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Review Article

A REVIEW ON CALIBRATION OF ANALYTICAL INSTRUMENTS

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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. There are three main reasons for having instruments calibrated are to ensure readings from an instrument are consistent with other measurements, to determine the accuracy of the instrument readings, to establish the reliability of the instrument i.e. that it can be trusted. This review includes the information about the tests conducted for calibrating different analytical instruments and acceptance criteria. Out of calibration is the major thing during analysis. It gives in detail about the out of calibration also.

Keywords: calibration- UV, IR, HPLC-performance check-out of calibration.

INTRODUCTION

Calibration is a comparison between measurements one of known magnitude or correctness made or set with one device and another measurement made in as similar a way as possible with a second device. The device with the known or assigned correctness is called the standard. The second device is the unit under test, test instrument, or any of several other names for the device being calibrated. The formal definition of calibration by the International Bureau of Weights and Measures is the following: "Operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties (of the calibrated instrument or secondary standard) and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication.

Calibration may be called for

- a new instrument
- after an instrument has been repaired or modified
- when a specified time period has elapsed
- when a specified usage (operating hours) has elapsed
- before and/or after a critical measurement
- after an event, for example
  - after an instrument has had a shock, vibration, or has been exposed to an adverse condition which potentially may have put it out of calibration or damage it
  - sudden changes in weather
- whenever observations appear questionable or instrument indications do not match the output of surrogate instruments
- as specified by a requirement, e.g., customer specification, instrument manufacturer recommendation.

In general use, calibration is often regarded as including the process of adjusting the output or indication on a measurement instrument to agree with value of the applied standard, within a specified accuracy. For example, a thermometer could be calibrated so the error of indication or the correction is determined, and

adjusted (e.g. via calibration constants) so that it shows the true temperature in Celsius at specific points on the scale. This is the perception of the instrument's end-user. However, very few instruments can be adjusted to exactly match the standards they are compared to. For the vast majority of calibrations, the calibration process is actually the comparison of an unknown to a known and recording the results.

Calibration of analytical balance
Measure the pan position error of the balance
Daily calibration
The maximum and minimum weight limits of the balance are taken and divided into four to five parts and single standard weight is selected in those four or five different regions for the purpose of daily calibration. The variation allowed from the standard weight used for measuring is NMT ±0.1% of standard weight. Drift check from day to day is carried out using any particular weight and the deviation allowed should not be more than **±**1% of that weight.

Monthly calibration
All the standard weights in the maximum and minimum weighing limits are used in monthly calibration and the deviation allowed should not be more than ±0.1% of standard weight used. (The standard weights that are used are E1 and E2 weights)

Measurement of uncertainty
For measurement of uncertainty any single standard weight is taken and is weighed for 10 times the readings are noted and the standard deviation for all the readings is calculated. And measurement of uncertainty can be measured using the formula

$$Measurement\ of\ uncertainity = \frac{S.D \times 3}{Standard\ weight\ taken}$$

Calibration of UV-spectrophotometer
The calibration of U.V – Visible Spectrophotometer consists of five tests. It is done in intervals of every 3 months or after every heavy usage of the instrument. The tests are as follows

1. Baseline Flatness
2. Control of Wavelengths
3. Control of Absorbance
4. Resolution
5. Stray light

Baseline Flatness
This test is performed to ensure that the baseline does not interfere with the obtained absorbance peaks. The test specifications are as follows:

- Scan speed : Slow
- Wavelength range : 200 – 800 nm
- Slit Width : 2
- Acceptable Absorbance : ±0.001

We scan in the range of 200 – 800nm without placing anything in the sample or reference compartment. The obtained absorbance should be within ±0.001 AU (Absorbance units).

Control of Wavelengths
This test signifies the system precision. The holmium oxide is used as sample and is scanned in the range of 200-600nm using perchloric acid as blank. It should give four different absorbance maximas as shown in the table. The deviation allowed for the first two wavelengths is ±1nm and for rest two is ±2nm.
Holmium oxide has the rare ability to give sharp peaks in both uv and visible region and is also stable for long period after preparing the solution. The sample is prepared by taking 400mg of holmium oxide is taken into 100ml volumetric flask and dissolved in 1.4M perchloric acid using it to make upto the marked volume.

