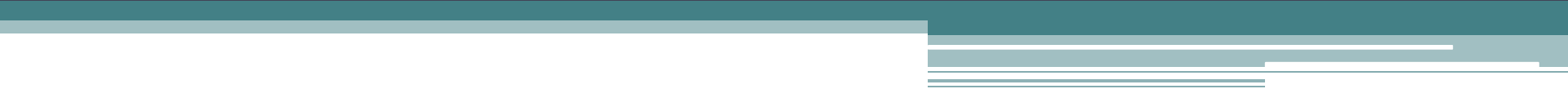


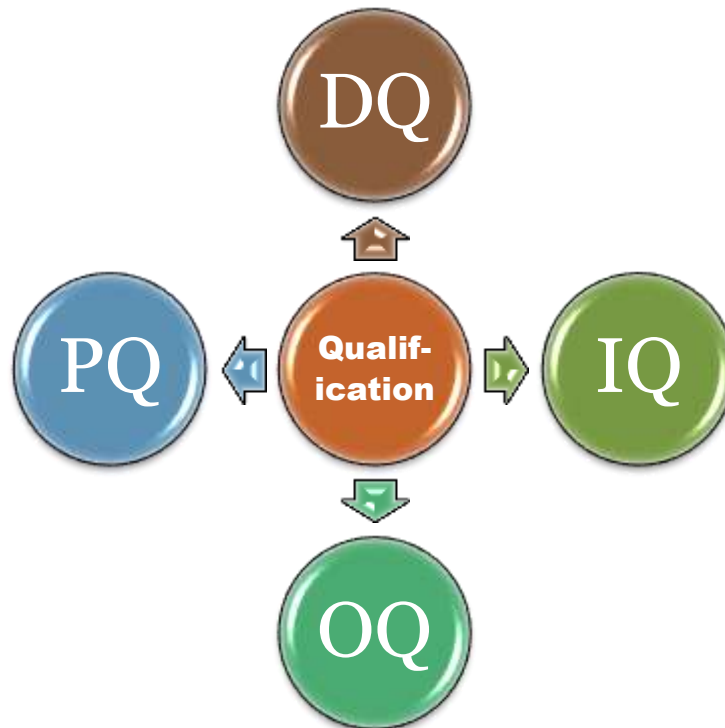
Qualification of UV - Visible Spectrophotometry, FTIR, DSC & HPLC

A series of horizontal lines in teal and light blue colors, with some lines having a slight 3D effect, spanning the width of the slide below the title.

INTRODUCTION

- ❖ **VALIDATION:** It is a act of demonstrating and documenting that the process operates effectively and reproducibly to produce a product meeting its predetermined specifications and quality attributes.
- The aim of validation is to show that the critical steps are under control and lead continuously to the desirable quality.
- ❖ **CALIBRATION:** 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.

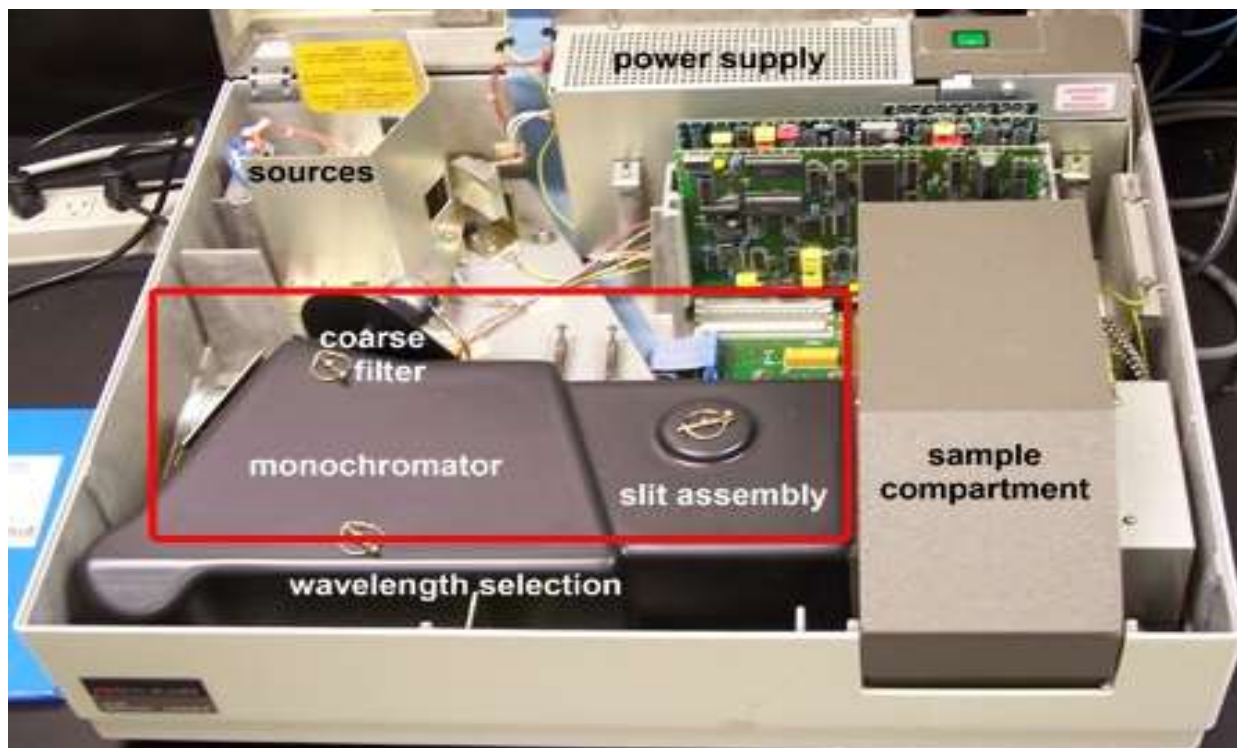
❖ **QUALIFICATION:** Qualification is an act or process to assure something complies with some conditions, standard or specific requirements.



