Use and Maintenance of HPLC column

Welch Materials, Inc.

Email: info@welchmat.com
Web: www.welch-us.com

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Part One

- Reasons of abnormal use of HPLC columns

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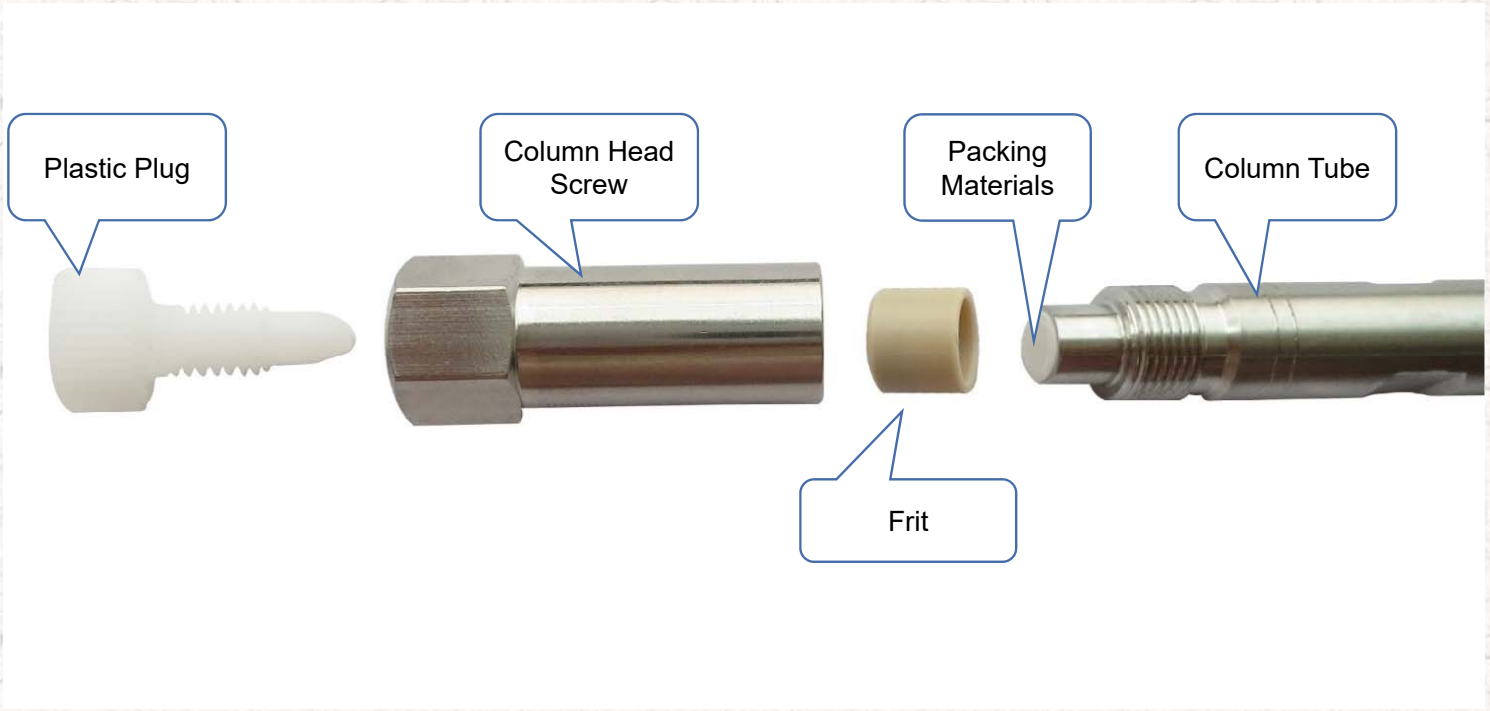
HPLC column Structure

- Appearance



Bridging Separation Techniques

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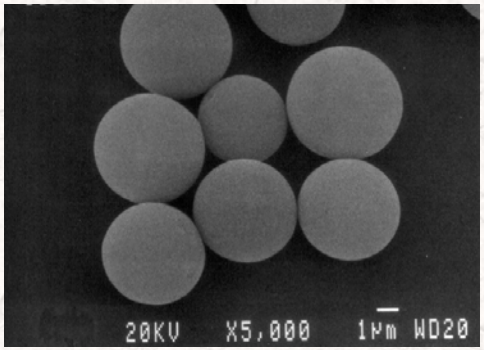
HPLC column Structure

- Packing Materials



SEM Picture of
Packing Materials

Appearance

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HPLC column Structure

- Packing Materials

Picture of column tube
(without column head screw)

Clear picture of Frit

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HPLC column parameters

- **Column length, Inner diameter(ID):** e.g. 250×4.6 mm etc.
- **Particle size:** e.g. 1.8μm, 3μm, 5μm, 10μm etc.
- **Pore Size**: e.g. 60Å , 120Å, 200Å, 300Å etc.
- **Surface Area:** e.g. 180m2/g, 350m2/g etc. (Ultisil: 320m^2/g)

Physical Properties

- **Matrix**: Silica, Polymer
- **Silica gel surface property**
  - Carbon Loading: 10%, 20% etc. (Ultisil XB-C18: 17%)
  - Nitrogen Loading: 4%
- **Bonding phase:** C18,C8, CN etc.
- **Endcapping**: double endcapping
- **pH stability**: Xtimate pH range: 1.0-12.5

Chemical properties

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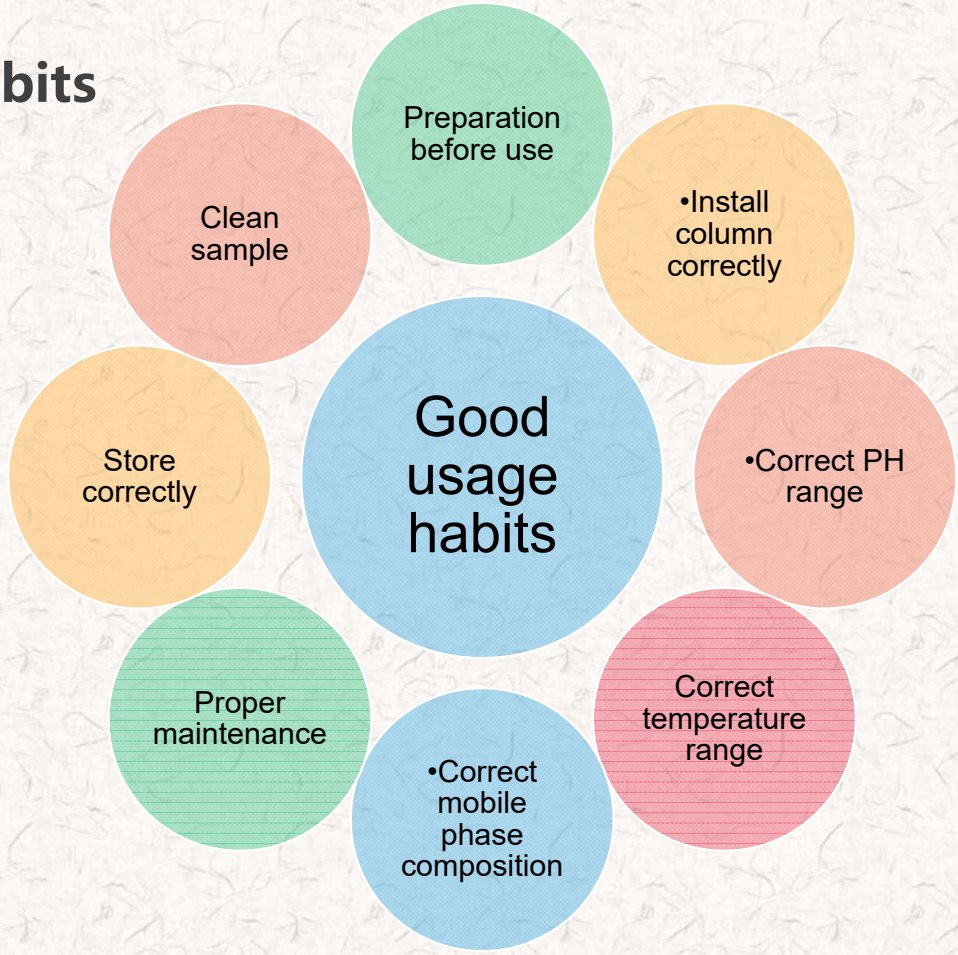
Good usage habits



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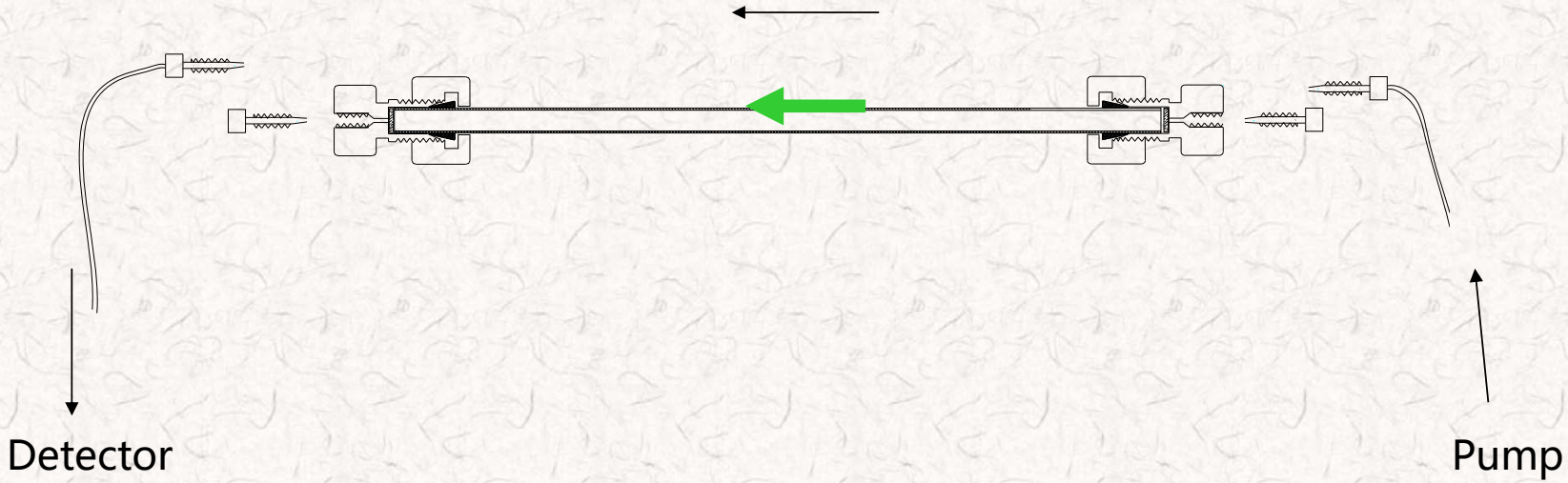
HPLC Column Installation



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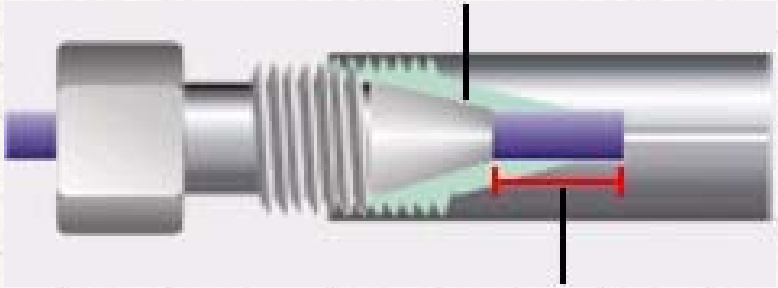
HPLC Column Connection

Nut slope does not match, poor sealing



If the length of the extended pipeline is too long, it may leak,

And the connecting piece may even get stuck in the column head

Dead volume

If the length of the extended pipeline is not enough, dead volume may be generated;

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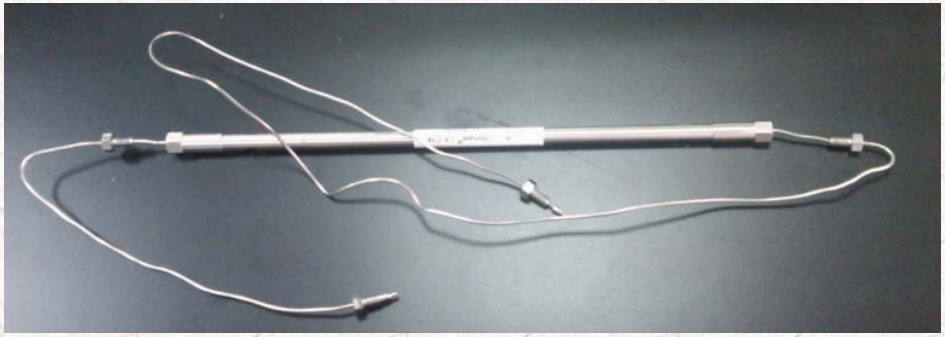
Customer's treatment after the connecting piece is stuck into the column