Absorbance Maximum	Acceptable range
241.15	±1nm
287.15	±1nm
361.5	±2nm
586.3	±2nm

Control of Absorbance
This test signifies the system precision. Potassium dichromate is used as the sample and its absorbance is measured at four different wavelengths using 0.005M sulphuric acid solution. The obtained values should be within the mentioned tolerance limits range mentioned in the table.

Wavelength (nm)	A(1% 1cm)	Tolerance limits
235	124.5	122.9 – 126.2
257	144.0	142.4 – 145.7
313	48.6	47.0 – 50.3
350	106.6	104.9 – 108.2

57.63mg of potassium dichromate is taken and dried at 130°C for about 1hr and taken into 100ml volumetric flask. It is dissolved in 0.005M sulphuric acid solution and made upto the volume

with it. The obtained absorbance at the four mentioned wavelengths are recalculated for 1%concentration and with 1cm width using the formula

$$A(1\%\ 1cm) = \frac{\text{Absorbance} \times 1 \times 100}{\text{Weight of Pot. dichromate (g)} \times 100}$$

Resolution

Resolution is performed to determine the spectral band within the UV region. 0.02% of toluene in hexane solution is taken and its absorbance is measured at 266 and 269 nanometers. The ratio of these absorbance should not be less than 1.5.

$$\text{Ratio} = \frac{\text{Absorbance at 266nm}}{\text{Absorbance at 269nm}}$$

Stray Light

This test is performed to know it there is any interference of stray light in the absorbance. For this test we take 1.2% potassium chloride solution and measure the absorbance at 198nm. The absorbance should not more than 2.0. the potassium chloride of 1.2%w/v concentration does not absorb radiation at 198nm.

Cells

The absorbance of the cells intended to contain the solution to be examined and the reference liquid when filled with the same solvent should be identical. If this is not the case, an appropriate correction must be applied. The tolerance on the path length of the cells used is not more than 0.005 cm. Cells should be cleaned and handled with greater care.

Calibration of IR spectrophotometer

The calibration of IR Spectrophotometer consists of five tests. It is done in intervals of every 3 months or after every heavy usage of the instrument. The tests are as follows

1. Power spectrum
2. Wavenumber accuracy test
3. Resolution
4. Wavenumber reproducibility
5. Transmittance reproducibility

Power spectrum

This test is to evaluate the basic performance of IR Spectrophotometer. We do not place any sample in the sample compartment and run the instrument it should show the respective standard power percentage at the mentioned wavenumbers an in the given table.[3]

Wavenumber (cm^{-1})	Standard Value
4600	10 %
4000	25 %
3000	50 %
Max. value	50.0 or more
700	10 %
500	2 %
403	0.5 %
351	0.01 %

Wavenumber accuracy test

0.04mm thick polystyrene film is taken and placed in the sample compartment. The instrument is allowed to run. It should give peaks at the specified wavenumbers as mentioned below in the table. and the transmittance should be within the specified limits. This serves as standard and any deviation indicates system inefficiency.

Wavenumber (cm^{-1})	Transmittance Acceptance range (cm^{-1})
3060.0	± 1.5
2849.5	± 1.5
1942.9	± 1.5
1601.2	± 1.0
1583.0	± 1.0
1154.5	± 1.0

Resolution

The measured absorption spectrum should have a transmittance (%T) difference of18% or more between a minimum of approximately 2870 cm^{-1} and a maximum of approximately 2850 cm^{-1}. It should also have a transmittance (%T) difference of 12% or more between a minimum of approximately1589 cm^{-1} and a maximum of approximately 1583 cm^{-1}. If the measured absorption spectrum has both transmittance differences higher than the standard value, the results are labeled "PASS."

