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Calibration and Validation

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Abstract: *Analytical instruments are used for a specific analysis of drugs and pharmaceuticals. So, regular performance verification are made to ensure that the instruments used in the analytical purpose should be properly validated and calibrated "To demonstrate that it is suitable for its intended use".*

Keywords: Calibration

I. INTRODUCTION

Analytical instruments are used for a specific analysis of drugs and pharmaceuticals. So, regular performance verification are made to ensure that the instruments used in the analytical purpose should be properly validated and calibrated "To demonstrate that it is suitable for its intended use".

1.1 Calibration

- Calibration of an instrument is the process of determining its accuracy. The process involves obtaining a reading from the instrument and measuring its variation from the reading obtained from a standard instrument".
- Calibration of an instrument also involves adjusting its precision and accuracy, So that its reading comes in accordance with the established standard.
- This is important for justifying the process of Qualification and Validation.
- The instrument or equipment with the known accuracy is known as standards. All the other instruments are measured against this standard.
- It is important to know that the standards vary from one country to the other depending upon the type of industry.

Calibration achieves two main objective:

- It checks the accuracy of an instrument calibration and validation.
- It determines the traceability of the measurement.

1.2 What is Instrumental Calibration?

Instrument calibration can be defined as the process of comparing the measurements made by the instrument to be calibrated against a known measurement of either standards or an instrument known to be making measurements that exceed the acceptable limits of Accuracy and Precision.
Usually, calibration labs prefer a standard with 10 times the accuracy, however, most regulating organisations and authorities also accept a 3:1 accuracy ratio.

1.3 Types of Calibration

- Temperature Calibration
- Mechanical calibration
- Electrical calibration
- Pipette Calibration
- Flow Calibration

II. VALIDATION

Validation is an integral part of quality assurance; it involves the systematic study of systems, facilities and processes aimed at determining whether they perform their intended functions adequately and consistently as specified. A

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validated process is one which has been demonstrated to provide a high degree of assurance that uniform batches will be produced that meet the required specifications and has therefore been formally approved, Validation does not improve processes but confirms that the processes have been properly developed and under Control.

1.5 Definitions

- **According to ISO: "**Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled."

According to the US Food and Drug Administration (FDA), the goal of validation is to

- Product liability
- Control production cost

There are 4 main types of validation:

- Prospective Validation.
- Concurrent Validation.
- Retrospective Validation.
- Revalidation (Periodic and After Change)

2.1 Hot Air oven (Bio-techniques India, Model BTI 29)

Fig. 1 HOT AIR OVEN

2.2 SOP of Hot Air Oven

1. Confirm that the instrument is clean**,** Open the ventilation knob provided on top of the oven.
2. Turn "ON" the power supply, Electronic temperature controller displays the chamber temperature.
3. Set the required temperature by pushing the "PUSH" switch and first potentiometer knob clockwise until the temperature comes to set one.
4. Set the temperature with the help of a second potentiometer knob,
5. Release the "PUSH" switch.
6. Indicator bulb glows indicates that the power to the heater is "ON".
7. Switch "ON" the fan switch for air circulation.
8. Use a rotary switch for precise control of temperature.
9. Four positions of rotary switch are available.
10. Keep the switch on suitable markings as per requirements of temperature.

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2.2 Volumetric Glasswares

Calibration of Glassware Generally we use following glassware

Make	Commodity mfg.
HIMEDIA	Kit Items
BOROSIL	Glassware-Quoted Rates
AGARWAL	Borosilicate Glass- Quoted Rates
J-SIL	Borosilicate Glass- Quoted Rates

A. Pipette(Borosilicate Glass)

Fig.2 Pipette

The pipette is used to transfer quantitatively a known volume of solution from one container to another. There are two common types of pipettes, the volumetric pipette and measuring pipette.