- **Design Qualification:** Documented evidence which shows that the plant design agrees with the design specifications of the customer.
- **Installation Qualification:** Written evidence is given that all parts of equipment are installed according to the equipment supplier's and purchase specifications.
- **Operational qualification:** Documented evidence which shows that all parts of the plant and equipment work within their specifications and process parameters.
- **Performance Qualification:** Provides documented evidence that all parts of a plant and other processes produce products of specified quality under conditions of normal production for a longer period of time.

UV-VISIBLE SPECTROPHOTOMETER

- UV-Visible spectroscopy is concerned with ultra violet and visible regions which ranges from 200-780 nm.



INSTALLATION PROCEDURE:

- While the UV instrument was shipped after the precise adjustment and inspection at the factory, it is recommended to install according to the following procedures so as to provide its optimum performance and to meet the user's demands.

INSTALLATION SITE:

- Room temperature during use of 15 to 35 °C.
- Out of direct sunlight.
- No strong vibration or continuous weak vibration.
- No strong magnetic fields or electromagnetic fields.
- Humidity of 45 to 80%.
- No corrosive gases or organic or inorganic gases with absorptivity in the ultraviolet range.
- Small amount of dust.

ACCEPTANCE PROCEDURES:

Item to be checked	Specification
Appearance	No defect
Number of parts	No missing parts
ROM check	Latest version
Linearity of Absorbance	Bent: ± 0.002 Abs (Shock noise: ± 0.004 Abs)
Noise level	Noise width: ± 0.002 Abs (Shock noise: ± 0.004 Abs)
Accuracy of wavelength	± 0.5 nm
Repeatability of wavelength	± 0.1 nm

PERFORMANCE QUALIFICATION

- **Wavelength accuracy**

It is defined as the deviation of the wavelength reading at an absorption band and emission band from the wavelength of the band.

✓ **Acceptance:** $\pm$ nm in UV range (200-380 nm) and
 $\pm$ nm in visible range (380-800 nm)

Three repeated scan of the same peak should be with in
 ± 0.5 nm

- **Stray light**

Stray light is defined as the detected light of any wavelength that is outside the band width of the wavelength selected.

- ✓ **Acceptance:** the transmittance of the solution in a 1cm cell should be less than 0.01 or the absorbance value should be greater than 2

- **Resolution power**

The resolution of the UV-VIS spectrometer is related to its spectral band width. The smaller the band width the finer the resolution. The SBW depends on the slit width and the dispersive power of the monochromator.

- ✓ **Acceptance:** The ratio of the absorbance at 269 nm and absorbance at 266 nm should be greater than 1.5

- **Noise**

Noise is the measurement affects the accuracy at the both end of the absorbance scale. Photon noise from the light source affects the accuracy of the measurement leads to low absorbance.

- ✓ **Acceptance:** The RMS noise should be less than 0.001 AU

- **Baseline flatness**

The flat baseline test demonstrates that the ability of the instrument to normalise the light intensity measurement and the spectral out put at different wavelength throughout the spectral range.

- ✓ **Acceptance:** The measurement is typically less than 0.01 AU

- **Stability**

The lamp intensity is a function of the lamp age, temperature fluctuation and wavelength of the measurement. These changes can lead to errors in the value of the measurements, over an extended period of time.

- ✓ **Acceptance:** The deflection is less than 0.002 AU/ hr

- **Photometric accuracy**

Photometric accuracy is determined by comparing the difference between the measured absorbance of the reference material and the established value.

- ✓ **Acceptance:** Six replicate measurements of the 0.006% w/v of the potassium dichromate solution at 235, 257, 313 and 350 nm should be less than 0.5% RSD.

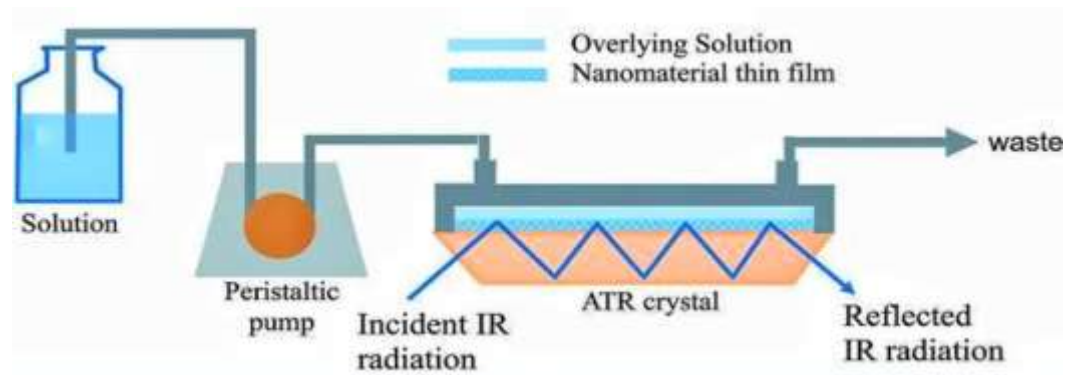
- **Linearity**

The linear dynamic range of measurement is limited by stray light at high absorbance and by noise at low absorbance. The accuracy of the quantification of the sample depends on the precision and linearity of the measurements.