Wavenumber reproducibility

The wavenumber reproducibility should satisfy 5 cm^{-1} around 3000 cm^{-1} of the polystyrene absorption wavenumber, 1 cm^{-1} around 1000 cm^{-1} when several points of polystyrene absorption, from 3000 cm^{-1} to 1000 cm^{-1}, are measured twice.

Wavenumber (cm^{-1})	Tolerance (cm^{-1})
2849.5	± 5.0
1601.2	± 1.0
1028.3	± 1.0

Transmittance reproducibility

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.

Wavenumber (cm^{-1})	Tolerance of % Transmittance
2849.5	± 0.5%
1601.2	± 0.5%
1028.3	± 0.5%

Calibration of HPLC

Initially before starting with calibration flush all the channels with water at 2ml/min for 1hr and allow the lamps to warm up. Use all HPLC grade chemicals and milli – Q water only throughout process. Calibration of HPLC is divided into four parts based on the parts. They are

1. Tests for pump.
2. Tests for autosampler.
3. Tests for detector.
4. Tests for heating system.

Tests for pump

1. **Flow rate accuracy;** this test is performed to check if the pump is delivering the set flow rate or not. Chromatographic conditions are
   a. Mobile Phase : filtered and degassed water
   b. Flow rates : 0.5, 1, 2, 5 (ml/min)
   c. Run time : 5 min

We set different flow rates using mobile phase as water. Disconnect the column and collect the mobile phase into volumetric flask. Take weight of volumetric flask before and after weighing. Deviation allowed in this test is ±5% from the set flow rate. Now calculate flow rate from the formula

$$\text{Flow Rate} = \frac{\text{Post measurment wt.} - \text{Pre measurement wt.}}{\text{Time} \times \text{Density of Water}}$$

2. **Gradient composition accuracy;** significance of this test is to assess the accuracy and stability of online solvent mixing. Chromatographic conditions here are:
   a. Mobile Phase A & C : Milli Q Water , B & D : 0.5% Acetone
   b. Flow rate : 1ml/min
   c. Run time : 40 min
   d. Column : restricted capillary tube
   e. Detection λ : 265 nm

Equilibrate the column by running mobile phase for 30min at flow rate of 2ml/min. After that set the gradient program as shown in the table

TIME	MOBILE PHASE (A)	MOBILE PHASE (B)
0.00	100	0
0.10	90	10
10.00	90	10
10.10	50	50
20.00	50	50
20.10	10	90
30.00	10	90
30.10	0	100
40.00	0	100

Now calculate the concentration of Mobile phase B at 10%, 50%, 90% concentrations. The deviation allowed here is ±1% from the set concentration. Formula

$$B = \frac{\text{B conc. at set value} \times 100}{\text{B conc. at 100\%}}$$

Tests for auto sampler

1. **Injection volume accuracy;** this test is performed to check if the injector is delivering the set injection volume or not. Chromatographic conditions are
   a. Mobile Phase : filtered and degassed water
   b. Flow rate : 1ml/min
   c. Injection volumes : 10, 20, 50, 100 µl's each 10 times
   d. Run time : 0.1 min

Fill the sample vials with water and take the weights now run the test and take the weight of the vials again. Calculate the difference in weight. The Deviation allowed in this test is ±5% from the set volume. Now calculate the injection volume form the formula

$$\text{Injection Volume} = \frac{\text{Weight difference} \times 1000}{\text{Density of water} \times \text{No. of injections}}$$

2. **Carry over test;** significance of this test is to assess the amount of carryover from one sample to the other. Chromatographic conditions are:
    a. Caffeine : 25µg/ml
    b. Injection volume : 100 µl's
    c. Blank : Water
    d. Column : C18 – ODS Column
    e. Wavelength : 273nm
    f. Run time : 10min
    g. Flow rate : 1ml/min
    h. Mobile phase : Acetonitrile : water (15:85)
    i. Oven temperature : 40°C
    j. Order of Injection : Blank, Caffeine, Blank 1, Blank 2
    k. Carry over in Blank 1 : NMT 0.030%
    l. Carry over in Blank 2 : NMT 0.015%