Pipette can be calibrated as follows:

- Clean and rinse the pipette thoroughly and dry it.
- Weigh to nearest milligram a receiver generally Erlenmeyer flask
- Fill the pipette to a level above the etched line using distilled water at the laboratory temperature.Remove any liquid on the outside and release the pressure to allow the liquid to fall to the etched line.
- Discharge the contents of the pipette to a previously weighed receiver. Allow the pipette to drain completely for 20 to 30 seconds.
- Stopper the container and reweigh it. Calculate the volume of water delivered by the pipette from the weight and see the apparent volume from the table.
- Calibration of pipette is repeated as a check on work and duplicate results should not differ by more than 1 mg

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Determination of % Error:-

One mark Pipette			
Normal Capacity ml.	10	25	50
Tolerance +/- ml.	0.02	0.03	0.05
Graduated Pipette			
Normal Capacity ml.	1	5	10
Subdivision ml.	0.01	0.05	0.1
Tolerance +/- ml.	0.006	0.03	0.05

1. Temperature 22° c
2. Empty weight of beaker = 49.88
3. Beaker + water = 59.88
4. w1-w2 = 59.90 - 49.88 = 10.02
5. Actual volume = (w2 – w1)/specific gravity
6. Actual volume of water(Y) = 10.02/0.9982 = 10.03
7. Error (Y-X) =10.03-10 = **0.03 ml**
8. % Error = Error/X * 100 = 0.03/10 * 100 = 0.3%

Result

The error of 10 ml pipette = **0.03 ml**
The % Error of 10 ml pipette = **0.3 %**

Inference:

From above data we conclude that given pipette working with appropriate calibration and validation manner.

B. Burette (Borosilicate Glass)

Fig. 3 Burette

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Calibration of Burettes

- Clean the burette thoroughly and lubricate the stop cock properly.
- Fill with water and test for leakage and wait for at least 5 minutes.
- During the waiting period weigh a suitable receiver (generally an Erlenmeyer flask) to the nearest milligram Record this weight.
- Fill the burette with distilled water. Measure and record the temperature Sweep any air bubble from the tip of the burette now withdraw water more slowly until meniscus is at or slightly below the zero mark on the burette. After drainage is complete read the burette to the nearest 0.01 ml. Record the initial reading Remove the hanging drop.
- Now run about 10 mil of water from the burette into a previously weighed flask. Quickly stopper the flask and weigh it to the nearest milligram. Record this weight.

Now, refill the burette and obtain another initial reading. Run about 20 al of water into the flask. Obtain the final burette reading and reweigh the flask. Note that we are calibrating the burette in 10 ml intervals but starting each time from the initial reading as titration starts generally from zero. This process is repeated for 30, 40, 50 ml. Volumes
The difference between actual volume and apparent volume is of course the correction
Calibration of burette is repeated as a check on work and the duplicate results should agree within 0.04 ml.

Standard

Normal capacity ml	**10**	**25**	**50**
Subdivision ml	0.05	0.03	0.1
Tolerance +/- ml	0.01	0.03	0.05

Calculation

- Temperature of water = 27° c
- Specific gravity of water= 0.9982 gm/ ml
- Empty weight of beaker = 55.25
- Empty weight of beaker + 10 ml water withdraw from burette= 65.43gm
- Empty weight of beaker + 20 ml water withdraw from burette= 75.40 gm
- Empty weight of beaker + 30ml water withdraw from burette= 85.64 gm
- Empty weight of beaker + 40 ml water withdraw from burette= 95.47 gm
- Empty weight of beaker + 50ml water withdraw from burette= 105.27 gm

Observation Table

Sr. No	**Water delivered from burette (X) ml**	**Weight of empty beaker + water (w1) gm**	**Weight of water (w2 – w 1) gm**	**Actual volume of water = (w2- w1) Specific gravity of water (Y) ml**	**Error +/- (y-x) ml**	**% error = Error X *100**
1.	10	65.43	10.18	10.19	0.19	1.9
2.	20	75.40	20.15	20.18	0.18	0.9
3.	30	85.64	30.39	30.44	0.44	1.4
4.	40	95.47	40.22	40.29	0.29	0.7
5.	50	105.27	50.02	50.11	0.11	0.2

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Sr. No	Water delivered from burette (X) ml	Weight of empty beaker + water (w1) gm	Weight of water (W2-w1) gm	Actual volume of water = (w2-w1) Specific gravity of water (Y) ml	Error +/- (y-x) ml	% error= Error X * 100
1.	10	67.35	10.20	10.21	0.21	2.1
2.	20	77.56	20.17	20.20	0.20	2
3.	30	86.65	30.43	30.48	0.48	4.8
4.	40	96.56	40.30	40.37	0.37	3.7
5.	50	107.34	50.15	50.24	0.24	2.4

Result:
Average % Error of 50 ml burette is **1.024 ml**
Average % Error of 50 ml burette is **3 ml**

Inference:
From above data we conclude that given Burette working with appropriate calibration and validation manner.

