Attachment 13

Analytical Equipment Calibration Certificate

COMPANY NAME
SITE / GROUP

STANDARD OPERATING PROCEDURE

Title	HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING UV/VIS ABSORBANCE AND FLUORESCENCE BASED DETECTION
Purpose	This Standard Operating Procedure (SOP) describes the operation, calibration and maintenance of a high performance liquid chromatography (HPLC) system.
Owner	
Areas Involved	QC Laboratories

Document Information

Master Production Records	N/A

Contents

Revision History

Author	Rev	Reason for Revision

References

Controlled Documents

This SOP refers to or relies on the following controlled documents:

Title	Reference No.
Laboratory Equipment	
Deviation Management	
Degassing and Sparging of Liquids	
Analytical Equipment Qualification	
Analytical Equipment Change Control	

Forms

This SOP refers to the following forms:

Form Title	Controlling SOP
Auto Injector Precision Check	
Pump Flow Rate	
U.V Detector Linearity Check	
U.V Detector Wavelength Accuracy Check	
Visible Detector Wavelength Accuracy Check	
Visible Detector Linearity Check	
Pump Gradient Accuracy Check	
Column Oven temperature check	
Cooled Auto sampler temperature check	
Auto Injector Precision Check (Fluorescence System)	
Fluorescence Detector Linearity Check	
Fluorescence Detector Wavelength Accuracy Check	
Reaction Coil Oven temperature check	
Record of Fault	

Document Templates

Title
HPLC System 12 Month Preventive Maintenance

External Standards

The following standards documents apply to this SOP:

Title	Reference
N/A	N/A

ISO Clause

4.2.4

Global Quality Standards

Title	Reference
Analytical Equipment	

Overview

When to use this Standard Operating Procedure

Use this Standard Operating Procedure (SOP)

For the operation, calibration and maintenance of a high performance liquid chromatography (HPLC) system.

Who Should Use This Standard Operating Procedure

This SOP is for

Laboratory personnel

Maintenance personnel

Training Requirements

Recommended Prerequisite Training for SOP Users

SOP 111 'Laboratory Equipment'

SOP 222 'Degassing and Sparging of Liquids'

SOP 333 ' Naming Conventions, Data Acquisition and Reporting'

Initial Training Delivery Method

The initial training method for this SOP Leader Led training because practical training is required due to its complex nature.

Retraining Delivery Method

There is a Change Notification/Delta for personnel trained in the previous revision of this procedure because there have been modifications which are intuitive by reading alone.

Approval Requirements for This SOP

This SOP requires approval from the document review board.

Record Retention Requirements for This SOP

Refer to the Global Retention Schedule for the official retention periods.

General Equipment Information

Introduction

GMP Classification	This equipment is classified as **E3a** and is therefore GMP critical.
Equipment Traceability	Equipment traceability **is required.** Record equipment ID number when used.
Location	Located in the QC laboratories and are f**ixed**.

Definitions

Analytical Column

HPLC columns are usually stainless steel columns with end fittings to retain packing material. These are most commonly packed with micro-particulate silica. The packing material is the stationary phase.

Gradient Elution

A predetermined change in mobile phase composition during separation e.g. 40% methanol/60% water increasing in a linear step to 80% methanol/20% water during the run.

Isocratic Elution

When there is constant solvent composition throughout chromatographic separation.

HPLC Analytical System

An analytical system consists of solvent reservoir(s), high/low pressure pump(s), an injection system, an analytical column, a UV/Vis absorbance and/or fluorescence based detector, data acquisition software and where required a column oven, sample cooler and/or a gradient controller.

High Performance Liquid Chromatography (HPLC)

This technique can be used for almost any sample that dissolves in a solvent. By choosing an appropriate column and mobile phase, desired separations can be achieved. The technique involves separation due to differences in the equilibrium distribution of sample components between two different phases. One of these phases is a solid stationary phase (the column), while the other is a moving liquid (the mobile phase). The samples migrate through the chromatography system only when they are in the mobile phase. Separation results from different velocities of migration as a consequence of differences in equilibrium distributions.

Mobile Phase

Mobile Phases used in HPLC may be water, organic solvents or buffers either on their own or mixed with one another. This acts as a carrier for the sample in the HPLC system.

Normal Phase HPLC

This mode of HPLC is used for separating compounds containing polar functional groups. Separation is due to differences in the partition coefficients of solutes between the relatively polar stationary phase and the relatively non-polar mobile phase.

