EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 5
146-153
Year: 2017

"CALIBRTION OF SPECTROSCOPIC PHARMA ANALYTICAL INSTRUMENTS"

[1]Avinash M. Bhagwat*, [2]Anand P. Khadke, [3]Anuradha M. Patil and [4]Rucha J. Kulkarni

[1,2]Assistant Professor, YSPM's Yashoda Technical Campus, Satara.
[3]Jayavantrao Sawant Institite of Pharmacy, Pune.
[4]YSPM's Yashoda Technical Campus, Satara.

***Corresponding Author: Avinash M. Bhagwat**
Assistant Professor, YSPM's Yashoda Technical Campus, Satara.

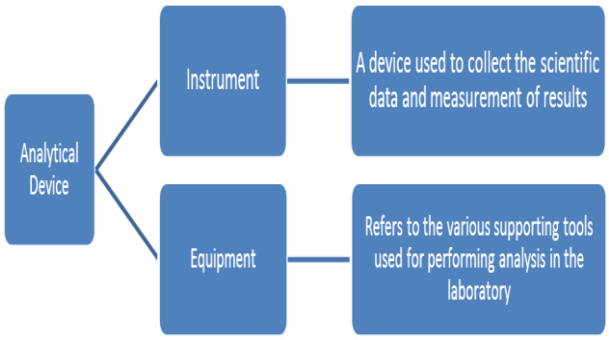
Article Received on 25/02/2017 **Article Revised on 17/03/2017** **Article Accepted on 06/04/2017**

ABSTRACT
Instrument calibration is one of the primary processes used to maintain instrument accuracy. Calibration is the process of configuring an instrument to provide a result for a sample within an acceptable range. Eliminating or minimizing factors that cause inaccurate measurements is a fundamental aspect of instrumentation design. Although the exact procedure may vary from product to product, the calibration process generally involves using the instrument to test samples of one or more known values called "calibrators." The results are used to establish a relationship between the measurement technique used by the instrument and the known values. The process "teaches" the instrument to produce results that are more accurate than those that would occur otherwise. The instrument can then provide more accurate results when samples of unknown values are tested in the normal usage of the product. Calibrations are performed using only a few calibrators to establish the correlation at specific points within the instrument's operating range. The instrument will provide the best performance when the intermediate points provided in the manufacturer's performance specifications are used for calibration; the specified process essentially eliminates, or "zeroes out", the inherent instrument error at these points. This article provides the information of calibration of spectroscopic instruments which is need pharma industry.

KEY WORDS: Calibration, Calibrator, accuracy, pharma industry.

INTRODUCTION

All analytical devices can be classified under 2 categories viz. Equipment and Instrument.



Classification of instruments

1) Group A
No measurement capability or requires calibration.

2) Group B
Providing measurements or controlling physical parameters such as temperature, pressure, etc Balance, thermometer, pH meter.

3) Group C
Sophisticated instruments such as HPLC, GC, etc.

Qualification - Four Qs

Design Qualification (DQ)
Installation Qualification (IQ)
Operational Qualification (OQ)
Performance Qualification (PQ)
Calibration is part of Performance Qualification.

Unplanned Calibration

"Unplanned" Calibration can be called for:
When the instrument is physically moved to a different location within a lab. Questionable or ambiguous results are obtained – perhaps as a part of investigation

Calibration Specification

Instrument calibration tolerance limits should be established so problems are identified and corrected in a timely manner.

When assigning tolerances, considerations given to:
Capability of the instrument being calibrated (what the manufacturer claims the instrument can achieve).

Parameters at which the instrument operates (ex: if testing accuracy of + 0.5% is required, the instrument calibration tolerances should be <0.5%).

Work environment - environmental conditions can affect the performance of the instrumentation.