C. Volumetric flask

Figure 4: Volumetric flask

- Volumetric flask is cleaned, rinsed, and then clamped in an inverted position to dry it.
- Stopper the flask and weigh the nearest milligram and record this weight.
- Fill the flask with distilled water at room temperature. Adjust the lower meniscus of water to the etched level mark by the means of pipette or dropper.
- Stopper the flask and reweigh to the nearest milligram. Difference in weight gives the apparent volume of water contained and from the weight of water. Calculate the actual volume.
- Calibration should be checked by repeating the procedure. Duplicate results should agree within 0.3ml for the flask.

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Acceptance criteria

Normal capacity ml	10	25	50	100
Tolerance	0.02	0.03	0.04	0.06

Volumetric Flask (Calibration & validation)

Initial volume(ml)	W 1(gm)	W 2(gm)	Apparent volume	Deviation
10	22.84	32.65	9.81	-
10	22.95	32.77	9.82	0.01
10	23.07	33.64	9.5	0.32

Initial volume(ml)	W 1 (gm)	W2(gm)	Apparent volume	Deviation
100	65.04	165.40	100.36	-
100	65.24	165.65	100.41	0.05
100	65.36	165.30	99.94	0.47

Mean mass of water = 9.81+9.82+9.5/3 =9.71
Volume = Mass/Density = 9.7/1 = 9.7
Tolerance = 10 - 9.7 = 0.3
For 100 ml
Mean mass of water=100.36+100.41+99.91/3 = 100.23 Volume = Mass / density = 100.23/1 == 100.23
Tolerance = 100 - 100.23 = 0.23

Inference
From above data we conclude that given Volumetric flask working with appropriate calibration and validation manner.

Tolerance
The tolerance of given 10 ml volumetric flask is **0.3**
The tolerance of given 100 ml volumetric flask is **0.23**

2.3 pH Meter (Labtronics, Model:LT-11)

Fig 5: pH Meter

Calibration of pH meter: Daily (Prior to use)

1. Before starting the calibration make sure that the correct measurement mode is selected.
2. Wash the electrode thoroughly with deIonized water or a rinse solution. Do not wipe the electrode; this causes a build-up of electrostatic charge on the glass surface.

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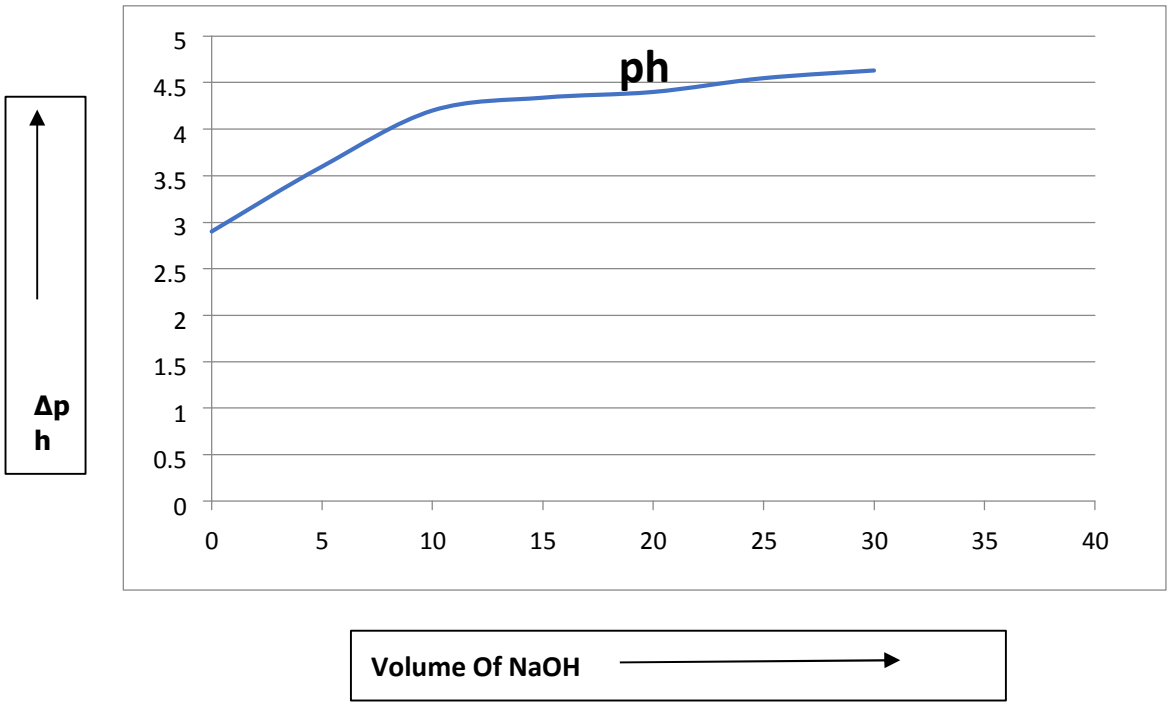
3. Maintain the temperature of the buffers to 25°C ± 2°C, unless otherwise specified in the individual monograph and dip the temperature sensor into the buffers.
4. Perform the five-point calibration using standard buffers of pH 1.68, 5.01/5.00, 7.00/7.01 10.00/10.01

