RECOMMENDED PROCEDURES FOR CALIBRATION OF TEST AND MEASURING EQUIPMENT USED IN FORENSIC SCIENCE LABORATORIES

DIRECTORATE OF FORENSIC SCIENCE SERVICES
MINISTRY OF HOME AFFAIRS, GOVT. OF INDIA
BLOCK NO. 9, 8TH FLOOR, CGO COMPLEX
NEW DELHI – 110 003
Website: dfs.nic.in, Email: cfs-dfss@nic.in

Tel No. 011-24362676

Fax No. 011-2436281

राष्ट्रीय न्यायालयिक विज्ञान विश्वविद्यालय
(राष्ट्रीय महत्व का संस्थान, गृह मंत्रालय, भारत सरकार)
National Forensic Sciences University
(An Institution of National Importance, MHA, Government of India)
सत्यमेव जयते
विद्यया अमृतं अश्नुते

Dr. J. M. Vyas
Vice Chancellor
Padma Shri awardee

Date: 10/11/2022

FOREWORD

I feel extremely happy to place on record that a long awaited document, first of its kind in the country, **'RECOMMENDED PROCEDURES FOR CALIBRATION OF TEST AND MEASURING EQUIPMENT USED IN FORENSIC SCIENCE LABORATORIES'** is now available to all of us. I appreciate the initiative of Directorate of Forensic Science Services, MHA, GoI in preparing this exclusive document.

As a matter of fact, Equipment calibration is essential for establishing confidence in the results that are generated during qualitative and quantities analysis of forensic samples.

I am sure; it will provide advanced level of information to forensic scientists and will act as a manual for them.

The aim of preparation of this booklet is to provide documented, verified and validated procedures for calibration of general and sophisticated equipment routinely used in forensic science laboratories. The Experts have also devised various logs, which will help in maintenance of calibration, calibration plan and verification of calibration.

I am confident that this initiative of DFSS will definitely play an important role in our journey towards building quality Forensic Services in our Country. My best wishes to DFSS and its team for creating much needed literature which will go a long way in further improving quality standards of FSL's of the country.

National Forensic Sciences University ★ Gandhinagar ★

(Dr. J. M. Vyas)
Vice Chancellor
NFSU, Gandhinagar

Sector-9, Gandhinagar-382007, Gujarat, India.
Tel. (0) +91 79 23977102 • Fax +91 79 23247465 • E-mail : vc@nfsu.ac.in • Web : www.nfsu.ac.in

डॉ. एस. के. जैन
निदेशक–सह–मुख्य न्यायालयिक वैज्ञानिक

Dr. S.K. Jain, M.Sc Ph.D
Director-cum-Chief Forensic Scientist

सत्यमेव जयते

न्यायालयिक विज्ञान सेवा निदेशालय,
गृह मंत्रालय, भारत सरकार
ब्लॉक–9, तल नं. 8 , केन्द्रीय कार्यालय परिसर
लोधी रोड, नई दिल्ली–110 003

Directorate of Forensic Science Services,
Ministry of Home Affairs, Govt. of India
Block No. 9, 8th Floor, C.G.O. Complex
Lodhi Road, New Delhi-110 003 (India)
Tel. : 011-24362676 Fax : 011-24362819
E-mail : cfs-dfss@nic.in

November 15, 2022

PREFACE

Forensic Science applies scientific methods to the recovery, analysis, and interpretation of relevant materials and data in criminal investigation and court proceedings. It is both an intelligence and evidential tool to assist in the delivery of Justice. In many criminal cases forensic evidence is pivotal. The delivery of justice depends on the integrity and accuracy of that evidence, and the trust that society has in it.

Forensic science capabilities and facilities, from the crime scene to the court room, provide accurate, objective and timely information not only to law enforcement agencies and the criminal justice system, but also to regulatory authorities. The constantly changing pattern in both conventional and organized crime, including all forms of trafficking, has led in recent years to increased interest on the part of Governments and the international community in establishing or strengthening quality forensic science services at the national level. While examining existing resources to ensure the availability of both the skills and equipment necessary for effective forensic science facilities, the Directorate continue to receive a range of requests for advice and assistance the selection of appropriate equipment, calibration of test and measuring equipment and reference material.

Most of the laboratories are accredited or being accredited as per ISO/IEC 17025 (2017) and calibration of test and measuring parameters is one of the important clauses (e.g. Clause 7). It is observed that a lot of difficulties are faced by the laboratories to get the equipment and tools calibrated. Beside this, there are some tools, the calibration of which requires to be checked every time before use. Keeping this in view, DFSS formed a Committee of Experts to prepare the procedure for calibration of test and measuring equipment used in FSLs under the Chairmanship of Prof. R M Sharma, Advisor (Forensics)Rajiv Gandhi National University of Law, Patiala and Formerly Professor of Forensic Science and Dean, Faculty of Physical Sciences, Punjabi University, Patiala. We are grateful for Dr Sukhminder Kaur, Director, CFSL Chandigarh; Dr Rajeev Jain, Sc. B, Dr. Shivani Sharma, Jr. Scientific Officer and Sh. Rahul Gupta, SSA of CFSL Chandigarh, who immensely contributed with the committee for preparation of this Document.

I am sure that the document will play an important role in our journey towards building quality Forensic Services in our Country. This is first edition on the subject and we are looking forward for your comments and suggestions for future editions of this document.

15/11/2022

(Dr S K Jain)
Director-cum-Chief Forensic Scientist

Table of content

General guidelines and definitions related to calibration

I. Introduction:

Forensic Science Laboratories are institutions of National Importance and have a vital role in solving crime with the scientific approach by using hi-tech sophisticated instruments and tools. All these equipment and tools can give satisfactory and unambiguous results only if they are properly calibrated. Calibration ensures that measuring equipment / devices and processes used in the testing of crime exhibits meet the expected performance specifications within universally acceptable levels and accuracy.

In India, there are 7 Central Forensic Science Laboratories (CFSLs), 32 State Forensic Science Laboratories (SFSLs), and 90 Regional Forensic Science (RFSLs). Most of the laboratories are accredited as per ISO/IEC 17025 (2017) and calibration of test and measuring parameters is one of the important clauses (e.g. Clause 7). During various conferences and panel discussions, it is also observed that a lot of difficulties are faced by the laboratories to get the equipment and tools calibrated. Sometimes instruments and tools get calibrated by outside agencies and/or suppliers but verification of calibration poses a real problem. Beside this, there are some tools, the calibration of which requires to be checked every time before use.

The instruments and tools used in forensic laboratories are broadly classified into following major categories:

1. Measurement of physical features by using instruments such as micrometer, temperature indicator, weighing balance, volumetric flasks etc.
2. Calibration of sophisticated analytical instruments and methods used in various disciplines of forensic science such as:
    - Chemical Sciences: GC, GC-MS, HPLC, HPTLC, IC, LC-MS/MS, UV-Visible Spectrophotometer, FTIR etc.
    - Biological Sciences: PCR, RT-PCR, Genetic Analyzer
    - Physical Sciences: Comparison microscope, GRIM, XRD, EDXRF, HRSC etc.
    - Forensic Electronics: Various software and tools used for solving cyber cases.

The sole aim of this manual is to provide documented, verified and validated procedures for calibration of general and sophisticated equipment routinely used in forensic science laboratories. An attempt has also been made device various logs which will help in maintenance of calibration, calibration plan and verification of calibration.

II. Some of the important definitions related to calibration:

1. **Calibration:** "A set of operations to establish the relationship between value of quantity indicated by measuring instrument and system under specific conditions"

2. **Traceability:** "The concept of establishing valid calibration of a measuring standard or instrument by step – by – step comparison with better standards up to an acceptable International / National standard.

3. **Standard:** A standard is a material measure or physical property that defines or reproduces the unit of measurement of base or derived quantity.

4. **Absolute Standard:** The standard whose value has been established without recourse to another standard of the same quantity e.g. second, minute, kilograms, ampere.

5. **International Standards:** One recognized by international agency on the basis for fixing value of all other standards of given quantity.

6. **National of Primary Standard:** It is one which establishes the value of all other standards of a given quantity within a particular country.

7. **Secondary standard:** One whose value has been established by comparison with primary standard.

8. **Working standard:** A secondary standard used to verify measuring instruments at their locations including filed locations.

9. **Reference Standard:** The standard of highest purity from which measurement made at a location is derived.

10. **Reference material:** Sufficiently homogenous stable material with respect to one or more specified quantities used for calibration of a measuring system for the assessment of measurement process.

11. **Certified Reference Material (CRM):** Reference material with authenticated certificate having each specified quantity a value, measurement uncertainty and stated meteorological traceability chain.

12. **Accuracy:** It is the closeness of agreement between results of measurement and true value of measurand.

13. **Precision:** Closeness of agreement between the results of successive measurement of the same measurand carried out under the same conditions of measurement.

14. **Robustness:** Closeness of the agreement between the results and measurement of the same measurand carried out under minute and deliberately changed condition of measurement.

15. **Least Count:** It is the minimal value of a unit that can be read in an instrument or device.

16. **Resolution:** It is the smallest difference indications of displaying device that can be meaningfully distinguished.

17. **Uncertainty:** It is the parameter associated with the results of measurement which characterizes the dispersion of value that could reasonably be attributed to the measurand.

18. **Confidence level:** It is the probability that is expressed in decimal or percentage that the true value lies in the specified range of value.

III. Need of periodicity of calibration:

Calibration of the measuring instrument ensured that the displayed value is accurate and repeatable with respect to traceable standards. However, it cannot be assumed that the instrument once calibrated will always give accurate results. Instruments, whether small or sophisticated need to be reviewed periodically for affirmation and require re-calibration when the instrument undergoes maintenance and once out of order or gets repaired.

Periodicity of calibration is based on multiple factors such as:

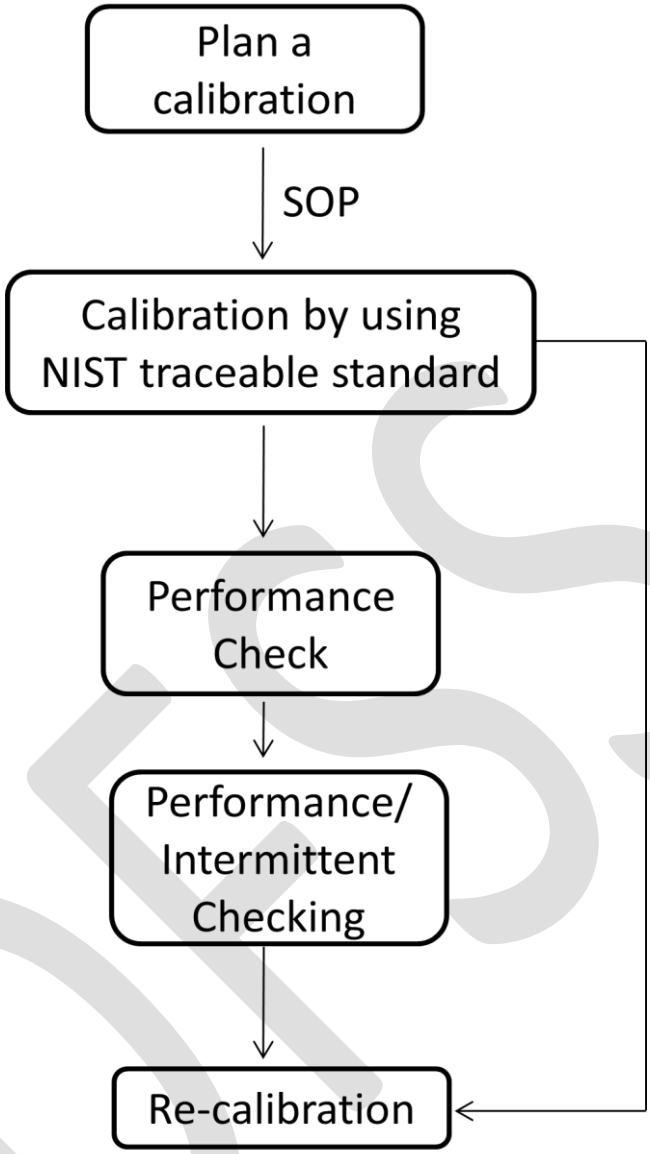
- Frequent use of the instrument
- Influence of environment
- Deviation in the allowable variation of measurand
- Check of uncertainty

There cannot be one universal method to ascertain the periodicity. The experiment and knowledge of the operators play a major role. The recommended operating procedures of calibration of following various instruments and tools generally used in forensic laboratories along with periodicity of calibration are given in Annexure 1 to 21.

