BIKAI

Standard Operating Procedure (SOP) for BIKAI 1511 Pro HPLC System

1. Purpose

To standardize the operation, use, cleaning and daily maintenance procedures of the BIKAI 1511 Pro high performance liquid chromatography (HPLC) system and its supporting chromatographic workstation, unify operating standards, avoid operational risks, ensure stable operation of equipment, accurate and reliable test data, extend the service life of equipment, and meet the quality control requirements of laboratory testing.

2. Scope of Application

This procedure is applicable to all working scenarios of the BIKAI 1511 Pro HPLC in the laboratory, including the entire system (liquid delivery system, sampling system, column oven, detector, column) and supporting chromatographic workstation for daily operation, sample testing, system cleaning, system shutdown, daily maintenance and instrument usage log.

3. Job Responsibilities

3.1 Instrument Operators

Conduct experimental operations in strict accordance with this procedure, be responsible for pre-experiment equipment inspection, whole-process monitoring during experiments, post-experiment system cleaning and shutdown, truthfully fill in instrument usage records, and complete daily equipment cleaning and basic maintenance.

3.2 Equipment Administrators

Responsible for regular instrument calibration, consumable replacement, fault troubleshooting, implementation of maintenance plans and account filing, and supervise the implementation of operating procedures.

4. Operating Environmental Requirements

Environmental Parameters	Standard Requirements
Ambient Temperature	5℃~35℃, temperature fluctuation ≤2℃/h; optimal operating temperature: 25±2℃
Relative Humidity	40%~70%, no condensation and no damp water accumulation
Power Supply Conditions	220V±10%, 50/60Hz, independent power supply circuit with reliable grounding to avoid voltage fluctuation and electromagnetic interference
Environmental Conditions	Dust-free, free of corrosive gas, strong vibration and direct sunlight, far away from heat sources, magnetic fields and high-power electrical equipment

5. Preparation Before Analysis

5.1 Reagent and Consumable Preparation

5.1.1 Mobile Phase

Prepare with high-performance liquid chromatography (HPLC) grade reagents. After thorough mixing, perform vacuum filtration with corresponding 0.22μm or 0.45μm microporous filter membranes (aqueous/organic) according to solvent properties to remove impurities and particles. Conduct ultrasonic degassing for 20 minutes after filtration to eliminate air bubbles in pipelines. Mobile phases containing buffer salts, acid or alkali systems shall be prepared and used freshly, and overnight use is prohibited.

5.1.2 Needle Wash Solution and Seal Wash solution

10% methanol aqueous solution (HPLC grade) is routinely selected. The prepared solution shall be filtered through a 0.22μm filter membrane and ultrasonically degassed for 20 minutes for standby. The needle washing system can be adjusted according to the solubility of special samples.

5.1.3 Sample and Standard Solutions

Prepare samples and gradient concentration standard solutions in accordance with test methods. All solutions shall be filtered through 0.22μm/0.45μm syringe filters to remove particulate impurities and prevent blockage of columns and pipelines.

Note: Filter membranes are strictly prohibited from mixed use. Aqueous filter membranes are only applicable to pure water and buffer salt systems, while organic filter membranes are suitable for organic solvents such as methanol and acetonitrile, so as to avoid mobile phase and sample contamination caused by filter membrane dissolution.

5.2 Equipment Pre-inspection

a) Check that the power cord, data cable and liquid delivery pipeline of the entire instrument are firmly and correctly connected without looseness, falling off, bending or damage.

b) Verify that the waste liquid pipeline is unobstructed, and the waste liquid bottle is stably placed with sufficient capacity.

c) Confirm that the column is installed correctly without liquid leakage at the interface, the column oven is in normal condition, and the sampling system is clean without residue.

d) Ensure that the laboratory environment and power supply conditions meet the standards without potential safety hazards.

e) Replace the appropriate column and sample loop according to the requirements of test methods.

5.3 Pump Channel Configuration Instructions

✧ For systems equipped with P20/P22 binary high-pressure gradient pump: Pump A delivers aqueous phase, and Pump B delivers organic phase.

✧ For systems equipped with P40/P42 quaternary low-pressure gradient pump: Channel A delivers aqueous phase, Channel B delivers organic phase, and Channels C and D are user-definable.

6. Power-on Operation Procedure

Follow the power-on sequence of **hardware first, software later** strictly to avoid equipment startup faults:

Turn on the power of BIKAI 1511 Pro detector, column oven, automatic sampler and pump in sequence, and wait for the completion of hardware self-inspection (no self-inspection errors and normal indicator lights).

Turn on the computer host and display, start the Chromcore chromatographic workstation software, complete the communication connection between the instrument and the software, and confirm that the workstation successfully identifies all instrument modules without connection abnormality prompts.