Success of Calibration

Success of calibration depends on the following:

a. Consistency of results obtained
b. Recognition and mitigation of outlier/potential outliers
c. Scientifically designed calibration frequency

Calibration Process

1. Written calibration procedures that use traceable calibration standards or calibration equipment.
2. Qualified individuals (having the appropriate education, training, background and experience) responsible for calibrating & maintaining instrumentation
3. Second person check of all calibration tests
4. Qualified individuals responsible for monitoring the calibration
5. Ensure the calibration program and procedures are reviewed and approved by Quality

Each calibration & maintenance procedure should include the following:

1. Identification of department responsible to perform the calibration or maintenance
2. Step-by-step calibration instructions, reference to appropriate calibration procedures or instrument manuals
3. Methods for preventive maintenance or reference to appropriate instrumentation manuals
4. Calibration equipment used in the calibration are valid (e.g. spectroscopy filters, voltmeters, digital thermometers, etc)
5. Calibration parameter and tolerance (+)

Records for Calibration

1. All calibration records must be retained per document retention procedures
2. Should include “as found” measurements, results of adjustments (“as left”) and appropriate review & approval of all results
3. Tolerance or limit for each calibration point
4. Identification of standard or test instrument used
5. Identification of persons performing the work and checking the results with dates.

After Calibration

1. Review must ensure the approved activities have been completed and all results have passed the established acceptance criteria
2. Actions to be taken if instrumentation cannot be calibrated (e.g. contact appropriate service people, label and remove from service)
3. A step to record all calibration & maintenance activities
4. Periodic review of historic calibration & maintenance data to evaluate appropriateness of established frequencies.

Type of Equipment/Instrument/ Calibration Item	**Recommended Frequency of Calibration**	**Intermediate Checks**	**Remarks**
UV-Visible / IR / FT-IR Spectrophotometer / Colorimeter	Quarterly-Photometric Absorbance and wavelength accuracy for the working range	-	Using CRM
Atomic Absorption Spectrophotometer	Performance check by f Cu CRM as per manufacturer’s instruction.	When used, Standard solution of specific element	Generally the performance check is done against the initial value checked at the time of installation.

- **INSTRUMENT NAME**

Atomic Absorption Spectrophotometer (AAS).

Fig. Atomic Absorption Spectroscopy

Purpose

To check performance of the equipment for reliable and accurate results.

Procedure

Frequency: Once in a month

1. Operate the instrument as per respective standard operating procedure.
2. Use Cu standard for radiations.
3. Prepare 0.5 mcg/ml, 1.0 mcg/ml, 1.5 mcg/ml, 2.0 mcg/ml and 2.5 mcg/ml in water.
4. Aspirate the above solution and measure the absorbance.
5. Calculate the correlation coefficient by the instrument/computer.
6. Correlation coefficient should not less than 0.9900%.
7. The performance of the instrument is satisfactory if the obtained correlation coefficient is within the given limit otherwise follow the S.O.P.