Sr. No.	Chemical Science		Physical Science		Biological Science	
	Instrument	**Periodicity (months)**	**Instrument**	**Periodicity (months)**	**Instrument**	**Periodicity (months)**
1.	Analytical Balance	12	Glass Refractive Index Measurement (GRIM)	12	Thermal Cycler	12
2.	pH Meter	12	Vernier Caliper	12	Genetic Analyzer	12
3.	Laboratory glassware	24	Screw Gauge	12	Real Time PCR	12
4.	UV-Visible Spectrophotometer	12	Atomic Absorption Spectrophotometer	12		
5.	FTIR Spectrophotometer	12	SEM-EDXA	12		
6.	HPLC System	12	Comparison microscope	12		
7.	GC-FID System	12				
8.	GC-MS System	12				
9.	HS-GC-FID System	12				
10.	Ion chromatography (IC)	12				
11.	HPTLC System	12				

Once the periodicity of calibration is decided, it is easy to plan and process the calibration by adapting the standard calibration procedures. Various log / forms which are generally required to plan a calibration of instrument, its performance check and to maintain a calibration record is given in Annexure I – IV.

The flow chart for calibration is as given below:


Plan a calibration
SOP
Calibration by using NIST traceable standard
Performance Check
Performance/ Intermittent Checking
Re-calibration

Various calibration logs

Annexure – I

Calibration plan

S. No.	Name of the Instrument	Make & Model	In house/ outside	Periodicity	Next Calibration due on	SOP reference	Traceable standard

Annexure – II

Performance check

Name of the Instrument		
Performance check material		
SOP reference No.		
Parameter checked	+/- value deviation	Acceptable/Not acceptable

Annexure - III

Calibration Record

S.no.	Name of the Instrument	Make & Model	Calibrated on	Method SOP no.	Traceability of standard	Details of Parameters acceptability range (Yes/No)

Calibration → OK / with deviation

Deviation, if any:

1 …….

2 …….

3 …….

Note: All printouts of instrument output, calibration graphs and recorded values are to be kept in record.

Annexure – IV

Calibration Certificate

Instrument:

System ID :
Manufacturer :
Make & Model :
Serial No. :

Environmental Conditions:

Temperature :
Relative Humidity :
Any other specific Conditions :

Specific Comments (example)

- Instrument was allowed to stabilize for at least 12 hours before calibration

Calibration

Date of calibration:

The instrument was calibrated by using Standard Operating Procedure (Ref. No.) _____ against _____ standard whose values are traceable to recognized _____ / National / International Standards. All the values of measured parameter are in allowable range of values / acceptable criteria.

Next calibration due on:

Signature of analyst

Approved by

Technical Manager **Quality Manager** **Director**

Note: The observed values should meet the acceptance criteria. Any deviation to acceptance criteria has to be recorded.

Validation of equipment and software for Digital Forensic Laboratories

Good software engineering includes phases for setting requirements, designing, constructing, testing, installing, validating, documenting, performing operations and maintenance, and retiring the software when appropriate. All phases are important aspects to consider when validating software.

- All equipment and software used by digital forensic lab personnel must first be tested and validated to confirm that it is operating as designed and producing accurate, valid results. Testing and validation must be repeated each time the equipment, firmware, and software are upgraded, reinstalled, or modified.
- The results of all testing and validation will be recorded and kept on file in order to document that all equipment being used in the collection and processing of digital evidence is functioning within the manufacturer's specifications and the examiner's expectations based on training and experience.
- In order to determine, if hardware or software is working properly, it must be tested by the user and found to perform consistently over time and deliver repeatable results in line with a known dataset each time it is used.
- Testing steps will be clearly detailed and should be followed in order. It is recommended that as each step in the testing and validation is completed, the person performing the testing and validation should acknowledge the completion of the step in writing with initials or another identifiable mark. Validation testing will be conducted by all examiners who use any of the collection and processing hardware or software, in any fashion or to any degree, to collect or process digital evidence in the digital forensic lab or at a crime scene.

2.1. VALIDATION PROCEDURES:

2.1.1. Once the software has been approved for operational use, routine maintenance may be performed to remove errors, to respond to new or modified equipment, or to adapt the software to changes in the operating environment. All planned changes must be approved by

the Laboratory Director or Technical Manager before work is started. After any modifications, software must be verified and validated again. No forensic equipment will be used in the digital forensic lab prior to being tested and validated by lab personnel and approved by the lab Director.

2.1.2. All hardware and software will be registered in the lab's name. If the registration is in the name of an individual, approval from the lab director will be obtained in a memo format, with a copy of the memo maintained on the lab server (if available) and in the lab validation report binder.

2.1.3. An updated version of the software and a high-end configuration system should be require to execute the digital forensic software otherwise software can be crash /hang or produce inadequate results.

2.1.4. Every tool (S/W & H/W) has unique specifications and examiner should have knowledge regarding the abilities of the S/W & H/W and can work on the tool based on their requirement.

2.1.5. Examiners will test each item of hardware and software in a manner consistent with the manufacturer's specifications of usage. Testing will be performed using the same datasets for standardization. All results and anomalies will be documented in calibration record.

2.1.6. Examiners performing the validation testing on all forensic hardware and software will use a standardized testing and report form, including the date of validation, product name, version number, manufacturer, and cost. The lab director will approve all of the validation reports for each item of hardware and software before those items are used in the lab, and all the reports will be maintained on the digital forensic lab server (if available) and also in paper format in a binder maintained within the lab.

2.2. MAINTAINING VALIDATIONS

When a piece of equipment becomes damaged or is showing signs of wear or age, it should be tested to verify that it is still operating within the manufacturer's specifications. After the initial validation of a piece of software, subsequent validations will be done whenever an update to the software is installed on the lab equipment — including the computers used to examine digital evidence (examination machines). Subsequent hardware validations will be performed each time when existing hardware is updated, including firmware updates or installing a new or replacement item, such as a write-blocker. Any hardware upgrades done to an examiner's computer, however minor, should be documented and tested to ensure that they do not affect the performance of the forensic software installed on the computer.

2.3. SOFTWARE UPDATES

Updates, patches, or operating system service packs should be installed to the examiner's computer as necessary. The updates, patches, or service packs should be downloaded using a system connected to the Internet but isolated from the examination machine and the forensic network. After performing any updates to forensic software, the hard disk drive may be imaged and the image may be maintained for future use in restoring the examiner's computer.

When the software product is in use and not functioning properly then it can be subjected to the service, maintenance, support and performance check.

When a new version of the software product is taken into use, the effect on the existing system is carefully analyzed and the degree of revalidation decided.

It is also very much pertinent to consider how (and when) to discontinue the use of the software product. The potential impact on existing systems and data are needed to be examined prior to withdrawal.

Annexure - 1

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF ANALYTICAL BALANCE

1. Description of the item to be calibrated

- **Name:** Analytical Balance
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined:

- Accuracy
- Uncertainty

3. Reference standard and reference material:

- NIST traceable analytical standard weights

4. Environmental conditions and stabilization period

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** 5 - 10 min

5. Any safety measures / precautions to be observed

- Ensure to place the balances in an area with controlled humidity and temperature. They should not be exposed to direct sunlight since it can cause temperature variations inside the weighing chamber.
- Place the analytical balance on a vibration resistant table.
- Don't place the balances next to doors or windows since opening or closing them will result in air drafts.
- Ensure to weigh the samples only after closing the weighing chamber doors. Lastly, keep the weighing chamber clean to prevent cross-contamination of samples and erroneous readings.
- Never touch the weights with bare hands as hand grease can cause errors in the readings. Always use a pair of clean forceps while placing the sample.
- Always store the weights in a room free of moisture, corrosive gases, and dust.

6. Procedure:

- **Accuracy:** 1 mg, 2 mg, 5 mg, 10 mg and 20 mg calibration standard (NIST traceable) weights should be placed separately on the top pan balance after the auto calibration process, and the measurements noted in the performance check log.
    - **Acceptance criteria**: measurements need to remain within the 0.1% of the actual mass value of each weight.
- **Uncertainty:** Calculate the average observed weight for 10 measurements of certified weight e.g. 10 mg and determine the random error (3 × standard deviation). Calculate uncertainty by following formula: MU = (random error × 100)/actual weight of certified weight.
    - **Acceptance criteria:** MU shall be less than 0.1 %

7. Data to be recorded and presentation*:

Accuracy

Actual weight (mg)	Observed weight (mg)	Deviation (%)
1		
2		
5		
10		
20		

*for illustrative purpose only

Ratio and intensities may vary according to instrument make and model and can be determined by the softwares provided by the manufacturer.

8. Results:

Annexure - 2

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF pH METER

1. Description of the item to be calibrated

- **Name:** pH Meter
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined:

- Accuracy

3. Reference standard and reference material:

- NIST traceable analytical buffers or standard buffers.

4. Environmental conditions and stabilization period

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** 5 - 10 min

5. Any safety measures / precautions to be observed

- Electrodes are fragile and can be damaged easily, Use caution when handling the electrodes. Keep the glass electrodes in the storage solution when not using.
- Handle buffer solutions with care. The solutions may cause eye and skin irritation, and may be harmful if swallowed or inhaled.
- Always wear gloves, goggles, and lab coats while handling solutions.
- Buffers should always be read at accurate pH.
- Do not immerse electrodes in the buffer solutions before rinsing the electrodes thoroughly with deionized water.
- Do not mix buffers with other solutions or contaminate with samples.

6. Procedure:

- Commercially-prepared NIST traceable buffers or standard buffers (as specified in a pharmacopoeia) are used according to the manufacturer's instructions. Commonly pH buffers of

4.01, 7 and 10.01 are used for calibration of pH meters. Maintain the temperature of sample at 25 °C ±2 °C. For detailed procedure, refer to the working guidelines provided by manufacturer.

- **Acceptance criteria**: Deviation ±0.5.

7. Data to be recorded [*]:

Accuracy at 25 °C

Standard buffer solution	Observed pH at 25 °C	Deviation (%)
4.01		
7		
10.01		

*for illustrative purpose only

Ratio and intensities may vary according to instrument make and model and can be determined by the softwares provided by the manufacturer.

8. Results:

Annexure - 3

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF LABORATORY GLASSWARE

1. Description of the item to be calibrated

- **Name:** Volumetric flask / pipette /measuring cylinder
- **Make:**

2. Measurement parameters, quantities and ranges to be determined:

- Accuracy

3. Acceptance criteria:

- Labeled Tolerance on glassware in mL (e.g. ±0.04 mL)

4. Material required:

- Calibrated analytical balance.
- Calibrated digital thermometer.
- Distilled water.

5. Environmental conditions and stabilization period

- **Temperature:** ~25 °C

6. Any safety measures / precautions to be observed

- The variation in the temperature shall not be more than ±0.5 °C

7. Procedure:

- Place the required amount of distilled water in the beaker.
- Allow the temperature of the water to be equilibrated at room temperature (25°C ±2°C)
- Weight the dry empty glassware and note down the weight (W1).
- Fill the glassware to nominal volume with distilled water.
- Again weight the glassware and note down the weight (W2).
- Calculate the difference in weight (W2-W1).
- Convert weight difference in terms of mL of water.
- Repeat the above step in triplicate.

8. Data to be recorded and presentation*:

Flask 1 (50 mL)	1st	2nd	3rd
Tare mass (g), W1	24.3398	24.3398	24.3398
Filled mass (g), W2	74.3634	74.3630	74.3627
Mass of water (g), W2-W1	50.0236	50.0232	50.0229
Volume of water (mL)	50.0386	50.0382	50.0379
Accuracy (%)	100.077	100.076	100.075
Tolerance	+0.038	+0.038	+0.037

*for illustrative purpose only

8. Results:

Annexure - 4

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF GLASS REFRACTIVE INDEX MEASUREMENT SYSTEM (GRIM)

1. Description of the item to be calibrated

- **Name:** GLASS REFRACTIVE INDEX MEASUREMENT SYSTEM (GRIM)
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined

Refractive index (RI) of glass samples in the Trace Unit

3. Reference standard & reference material

Set of Locke Scientific Glass Standards

- A1 Locke Scientific Glass Standard
- A2 Locke Scientific Glass Standard
- A3 Locke Scientific Glass Standard
- A4 Locke Scientific Glass Standard
- A5 Locke Scientific Glass Standard
- B2 Locke Scientific Glass Standard
- B3 Locke Scientific Glass Standard
- B4 Locke Scientific Glass Standard
- B6 Locke Scientific Glass Standard
- B7 Locke Scientific Glass Standard
- B8 Locke Scientific Glass Standard
- B9 Locke Scientific Glass Standard
- B10 Locke Scientific Glass Standard
- B11 Locke Scientific Glass Standard
- B12 Locke Scientific Glass Standard
- C1 Locke Scientific Glass Standard
- Immersion liquids, such as Locke Silicone Oils Type A, B, and C

Acetone

4. **Environmental conditions and stabilization period**

- **Temperature:** 21 – 40 °C
- **Humidity:** <20% maximum relative humidity
- **Stabilization period:** ~ 15 min

5. Any safety measure to be observed

- This method will not differentiate between glasses whose refractive indices differ by fewer than ± 0.00003.
- Glass slides are sharp.
- High temperatures may be produced. Care shall be exercised when using this process.
- All standards shall be packaged separately and stored at room temperature next to the instrument.

