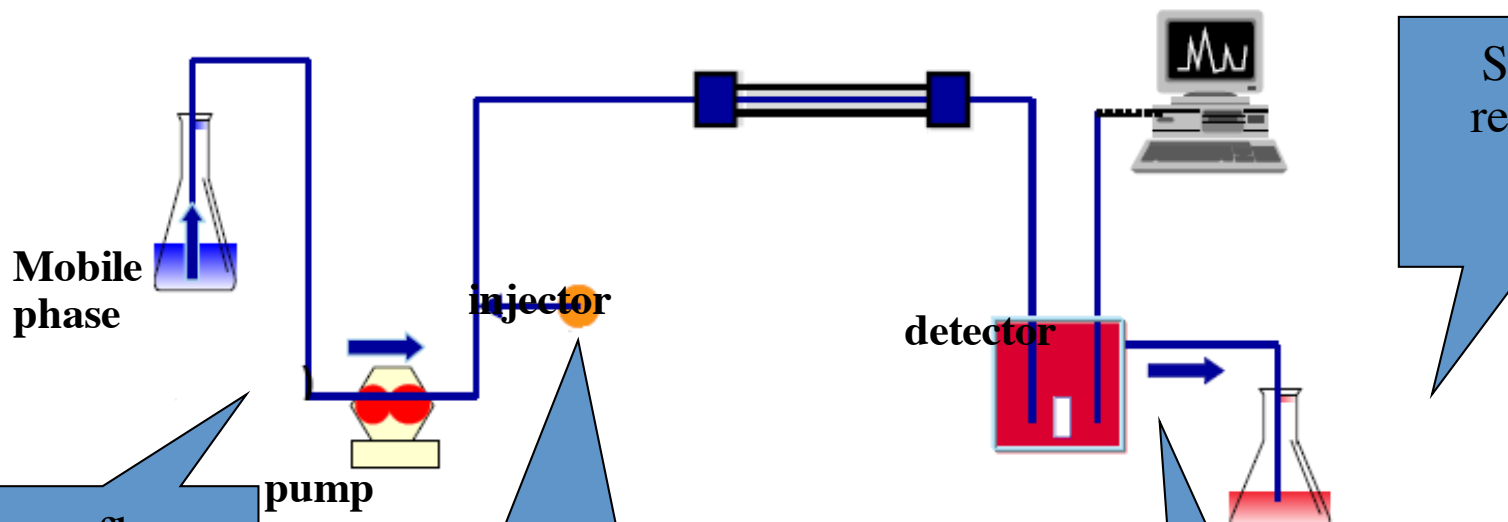
Preparative system

Larger tank

≥10mm flange column DAC

Work station

column



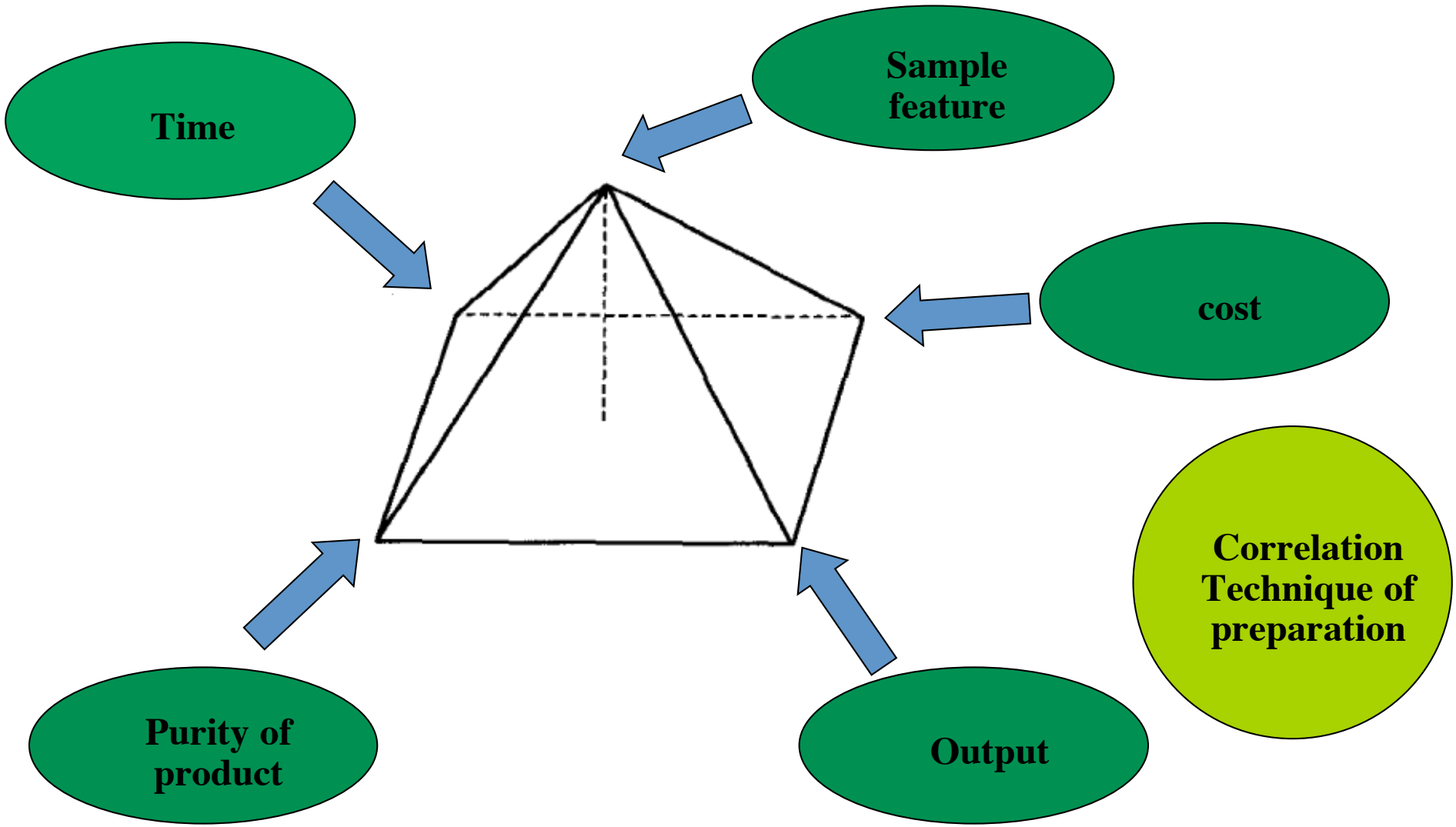
Solvent recovery

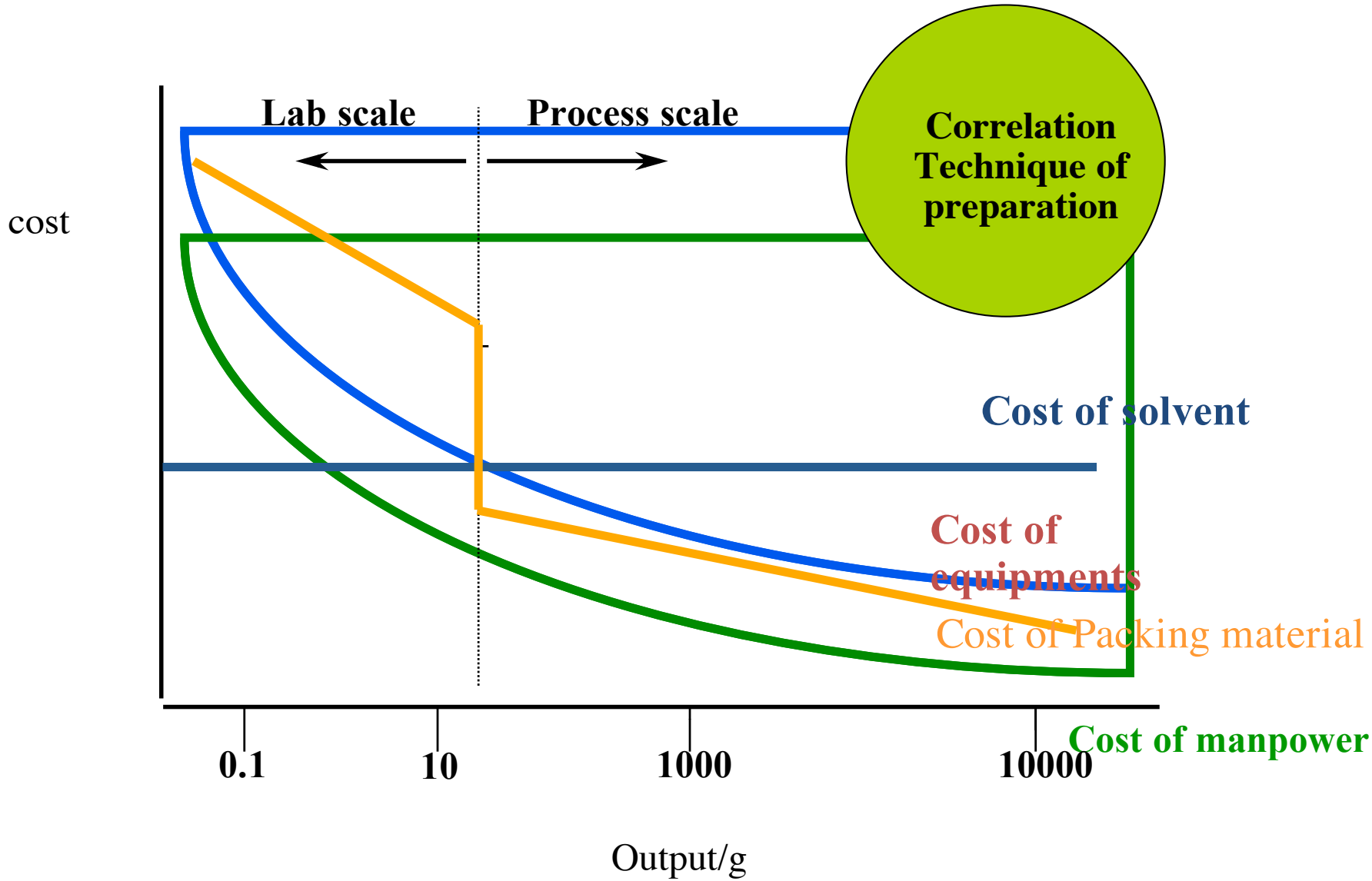
Larger flow rate pump ≥50ml

Sample loop ≥1ml
Injection pump≥50ml