✓ **Acceptance:** Correlation coefficient $r \geq 0.999$

FTIR

- **Fourier transform infrared spectroscopy (FTIR)** is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas.
- An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time.



CALIBRATION

- **WAVE NUMBER PRECISION:**
- This is performed for substances with well known peak wave number(s) position such as carbon dioxide, water vapour, polystyrene ,ammonia.
- Test is performed to know whether the exact peak wave numbers are shown at that time of validation.
- Thus the result is obtained from the difference between the peak wave numbers position for a substance with a well known peak wave numbers and the values indicated by the system.

0% TRANSMITTANCE:

- A sample which do not allow the transmission of light is measured in order to investigate the 0% transmittance.
- This test thus can be used to find out error caused by stray light and secondary emission spectra.

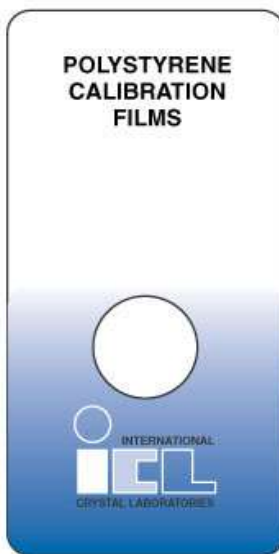
100% TRANSMITTANCE

- This is investigated by performing analysis with out a sample.
- By performing analysis with out the sample 100% transmittance can be investigated.

- **LINEARITY OF CURVE:** A calibration curve for the % transmittance and the concentration is created and the linearity of the inspected.
- **REPRODUCIBILITY:** A stable sample is measured twice with in a short period and confirmed whether the variation in the measurement values such as wave numbers and transmittance are obtained.

VALIDATION OF FTIR:

- To perform FTIR validation and to confirm that it is operating properly, diverse IR inspection was performed by measuring the spectra of polystyrene film.



INSTALLATION OF VALIDATION PROGRAMME

- Software validates the Shimadzu Fourier transform infrared spectrophotometer IRPrestige-21/IRAffinity-1/FTIR-8000 series by the IRsolution software to control them and data processing
- The Validation program is automatically installed when the IRsolution is installed, therefore the Validation program does not need to be separately installed.

PERFORMANCE QUALIFICATION

- **POWER SPECTRUM**

- Power spectrum gives the plot of portion of signal's power(energy per unit time) falling within the given frequency bins.
- This test estimates the intensity of power spectrum at a specified wave numbers .
- When the measured intensity is equal to or larger than the criterion value, the test is passed.

ACCEPTANCE CRITERIA

Wave number (cm-1)	Standard Value FTIR-8400S.
4600	10% or min of max
4000	25% or min of max
3000	50% or min of max
Power max value	50.0
700	10% or min of max
500	2% or min of max
403	0.5% or min of max
351	0.01% or min of max

RESOLUTION

- The resolution is checked by recording the spectrum of polystyrene film of approximately $35\mu\text{m}$ in thickness.
- The difference between percentage transmittance at the absorption maximum A at 2870 cm^{-1} and absorption minima B at 2849.5 cm^{-1} must be greater than 18.
- The difference between percentage transmittance at the absorption maximum C at 1589 cm^{-1} and absorption minima D at 1583 cm^{-1} must be greater than 12.

WAVE NUMBER ACCURACY

- The wave number scale is usually calibrated by the use of several characteristic wave numbers of a polystyrene film.

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}

- ❖ The software then judges whether the values are within in the allowable range.
- ❖ The program labels the results “PASS” if all the peak numbers are within the range.
- ❖ In case of dispersive spectrophotometer the permissible level of frequency at 1601.2 cm^{-1} and at 1028.3 cm^{-1} should be with in $\pm 2.0\text{ cm}^{-1}$.

WAVE NUMBER REPRODUCIBILITY

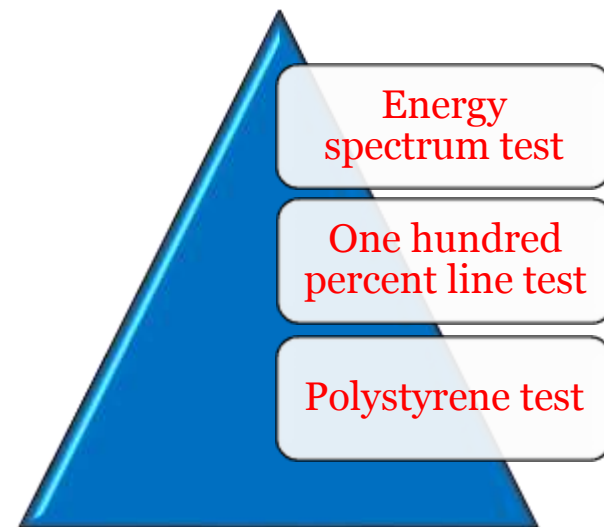
- This program specifies three points to measure the peak wave numbers.
- Then it obtains the actual peak wave numbers at each point by measuring the polystyrene film twice
- It should satisfy 5 cm^{-1} around 3000 cm^{-1} of polystyrene absorption wave number, 1 cm^{-1} around 1000 cm^{-1} .
- The software determines whether the differences between each of two measurements are within the allowable range and it labels the result PASS if they are within the range.
- EP 4.0 doesn't include this inspection