Standard	Observed
2	1.06
5	5.03
7	7.02
10	10.09

Validation of pH Meter

Volumetric of NaOH ml	PH	ΔpH	ΔpH/ΔV
00	2.90	-	-
05	3.60	0.90	0.18
10	4.20	0.22	0.022
15	4.34	0.16	0.01
20	4.40	0.19	0.009
25	4.55	0.13	0.005
30	4.63	0.11	0.003
35	4.17	0.08	0.002



Graph of pH meter

Inference

From above data we conclude that given pH Meter working with appropriate calibration and validation manner

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2.4 Conductometer

Fig .6 Conductometer

Calibration of conductometer:

1. Before starting calibration make sure that the instrument is in the correct measurement mode.
2. Wash the electrode with deionised water after and before use and store it dry.
3. Change buffer after 1 week or when required.
4. Perform calibration using a standard buffer of 1413 uS and record the details.
5. Press the mode key to select conductivity mode; the conductivity indicator appears in the upper right hand corner of the display.
6. Rinse the electrode thoroughly with deionised water or rinsing solution, do not wipe the probe this causes "electrostatic charge" on the glass surface.
7. Deep the electrode into calibration buffer, the end of probe must be completely immersed into the sample. Stir the probe chain key to homogenize the sample.
8. 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 conductivity standard buffer solution.
9. Press HOLD/ENTER key to confirm calibration. The meter is now calibrated to the current buffer.
10. Rinse the electrode with deionized water or rinse solution and store it dry.

Preparation of standard solution: Reparation of standard solution :
Dissolve 0.7456 gm of KCl (chemically pure) in 1 litre of double distilled water. The solution
Reference Standard

Sr No	Solvent	Std. Conductance ms/cm	Observed conductance
1.	0.1 N NaoH	0.22	0.018
2.	HCl	750	755
3.	Drinking water	200-800	255

Validation

Volume of NaoH added (ml)	Conductivity (ms/CM)
0	0.945
1	0.955
2	0.967
3	0.978
4	0.986
5	0.994
6	1.002
7	1.017