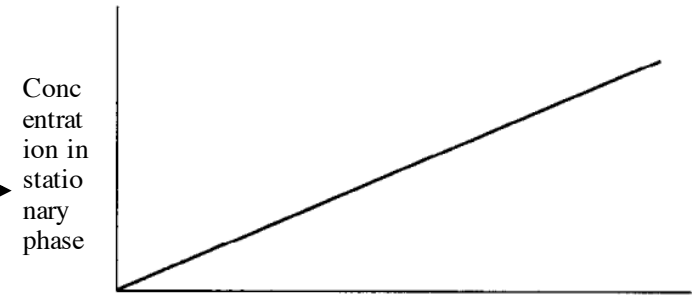
Bigger volume flow cell

The five elements for preparative process



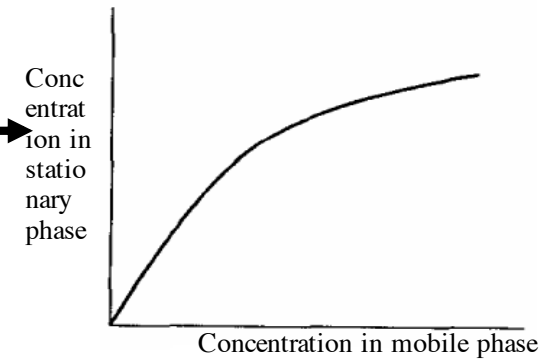

Lab scale
Process scale
Correlation Technique of preparation
cost
Cost of solvent
Cost of equipments
Cost of Packing material
Cost of manpower
0.1
10
1000
10000
Output/g