Reverse Phase HPLC

This mode of HPLC is used for separating non-polar and moderately polar compounds. Separation is due to differences in the partition coefficients of solutes between the relatively non-polar stationary phase and the relatively polar mobile phase.

Use

A HPLC system is used for the analysis and determination of sample components.

Visuals

Shown below is a typical layout of a HPLC System:

[insert appropriate graphic.]

Precautions

A high performance liquid chromatography system can involve liquids being pumped at high pressure. Therefore, if the pump system requires maintenance, ensure the pump(s) are off or that the eluting solvent flow rate is zero.

Refer to CoSHH assessments and Material Safety Data Sheets for handling requirements needed for compounds being tested.

All electrical instruments are potentially hazardous; ensure that PATS testing has been carried out on the instrument and that the mains leads and connections are in good condition.

Before Use

Procedure for Changing Mobile Phase System

When changing from Reverse Phase to Normal Phase operation

Wash the system with methanol: water 50:50 or wash as recommended in the method for 30 minutes.

Remove the analytical column.

Insert a suitable connector in place of the column.

Draw through methanol and wash with 100% methanol for 30 minutes.

Draw through dichloromethane: methanol, 50:50 and wash for 30 minutes.

Draw through the Normal Phase eluant and wash for 30 minutes.

Insert normal phase analytical column.

Equilibrate with eluting solvent for a period of 30 minutes, or until a stable baseline is obtained.

Note that if changing a suitable column (e.g. NH_2) from reverse phase to normal phase, then leave column in place during change over.

When changing from Normal Phase to Reverse Phase operation

Wash the system with recommended wash solvent for 30 minutes.

Remove the analytical column.

Insert a suitable connector in place of the column.

Draw through dichloromethane: Methanol 50:50 and wash for 30 minutes.

Draw through and wash with 100% methanol for 30 minutes.

Draw through and wash with methanol: water, 50:50 for 30 minutes.

Draw through and wash with Reverse Phase eluant and wash for 30 minutes.

Insert reverse phase analytical column.

Equilibrate with eluting solvent for a period of 30 minutes or until a stable base-line is obtained.

Note that if changing a suitable column (e.g., NH_2) from Normal Phase to Reverse Phase, then leave column in place during change over.

Operating Procedure

Overview

For specific details of each individual instrument, refer to the instrument manufacturer's user manual.

Prepare mobile phase as stated in the analytical test method.

Ensure that the mobile phase remaining in the system (from previous run) is compatible with that to be used i.e. both reverse phase or both normal phase. Mixing aqueous and non-polar solvents can result in problems due to the immiscibility of the liquids.

If aqueous buffers have been used then the HPLC system/column needs to be flushed with a solvent composition excluding the buffer. This prevents precipitation of buffer in the system with pure organics.

Draw through the solvent(s) from the solvent reservoir(s) to the pump with the purge valve open, ensuring to pull through any bubbles trapped in the solvent line(s). For systems with degasser modules, it takes at least 40ml for the solvent to exit the degasser and reach the pump.

When drawing through more than one solvent for gradient elution, ensure that the proportioning valve or pump has switched to the correct reservoir. This can be done by turning on the flow at 100% A, B, etc. in turn.

Select an appropriate analytical column as stated in the analytical test method and record in OLDD. This data may also be recorded in the HPLC computer system if required.

Ensure that the column storage solvent is compatible to the mobile phase to be used.

Attach the auto sampler outlet to the column inlet and the detector inlet to the column outlet so that the flow will be in the direction of the arrow on the column. If using a column without an arrow, mark an arrow on the column indicating the flow direction used for future reference.

Set the system parameters as stated in the method, i.e. injection volume, column temperature, detector wavelength and the gradient programme etc.

Allow the mobile phase to run through the column for a minimum of 30 minutes to equilibrate the system prior to any injections.

The chromatographic data system should show the baseline to level out into a flat, horizontal line.

Using the XYZ Systems Controller

[Insert instructions for using a systems controller (if applicable).]