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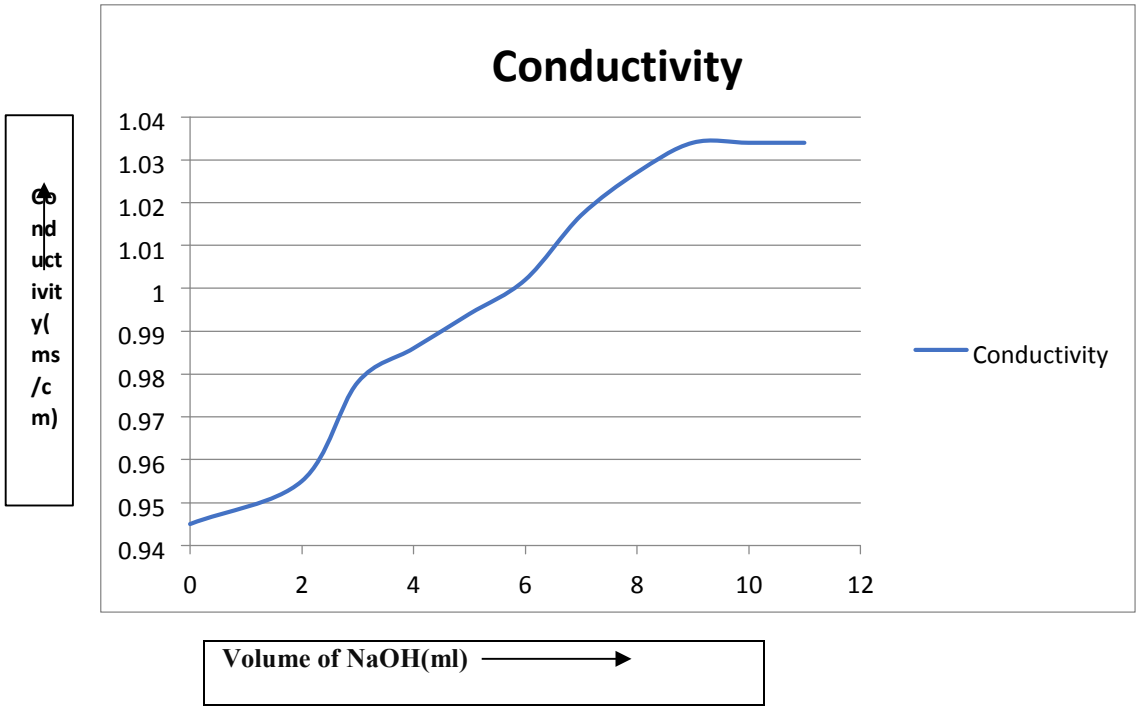
8	1.027
9	1.034
10	1.034
11	1.034

Calculations
N1 V1 == N2 V2
0.1x 10== N2 x 7
N2 == 0.14



Graph of conductometer Inference:
From above data we conclude that given Conductometer working with appropriate calibration and validation manner Normally of given sample is found to be N= **0.14**

2.5 Refractometer(Dolphin kf-33)

Fig.7 Refractometer

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A. Calibration

To achieve accuracy the instrument should be calibrated against distilled water which has a refractive index of 1.3325 at 250C against the reference liquids given below:

Carbon Tetrachloride: 1.4603

Toluene: 1.4969

Water: 1.3325

Frequency of Calibration = monthly

Sample	Standard Refractive index	Observed Refractive index
Distilled water	1.33	1.3325
Toluene	1.49	1.457
Carbon tetrachloride	1.46	1.445
Benzene	1.49	1.467

Validation:

R1 = Water - 1.33

CCL4 - Std – 1.46 & Test – 1.445

Benzene – Std – 1.49 & Test – 1.467

Toluene – Std – 1.49 & Test – 1.457

Corrective Factor = Std Value – Test Value

For CCL4 = 1.46 – 1.445

CCL4 = 0.0015

For Benzene = 1.49 – 1.467

Benzene = 0.023

For Water = 1.3325 – 1.33

Water=0

Molar Refract for Benzene

MRC

$$_6H_6 = \left(\frac{n^2-1}{n^2+2}\right)\frac{M\omega}{d}$$

$$\left[\left(\frac{1.467^2-1}{1.467^2+2}\right)\right]\times\frac{84}{876}$$

$$\frac{2.1520-1}{2.1520+2}\times\frac{84}{876}$$

$$\frac{1.1520}{4.1520}\times\frac{84}{876}$$

$$= 0.2774 \times 0.095 = 0.02635$$

5. Molar Refract For CCL4

$$\text{MRCCL4} = \left(\frac{n^2-1}{n^2+2}\right)\frac{M\omega}{d}$$

$$\left[\left(\frac{1.44^2-1}{1.44^2+2}\right)\right]\times\frac{153.82}{1.59}$$

$$\left[\frac{2.0736-1}{2.0736+2}\right]\times 96.74$$

$$\frac{1.0736}{4.0736}\times 96.74$$

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MRCCL4 = 25.49

Inference:
From above data we conclude that given Refractometer working with appropriate calibration and validation manner.

