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REVIEW ARTICLE: SYSTEM SUITABILITY PARAMETERS AND SPECIFICATIONS

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ABSTRACT
System suitability test (SST) is a test to determine the suitability and effectiveness of chromatographic system prior to use. The performance of any chromatographic system may continuously change during their regular use, which can affect the reliability of the analytical results. For this reason different parameters which are related to the operation of the whole chromatographic system can be monitored and investigated to find out the integrity and reliability of the whole HPLC system. The parameters that can be used to determine system suitability prior to analysis includes the number of effective theoretical plates, resolution, asymmetry/tailing factor, selectivity ,capacity factor ,relative retention and relative standard deviation(RSD). After checking the parameters, the system is then only can be declared suitable if the responses are within permitted limits. This study was performed to set system suitability criteria for development and validation of the HPLC analysis. In very simpler way, we also discuss about the measurement and calculations of different SST parameters.

KEYWORDS: HPLC, System suitability test, ICH, USP.

INTRODUCTION

Prior to the analysis of samples each day, the operator must establish that the HPLC system and procedure are capable of providing data of acceptable quality. This is accomplished with system suitability experiments, which can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation.

System suitability parameter is an integral part of many analytical procedures. The tests are based on the concept that the equipment, analytical operations and samples are the integral part of the system that can be evaluated as such. SST provide the added assurance that on a specific occasion the method is giving accurate and precise results. SST are run every time a method is used either before or during analysis. The results of each system suitability tests are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion. In case of HPLC methods, SST ensure the adequacy for performing the intended application on daily basis. The chromatographic system used for most pharmaceutical analysis such as assays of the active ingredients, impurity determinations and dissolution testing must pass a set of predefined acceptance criteria(SST limits) before sample analysis can commence.

The primary SST parameters considered are resolution(Rs), repeatability (%RSD of peak response and retention time), relative retention, column efficiency(N), tailing factor(T_F) and the signal to noise ratio. The other SST parameters include retention factor (K) and separation factor.

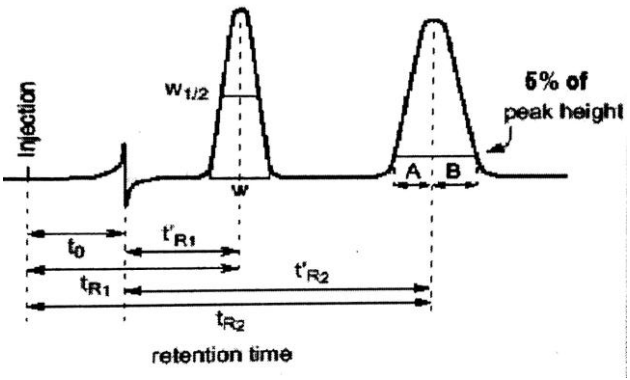


FIG: Fundamental parameters of HPLC.

Where,
$w_{1/2}$: peak width at half height
w: band width of the peak.
A: peak front at 5% of peak height to peak maximum
B: peak maximum to peak end at 5% of retention time.
t0: dead time of a column.
t_{R1}, t_{R2}: retention time of components 1and 2
t'_{R1}, t'_{R2}: net retention time of component 1 and 2.

Systeam Suitability

United State Pharmacopoeia

The USP states, SST are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. The USP defines parameters that can be used to determine system suitability prior to analysis. These parameters include plate number (N), tailing factor, k and/or α, resolution (Rs) and relative standard deviation(RSD) of peak height or peak area for repetitive injections.

ICH Guidelines

Q2B: Validation of Analytical Procedures: Methodology", has a section devoted to system suitability testing. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.[5]

Indian Pharmacopoeia

System suitability is an integral part of liquid chromatographic method for assuring adequate performance of the system. Because of the normal variations in equipment, supplies and techniques, a system suitability test is required to ensure that a given operating system may be generally applicable. System suitability also verifies that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done.