During equilibration

1	Check for any system leaks
2	Ensure that the pressure of the pump is a steady value; if this is not the case then flush the pump
3	If the baseline on the chromatogram does not stabilise and exhibits erratic behaviour (particularly shooting off scale), then there is likely to be air in the detector cell. This should be primed by gently pushing mobile phase through the cell using a syringe
4	If the baseline exhibits regular stepping patterns, either peaks or troughs, this means that the detector lamp is deteriorating and requires changing
5	If there is an absolutely straight baseline ensure that all electric connectors are in place and that the detector lamp is lit
6	If the expected profile is not exhibited during a blank gradient run. Ensure that the correct reservoirs are being used Check that the programme has been set correctly and the correct wavelength is being used Ensure the pump(s) and the mixing valve are in working order Ensure that the correct absorbance wavelength and/or the correct emission and excitation wavelength are selected for UV/Vis absorbance and/or fluorescence detection respectively
7	Prepare system suitability/standards/control/samples as directed in the appropriate analytical method

System suitability

System suitability is used as an instrument check as it monitors the method's performance on that instrument including column. Refer to relevant analytical test method.

Starting a run

1	Set up the sample run using the appropriate sample set method
2	Check that the HPLC conditions are stable and ready for testing to commence
3	If the instrument is ready, start the run

After Use

1	Calculate the results from the collected data as directed in the appropriate analytical method
2	After all work is complete, flush the HPLC system with an appropriate wash solvent (refer to Analytical Method)
3	If the system is not going to be used the next day, then switch off the detector lamp, column heater and pump flow

Maintenance and Qualification

Preventive Maintenance (PM)

When assays are completed, flush the HPLC system with a suitable wash solvent (refer to Analytical Method).

If the system is not going to be used the next day, then switch off the detector lamp, column heater and pump flow.

If the system over-pressurises (it will display an error message to this effect) or the pressure doesn't drop to zero with purge valve open, then replacement of the Purge Valve Frit is required (refer to Manufacturer's manual of how to replace the frit).

If a column fault is suspected e.g. peak broadening, follow instructions for regeneration of HPLC columns supplied by column manufacturers.

Corrective Maintenance (CM)

All corrective maintenance activities must be documented in detail as described in SOP 123.

If a fault or malfunction occurs complete a copy of the Record of Fault form as per SOP 123, describe the fault in detail and if known, the possible cause. Record the action taken and the parts cleaned or replaced.

Re-qualification

Re-qualification of the HPLC systems must be conducted every twelve months.

Refer to attachments A to M for the relevant Qualification documentation.

Relocation

If relocated, the following actions must be taken:

Raise a change control using the appropriate system

Carry out installation qualification (IQ)

Carry out operational qualification (OQ)

Calibration and Service

Calibration

Calibration is carried out as part of the annual service/qualification.

Service

HPLC instruments will be serviced on an annual basis by the Equipment Group. Following service, the instruments will be re-qualified.

Refer to templates for relevant servicing documentation.

Checks required after replacement or maintenance

Component	Part Repaired/Replaced	RQ test to be performed
Auto sampler	Needle Fixed volume sample loop Stator/rotor seal	Auto sampler Precision Check
VWD/MWD/ Fluorescence Detector	Flow cell	None
VWD/MWD/ Fluorescence Detector	Lamp(s)	Detector Linearity Check Wavelength Accuracy Check
Thermostatted Auto sampler	Cooler Unit Fan(s)	Temperature Check
Column Oven	Peltier Elements	Temperature Check
Iso/Quat/Binary Pump	Proportioning valve/solenoid	Pump Gradient Accuracy and Flow Rate Check
Iso/Quat/Binary Pump	Check valves/pump head/pump seals	Pump Flow Rate Check

Attachments

All forms are controlled and must be obtained from the Equipment Group.

Auto Injector Precision Check	1
Pump Flow Rate	2
U.V Detector Linearity Check	3
U.V Detector Wavelength Accuracy Check	4
Visible Detector Wavelength Accuracy Check	5
Visible Detector Linearity Check	6
Pump Gradient Accuracy Check	7
Column Oven temperature check	8
Cooled Auto sampler temperature check	9
Auto Injector Precision Check (Fluorescence System)	10
Fluorescence Detector Linearity Check	11
Fluorescence Detector Wavelength Accuracy Check	12
Reaction Coil Oven temperature check	13

Auto Injector Precision Check

Instrument set up and procedure:

Mobile phase	Water
Detector wavelength	265nm
Flow rate	1.00 mL min^{-1}
water system number	
Acetone lot number	
Approximate % v/v acetone solution used	

If the auto injector is capable of injecting variable volumes, perform 2 checks that cover the range of injection volumes to be used. If a fixed loop auto injector is used only one check needs to be performed.

