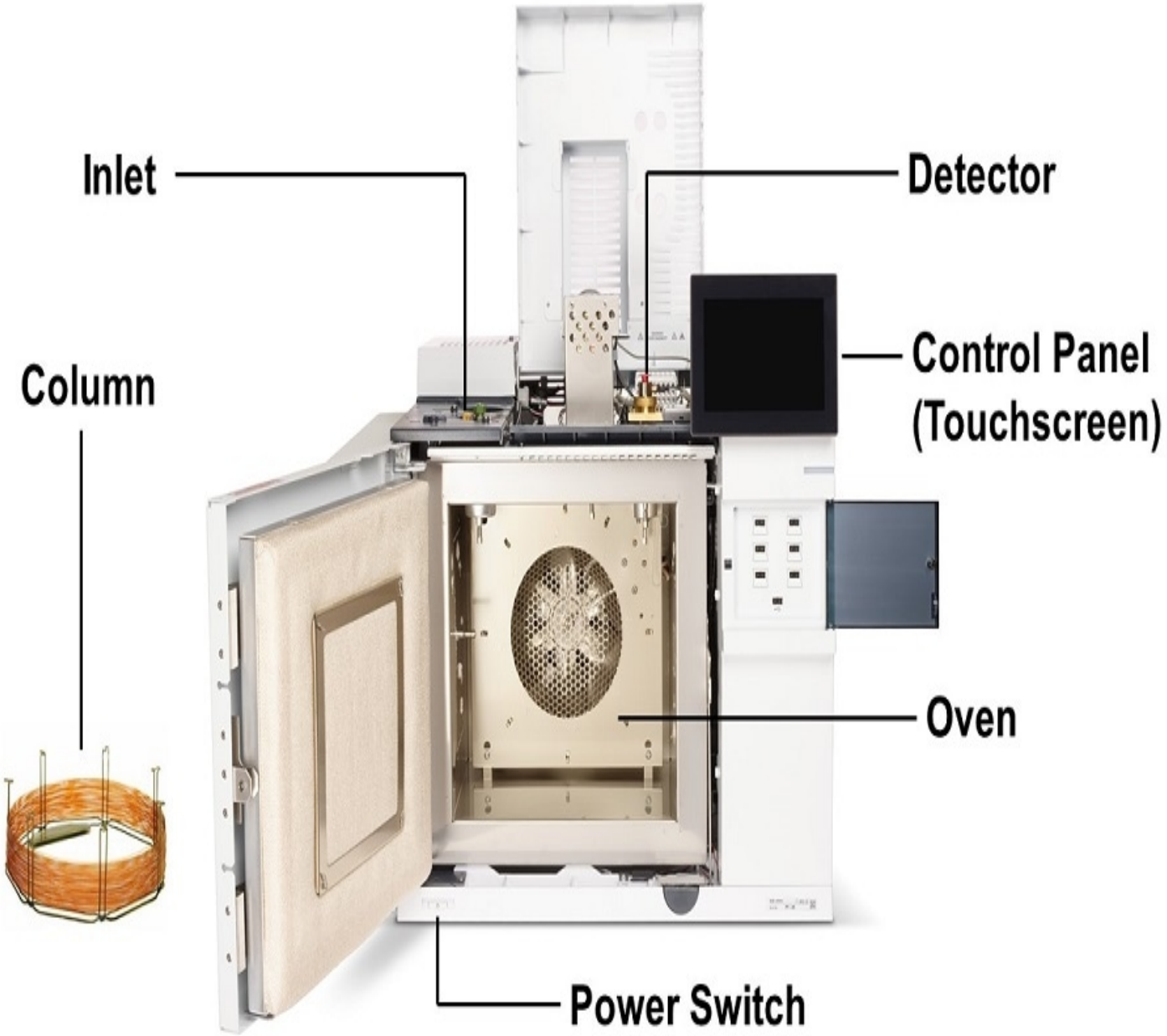
QUALIFICATION OF GAS CHROMATOGRAPHY



Contents :

- Introduction
- Instrumentation of GC
- Qualification of GC
- GC applications in pharmaceutical analysis

CHROMATOGRAPHY

•Is a technique used to separate and identify the components of a mixture.
•Chroma -"color" and graphein - "to write"

PRINCIPLE

•Physical method of separation that distributes components to separate between two phases moves in a definite direction.
• Substances are separated based on their differential distribution between two phases.
•Substances will move with the mobile phase at different rate depending upon their Partition or Distribution co- efficients.

CHROMATOGRAPHY TERMS

• Chromatograph - equipment that enables a sophisticated separation
• EX. Gas chromatography or Liquid chromatography

•Eluent - Fluid entering column/ solvent that carries the analyte.
• Eluate - Mobile phase leaving the column.

GAS CHROMATOGRAPHY

•Gas chromatography (GC) is a widely used technique for separation & analysis of gaseous & volatile substances which are difficult to separate & analyze.
•In performing gas chromatographic separation, the sample is vaporized & injected onto the head of a chromatographic column.
•Elution is brought about by the flow of an inert gaseous mobile phase.
•In GC gas as a moving phase is passed through a column containing solid adsorbent or liquid adsorbent. Thus adsorption or partition is possible.