6. Procedure:

1. Turn "ON" the power switch.
2. Turn "ON" the power source to the phrase contrast microscope.
3. Turn on the computer.
4. Allow the system to warm up and stabilize for one (1) hour before taking readings.
5. Open the software by double clicking A calibration curve with the B Oil shall be performed annually using the B2, B3, B4, B6, B7, B8, B9, B10, B11 and B12 Locke Scientific Glass Standards.
6. Calibration curves with the A and C oils shall be done upon use when the A and C oils are required for the examination.
7. Go to the menu bar and select: File/open from the dialog box select: Sbi/gfw, then click on ok.
8. Return to the menu bar and select: Recalibrate From the dialog box select: Oil B & Wave 589.
9. The calibration page will appear and it will have a previously entered data. You can replace this data with current data as you proceed with calibration.
10. Crush a sample of B1 glass and immerse in B liquid.

11. Click on first match temperature box and then click on Measure B1.
12. This will bring you to the hotstage screen.
13. Choose an edge with sufficient contrast and select F9 Setup. Position the analysis box over the edge of sharp contrast.
14. Adjust temperature to 5 degrees above previous mean temperature.
15. Click on F4 Auto to record match temperature.

    Note: Edge count is a measure of contrast. The edge count should always be above 10 and optimally be at 99. 16.
16. If analysis is acceptable select F10 Accept. 17.
17. If not acceptable, select F9 Setup to reposition analysis box.
18. In calibration dialog select Save to save value.
19. Repeat steps 12-18 for fragments 2-5.
20. Repeat steps 11-19 for glass standards B2-B12.

    Note: During analysis should you decide to exit the calibration box you must select Calibrate in order to save the current values. This command will adjust the match temperature values to the current entries.

7. Data to be recorded and presentation*:

S. No	Glass Sample*	Immersion Liquid	Temperature			Refractive Index
1	B2	Immersion liquids such as Locke Silicone Oil Type B				
2	B3					
3	B4					
4	B5					

*For illustration purpose only.

8. Results:

Annexure - 5

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF VERNIER CALIPER

1. Description of the item to be calibrated

- **Name:** VERNIER CALIPER
- **Make:**
- **Model No: NA**

2. Measurement parameters, quantities and ranges to be determined:

- Accuracy

3. Reference standard and reference material:

- Standard length rods e.g. 25 mm, 50 mm, 75 mm and 100 mm.

4. Any safety measures / precautions to be observed

- The jaws of calliper are sharp and can injure the user. Care should be taken when handling it.
- Vernier Calliper is a precision instrument. It shall be handled carefully. Care to be taken to prevent it from falling and causing damage to its jaws.
- The calliper should not be used in corrosive environment.
- The calliper should be cleaned with dry cloth and kept securely in its box after use.
- The calliper should be used in the normal temperature range, as specified. Measurements done at too high or too low temperatures will affect its accuracy.
- In case of a digital calliper, it would be prudent to remove its battery if the calliper is not used frequently; to avoid damage in the event of battery leakage.

5. Procedure:

- Perform a zero check on calliper. Keeping the calliper in position as both jaws touching each other, the scale should read 0 (zero) mm, or inch.
- NIST standard shall be measured with the Vernier calliper at the specified temperature range. The Vernier calliper reading(s) shall be compared to the standard's dimensions.
- **Acceptance criteria**: Tolerance: ±0.05 mm.

6. Data to be recorded and presentation*:

Sr. No.	Standard length rods (mm)	Reading of measurement jaws at position (mm)			Average reading (mm)	Limit (mm)	
		Upper	Centre	Lower		Min.	Max.
1	25					24.95	25.05
2	50					49.95	50.05
3	75					74.95	75.05
4	100					99.95	100.05

8. Results:

Annexure - 6

STANDARD OPERATING PROCEDURE FOR CALBRATON OF SCREW GAUGE

1. Description of the item to be calibrated

- **Name:** SCREW GAUGE
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined:

- Accuracy

3. Reference standard and reference material:

- Standard slip gauge e.g. 1.04 mm;

4. Any safety measures / precautions to be observed

- Screw gauge is a precision instrument. It shall be handled carefully. Care to be taken to prevent it from falling.
- Care should be taken to unlock the gauge before using, else the ratchet can get damaged.
- The specimen to be measured should be placed such that the anvil and spindle just touch it 9the specimen). Too much tightening of spindle on the specimen may damage the softer material (specimen or edges of spindle). Final adjustment of thimble shall be done with the ratchet, and locked, to get accurate result.
- The screw gauge should not be used in corrosive environment.
- The screw gauge should be cleaned with dry cloth and kept securely in its box after use.
- The screw gauge should be used in the normal temperature range, as specified. Measurements done at too high or too low temperatures will affect its accuracy.

5. Procedure:

- Perform a zero check on screw gauge. Keeping the screw gauge in position as its spindle touches the anvil, the scale should read 0 (zero) mm.
- NIST standard shall be measured with the screw gauge at the specified temperature range. The screw gauge reading(s) shall be compared to the standard's dimensions.

- **Acceptance criteria**: As per manufacturer instructions

5. Data to be recorded and presentation*:

Sr. No.	Slip gauge reading (mm)	Main Scale Reading, MSR (mm)	Vernier / Circular Scale Reading (mm)	Total Reading [MSR+(No. of division on circular scale × Least Count)]	Error
1	1.04				
2	1.1				
3	1.9				

*For illustrative purpose only

Note: Least Count = Pitch / No. of division on the circular scale

8. Results:

Annexure - 7

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF UV-VISIBLE SPECTROPHOTOMETER

1. Description of the item to be calibrated:

- **Name:** UV-Visible Spectrophotometer
- **Make:**
- **Model No:**

2. **Measurement parameters, quantities and ranges to be determined**:

- Wavelength accuracy
- Photometric accuracy
- Quantity & ranges: Concentration of standard in the range of ppm (µg/mL) & ppb (ng/mL)

3. Reference standard & reference material

- Certified Reference Material with NIST traceability or analytical standard with ≥99 **%** purity e.g. paracetamol

4. Environmental Conditions and stabilization period:

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** ~ 30 min

5. Any safety measure to be observed

- The solution in the cuvette should be 2/3～4/5 of the height of the cuvette, and it should not be too full to prevent the liquid from overflowing and corroding the instrument.
- Keep the cuvette clean during the measurement, and wipe the liquid droplets on the wall with lens cleaning paper. Do not pinch the translucent surface with your hands. When measuring the ultraviolet wavelength, a quartz cuvette is required.

- During the measurement, it is forbidden to put reagents or liquid substances on the surface of the instrument. If the UV-Vis spectrophotometer has solution overflow or other reasons, the sample tank should be cleaned up as soon as possible.
- After the experiment, pour out the solution in the cuvette, then rinse the cuvette with distilled water or organic solvent until it is clean, and dry it upside down. Turn off the power, put the desiccant into the sample chamber, cover the dust cover, and register for use

6. Procedure

- UV absorption wavelengths are checked with NIST traceable holmium and didymium filters, which should be supplied by the manufacturer.
- Scan range: 200 – 800 nm
- With empty cell holder take base line scan.
- Now put holmium oxide glass filter into the cell holder and take full scan UV-Vis spectra
- Refer calibration certificate of holmium oxide glass filter and match the observed wavelengths with those provided in calibration certificate by the manufacturer.
    - **Acceptance criteria**: ±1.0 nm.
- For photometric accuracy, compare the absorbance at particular wavelengths provided in calibration certificate with observed absorbance at corresponding wavelengths.

7. Data to be recorded and presentation:

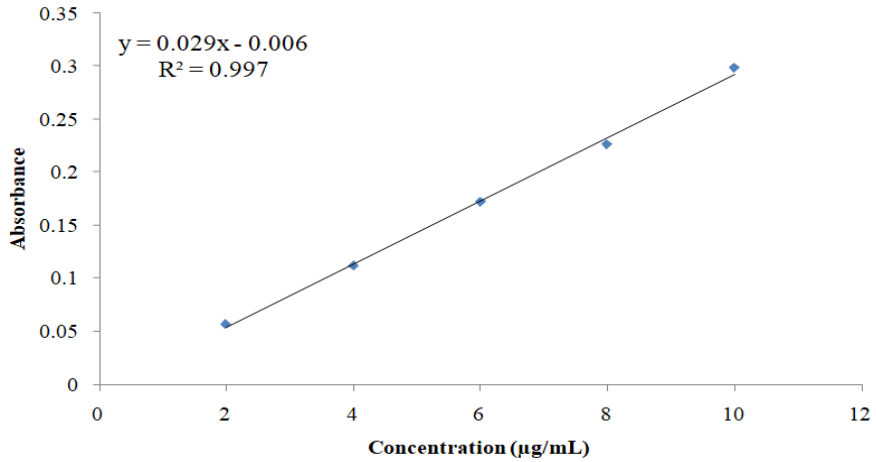
Sr. No.	Wavelength in calibration certificate* (nm)	Observed wavelength (nm)	Deviation (±nm)
1	241		
2	279		
3	361		
4	453		
5	536		
6	638		

*For illustrative purpose only.

8. Calibration plot and accuracy check*

Concentration (µg/mL)	Absorbance	Calculated Concentration (y = mx+c)	Accuracy [(Observed value/True Value)*100]
2	0.0566	2.15	107.9
4	0.112	4.0	101.7
6	0.172	6.13	102.2
8	0.226	8.0	100
10	0.2982	10.4	104.8

*For illustrative purpose only.



8. Results:

Annexure - 8

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF FTIR SPECTROPHOTOMETER

1. Description of the item to be calibrated

- **Name:** FTIR spectrophotometer
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined

- Resolution
- Wave number accuracy

3. Reference standard & reference material

- Certified Reference Material with NIST traceability of polystyrene film

4. **Environmental conditions and stabilization period**

- **Temperature:** 21 – 40 °C
- **Humidity:** < 20% maximum relative humidity
- **Stabilization period:** ~ 15 min

5. Any safety measure to be observed

- The FTIR spectrophotometers use laser lights and electrical power to perform their functions. Improper use can lead to body injuries and damage to the machine.
- Never look or use optical instruments to look into the laser beam. Direct viewing of the laser light can cause permanent eye damage.
- Keep the machine dry and avoid any contact with water

6. Procedure:

- In order to calibrate resolution of the instrument; the total range of the instrument is scanned using a polystyrene film (NIST traceable).
    - **Acceptance criteria**: The absorption peak at 3095 cm^{-1} should be resolved from that at 3080 cm^{-1} and the absorption at 3020 cm^{-1} should be resolved from that at 3015 cm^{-1}.

- In order to calibrate wave number accuracy; a polystyrene film is scanned and the accuracy of the peaks at wave numbers 2852, 1602 and 1028 is checked.
    - **Acceptance criteria:** The accuracy should be within ±3-5 cm^{-1} in the rang 4000-2000 nm and ± 1.5-2.5 cm^{-1} in the range below 2000 cm^{-1}

7. Data to be recorded and presentation *:

Resolution

	Difference between %T at 3095 cm^{-1} & 3080 cm^{-1}	**Difference between %T at 3020 cm^{-1} & 3015 cm^{-1}**
Limit	Not less than 15 %	Not less than 12 %
Actual		

Wave number accuracy

Wave numbers to be checked (cm^{-1})	**Observed wave numbers (cm^{-1})**	**Deviation (±cm^{-1})**
1028		
1602		
2852		

*For illustration purpose only.