Abbreviations

1. % = Percentage
2. mcg= microgram
3. ml= millilitre
4. cu= copper

- **INSTRUMENT NAME**

Fourier Transform Infra Red Spectrometer

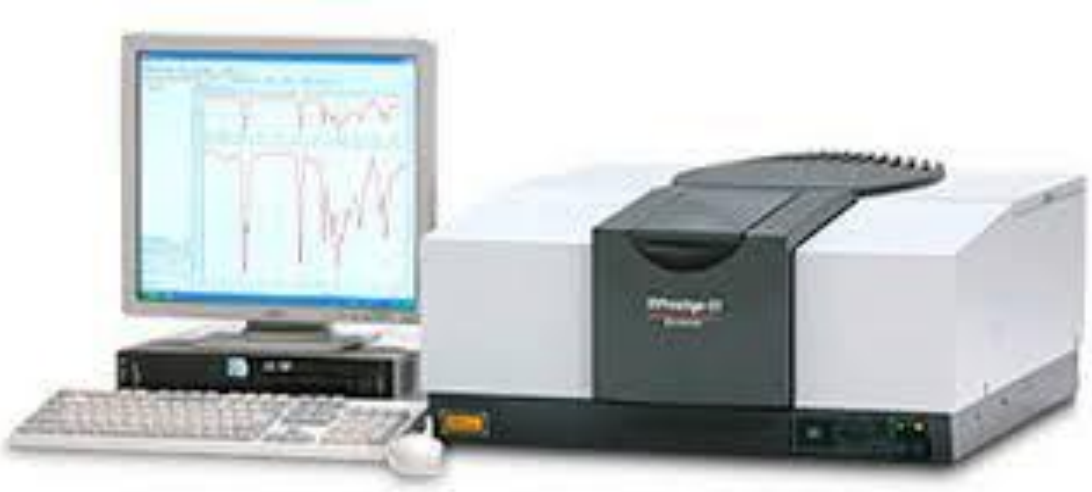


Fig. FTIR Spectrophotometer (Mech: Shimadzu)

Parameters to be calibrated

1. Power Spectrum
2. Resolution
3. Wave number Accuracy
4. Wave number Reproducibility
5. Transmittance Reproducibility.

Purpose

To check the above parameters are working according to standard.

Procedure

Frequency: Every three months.

Power Spectrum

Resolution (As Per Japanese Pharmacopoeia)

1. The resolution is validated by measuring the absorption spectrum of polystyrene film with a thickness of approximately 0.04 mm.
2. The measured absorption spectrum should have a transmittance (%T) difference of 18% or more between a minimum of approximately 2870 cm^{-1} and a maximum of approximately 2850 cm^{-1}.
3. It should also have a transmittance (%T) difference of 12% or more between a minimum of approximately 1589 cm^{-1} and a maximum of approximately 1583 cm^{-1}.
4. If the measured absorption spectrum has both transmittance differences higher than the standard value, the results are labelled "PASS."

Resolution (As Per European Pharmacopoeia)

1. When the spectrum of polystyrene film about 35 μm thick is recorded, the difference between the absorbances at the absorption minimum at 2870 cm^{-1} and the absorption maximum at 2849.5 cm^{-1} is greater than 0.33.
2. The difference between the absorbances at the absorption minimum at 1589 cm^{-1} and the absorption maximum at 1583 cm^{-1} is greater than 0.08.
3. If the measured absorption spectrum has both absorbance differences higher than the standard value, the results are labelled "PASS."

Wave number Accuracy (As per Japanese Pharmacopoeia)

1. Evaluating wave number accuracy is described in the Japanese Pharmacopoeia as follows:
2. The wave number scale is usually calibrated by using some of the following absorption bands of polystyrene film shown below.
3. The numbers in parentheses indicate the allowable range for them.
4. This program uses seven wave number points for judging, and it obtains the peak wave numbers from the measured spectrum of polystyrene film.
5. The software then judges whether the values are within the allowable range.
6. The program labels the results "PASS" if all the specified peak wave numbers are within the allowable range.

3060.0 (+/- 1.5) cm^{-1}
2849.5 (+/- 1.5) cm^{-1}
1942.9 (+/- 1.5) cm^{-1}
1601.2 (+/- 1.0) cm^{-1}
1583.0 (+/- 1.0) cm^{-1}
1154.5 (+/- 1.0) cm^{-1}
1028.3 (+/- 1.0) cm^{-1}

Wave number Accuracy (As per European Pharmacopoeia)

1. Evaluating wave number accuracy is described in the European Pharmacopoeia 5.0 as follows:
2. "The wave number (cm^{-1}) scale may be verified using a polystyrene film, which has transmission minima (absorbance maxima) at the wave numbers (in cm^{-1})."
3. This program uses seven wave number points for judging, and it obtains the peak wave numbers from the measured spectrum of polystyrene film.
4. The software then judges whether the values are within the allowable range.
5. The program labels the results "PASS" if all the specified peak wave numbers are within the allowable range.