TRANSMITTANCE REPRODUCIBILITY

- This program specifies peak wave number at three points and the transmittance at each point is measured is twice.
- 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.
- Then it is determined whether the differences between the two data are within the allowable range and it labels the result PASS if they are within the range.
- All of the above furnished data should be represented in

AS PER ASTM E1421-94 Level Zero

- This software complies with the description in the ASTM (American Society for Testing and Materials).
- The FTIR abnormalities or large changes over short term and long term are assessed by these tests.
- The three parameters checked by this program are:



- **ENERGY SPECTRUM TEST**

Power spectra obtained in the inspection are compared with reference data and the spectra are checked for changes over long periods.

- **ONE HUNDRED PERCENT LINE TEST**

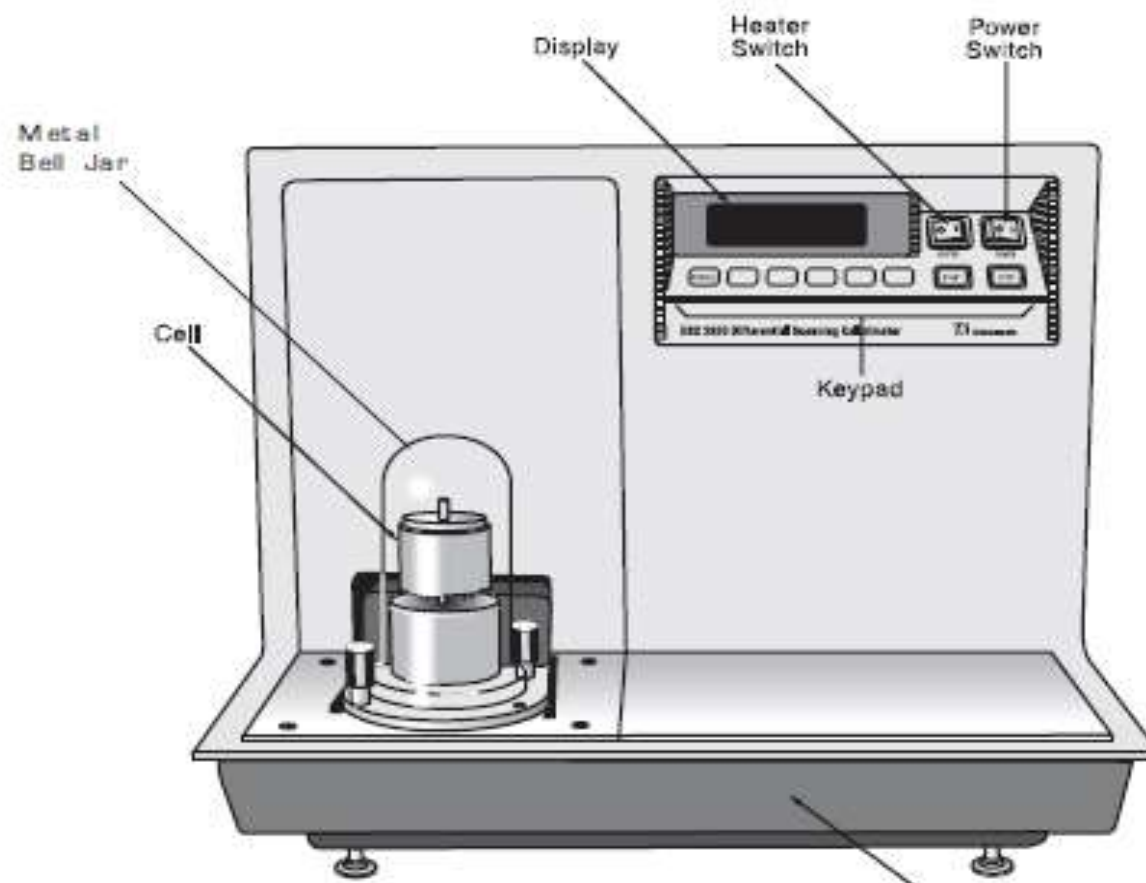
100% T line spectra are calculated for power spectra & are measured continuously in inspection and the spectra are checked for changes over short periods.

- **POLYSTYRENE TEST**

- Evaluation is performed using differences between spectra obtained for polystyrene film in inspection and the stored reference data.
- When the differences are within the standard, "pass" results.
- All of the above furnished data should be represented in Validation Report.

Differential Scanning Calorimeter

- Differential Scanning Calorimeter (DSC) measures the temperatures and heat flows associated with transitions in materials as a function of time and temperature in a controlled atmosphere.
- The DSC has two major parts: the instrument which contains the system electronics, and the cell, which contains its own thermocouples (temperature sensor) for monitoring differential heat flow and temperature.