Based on stationary phase used in column, G.C is of 2 types :

a. Gas solid chromatography (GSC)
b. Gas liquid chromatography (GLC).

a. GSC : Mobile phase – gas
Stationary phase – solid

b. GLC : Mobile phase – gas
Stationary phase – liquid

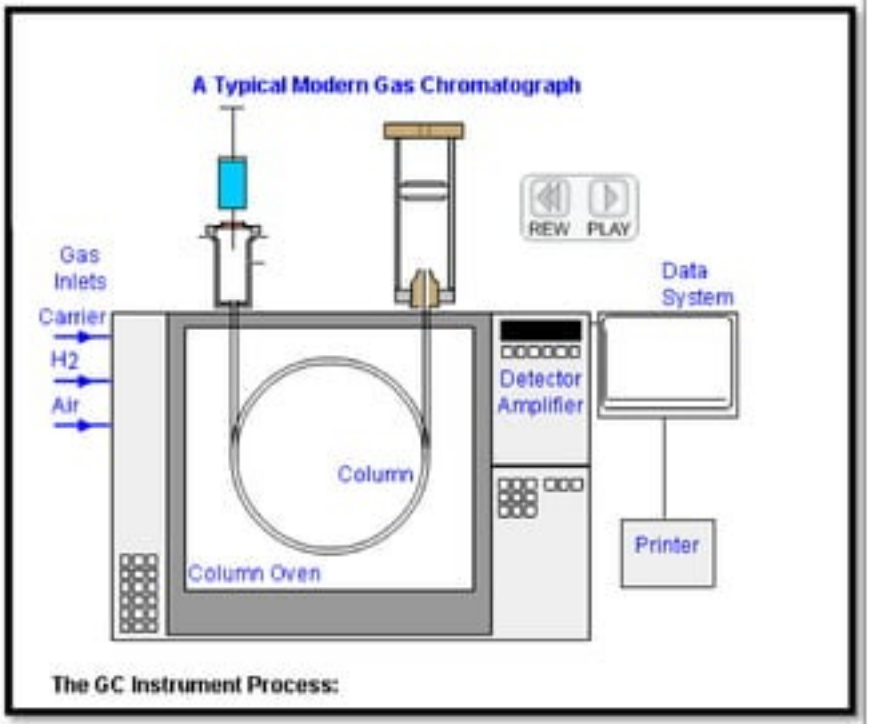
Criteria for compounds to be analyzed by GC :

- Volatility
- Thermo stability
- The analyte should have a measurable vapor pressure at the temperature employed.

Instrumentation :

It consists of:

1. Carrier gas tank
2. Flow regulators
3. Sample injection system
4. Column & Stationary phase
5. Detectors



CARRIER GAS

- The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, oxygen, air .
- The choice of carrier gas is often dependant upon the type of detector which is used.
- The carrier gas system also contains a molecular sieve to remove water and other impurities.

FLOW REGULATORS:

- Used to maintain the uniform pressure and flow rate.
- Rotameter
- Soap Bubble Flow Meter

SAMPLE INJECTION PORT

- For optimum column efficiency, the sample should not be too large.
- The most common injection method is where a micro syringe is used to inject sample through a rubber septum into a flash vaporizer port at the head of the column.
- The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample.

INJECTION DEVICES:

Gas: Valve Devices
Liquid: Loop Devices
Solid: Dissolve the substance in suitable solvent and then inject.

TEMPERATURE CONTROLLING DEVICES:

The devices are very important role playing.

- Pre heaters (for vapour formation)
- Thermostatically controlled oven .

COLUMNS : Two general types of columns are encountered in GC

A. Packed columns

Have inside diameter of about 2 to 4mms.

B. Capillary columns or open tubular columns.

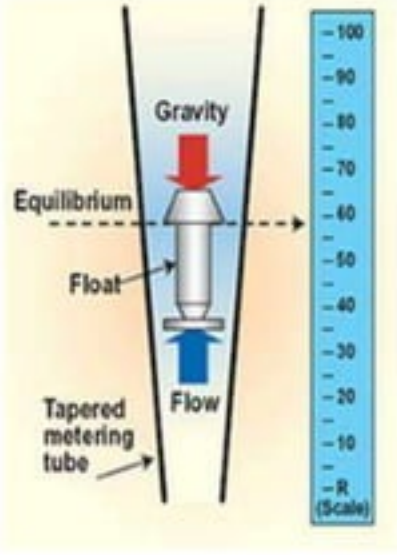
Inside diameter --- 1mm.

Capillary columns are of 2 basic types :

a) Wall-coated open tubular columns (WCOT)
b) Support-coated open tubular columns (SCOT).

