BIKAI

Service Note:

Standard Operation Guideline for HPLC with Acetonitrile and High-Salt Mobile Phase

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1. Document Overview

1.1 Basic Information

Document Type: Professional HPLC Technical Service Note

Applicable Instrument: High Performance Liquid Chromatography (HPLC) equipped with infusion pump system

Applicable Condition: Detection scenarios using acetonitrile organic phase, high-salt aqueous buffer (phosphate, citrate, etc.), and acetonitrile-water gradient elution

Effective Date: 2026-06-17

Issuing Department: Technical After-Sales Department

1.2 Service Purpose

This guideline standardizes the routine operation and maintenance of HPLC instruments under acetonitrile and high-salt mobile phase conditions. It stabilizes system pressure and baseline performance, reduces wear, crystallization and blockage of core consumables including check valves, pump heads and seals, minimizes unexpected downtime, and ensures long-term stable and efficient operation of customer instruments.

2. Standard Operation & Maintenance Specifications

To avoid common risks such as high-salt crystallization, acetonitrile deterioration and gradient pressure fluctuation, customers are recommended to strictly follow the standardized operations below. All specifications are verified by comparative instrument tests to optimize overall equipment performance.

2.1 Differential Filtration Specifications for Mobile Phases

All mobile phases must be vacuum-filtered before use. Unfiltered mobile phase is prohibited. Filter membranes shall be selected according to solvent type to avoid instrument abnormalities caused by mismatched media:

- **Organic System**: Pure acetonitrile and acetonitrile-water mixtures with acetonitrile proportion ≥50%: use 0.22μm organic filter membrane
- **Aqueous / High-Salt System**: Pure water, high-salt buffer solutions, and mixtures with acetonitrile proportion ＜50%: use 0.22μm aqueous filter membrane
- **Vacuum Degree Control**: Maintain vacuum pressure at -0.06~-0.08 MPa. Excessively high negative pressure will cause premature salting-out and crystallization of high-salt mobile phase, resulting in pipeline and pump blockage.

In case of baseline drift during detection, perform an additional 15-minute ultrasonic degassing after filtration. Allow the solvent bottle to reach uniform temperature before sample injection.

2.2 Check Valve Upgrade & Full-Cycle Maintenance

To resolve pressure fluctuation during acetonitrile-water gradient elution, it is recommended to upgrade the original ruby inlet/outlet check valves to **ceramic check valves**.

<table>
<tr><th colspan="2">Check valve flow direction</th><th colspan="2">The check valve is assembled to the sleeve</th><th>Sleeve Installation</th></tr>
<tr>
<td> 
Inlet</td>
<td> 
outlet</td>
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Inlet</td>
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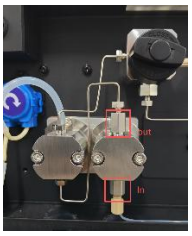
Outlet</td>
<td>  </td>
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</table>

Ceramic valves effectively buffer gradient pressure shocks and improve operational stability. Relevant maintenance procedures are specified below:

2.2.1 Routine Maintenance after Each Test

After detection, flush the chromatographic column and the entire pipeline system with 20% methanol aqueous solution for 30 minutes to eliminate residual mobile phase and prevent salt and organic deposition.

2.2.2 Monthly Deep Maintenance

Perform systematic deep cleaning monthly:

- Step 1: Flush the pump, autosampler and column oven (do NOT flush the detector) with 90–100℃ pure water for 60 minutes.
- Step 2: Use 50% isopropanol aqueous solution to flush the pump, autosampler, column oven and detector for 60 minutes with the column oven set at 45℃.
- Step 3: Perform full-system solvent replacement with pure methanol for system preservation.

2.2.3 Emergency Treatment for Blocked Check Valves

If check valve blockage occurs, prepare a mixed solution of pure water, methanol and isopropanol at a volume ratio of 1:1:1. Ultrasonically clean the check valve assembly in the mixture for 5 minutes, then reinstall after cleaning.

2.3 Enhanced Post-Pump Flushing for Anti-Crystallization

High-salt mobile phases tend to crystallize in pump cavities and check valve gaps. Optimize post-pump flushing settings to enhance residue removal:

- Shorten the default post-pump flushing interval to increase flushing frequency and suppress salt crystallization accumulation
- Use either 10% methanol aqueous solution or 20% isopropanol aqueous solution as the standard post-flush solvent
- Replace the flushing solution completely every 3–4 days under circulating mode to ensure cleaning efficiency
- The standard 250 mL solvent bottle is sufficient for regular use; replace with a larger bottle for long-term continuous operation. **Never leave the flushing bottle empty**.
- After unattended operation, flush the system with 15% methanol water with a volume of no less than 30 column volumes (one column volume ≈ 4.15 mL for 5μm 250×4.6mm column). Appropriately raise the column oven temperature to completely remove residual salt deposits.