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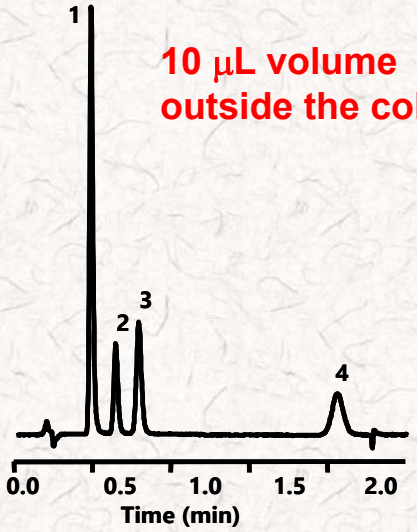
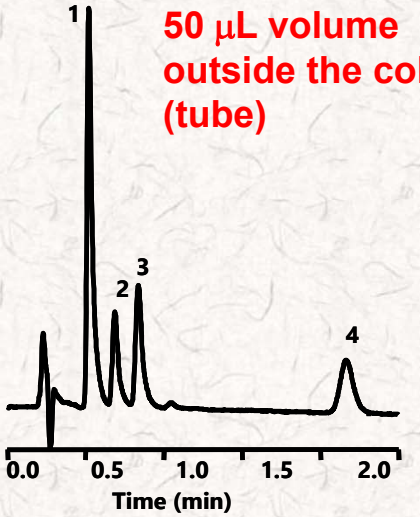
HPLC Column Connection

- Connection Tube



10 μL volume outside the column



50 μL volume outside the column (tube)

Sample:

1. Phenylalanine 2. 5-phenyl-3, 6-dioxo-2-piperazine acetic acid
3. Asp-Phe 4. Aspartame

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Incorrect use of guard column and inline filter

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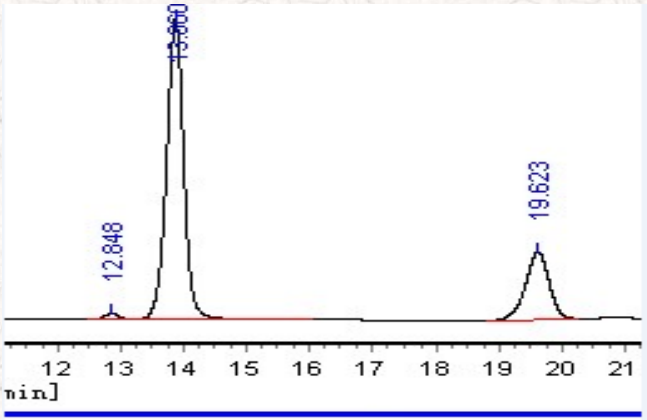
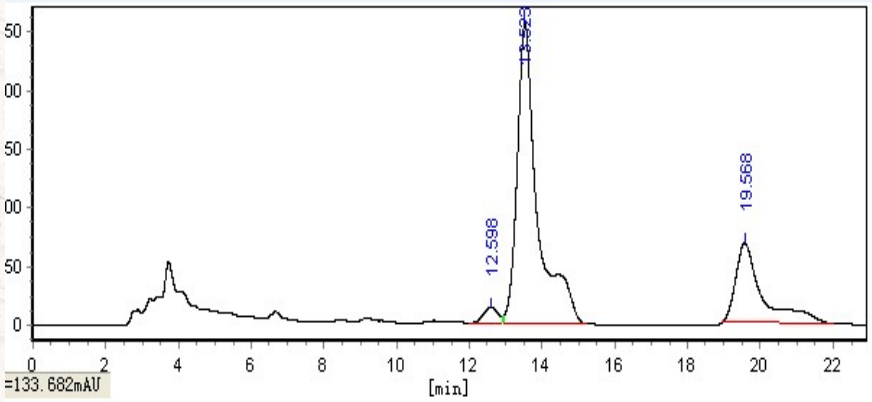


将柱参数保存到仪器条件中

容量因子	理论塔片	有效塔片	理论塔片/米
	13352	0	53
.07874	13301	71	53
.5273	13656	1628	55



柱参数： 柱长[mm] 250 死时间[min] 0.000 将柱参数保存到仪器条件中

	保留时间	半高峰宽	容量因子	理论塔片	有效塔片	理论塔片/米	有效塔片/米	分离度	拖尾因
	12.598	0.44575	0	4425	0	18	0	0	1.039
	13.523	0.46582	0.07342	4669	22	19	0	1.197	1.897
	19.568	0.59601	0.5532	5972	758	24	3	6.718	2.452

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When to change it?

- 150 Injections
- 1500 column volumes used for mobile phase

Depending on the chromatogram

- Column efficiency decreases＞10%
- Column pressure increased ＞10%
- Resolution decreased＞10%

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Two Questions when using a new column

Some columns' performance is bad just after being installed, the more used the better the performance;

Some columns' performance is good just after being installed, and it becomes bad after tens of injections.

Why?

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Priming Injection

■ Also called Saturation, Aging or Doping

Before the formal analysis, **pre-sample Priming** is required so that the Buffer and the organic solvents accumulate on the column and occupy the active sites in the chromatographic system such as the surface of the packing materials, frits, and pipelines.

Most compounds in reversed-phase chromatography interact with the active site, and their equilibration time is short, so the peak area and Retention Time is stable after the first injection.

But for the analyte with **large molecular weight**, especially those with **alkaline polarity (Basic Compounds)**, it is often necessary to do a bit longer conditioning.

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The method established after packing materials was modified

- Low pH leads to the hydrolysis of small end-capping reagents;
- The force between the ion pair reagent and the hydrophobic functional group is too strong;
- The silica gel matrix has weak dissolution;
- The packing materials absorbs substances of the same polarity;

etc.

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Why is it important to start method development with a new column?

- Depending on the usage conditions and sample cleanliness, most columns can undergo potential changes such as chemical contamination, surface changes, and partial Disturbance of the Silica material during use (Void).
- Such changes can lead to Deterioration of the packing materials in the column, Which reduces the separation selectivity of certain compounds. Such special separation selectivity is often impossible to reproduce.
- Methods established on such old columns will not be able to be reproduced on new columns or other old columns, resulting in the need to re-develop methods or perform a lot of unnecessary diagnostic work for instrument systems, chromatographic columns and mobile phases in the future

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Premature Deterioration of column

e.g.
A C8 column performs gradient separation at 40 °C, varying from methanol/water to THF/water. The mobile phase contains 0.05% trifluoroacetic acid and a guard column is used. The analyte is the hindered amine in the polymer extract, which is dissolved in toluene and precipitated in methanol. Before injection, all samples are filtered. A nitrogen chemiluminescence detector is used. After about 50 injections, the chromatographic peak of amine disappeared.

Solution:
After flushing the column with a mixture of dichloromethane and 0.2% trifluoroacetic acid, about half of the amine chromatographic peak signal was recovered; then flushing with 0.2% trifluoroacetic acid and toluene solvent, 100% of the amine chromatographic peak was recovered.

Conclusion:
In some cases, the new column must be stuck with some analytes to obtain stable analysis results; on the contrary, in the second situation, because too many impurities are stuck on the column, it will also cause trouble.

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Proper use of HPLC columns

- **Activation or Conditioning**

It is recommended to Activate or Condition new columns and columns that have not been used for a long time;

■ The importance of activation:

1) Wetting of the packing materials, which might have dried up during transportation or storage, as much as possible to restore the activity of the packing materials.

2) The difference between different brands of solvents may cause increased noise and drift, so replace the storage solvent as much as possible.

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Proper use of HPLC columns

- **Preparation of mobile phase**

■ Purity:

Organic solvent: chromatographically pure
Water: ultrapure water
Acids: superior pure grade
Buffer salts: analytically pure
Ion pair reagents: chromatographically pure (purity>98%)

■ The mobile phase should be filterd before use;
The mobile phase should be Freshly prepared for use for every Experiment !

The mobile phase containing organic solvent does not grow bacteria!

The water is absolutely clean!

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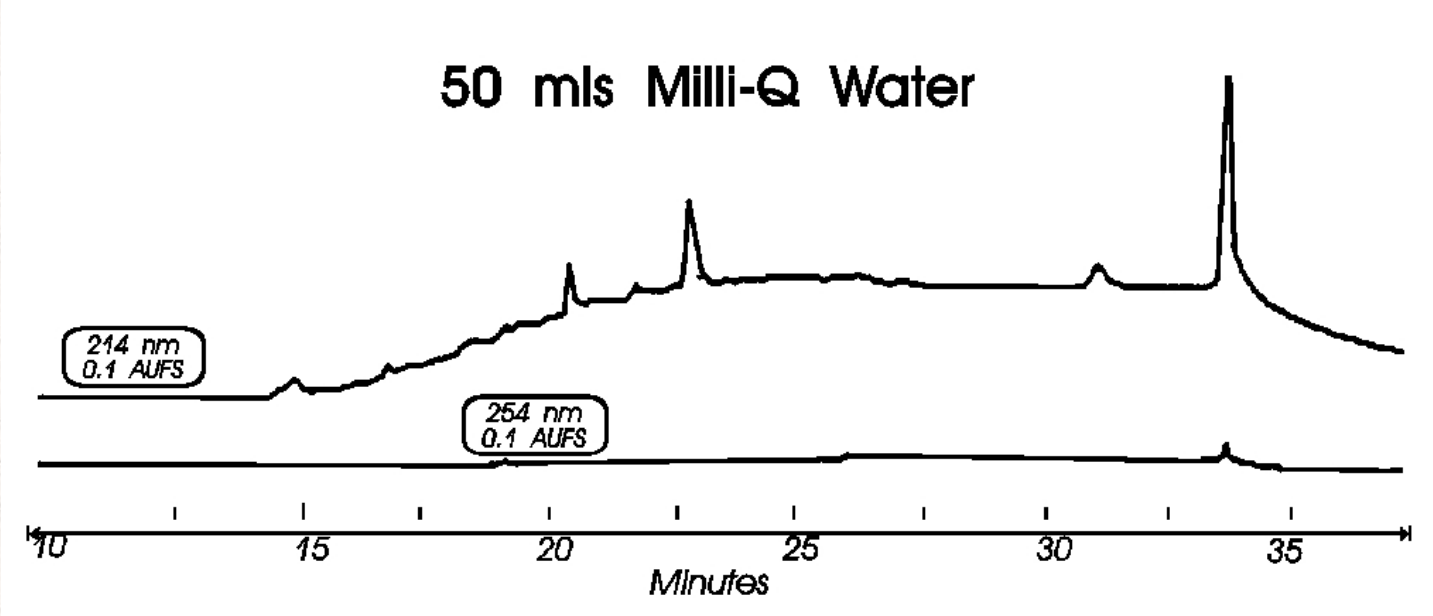
Experimental water of different quality