Inject the prepared % (v/v) acetone solution 6 times onto the system.

Instrument Number	
Auto sampler make and model	
Auto sampler serial number	

Sample Set		
Max. syringe volume (L)		
Syringe volume used (L)		
Peak Area of injection	1)	7)
Peak Area of injection	2)	8)
Peak Area of injection	3)	9)
Peak Area of injection	4)	10)
Peak Area of injection	5)	11)
Peak Area of injection	6)	12)
Mean Area		
% R.S.D.		
% R.S.D. acceptance limit	1.0 % R.S.D.	
Pass / fail		

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Pump Flow Rate Check

Reference: SOP 123 (Page 1 of 1)

Instrument set up and procedure:

Mobile phase	Water
Other equipment	Dry 10.0 mL volumetric flasks or flow-meter
water system number	

Three flow rate checks should be performed on the pumping system at 0.50, 1.00, and 2.00 ml/min.

Instrument Number	
Pump make and model	
Pump Serial Number	

	1	2	3
Set flow rate mL min^{-1}			
Volume (mL)			
Time			
FLOWMETER USED:			
Measured flow rate mL min^{-1}			
Flow Rate Ratio: Actual/set			
Flow rate ratio limit	0.95 to 1.05		
Pass / fail			

SIGNED (INITIATOR)	DATE

SIGNED (SECOND PERSON VERIFIER)	DATE

UV Detector Linearity Check

Instrument set up and procedure:

Mobile phase	Water
Flow rate	1.0 mL min^{-1}
Detector wavelength	265 nm
Record injection volume (20 L is recommended)	L
Acetone lot number	
water system number	

Ensure that the highest acetone standard solution gives a response slightly less than 1 unit. Inject the range of 5 acetone solutions and record their peak area. Calculate the correlation coefficient for the plot of peak area against % v/v acetone concentration.

Instrument Number	
Detector make and model	
Detector Serial Number	

Sample Set		
Inj. #	Acetone % v/v concentration	Peak area
1		
2		
3		
4		
5		
Correlation coefficient		
Correlation Coefficient limit		0.990
Pass / fail		

SIGNED (INITIATOR)	DATE

SIGNED (SECOND PERSON VERIFIER)	DATE

U.V Detector Wavelength Accuracy Check

Instrument set up and procedure:

Caffeine lot number	
water system number	

Caffeine has two known absorbance maxima ($_{max}$) in the UV region of the electromagnetic spectrum. These are 205nm and 273nm.

The detector should be zeroed at the expected $_{max}$ with water. It may take a while for the system to stabilise. Then pump in the caffeine solution (this may be performed by direct injection into the detector inlet connector) until a steady absorbance reading is obtained.

Then take absorbance readings on each side of 205 and 273 nm to find the UV maxima. Each make and model of detector will have a slightly different way of doing this. The detector manual should be consulted for the best approach to take.

When all of the readings have been taken, determine the wavelength of maximum absorption.

Instrument Number	
UV Detector make and model	
UV Detector Serial Number	

Wavelength (nm)	Absorbance
200	
201	
202	
203	
204	
205	
206	
207	
208	
209	
210	
Measured wavelength (nm) peak maxima	
Acceptance limits	200 to 210 nm
Pass / fail	

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Instrument Number	
UV Detector make and model	
UV Detector Serial Number	

Wavelength (nm)	Absorbance
268	
269	
270	
271	
272	
273	
274	
275	
276	
277	
278	
Measured wavelength (nm) peak maxima	
Acceptance limits	268 to 278 nm
Pass / fail	

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Visible Detector Wavelength Accuracy Check

Instrument set up and procedure:

Holmium Perchlorate Lot Number	
water system number	

Holmium has absorbance maxima ($_{max}$) at 241, 278, 288, 361, 452, 468, 485, 536 and 641 nm. Note that absorbance maxima also occur at wavelengths close to the ones stated above and should not be confused with them.

Either choose 2 absorbance maxima that bracket the wavelength required for assay or the nearest single appropriate maxima wavelength.

The detector should be zeroed at the expected $_{max}$ with water. It may take a while for the system to stabilise. Then pump in the Holmium Perchlorate solution (this may be performed by direct injection into the detector inlet connector) until a steady absorbance reading is obtained.