2.4 Storage Specifications for Acetonitrile Mobile Phase

Acetonitrile is chemically active. Under strong light, UV irradiation, aerobic conditions or trace metal catalysis, it is prone to cracking, oxidation and coupling reactions, leading to solvent deterioration and unstable baseline and test data. Therefore, acetonitrile mobile phase must be stored in **brown opaque reagent bottles**. Avoid open and light-exposed storage. Prepare mobile phase on-demand for short-term use.

3. Instrument Performance Verification Test (For Customer Reference)

A dedicated comparative test was conducted to verify the adaptability and stability of ceramic check valves under acetonitrile-water gradient conditions. The test data fully supports the optimization recommendations in this service note.

3.1 Test Instrument Information

Model	Serial No.	Inlet Check Valve Material	Outlet Check Valve Material
BIKAI 1511 Pro P40	BS-001	Ruby	Ruby
BIKAI 1511 Pro P40	TC-4001	Ceramic	Ceramic

3.2 Test Equipment & Reagents

Test Equipment: Vacuum filtration device, ultrasonic cleaning device, stainless steel union connector, backpressure coil

Test Reagents: HPLC-grade acetonitrile (CONCORD TECHNOLOGY), Watsons ultrapure water

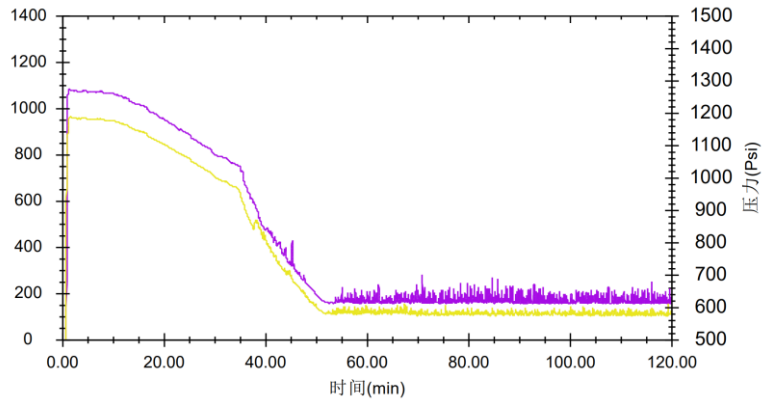
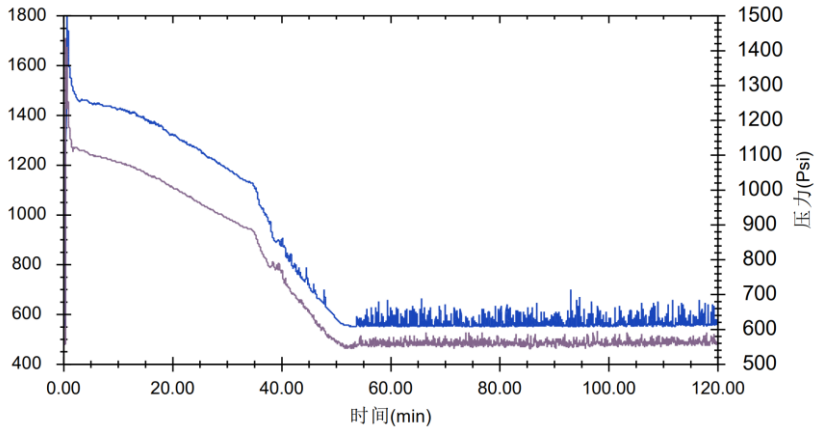
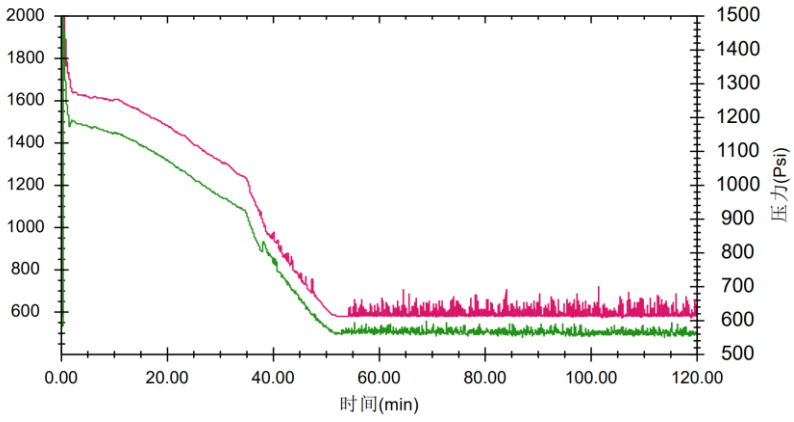
3.3 Chromatographic Test Conditions