Run the test and calculate the carryover from the formula

$$\text{CO in Blank 1} = \frac{\text{Area of caffeine peak in B1} \times 100}{\text{Area of Peak in caffeine Injection}}$$

3. **Precision and linearity of injector;** this test helps us to determine the system precision based on the precision and linearity of injector. Chromatographic conditions are:
    a. Injection volume : 10, 20, 50, 100 µl's each 5 replicates
    b. Blank : Water
    c. Column : C18 – ODS Column
    d. Wavelength : 273nm
    e. Run time : 10min
    f. Flow rate : 1ml/min
    g. Mobile phase : Acetonitrile : water (15:85)
    h. Oven temperature : 40°C
    i. Caffeine : 25µg/ml

Now run the test and determine the theoretical plates, tailing factor, peak areas, avg. peak area, retention time, linearity coefficient. The deviations allowed are

- At 20µl : NLT 3000 Theoritical plates and Tailing Factor NMT 2.0
- % RSD of Peak Areas : NMT 2% at each level
- Average Peak Area : NMT 2% at each level
- % RSD of Retention time : NMT 1.5% at each level
- Linearity coefficient "r" : NLT 0.999 at each level

Tests for detector

1. **Precision and linearity of detector;** detector linearity is a critical parameter which helps us establish for the reliable and accurate quantitative results. The Chromatographic conditions in this test are:
    a. Injection volume : 20 µl's
    b. Blank : Water
    c. Column : C18 – ODS Column
    d. Wavelength : 273nm
    e. Run time : 10min
    f. Flow rate : 1ml/min
    g. Mobile phase : Acetonitrile : water (15:85)
    h. Oven temperature : 40°C
    i. Caffeine: 0.5, 2, 25, 50µg/ml each 5 replicates.

Now run the test and determine the theoretical plates, tailing factor, peak areas, avg. peak area, retention time, linearity coefficient. The deviations allowed are

- At 25µg/ml : NLT 3000 Theoretical plates and Tailing Factor NMT 2.0
- % RSD of Peak Areas : NMT 2% at each level
- Average Peak Area : NMT 2% at each level
- % RSD of Retention time : NMT 1.5% at each level
- Linearity coefficient "r" : NLT 0.999 at each level

2. **Wavelength accuracy;** is performed to check the detector response. It's essential in both qualitative and quantitative analysis. The Chromatographic conditions are :
    a. Caffeine : 250µg/ml
    b. Column : Restricted Capillary tube
    c. Flow rate : 1ml/min
    d. Injection volume : 10µl
    e. Run time : 1min
    f. Erbium Perchlorate : 250µg/ml

Run the test by measuring the absorbances at specified wavelengths as mentioned in the table. The deviation should not be more than ±2nm from that of specified wavelength maximum.

Drug	Wavelength Range(nm)	Acceptable λ_{max} value
Caffeine	202 – 208	205 ±2nm
	242 – 248	245 ±2nm
	270 – 275	273 ±2nm
Erbium Perchlorate	252 – 258	255 ±2nm
	376 – 382	379 ±2nm
	519 – 525	522 ±2nm

3. **Drift and Noise test;** test significance is to determine the detector stability and sensitivity. Before starting the test purge the channels and allow the system to stabilize. The Chromatographic conditions are :
    a. Column : Restricted Capillary tube
    b. Mobile Phase : filtered and degassed water
    c. Flow rate : 1ml/min
    d. Wavelength : 254nm
    e. Run time : 60 min
    f. Column oven temperature : 25°C

Run test according to given conditions and determine the noise and drift values detector noise should not be more than 0.000125 AU and detector drift should not be more than 0.005 AU/hr.

Noise is the short term variation from the baseline due to instability of detector, temperature fluctuations or electric signal fluctuations or dirty mobile phase. While drift is the long term variation from the baseline due to solvent or mobile phase absorbance or it will be observed in the first one hour when the detector is heating up.