7. Reagent Replacement and System Degassing

7.1 Mobile Phase Replacement and Pipeline Degassing

a) Take out the mobile phase filter, immerse it in a beaker containing a small amount of new mobile phase, shake gently to replace the residual old mobile phase in the mobile phase filter, then place it into the new mobile phase solvent bottle, ensuring the filter head is completely immersed without suspension.

b) Rotate the purge valve knob of the pump counterclockwise by 90°~180° to fully open the purge valve; if there are excessive residual air bubbles in the pipeline, use a matching large syringe to connect the purge valve outlet tube for auxiliary liquid extraction and degassing.

c) Log in to the chromatographic workstation, enter the pump monitoring interface, right-click the "Flush" function, set the flow rate of the selected channel to 5mL/min, and start purging. Stop the pump when no air bubbles are observed in the pipeline and the liquid flows continuously and stably (the default purging duration is 5 minutes, which can be adjusted as required).

d) Tighten the purge valve clockwise after venting to ensure complete sealing without liquid leakage risks.

Warning: It is strictly prohibited to start purging without opening the purge valve! High flow rate operation of closed pipelines will cause a sharp rise in system pressure, resulting in pipeline liquid leakage, joint damage, pump failure and other equipment damages.

7.2 Seal Wash solution and Needle Wash Solution Replacement and Degassing

a) Replace the seal wash solution (10% methanol aqueous solution or 10% isopropanol aqueous solution) and complete simple pipeline flushing.

b) Replace the needle wash solution and start the needle washing pipeline degassing procedure to remove residual air bubbles and old liquid in the pipeline.

8. Test Method Setting

8.1 Create or Select Methods

Select existing methods through the BIKAI chromatographic workstation, or create new methods in accordance with the *BIKAI Chromatographic Workstation User Manual*, and set the parameters of each instrument module (flow rate, detection wavelength, column temperature, gradient program, etc.), integration parameters and calibration parameters in sequence.

8.2 Set Pressure Protection Range

Elution Mode	Upper Pressure Limit	Lower Pressure Limit
Isocratic Elution	1.5 times the normal pressure	0.5 times the normal pressure
Gradient Elution	1.5 times the maximum gradient pressure	0.5 times the minimum gradient pressure

8.3 Sequence Setting and Method Delivery

If equipped with an automatic sampler, set the sample sequence parameters (vial number, injection volume, sample name, save path, etc.) in the workstation. Click "Send Method" to enable the system to start balancing automatically.

9. System Balancing

9.1 Liquid Leakage Inspection

Check pipelines, column joints and all system parts for liquid leakage, and eliminate faults in accordance with the guidelines of each component manual if leakage occurs.

9.2 Pressure Monitoring

The pressure fluctuation shall be within 5%~10% of the average value. If the fluctuation exceeds this range, it indicates residual air bubbles in the pre-column pipeline, and the degassing operation shall be repeated.

9.3 Baseline Monitoring

- Ensure that at least 5~10 column volumes of mobile phase pass through the

system.

- The system is considered balanced and ready for sample injection when the baseline drift is less than 10^{-4} AU/min, the noise is at the magnitude of less than 10^{-4} AU, and the pressure is stable.

10. Sample Injection Operation

10.1 Manual Injection

a) Rinse the injection needle 3 times with mobile phase, and then rinse once with sample solution.

b) Extract the required volume of sample and eliminate air bubbles in the injection needle.

c) Turn the injection valve handle to the Load position, fully insert the injection needle into the injection valve inlet, and inject the sample solution smoothly.

d) Quickly turn the injection valve handle to the Inject position; the instrument will operate automatically to collect data and record chromatograms.

e) Keep the Inject state until the next injection, then switch the injection valve back to the Load position and pull out the injection needle.

For full-loop injection, the injection volume shall be at least 3~5 times the loop volume; for partial injection, the injection volume shall be less than 50% of the loop volume with accurate injection volume for each test. For testing standard samples with different concentrations, inject samples from low to high concentration.

10.2 Automatic Injection

a) Run the automatic sampler cleaning function of the chromatographic workstation.

b) Confirm that the injection tray is placed in place and the sampling needle is free of bending and blockage.

c) Verify all parameters in the "Sequence" interface and click the "Run" button; the instrument will automatically complete sampling, injection and data collection.

For gradient methods, it is recommended to perform 1~2 blank gradient runs before sample injection. For unattended continuous automatic injection, strictly check the correctness of sequence settings and sufficient mobile phase volume to avoid abrasion of sealing rings and plunger rods caused by empty liquid circuit operation.