DESIGN SPECIFICATIONS

Principle	Heat Flux Type
Heat Flow Range	$\pm 40\mu\text{W}$
Hold Time	0 - 999 min, hour
Noise level	$1\mu\text{W}$
Size (mm)	300Wx 490Dx 290H
Temperature Range	-150 to 600° C
Program Rate	0 - 99°K/min, °K/hour
Cooling Time	about 6 min from 600°C to 40°C with LN2
Atmosphere	Inert gas or air
Power supply	100/120 VAC 800AV

INSTALLATION QUALIFICATION

The DSC should be:

In

- A temperature-controlled area(15°C to 30°C is recommended).
- A clean environment.
- An area with ample working and ventilation space.

on

- A stable, heat-resistant, and fire-resistant work surface.

- **Near**

- A power outlet (120 Vac, 50 or 60 Hz, 15amps). A step up/down line transformer may be required if the unit is operated from a higher or lower line voltage.
- Your controller.
- A compressed lab air and purge gas supply for use during cooling, sub ambient, and high temperature experiments.

- **Away from**

- Dusty environments.
- Exposure to direct sunlight.
- Direct air drafts (fans, room air ducts).
- Poorly ventilated areas.
- Flammable materials

PERFORMANCE QUALIFICATION

- The most common procedure is to run an indium standard under the normal test conditions and measure the heat of fusion value and melting onset temperature.
- These values are then compared with literature values and a check made against accepted limits.
- For many industries limits of—
 - ✓ $\pm 0.5^{\circ}\text{C}$ for temperature or 1% for heat of fusion may be accepted, though tighter limits of $\pm 0.3^{\circ}\text{C}$ and 0.1% may also be adopted.
 - ✓ The choice of limits depends on how accurate you need to be.
 - ✓ Indium is the easiest standard to use because of its stability and relatively low melting point of 156.6°C , which means it can often be reused, provided it is not heated above 180°C .

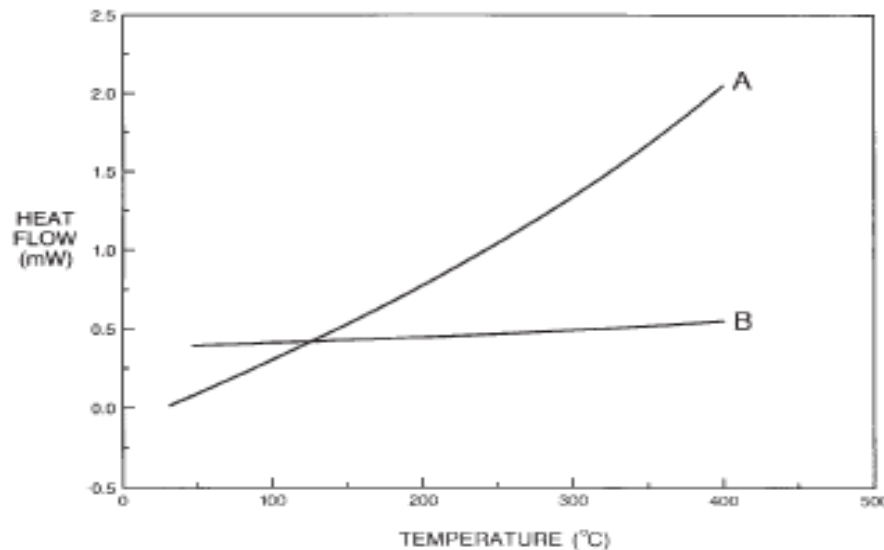
CALIBRATION

- To obtain accurate experimental results you should calibrate each standard DSC and dual sample DSC when you first install it.
- The calibration will be stored in the cell's memory and automatically entered when the cell is installed.
- For precise experimental results you will need to generate a new calibration file whenever you change one of the following parameters:

Baseline slope calibration:

- The baseline slope and offset calibration needs to be performed separately for each cell.
- This calibration involves heating an empty cell through the entire temperature range expected in subsequent experiments.
- The results may look similar to Figure below.
- This figure shows two example heat flow curves for an empty standard DSC cell run from 25°C to 400°C.
- Ideally, the heat flow signal should be zero, since there is no sample in the cell, and it should have minimum slope.

- The calibration program is used to calculate the slope and offset values needed to flatten the baseline and zero the heat flow signal.

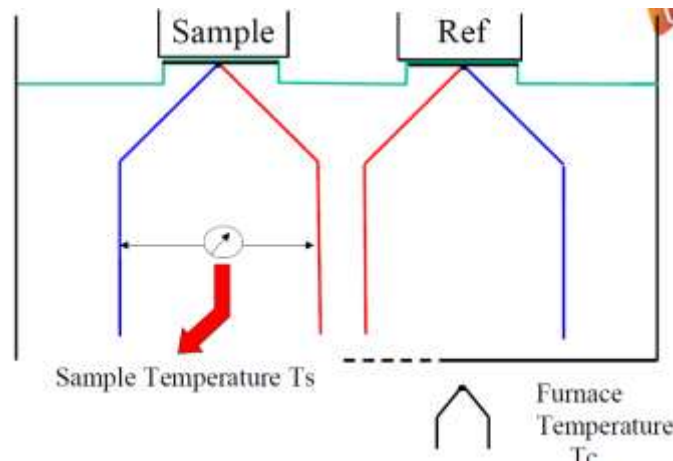


Baseline and slope calibration

Temperature Calibration:

- Establishment of the relationship between temperature measured T_{meas} and the true temperature T_{tr}

$$T_{\text{true}} = T_{\text{meas}} + \Delta T_{\text{corr}}(T_{\text{meas}})$$



Enthalpy calibration:

- Establishment of the relationship between enthalpy change measured ΔH_{meas} and the true enthalpy change $\Delta_{\text{trs}}H$ absorbed or released by the sample as a result of a transition at the transition temperature T_{trs} :

$$\Delta_{\text{trs}}H = K_{\text{H}}(T_{\text{tr}})\Delta H_{\text{meas}};$$

Heat flow rate calibration:

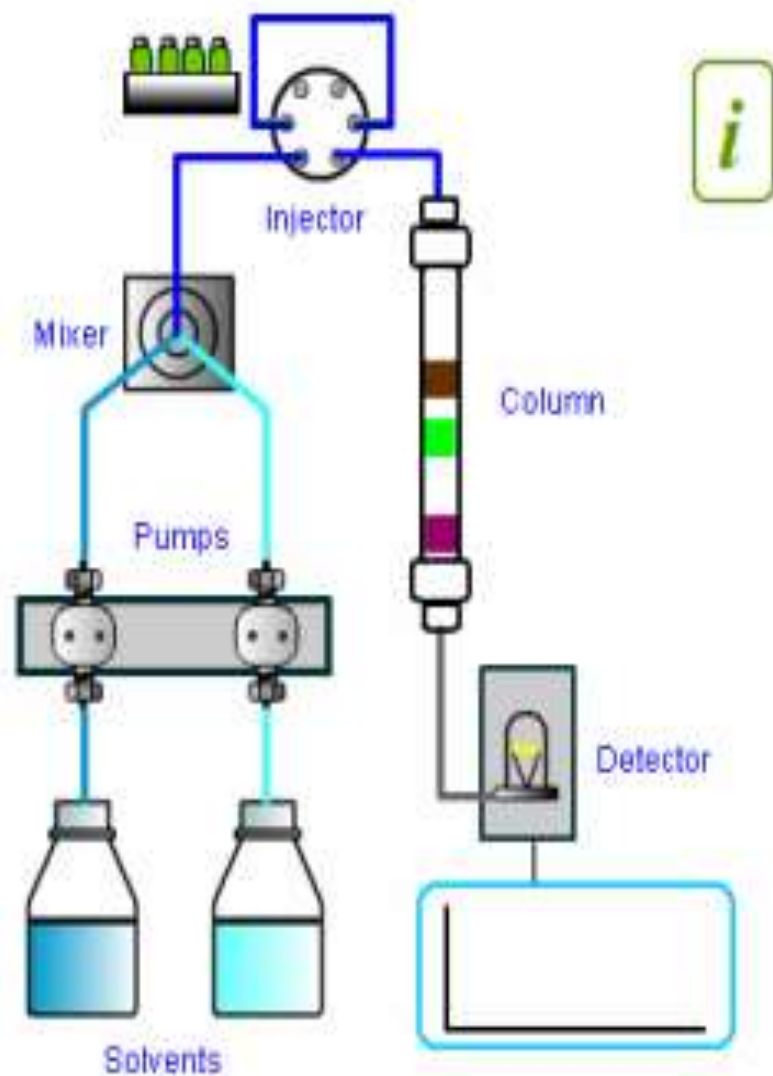
Establishment of the relationship between heat-flow rate Φ_{meas} indicated by the instrument and the heat-flow rate Φ_{tr} absorbed or released by the sample due to its heat capacity (at constant pressure) Cp :