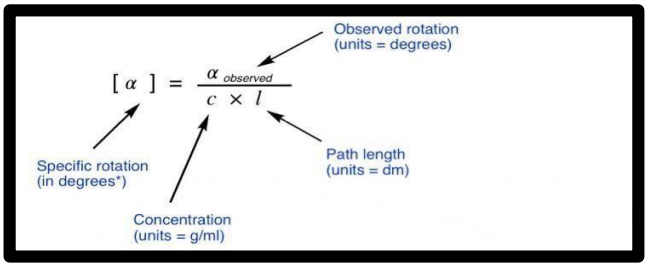
2.6 Polarimeter (Coslab, Model pm 54)

Fig.8 Polarimeter

Calibration of Polarimeter :

1. Switch on the mains.
2. Weight till the sodium lamp glows with full intensity of yellow light.
3. Rinse the polarimeter tube with distilled water.
4. Adjust the Vernier scale and main dial scale to zero.
5. If two halves intensity is not metering to then adjust it by turning the knurled screw of the prism box slightly. If not possible then adjust the field by rotating milled knob and Vernier scale note the reading (+) or (-) side as a zero and to be considered while calibrating.
6. Fill the distilled water in a Polarimeter tube.
7. Clean the sides of the tube with tissue paper.
8. Entrap any air bubble in space at the center of the tube.
9. Keep the polarimeter tube in the place provided in the polarimeter.
10. Adjust the zero with help of "control wheel"
11. Take an average of the five readings.

Formula:-



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Observation table:

Sample	Standard specific rotation	Angle of rotation	Specific rotation
10% sucrose	66.37	13.5	9.2
20% sucrose	66.37	13.9	8.7

Calculations for 10 % sucrose

$$[\alpha] = \frac{\alpha_{observed}}{c \times l}$$

= 92 /10x1 = 9.2°

The specific angle of rotation is **9.2°**

For 20% sucrose

$$[\alpha] = \frac{\alpha_{observed}}{c \times l}$$

= 174/20*1 = 8.7 °

The specific angle of rotation is **8.7° Specific angle of rotation:-**

The specific angle of rotation of 10 % sucrose is found to be **9.2°**The specific angle of rotation of 10 % sucrose is found to be **8.7°**

Inference

From above data we conclude that given Polarimeter working with appropriate calibration and validation manner.

2.7 UV-Visible Spectrophotometer (Bio Era)

Fig. 9 UV-Visible Spectrophotometer

Calibration:

Calibration of UV-VIS Spectrophotometer is done in following steps

1. Control of Absorbance
2. Limit of Stray
3. Light Resolution Power
4. Operate the instrument as per above SOP

Control on absorbance:-

1. Dry a quantity of the Potassium dichromate by heating to constant weight at 1300 C.
2. Weight within the rangely about 60 mg of dried potassium dichromate and dissolve it in 0.005M sulphuric acid solution. Make up to 1000ml with the same solvent. Mark the solution as (A).
3. Weight within the rangely 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).

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

1. After selecting the file press Reference button for baseline correction. Then fill the cuvette with 0.005M Sulphuric acid for blank and put in both sample cubicle and Press reference to zero.

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2. After auto zero put the Potassium Dichromate solution labelled as solution 'A' in sample cubicle then press start key taking absorbance individually for first four wavelengths mentioned in 'Table I'.
3. Now take absorbance at 430 nm for solution 'B'.
4. Note the absorption maxima of Potassium Dichromate solution at a different wavelength and calculate the absorbance, tolerance is given in below table.