The state of organic contamination of ultrapure water

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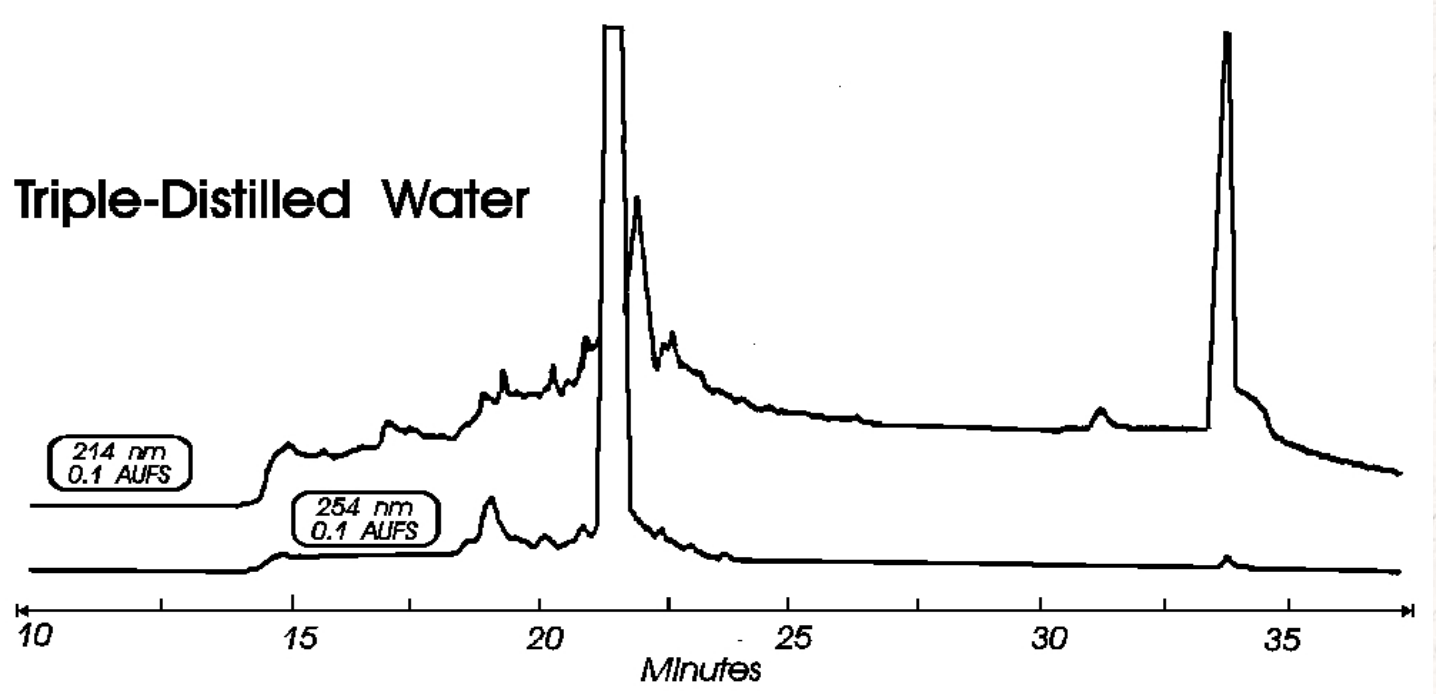
Experimental water of different quality



"Triple-distilled water" has a lot of organic pollutants and is not suitable for gradient analysis

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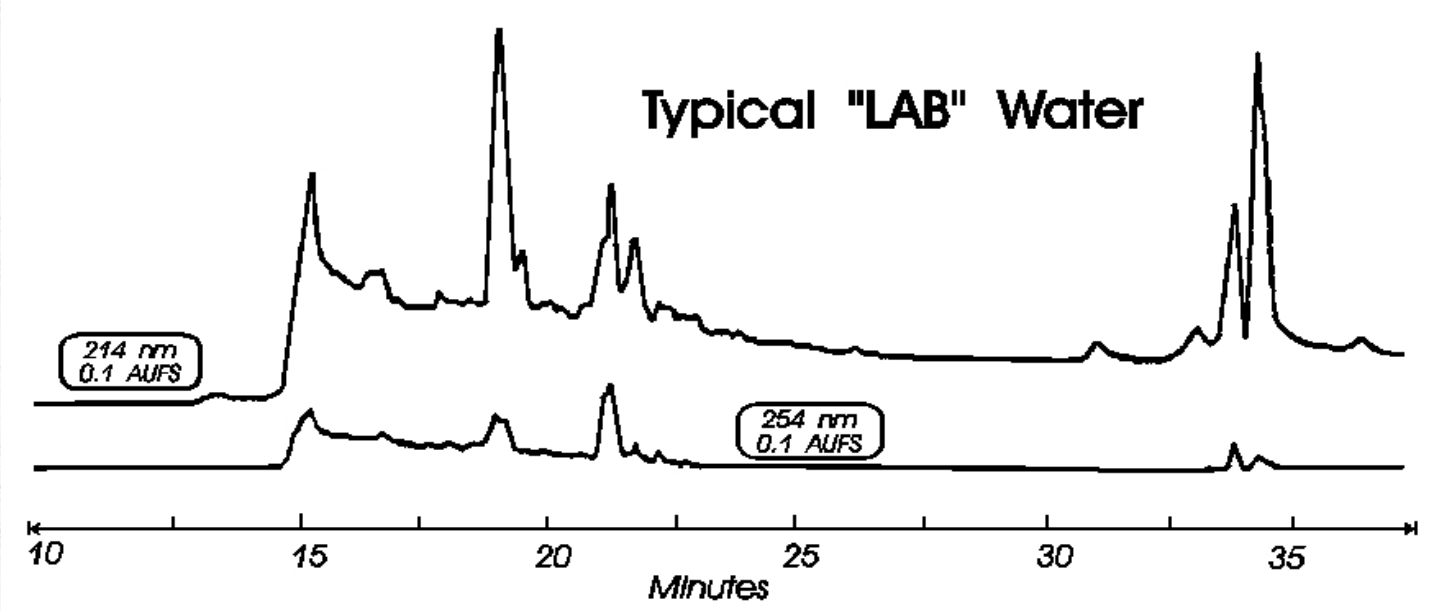
Experimental water of different quality



Typical "lab" water has a large amount of organic pollutants and is not suitable for high sensitivity gradient analysis

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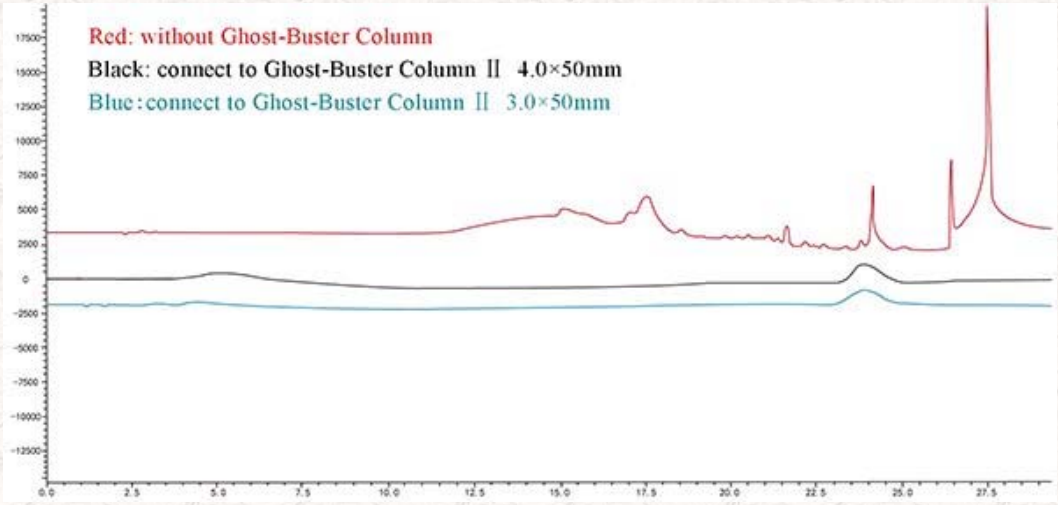
How to Solve “Ghost Peaks”?

Column: Ultisil AQ-C18 4.6×250mm,5μm
Ghost-Buster Column II, 3.0×50mm
Ghost-Buster Column II, 4.0×50mm
Mobile phase:
A: 0.05% phosphoric acid in water
B: 100% acetonitrile

Time/min	0	3	15	20	20.1	30
A/%	95	95	15	15	95	95
B/%	5	5	85	85	5	5



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How to prepare mobile phase?

- **C18 column, flow rate: 1ml/min**

A: methanol / B: water = 60/40; Prepare 500ml mobile phase

Mixed ways:

A: Use 500ml graduated cylinder, measure 300ml methanol and add 200ml water --- the elution capacity is weakened

B: Use 500ml graduated cylinder, measure 200ml water and add 300ml methanol --- the elution capacity is increased

C: Use 500ml graduated cylinder, measure 300ml methanol and 200ml water respectively and mixed --- the most reasonable and most commonly used way

D. Quantitatively transport water and methanol through double pumps respectively, and mix under high pressure --- when the flow rate is less than 1ml/min, same as C

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How to prepare mobile phase?

- **pH of mobile phase**

a. Increase organic solvent to adjust pH
For acidic buffer salt, pKa increased by 1 unit; alkaline buffer salt, pKa changed by 0.5 unit;
b. Adjust the pH in the water phase and mix the organic solvent 

Correct expression:
"20mM KH_2PO_4/K_2HPO_4 buffer, pH7.0/methanol= 40/60" or
"20mM KH_2PO_4/K_2HPO_4 buffer, pH7.0 and 60% methanol"

A confusing expression:
Methanol/20mM KH_2PO_4/K_2HPO_4 buffer, pH7.0, 60/40

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Proper use of HPLC columns

- **Column Temperature**

Suitable column temperature:

- 5℃ above room temperature, below 40℃ is recommended;
- Good retention time reproducibility
- Increasing the column temperature properly can reduce the viscosity of the mobile phase and reduce the back pressure of the column;
- Improve column efficiency to a certain amount;
- Increase the resolution;

etc.

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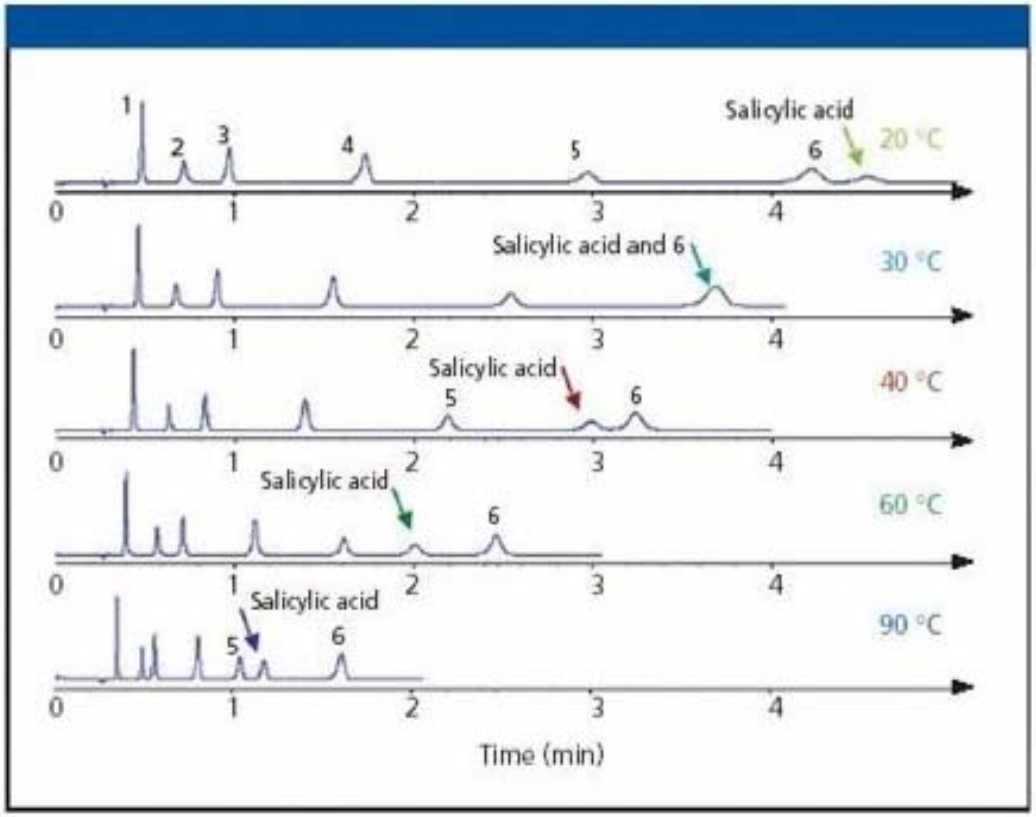
Proper use of HPLC columns

- **Column Temperature**

Comparison of the separation of seven analgesics at a column temperature of 20-90°C



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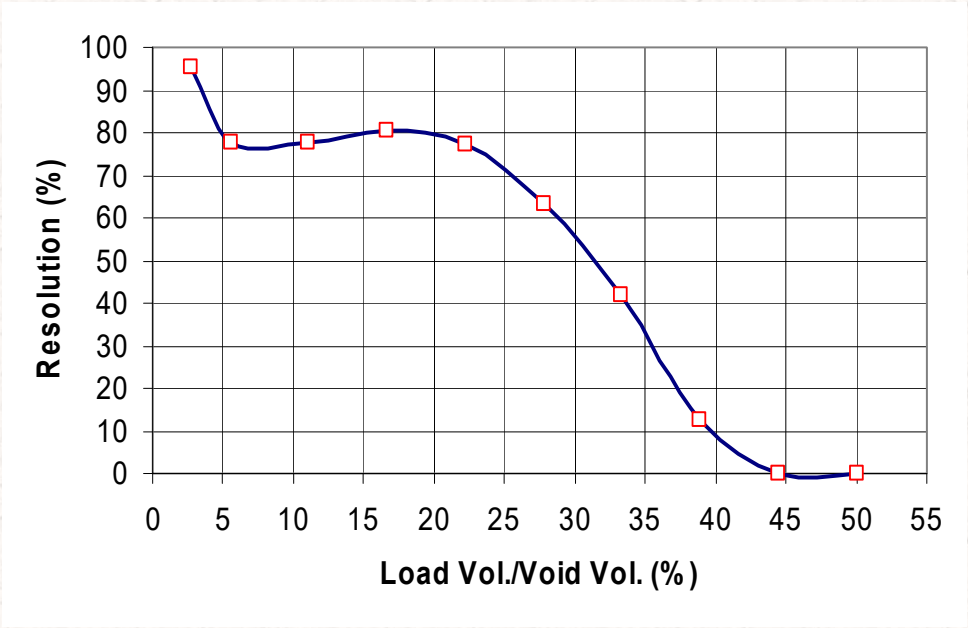
Proper use of HPLC columns

- **Injection Volume**



- When the loading volume exceeds 20% of the column volume, the resolution will decrease

- 4.6×250mm column volume: about 3ml
  Sample load volume: maximum - 600 µl

Keep the load volume less than 20% of the column volume.