$$\Phi_{\text{tr}} = K_{\Phi}(T)\Phi_{\text{meas}}$$

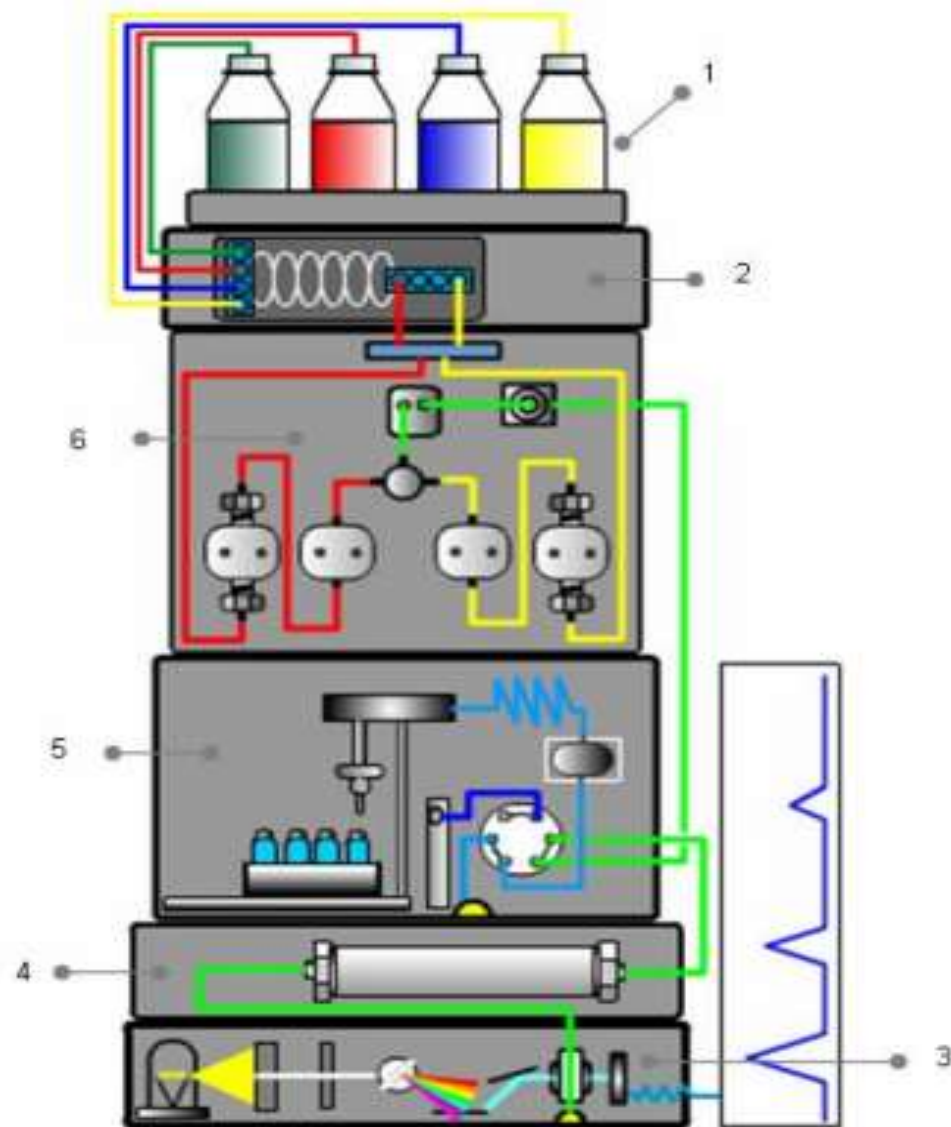
where Φ_{tr} is equal to $Cp\beta$

HPLC

The technique of high performance liquid chromatography is so called because of its improved performance when compared to classical column chromatography. It is also called high pressure liquid chromatography since high pressure is used when compared to classical column chromatography



Liquid Chromatographic Process



A typical Liquid Chromatograph

Design Elements	Examples
Intended use	Analysis of drug compound and impurities
User requirement specification for the hplc analysis	• Up to 100 samples per day. • Automated over night analysis. • Limit of Quantitation:0.1% • Automated conformation of peak identity and purity with diode array detection. • Automated compound quantitation and printing of report .
Functional specifications 1. Pump 2. Detector 3. Auto sampler. 4. Column compartment. 5. computer	• Binary or higher gradient • Uv /vis diode – array, 190 to 900 nm • 100 samples, 0.5µl to 5ml sample volme • 15 to 60 Deg C,Peltier controlled. • System control, data acquisition for signals and spectra, peak integration and quantitation , xpectral evalution for peak purity and compound conformation • Electronically save all chromatograms generated by the system.

INSTALLATION QUALIFICATION

BEFORE INSTALLATION

INSTALLATION ENVIRONMENT:

- Temperature: 10 to 35°C when CLASS-VP is used
5 to 35°C when C-R7A plus or the C-R8A is used
- Humidity : 20 to 85%.
- Allow sufficient shelf space for the equipment, sop, operating manuals and software

➤ Avoid exposure to :

- Direct currents from air conditioners
- Direct sunlight
- Vibration
- Instruments that can generate strong magnetic fields
- The installation site shall be clean and free from corrosive gas and dust.

➤ **DURING INSTALLATION**

- Compare equipment , as received with purchase order (including software , accessories , spare parts)
- Check documentation for completeness (operating manuals , maintenance instructions, sop for testing , safety and validation certificates)
- Check equipment for any damage
- Install hardware (computer ,equipment, fittings and tubings for fluid connections , columns in HPLC, power cables , data flow and instrument control cables)

- Switch on the instruments and ensure that all modules power up and perform an electronic self –test
- Identify and make a list with a description of all hardware , include drawings where appropriate
- Run test samples and compare chromatogram print out with reference chromatogram
- List equipment manuals and sop
- Prepare an installation report

OPERATIONAL QUALIFICATION

- **REPEATABILITY TEST:** analyze a standard solution 6 times using an isocratic system and calculate the repeatability of retention times and peak areas.
- ✓ **ACCEPTENCE CRITERIA:**
 - Peak area : $CV\% \leq 1.00$
 - $CV\% \leq 0.50$
- **CARRY OVER TEST:** measures the amount of residual sample in the autosampler based on the peak area ratio obtained by injecting a high concentration sample, followed by injecting a blank.
- ✓ **ACCEPTANCE CRITERIA:** carryover $\leq 0.010\%$

Performance Qualification

- **Flow rate accuracy**

1. Prime all the solvent lines with Milli Q water.
 2. Set the flow rate to 0.500 ml/m.
 3. Wait for about 15 m to stabilize the system and ensure that the pressure is stable.
 4. Insert the outlet tubing into a 10 ml volumetric flask and start the stop watch simultaneously.
 5. Stop the stopwatch when the lower meniscus reaches the 10 ml mark on the flask.
 6. Record the elapsed time.
 7. Similarly check the flow for 1.0 ml/m and 2.0 ml/m.
- ✓ **Acceptance criteria:** The time taken to collect the water should be within $\pm 2.0\%$ of the actual value.