Then take absorbance readings on each side of the chosen wavelengths to find the absorbance maxima. Each make and model of detector will have a slightly different way of doing this. The detector manual should be consulted for the best approach to take.

Instrument Number	
Detector make and model	
Detector Serial Number	

Selected Wavelength =	
Wavelength (nm)	Absorbance
Measured wavelength (nm) peak maxima	
Acceptance limits: 8 nm of theoretical maxima	
Pass / fail	

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Visible Detector Linearity Check

Reference: SOP 123 (Page 1 of 1)

Instrument set up and procedure:

Mobile phase	Water
Flow rate	1.0 mL min^{-1}
Detector wavelength	
Holmium Perchlorate Lot Number	
Record injection volume (20 L is recommended)	L

Ensure that the highest holmium perchlorate standard solution gives a response slightly less than 1V. Inject the range of holmium perchlorate solutions and record their peak area. Calculate the correlation coefficient for the plot of peak area against the holmium perchlorate concentration.

Instrument Number	
Detector make and model	
Detector serial number	

Sample Set		
Inj. #	Holmium Perchlorate concentration	Peak area
1		
2		
3		
4		
5		
Correlation coefficient		
Corr. Coeff. limit		0.990
Pass / fail		

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Pump Gradient Accuracy Check

Instrument set up and procedure:

Mobile phase A	Water
Mobile phase B (Acetone in Water)	% v/v Acetone =
Flow rate	2.0 mL min^{-1}
Acetone lot number	
water lot number	

For binary gradient systems, pumps A and B should be checked. For tertiary gradient systems, firstly pumps A and B should be checked and then pumps B and C should be checked. For quaternary gradient systems, firstly channels A and B should be checked and then channels C and D should be checked.

Establish a stable baseline using 100% of mobile phase A. Use the following gradient table to perform the pump gradient accuracy check.

Time / min.	**% A**	**%B**
0.00	100	0
25.00	0	100
30.00	0	100
30.10	50	50
35.00	50	50
35.10	100	0
45.00	100	0

Obtain a plot of the gradient conditions and calculate the plateau response in mV at 100% (H1) and 50% (H2); see Example 1. Then use the following equation to calculate the ratio.

Ratio = 2 H2 / H1

Pump Gradient Accuracy Check

Calculate the dwell volume of the system this is for information purposes only. To do this, zoom in on the first couple of minutes of the plot; see Example 2. The dwell time t_d is estimated from the intersection of the line of best fit through the linear ramp and the baseline. The following equation is used to calculate the dwell volume.

Dwell volume (mL) = dwell time (min) flow rate (mL min^{-1})

Dwell volume (mL) = t_d (min) 2.00

If more than 1 combination is tested for the gradient check, a full scale overlay plot should be obtained for each combination (they should overlay as in Example 3). Visually inspect the linear ramps from 100% A to 100%B with the initial baseline at 100% A for any significant departure from theoretical linear profile.

Attach the following plots to the protocol:

Full scale gradient accuracy test, one for each combination tested.

Dwell volume plot.

Full scale overlay if more than one combination is checked for accuracy.

Instrument Number	
Pump make and model	
Pump Serial Number	

Sample Set	
Pumping combination e.g. A/B	
100 % plateau height (H1) / µV	
50 % plateau height (H2) / µV	
2 H2 / H1	
Acceptance limits for 2 H2 / H1	0.98 to 1.02
Pass / fail	

Sample Set	
Pumping combination e.g. C/D	
100 % plateau height (H1) /µ V	
50 % plateau height (H2) / µV	
2 H2 / H1	
Acceptance limits for 2 H2 / H1	0.98 to 1.02
Pass / fail	

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Instrument Number	
Pump make and model	
Pump Serial Number	

Sample Set	
Dwell time t_d /min	
Flow rate / mL min^{-1}.	
Dwell volume = t_d (min) flow (mL min^{-1})	
If more than one pump combination is used obtain a full scale overlay of these (list combinations)	
State whether overlay is satisfactory / unsatisfactory	

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Column Oven Temperature Check

Reference: SOP 123 (Page 1 of 1)

Instrument Number	
Oven make and model	
Oven Serial Number	

Thermometer Make/Model	
Serial Number	

Set Temperature (°C)	20.0	60.0
Time (Min)	Temperature Reading (°C)	
0		
10		
20		
30		
40		
50		
60		
Minimum Temperature		
Maximum Temperature		
Acceptance Criteria	2°C of Set Temperature	
Pass / Fail		