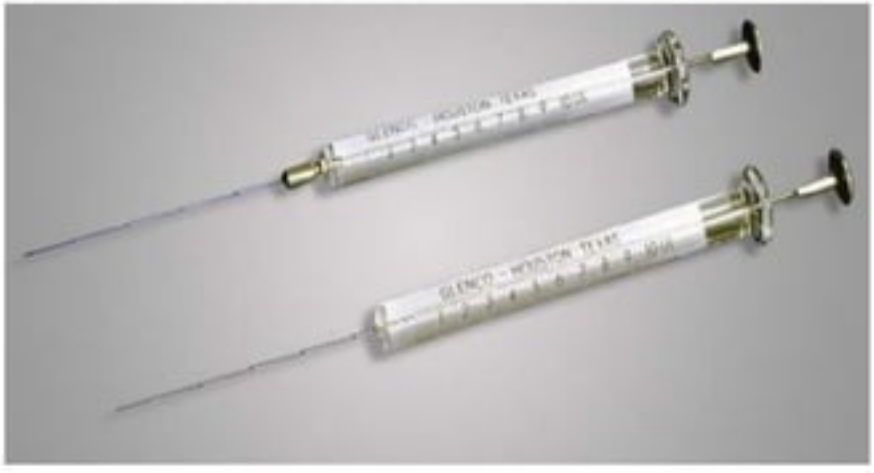
DETECTOR

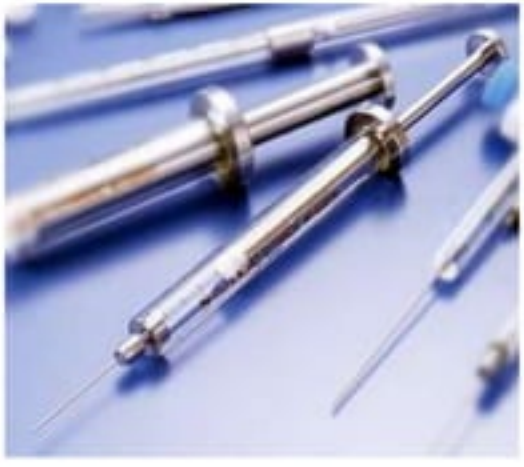
- Flame ionization (FID)
- Thermal conductivity detector (TCD)
- Electron capture (ECD)