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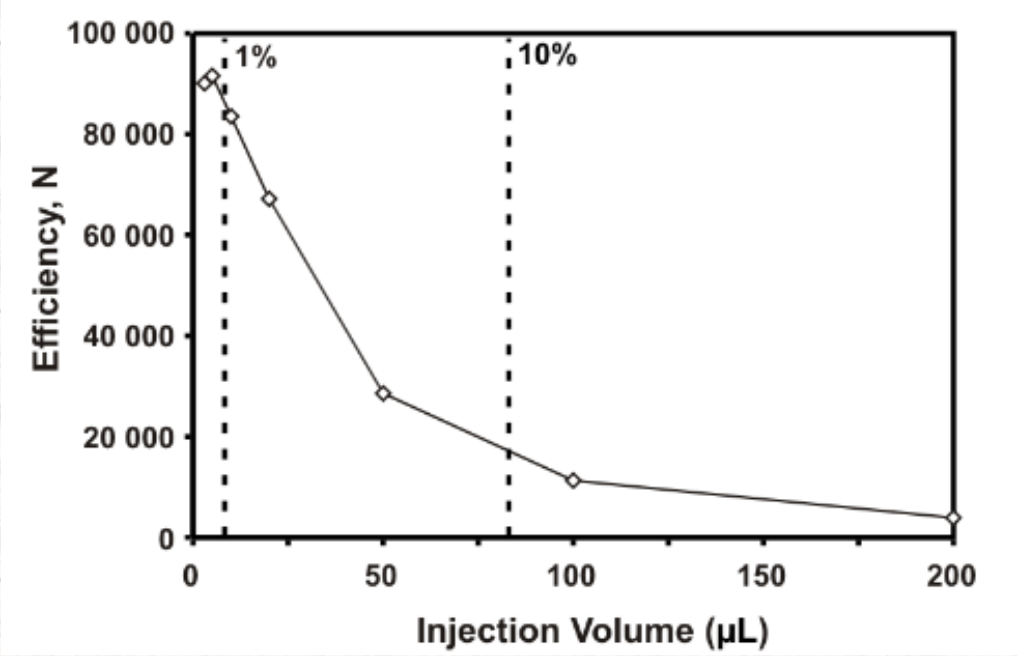
Injection Volume

- **Effect of injection volume on column efficiency**



Analyte k=1.6
Column: Ultisil XB-C18 4.6×50mm, 5um

The injection volume should not exceed 1% of the column volume

4.6×250mm column volume: about 3ml
Sample load volume: maximum - 30 µl

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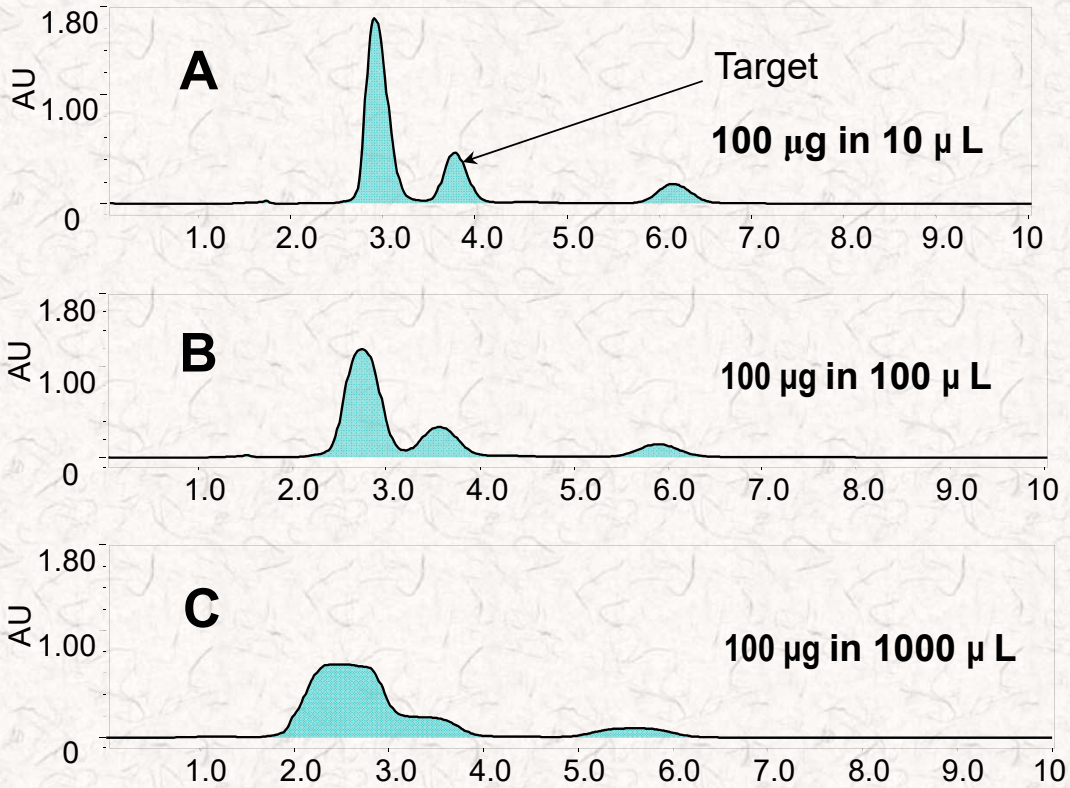
Injection Volume

- **Effect of injection volume on resolution**



As the injection volume increases, the resolution decreases

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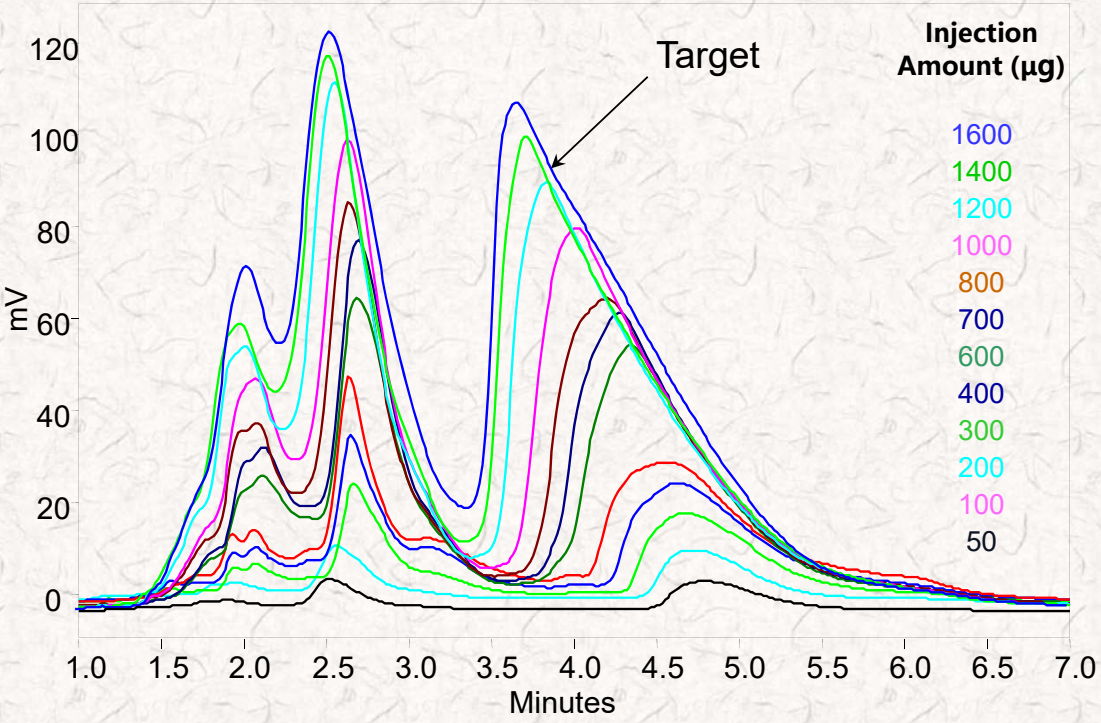
Injection Amount



- The injection volume remains unchanged (20 µL), dissolve the sample with 80% acetonitrile aqueous solution
- Increase the amount of the sample:
  -Peak forward
  -Increased overlap

The sample amount is determined by the inner diameter of the column

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Column Type	ID(mm)	Sample Loading	Regular flow rate	Sensitivity	Applications
Analytical column	4.6	0.1-1.5mg	0.5-3 mL/min		Standard separation
Solvent Saving column	3.0	150-500μg	0.3-1.5 mL/min	+	Solvent saving, used at standard HPLC system
Narrow bore column	2.1	50-120μg	0.1-0.5 mL/min	++	High sensitivity, limit sample loading, LC/MS, solvent saving
Micro bore column	1.0	10-50μg	10-100μL/min	++++	High sensitivity, limit sample loading, LC/MS
Capillary column	0.5, 0.3	1-10μg	1-15μL/min	+++++	Extremely high sensitivity, LC/MS, peptide and protein
Nano column	0.1, 0.075	100-200ng	200-500nL/min	++++++	Extremely high sensitivity, LC/MS, peptide and protein
Semi-prep column	9.4	1-10mg	5-10 mL/min		Mg grade preparation
Prep column	21.2	20-250mg	20-60mL/min		Hundreds of milligram to 1 gram

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Part Two

- Maintenance of HPLC columns

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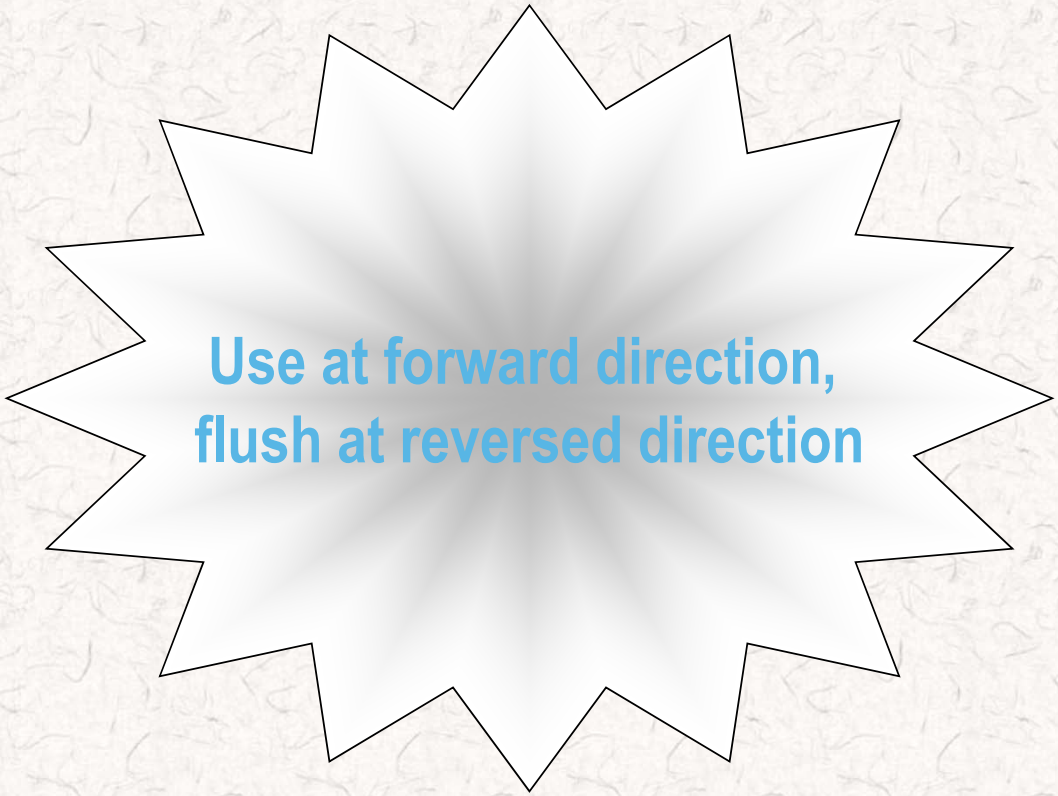
Non-Welch column please ask your column manufacturers!