Comments

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Cooled Auto sampler Temperature Check

Reference: SOP 123 (Page 1 of 1)

Instrument Number	
Auto sampler make and model	
Auto sampler Serial Number	

Thermometer Make/Model	
Serial number	

Auto sampler Set Temperature (°C):					
Vial Position					
Temperature Reading (°C)					
Acceptance criteria	± 3°C				
Pass/Fail					

Comments

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Auto Injector Precision Check (Fluorescence System)

Reference: SOP 123 (Page 1 of 1)

Instrument set up and procedure:

Mobile phase	Water
Detector Wavelength (Ex/Em)	Ex 278nm / Em 337nm
Flow rate	1.00 mL min^{-1}
water system number	
Caffeine lot number	
Approximate % v/v caffeine solution used	

If the auto injector is capable of injecting variable volumes, perform 2 checks that cover the range of injection volumes to be used. If a fixed loop auto injector is used only one check needs to be performed.

Inject the prepared % (v/v) caffeine solution 6 times onto the system.

Instrument Number	
Auto sampler make and model	
Auto sampler Serial Number	

Sample Set		
Max. syringe volume / L		
Syringe volume used / L		
Area of injection	1)	7)
Area of injection	2)	8)
Area of injection	3)	9)
Area of injection	4)	10)
Area of injection	5)	11)
Area of injection	6)	12)
Mean Area		
% R.S.D.		
% R.S.D. acceptance limit	1.0 % R.S.D.	
Pass / fail		

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Fluorescence Detector Linearity Check

Reference: SOP 123 (Page 1 of 1)

Instrument set up and procedure:

Mobile Phase	Water
Flow Rate	1.0 ml/min
Detector Wavelength (Ex/Em)	Ex 278nm / Em 337nm
Caffeine Lot Number	
Water System Number	

Prepare a series of Caffeine solutions in water. Inject the range of solutions and record their peak area. Calculate the correlation coefficient for the plot of peak area against the caffeine concentration.

Instrument Number	
Detector Make and Model	
Detector Serial Number	

Sample Set		
Inj #	Caffeine Concentration	Peak Area
1		
2		
3		
4		
5		
Correlation Coefficient		
Correlation Coefficient Limit		≥ 0.990
Pass/ Fail		

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Fluorescence Detector Wavelength Accuracy Check

Instrument set up and procedure:

Mobile Phase	Water
Flow Rate	1 ml/min
Fluorescence Grade Water	

Excitation Wavelength Accuracy (Raman Band of Water) Test

The excitation wavelength accuracy (Raman Band of Water) test checks the calibration of the excitation monochromator. Water emits light at a wavelength peak of approximately 397nm when the wavelength accuracy of the excitation light is 350nm. Water can be used to check the accuracy of the monochromator by looking for a peak in the emission scan.

To perform the excitation wavelength accuracy test:

Flush the flow cell with HPLC quality water.

Using the handheld controller, enter the following parameter values:

Select ‘Settings’ followed by ‘FLD’. In this screen, set;

Ex λ = 350 nm

Em λ = 397 nm

MW Settings = MULTI EM

Then select ‘more…’ followed by ‘scan’. In this screen, set;

Emission = 377 to 417 in 1nm steps

Store Spectra = all w/o signals

Pump water through the cell until a steady reading is obtained. In the ‘Settings’ then ‘FLD’ screen select ‘spectrum’ and take intensity readings on either side of 397 nm to find the emitted light maxima.

Instrument Number	
Detector Make and Model	
Detector Serial Number	

Wavelength (nm)	Intensity
393	
394	
395	
396	
397	
398	
399	
400	
401	
Measured wavelength (nm) peak maxima.	
Acceptance Limits	397 ± 3nm
Pass / Fail	

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE

Reaction Coil Oven Temperature Check

Reference: SOP 123 (Page 1 of 1)

Instrument Number	
Oven make and model	
Oven Serial Number	

Thermometer Make/Model	
Serial number	

Set Temperature (°C)	130°C
Time (Min)	Temperature Reading (°C)
0	
10	
20	
30	
40	
50	
60	
Minimum Temperature	
Maximum Temperature	
Acceptance Criteria	4°C of Set Temperature
Pass / Fail	

Comments

SIGNED (INITIATOR)	DATE
SIGNED (SECOND PERSON VERIFIER)	DATE