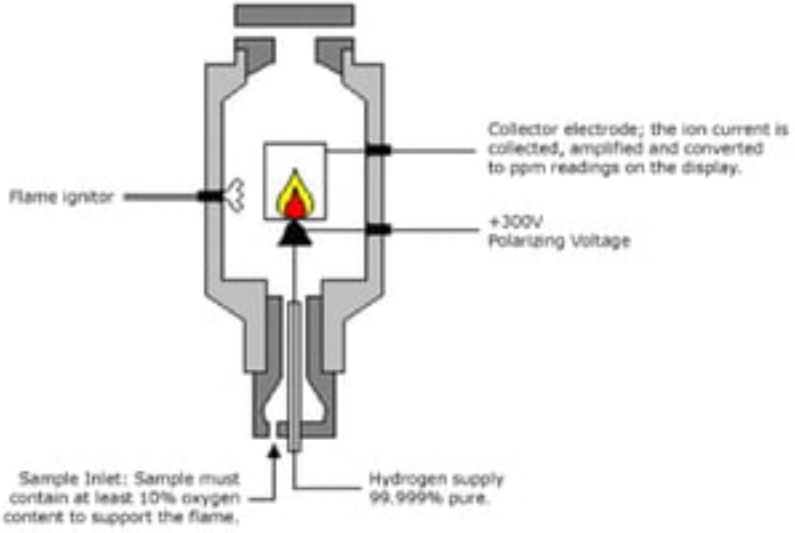


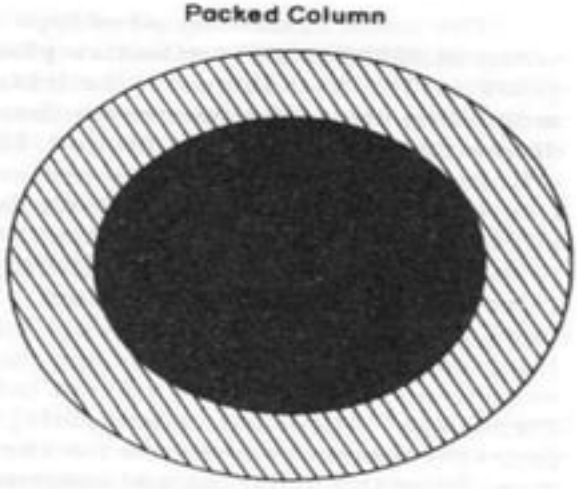
Rotameter

Soap bubble flow meter

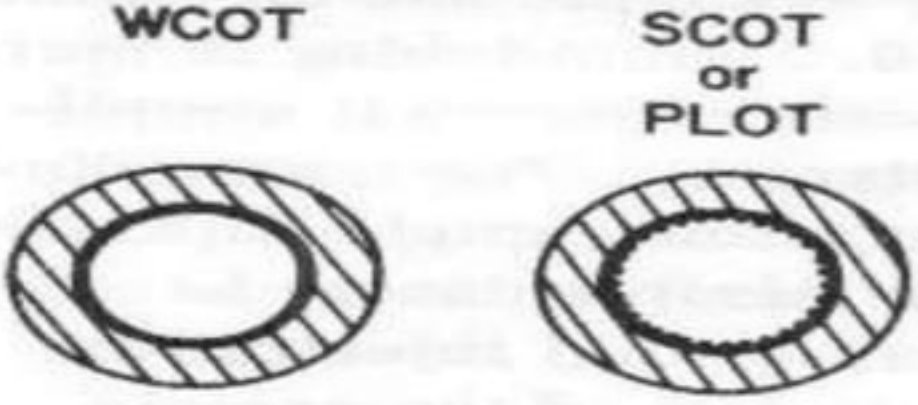








Flame ionisation detector



QUALIFICATION

DEFINATION

Action of proving and documenting that equipment or ancillary systems are properly installed, work correctly, and actually lead to the expected results.

- Qualification is part of validation,

  but the individual qualification steps alone do not constitute process validation.

Types Of Qualification:

1. Design qualification
2. Installational qualification
3. Operational qualification
4. Performance qualification

1. DESIGN QUALIFICATION (DQ)

- It describe the user requirements and defines the functional and operational specifications of the instrument.

2. INSTALLATION QUALIFICATION (I.Q):

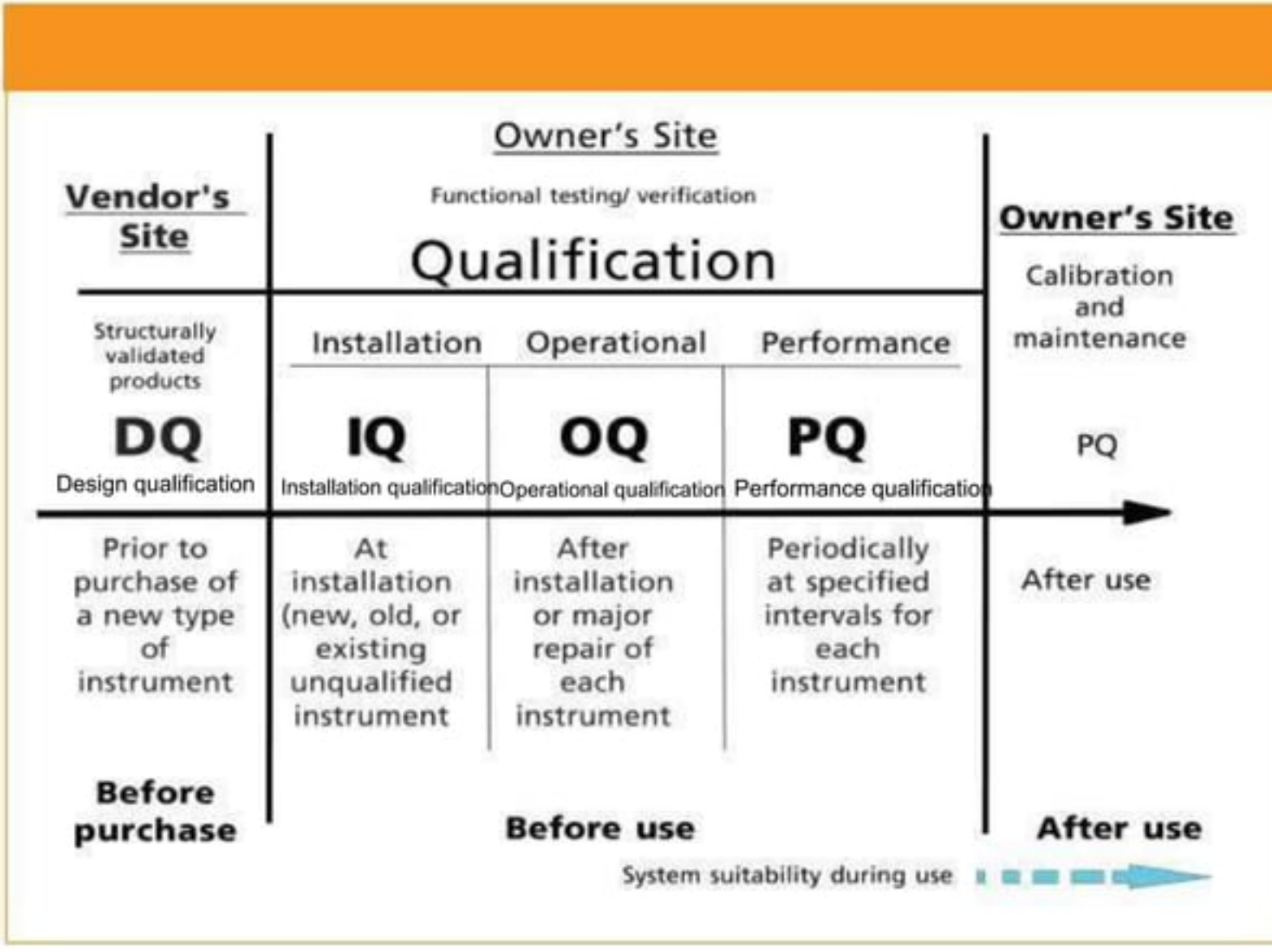
- The purpose of I.Q is to check the installation site/ environment, confirms equipment specifications and verifies the condition of installed equipment.