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Routine Maintenance

- No buffer in mobile phase

  Flush the column with mobile phase until all samples come out ⟶ flush with 20 column volume of 90% organic solvent

- Buffer in mobile phase

  Flush the column with mobile phase until all samples come out ⟶ flush with 20 column volume of transitional mobile phase flush with 20 column volume of 90% organic solvent

- Ion-pair solvent in mobile phase

  Flush the column with mobile phase until all samples come out ⟶ flush with 20 column volume of transitional mobile phase ⟶ flush with 20 column volume of 50% methanol ⟶ flush with 20 column volume of 90% organic solvent

Transitional mobile phase: ratio of water and organic solvent in transitional mobile phase is the same or higher than analysis mobile phase, no buffer salts.

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Common Problems

- **High Pressure**
- **Low column efficiency**

etc.

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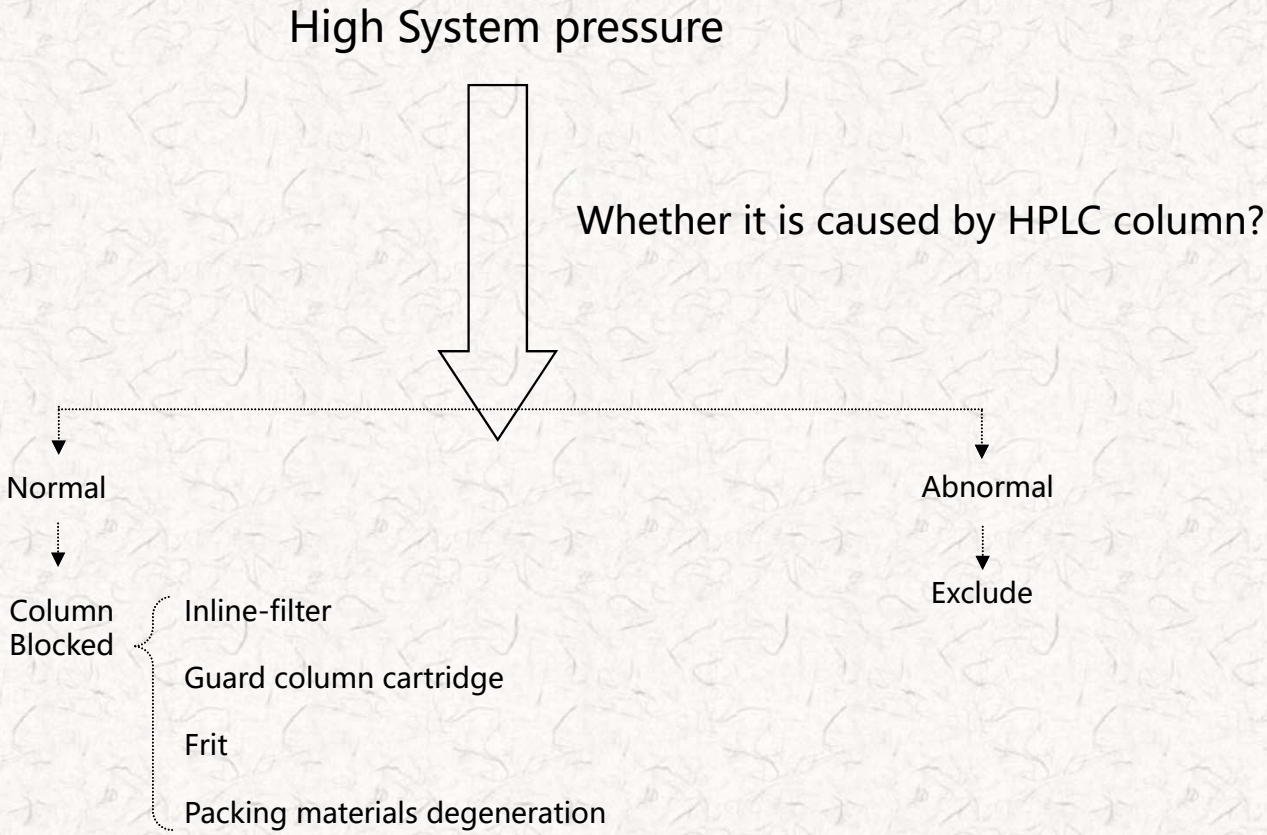
Solutions to solve high column pressure



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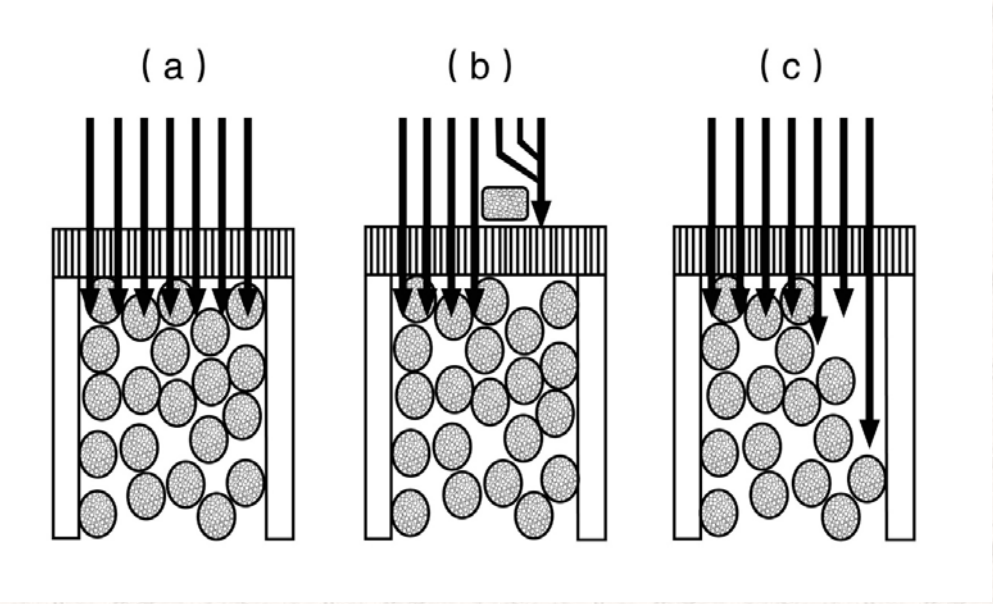
High column pressure

- **Frit blocked**

High pressure, reduced column efficiency, poor resolution, and ugly peak shape are mainly due to clogging of the frit, resulting in the sample separation and distribution is not good at the beginning.



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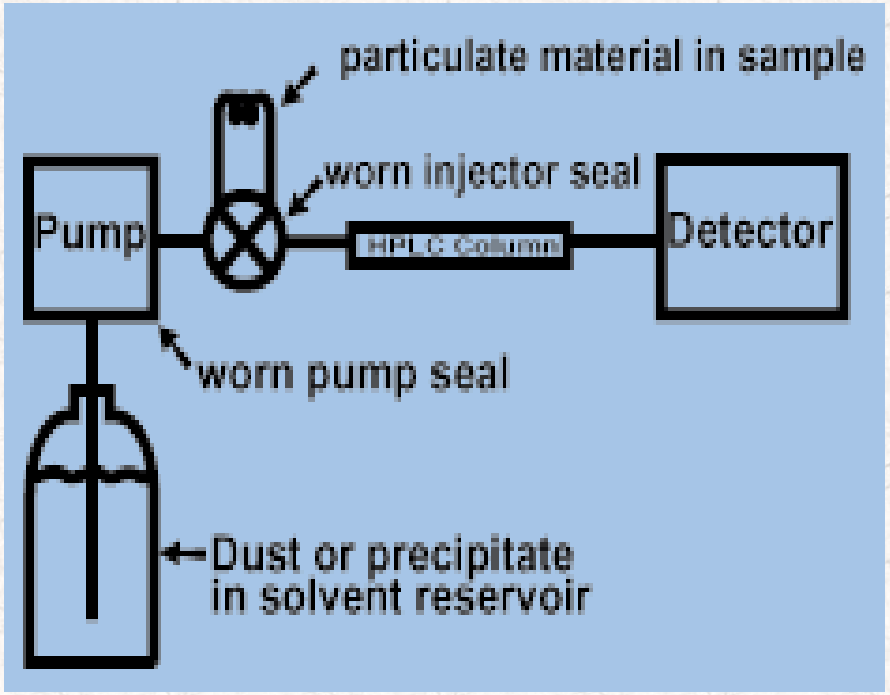
High column pressure

- **Possible reasons of blocked frits**

Exogenous impurities:
Unfiltered impurities in sample and mobile phase;

Precautions:
High purity solvents
Specific pretreatment of samples
All the sample and mobile phase filter with micropore membrane
Use in-line filter



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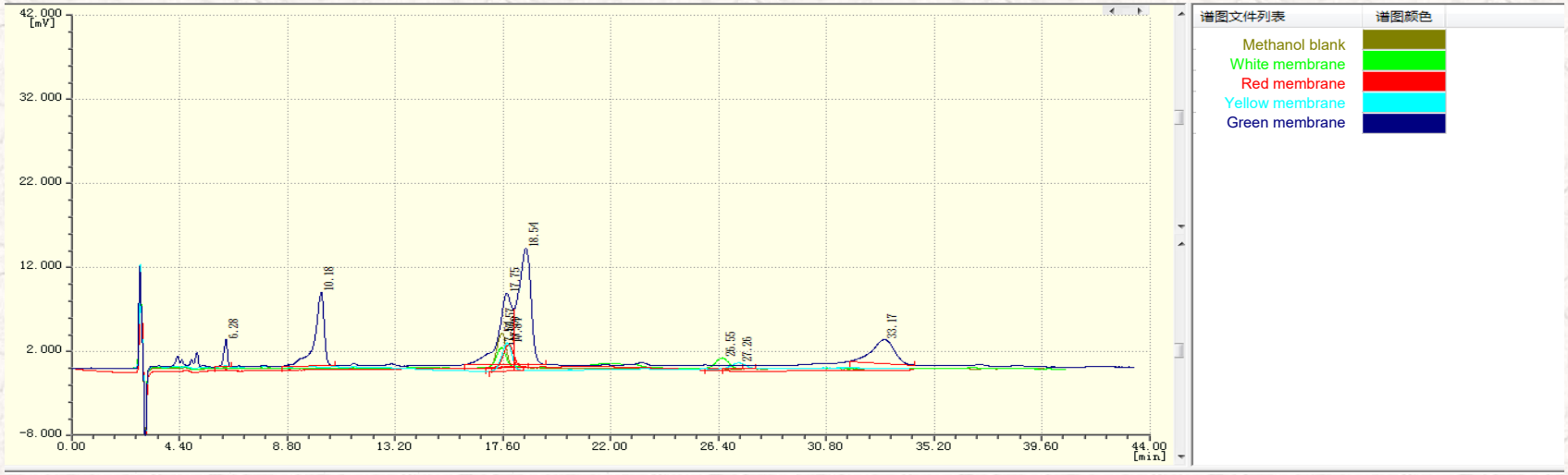
High column pressure

- **Possible reasons of blocked frits**

Column: Ultisil XB-C18, 4.6×250mm, 5μm;
Sample: Methanol passes through different membranes;
Detection wavelength: 254nm;
Mobile phase: methanol-water=75:25;
Temperature: 25 degrees;
Flow rate: 1.0mL/min;
Injection volume: 20μL



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High column pressure

- **Possible reasons of blocked frits**

■ Endogenous impurities

1) Fragments from worn pump plunger lever and seal；
2) particles from old PEEK tubes；
3) impurities from old injection valve seal ；

