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A Review Paper On: Validation and Calibration of Analytical Instruments

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ABSTRACT: Analytical instruments are a large class of instruments used for analytical applications in chemical, pharmaceutical, clinical, food processing laboratories and oil refineries. The Instruments helps in qualitative and quantitative analysing. When analytical method is utilised to generate results about the characteristics of drug related samples it is essential that the results are trustworthy. To maintain the quality and quantity of instruments Validation and Calibration is most important. Validation is a process of establishing documentary evidence demonstrating that a procedure, process or activity carried out in testing and then production. Calibration is the comparison of measurement values delivered by a device under test with those of a calibration standard of known accuracy.

Keywords: Analytical instruments, Calibration, Validation, Quality, Instrumentation.

I. INTRODUCTION:

Analytical Instruments are used for a specific analysis of drugs and pharmaceutical products. The use of instrumentation is an exciting and fascinating part of chemical analysis that interacts with all the areas of chemistry and with many other fields of pure and applied science.

Analytical instrumentation plays an important role in the production and evaluation of new products and in the production of consumers and the environment. The instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air. The manufacture of materials whose composition must be known precisely, such as the substance used in integrated circuit chips, is monitored by analytical instruments.

Regular performance verification are made to insure that the instruments used in the analytical purpose should be properly validated and calibrated, ‘’ To demonstrate that it is suitable for its intended purpose.’’

It may defined that analytical chemistry is the study of separation, quantification and chemical components identification of natural and artificial materials constituted with one or more compounds or elements. Pharmaceutical analysis plays a very outstanding role in the examination of pharmaceutical formulations and bulk drugs regarding the quality control and assurance. Rapid increase in pharmaceutical industries and production of drug in and around the world bring forward a rise in inevitable demand to seek novel and systematic analytical techniques in the pharmaceutical industries.

Analytical Method development finally results in official test methods. Consequently quality control laboratories used these methods to check the efficacy, identity, purity, safety as well as performance of products of the drug.

The prime objective of any pharmaceutical plant is to manufacture products of requisite attribute and quality consistently, at the lowest possible cost.

Guided by pharmacology and clinical science, and driven by chemistry, pharmaceutical research in the past has played a crucial role in the progress of development of pharmaceuticals.

CALIBRATION:

Calibration is the process of comparing the reading of the reading of one instrument or equipment with a standard instrument. The reference instrument is already calibrated and referenced to a known set of parameters. The reference instrument should itself be directly traceable to equipment that is calibrated. Uncertainty in measurement by ensuring the accuracy of test equipment.

For most standard organisations accuracy ratio will be 3:1. The main objective of

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calibration is checking the accuracy of the given instrument and determining the traceability of measurements.

NEED FOR CALIBRATION:
-With new instruments.
-With a specified time period is elapsed.
-When a specified usage has elapsed.
-Sudden change in weather.
-Whenever observation appears questionable.
-Proper calibration of instruments is essential in obtaining accurate analyses. The choice of a calibration technique is affected by the instrumental method, instrument response, interferences present in the sample matrix, and number of samples to be analysed. Three of the most commonly used calibration techniques are the analytical or working curve, the method of standard additions, and the internal standard method.
-Calibration demonstrates that a particular instrument produces results within specified limits by comparisons with those produced by a reference or traceable standard over an appropriate range of measurements.

VALIDATION:

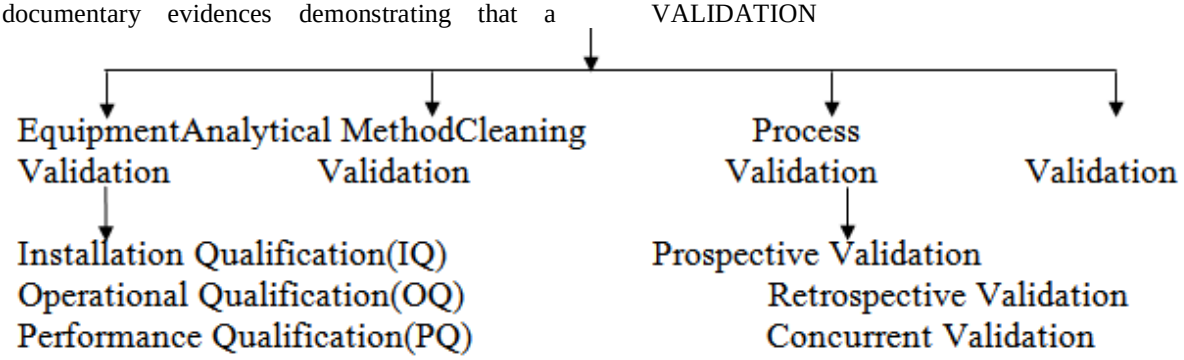
Validation is a process of establishing documentary evidences demonstrating that a procedure, process or activity carried out in testing and then production maintains the desired level of compliance at all stages.

Validation is a systematic approach to identify, evaluate, measure, document and re-evaluate critically all events of process of manufacturing which need control to make sure the reproducibility of final product.

Validation is a detailed process of confirming that the instrument is installed correctly, that it is operating effectively and that it is performing without error. The word validation simply means assessment of validity or action of proving effectiveness, validation is a team effort where it involves people from various disciplines of the plant.

IMPORTANCE OF VALIDATION
-Assurance of quality.
-Time bound.
-Process optimization.
-Reduction of Quality cost.
-Reduction in rejections.
-Increased output.
-Avoidance of capital expenditures.

TYPES OF VALIDATION



PROCESS VALIDATION:

Process validation is establishing documented evidences which provides a high degree of assurance that specific processes consistently produce a product meeting its predetermined specifications and quality attributes

ANALYTICAL METHOD VALIDATION:

The biggest advantage of analytical method validation is that it builds a degree of confidence, not only for the developer and also to the user. Although the validation exercise may appear costly and time consuming, it results in expensive, eliminate frustrating repetitions and leads to better time management in the end.

WRITING A TEST METHOD VALIDATION PROTOCOL:
Analytical method validations should contain the following information in detail

PURPOSE:

This section provides a short description of what is to be accomplished by the study. Identify the test methods and which products are within the scope of the validation.

OVERVIEW:

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This section contains the following- A general description of the test method, a summary of the characterization studies, identification of method type and validation approach test method applications and validation protocol, the intended use of each test method application and the analytical performance characteristics for each test method application.

RESOURCES:

This section identifies the following- End user laboratory where the method validation is to be performed; equipment to be used in the method validation; Software to be used in the method validation; materials to be used in the method validation; special instructions on handling, stability and storage for each material.

APPENDICES:

This section contains references, signature and a review worksheet for all personnel, their specific tasks and the documentation of their training. Listings of all equipment and software necessary to perform the method validation should be found here along with document and materials worksheets used in the method validation and in the test method procedures.

PARAMETERS FOR METHOD VALIDATION:

The various validation parameters are,

1. Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

TEST PROCEDURE:

Spiked samples will be prepared at three concentrations over the range of 50 to 150% of the target concentration. Three individually prepared replicates at each concentration will be analyzed. When it is impossible or difficult to prepare known placebos, use a low concentration of a known standard.

2. Precision:

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. Precision may be considered at three levels- Repeatability, Intermediate precision and reproducibility.

TEST PROCEDURE FOR REPEATBILITY:

One sample solution containing the target level of analyte will be prepared. Ten replicates will be made from this sample solution according to final method proceed record.

TEST PROCEDURE FOR INTERMEDIATE PRECISION:

Intermediate precision will be demonstrated by to analysts, using two HPLC systems on different days and evaluating the relative percent purity data across the two HPLC systems at three concentration level (50%, 100%, 150%) that cover the analyte assay method range 80 to 120%.

3. Linearity:

The linearity of analytical procedure is its ability of obtain test results which are directly proportional to the concentration of analyte in the sample. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content.