3. OPERATIONAL QUALIFICATION (O.Q):

- O.Q includes procedures and documentation of O.Q of analytical instrument.
- When all procedures are executed and all items pass the inspection, it is verified that the system operates to satisfy the intended purpose.

4. PERFORMANCE QUALIFICATION (PQ):

- The objective is to ensure that the instrument is performing within specified limits.
- Hence documented verification that the equipment and ancillary systems, as connected together, can perform effectively and reproducibly based on the approved process method and specifications.


Owner's Site
Vendor's Site
Functional testing/ verification
Qualification
Owner's Site
Calibration and maintenance
Structurally validated products
Installation
Operational
Performance
DQ
IQ
OQ
PQ
PQ
Design qualification
Installation qualification
Operational qualification
Performance qualification
Prior to purchase of a new type of instrument
At installation (new, old, or existing unqualified instrument
After installation or major repair of each instrument
Periodically at specified intervals for each instrument
After use
Before purchase
Before use
After use
System suitability during use

CALIBRATION

•It is set of operation which is established under specified conditions.

•It is necessary to ensure the accuracy of the data produced during the process

•Calibration is the process by which ensure that an instrument readings are accurate the reference to establish standard. Calibration is performed by using primary standard. It is done to check the zero error deflection by using standard reference.

NEED FOR CALIBRATION:

- With a new instruments
- When a specified time period is elapsed
- When a specified usage (operating hours) has elapsed
- When an instrument has had a shock or vibration which potentially may have put it out of calibration
- Sudden change in weather
- Whenever observation appears questionable

QUALIFICATION OF GC EQUIPMENT

Introduction

- The present document is the core document of "Qualification of Equipment", and it should be used in combination with it when planning, performing and documenting the GC equipment qualification process.

- The core document contains the general introduction and the Level I and II of qualification, common to all type of instruments, and the present annex contains GC instrument-related recommendations on parameters to be checked and the corresponding typical acceptance limits, as well as practical examples on the methodology that can be used to carry out these checks.

- The tests proposed in the Level III and IV of qualification are based on an overall approach, in which several parameters are checked at the same time in a combined test procedure, to obtain information on the overall system performance (e.g. peak area precision, retention time precision, temperature programme reproducibility, etc).

- Nevertheless, it should be noted that it is also acceptable to check these parameters individually by using other well- defined procedures

Level I. Selection Of Instruments & Suppliers

- At level I of the qualification of a gc equipment(selection of instruments and suppliers)
- It is recommended to select a manufacture of gc that can satisfy the needs of the laboratory and worksunder ISO9001 certification

Level II of Equipment Qualification: Installation and release for use

- It is recommended to check all requirements set during the selection of the instrument, and calibration should be performed before putting into service by an accredited external service supplier, or
- Internally by appropriately qualified personnel, using certified reference buffers according to an approved procedure.
- It establishes that instrument is properly installed in the selected environment and that environment should be suitable for operation of the instrument.
- Run of test samples verifies correct installation of all modules, electrical and fluid connections

COLUMN OVEN

Temperature Range	(Ambient + 10°C) ~ 400°C (using liquid CO_2 gas: -50°C - 400°C)
Dimensions	250 (W) x 360 (H) x 175 (D) mm
Oven Capacity	15.8L
Temperature Accuracy	Set value (K) ±1% (calibration at 0.01°C increments)
Temperature Deviation	2°C max. (on 200mm dia. circumfrence 30 mm from rear)
Temperature Variation Coefficient	0.01°C/°C
Temperature Program Steps	Up to 20 (cooling program possible)
Programmed rate setting range	-250°C ~ 250°C/min
Total time for all steps	9999.99 minutes max.
Linear Heating Range	30°C/min up to 150°C 20°C/min up to 250°C 10°C/min up to 380°C 7°C/min up to 400°C (at 25°C ambient temperature)
Cooling Rate	300°C ~ 50°C in 6 min max. (at 25°C ambient temperature)
Columns Accepted	Capillary Columns: 2 Packed columns for GC14B: 4 (Glass columns:2)

SAMPLE INJECTION UNIT	
Temperature Range	Up to 400°C
Heating Settings	1°C steps
No. of units installed simultaneously	Up to 3 units
Sample injection unit types	Dual packed, single packed, split/splitless

CARRIER GAS FLOW CONTROLLER	
For Packed/Dual	
Flow rate setting range	0 ~ 100mL/min
Programmable Steps	7
Programmed rate setting range	-400 ~ 400mL/min
Correction Function	Maintains column flow rate during column oven heating