Tests for heating system

1. **Temperature accuracy in column compartment ;** set different temperatures from [5, 10, 20] 30, 40, 50, 60°C and measure the temperature using an external calibrated thermometer when the temperature is obtained the deviation allowed is ± 3°C.
2. **Temperature accuracy in sample compartment ;** set different temperatures from [5, 10, 25] 30, 40, 50, 60°C and measure the temperature using an external calibrated thermometer when the temperature is obtained the deviation allowed is ± 2°C.

The calibration of HPLC is done at time intervals of every 6 months or after every heavy usage of the instrument.[4]

Pressure test

The performance of the LC pump depends on the proper functioning of the check valves and the proper connection of the tubing. Properly functioning check valvesand tubing connections (seals) are important in maintaining stable mobile-phase flow and system pressure. For pump systems that output the pressure reading in the pump head over time, a simple pressure test can be a useful qualitative test to check the condition of the check valves and to determine whether or not there are any leaks in the system. The first step of the pressure test is to plug the outlet of the pump using a dead-nut and by setting the automatic pump shutdown pressure to 6,000 psi.

The pump-head pressure signal output is connected to a recorder. Pressurize the pump by pumping methanol at 1 mL/min. The pressure inside the pump head increases quickly as the outlet of the pump is blocked. As the pressure increases to about 3,000 psi, the flow rate is reduced to 0.1 mL/min. The pressure will gradually rise to the shutdown pressure if the check valves are able to hold the mobile phase in the pump chamber as would be normally expected. If the check valve is not functioning properly, the pressure will fluctuate at about 3,000 psi instead of reaching the shutdown pressure. The pressure in the pump head decreases slowly over time after the automatic shutdown. A steep decrease in pressure over time implies poor check-valve performance or leaks within the pumping system.

Linearity of Response

Since the analytes of interest may vary in concentration, the ability of a detector to produce a linear response to concentration variation within a reasonable range is crucial to the accuracy for peak-area and peak-height comparison between standards and samples. The linearity of the detector response can be checked by injecting or by filling the flow cell with a series of standard solutions of various concentrations. Aqueous caffeine solutions are convenient for the linearity measurement. The concentration range typically should generate responses from zero to

at least 1.0 AU. Absorbencies beyond 1.5 AU are more prone to deviation due to stray light. From the plot of response versus the concentration of the solutions, the correlation coefficient between sample concentration and response can be calculated to determine the linearity. Noise and Drift (Not all vendors perform this test. Older systems may not be able to meet the same signal-to-noise ratio specified for the new equipment.) Electronic, pump and photometric noise, poor lamp intensity, dirty flow cell, and thermal instability contribute to the overall noise and drift in the detector. Excessive noise can reduce the sensitivity of the detector and hence affect the quantitation of low-level analytes. Detector drift may affect the baseline determination and peak integration. Nowadays, most chromatographic software is capable of calculating the detector noise and drift. Typically, the detector should be warmed up prior to the test, and any temperature fluctuations should be avoided during the test. For a dynamic testing condition, methanol is passed through the flow cell at 1 mL/min. A backpressure of about 500 psi is maintained to prevent bubble formation.

Calibration of Gas Chromatography

Various Calibration parameters are:
Flow rate accuracy
Column oven temperature accuracy
System precision
System precision for head space auto sampler
Detector linearity
Detector noise and drift test.

Flow rate accuracy

Connect the digital flow meter to the detector outlet port. Set the carrier gas (Helium) flow and wait till it reaches the set flow. Note the observed flow in replicate. Repeat the procedure for other carrier gases such as Hydrogen and Air. Record the result in GC calibration protocol. Acceptance criteria: The flow rate of carrier gas should be ±10% of set flow.

Column Oven Temperature Accuracy

Connect the column to the detector port. Place the thermometer probe in the column oven and set the column oven temperature at 40°C.Wait till the temperature stabilizes. Note the observed temperature as read by the probe in triplicate over a period of 10 m. Repeat the procedure for 100°C, 150°C and 190°C. Acceptance criteria: The resulting oven temperature from the thermometer display should be within ±2°C of the set temperature

System Precision

Preparation of Standard solution: Transfer 20 ml of Methanol, Ethanol and Acetone into 100ml volumetric flask and make up with Ethyl acetate.
Procedure: Inject blank, followed by Standard preparation in 6 replicates. Note down the areas and Retention times. Acceptance criteria: The %RSD of retention time should be not more than 1.0%& peak area should be not more than 5.0%.