8. Results:

Annexure - 9

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1. Description of the item to be calibrated

- **Name:** High Performance Liquid Chromatography (HPLC)
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined:

- **Pump:** Flow rate accuracy
- **Injector:** Precision and linearity
- **Quantity & Ranges:** Concentration of standard in the range of ppm (µg/mL) and ppb (ng/mL)

3. Reference standard and reference material:

- Certified reference material (CRM) with NIST traceability or analytical standard ≥99% purity e.g. caffeine.

4. Environmental conditions and stabilization period

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** ~ 30 min

5. Any safety measure to be observed

- The chemical hazards vary depending on the experiment. Flammable organic liquids are often used as the mobile phase for HPLC. The sample that is being analyzed may be toxic, biohazardous, flammable, etc. All the necessary precautions for these compounds should be taken into account when conducting work with an HPLC. Always read the SDS for any compound that is being used as a solvent or as the analyte for HPLC.
- Prepare any solutions for HPLC in a fume hood to ensure adequate ventilation and prevent inhalation.

- Ensure that solvent reservoirs and waste container are air-tight, in secondary containment and do not allow the solvent vapors to enter the room.
- Ensure that the pressure is behaving normally and is well below the maximum pressure for the HPLC system.

6. Procedure:

- In order to check flow rate accuracy the column affluent (mobile phase) is collected in a calibrated measuring cylinder or volumetric flask for a particular time interval.
    - **Acceptance criteria:** ± 2%
- In order to check the precision of injector, a set of regularly used NIST traceable standard (e.g. caffeine) is injected 3–5 times and the percent relative standard deviation (%RSD) is calculated for retention time and peak areas.
    - **Acceptance criteria**: % RSD ≤ 1%
- Similarly, for checking the linearity of the injector, different volumes of the standard are injected (e.g. 5 µL, 10 µL, 20 µL, 50 µL and 100 µL) and coefficient of determination (R^2) is calculated.
    - **Acceptance criteria:** $R^2 \geq 0.999$

7. Data to be recorded and presentation*:

Flow rate accuracy

Set flow rate	Observed flow rate	Deviation
2 mL min^{-1}		

Precision of injector:

Injection number	RT (Peak area)
1	
2	
3	
4	
5	
%RSD	

Linearity of injector:

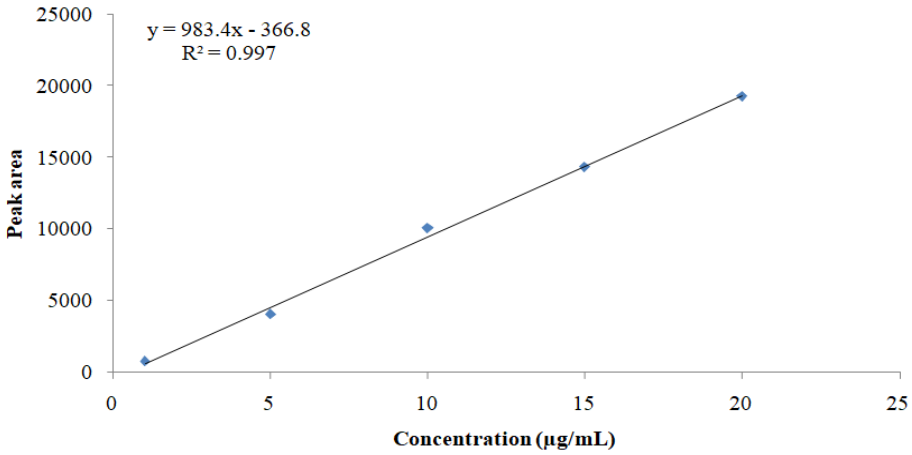
Injection volume (µL)	Peak area
5	
10	
20	
50	
100	
R^2 Value	

*for illustrative purpose only

8. Calibration plot and accuracy check*:

Concentration (µg/mL)	Peak area	Calculated Concentration (y = mx+c)	Accuracy [(Observed value/True Value)*100]
1	760	1.14	114.5
5	4048	4.39	87.8
10	10014	10.5	105.1
15	14257	14.8	99.1
20	19245	19.9	99.9

*For illustrative purpose only



8. Results:

Annexure - 10

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF GAS CHROMATOGRAPH-FLAME IONIZATION DETECTION (GC-FID) SYSTEM

1. Description of the item to be calibrated

- **Name:** Gas chromatograph – flame ionization detection (GC-FID)
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined:

- Linearity
- Accuracy
- Precision
- Quantity and ranges: Concentration of standard in the range of ppm (µg/mL) and ppb (ng/mL)

3. Reference standard and material:

- Certified reference material with NIST traceability of analytical standard with ≥99% purity e.g. hydrocarbons or pesticide mixture.

4. Environmental conditions and stabilization period

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** 30-60 min

5. Any safety measure to be observed

- Perform periodic visual inspections and pressure leak tests of the sampling system plumbing, fittings and valves.
- Never open oven and touch the injection port during operating conditions of GC.
- Follow the manufacturer's instructions when installing columns. Glass or fused capillary columns are fragile: handle them with care and wear safety glasses to protect eyes from flying particles while handling, cutting or installing capillary columns.

- Turn off and allow heated areas such as the oven, inlet and detector, as well as connected hardware, to cool down before touching them
- Turn off the hydrogen gas supply at its source when changing columns or servicing the instrument.
- When using hydrogen as fuel (flame ionization FID and nitrogen-phosphorus detectors NPD), ensure that a column or cap is connected to the inlet fitting whenever hydrogen is supplied to the instrument to avoid buildup of explosive hydrogen gas in the oven.

6. Procedure:

- In order to check the relation between detector response and concentration of analytes, a set of regularly-used NIST traceable standard(s) (e.g. mixture of hydrocarbons) are injected in different concentration at fixed injection volume. A linear regression curve is plotted between peak area and concentration of analyte and coefficient of determination (R^2) is calculated.
    - **Acceptance criteria:** $R^2 \geq 0.999$
- In order to determine the accuracy of the GC system, following formula is used: *(measured concentration / true concentration)*100*.
    - **The acceptance criterion:** ± 10%.
- Precision or repeatability is determined by analyzing 3 – 5 injections of same standard of same concentration.
    - **Acceptance criterion**: %RSD ≤ 5 %.

7. Data to be recorded and presentation*:

Linearity and accuracy:

True concentration (µg mL^{-1})	**Peak area**	**Measured Concentration (µg mL^{-1})**	**% Accuracy**
1			
10			
20			
30			
50			
R^2		**-NA-**	**-NA-**

Precision:

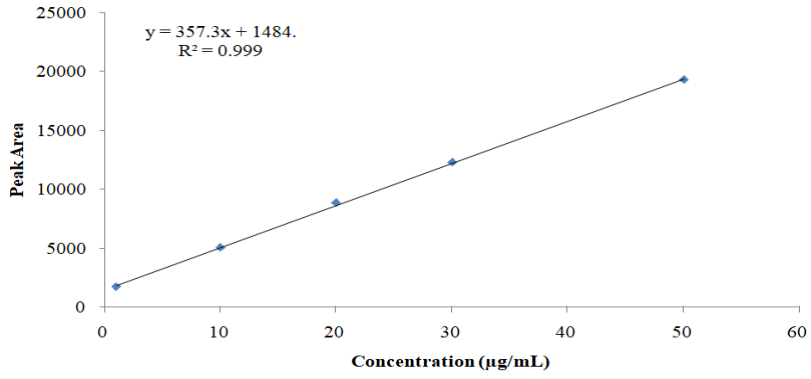
Injection number	Pear Area	Retention time
1		
2		
3		
4		
5		
%RSD		

*for illustrative purpose only

8. Calibration plot and accuracy check*

Concentration (µg/mL)	Peak area	Calculated Concentration (y = mx+c)	Accuracy [(Observed value/True Value)*100]
1	1702	0.61	61.01
10	5048	10.18	101.8
20	8841	20.71	103.5
30	12257	30.19	100.6
50	19245	49.58	99.1

***For illustrative purpose only**



8. Results:

Annexure - 11

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF GAS CHROMATOGRAPH-MASS SPECTROMETER (GC-MS) SYSTEM

1. Description of the item to be calibrated

- **Name:** Gas chromatograph – flame ionization detection (GC-FID)
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined:

- Linearity
- Accuracy
- Precision
- Quantity and ranges: Concentration of standard in the range of ppm (µg/mL) and ppb (ng/mL)
- Source tuning and mass calibration

3. Reference standard and material:

- Certified reference material with NIST traceability of analytical standard with ≥99% purity e.g. hydrocarbons or pesticide mixture for GC and NIST traceable heptacosafluorotributylamine (perfluorotributylamine) for MS.

4. Environmental conditions and stabilization period

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** 30-60 min

5. Any safety measure to be observed

- Perform periodic visual inspections and pressure leak tests of the sampling system plumbing, fittings and valves.
- Never open oven and touch the injection port during operating conditions of GC-MS.
- Follow the manufacturer's instructions when installing columns. Glass or fused capillary columns are fragile: handle them with care and wear safety glasses to protect eyes from flying particles while handling, cutting or installing capillary columns.
- Always changes septa time to time in order to avoid carry over effect.

- Always check for washing solvent vial (it should be filled with solvent) and waste vial (it should be empty) before each analysis.
- Turn off and allow heated areas such as the oven, inlet and detector, as well as connected hardware, to cool down before touching them
- Turn off the helium gas supply at its source when changing columns or servicing the instrument.

6. Procedure:

For GC: Please refer to procedure for GC-FID

For MS:

Calibrations compound such NIST traceable heptacosafluorotributylamine (perfluorotributylamine) is introduced to the spectrometer using a direct inlet device. The source is tuned using selected fragment ions to give optimum sensitivity and peak shape, and obtain peak ratios (for example, of m/z 69, 131, 219, 414, 502 and 614 in the perfluorotributylamine spectrum) usually determined by the manufacturer. Spectra are recorded and compared with the reference spectrum with respect to mass assignments and relative peak intensities.

7. Data to be recorded and presentation*

For MS:

m/z	Ratio#	Intensity#
69		
131		
219		
414		
502		
614		

*for illustrative purpose only

Ratio and intensities may vary according to instrument make and model and can be determined by the softwares provided by the manufacturer.

Linearity and accuracy:

True concentration (µg mL^{-1})	Peak area	Measured Concentration (µg mL^{-1})	% Accuracy
0.5			
1			
2			

5			
10			
R^2		**-NA-**	**-NA-**

Precision:

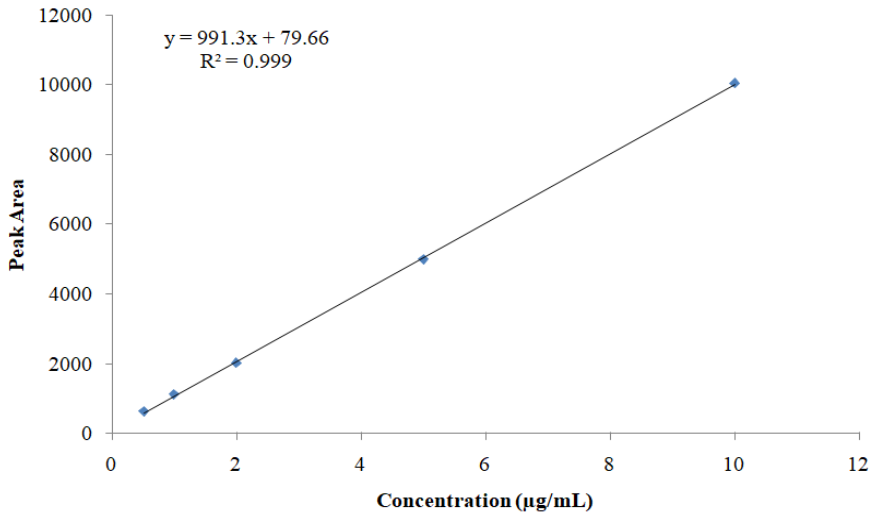
Injection number	Pear Area	Retention time
1		
2		
3		
4		
5		
%RSD		

*for illustrative purpose only

8. Calibration plot and accuracy check*

Concentration (µg/mL)	Peak area	Calculated Concentration (y = mx+c)	Accuracy [(Observed value/True Value)*100]
0.5	614	0.53	107.8
1	1102	1.03	103.3
2	2014	1.95	97.5
5	4985	4.94	98.6
10	10024	10.03	100.3

***For illustrative purpose only**



8. Results:

Annexure - 12

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF HEADSPACE-GAS CHROMATOGRAPH-FLAME IONIZATION DETECTION (HS-GC-FID) SYSTEM

1. Description of the item to be calibrated

- **Name:** Head space-Gas chromatograph – flame ionization detection (GC-FID)
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined:

- Linearity
- Accuracy
- Precision
- Quantity and ranges: Concentration of standard in the range of mg%

3. Reference standard and material:

- Certified reference material with NIST traceability of analytical standard with ≥99% purity e.g. ethyl alcohol.