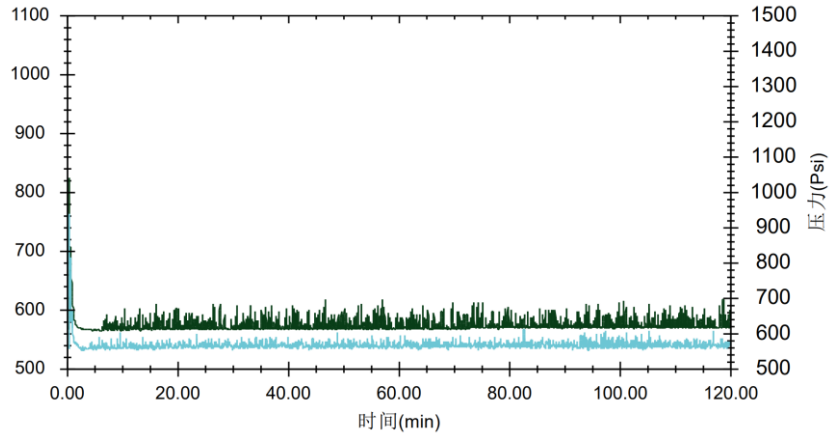
The chromatographic column was removed and replaced with a stainless steel union. A backpressure coil was installed at the detector outlet to simulate a stable system backpressure of approximately 700 psi. Acetonitrile-water gradient elution parameters are shown below:

Time (min)	Flow Rate (mL/min)	Phase A (Water, %)	Phase B (Acetonitrile, %)
0.01	1.0	90	10
10.00	1.0	80	20
30.00	1.0	50	50
50.00	1.0	20	80
120.00	1.0	20	80

3.4 Test Conclusion

Pressure curve:

Legend	Pressure Diagram
No. 1 Purple: Ruby Yellow: ceramic	
No. 2 Blue: Ruby Brown: ceramic	
No. 3 Red: Ruby Green: Ceramic	

No. 4 Green: Ruby Blue: Ceramic Mpbile phase: CAN: Water=80:20	

Multiple parallel gradient tests prove that ceramic check valves provide significantly better pressure stability than traditional ruby check valves under acetonitrile-water gradient switching conditions. Ceramic valves effectively eliminate abnormal pressure fluctuations caused by gradient changes without instrument failure or valve sticking. Upgrading to ceramic check valves greatly improves overall instrument operational stability.

4. Quick Troubleshooting for Common Abnormalities

4.1 Sudden System Pressure Rise

Stop the pump immediately and perform 40-minute extended post-pump flushing. Check whether filter membranes are misused and whether high-salt mobile phase has crystallized and blocked the pipeline.

4.2 Continuous Baseline Drift

Verify mobile phase filtration compliance. Perform an additional 15-minute ultrasonic degassing. Let the solvent bottle reach uniform temperature before restarting analysis.

5. After-Sales Technical Support

If instrument abnormalities including pressure fluctuation, baseline instability or component blockage still occur after strictly following the specifications in this Service Note, please contact our Technical After-Sales Department. We provide remote technical guidance and on-site maintenance services to ensure stable instrument operation.

6. Recommended Consumable List

Consumable Name	Part No.	Specification	Recommended Quantity
Ceramic Inlet Check Valve	BK100675	ceramic ball/ ceramic seat	1 PC
Inlet Check Valve sleeve	BK100676	-	1 PC
Ceramic Outlet Check Valve	BK100677	ceramic ball/ ceramic seat	1 PC
Outlet check valve sleeve	BK100678	-	1 PC
Vacuum Filtration Device	BK200143	Compatible with 0.22μm aqueous/ organic filter membranes	1 SET