System precision for head space auto sampler

Preparation of standard solution: Prepare a standard mixture solution by taking Methylene dichloride (0.6g), Chloroform (0.06g), Trichloroethane (0.08g),1,4-Dioxane (0.38g) in 50ml volumetric flask containing about 40ml of Dimethyl formamide.Finally makeup to volume with DMF(Solution-A). Procedure: Take 0.5 ml of standard solution-A in 6 different vials and seal with septum, then magnetic caps and crimp. Place these vials on head space sampler; prepare a blank vial also. Load the vials in head space sampler tray. Blank vials followed by the standard vials. Acceptance criteria: The %RSD of retention time should be NMT 1.0%& peak area should be NMT 15.0%.

Detector linearity

Preparation of standard solutions: Detector linearity solution A: Transfer 10ml of each Methanol, Ethanol and Acetone into a 100ml volumetric flask and dilute to the volume with Ethyl acetate. Detector linearity solution B: Transfer 15ml of each Methanol, Ethanol and Acetone into a 100ml volumetric flask and dilute to the volume with Ethyl acetate.
Detector linearity solution C: Transfer 20ml of each Methanol, Ethanol and Acetone into a 100ml volumetric flask and dilute to the volume with Ethyl acetate.
Detector linearity solution D: Transfer 25ml of each Methanol, Ethanol and Acetone into a 100ml volumetric flask and dilute to the volume with Ethyl acetate.
Detector linearity solution E: Transfer 30ml of each Methanol, Ethanol and Acetone into a 100ml volumetric flask and dilute to the volume with Ethyl acetate. Procedure: Inject blank, followed by Detector linearity solutions and record the peak responses .Draw a standard plot between the concentrations Vs the peak responses. Acceptance

criteria: The plot should be linear and regression coefficient (R2) should not be less than 0.99.

Detector Noise and Drift Test

After GC is ready run the system up to 15 m through single run. After completion of run calculate noise and drift through software. Acceptance criteria: Noise NMT: 100 µV Drift NMT: 2500 µV/hr.

Chromatographic Conditions for System Precision

Column -30m × 0.32mm, 1.8µ, DB-624
Detector -Flame ionization detector
Injector temperature -180°C
Detector temperature- 250°C
Flow mode- Pressure
Carrier Gas flow rate -Helium 25 kpa
Oven program -50°C (hold 5 m) raise to 10°0C
Split ratio -1:10
Injection volume -0.2 µl
Hydrogen flow -40 ml/m
Air flow- 400 ml/m

Chromatographic Conditions for Head Space Auto Sampler

Column - 30m × 0.32mm, 1.8µ, DB-624
Detector- Flame ionization detector
Injector temperature - 220°C
Detector temperature - 260°C
Flow mode - Pressure
Carrier Gas flow rate- Helium 25 kpa
Oven program - 40°C (hold 5 m) raise to 200°C (hold 5 m)
Split ratio - 1:10
Injection volume - 0.2 µl
Hydrogen flow- 40 ml/m
Air flow - 400 ml/m

Head Space Conditions

Vial equilibrium- 22 m
Vial pressure - 0.5 m
Loop fill- 0.5 m
Loop equilibrium - 0.05 m
Inject - 1.00 m
GC cycle time - 38 m
Oven temperature - 80°C
Loop temperature - 100°C
Vial pressure - 10.8 psi [5]

Performance check of HPLC column

Unpacking the Column

Each column is packaged in foam to reduce shipping vibrations, and accompanied by a test chromatogram which details the test conditions and the results obtained for your column. This information is useful in initial verification of column performance and also for troubleshooting as the column ages. The test chromatogram also contains information regarding the shipping solvent, upper pressure limit, lot number of packing material, pH range, column catalog number and serial number. The serial number is used to access information such as the original column performance, packing method, and date of manufacture.