4. Environmental conditions and stabilization period

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** ~30 min

5. Any safety measure to be observed

- Perform periodic visual inspections and pressure leak tests of the sampling system plumbing, fittings and valves.
- Never open oven and touch the injection port during operating conditions of GC.
- Follow the manufacturer's instructions when installing columns. Glass or fused capillary columns are fragile: handle them with care and wear safety glasses to protect eyes from flying particles while handling, cutting or installing capillary columns.

- Turn off and allow heated areas such as the oven, inlet and detector, as well as connected hardware, to cool down before touching them
- Turn off the hydrogen gas supply at its source when changing columns or servicing the instrument.
- When using hydrogen as fuel (flame ionization FID, ensure that a column or cap is connected to the inlet fitting whenever hydrogen is supplied to the instrument to avoid buildup of explosive hydrogen gas in the oven.

6. Procedure:

- In order to check the relation between detector response and concentration of analytes (e.g. ethyl alcohol), a set of regularly-used NIST traceable standard(s) (e.g. mixture of hydrocarbons) are injected in different concentration at fixed injection volume. A linear regression curve is plotted between peak area and concentration of analyte and coefficient of determination (R^2) is calculated.
    - **Acceptance criteria:** $R^2 \geq 0.999$
- In order to determine the accuracy of the GC system, following formula is used: *(measured concentration / true concentration)*100*.
    - **The acceptance criterion:** ± 10%.
- Precision or repeatability is determined by analyzing 3 – 5 injections of same standard of same concentration.
    - **Acceptance criterion**: %RSD ≤ 5 %.

7. Data to be recorded and presentation*:

Linearity and accuracy:

True concentration (mg%)	Peak area	Measured Concentration (µg mL^{-1})	% Accuracy
10			
50			
100			
200			
300			
R^2		**-NA-**	**-NA-**

Precision:

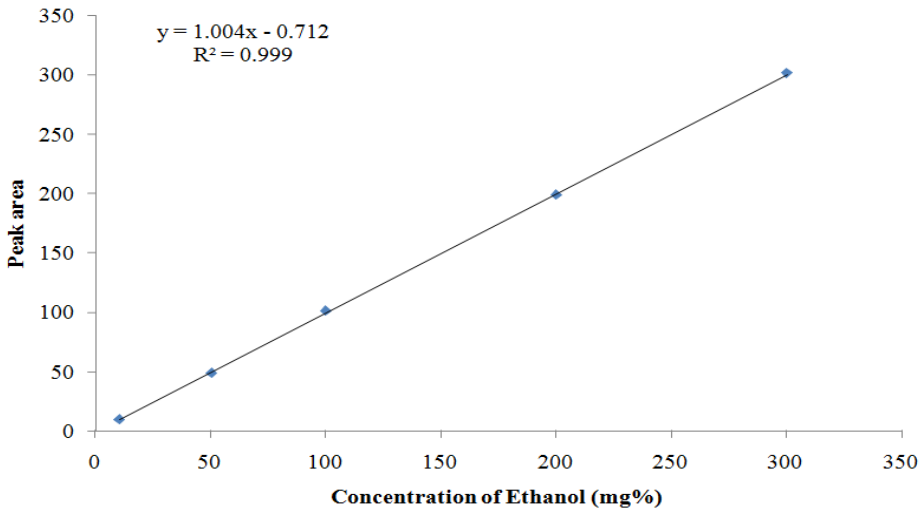
Injection number	Pear Area	Retention time
1		
2		
3		
4		
5		
%RSD		

*for illustrative purpose only

8. Calibration plot and accuracy check*

Concentration (µg/mL)	Peak area	Calculated Concentration (y = mx+c)	Accuracy [(Observed value/True Value)*100]
10	9.8	10.4	104.7
50	48.5	49.01	98.0
100	101.2	101.5	101.5
200	198.6	198.5	99.2
300	301.5	301.0	100.3

***For illustrative purpose only**



8. Results:

Annexure - 13

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF LIQUID CHROMATOGRAPH-MASS SPECTROMETRY (LC-MS) SYSTEM

1. Description of the item to be calibrated

- **Name:** Liquid chromatography-mass spectrometry (LC-MS)
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined:

- Linearity
- Accuracy
- Precision
- Quantity and ranges: Concentration of standard in the range of mg%
- Mass Accuracy for MS

3. Reference standard and reference material:

- Certified reference material (CRM) with NIST traceability or analytical standard ≥99% purity e.g. caffeine for LC
- NIST traceable polypropylene glycols (PPGs) and polyethylene glycols (PEGs) for MS.

4. Environmental conditions and stabilization period

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** ~ 30-60 min

5. Any safety measure to be observed

- The chemical hazards vary depending on the experiment. Flammable organic liquids are often used as the mobile phase for HPLC. The sample that is being analyzed may be toxic, biohazardous, flammable, etc. All the necessary precautions for these compounds should be taken into account when conducting work with an HPLC. Always read the SDS for any compound that is being used as a solvent or as the analyte for HPLC.

- Prepare any solutions for HPLC in a fume hood to ensure adequate ventilation and prevent inhalation.
- Ensure that solvent reservoirs and waste container are air-tight, in secondary containment and do not allow the solvent vapors to enter the room.
- Ensure that the pressure is behaving normally and is well below the maximum pressure for the HPLC system.

6. Procedure:

- Polymers, such as NIST traceable polypropylene glycols (PPGs) and polyethylene glycols (PEGs) are the preferred calibrant for many small molecule applications. The PPG ions in general used for calibration in positive mode are: 59.0 (solvent and fragment ion), 175.1 (fragment ion), 616.5, 906.7, 1254.9, 1545.1, 2010.5, and 2242.6.
    - **Acceptance criteria**: difference between predicted and actual mass should not be more than 0.05 amu.
- The PPG ions used for calibration in negative-ion mode are 45.0 (solvent ion), 585.4, 933.6, 1223.8, 1572.1, 1863.3, 2037.4, and 2211.6.
    - **Acceptance criteria**: difference between predicted and actual mass should not be more than 0.05 amu.

7. Data to be recorded and presentation*:

For positive ESI Mode

Actual m/z	Observed m/z	Deviation (amu)
59.0		
175.1		
616.5		
906.7		
1254.9		
1545.1		
2010.5		
2246.6		

For negative ESI Mode

Actual m/z	Observed m/z	Deviation (amu)
45.0		
585.4		
933.6		
1223.8		
1572.1		
1863.3		
2037.4		
2211.6		

*for illustrative purpose only

Ratio and intensities may vary according to instrument make and model and can be determined by the softwares provided by the manufacturer.

Linearity and accuracy:

True concentration ($\mu g\ mL^{-1}$)	Peak area	Measured Concentration ($\mu g\ mL^{-1}$)	% Accuracy
0.5			
1			
2			
5			
10			
R^2		**-NA-**	**-NA-**

Precision:

Injection number	Pear Area	Retention time
1		
2		
3		
4		
5		

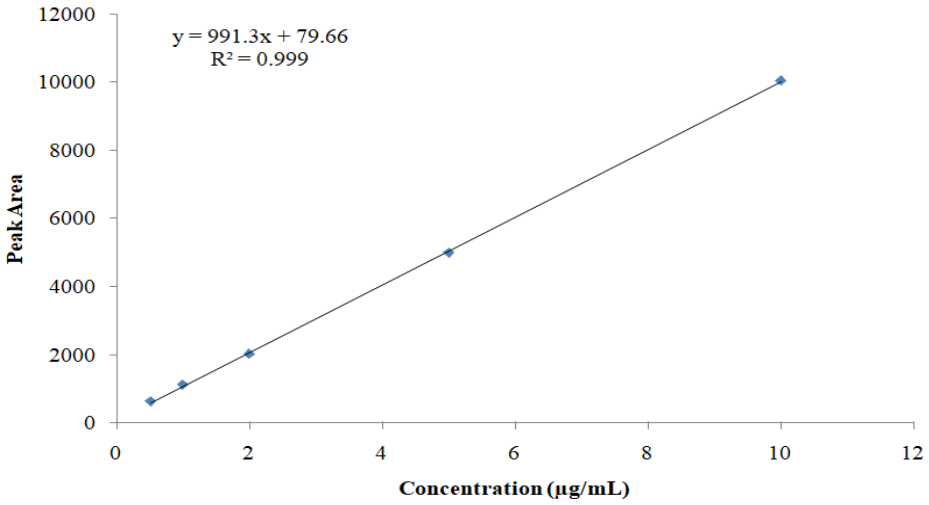
%RSD		

*for illustrative purpose only

8. Calibration plot and accuracy check*

Concentration (µg/mL)	Peak area	Calculated Concentration (y = mx+c)	Accuracy [(Observed value/True Value)*100]
0.5	614	0.53	107.8
1	1102	1.03	103.3
2	2014	1.95	97.5
5	4985	4.94	98.6
10	10024	10.03	100.3

***For illustrative purpose only**



8. Results:

Annexure - 14

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF ION CHROMATOGRAPHY (IC) SYSTEM

1. Description of the item to be calibrated

- **Name:** Ion Chromatography
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined:

- **Pump:** Flow rate accuracy
- **Injector:** Precision and linearity
- **Quantity & Ranges:** Concentration of standard in the range of ppm (µg/mL) and ppb (ng/mL)

3. Reference standard and reference material:

- Certified reference material (CRM) with NIST traceability or analytical standard ≥99% purity e.g. commercial $SO4^{2-}$.

4. Environmental conditions and stabilization period

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** ~ 30 min

5. Any safety measure to be observed

- If the vial has a septum type closure, be careful of the following points to prevent contamination.
    1. Do not touch the septum with bare hands (especially if covered in perspiration).
    2. Rinse the vial interior with ultrapure water, and then rinse with the sample solution.
- The surfaces of ion exchange columns are hydrophilic, even if a hydrophobic substance is adsorbed; it can be rinsed with a low concentration organic solvent.
- Follow column instruction manuals when rinsing the column.

6. Procedure:

- In order to check flow rate accuracy, the column eluent is collected in a calibrated measuring cylinder or volumetric flask for a particular time interval.
    - **Acceptance criteria:** ±2%

- In order to check the precision of injector, a set of regularly used NIST traceable standard (e.g. $SO4^{2-}$) is injected 3–5 times and the percent relative standard deviation (%RSD) is calculated for retention time and peak areas.
    - **Acceptance criteria**: % RSD ≤ 1%

- Similarly, for checking the linearity of the injector, different volumes of the standard are injected (e.g. 5 µL, 10 µL, 20 µL, 50 µL and 100 µL) and coefficient of determination (R^2) is calculated.
    - **Acceptance criteria:** $R^2 \geq 0.999$

7. Data to be recorded and presentation*:

Flow rate accuracy

Set flow rate	Observed flow rate	Deviation
1.5 mL min^{-1}		

Precision of injector:

Injection number	Electric conductance (Area)
1	
2	
3	
4	
5	
%RSD	

Linearity of injector:

Injection volume (µL)	Electric conductance (Area)
5	
10	
20	
50	
100	

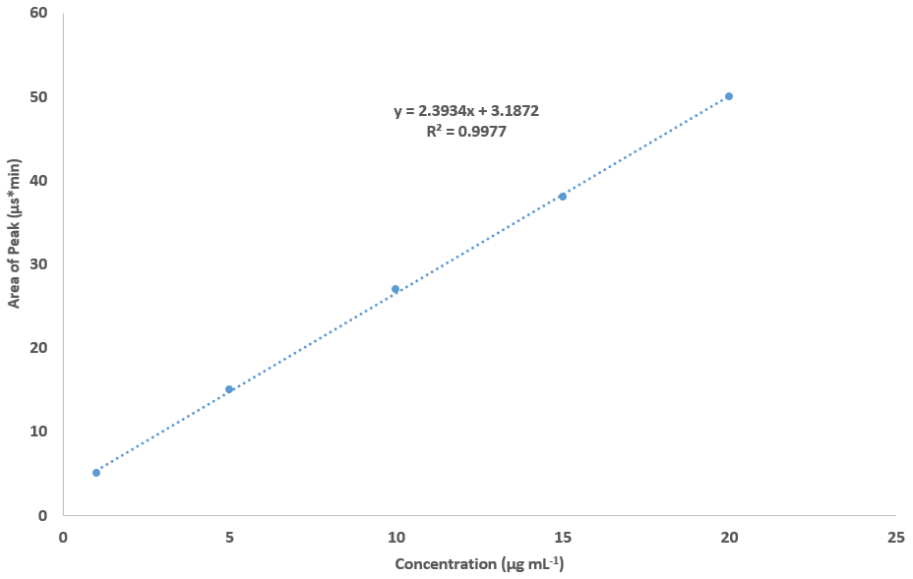
R^2 Value	

*for illustrative purpose only

8. Calibration plot and accuracy check*:

Concentration (µg/mL)	Electric conductance (Area)	Calculated Concentration (y = mx+c)	Accuracy [(Observed value/True Value)*100]
1	5	0.75	75.7
5	15	4.9	98.7
10	28	10.36	103.6
15	40	15.38	102.5
20	50	19.55	97.7

***For illustrative purpose only**



8. Results:

Annexure - 15

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF

HIGH PERFORMANCE THIN- LAYER CHROMATOGRAPHY (HPTLC) SYSTEM

1. Description of the item to be calibrated

- **Name:** High performance thin-layer chromatography (HPTLC)
- **Make:**
- **Model No:**
- **Date of installation:**
- **Date of last calibration:**

2. Measurement parameters, quantities and ranges to be determined:

- Linearity
- Accuracy
- Precision
- Quantity and ranges: Concentration of standard in the range of ppm (µg/mL) and ppb (ng/mL)

3. Reference standard and material:

- Certified reference material with NIST traceability of analytical standard with ≥99% purity e.g. pesticide mixture.