Analysis → **Linear chromatography** →

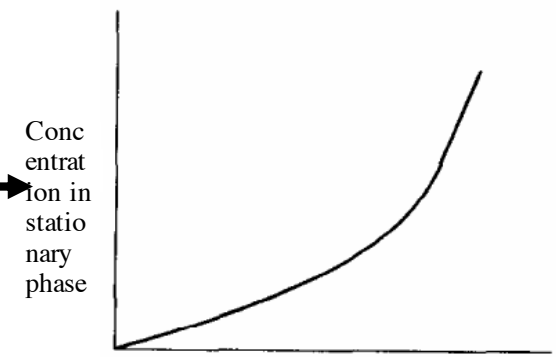


Correlation Technique of preparation

preparation → **Non-Linear chromatography** →

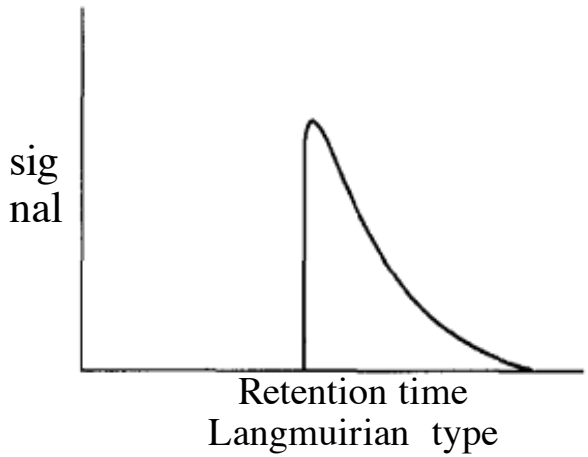


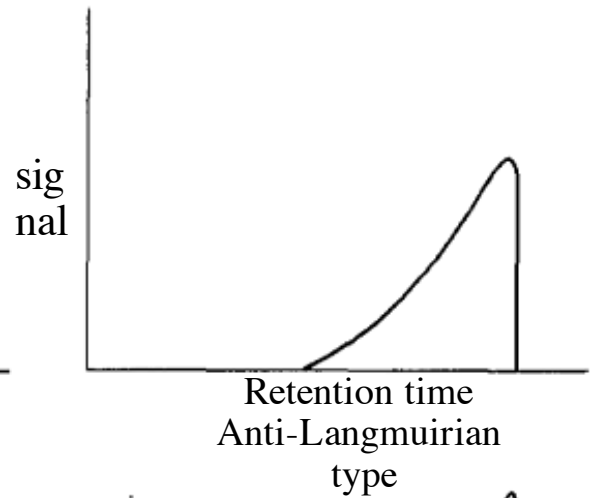
Langmuirian type

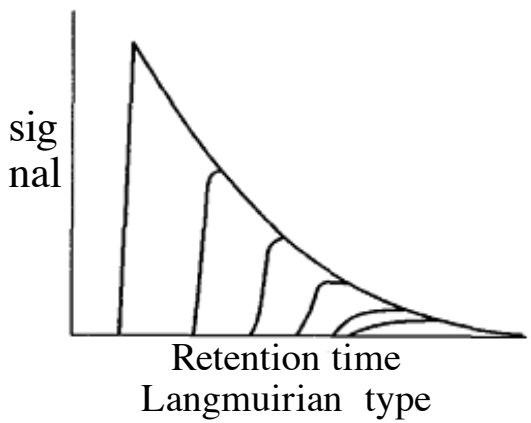


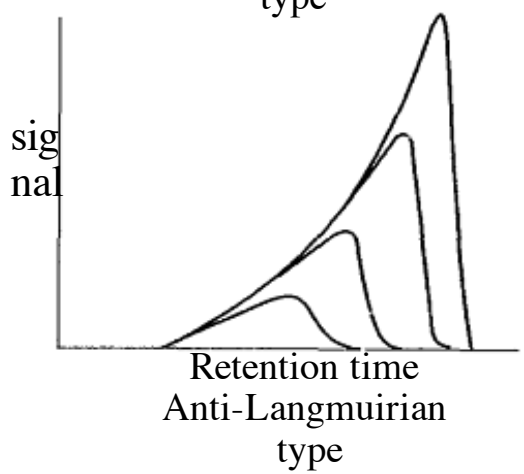
Anti-Langmuirian type

The peak shape of preparative chromatography









Correlation Technique of preparation

Asymmetric peak shape

The retention time of peak is modified by increasing sample concentration

The peak height (or peak area) is not proportional to sample concentration

Feature Of Non-linear chromatography

Correlation Technique of preparation

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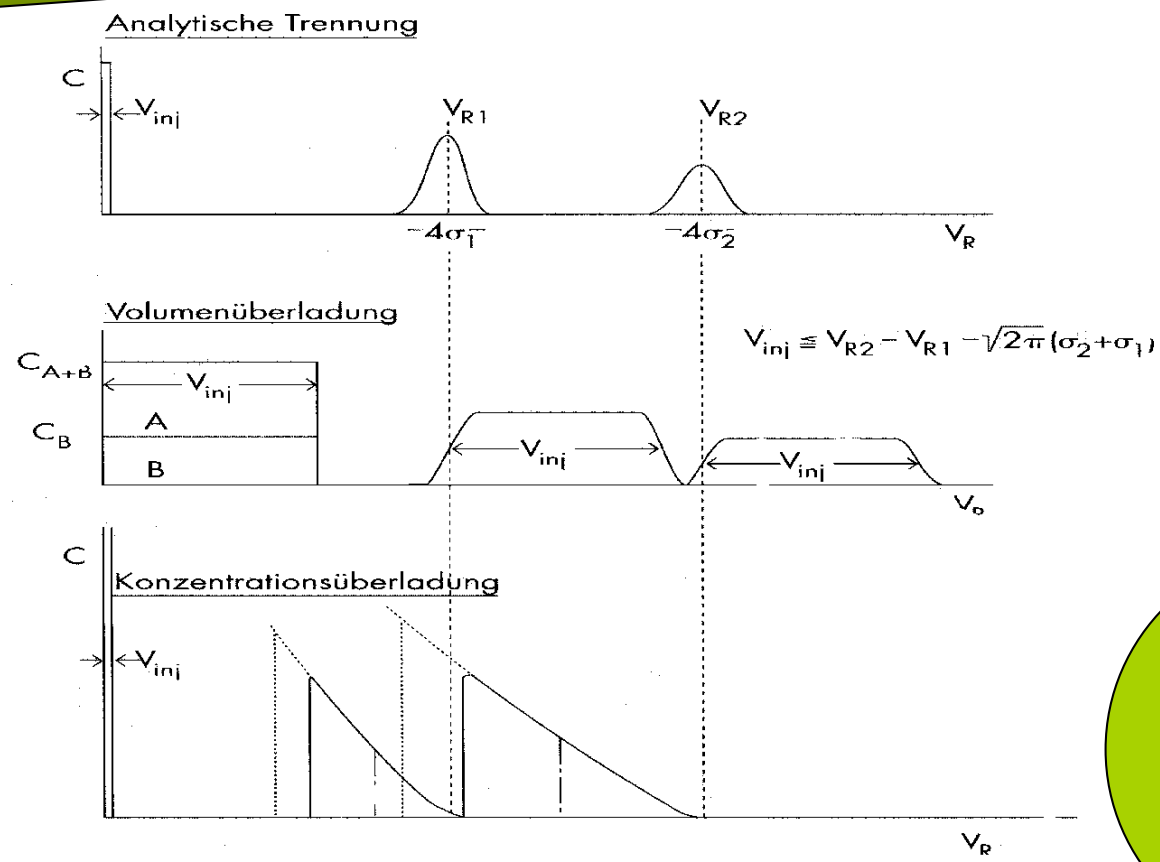
The most important thing in preparative chromatography:

Sample overload



Correlation Technique of preparation

Langmuir Type: the capacity factor decreases; the peak shape changes from Gaussian curve to triangle

Invariant peak height;

the peak width increase and present rectangle

Concentration overload

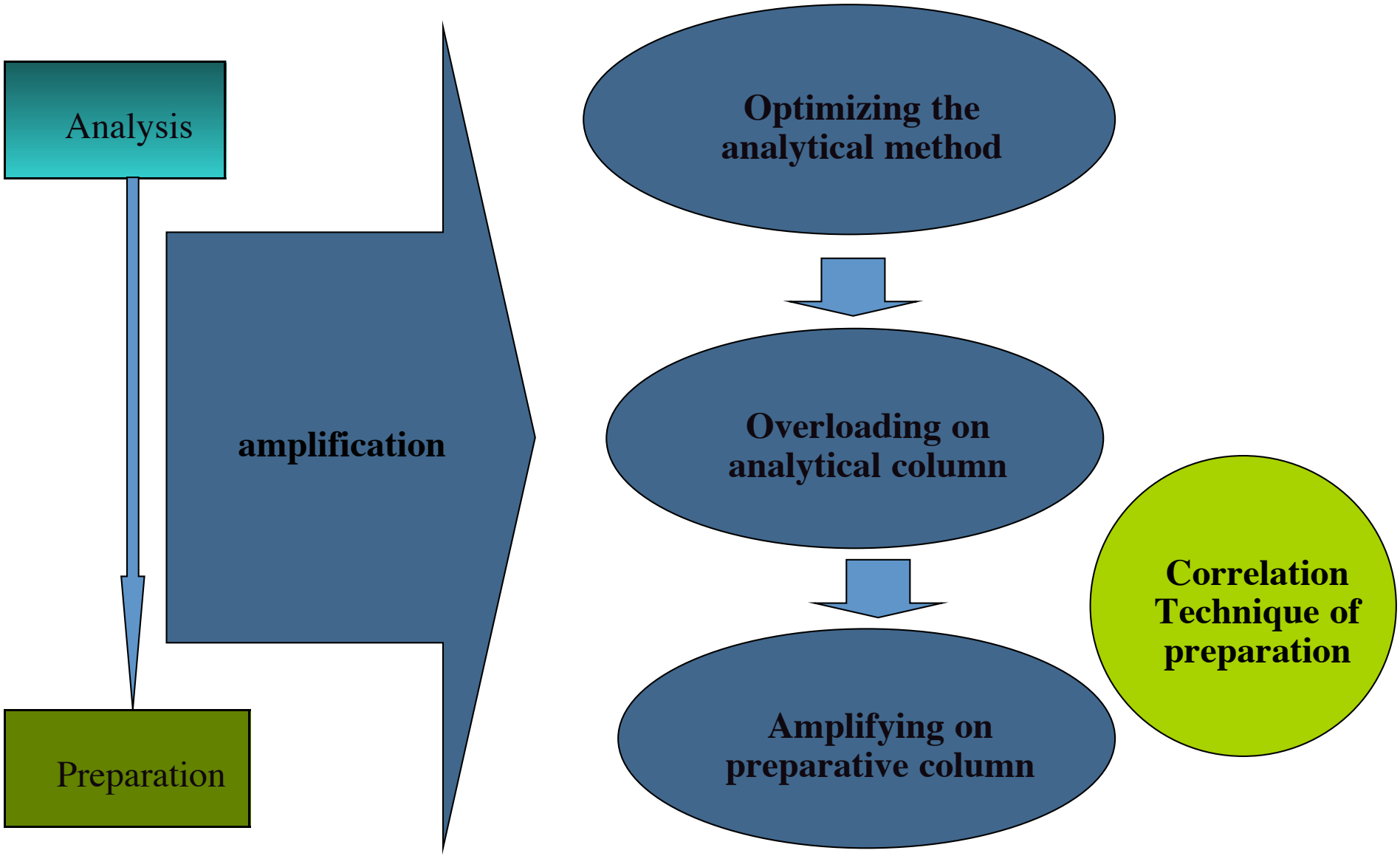
PK

Volume overload

Correlation Technique of preparation

Increasing sample concentration and keeping the inject volume invariantly; the sample size depends on the solubility of sample in mobile phase; the output depends on selectivity and the particle size of packing material has little influence on it