For Capillary Split / Split less (Split /Split less injection mode)	
Pressure Setting Range	0 ~ 970kPa
Programmable Steps	7 (pressure-decreasing program possible)
Programmed rate setting range	-400 ~ 400kPa/min
Total flow rate setting range	0 ~ 200 mL/min
Correction Function	Maintains column average linear velocity during column oven heating (for capillary only)
(Pressure mode direct injection)	
Pressure Setting Range	0 ~ 970kPa/min
Programmable Steps	7
Programmed rate setting range	-400 ~ 400kPa/min
(Flow-rate mode direct injection)	
Flow rate setting range	0 ~ 1200mL/min
Programmable Steps	7
Programmed rate setting range	-400 ~ 400mL/min
Correction Function	Maintains column average linear velocity during column oven heating (for capillary only)

Level III. Periodic and motivated instrument

Checks Examples of requirements for GC instruments with FID

Instrument module	Parameter to be checked	Typical tolerance limit
1. Inlet system	1.1 Injector leak test	Pressure drop ≤ 15 kPa within 5 minutes Covered by overall test 1
	2.Pressure/flow accuracy and stability	
	3.Repeatability of injection (overall test 1) - In split mode -In split less mode	RSD ≤ 3.0% RSD ≤ 3.0%
	4.Injector temperature accuracy and stability	Covered by overall test 2
	5. Carry-over (overall test 3)	
2. Oven	2.1. Repeatability of oven temperature characteristics	≤ 0.2% Covered by overall test 2

Instrument module	Parameter to be checked	Typical tolerance limit
3. FID detector	1.Linearity (overall test 3) 2.Constant detector response 3. Noise 3.3. Drift	r 2 ≥ 0.999 Covered by overall test 1 or 2 See Annex I See Annex I

- Practical examples of tests and their associated tolerance limits for several parameters related to the performance of the different modules of a GCare presented below.

- These examples can be considered by the OMCLs as possible approaches to perform the Level III of the equipment qualification process: "Periodic and motivated instrument checks".

- Several tests are proposed to check various parameters at the same time (overall tests).

- In order to run the tests in a more economical way, other suitable solutions can be used, as for example, the "Grob Test" mixture, available from different suppliers (e.g. Alltech, Sigma, Thames Restek).

- This commercial solution should be appropriate to the column material used.
- It is recommended to run the overall tests by using always the same test column, exclusively dedicated to qualification purposes, to guarantee reproducible conditions.

1. Inlet system

The following tests are proposed for the periodic and motivated check of the GC Inlet System.

1.1. Injector leak test method

If it is not specified by the instrument manufacturer, the leak test is carried out according to the procedure laid down in the instrument manual or by the built in automatic leak check procedure of the instrument.

Otherwise use the test described below:

- Disconnect the column from the injector and close the injector outlet with a sealed cap.
- Close the septum purge and the bypass.
- Adjust the flow and pressure controller to the maximal
- possible value of the pressure gauge.
- Adjust the flow controller to zero.
- Read the pressure after 1 minute and record the value.
- Record the pressure after 5 minutes.

- **Limits:**
- Pressure drop ≤ 15 kPa within 5 minutes.

1.2. Inlet pressure / flow accuracy and stability

- Adirect measurement of these parameters was not deemed practical or necessary, but the optimal conditions of flow/pressure can be verified by the overall test 1.

Limits: Refer to overall test 1.

1.3. Repeatability of injection

- The verification of this parameter is covered by the overall test 1.
- This test is to be performed in both split and split less mode.

Limits: Refer to overall test 1.

1.4. Injector temperature accuracy and stablity

- Due to the fact that the temperature cannot be reliably measured without opening and modifying the system and due to the difficulties of introducing a probe inside this module, the verification of this parameter is considered to be covered by the overall test 2.

Limits: Refer to overall test 2.

1.5 Injector carry over

- After having injected the solutions for the linearity test of the FID detector, in increasing order, inject the blank and measure the peaks that correspond to the major peaks (= analytes) in the linearity solutions.
- The verification of this parameter is covered by the overall test 3.

Limits: Refer to overall test 3.

2. OVEN

Repeatability of the oven temperature characteristics

- Due to the fact that the temperature cannot be reliably measured without opening and modifying the system conditions and that even when introducing a probe inside the oven, its location would not reflect the real temperature conditions at all points, the verification of this parameter is covered by the overall tests 2A and 2B.

Limits: Refer to overall test 2.

3. FID detector

The following tests are proposed for the periodic and motivated check of the GC FID detector.

3.1 . FID detector linearity

- Increasing amounts of analyte are injected and a linear response should be obtained.
- The verification of this parameter is covered by the overall test 3.

Limits: Refer to overall test 3.

3.2. Constant FID detector response

- The proper and reproducible functioning of the FID can be demonstrated by checking the peak areas obtained from a pre- defined standard solution.
- The verification of this parameter is covered by the overall test 1 or 2.

Limits: Refer to overall test 1 or 2.