TEST PROCEDURE:

The standard solutions will be prepared at six concentrations typically 25, 50, 75,100, 150 and 200% of target concentration. Three individual prepared replicates at each concentration will be analysed. The method of standard preparation and the number of injections will be same as used in a final procedure.

4. Range:

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of Precision, accuracy and linearity.

TEST PROCEDURE:

The data obtained during the linearity and accuracy studies will be used to access the range of the method.

5. Limit of Detection:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. approaches other than those listed below may be applicable - Based on visual is evolution.

Based on signal to noise.

Based on standard deviation of the response and the slope.

TEST PROCEDURE:

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The lowest concentration of the standard solution will be determined by sequentially diluting the sample.
Six replicates will be made from the sample solution.

6. Limit of Quantitation:
The quantitative given of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable Precision and accuracy. Approaches other than those listed below may be applicable –
Based on visual evaluation.
Based on signal-to-noise approach.
Based on the standard deviation of the response and the slope.

TEST PROCEDURE:
Establish the lowest concentration at which an analyte in the sample Matrix can be determined with the accuracy and procedures required for the method in question. This value may be the lowest concentration in the standard curve. Make six replicates from this solution.

7. Specificity:
Specificity is the ability to accept unequivocally the analyte in the presence of component which may be expected to be present. Typically these might include impurities, Degradants, matrix, etc.

TEST PROCEDURE:
The specificity of the assay method will be investigated by injecting of the extracted placebo or demonstrated the absence of interference with the elution of analyte.

8. Robustness:
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an identification of its reliability during normal usage. The evolution of robustness should be considered during the development phase and depends on the type of procedure under study.

9. Ruggedness:
Ruggedness is a measure of reproducibility test result under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst.

10. System suitability studies:
System suitability testing is an integral part of many analytical procedures. The tests are based on a concept that the equipment, electronics, analytical operations and samples to be analysed constitute and integral system that can be evaluated.

TEST PROCEDURE:
System suitability test will be performed on both HPLC system to be determined the accuracy and Precision of the system by injecting six injections of a solution containing analyte at 100% of the test concentration. The following parameters will be determined- plate count, tailing factors, resolution and reproducibility.

BASIC FUNCTION OF INSTRUMENTATION:
Pharmaceutical instrumentation encompasses a wide variety of products, equipment, and machinery. Each type of instrumentation plays an important role in each unique manufacturing process. Instrumentation for the pharmaceutical industry requires the highest level of precision, reliability, and quality.
Product and service providers for companies in the pharmaceutical sector understand the importance of providing precision measuring equipment in order to deliver accurate dosages of medicine and provide other types of medical care.
The pharmaceutical industry requires a wide variety of equipment and technologies to create and test compounds, document results, and share findings. Many vendors specialize in particular components associated with each part of pharmaceutical development process. For example, pharmaceutical incubators are used to grow cell cultures and micro-organisms in a controlled environment for development and testing. What is most important is the ability to control variables so the result is repeatable and accurate.