The sample size depends on inject volume; the output depends on column ID; it needs small particle size packing material


Analysis
Preparation
amplification
Optimizing the analytical method
Overloading on analytical column
Amplifying on preparative column
Correlation Technique of preparation

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Key point 1

Adjust sample size:

$$mp/ma = lp/la * rp^2/ra^2$$

L: column length；m：sample size；
r：column ID p: preparative column；
a：analytical column

Correlation Technique of preparation

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Key point 2

Adjust flow rate：

$$Fp/Fa = Vp/Va = Lp/La^{2} * rp^{2}/ra$$

F ：flow rate ；V:column volume；
L: column length；r：column ID；
p: preparative column；a：analytical column

Correlation Technique of preparation

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Key point 3

Adjust gradient：

tp/ta = Vp/Va * Fp/Fa

F ：flow rate ；V:column volume；
t:gradient elution time；
p: preparative column；a：analytical column

Correlation Technique of preparation

Analytical Technologies 3000 Series
HPLC System

10ml 3000 Series
analytical HPLC
system

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3000 Series Semi PREP system

50ml binary 3000 Series **Semi-preparative** system

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6000 Series PREP system

100~1000ml 6000 Series **Preparative** system

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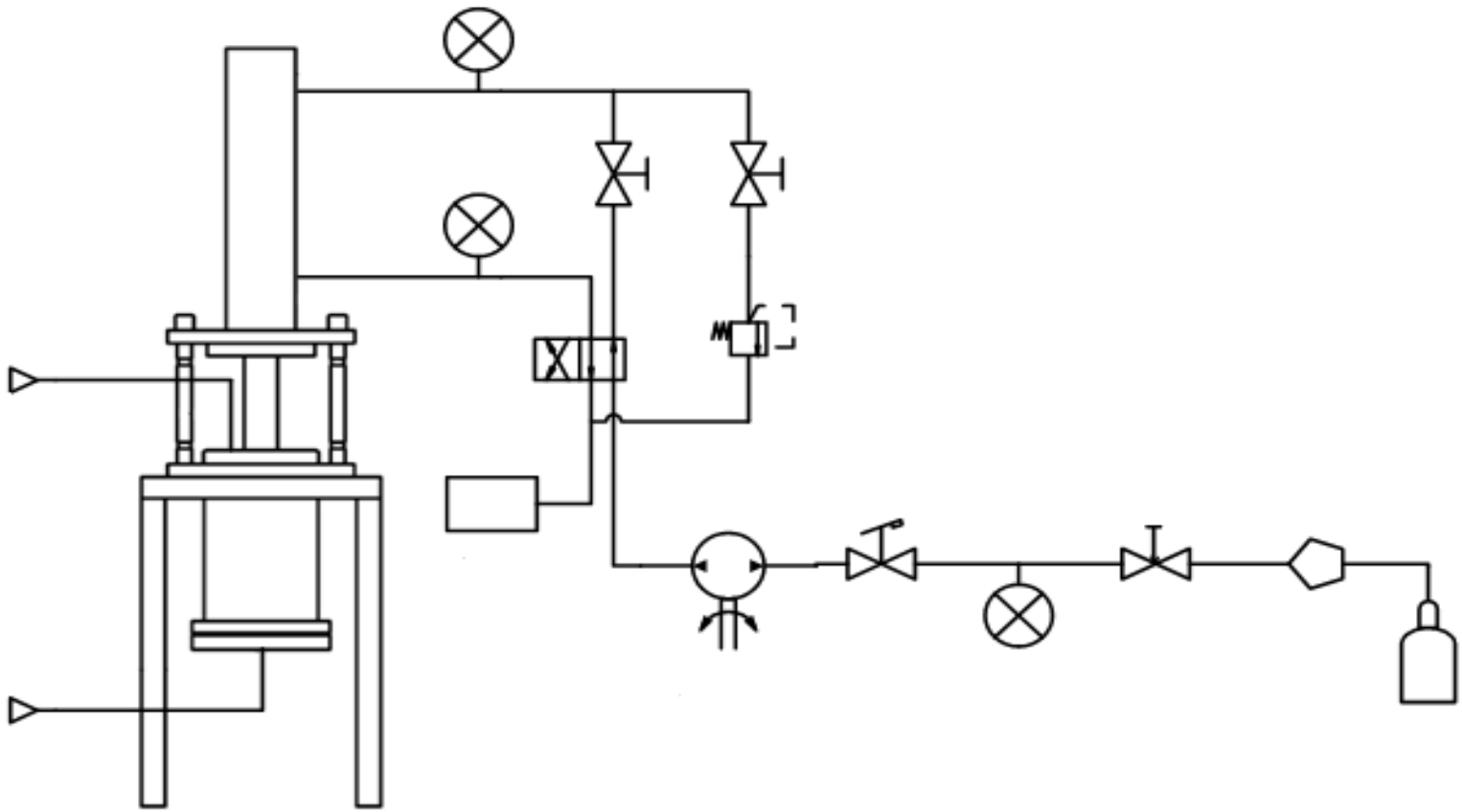
6000 Series
100~1000ml
Preparative
system