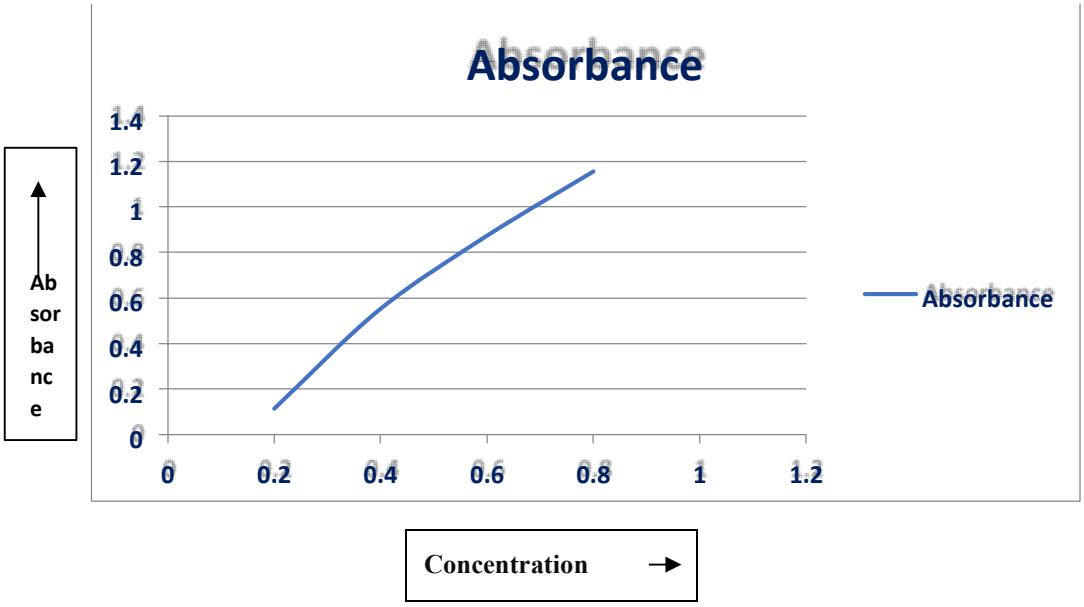
Observation table

Sr no	Wavelength (nm)	Absorbance E(1%/1cm)	Maximum tolerance
1	235nm	123.6	112.9 – 126.2
2	257 nm	145.1	142.8-145.7
3	313nm	49.6	47.0-50.3
4	350nm	106.7	104.9-108.2
5	430nm	15.8	15.7-16.1

VALIDATION

λmax of paracetamol =257 nm

Concentration (mg/ml)	Absorbance
0.2	0.114
0.4	0.552
0.6	0.873
0.8	1.156
1.0	1.587



EFFECT OF PH ON ABSORBANCE

The increasing pH values resulted in the greater wavelength of absorbance peak, suggesting the increased aromaticity and conjugated degree, and the novel fluorescence peak was found in λ(ex/em) = 250/450.

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Aniline	Wavelength
Acidic	224 nm
Basic	230nm

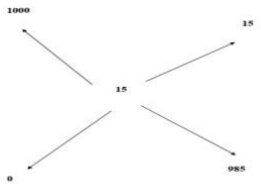
Inference:
From above data we conclude that given UV Visible Spectrophotometer working with appropriate calibration and validation manner.

2.8 Flame Photometer(Model EQ 850 A)

Fig 10. Flame photometer Preparations of Standards 1.Sodium (1000 PPM)
Dissolve 2.542 grams of AR grade sodium chloride in double distilled water to make 1 liter of solution.

A. Potassium(1000 PPM)
Dissolve 1.909 grams of AR grade potassium chloride in double distilled water to make 1 liter of solution.

B. Calcium (1000 PPM)
Dissolve 2.497 grams of AR grade calcium carbonate in just minimum quantity of dilute AR grade HCI (add drop by drop till calcium carbonate dissolves) and add double distilled water to make 1 liter of solution.
Validation: For further calculations, we convert sodium chloride 1000 ppm to 1 ppm for that we use ALIGATION METHOD.



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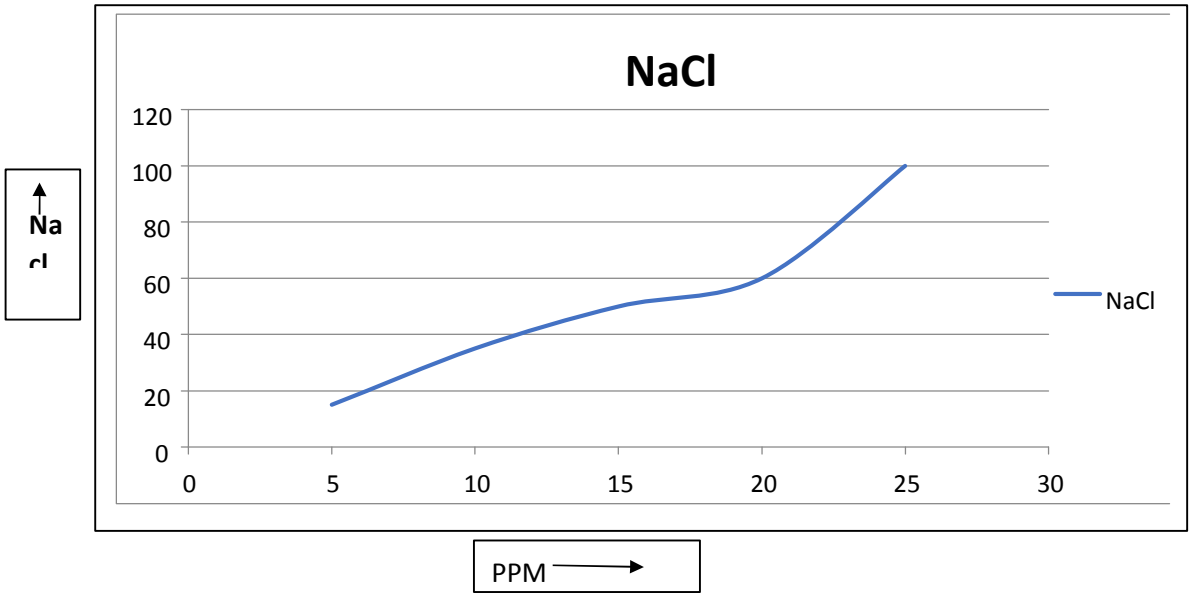
Observation table