3060.0 (+/- 1.0) cm^{-1}
2849.5 (+/- 1.0) cm^{-1}
1942.9 (+/- 1.0) cm^{-1}
1601.2 (+/- 1.0) cm^{-1}
1583.0 (+/- 1.0) cm^{-1}
1154.5 (+/- 1.0) cm^{-1}
1028.3 (+/- 1.0) cm^{-1}

Wave number Reproducibility (As per Japanese Pharmacopoeia)

1. The Japanese Pharmacopoeia describes the wave number reproducibility as follows:
2. "The wave number reproducibility should satisfy 5 cm^{-1} around 3000 cm^{-1} of the polystyrene absorption wave number, 1 cm^{-1} around 1000 cm^{-1} when several points of polystyrene absorption, from 3000 cm^{-1} to 1000 cm^{-1}, are measured twice."
3. This program specifies three points to measure peak wave numbers.
4. It then obtains the actual peak wave numbers at each point by measuring the polystyrene film twice.
5. The software determines whether the differences between each of the two measurements are within the allowable range.
6. The software labels the result "PASS" if all the specified peak wave numbers are within the allowable range.

Wave number Reproducibility (As Per European Pharmacopoeia)

1. European Pharmacopoeia 5.0 does not have this test, but this validation program inspects based on the Japanese Pharmacopoeia.
2. The Japanese Pharmacopoeia describes the wave number reproducibility as follows:
3. "The wave number reproducibility should satisfy 5 cm^{-1} around 3000 cm^{-1} of the polystyrene absorption wave number, 1 cm^{-1} around 1000 cm^{-1} when several points of polystyrene absorption, from 3000 cm^{-1} to 1000 cm^{-1}, are measured twice."
4. This program specifies three points to measure peak wave numbers. It then obtains the actual peak wave numbers at each point by measuring the polystyrene film twice.

5. The software determines whether the differences between each of the two measurements are within the allowable range.
6. The software labels the result "PASS" if all the specified peak wave numbers are within the allowable range.

Transmittance Reproducibility (As Per Japanese Pharmacopoeia)

1. The Japanese Pharmacopoeia describes the transmittance reproducibility as follows:
2. "The transmittance reproducibility should satisfy 0.5%T when the several points of polystyrene absorption from 3000 cm^{-1} to 1000 cm^{-1} are measured twice."
3. This program specifies the peak wave number at three points and the transmittance at each point is measured twice.
4. Then it is determined whether the differences between the two data are within the allowable range or not.
5. If all the transmittance differences satisfy the allowable range, the results are labelled "PASS".

Transmittance Reproducibility (As Per European Pharmacopoeia)

1. European Pharmacopoeia 5.0 does not have this test, but this validation program inspects based on the Japanese Pharmacopoeia.
2. Tolerance is specified of absorbance which is nearly equivalent to 0.5% transmittance at the wave number.
3. The Japanese Pharmacopoeia describes the transmittance reproducibility as follows:
4. "The transmittance reproducibility should satisfy 0.5%T when the several points of polystyrene absorption from 3000 cm^{-1} to 1000 cm^{-1} are measured twice.
5. "This program specifies the peak wave number at three points and the absorbance at each point is measured twice.
6. Then it is determined whether the differences between the two data are within the allowable range or not.
7. If all the absorbance differences satisfy the allowable range, the results are labelled "PASS."

- **INSTRUMENT NAME**

Infra Red Spectrometer

Fig. IR Spectrophotometer (Mech: Shimadzu)

Parameters to be calibrated

1. Wave number setting
2. Resolution Performance

Purpose

To check the above parameters are working according to standard.