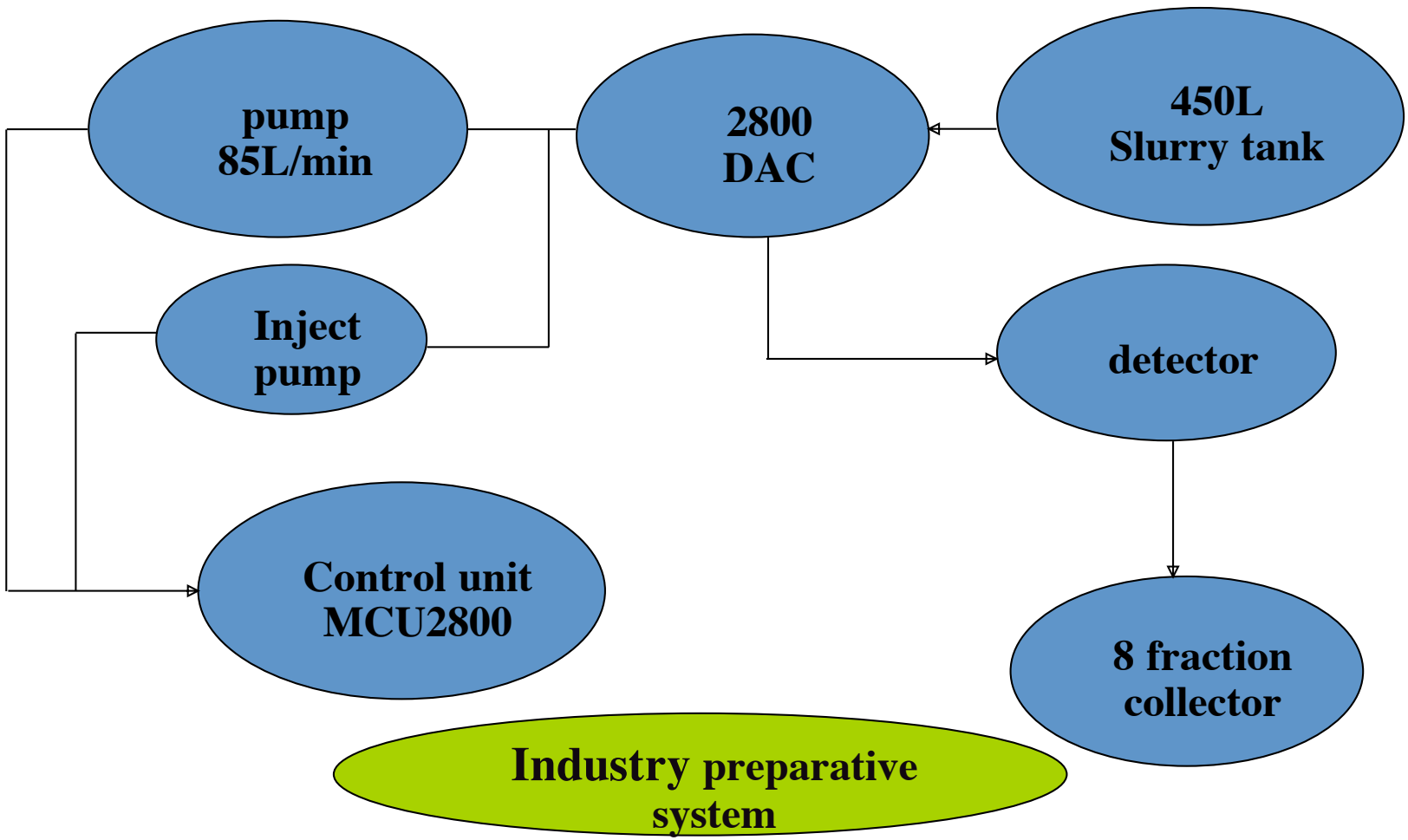
50~200ml
DAC

PREP with DAC system

3.Industry preparative system



Composition of 2800 DAC system



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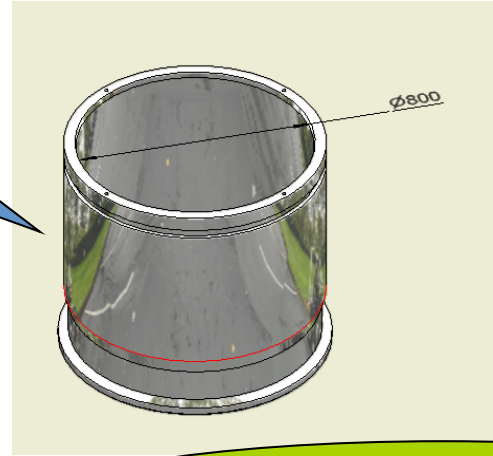
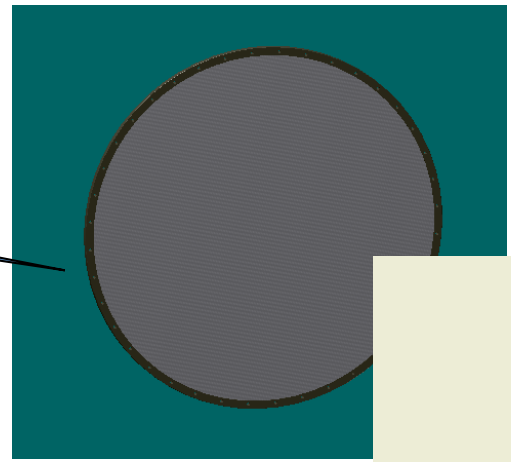
Column body

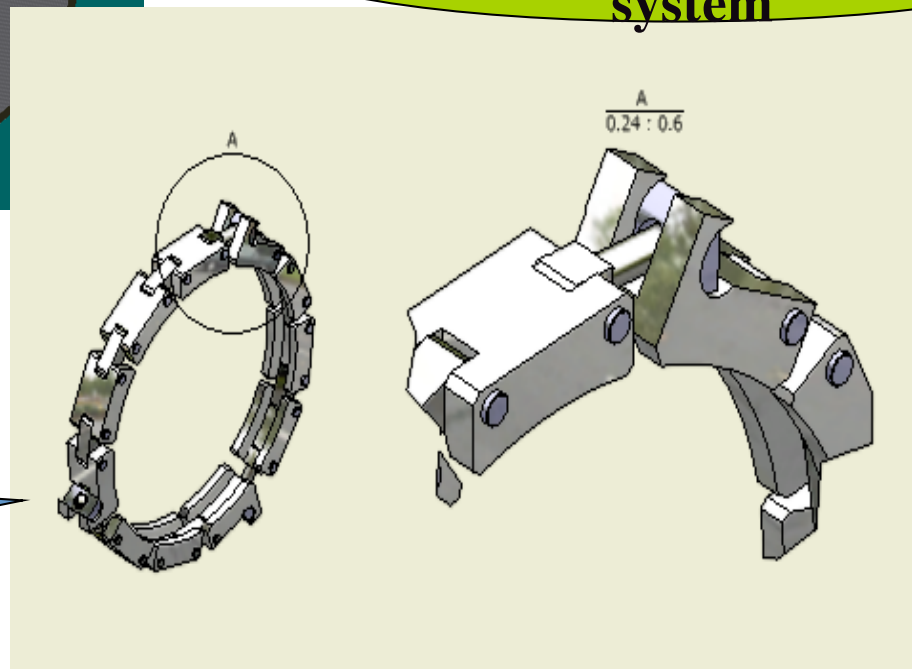


Sieve plate



Industry preparative system

Flange of column bottom



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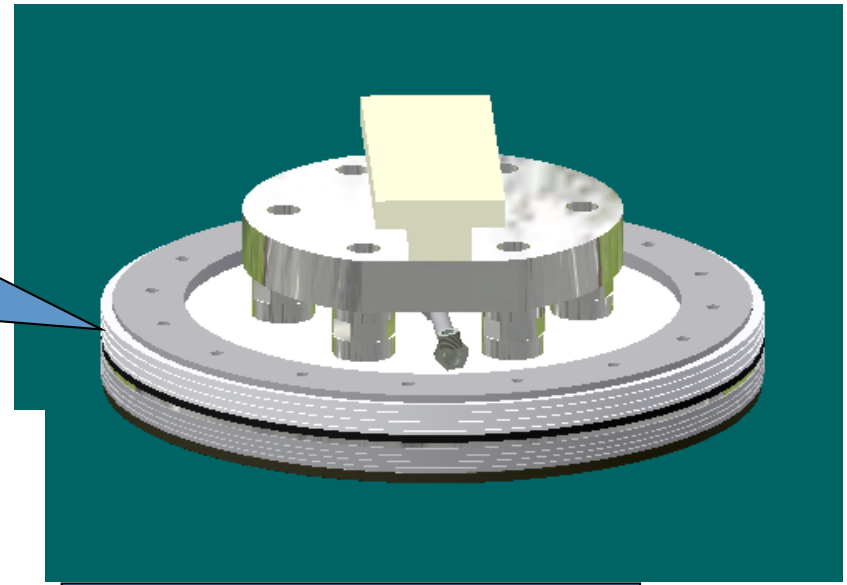
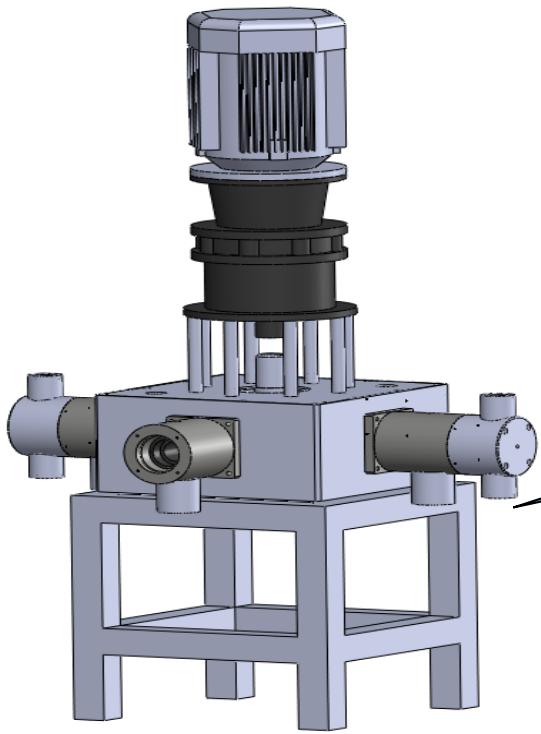
2800DAC
Piston head