11. Data Processing and Report Output

a) Perform peak integration, draw calibration curves, and conduct qualitative and quantitative component analysis in accordance with the BIKAI chromatographic

workstation manual.

b) Set the report format, including core contents such as sample information, test parameters, retention time, peak area and calculation results.

c) Save electronic data and print paper reports after confirming data accuracy, and complete data backup and filing.

Note: Arbitrary tampering with original test data is prohibited.

12. System Cleaning and Maintenance

12.1 Automatic Sampler Cleaning

Run the automatic sampler cleaning function of the chromatographic workstation for instruments equipped with an automatic sampler.

12.2 Manual Injection Valve Cleaning

After the experiment, keep the injection valve in the Inject state, and flush the injection valve sequentially with mobile phase, ultrapure water-methanol (80:20) and methanol using a syringe. Switch the injection valve back to the Load state after cleaning and seal the injection port with a blocking cap.

12.3 System and Column Cleaning

12.3.1 Reversed-phase System

- Salt-free mobile phase: Flush with methanol for 30 minutes.
- Salt-containing mobile phase: Flush with ultrapure water-methanol (80:20) at 1mL/min for more than 40 minutes first, then flush with methanol for 20 minutes.

12.3.2 Normal-phase System

Flush with appropriate normal-phase solvent according to chromatographic methods.

12.3.3 Storage Recommendations

- Short-term storage (overnight or weekend): Store with salt-free mobile phase to shorten the next balancing time.
- Only for chromatographic column flushing: Turn off the detector deuterium lamp and tungsten lamp to extend lamp service life.

Warning: do not leave the pump with pure water, acetonitrile, acid-base or salt mobile phases for extended periods. Pure water will breed bacteria and block pipelines; acetonitrile may cause adhesion of one-way valve balls and seats leading

to pump faults; acid-base and salt systems will corrode precision equipment components.

13. Shutdown Operation Procedure

a) Click "Stop Pump" in the chromatographic workstation to reduce the flow rate to 0 after cleaning is completed.

b) Exit the workstation software.

c) Turn off the power of all system modules, computer host and display in sequence.

14. Experiment Completion and Account Recording

Truthfully and completely fill in the *Instrument Usage Record Form* in accordance with laboratory management requirements, specifying the usage date, experimental items, operation duration, equipment status, consumable consumption, abnormal problems and other information.

Standardize the disposal of experimental waste liquid, waste filter membranes, sample residues and other wastes with classified collection and compliant disposal. Seal and cap all mobile phase bottles and organic solvent bottles for light-shielded storage.

Inspect the laboratory carefully before leaving, and turn off the power of unused instruments and equipment.

15. Daily Instrument Maintenance and Upkeep

a) Ultrasonically clean the mobile phase filter every half month to ensure smooth liquid absorption.

b) Inspect the energy of deuterium lamp and tungsten lamp as well as the liquid delivery volume of sealing rings every three months.

c) Regularly replace wearable consumables such as pump seals, one-way valves, injection needles and filter membranes in accordance with the maintenance plan.

d) Regularly calibrate detector wavelength, system pressure and injection precision, and complete annual equipment metrological verification.

e) For long-term unused chromatographic columns, flush thoroughly with the storage solvent recommended in the chromatographic column manual, remove from the system, seal both ends with special plugs, and store at room temperature in a dry and light-shielded environment.

f) For long-term instrument shutdown, power on and run the instrument regularly, and flush the system with methanol to prevent pipeline aging and component jamming.

g) To ensure long-term stable operation of the instrument, BIKAI original consumables and accessories are recommended.

16. Abnormality Handling

Abnormality Type	Phenomenon	Handling Measures
Pressure Abnormality	Sharp pressure rise	Stop the pump immediately and troubleshoot pipeline and column blockage
Pressure Abnormality	Sharp pressure drop	Check for liquid leakage, pipeline disconnection and pump failure
Baseline Abnormality	Baseline drift and excessive miscellaneous peaks	Check mobile phase purity, pipeline air bubbles and detection cell contamination, and rebalance the system
Injection Abnormality	Poor repeatability and unsmooth sampling	Clean the injection needle and sample loop, and check the tightness of the sampling system
Equipment Error	Fault code display	Stop the instrument immediately, record the error information, report to the equipment administrator for troubleshooting and maintenance, and do not disassemble the instrument without authorization

This procedure is a general operating procedure for the BIKAI 1511 Pro HPLC system. Special test items can be supplemented and refined with parameter requirements based on exclusive test methods. All operators must pass training and assessment before taking up their posts.

BIKAI Technology (Suzhou) Co., Ltd.

Add: 2-B702, Dao’an Rd, Suzhou, China

Email: info@bikaicorp.com

Tel: +86 18613365565