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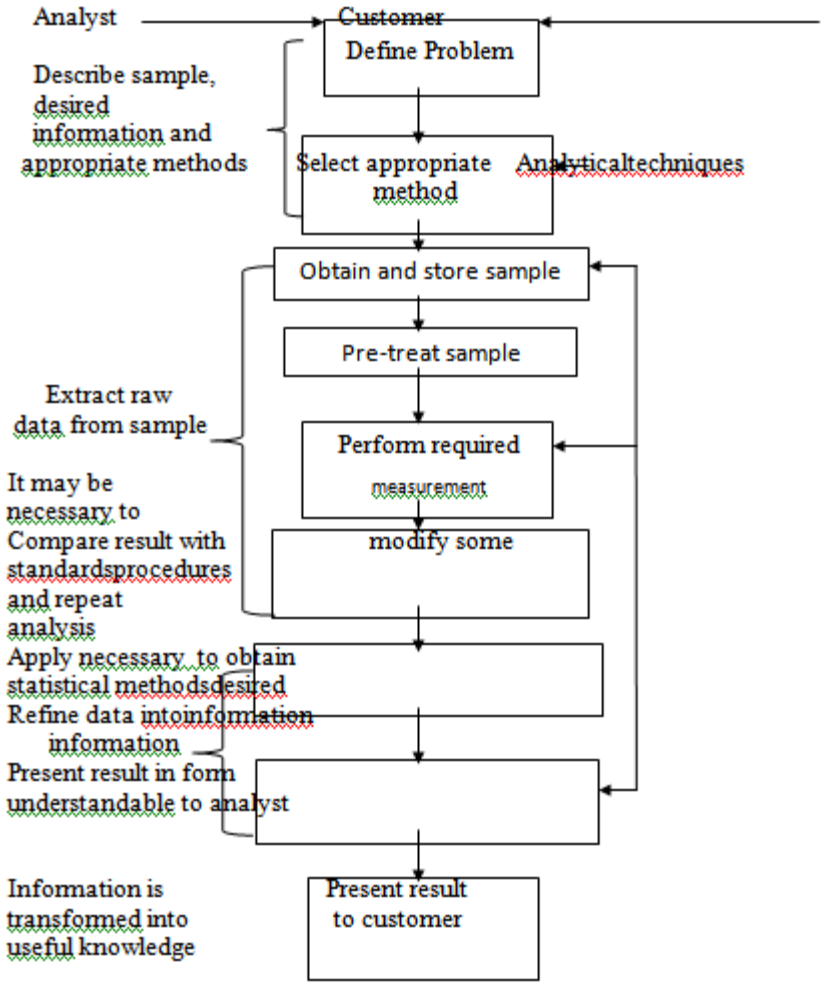
MAJOR STEPS IN SOLVING THE ANALYTICAL PROBLEM:



PRINCIPAL TYPES OF CHEMICAL INSTRUMENTATION:

A. SPECTROSCOPIC TECHNIQUES-
1) Ultraviolet and visible spectrophotometry
2) Fluorescence and phosphorescence spectrophotometry
3) Atomic spectrometry
4) Infrared spectrophotometry
5) Raman spectroscopy
6) X-ray spectroscopy
7) Radiochemical techniques including activation analysis
8) Nuclear magnetic resonance spectroscopy
9) Electron spin resonance spectroscopy

B. Electrochemical techniques-
1) Potentiometry (pH and ion selective electrodes)
2) Voltammetry
3) Voltametric techniques
4) Stripping techniques
5) Amperometry techniques
6) Coulometry
7) Electrogravimetry
8) Conductance techniques

C. Chromatographic techniques-
1) Thin layer chromatography
2) Paper chromatography
3) Gas chromatography
4) Column chromatography
5) High performance liquid chromatography
6) High performance thin layer chromatography
7) Ion-exchange chromatography

D. Miscellaneous techniques-
1) Thermal analysis
2) Mass spectroscopy
3) Kinetic techniques

E. Hyphenated techniques-
1) Gas chromatography- mass spectroscopy (GC-MS)

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2) Gas chromatography- Infrared spectroscopy (GC-IS)
3) Mass spectroscopy- mass spectroscopy (MS-MS)
4) Inductively coupled plasma- mass spectroscopy (ICP- MS)

STANDARD OPERATING PROCEDURE FOR CALIBRATION OF GIVEN INSTRUMENTS:

1) pH meter:

i. Operate the instrument as per respective standard operating procedures
ii. Use certified standard pH buffer to set the instrumentat pH 7.00 (with 7.00 with buffer) and pH 4.00 (with 4.00 wit buffer) at 25°C (room temperature).
iii. Take 100ml buffer pH 4.0 and pH 7.0 from the certified buffer bottle in glass bottle. Discard the standard pH buffer after one month.
iv. Prepare different pH solution of 0.05M potassium bi-phthalate, 0.05M equimolal phosphate, 0.01M sodium tetraborate and sodium hydroxide (saturated solution at 25°C).