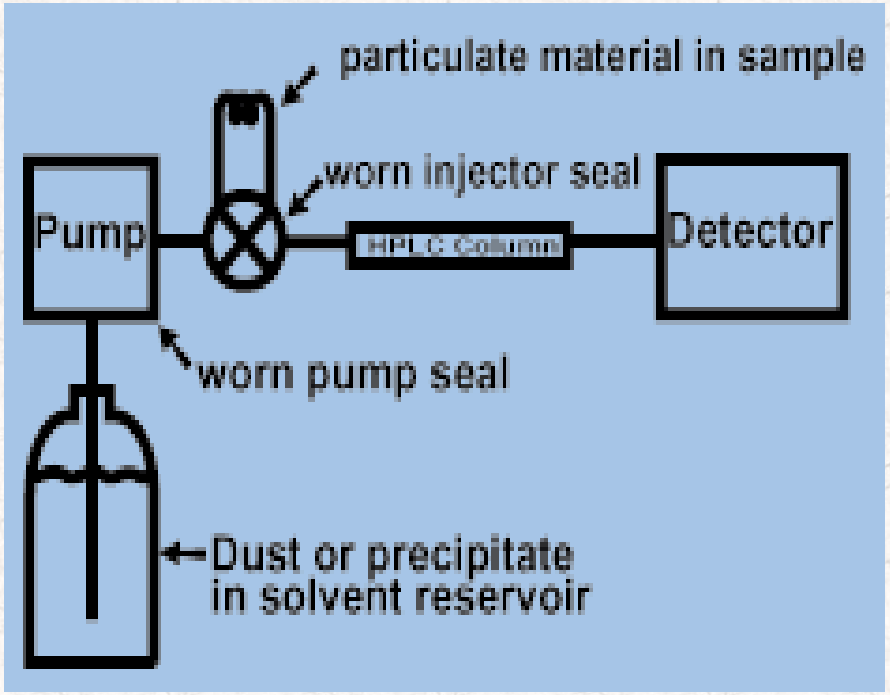
■ Precautions

1)Routine maintenance and replacement of seals, plunger lever and other damageable parts

2) Avoid using solvent which accelerate the aging of plastic parts(such as THF)

3)Timely replacement of peek joints

4)Use online filter



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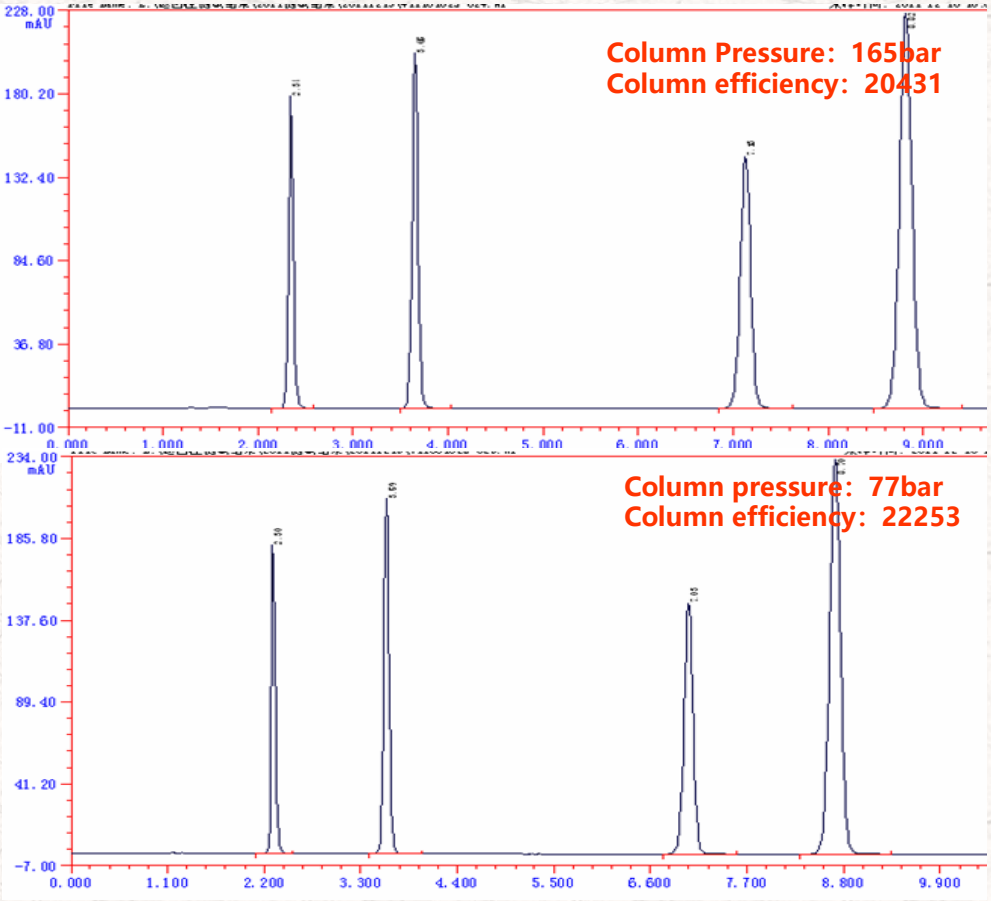
High column pressure

- **Frit blocked**

HPLC column: Xtimate C18, 4.6×250mm, 5μm
Wavelength: 254nm
Mobile phase: acetonitrile-water=65:35
Temperature: 25 ℃
Flow Rate: 1.0mL/min
Injection Volume: 20μL

Frit changed



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High column pressure

- **Packing materials denaturation-accumulation of protein samples**

Solutions：

Use SPE or other more efficient pretreatment method
Column rinse, protein remove solvent is recommended(acetonitrile-water-TFA= 50-50-0.1)
Guard column
Etc.

Together with
Column efficiency decline
Resolution decline
Abnormal peak shape

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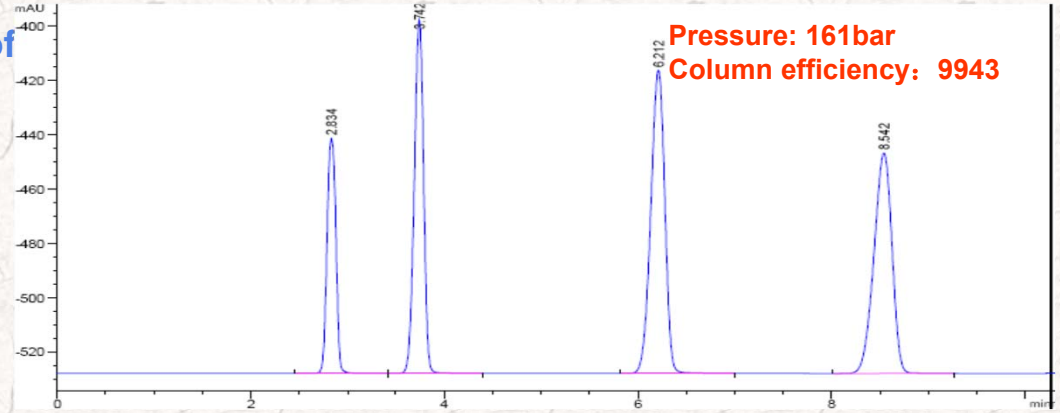
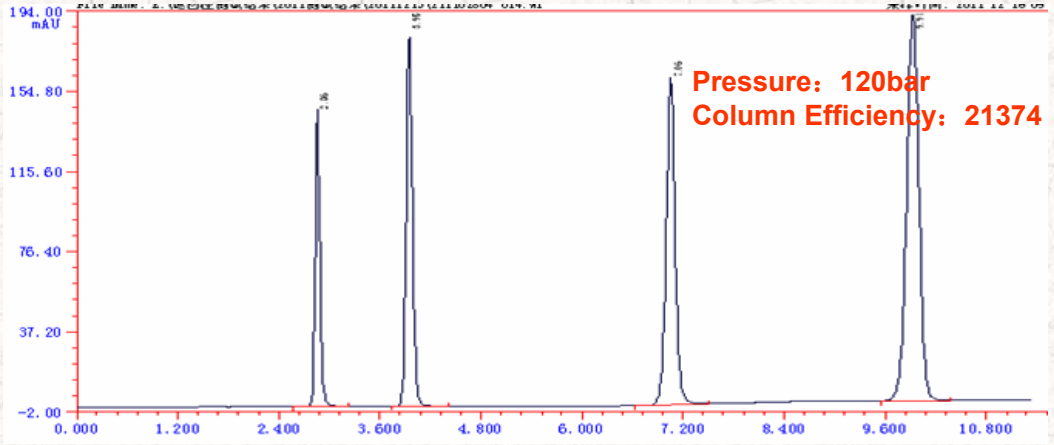
High column pressure

- **Packing materials denaturation-accumulation of protein samples**

HPLC column: Ultisil XB-C18，4.6×250mm，5μm；
Wavelenghth: 254nm；
Mobile Phase: methanol-water=75:25；
Temperature: 25℃；
Flow rate: 1.0mL/min；
Injection Volume: 20μL

After flushing with TFA water solution





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High column pressure

- **Packing materials denaturation- accumulation of strongly retained materials**

Solutions：

Use SPE or other more efficient pretreatment method

Back flush the column with solvent which could dissolve contamination for a long time(more than 60 column volume)

Replace the contaminated packing material at the end of the column

Flush the column with solvent which is more eluent than mobile phase

Special column for special use, contamination knowable

Etc.

Together with
Column efficiency decline
Resolution decline
Abnormal peak shape

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High column pressure

- **Packing materials denaturation- accumulation of strongly retained materials**

Flush the column with solvent

which is more eluent than mobile phase

- Reversed HPLC (RP-18,RP-8), choose low polarity solvent

  water⇨methanol⇨acetonitrile ⇨isopropanol ⇨ **dichloromethane** ⇨ isopropanol ⇨ acetonitrile

- Normal HPLC (SiO_2, NH_2, CN, DIOL) , choose high polarity solvent

  n-hexane⇨dichloromethane⇨ethyl acetate⇨isopropanol⇨ acetonitrile

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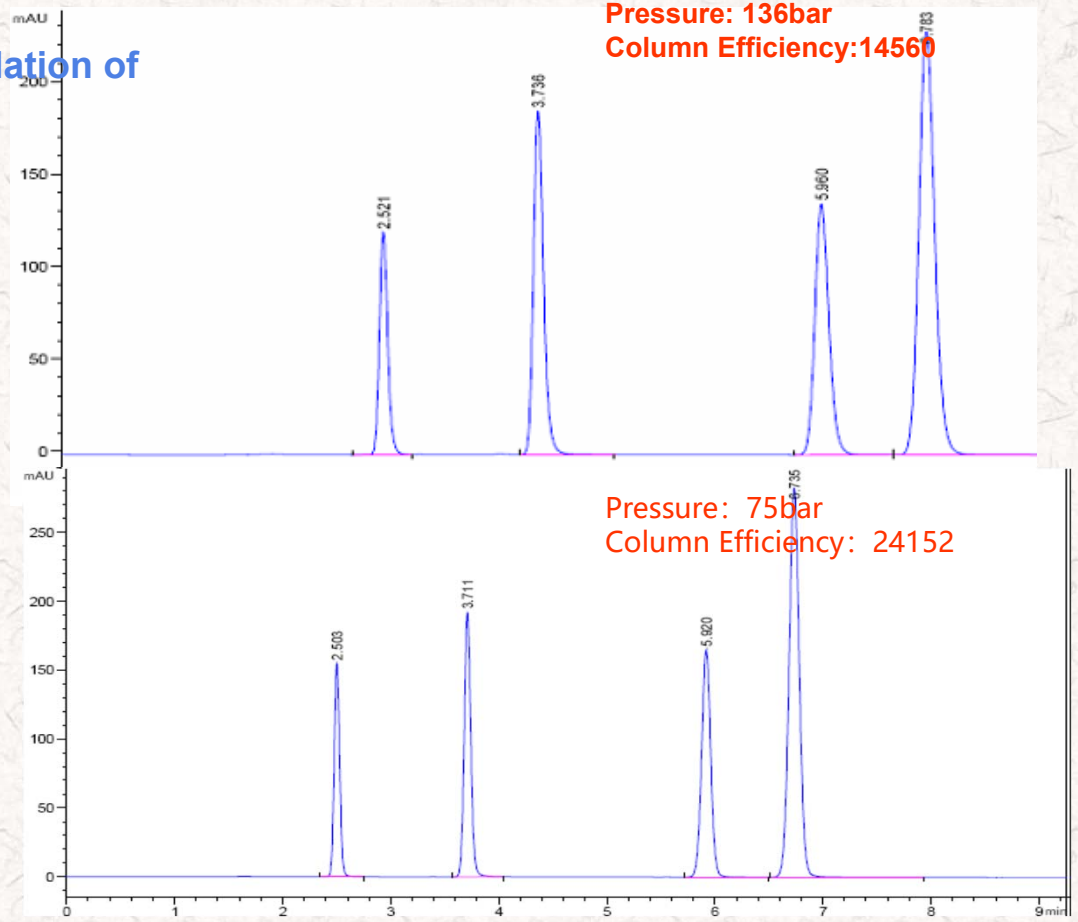
High column pressure

- **Packing materials denaturation- accumulation of strongly retained materials**

HPLC Column: Xtimate C8, 4.6×250mm, 5μm
Wavelength: 254nm
Mobile Phase: Acetonitrile-water=65:35
Temperature: 25 ℃
Flow Rate: 1.0mL/min
Injection Volume: 20μL

Packing material in column end turned to grey



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High column pressure

- **Packing materials denaturation- buffer out**

Solutions：

Back flush the column with methanol/water=10/90 60 column volume at analysis flow rate, temperature 35℃；
Back flush the column with methanol/water=10/90 at 0.2ml/min overnight；

“ Make transition before using!
Flush column after using!”