Graph

Ppm	NaCl
5	15
10	35
15	50
20	60
25	100

Observation table:

Ppm	KCl
5	10
10	25
15	34
20	62
25	100

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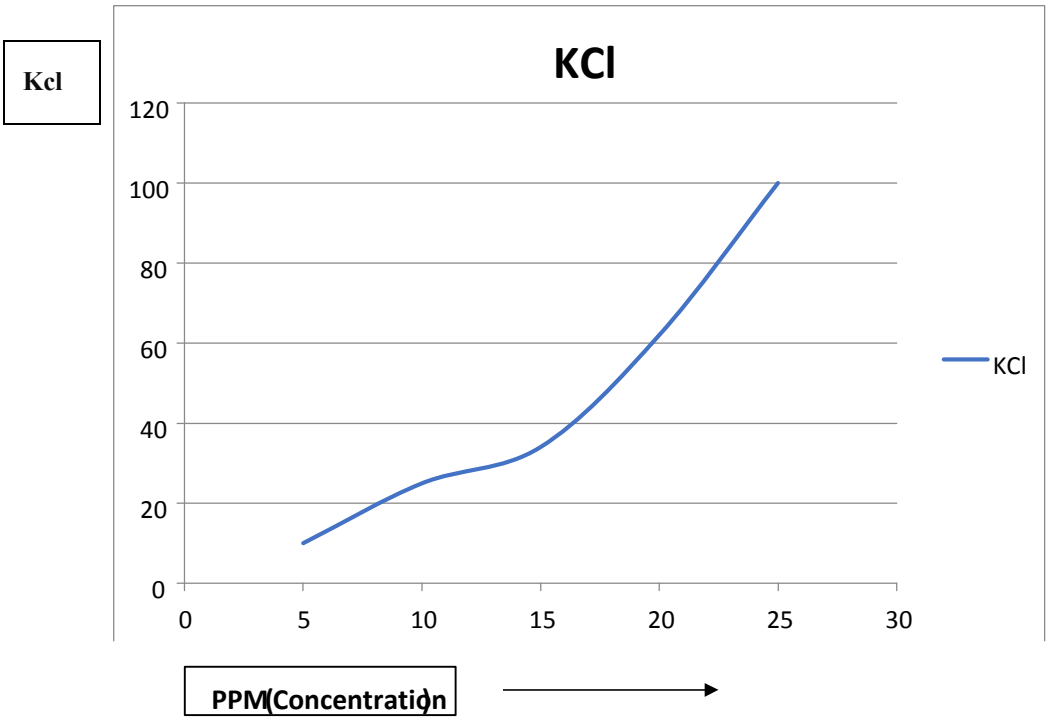

Inference:
From above data we conclude that given Flame Photometer working with appropriate calibration and validation manner

III. CONCLUSION

From this Practice school we have learnt a lot of things which are applicable in industries. The importance of instrument calibration in different industries cannot be emphasised enough.
It is the most basic, yet crucial maintenance requirement and is an established procedure that should be conducted by any industry that uses instruments and machinery to manufacture products.
Calibration should be conducted periodically or on-demand as required by the instrument and its importance to the process, but it must not be skipped for any reason.
With proper instrument calibration, end users get improved efficiency and products that will always achieve the desired quality