• **Injector accuracy**

1. Connect the pump and detector inlet with union.
 2. Prepare mobile phase consisting of a mixture of water and Methanol (70:30 v/v)
 3. Set a flow rate of 0.5 ml/m and a run time of 1 m.
 4. Set the column temperature at $25 \pm 2^{\circ}\text{C}$.
 5. Fill a standard HPLC vial to 2/3rd with Milli-Q water. Seal the vial properly with a cap.
 6. Weigh the vial and record the weight as W1 grams.
 7. Place the vial in the chromatographic system and perform 6 injections of 50 μl volume from this vial.
 8. Weigh the vial again and note the weigh after the injections as W2 grams.
- Calculate the mean volume injected per injection as follows:
Mean injected volume (μl) = $(W1 - W2) \times 100/6$
 - ✓ **Acceptance criteria:** The mean injected volume should be 50.0 ± 1.0 μl .

■ Auto sampler precision

The precision of the injector was demonstrated by making six replicate injections from a sample (caffeine 0.05 mg/mL in methanol). The relative standard deviation (RSD) of the response of the injections was calculated to evaluate the precision

■ System precision

Standard Preparation:

Accurately weigh and transfer about 60mg of Caffeine into a 100ml volumetric flask. Dissolve and dilute to the volume with mobile phase. Transfer 10ml of this solution into a 100ml volumetric flask and dilute to the volume with mobile phase.

❖ Procedure:

- Inject blank, followed by standard preparation in 6 replicates. Note down the areas and retention times.
- Now calculate the %RSD of retention time and peak areas for 6 replicates injections.
- ✓ **Acceptance criteria:** The %RSD of retention time & peak area should be $<1.0\%$

■ Wavelength accuracy

Wavelength accuracy is defined as the deviation of the wavelength reading at an absorption or emission band from the known wavelength of the band

- ✓ **Acceptance criteria:** The maximum absorbance should be $\pm 2\text{nm}$.

■ Autosampler carry over test

Carryover was evaluated by injecting a blank (methanol) after a sample that contains a high concentration of analyte ((0.2 mg/mL of caffeine in methanol). The response of the analyte found in the blank sample expressed as a percentage of the response of the concentrated sample was used to determine the level of carryover.

$\text{Carryover}(\%) = (\text{blank peak area} / \text{sample peak area}) \times 100$

- ✓ **Acceptance criteria:** $\text{carryover} \leq 0.010\%$

▪ **Detector linearity**

Inject blank, followed by Detector linearity solutions and record the peak responses of Caffeine standard plot between the concentration (x-axis) Vs the peak responses

(y axis)

✓ **Acceptance criteria:** The plot should be linear and regression coefficient should not be less than 0.99.

■ Injector linearity

- Standard Preparation:

Accurately weigh and transfer about 60mg of Caffeine into a 100ml volumetric flask. Dissolve and dilute to the volume with mobile phase. Transfer 10ml of Standard Preparation into a 100ml volumetric flask and dilute to the volume with mobile phase.

- Procedure:

1. Inject 5 μ l of the mobile phase as blank injection.
 2. Inject 5 μ l, 10 μ l, 20 μ l, 50 μ l and 80 μ l of the Standard Preparation and record the peak areas.
 3. Plot a curve for the volume injected Vs peak area.
- ✓ **Acceptance criteria:** The plot should be linear and regression coefficient should not be less than 0.99.

■ Gradient performance check

- Add 5ml of acetone to 1000ml of methanol ,filter and degas.
- Connect the pump and detector inlet with union.
- Set the detector wave length at 254 nm.
- Place Channels A and C in methanol and channel B and D in 0.5% acetone in methanol.
- Set binary gradient with a total flow rate of 2.0ml/m.
- Set gradient program as shown below for channels A, B and C, D individually and Purge all the channels at a flow rate of 2ml/m for about 5 m.
- Set the flow rate at 2ml/m and wait until the base line is stable.

- Set the gradient profile for A and B and run the gradient profile by injecting methanol.
- Record the height of the peaks.
- Perform the gradient performance check similarly for channels C and D.
- ✓ **Acceptance criteria:** The calculated percentage composition (Height (%)) should be within $\pm 1.0\%$ of the set composition.

■ **Column oven temperature accuracy**

- It is evaluated with a calibrated digital thermometer at **30°C and 60°C** .
- Place the thermometer probe in the column oven and set the column oven temperature at 30°C. Wait till the temperature stabilizes.
- Record the temperature displayed on the thermometer.
- Similarly performs the column oven temperature accuracy test at 60°C.
- ✓ **Acceptance criteria:** The resulting oven temperature from the thermometer display should be within $\pm 2^{\circ}\text{C}$ of the set temperature.

CONCLUSION

- ✓ Qualification of equipment is pre-requisite for validation of the process in which the equipment is being used. Many types of equipment have measuring devices on them.
- ✓ Calibration of measuring devices is a part of qualification. Calibration of measuring devices is important, as the data is often collected through them.
- ✓ If the data collected is not from a measuring devices that have been calibrated, the data can not be relied upon. Thus the whole validation exercise can be questioned.

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THANK YOU