3.3. FID detector noise and drift

- if the instrument has a built-in automatic system for the verification of the noise and drift, follow the manufacturer's instructions and apply the defined acceptance criteria.
- Otherwise, use the test described below:

Settings:

- Column installed
- Suitable flow, depending on column length/diameter
- No injection
- Oven temperature: 40°C
- Detector on and heated at working temperature (270- 300°C)

Method:

- After stabilisation of the system, record the signal for 15 minutes.
- Noise: evaluate 10 periods of 1 minute and calculate the mean value.
- Drift: Evaluate the slope of the baseline over the 15 minutes.

Limits:

- The acceptance criteria for these parameters have to be chosen in accordance with the instrument vendor's instructions and the intended use of the instrument.
- If no instructions are given, the user has to pre-define these acceptance criteria by taking into account the previous experience and the intended use of the instrument.
- No fixed values can be pre-defined in this guideline due to the high variety of integration systems used and consequently the acceptance criteria may be expressed in different units (voltage, current, arbitrary units per time).

Level IV. In-use instrument checks Examples of requirements for GC instruments with FID

Parameter to be checked	Typical tolerance limit
1. System suitability check for the method	According to Ph. Eur. or MAH dossier or validated in-house method
2. Peak area precision	RSD≤ 3.0% unless otherwise prescribed*
3. Retention time repeatability	RSD≤2.0%
4. Sensitivity (where relevant, e.g. for related substances tests)	According to Ph. Eur. or MAH dossier or validated in-house method

*This is to be defined in conjunction with the target concentration of the analyte

Do you think that

the instrument is Qualified
?

OVERALL TEST 1

- The overall test 1 covers the following parameters:

  - *Pressure/flow accuracy and stability in the inlet system: Retention time repeatability*
  - *Repeatability of injection: peak area precision*

  a) In split mode

  b) In split less mode

  The test may be combined with overall test 3.

a) Split mode:

- Test solution: 1-octanol in n-hexane 1%(v/v).

Settings:

- Column: SPB-1 (30m x 0.32mm ID x 0.25µm film)
- Carrier gas: He
- Velocity: 25cm/sec
- Split: 1:100
- Injection: 1µl
- Injector temperature: 220°C
- Oven temperature: 100°C isotherm
- Detector temperature: 300°C

- Runtime: 8 min
- Retention time of 1-octanol: about 5 min

b) Split less mode:

- Stock solution: 1-octanol in n-hexane 1% (v/v)
- Test solution: Dilute 10 ml of the stock solution with n- hexane to 100 ml (corresponds to 1µl/ml of 1-octanol in n- hexane)

Settings:

- Column: SPB-1, 30m, 0.32mm ID, 0.25µm film
- Carrier: He
- Velocity: 30cm/sec
- Split less injection: purge valve closed during 2 min
- Injection: 0.2µl of the test solution
- Injector Temperature: 220°C
- Oven Temperature: Initial 60°C for 4 min, 15°C/min. up to 135°C, final time 1min
- Detector temperature: 300°C
- Runtime: 9.5 min
- Retention time of 1-octanol: about 8 min

Method:

- Carry out 6 consecutive injections of the test solution and calculate the RSD of the different peak areas and retention times.

Limits:

- Retention time repeatability: the RSD of the retention times should be ≤ 2.0%
- Peak area precision (split and split less mode): the RSD of the peak areas should be ≤ 3.0%

OVERALL TEST 2

The overall test 2 covers the following parameters:

Injector, oven and detector temperature accuracy and stability: retention time repeatability

Test solution:

- 0.035 ml 1-octanol
- 0.035 ml 2-octanone
- 0.035 ml 2,6-dimethylanilin
- 0.035 ml n-tridecane
- 0.035 ml n-tetradecane
- 35 mg n-eicosane
- Dissolved in 50 ml Dichloromethane

Settings:

- Column: SPB-1 (30m x 0.32mm ID x 0.25µm film)
- Carrier gas: Helium
- Velocity: 25 cm/s
- Split: 1:100

- Injection volume: 1 µl
- Injector temperature: 220°C
- Detector: FID
- Detector temperature: 300°C
- Gradient program : 60°C (4 min), 5°C/min, 270°C (3 min)

- **Method:**

- ✓ Inject the solution twice and calculate the relative retention times in relation to n-eicosane (RRT=1)
- ✓ The following table shows the approximately expected relative retention times.

Analyte	1-octanol	2-octanone	2,6-dimethylaniline	n-tridecane	n-tetradecane
RRT	0.30	0.22	0.37	0.52	0.60

Limits:

The RSD of each RRT from two consecutive injections should be ≤ 1.0%

OVERALL TEST 3

- This test is a *modified version* of the *overall test 1* to be used for the verification of:
  - *Detector linearity: linearity of the areas recorded*
  - *Injector carry-over*: area recorded in the blank run
- It is described for both split and split less mode and may be combined with overall test 1.

Split mode:

Prepare further reference solutions by diluting the test solution as described below.

Settings:

see overall test 1

- Injection sequence:
- 5.0 ml of the test solution diluted to 25.0 ml with n-hexane (2 µl/ml): 2 injections
- Test solution: 1-octanol in n-hexane 1% (v/v)
- 10.0 ml of the test solution diluted to 25.0 ml with n-hexane (4 µl/ml): 2 injections
- 15.0 ml of the test solution diluted to 25.0 ml with n-hexane (6 µl/ml): 2

Split less mode:

•Stock solution: 1-octanol in n-hexane 1% (v/v)

•Test solution: Dilute 10 ml of the stock solution with n- hexane to 100 ml (corresponds to 1µl/ml of 1-octanol in n- hexane).