Installing the Column

Extra-column dead volume can make high efficiency columns look inefficient. To minimize extra-column dead volume, your system should be equipped with short pieces of small bore tubing (0.005"-0.010"i.d.), and zero dead volume fittings. Keep in mind that as the i.d. of the HPLC column decreases, extra-column effects become more detrimental and obvious. The stop depth on the connecting tubing must match the stop depth in the fitting. A mismatch will cause either dead volume or a leak Purge the pump and fluid lines with the mobile phase to ensure contaminants and bubbles are removed from the system. Once this is completed, connect the column. HPLC pressure requirements dictate that stainless steel, PEEK, or carbon-reinforced PEEK fittings be used throughout the system. PEEK fittings are more convenient and easier to use. Often these are designed with a knurled nut which requires only hand tightening to obtain a leak-free seal. When loosened, the ferrule returns to its original shape allowing it to reposition itself to the appropriate stop depth each time a connection is made. PEEK ferrules and fittings can be re-used many times and can be placed into ports with different stop depths without causing leaks or extra dead volume. Unlike PEEK ferrules, stainless steel ferrules can only be used on stainless steel tubing and will swage onto the tubing with the first connection. This ferrule-tubing connection now has a fixed stop depth and must be used only on the type of port on which it was swaged**.**

Performance check of gas column

1. Check traps, carrier gas, septum and liner

Check gas traps for expiration and replace if necessary. Install a new septum in the inlet. If needed, clean or replace the inlet liner and gold-plated inlet seal, especially after injecting dirty samples or when analyzing active compounds. The liquid phase in the column is easily damaged by oxygen at temperatures above

ambient. Use traps on the carrier gas lines to extend column lifetime and minimize background noise. A high capacity oxygen trap and an indicating oxygen trap are highly recommended. An indicating moisture trap before the oxygen trap prolongs the life of the oxygen trap and reduces background noise. An oxygen trap on the ECD makeup gas line is recommended.

2. Place nut and ferrule on the column, carefully cut column end

Place the column nut and ferrule over one end of the column. There is no front or back of the column; however, the posts of the column cage usually point towards the oven door. Cut the end of the column after nut and ferrule placement. Hold the section of column to be cut against a finger. In one motion, scribe the outside of the column using a suitable cutting tool. Do not cut completely through the tubing. Grasp the column on each side of the scribe mark and bend away from the mark. Inspect the column end with amagnifier. Ensure the cut is at a right angle to the tubing wall and free of chips, burrs, or uneven areas. Recut if necessary.

3. Install column into the inlet

Place the column on the GC oven column hanger. Make sure the column tubing does not touch the sides of the oven. Unwind enough column to obtain a smoothly curved section of tubing connected to the inlet. Avoid tight bends as this stresses the tubing and could cause breakage. Make sure that column tags, sharp edges, or other items do not rub against the column. The optimal insertion distance of the column into the inlet depends on the inlet type. Consult the GC instruction manual for the proper insertion depth and technique. With the column at its proper position, finger tighten the column nut. Use a wrench to tighten an additional 1/2 turn. If the column can be moved in the fitting, tighten another 1/4 turn. Failure to achieve a leak free seal will cause rapid and permanent column damage. Do not move the column while tightening the nut. Ferrules, especially those made of graphite/Vespel™, will change shape slightly upon heating. If the column was installed while the inlet and detector were cool, retighten the fitting. It is also good practice to make sure the column nuts are tight after conditioning the column.[7]

Out of calibration

This can simply be defined as „failure to meet the acceptance criteria during the calibration processes". The departing of calibration results may happen in any measuring instrument or a part thereof. The examples are gauges, calibration curves, thermometers, temperature sensors, temperature indicators, different displays, etc. The departing may be due to mishandling of instrument under calibration, master equipment, insufficient training, errors in the instrument itself, faulty machinery parts, incorrect procedure, improper calibration, improper storage conditions, improper environmental conditions, etc. In GMP and GLP environment, any objectionable result or deviation or departing values like Out of specification results or out of trend results shall be investigated. Similarly, any calibration result going out of limit shall also be thoroughly investigated to find the root cause, its impacts and for taking corrective and preventive action to avoid recurrence of such an incidence.