4. Environmental conditions and stabilization period

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** ~ 20 min

5. Any safety measure to be observed

- Wear chemical splash-proof goggles and appropriate attire at all times.
- Acetone, dichloromethane, ethyl acetate, hexane, methanol and toluene are flammable liquids.
- Keep the chamber covered as much as possible in order to keep the chambers saturated with solvent vapors and to prevent the inhalation of hazardous or smelly chemicals.
- Do not touch the surface of the plates with your fingers.

6. Procedure:

- In order to check linearity of spotting, apply 2 µL, 4µL, 6 µL, 8µL and 10 µL of solution on HPTLC plate with spotter.
    - **Acceptance criteria:** ±2%
- In order to check the reproducibility of spotting, apply 10 µL solution on HPTLC plate for five times in sequence.
    a. **Acceptance criteria**: % RSD ≤ 3%

7. Data to be recorded and presentation*

Linearity:

Spotting volume (µL)	Peak area
2	
4	
6	
8	
10	
R^2	

*for illustrative purpose only

Precision:

Spot number	Applied volume (µL)	Pear Area
1	10	
2	10	
3	10	
4	10	
5	10	
%RSD		

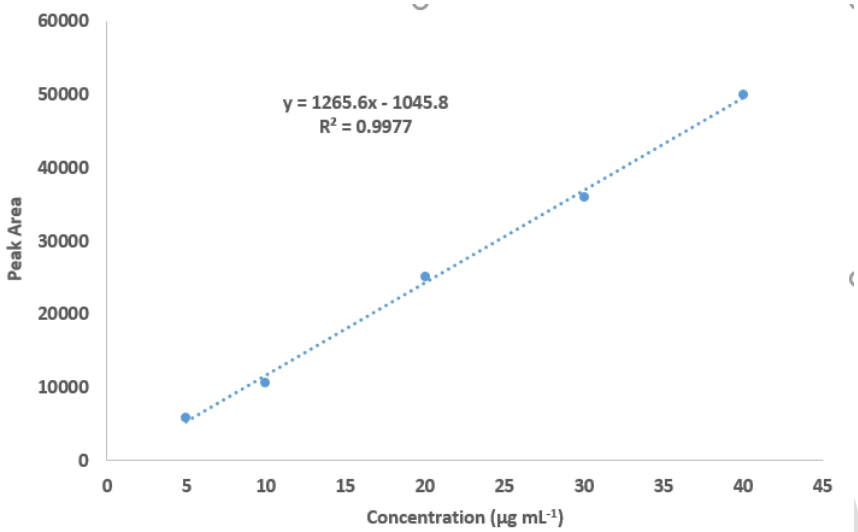
*for illustrative purpose only

8. Calibration plot and accuracy check*

Concentration (µg/mL)	Peak area	Calculated Concentration (y = mx+c)	Accuracy [(Observed value/True Value)*100]
5	57870.304	5.464684	109.2937
10	61649.07	9.240574	92.40574
20	69140.726	20.691	103.455
30	77998.522	29.27017	97.56722

40	79000.538	40.33371	100.8343

*For illustrative purpose only



8. Results:

Annexure - 16

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF ATOMIC ABSORPTION SPECTROSCOPY (AAS)

3. Description of the item to be calibrated:

- **Name:** Atomic Absorption Spectroscopy
- **Make:**
- **Model No:**

4. **Measurement parameters, quantities and ranges to be determined**:

- Accuracy
- Precision
- Quantity& ranges: Concentration of standard in the range of ppm (µg/mL)

3. Reference standard & reference material

- Certified Reference Material with NIST traceability or analytical standard. The standard solution for a calibration curve can be used for analysis after it has been diluted.

4. Environmental Conditions and stabilization period:

- **Temperature:** 21 – 40 °C
- **Humidity:** < 85% maximum relative humidity
- **Stabilization period:** ~ 30 min

5. Any safety measure to be observed

- Use only instrument grade gas for air flame such as instrument grade acetylene.
- Check the pressure of gas cylinder.
- Foe checking of instrument performance, analysis with Cu- 5ppm solution may be done and expected absorbance would be near 0.700abs.
- Hollow cathode lamps and flame emit UV radiations. Do not look at these sources without the aid of safety glasses or the flame shield.

6. Procedure

1. Switch on the system Atomic Absorption Spectroscopy (AAS).
2. Operate the instrument as per respective standard operating procedure.
3. Use stock standard as per NIST standard (acid or alkaline).
4. Aspirate the above solution and measure the absorbance.
5. Calculate the correlation coefficient by the instrument.
6. Correlation coefficient should not be less than 0.9900%.

7. Data to be recorded and presentation:

Calibration of AAS from Copper*

Preparation of Standard Solution: Dissolve 1.0 g of copper in 50 ml of 6N Nitric acid solution and further dilute to 1000 ml with distilled water to give 1000 µg/ml of Cu. Dilute quantitatively to get standard concentration of copper solution.

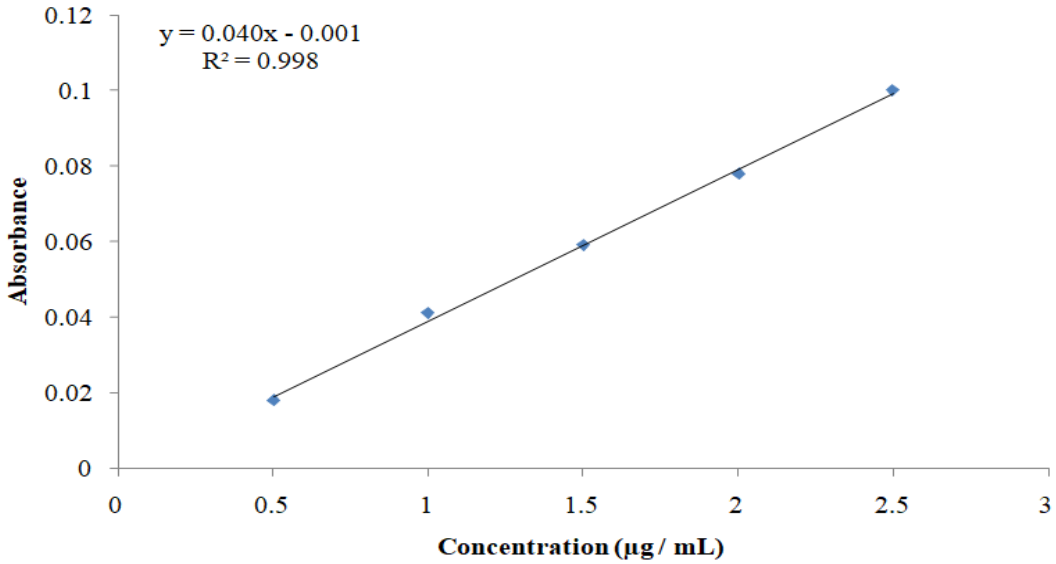
Number of analysis (conc. 1 µg / mL)	Absorbance
1	
2	
3	
4	
5	
Standard Deviation	
%RSD	

*For illustrative purpose only

8. Calibration plot and accuracy check*

Concentration (µg/mL)	Absorbance	Calculated Concentration (y = mx+c)	Accuracy [(Observed value/True Value)*100]
0.5	0.018	0.475	95
1	0.041	1.05	105
1.5	0.059	1.5	100
2	0.078	1.975	98.75
2.5	0.1	2.525	101

*For illustrative purpose only.



8. Results:

Annexure - 17

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF SCANNING ELECTRON MICROSCOPE-ENERGY DISPERSIVE X-RAY ANALYZER (SEM-EDXA)

1. Description of the item to be calibrated

- **Name:** SCANNING ELECTRON MICROSCOPE-ENERGY DISPERSIVE X-RAY ANALYZER (SEM-EDXA)
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined

- Elemental analysis

3. Reference standard & reference material

- Certified Reference Material with NIST traceability

4. **Environmental conditions and stabilization period**

- **Temperature:** 21 – 40 °C
- **Humidity:** < 20% maximum relative humidity
- **Stabilization period:** ~ 15 min

5. Any safety measure to be observed

- Perform procedural analysis protocols in an appropriate fume hood wearing personal protective equipment (PPE) including lab coats, appropriate gloves, and eye protection.
- Samples must be solid and they must fit into the microscope chamber.
- High vacuum environment needs to be maintained in specimen chamber.
- Specimens must be electrically conductive, at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge (non-metal samples should be coated with a conductive material during SEM sample prep to make them compatible with SEM)
- For SEM, a specimen is normally required to be completely dry, since the specimen chamber is at high vacuum.
- A clean sample is essential for image clarity.
- Biological specimens should be chemically fixed to preserve and stabilize their structure.

- Care should be taken that the adhesive from the office tape does not adhere to the sample surface, If necessary, a fresh surface may be exposed by scraping or cutting with a fine scalpel blade.
- Store the sample and stubs in a dry, clean environment. Use clean forceps and gloves whenever you handle the stubs.
- When comparing samples, all data should be collected in the same manner with the same conditions.

6. Procedure:

1. Switch on the systems SEM along with EDXA.
2. Open the "SEM Smart User Interface" software in the SEM system and "INCA" software in the EDXA system
3. Create Vacuum by pressing the **PUMP** Tab.
4. Set the stage in the center position i.e at no. 9 having standard samples of Cobalt, Gold, Rhodium and Carbon using **Stage Navigation** Tab.
5. Select any one of the above standard samples
6. Select "Point & ID" in the INCA software
7. Create a new Project
8. Select "Site of Interest" and then "Confirm elements".
9. Note the reading

7. Data to be recorded and presentation *:

S. No	Standard Sample	Calibrated Percentage	Observed Percentage			Deviation
			1	2	3	
1	Cobalt	100 %				
2	Gold	100 %				
3	Rhodium	100 %				
4	Carbon	100 %				

*for illustrative purpose only

8. Results:

Annexure - 18

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF COMPARISON MICROSCOPE

1. Description of the item to be calibrated

- **Name:** COMPARISON MICROSCOPE
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined

Length, breadth, height

3. Reference standard & reference material

- Certified Reference Material with NIST traceability

4. **Environmental conditions and stabilization period**

- **Temperature:** 21 – 40 °C
- **Humidity:** < 20% maximum relative humidity
- **Stabilization period:** ~ 15 min

5. Any safety measure to be observed

- Clean the microscope after each use
- Dust should be cleaned off with pressurized air or with a soft brush
- Clean smudges, fingerprints, oils, etc from the lens with clean lens paper or a soft clean cloth moistened with a small amount of absolute alcohol-ether mixture. If an alcohol-ether mixture is unavailable, use isopropyl alcohol
- Do not touch the optical lens with bare fingers
- Wipe the stage thoroughly before and after analysis.
- Turn off the light source when the microscope is not in use

6. Procedure:

i) Set the stage and put the calibration standard on the stage of the Comparison Microscope.

ii) Switch on the oblique or ring light.

iii) Switch on the computer attached with the Comparison Microscope and start the software.

iv) Select the magnification of the left lens at 0.4x and capture the image of the calibration standard.

v) Select the bar at 1mm on the MIC1 from the menu of the LAS V4 software on computer.

vi) Draw the straight line between 1mm scales on the image on computer and note the value measured by the computer software. Repeat the procedure on other magnifications for 1x, 2x, 4x and 8x.

vii) Follow the whole procedure for lenses on right side of the Comparison Microscope.