- Prepare further reference solutions by diluting the test solution with n-hexane.
- Settings: see overall test 1

Injection sequence:

- 5.0 ml of the test solution diluted to 25.0 ml with n-hexane (0.2 µl/ml): 2injections
- 10.0 ml of the test solution diluted to 25.0 ml with n-hexane (0.4 µl/ml): 2 injections
- 15.0 ml of the test solution diluted to 25.0 ml with n-hexane (0.6 µl/ml): 2 injections
- 20.0 ml of the test solution diluted to 25.0 ml with n-hexane (0.8 µl/ml): 2 injections
- If combined with overall test 1 for repeatability: test solution (1µl/ml): 6 injections

Limits:

- Linearity: coefficient of correlation of the calibration line obtained with the reference solutions and the test solution: $r^2 \geq 0.999$.
- Carry-over: the percentage of the peak area corresponding to the analyte in the blank solution should be ≤ 0.2% of the peak area of this analyte in the chromatogram obtained with the solution with the highest concentration within the sequence.

GC applications in pharmaceutical

APPENDIX Applications of Gas Chromatography in Pharmaceutical Analysis

Class and name of compound	Column	Column temperature (°C)	Derivatization	Detector	Purpose	Reference
I. Antibiotics						
A. Sulfonamides						
Sulfadiazine	1.82 m × 4 mm I.D. 5% OV-17	255	Diazomethane	ECD	Biological	221
B. Penicillins						
Penicillin G, penicillin V, phenethicillin, methicillin, oxacillin, cloxacillin, and dicloxacillin	61 cm × 3 mm I.D. 3% OV-17	215	HMDS + pyridine	FID	Formulation	222
C. Aminoglycosides						
Kanamycin	183 cm × 3 mm I.D. 3% OV-1	300	TMSIM + pyridine	FID	Formulation	223
Neomycin	61 cm × 3 mm I.D. 3% OV-1	290	TMSDEA + TMSIM + pyridine	FID	Formulation	224
Gentamicin	61 cm × 3 mm I.D. 3% OV-1	240	TMSDEA + TMSIM + pyridine	FID	Formulation	225
D. Others						
Chloramphenicol	1.22 m × 2 mm I.D. 3% OV-1	190 → 270	HMDS + TMCS + pyridine	FID	Biological	226
Tetracycline	185 cm × 3 mm I.D. 3% JX-R	260	BSA + TMCS + pyridine	FID	Formulation	227

GC applications in pharmaceutical analysis

a. It is a simple & inexpensive method, generally efficient with regard to separation.
b. The technique has a very high resolution power.
c. Small sample is needed – μLs.
d. Sensitivity of detection is very high (PPB or Picograms).
e. The speed of analysis is fast.
e. Qualitative & quantitative analysis at a time is possible. The area produced under each peak is proportional to that concentration.
f. Gas chromatograms are widely used to establish the purity of organic compounds.
Contaminants, if present, are revealed by the appearance of additional peaks.
g. The areas under these peaks provide rough estimate of the extent of contamination.

E.g. : Gas chromatography is used to determine the identity & composition of propellants that are widely used in aerosols.

h. The purity & acceptability of the propellants is tested with respect to moisture, halogen & non volatile residue determination by using GC.

Wait

Its not over still

Precautions:

•Do not heat the G.C. column without passing carrier gas through the column.

• Check the leakage of Hydrogen gas before putting the flame on.

•Ensure that no air bubble is entrapped in syringe while injecting the sample.

• If found any difficulty in operating the instrument or G.C. Solution software, and then refer the instrument manual.

• Inform to the Department Head, If any abnormality is observed while working with Gas chromatograph.

REFERENCES

- Ph.Eur.2.2.35 chromatography; *Gas chromatography*
- Guidance on equipment qualification of instrumental analysis
- Journal of Perkin Elmer life &analytical science
- **Validation and Calibration of Analytical Instruments,** *D.* Gowrisankar et al / J Biomed Sci and Res., Vol 2 (2), 2010,89-99 **(** *page no 94 to 96***)** .

THANK YOU

Software installation

- Connect the serial or USB cable to your computer and the GC. The serial port connection is on the left-hand side of the GC, and the USB connection is on the right-hand side.
- Locate your copy of the Peak Simple software just inside the front cover of your SRI manual. Insert the CD or floppy disks into your computer's appropriate drive.
- Double click on "My Computer," then on the appropriate drive to open it. Double click on the "setup.exe" icon.
- For USB, refer to "**Installing the USB Drivers for Model 302 USB Peak Simple Data System**"
- Double-click on the Peak Simple icon to launch the program.
- Open the Edit menu and choose Overall. In the dialog box that pops up, enter the number of the COM port to which you have connected the GC.