Regulatory requirements

Automatic, mechanical, or electronic equipment or other types of equipment, including computers, or related systems that will perform a function satisfactorily, may be used in the manufacture, processing, packing, and holding of a drug product. If such equipment is so used, it shall be routinely calibrated, inspected.

As per 21CFR 211.160 (Laboratory Controls)

The calibration of instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event accuracy and/or precision limits are not met. Instruments, apparatus, gauges, and recording devices not meeting established specifications shall not be used.Checked according to a written program designed to assure proper performance.

As per 21 CFR 820.72 (Inspection, measuring, and test equipment)

(a) ***Control of inspection, measuring, and test equipment.*** Each manufacturer shall establish and maintain procedures to ensure that equipment is routinely calibrated, inspected, checked, and maintained. The procedures shall include provisions for handling, preservation, and storage of equipment, so that its accuracy and fitness for

use are maintained. These activities shall be documented.

(b) ***Calibration.***Calibration procedures shall include specific directions and limits for accuracy and precision. When accuracy and precision limits are not met, there shall be provisions for remedial actionsto re-establish the limits and to evaluate whether there was any adverse effect on the device's quality.

As per ISO 13485 & ISO 9001, Clause 7.6, Control of monitoring and measuring devices

1. Measuring equipment shall be calibrated or verified at specified intervals, or prior to use, against measurement standards traceable to international or national measurement standards;
2. Measuring equipment shall be adjusted or re-adjusted as necessary
3. Measuring equipment shall be safeguarded from adjustments that would invalidate the measurement result

Since the result is out of calibration, following needs to be checked quickly for further decision making process.

1. Analyst / Instrument error
2. If there is an error (Thank God!), rectification and re-verification.
3. Impacts on products analyzed, approved and rejected using the instrument!

Investigation of analyst/ instrument error

If an error is observed in above investigation, calibration shall be repeated after approval from quality assurance. It shall be documented properly. Appropriate corrective and preventive actions shall be taken. If no error is found, the instrument may require be checking and servicing. In that case, the vendor's service engineer shall examine and opine. As per their recommendation, a small correction or major part replacement followed by appropriate re-calibration and/ or re-qualification shall be carried out. If required, based on the recommendations and our assessment, instrument may be discontinued with proper documentation.

Investigation of impacts and risk mitigation; It may not be possible to judge the exact date from which the instrument was not suitable. Therefore an effective risk assessment shall be carried out. The risk assessment shall be targeted with a question whether a failing batch is approved or vice a versa.

Investigation with respect to Product; Few points that are necessary for the investigation are listed below.

1. No. Of samples tested on the suspect instrument
2. No. Of samples passed in recent past
3. No. Of samples failed in recent past
4. Review of trend of analyzed product for confirming OOC period.
5. Testing methodology/ instrumentation used
6. Relevancy of testing methodology
7. System suitability / Instrument verification before startup of analysis.

Corrective actions

1. "Hold" all products tested on suspect instrument since earlier successful calibration
2. Segregate and quarantine products available in warehouse and label appropriately
3. Urgent analysis on alternate instruments or outside laboratory/ R & D whatever feasible
4. Information and procedure for recall
5. Available products shall be reprocessed / reworked after appropriate approvals and documentation procedure.

Preventive actions

1. Trending and monitoring of calibration data
2. Minimizing the calibration intervals
3. Minimizing the Preventive maintenance intervals
4. Unused/ less used instruments- calibrate before use
5. Instrument history cards to be sincerely maintained with all details
6. Proper training on calibration/ instrument handling
7. Analyst qualification calibration
8. Experienced person shall be responsible for calibration and related activities.

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