7. Data to be recorded and presentation *:

Sl. No.	Magnification	Calibration standard (mm)	Observed value on left side	Observed value on right side	Deviation ±mm (left side)	Deviation ±mm (right side)
1.	0.4x	1mm	1.001	1.000	-.001	+.000
2.	01x	1mm	1.000	1.000	+.000	+.000
3.	02x	1mm	1.002	1.001	-.002	-.001
4.	04x	1mm	1.000	1.002	+.000	-.002
5.	08x	1mm	1.001	1.001	-.001	-.001

*for illustrative purpose only

8. Results:

Annexure - 19

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF THERMAL CYCLER

5. Description of the item to be calibrated:

- **Name:** Thermal Cycler
- **Make:**
- **Model No:**

6. **Measurement parameters, quantities and ranges to be determined**:

- **Temperature Verification Test** – Checks the temperature of the sample blocks against specifications for temperature accuracy.
- **Temperature Non-Uniformity Test (TNU)** – Checks the temperature uniformity of the sample blocks.
- **Heated Cover Test** – Checks the heated cover temperature accuracy.

3. Reference standard & reference material

- Calibrated digital thermometer and multiprobe.

4. Environmental Conditions and stabilization period:

- **Temperature:** 21 – 20 °C
- **Humidity:** < 85% maximum relative humidity

5. Any safety measure to be observed

- Use only the multiprobe module that is designed for the sample block(s) you are testing.
- Do not crush the multiprobe module ribbon cable when the heated cover is closed.
- Use care when working around the heated cover and sample blocks to avoid being burned.
- A USB drive may be necessary if you intend to save the results to document compliance and do not have a printer connected. Please follow your internal quality systems requirements.

6. Procedure:

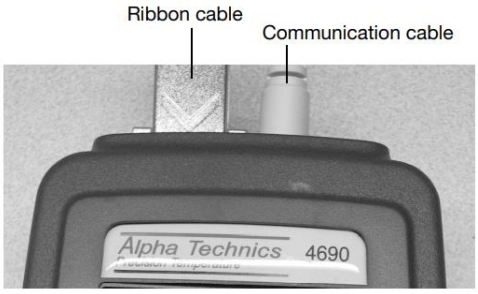
Requirements:

• A multiprobe module, 0.1-mL, 0.2-mL, or 0.02-mL size with ribbon cable

• A digital thermometer, Model 4690 with 9 V battery installed, and communication cable

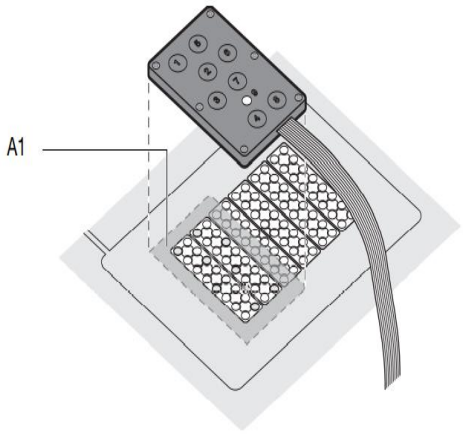
i. **Connection of Digital Thermometer**

- Connect the free end of the ribbon cable (from the multiprobe module) to the input connector port on the digital thermometer.
- Connect the round 8-pin DIN receptacle end of the communication cable to the thermometer communication port
- Connect the other end of the communication cable (9-pin) to the communication port of the instrument.



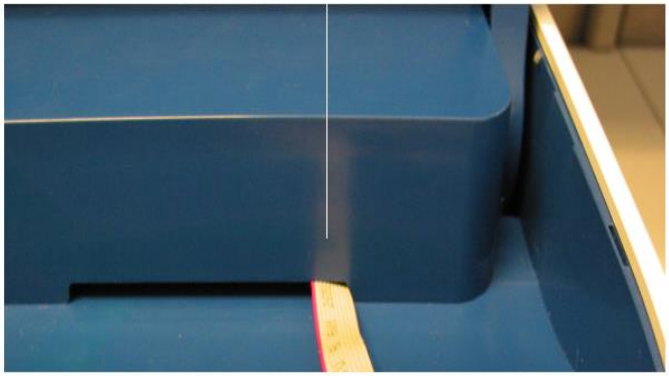
ii. **Set up of Multiprobe Module**

- If the heated cover is closed, lift the lever and place the heated cover in the fully opened position.
- Place the multiprobe module so that tip #1 sits in well A1.



- Ensure that the ribbon cable leads straight out from the module, lies flat, and is under the thermal cycler cutout.

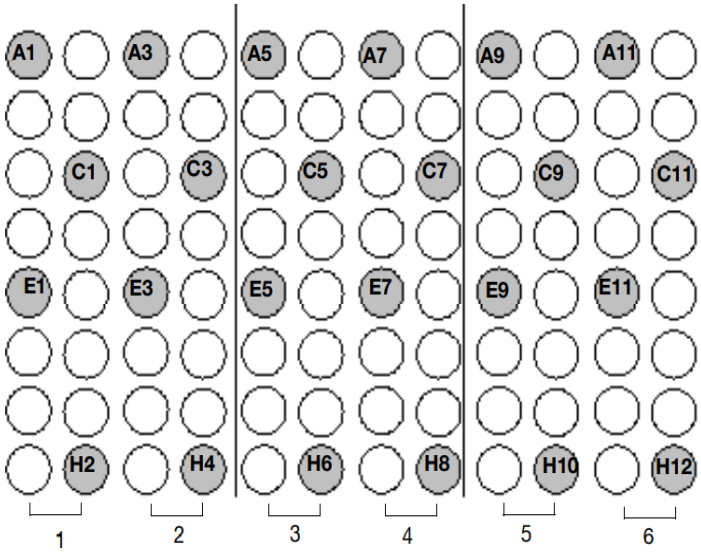
Ribbon cable placed under thermal cycler cutout



- Close the heated cover, then pull down the lever.

iii. <u>Temperature Verification Test:</u>

- This test determines the temperature accuracy of a thermal cycler by measuring sample well temperatures at two set points, 45 °C and 85 °C. To pass the test, the six sample blocks must be within ±0.25 degrees Celsius of the set points.
- The test wells (gray) are in the six sample blocks. Each sample block consists of two columns.



- Power on the thermal cycler, then power on the digital thermometer.
- Touch Main Menu > Tools Menu > Run Temperature Verification.
- The thermal cycler prompts to move the probes 3 times during the test

Testing Zones (Sample Blocks)	Well Position of Probe Tip #1
1 and 2	A1
3 and 4	A5
5 and 6	A9

- The test wizard refers to each sample block as a "zone." "Zone 1" refers to Sample Block 1, "Zone 2" refers to Sample Block 2, and so on.
- At the end of the test, the results are displayed. Print and save the results.

iv. <u>96-Well Temperature Non-Uniformity Test:</u>

- This test determines the temperature uniformity of the thermal cycler by measuring temperatures at 24 well locations.
- To pass the test, all six sample blocks must be within ±0.5 degrees Celsius of each set point temperature (95 °C and 60 °C) no later than 30 seconds after the set point temperature is changed.
- Power on the thermal cycler, then power on the digital thermometer.
- Touch Tools Menu>Run TNU Test.
- The thermal cycler prompts to move the probes 3 times during the test

Testing Zones (Sample Blocks)	Well Position of Probe Tip #1
1 and 2	A1
3 and 4	A5
5 and 6	A9

- At the end of the test, the results are displayed. Print and save the results.

v. <u>96-Well Heated Cover Verification Test:</u>

- This test determines if the heated cover holds temperature at 105 ±3 °C.
- Power on the thermal cycler, then power on the digital thermometer.
- Touch Tools Menu>Heated Cover Verification.
- Verify that the:
    - Probes are seated securely in the appropriate test wells in zones 3 and 4.
    - Cables are connected securely to the ports.
    - Ribbon cable lies flat under the thermal cutout and is not crimped.
    - Heated cover is closed and locked down.

- Follow the prompts in the touchscreen. When the test is finished, PASS or FAIL is displayed.

7. Data to be recorded and presentation*:

i. Temperature Verification Test:

Zone	Observed temperature values at 85 °C	Observed temperature values at 45 °C	Deviation (+/- 0.25 °C is acceptable)
1			
2			
3			
4			
5			
6			

ii. 96-Well Temperature Non-Uniformity Test:

Zone	Temperature Non-Uniformity at 85 °C	Temperature Non-Uniformity at 60 °C
1		
2		
3		
4		
5		
6		

Note: Six sample blocks (Zones) must be within ±0.5 degrees Celsius

*For illustrative purpose only

8. Results:

Annexure - 20

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF GENETIC ANALYZER

1. Description of the item to be calibrated

- **Name:** Genetic Analyzer
- **Make:**
- **Model No:**

. Measurement parameters, quantities and ranges to be determined

- **Spatial calibration**: The purpose of spatial calibration is to establish a relationship between the signal emitted by each capillary and the position where that signal falls on and detected by CCD camera. Spatial calibration needs to be done whenever the position of the capillary array get disturbed, mainly during replacement of capillary array.
- **Spectral calibration:** To create a de-convolution matrix that compensates for dye overlap in the 4-dye, 5-dye, 6-dye data stored in each sample file. Spatial calibration needs to be done, during usage of dye set that was not previously calibrated, change of capillary array, after re-aligning or replacement of laser or CCD camera, incorrect analyzed data (increased pull-up/pull-down in peaks).

3. Reference standard & reference material:

- Dye sets: J6 dye set for 6-Dye chemistry, G5 dye set for 5-dye chemistry

4. **Environmental conditions and stabilization period**

- **Temperature:** 21 – 25 °C
- **Humidity:** <20% maximum relative humidity

5. Any safety measure to be observed

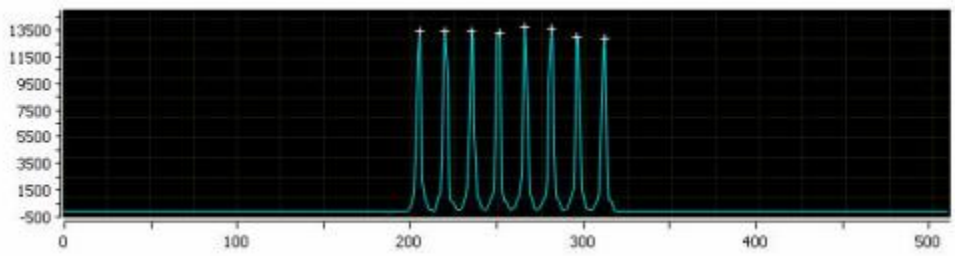
- Do not open the instrument door during spatial and spectral calibration run.
- Before spatial run, ensure buffer levels are at the fill lines, consumables in use are not expired.
- Pre-heat the oven for at least 30 minutes before start spectral run if the instrument is cold.
- Check the pump assembly for bubbles and run the Remove Bubble wizard if needed.

- If the reagents of any well contain bubbles or are not located at the bottom of the well, briefly centrifuge the plate, remove the plate from the centrifuge, and verify that each sample is positioned correctly in the bottom of its well.

6. Procedure:

i. <u>Spatial calibration:</u>

- Select Maintenance, then select Spatial Calibration in the navigation pane.
- Select No Fill, or select Fill to fill the array with polymer before starting the calibration.
- Click Start Calibration. The display updates as the run progresses.



If the average of any of the QC values exceeds the threshold, a Spatial QC check error message is displayed.