Avoid buffer coming out, as buffer is very hard to remove.

Together with
Column efficiency decline
Resolution decline
Abnormal peak shape

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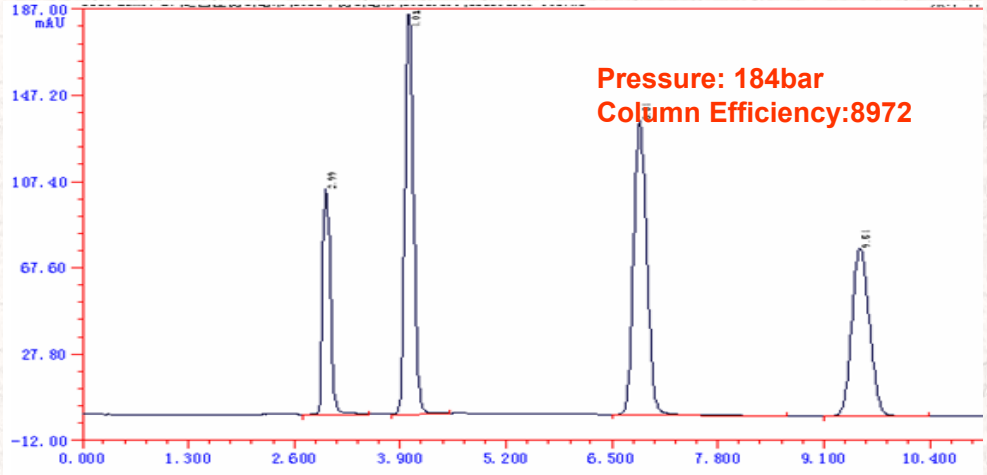
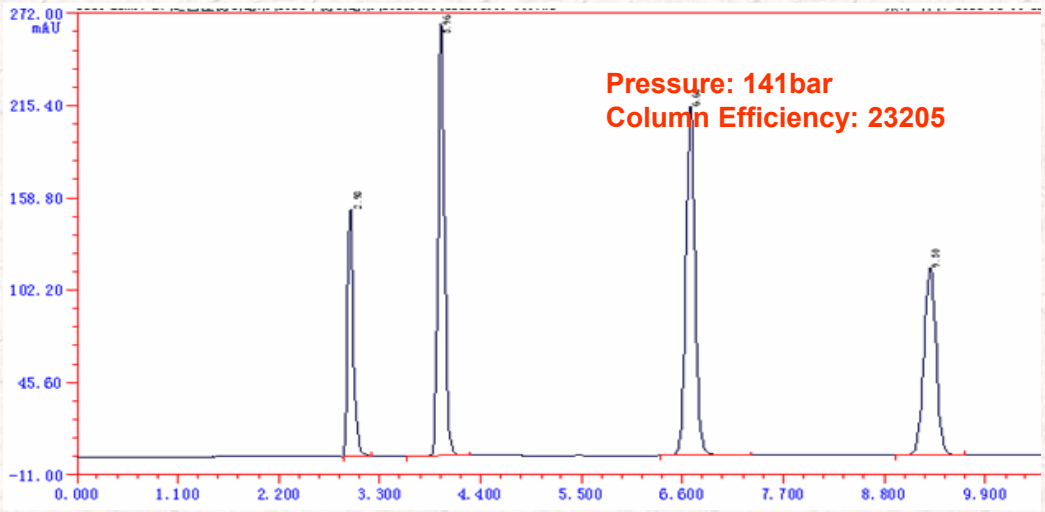
High column pressure

- **Packing materials denaturation- buffer out**

HPLC column: Ultisil XB-C18, 4.6×250mm, 5μm
Wavelength: 254nm
Mobile phase: methanol-water=75:25
Temperature: 25 ℃
Flow Rate: 1.0mL/min
Injection Volume: 20μL





Nothing unusual in column end packing materials

Buffer in mobile phase

Solution: flush with 10% methanol at 0.2ml/min overnight

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Don’t spend too much time in column regeneration, ensure regular analysis work first.

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High column pressure

- **Packing materials denaturation- loss of bonding phase**

Means of prevention:

Unless special instructions, pH should be no less than 2；
Non-polar bonding phase, avoid using non-polar solvent；
Moderate polar bonding phase, avoid moderate polar solvent；
Polar bonding phase, avoid using polar solvent；

Together with
Column efficiency decline
Resolution decline
Abnormal peak shape
Retention time drifting

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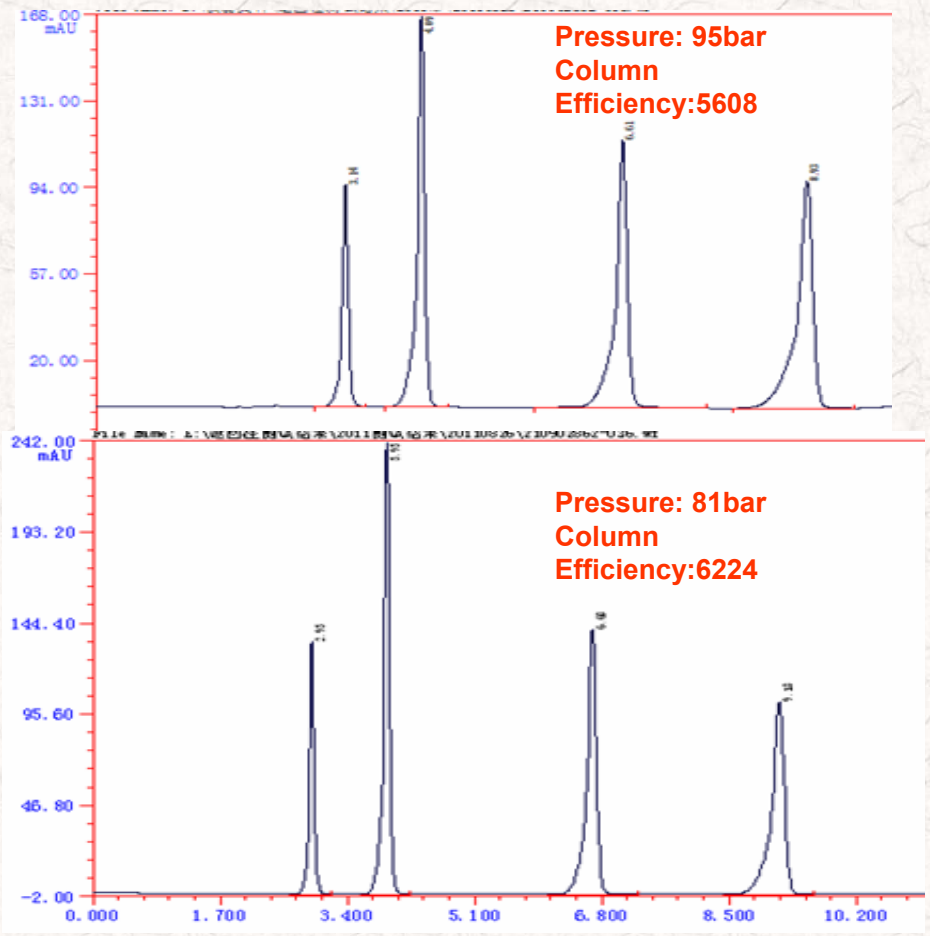
High column pressure

- **Packing materials denaturation- loss of bonding phase**

HPLC Column: XtimateTM -C18，4.6×250mm，5μm；
Wavelength: 254nm；
Mobile Phase: acetonitrile-water=65:35；
Temperature: 25 ℃；
Flow Rate: 1.0mL/min；
Injection Volume: 20μL

Packing is not tough (Voids in the packing)



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High column pressure

- **Packing materials denaturation- broken matrix**

Means of prevention:

Unless special instructions, pH should be no greater than 8 for silica based column;
Avoid high temperature；
Avoid pressure pulse, high pressure；
Etc.

Together with
Column efficiency decline
Resolution decline
Abnormal peak shape
Retention time drifting

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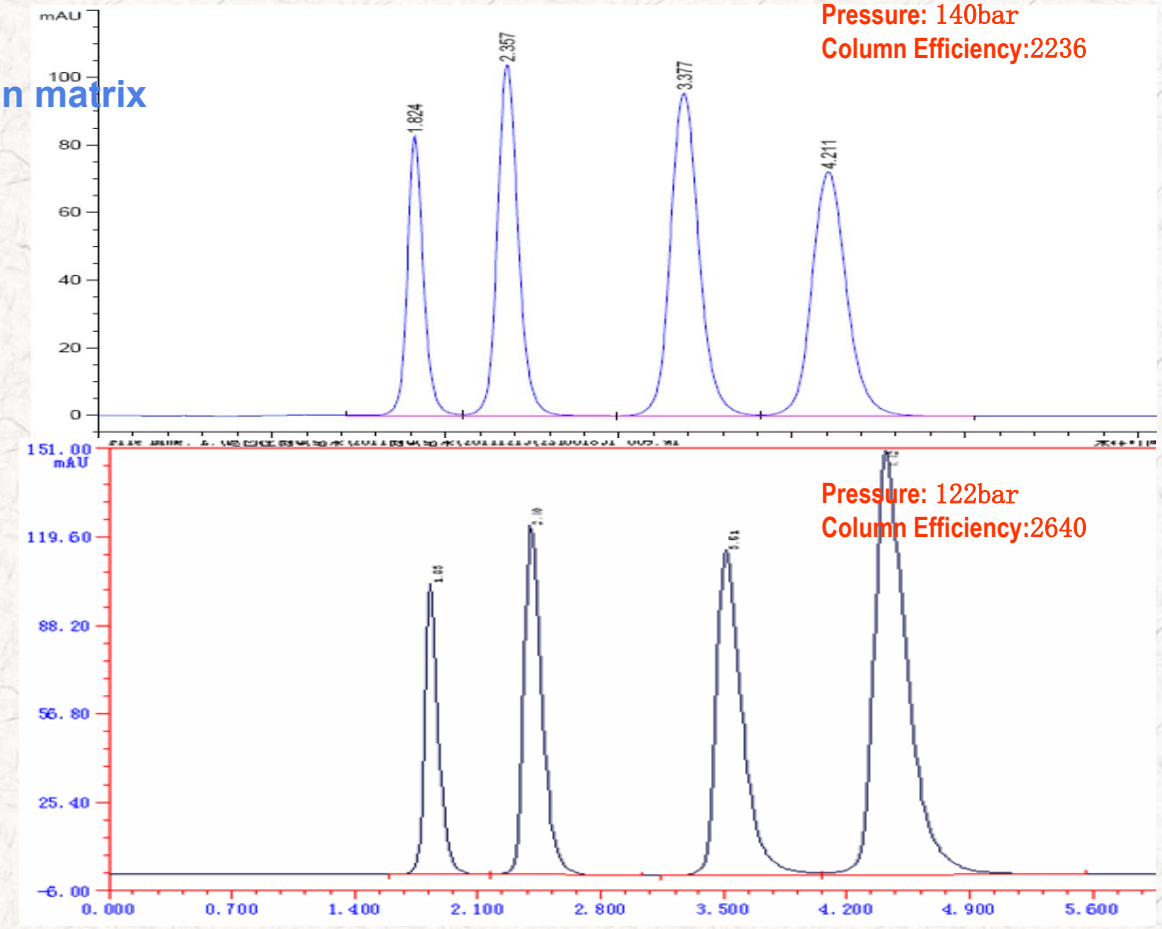
High column pressure

- **Packing materials denaturation- broken matrix**

HPLC Column: Ultisil XB -C8 ， 4.6×250mm，5μm；
Wavelength: 254nm；
Mobile Phase: methanol-water=75:25；
Temperature: 25 ℃；
Flow Rate: 1.0mL/min；
Injection Volume: 20μL

Silica dissolution, packing materials height is 3mm missed.