Solution A – Preparation of 0.05M potassium bi-phthalate (pH-4.01)

– Disable 10.12gm of $KHC_8 H_4 O_4$ (previously dried at 110°C for 1 hour) in water to make 1000ml.

Solution B – Preparation of 0.05M equimolal phosphate buffer
(pH-6.86)

- Dissolve 3.53gm of $Na_2 HPo_4$ and 3.39gm of $KH_2 Po_4$ (each previously dried at 120° for 2 hours) in water to make 1000ml.

Solution C - Preparation of 0.01M sodium tetraboratebuffer (pH-9.18)

- Dissolve 3.80gm of $Na_2 B_4 O_7 .10H_2 O$in water to make 1000ml protect the solution from absorption of carbon dioxide.

Solution D - preparation of saturated solution of calcium hydroxide at
25°C (pH 12.45).

-Shake an excess of calcium hydroxide with water and the
diluent at 25°Cbefore you protect the solution from absorption of carbon dioxide (prepare fresh solution when
required).

v. Check the pH of a buffer solutions. It should be within + 0.07. Record the value in the prescribed and annexure.
vi. Check the pH of certified buffer pH 4.0 and pH 7.0. It should be within +/- 0.02 pH limit of labelled value. Record the value as per annexure.
vii. Discard buffer solution A B and C after three months.
viii. Frequency of calibration is 15 days.
ix. Daily calibration.
x. Record the same procedure for daily calibration of pH metre using solution A B and solution C and record value as per annexure.
xi. If it is out of specified limit, follow the SOP.

2) Ultra Violet Spectrophotometer:

i. Calibration of UV-VIS Spectrophotometer is done in four steps.

A] Control of Wavelength
B] Control of Absorbance
C] Limit of Stray Light
D] Resolution Power

ii. Operate the instruments as per SOP.
iii. Control of Wavelength

a. Weight accurately 1.0 gm of Holmium Oxide and dissolve it in 1.4 M Perchloric acid solution. Makeup to 25 ml with the same solvent.
b. Select the method file of CONTROL OF WAVELENGTH in the instrument.
c. After selecting the file press Reference button for baseline correction.
d. Then fill the Cuvette with 1.4M Perchloric acid and put in the sample cubicle and press reference to zero.
e. After auto zero put the Holmium perchlorate solution in sample cubicle then press start key.
f. Scan it and verify the wavelength using absorption maxima of Holmium Perchlorate solution. The permitted tolerance is given in below table.

Table-

Sr. No.	Maxima Wavelength (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

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iv. Control of Absorbance

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

g. Select the method file of CONTROL OF ABSORBANCE in the instrument.

h. After selecting the file press Reference button for baseline correction.

i. Then fill the Cuvette with 0.005M Sulphuric acid for blank and put in both sample cubicle and press reference to zero.

j. After auto zero put the Potassium Dichromate solution labelled as solution 'A' in sample cubicle then press start key taking absorbanceindividually for first four wavelengths mentionedin 'Table I'.

k. Now take absorbance at 430 nm for solution 'B'.

l. Note the absorption maxima of Potassium Dichromate solution at a different wavelength and calculate the absorbance, tolerance is given inbelow table.

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

v. Limit of Stray light

a. Dry a quantity of the Potassium chloride by heating to constant weight at 130°C.

b. 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.

c. Select the method file of LIMIT OF STRAY LIGHT in the instrument.

d. After selecting the file press Reference button for baseline correction.

e. Check the absorbance of above solution using water as a blank at 200 nm.

f. Absorbance should be greater than 2.0

vi. Resolution power

a. Prepare 0.02%v/v solution of Toluene in Hexane UV.

b. Select the method file of RESOLUTION POWER in the instrument.

c. After selecting the file press Reference button for baseline correction.

d. Measure the absorbance of above solution at 266 nm and 269 nm using Hexane UV as blank solution.

e. The ratio of absorbance maxima at 269 nm to that of 266 nm minima should be more than 1.5

f. Note down the report in the internal calibration certificate and in Instrument Logbook.