80L
pump

Industry preparative system

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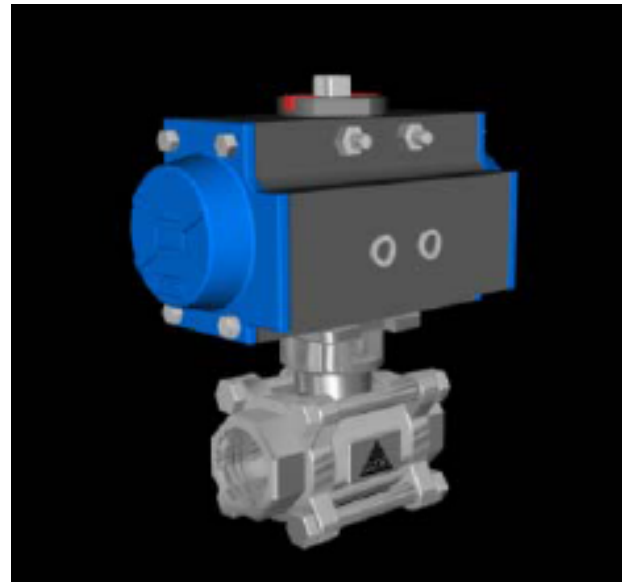
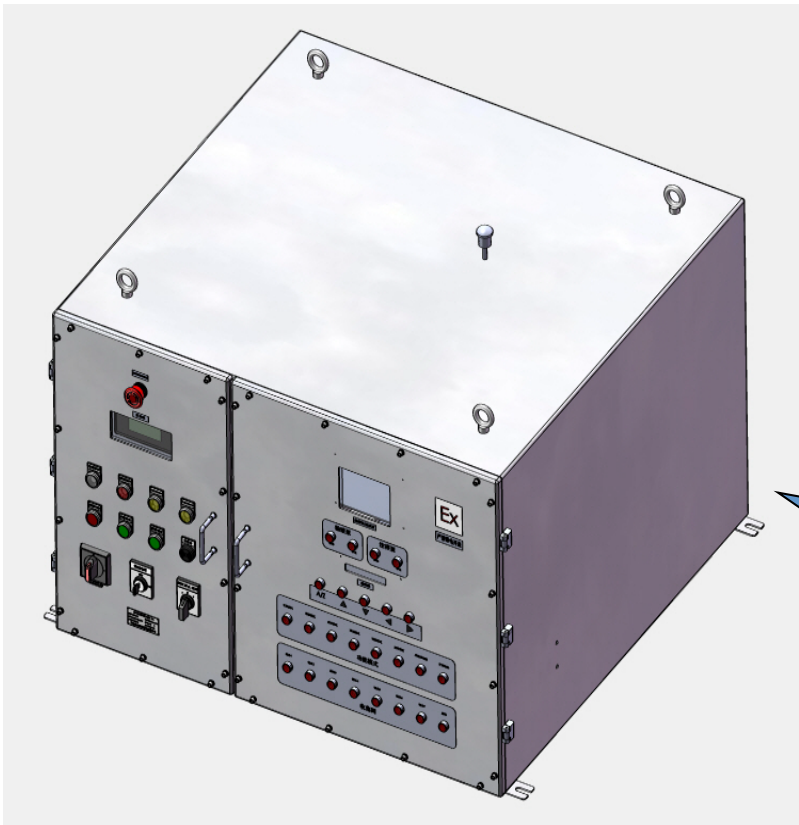
Pneumatic collector valve





explosion-proof equipment

Industry preparative system

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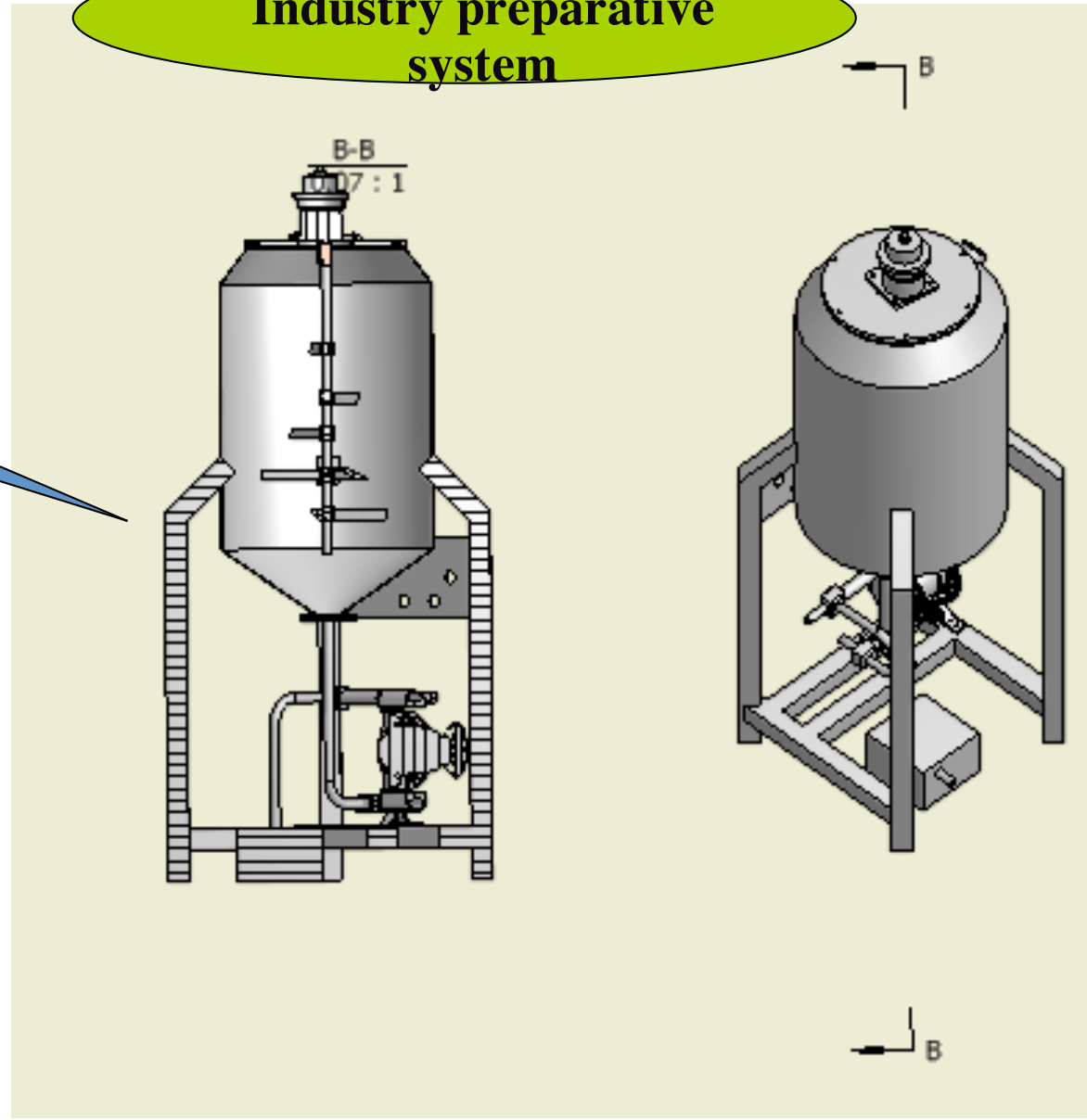
Industry preparative system

Automatic Slurry tank



4.A case of application

Step1:
Meet the requirements of custom

example

Sample ：
The row material contains 68% target compound. It is white solid powder and stability for hot and light (can tolerate 100°C).