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Other Reasons of High column pressure

* When changing mobile phase, not all the original mobile phase is replaced by the new one, different solvents(different nature, mutually immiscible) mixed together, which lead to high pressure (Increased Viscosity)；
* Frit gets rusty；
* When gradient elution Program, maximum viscosity number exists between different solvents, for example, methanol-water=45-55
* Sample Precipitates out in mobile phase；
Etc.

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Conclusion

* The new column is not as good as the old column --- The new column needs to be aged, some parameters of the new column packing change within the controllable range, and the method established after the old column is modified;

* New columns and new projects do not work well --- The selectivity is different, and the chromatographic conditions are inappropriate;

* Unsatisfactory column life --- Contamination of strongly retained substances (flush, improve with guard column),
* The bonding phase falls off and the silica matrix dissolves (change the column with a different bonding process).

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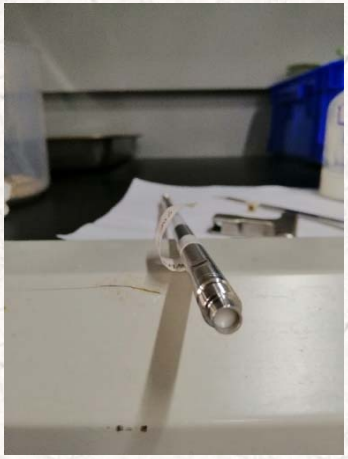
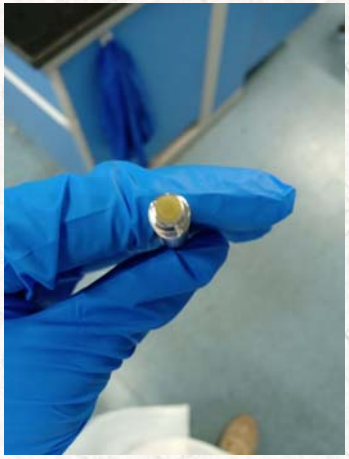
The "anatomy" of columns

Different damage situations
Packing materials dissolution
Packing materials Compaction
Packing materials Discoloration
Frit blocked
Packing Materials' appearance is normal


Possible reasons	Maintenance suggestions
The membrane material used is incorrect or of poor quality, and some impurities are introduced into the mobile phase or sample, causing the frit and packing materials to be contaminated.	Replace with more tolerant and better quality filter equipment
The instrument system has not been thoroughly cleaned for a long time.	The pump cleaning solvent and needle cleaning solvent should be replaced regularly. It is recommended to replace it once a week. The instrument system should be cleaned thoroughly with 40°C warm water and organic solvents regularly.
If the mobile phase is used for more than 12 hours at a time, it will deteriorate, and the resulting floccules will cause the frit to be blocked.	Replace the mobile phase and the storage bottle every 12 hours, no more than 24 hours
There are protein substances in the sample which are accumulated at at the head end of the column, leads to packing materials contamination and compaction.	Use a guard column. After removing the protein, the sample should be centrifuged and filtered at high speed. When the pressure is high, use acetonitrile-0.1% trifluoroacetic acid water = 1-1 to flush overnight at a low flow rate (refer to the method under abnormal conditions in the Welch column care and use manual)
The composition of the sample is complex, and it is easy to be adsorbed by the packing materials at the head end of the column and cause the packing to be contaminated.	Use a guard column and change the sample pretreatment method to a more targeted method to reduce the easily contaminated components in the sample solution; Polar RP column is recommended.
Except for special instructions, the long-term pH range of silica-based chromatographic columns is 2-8. The higher or lower pH of the mobile phase and sample solution will accelerate the shedding of the bonded phase and the broken silica gel.	The pH of the sample solution and mobile phase should be controlled in the long-term use range, if the pH of the sample solution is abnormal, use a guard column, or change to Ultisil LP series (tolerant pH≤2) or Xtimate series (tolerant pH≥8)
There is no transitional and effective flushing before and after use of buffer salts and ion pair reagents in the mobile phase, their precipitation blocked the packing materials and frit.	Use a transitional mobile phase to flush away the high organic solvent or high salt phase in the system before and after use (refer to the active method of a new column in the Welch column care and use manual)
In the process of gradient use, the organic phase ratio is higher than 60%, the conversion rate is faster, and there is a risk of buffer salt precipitation, causing blockage of frits and packing materials.	slowing the rate of change of mobile phase and reducing the proportion of the highest organic phase
The small group bonded phase is easy to fall off. It is a phenomenon of normal packing properties.	Refer to the storage conditions in the Welch column care and use manual.
The higher column temperature speeds up the buffer salt′ s attack on the silica matrix, causing the bond phase to fall off and the packing to collapse.	Without affecting the analysis effect, lower the column temperature, or choose the high temperature resistant LP series or Xtimate series
Failure to replace the guard column in time leads to contamination of the analytical column	Replace the guard cartridge in time. The column pressure increases> 10%; the column efficiency decreases> 10%; the resolution decreases> 10%, all of which are signals that the protection cartridge must be replaced.

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Solutions of common problems

Common Problem	Welch products	Features
Polluted by strong retained substances	Ultisil PLUS-C18, guard column needed	Low surface area, suitable carbon loading, week adsorbsion of pollutants
Loss of bonding phase due to low pH	Ultisil LP series	Side chain steric protection bonding process to prevent siloxane bond from hydrolysis at low pH condition
High pH, high temperature, high salt which lead to the broken silica	Xtimate series	Hybrid organic/inorganic technique to prevent the attacks to silica
Peak tailing when separate Alkaline compounds	Ultisil polar-RP Ultisil ALK-C18	Through the introduction of polar groups and the process of double encapped techiniques, the exposed hydroxyl group on the silicone surface is covered to a greater extent.
Substances which are not effectively retained in reversed and normal phases	Ultisil HILIC	Retention for highly polar, hydrophilic substances
Retention time is too long, want to shorten the retention time	Botimate	Core-shell silica gel can be used at high flow rate under normal pressure to obtain high column efficiency, high resolution and shorten retention time
The pH of solvent is too low or too high	Guard column needed	The guard column can well prevent the sample solvent from attacking the packing materials at the end of the column
Ghost peaks when run gradient program	Ghost buster column	Strong adsorption of organic pollutants in mobile phase makes the operating gradient drift gently and the impurity peak decrease effectively

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Part Three

- Six misunderstandings in the use of HPLC columns

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Six misunderstandings in the use of HPLC columns

1. The HPLC column cannot be reversed used:
   After reversed using, the packing materials will collapse and the bonded phase will not expand

False!

- Packing column pressure, about 7000psi;
- The packing direction is opposite to the use direction;
- Some manufacturers have inconsistent pore size of the front and rear frits
- The pore size of the frit at both ends of Welch column is the same.

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Six misunderstandings in the use of HPLC columns

2. The guard column does not affect the separation effect

False!

- Improper selection of packing materials can cause retention time drift or even different selectivity
- Improper installation, increased dead volume, worsened peak shape.

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Six misunderstandings in the use of HPLC columns

3. Increasing the temperature is beneficial to the separation effect

False!

- Make low-k substances almost no retention, which is difficult to quantify;
- Different compounds are inconsistent with temperature changes;
- Shorten the service life of the chromatographic column;
- The large temperature difference deforms the peak.

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Six misunderstandings in the use of HPLC columns

4. The higher the carbon loading, the better the reversed-phase column

False!

- The retention capacity depends on the relative hydrophobicity of the analyzed molecule;
- Prone to phase collapse;
- Poor reproducibility of retention time

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Six misunderstandings in the use of HPLC columns

5. The lower the column pressure of the new column, the better the column

False!

$$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{1 + k'_B}{k'_B}\right)$$

- Resolution (R), column efficiency (N), selection factor (α) and column pressure are not related

$$\Delta P = \frac{\eta F L}{K^0 \pi r^2 d_p^2}$$

- The denser the packing, the higher the column pressure;
- Packing pressure is about 8000psi

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Six misunderstandings in the use of HPLC columns

$$P = \frac{3000L\eta}{t_0 d_p{}^2}$$

P is the column back pressure (psi), L is the length of the column (cm), η is the viscosity of the mobile phase (cP), t0 is the column dead time, and dp is the diameter of the particle (μm)

- The uniform particle size results in a relatively low back pressure on the column.
- The pressure drop at both ends of the chromatographic column should not exceed 30% calculated by the formula.
- The particle size of silica gel has a great influence on the pressure of the chromatographic column; the smaller the silica particles, the greater the back pressure of the chromatographic column.
- The back pressure of the chromatographic column is also related to the viscosity of the mobile phase.

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Six misunderstandings in the use of HPLC columns

Comparison of column pressure of various brands (take C18 as an example)

Lowest: Dima Diamond Generation
Shimadzu VP-ODS Column

Lower: Welch (Welchrom C18)
Elite (Sino-Chrom C18)

Higher: Waters (Xterra, Xbrige, Symmetry),
Agilent (Zorbax)
Phenomenex (Luna, Gemini)
Dima II (Diamonsil C18 II)
Welch (Ultisil, Xtimate)

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Six misunderstandings in the use of HPLC columns

6. The chromatographic column should be tightly sealed to prevent the packing from being damaged by contact with air:

False!

- The diameter of the micropores at both ends of the column is less than 0.2 inches;
- Even Part of air enters column, it is still difficult to diffusely contact enough packing materials;
- A small amount of air will dissolve immediately under high pressure and flow out of the column.

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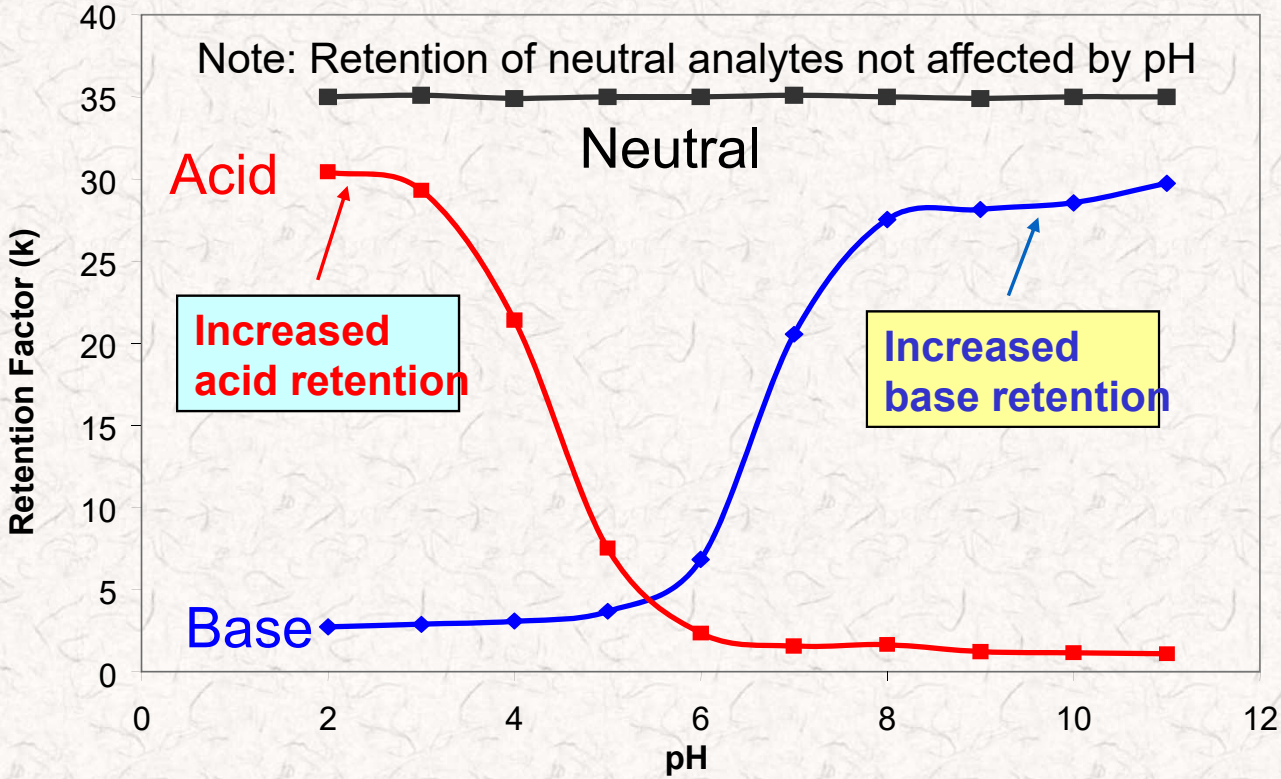
Handerson Hasselbalch Equation for pH and pKa

pH = pKa + log [A-]/[HA]



Bridging Separation Techniques

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Thank you!