3)High Performance Liquid Chromatography:

I. Check the performance of instrument in terms of

a. Linearity of spotting (Annexure-I)

b. Reproducibility of spotting (Annexure-II)

II. Prepare the mobile phase as per method given in Annexure-I.

III. Prepare the solution as mentioned in Annexure-I

IV. Use 10 X 10 cm. HPTLC plate (Kieselgel 60F254 or Identical)

V. For Linearity of spotting

a. Apply 2 µl, 4µl, 6µl ,8µl and 10 µl of solution on HPTLC plate with spotter.

b. Allow the plate to run in mobile phase.

c. Dry the plate with drier.

d. Scan the plate with scanner.

e. Check the linearity and corelation coefficient.

f. Fill in the Data as mentioned in Annexure-I

VI. Reproducibility of spotting

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a. Apply 10µl solution on HPTLC plate for five times in sequence
b. Allow the plate to run in mobile phase.
c. Dry the plate with drier.
d. Scan the plate with scanner.
e. Calculate the RSD for five track (RSD Limit: N.M.T 3.0%)
f. Fill in the data mentioned in Annexure-II

4)Conductivity Meter:

I. Before starting calibration makes sure that the instrument is in the correct measurement mode.
II. Wash the electrode with deionized water after and before use and Store it dry.
III. Change the buffer after one week or when required , and record the details.
IV. Perform the calibration using a standard buffer of 1413 µS and record the details.
V. Press the mode key to select conductivity mode the conductivity indicator appears in the upper right hand corner of the display.
VI. Rinse the electrode thoroughly with deionized water or a rinsing solution do not wipe the probe this causes the electrostatic charge on the glass surface.
VII. Dip the electrode into the calibration buffer, the end of the probe must be completely immersed into the sample. Stir the probe gently to homogenize the sample.
VIII. Press CAL/MEAS key to enter conductivity calibration mode. The CAL indicator will be shown. The primary display will show the measured reading while the smaller secondary display will indicate the conductivity standard buffer solution.
IX. Press HOLD/ENTER key to confirm calibration. The meter is now calibrated to the current buffer.
X. Rinse the electrode with de-ionized water or a rinse solution and store it dry.

5)Total Organic Counter:

I. Use reagent water as a blank solution having TOC level shall be less than 100 ppb.
II. Preparation of 500 ppb Sucrose solution:

a. Dry Sucrose at 105°C for 1 to 2 hours. Accurately weigh and transfer 29.75 mg of previously dried sucrose to a 100 ml volumetric flask (Solution A) and make up the volume with high purity reagent water. Dilute 1.0 ml of the solution A with high purity reagent water to 250 ml (500 ppb carbon), Dilute 0.5 ml of the solution A with high purity reagent water to 250 ml (250 ppb carbon) and Dilute 1.5 ml of the solution A with high purity reagent water to 250 ml (750 ppb carbon)
b. In Calibration window select – Calibration with constant volume – No of samples – 1 – Analytical Parameter – NPOC – constant sample volume 2000 microliter – preparation blank – Measure – feed the concentration in NPOC table – 0.500 mg/l – Click on measurement & follow the software instruction.
c. At the end of calibration Click on LINK WITH THE METHOD – Accept Values.

Now the calibration values are included in the method.

III. Calibration Frequency – Single Point calibration shall be carried out within a week.

Acceptance criteria: Regression coefficient 0.4 to 0.8

Four point calibration - Once in three months.

Acceptance criteria: Correlation coefficient greater or equal to 0.999

II. CONCLUSION:

This review paper provides and calibration and validation of analytical instruments which is most important part of pharmaceutical industry. It requires a different standard operating procedure (SOP) to measure and monitarised an analytical instrument.

Different calibration and validation methods are used depends on a analytical instruments which are operated or examine.

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