Custom requirement：
After the purification, the purity must reach greater than 99%; the single impurity must reach less than 0.8%; the recovery must reach 70%.

Step2:
The analysis of original sample

example

You must have an analysis method to detect the purity of the fraction and final product.

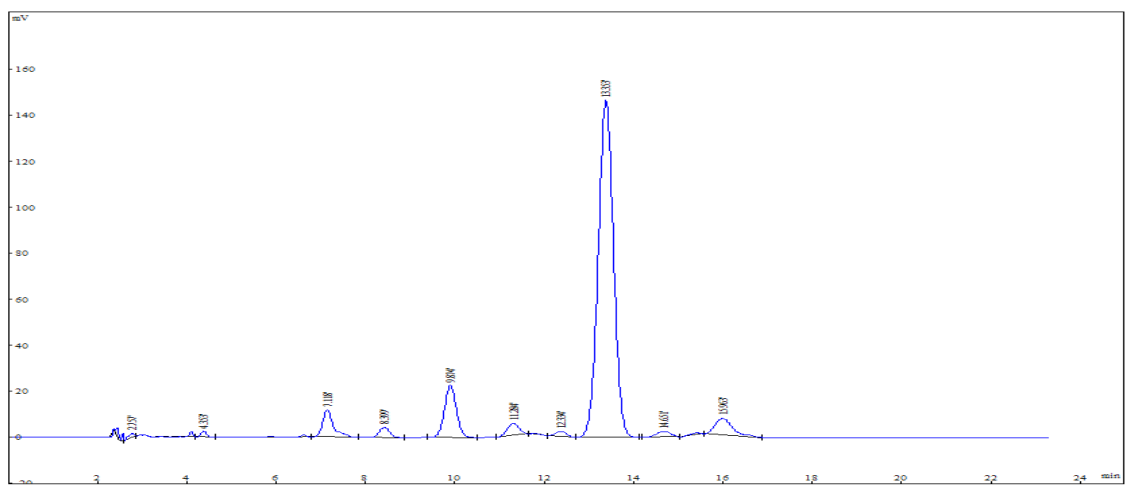
A case of application

example

Analytical condition：
methanol：acetonitrile：water=81：12：7
Wavelength ：246nm
Flow rate：1.0mL/min
Column ：C18， 5μm， 4.6*250mm

A case of application

result



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Step3:
Simulate preparation on analytical column

Particle size of packing material≥ 10um 4.6*250mm

No nonvolatile acid, alkali and salt in mobile phase

Select a suitable composition of mobile phase

Overloading when target and impurity has good resolution

Divide the target peak to about ten fractions and collect them

Detect the purity of the fraction and calculate the recovery

Further study of optimization

A case of application

example

Study of preparative method

Mobile phase

90%methanol、75%methanol、75%acetonitrile、70%acetonitrile、60%acetonnitile。

select

60%acetonitile

Packing material

Four kinds of different brand packing material

select

XX brand C8-10um-100A

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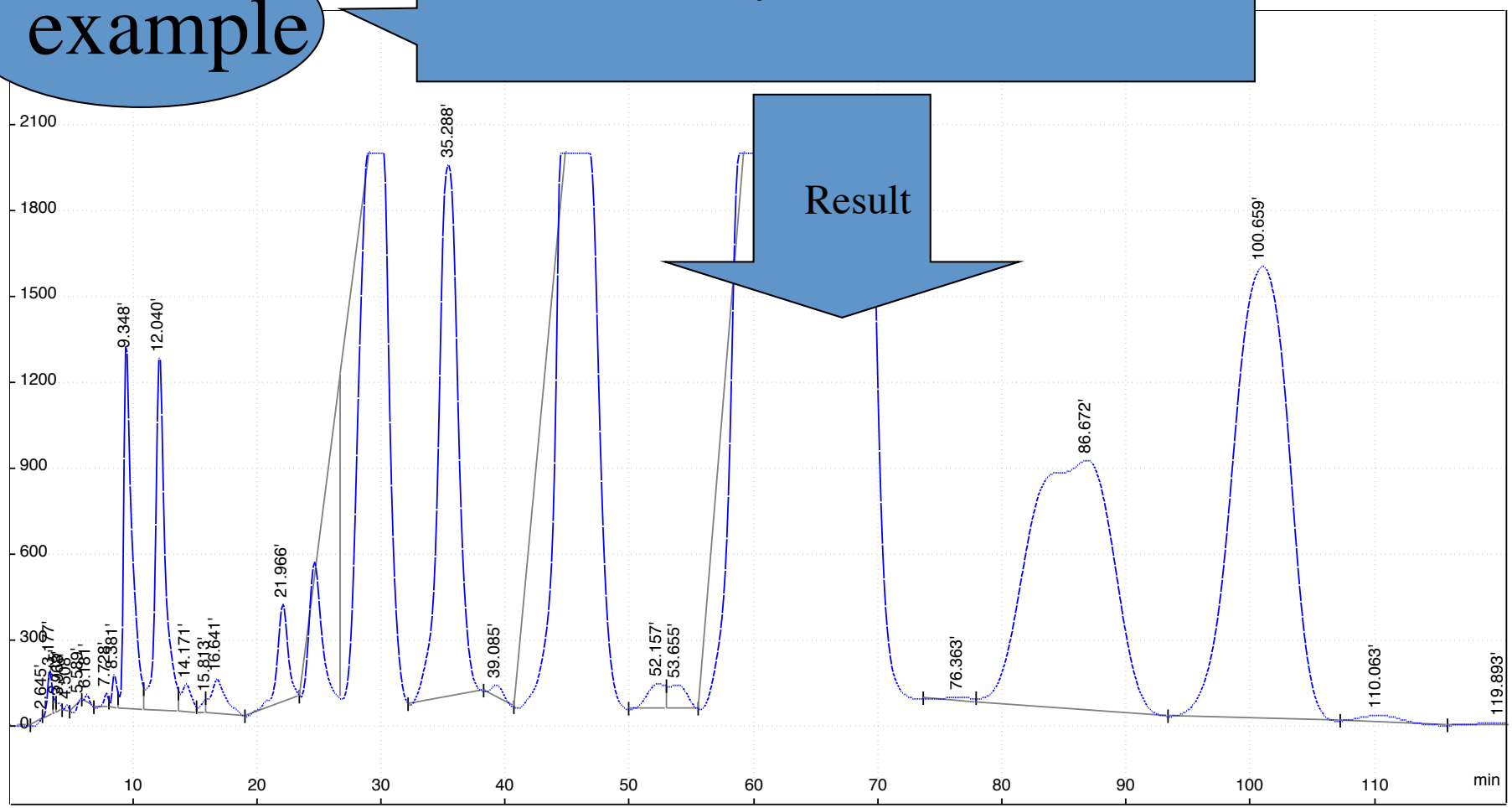
A case of application

example

The chromatogram of simulation on analytical column



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Step4:
Confirm the maximum sample size