- Evaluation and acceptance criteria of spatial calibration profile:
  - ✓ One sharp peak for each capillary. Small shoulders are acceptable.
  - ✓ One marker (+) at the apex of every peak. No off-apex markers.
  - ✓ An even peak profile (all peaks about the same height).
- If the data for all capillaries meet the acceptance criteria, click the Accept Results button.
- Export of spatial calibration results:
  - ✓ Export the spatial calibration results by enter an export file name. Results can be stored either in csv or text file.
  - ✓ The export contains the following parameters: Capillary Number, Spacing, Position (pixels), and Intensity.

ii. <u>Spectral calibration:</u>

- Preparation of instrument:
  - ✓ Check consumables status and buffer levels
  - ✓ Set the oven temperature to 60°C, then click Start Pre-heat.
  - ✓ Check the pump assembly for bubbles and run the remove bubble wizard, if needed

- Preparation of standard calibration plate:
  - ✓ Choose the appropriate Dye Set and Matrix Standards.
  - ✓ J6 dye set for 6-Dye chemistry, G5 dye set for 5-dye chemistry
- Take 294 ul of HIDI and 6 ul of J6/G5 dye set and load 10 µL of the spectral calibration mixture into each well.
  - ✓ For 8-capillary 96-well plate- A1 through H1
  - ✓ For 24-capillary 96-well plate- A1 through H1, A2 through H2, and A3 through H3
- Load the plate on the instrument.
- Click Maintenance on the dashboard. Click on Spectral under Calibrate in the left navigation pane.
- Select the number of wells and plate position on the instrument. Select the chemistry standard and dye set. Click start run.
- Passing and failing capillaries are shown in green and red respectively.
- To display the result for each capillary (spectral data, Quality Value, and Condition Number) below the run results table, click a capillary in the table.
- Evaluation of spectral calibration:
  - ✓ Spectral Quality Value: It is a measure of the consistency between the final matrix and the data from which it was computed. A Quality Value of 1.0 indicates high consistency, providing an ideal matrix with no detected pull-up/pull-down peaks.
  - ✓ Condition Number: Indication of amount of overlap between the dye peaks in the fluorescence emission spectra of the dyes in the dye set. If there is no overlap in a dye set, the condition Number is 1.0 (ideal conditions), the lowest possible value. The condition number increases with increasing peak overlap.

Dye Set	Quality Value Minimum	Condition Number Maximum
G5 (5-dye)	0.95	13.5
J6 (6-dye)	0.95	8.0

  - ✓ Click each and every capillary to display the spectral and raw data for a capillary.
- Evaluation and acceptance criteria of spatial calibration profile:
  - ✓ No extraneous peaks in the raw data profile.
  - ✓ No gross dips, overlaps or other irregular morphology.

- ✓ Spectral profile peaks are separate and distinct.
- ✓ Ideally, spectral peak heights should be approximately similar to the peak heights typically analyzed by the laboratory.

- If the data for all capillaries meet the acceptance criteria, click the Accept Results button.
- Export spectral calibration results:
  - ✓ Export the spatial calibration results by enter an export file name and save the results.
  - ✓ The export file contains the following parameters: Capillary Number, Quality Value, Condition Number, Peak Height, Scan Number, Reason For Failure (in case).

7. Data to be recorded and presentation*:

i. Spatial calibration:

Attribute	Description	Threshold	Observed value
Average peak height	sum of all peak heights/number of peaks	8-cap: 6400 RFU 24-cap: 3000 RFU	
Uniformity (peak height similarity)	standard deviation/ average peak height	0.2	
Capillary spacing	max spacing – min spacing	2 pixels	

ii. Spectral calibration:

Attribute	**Acceptance Criteria**	**Observation**
Order of the peaks in the spectral profile from left to right	• 4-dye: blue-green-yellow-red • **5-dye:** blue-green-yellow-red-orange	
Order of the peaks in the raw data profile from left to right	• Sequencing (matrix standard only): 4-dye: red-yellow-blue-green • Fragment analysis/HID: 4-dye: red-yellow-green-blue 5-dye: orange-red-yellow-green-blue	
Extraneous peaks in the raw data profile	**None**	

Peak morphology in the spectral profile	• No gross overlaps, dips, or other irregularities • Peaks separate and distinct	

*For illustrative purpose only

8. Results:

Annexure - 21

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF Real Time PCR (qPCR)

1. Description of the item to be calibrated

- **Name:** Real time PCR (qPCR)
- **Make:**
- **Model No:**

2. Measurement parameters, quantities and ranges to be determined:

Dyes calibration: ROI, background, Custom dyes- FAM, VIC, ROX, TAMRA, SYBR, ABY, JUN, MUSTANG PURPLE, NED and Cy5 dyes.

Custom dyes- ABY, JUN, Mustang purple are for HID workflow.

3. Reference standard and reference material:

Calibration plates for ROI, background, Custom dyes- FAM, VIC, ROX, TAMRA, SYBR, ABY, JUN, MUSTANG PURPLE, NED and Cy5 dyes.

4. Environmental conditions and stabilization period

- **Temperature:** 21 –25°C
- **Humidity:** < 85% maximum relative humidity

5. Any safety measure to be observed

- Remove calibration plate from its packaging, when to use. The fluorescent dyes in the wells of calibration plates are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dyes.

6. Procedure:

Requirements:

- Plate(s) for the calibration:
  - ROI/Uniformity plate (one ROI plate needed)
  - Background calibration plate
  - Dye calibration plates
- Centrifuge with plate adapter

i. Thaw, vortex, and centrifuge a calibration plate

- Remove the calibration plate from the freezer, then thaw the plate in its packaging. Keep plates protected from light until you perform the calibration.
- Thaw each plate for 30 minutes. Use each plate within 2 hours of thawing.
- Remove the calibration plate from its packaging and retain the packaging. Do not remove the optical film.
- Vortex the plate for 5 seconds, then centrifuge at 750 to 1,000 × g for 2 minutes.
- Confirm that the liquid in each well is at the bottom of the well and free of bubbles.

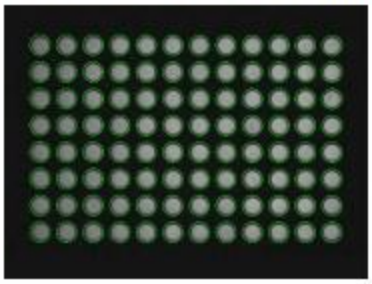
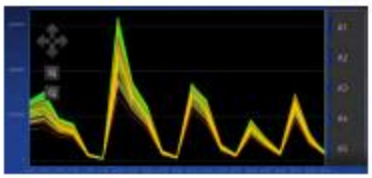
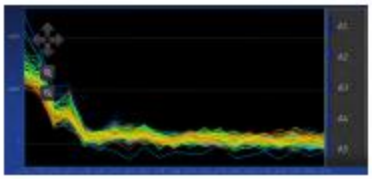
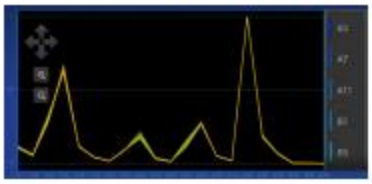
ii. Perform calibration

Calibration	Touch
ROI/Uniformity[1]	**Settings ▸ Maintenance and Service ▸ Calibrations ▸ ROI and Uniformity**
Background[2]	**Settings ▸ Maintenance and Service ▸ Calibrations ▸ Custom ▸ Background**
System Dye	**Settings ▸ Maintenance and Service ▸ Calibrations ▸ Dye**

- Follow the instructions on the screen to start the calibration.
- Select the Dye Plate to run, then touch Next.
- Load the plate into the instrument
- Touch Start. When the run is complete and the screen displays Calibration Complete, touch View Results to check the calibration status.
- Unload the plate from the instrument.
- Return the plate to its original packaging.

iii. View Calibration images after the background calibration is complete.

- In the Calibration Status screen, touch Details.
- In the Details screen, touch a calibration type to view its images and plots.

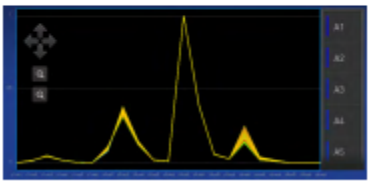
Calibration	Example results indicating successful calibration
ROI **Note:** Select the desired filter combination from the Filter Set dropdown list.	Green circles around all wells and bright well centers. 
Uniformity	Signals from each well following a uniform trend. 
Background	Few, if any, signals with abnormally high fluorescence. 
Dye	Signals from each well following a uniform trend, and each dye peaks at the correct filter. 

iv. Custom dye calibration for HID-validated workflow.-

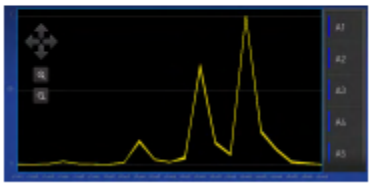
- Requirement:
    - ABY Spectral Calibration Plate, 96-Well 0.2-mL
    - JUN Spectral Calibration Plate, 96-Well 0.2-mL
- Thaw, vortex, and centrifuge the calibration plate as described in point no.1 of the procedure.
- Load the plate into the instrument

- In the instrument home screen, touch Settings>Maintenance and Service> Calibrations>Custom>Custom Dye.
- Touch the custom dye to calibrate.
- Review the custom dye information.
- Enter the calibration temperature.
- Touch Start.
- When the run is complete and the screen displays Calibration Complete, touch View Results>Details.
- Review the plot.
- Passing calibration results show uniform signals with peaks aligned with the dye wavelength.

Dye	Peak filter	Filter wavelength (nm)	
		Excitation	Emission
ABY-HID	x3-m3	550 ± 10	587 ± 10
JUN-HID	x4-m4	580 ± 10	623 ± 14



ABY-HID dye calibration plot



JUN-HID dye calibration plot

- Select an action depending on whether the custom dye calibration passed or failed.

Calibration status	Action
Passed	Touch **Accept Results** or **Reject Results**. **Note:** Accepting the results saves the calibration data to the instrument and overwrites existing data.

- Unload the plate from the instrument.

7. Data to be recorded and presentation*:

For custom dyes

Dye	Peak filter	Filter wavelength (nm)	
		Excitation	Emission
ABY-HID	x3-m3		
JUN-HID	x4-m4		

**For illustrative purpose only*

8. Results:

References:

1. "Guidance for the validation of analytical methodology and calibration of equipment used for testing of illicit drugs in seized materials and biological specimens" Laboratory and Scientific Section, United Nations Office on Drugs and Crime, Vienna; 2009.
2. Chan, Chung Chow, et al., eds. Analytical method validation and instrument performance verification. Vol. 18. ^ eNew Jersey New Jersey: John Wiley & Sons, 2004.

Ref. No.30(1)/2020-Pt. I/HM
Govt. of India, Ministry of Home Affairs
Directorate of Forensic Science Services
Block No. 9, 8th Floor, CGO Complex, New Delhi 110003

Dated: March 24, 2022

Office Memorandum

Standard list of equipment has been prepared and circulated by DFSS to all stake holders and the same has also been uploaded on DFSS web portal. As directed by the Hon'ble HM, Govt of India, it has been proposed that the equipment of Chemical, Biological, Physical Sciences are required to be calibrated, for which a committee has been constituted with following members:

(i)	Dr. R M Sharma. Retd. Prof. (FS), Panjabi University, Advisor Rajiv Gandhi National University of Law, Patiala	Chairman
(ii)	Dr S K Shukla, Campus Director, NFSU, Delhi Campus	Member
(iii)	Dr.S.O.Junare, Director NFSU, Gujarat	Member
(iv)	Prof. Dr. Vishal Sharma, HoD (FS) Punjab University	Member
(v)	Director: CFSL Guwahati , Pune, Bhopal and Chandigarh	Members
(vi)	Sh. K M Varsheny, Ex-Director, CFSL Hyderabad / Consultant, DFSS HQs	Member
vii)	Dr S Ahmed, SSO Gr. I (FS)	Member Secretary

First meeting of the said Committee will be held on March 25, 2022 in the presence of CFS, DFSS in online mode at 1430 hrs. It is requested to all the members to make it convenient to attend the same. A line of confirmation will be highly appreciated. Meeting link has been sent by emails.

This issues with the approval of CFS, DFSS.

(Dr S Ahmed)
Sr. Scientific Officer GR. I (FS)

Office Order Book

Copy for information to:-

- Dr. R M Sharma, Retd. Prof. (FS), Panjabi University, Advisor Rajiv Gandhi National University of Law, Patiala
- Prof. Dr. Vishal Sharma Prof. & HoD (FS), Punjab University
- Dr S K Shukla, Campus Director, NFSU, Delhi Campus
- Dr.S.O.Junare, Director National Forensic Sciences University Gujarat
- Sh. K M Varsheny, Ex-Director, CFSL Hyderabad Consultant, DFSS
- Director: CFSL Guwahati , Pune, Bhopal and Chandigarh

Sr. Scientific Officer GR. I (FS)