Procedure

Wave Number Accuracy Test

1. Switch on the System.
2. Set the instrument parameters as follows :

RESOLUTION 2.0
APODIZATION STRONG
RANGE 4000-400 cm^{-1}
MODE RATIO
NUMBER OF SCAN 16

3. Allow the instrument to warm for 30 minutes.
4. Operate the instrument as per operation SOP.
5. Open the sample compartment cover of FTIR 1600 and place the polystyrene film in the sample holder and the close the cover.
6. Click OK in the SCAN MODE.
7. The spectrum of the polystyrene film is displays on the screen.
8. PRINT the spectrum by going to print function.
9. When the printer of the spectra is completely go to DATA and then to PEAK.

10. The peak data's of the spectra of the polystyrene film is displays on the screen.
11. PRINT out these data's by going to print function.
12. Compare the wave function accuracy with the following limit :

Limit of wave Number Accuracy
3060.0(±1.5) 2849.5 (±1.5) 1942.9(±1.5) 1601.2(±1.0)
1583.0(±1.0) 1154.5(±1.0) 1026.3 (±1.0)

Resolution Performance Test

1. Record the spectrum of the polystyrene film 0.04 mm in thickness.
2. The different x between the percentage at the transmission maximum A at 2870 cm^{-1} (3.48µm) and the transmission minimum B at 2849.5 cm^{-1} (3.51 µm) should greater than 16. The difference y between the percentage transmission at the transmission maximum C at 1589 cm^{-1} (5.29 µm) and that at the transmission minimum D at 1583 cm^{-1} (5.32 µm) should be greater than 12.
3. Record the observation in the calibration record.
4. If test fails then label it as "Under Maintenance" and notify the defect to Engineer.

Abbreviations

1. SOP – Standard Operating Procedure
2. FTIR – Fourier Transforms Infra Red
3. µm – micrometer

- **INSTRUMENT NAME**

UV Visible Spectrophotometer

Fig. UV Visible Spectrophotometer

Purpose

To verify the performance by Control of Wave length, Control of Absorbance, Limit of Stray Light and Resolution Power tests.

Parameter to be calibrated

1. Control of Wave length
2. Control of Absorbance
3. Limit of Stray Light
4. Resolution Power

Procedure

Operate the instruments as per SOP.

Control of Wave Length

1. Weight accurately 1.0 gm of Holmium Oxide and dissolve it in 1.4 M Perchloric acid solution. Make up to 25 ml with the same solvent.
2. Select the method file of CONTROL OF WAVE LENGTH in the instrument.
3. After selecting the file press Reference button for baseline correction.
4. Then fill the Cuvette with 1.4M Perchloric acid and put in the sample cubicle and press reference to zero.
5. After auto zero put the Holmium perchlorate solution in sample cubicle then press start key.
6. Scan it and verify the wavelength using absorption maxima of Holmium Perchlorate solution. The permitted tolerance is given in below table.

Sr.No.	Maxima Wave length (nm)	Tolerance (nm)
1.	241.15nm	240.15nm to 242.15nm
2.	287.15nm	286.15nm to 288.15nm
3.	361.5nm	360.50nm to 362.50nm
4.	536.3nm	533.30nm to 539.30nm

7. Dry a quantity of the Potassium dichromate by heating to constant weight at 130°C.
8. Weight accurately about 60 mg of dried potassium dichromate and dissolve it in 0.005M sulphuric acid solution. Make upto 1000 ml with the same solvent. Mark the solution as (A). .3.3 Weight accurately about 60 mg of dried potassium dichromate and dissolve it in 0.005M sulphuric acid solution. Make up to 100 ml with the same solvent. Mark the solution as (B).

Control of Absorbance

1. Select the method file of CONTROL OF ABSORBANCE in the instrument.
2. After selecting the file press Reference button for baseline correction.
3. Then fill the Cuvette with 0.005M Sulphuric acid for blank and put in both sample cubicle and press reference to zero.
4. After auto zero put the Potassium Dichromate solution labeled as solution 'A' in sample cubicle then press start key taking absorbance individually for first four wavelength mentioned in 'Table I'.