Enlarge the sample size continuously until the recovery decreases obviously.
Theory sample size：3‰---8‰ of packing material

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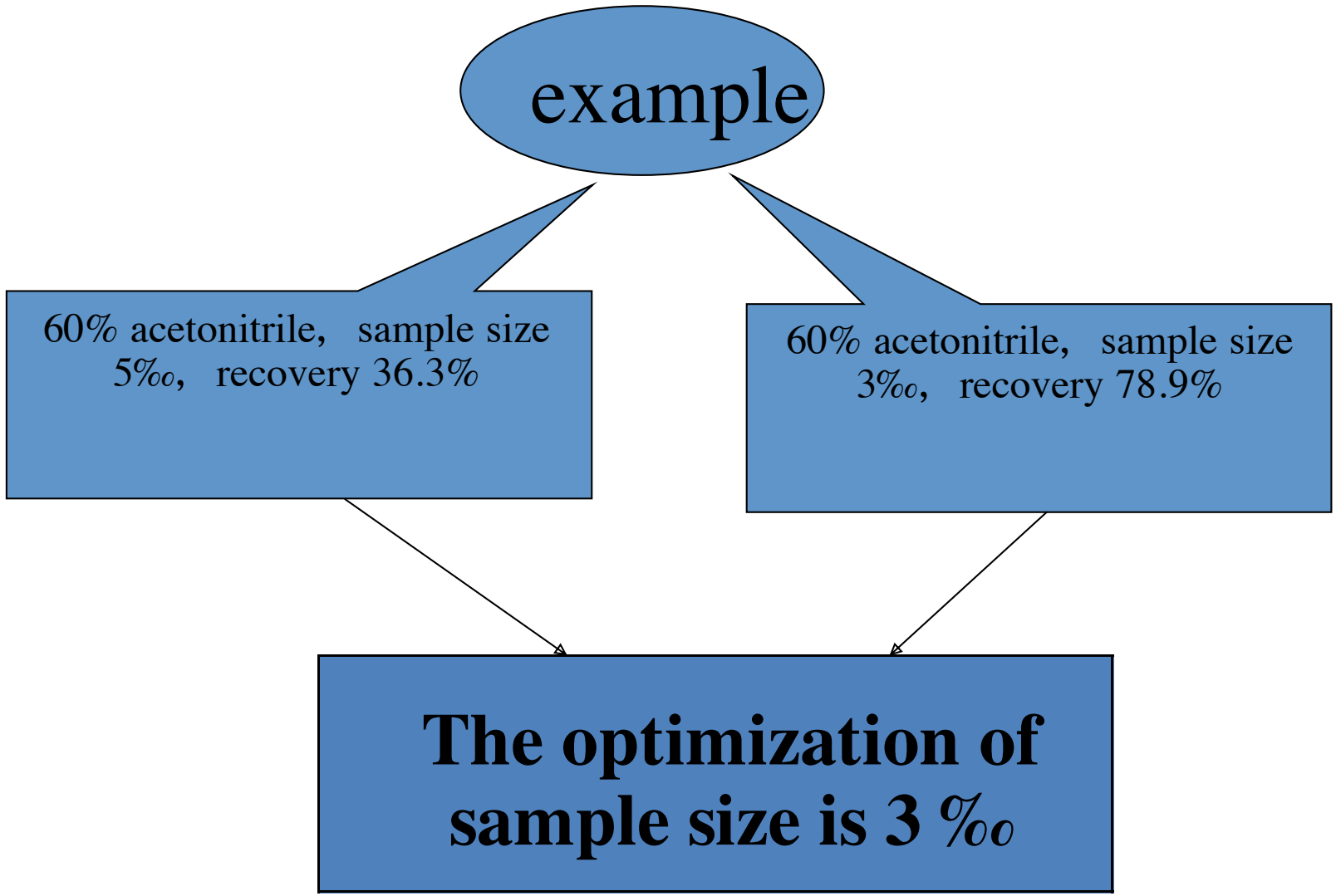
A case of application

A case of application



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A case of application

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The final preparative method：

Mobile phase：60% acetonitrile-water

Sample size ：3‰ of packing material weight

Packing material：XX brand-C8-10um-100A

Solvent of sample：mobile phase

Step5: Amplification

Linear amplification of the simulative preparative method on analytical column to preparative column

The formula is in front; the column size is decided by sample size

Divide the target peak to about ten fractions and collect them

Detect the purity of the fractions and calculate the recovery

Repeat this process several times, after the technology is mature, the target peak can be divided to only 4 to 6 fractions

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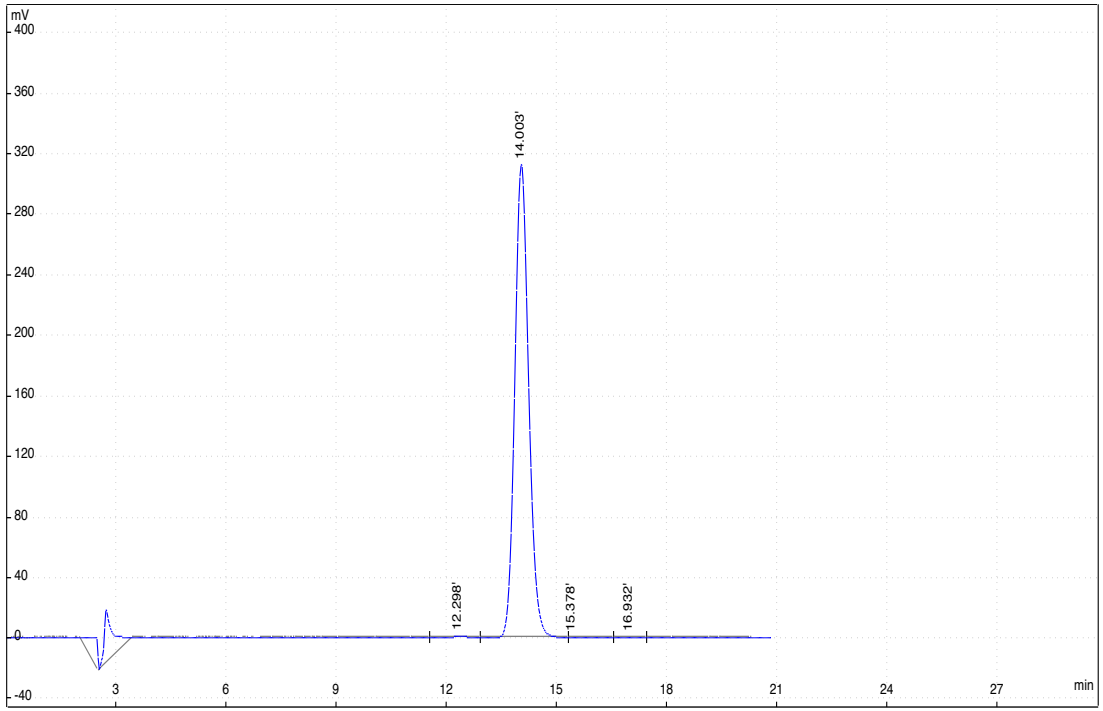
Chromatogram of the purification result



NO.	Retention time	percentage	peak area	resolution	theory plate	capacity factor	tailing
1	12.298	0.5167	42956	2.32	4218	-0.000	1.36
2	14.003	99.3	8255744	1.71	6614	0.139	1.14
3	15.378	0.1307	10865	1.74	4727	0.250	0.58
4	16.932	0.04702	3909	1.70	6200	0.377	0.95
total		100	8313474				

A case of application

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Optimization of preparative method is endless!

Thank you
For your attention!

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