Sr.No.	Wavelength (nm)	Absorbance E (1%1cm)	Maximum tolerance
1.	235	124.5	122.9 to 126.2
2.	257	144.0	142.8 to 145.7
3.	313	48.6	47.0 to 50.3
4.	350	106.6	104.9 to 108.2
5.	430	15.9	15.7 to 16.1

5. Now take absorbance at 430 nm for solution 'B'.
6. Note the absorption maxima of Potassium Dichromate solution at different wave length and calculate the absorbance, tolerance is given in below table.

Limit of Stray Light

1. Dry a quantity of the Potassium chloride by heating to constant weight at 130°C.
2. Weight accurately 1.20 g of dried potassium chloride and dissolve it in 50 ml distilled water. Make upto 100 ml with the same solvent.
3. Select the method file of LIMIT OF STRAY LIGHT in the instrument.
4. After selecting the file press Reference button for baseline correction.
5. Check the absorbance of above solution using water as a blank at 200 nm.
6. Absorbance should be greater than 2.0 5.5 Resolution power 5.5.1 Prepare 0.02%v/v solution of Toluene in Hexane UV.

Resolution Power

1. Select the method file of RESOLUTION POWER in the instrument.
2. After selecting the file press Reference button for baseline correction.
3. Measure the absorbance of above solution at 266 nm and 269 nm using Hexane UV as blank solution.
4. The ratio of absorbance maxima at 269 nm to that of 266 nm minima should be more than 1.
5. Note down the report in the internal calibration certificate and in Instrument Logbook.

Abbreviations

1. SOP - Standard Operating Procedure 6
2. nm - Nanometer
3. VIS - Visible
4. UV - Ultra Violet

CONCLUSION

By performing the calibration of spectroscopic instruments we can maintain the instrument accuracy and hence its long life. If the calibration is done time to time then the instrument gives accurate as well as precise results hence it will affects on the analytical tests which are to be performed on the instrument. Hence calibration impacts on the results. The calibration of an instrument is one of the key factor in the assay or analytical laboratory test in the pharmaceutical industry. It gives effect on the results. If an instrument is not calibrated it will gives wrong results which affects on the formulation results and ultimately on the health of the human beings. Hence calibration is moist important factor and it should be done time to time. This article helps to give information about the calibration of spectroscopic instruments. Its knowledge is important as it is need of accurate, precise results.

ACKNOWLEDGEMENT

We are thankful to President, Vice-President and Director of YSPM's Yashoda Technical Campus, Satara for providing facility for this work. We also thankful to Mr. Ajit Ekal of INSTA VISION LABORATORIES AND SERVICES for providing useful information about calibration.

REFERENCE

1. Indian Pharmacopoeia, 2010, Volume I, page no. 111, 112, 116, 117.
2. British Pharmacopoeia, 2009, A178, Appendix III B.
3. United States Pharmacopoeia / National Formulary, 2005, Page no. 2295, 2296.
4. Gurudeep Chatwal, S. K. Anand, Instrumental Methods of Chemical Analysis, page no 1.24.

5. Khadke AP et al, Synthesis and Biological Evaluation of Novel 2,4-Thiazolidinedione Derivatives as Antibacterial Agent,Asian Journal of Chemistry; Vol. 24, No. 11 (2012), 5305-5308.
6. www. USFDA. Com.
7. Survey report by World Health Organization.
8. ICH guidelines for Calibration of Instruments.
9. Annex 5 of the thirty-second report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations.
10. Instruction manual, operation guide, uv-1800, shimadzu spectrophotometer, Chapter 15.
11. Agilent 8453E UV-visible Spectroscopy System Operator's Manual, Service An Maintaince, Page No. 91.
12. Atomic Absorption Spectrophotometers AA-7000 Series Consumables by Shimadzu.
13. Agilent 5500 Series FTIR Operation Manual Chapter 2, page 17.