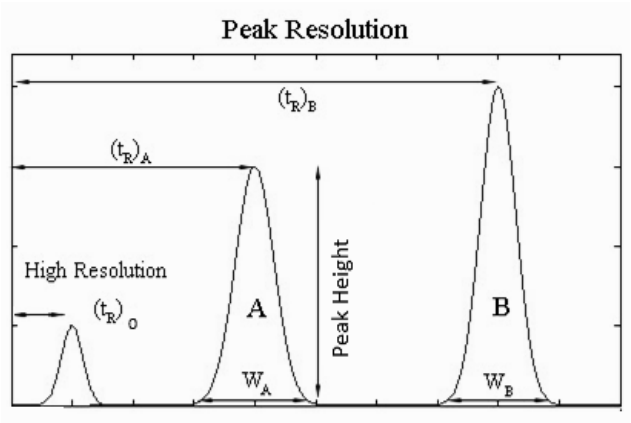
System Suitability Parameters

1. Resolution
2. Number of theoretical plates
3. Capacity factor
4. Symmetry factor/Tailing factor
5. Selectivity / separation factor
6. Column efficiency
7. Relative retention
8. Relative standard deviation
9. Signal to noise ratio.

Resolution

It is a measure of quality of separation of adjacent bands in a chromatogram; Rs is a measure of how well two peaks are separated. The Resolution Rs is a function of column efficiency, N and is specified to ensure that closely eluting compounds are resolved from each other, to establish the general resolving power of the system, and to ensure that internal standards are resolved from the drug.[3] Chromatographers measure the quality of separation by the resolution Rs of adjacent bands. Rs are equal to the distance between the peak centers divided by the average bandwidth. To increase resolution, either the two bands must be moved further apart, or bandwidth must be reduced. Two bands that overlap badly have a small value of Rs.[2] For realible quantitation, well separated peaks are essential for quantitation.

Resolution can be measured in 3 different ways:
1) Calculation based on equation.
2) Comparison with standard resolution curves.
3) Calculation based on the valley between the two bands.



$$Rs=2(t_2 - t_1)/w_1+w_2$$

An alternative approach gives more reliable values of Rs: Bandwidths at half height are measured for bands 1and 2, $W_{0.5,1}$ & $W_{0.5,2}$.

$$Rs = 1.18(t_2 - t_1)/W_{0.5,1} + W_{0.5,2}$$

The separation of any two bands in the chromatogram can be varied systemically by changing experimental conditions. Resolution Rs can be expressed in terms of three parameters (k, α and N) which are directly related to experimental conditions.

$$R_S = 1/4(\alpha-1)\, N^{1/2}\, K/1+K$$

Selectivity Efficiency Retention

Here,
k = average retention factor for the two bands
N =column plate number
α=separation factor; $\alpha=k_2/k_1$, where k_1 and k_2 are values of k for adjacent bands 1 and 2.

When conditions are changed so that k becomes smaller (earlier elution), resolution usually becomes worse. when k is made larger, resolution usully improves. If α is increased the two bands move apart, thereby increasing Rs significantly. When column efficiency N is increased, the bands become narrower and better separated , but their relative positions in the chromatogram do not change.

Factors affecting Resolution

Effect of solvent strength

Resolution increases when sample retention k increases; sample retention can be controlled by varying the solvent strength of the mobile phase. A strong solvent decreases retention and weak solvent increases retention. In most

cases, an intermediate solvent strength will be preferred so that 0.5<k<20 for all bands.

Effect of Selectivity
Changes in α can be created by a change in the mobile phase, a change in the type of column packing, or a change in temperature. once the selectivity increased , resolution ultimately increased.

Effect of column plate number
If resolution needs to be improved after adjusting k and α values, an increase in N is one option. Conversely, if the separation has more resolution than required (Rs>>2), this excess resolution can be decrease by reducing column length and increasing flow rate. N defines the ability of the column to produce sharp, narrow peaks for achieving good resolution of band pairs with small α values.

Sample size effects
As sample size is increased, peaks eventually broaden and the plate number N decreases, retention times decrease and sample resolution worsens. This situation refers as 'column overload'.
Ideally Resolution should be greater than **2. (Rs > 2)**

1) Number of Theoretical plates
A theoretical plate is an imaginary or hypothetical unit of a column where distribution of solute between stationary phase and mobile phase has attained equilibrium.It can also be called as functional unit of the column.[8]

The column plate number (N) is an important characteristic of column efficiency. N defines the ability of the column to produce sharp narrow peaks for achieving good resolution of band pairs with small α values. Number of theoretical plates characterizes the quality of a column packing and mass transfer phenomenon. In HPLC chromatography, there is equilibrium between stationary phase and mobile phase. During the time of elution, there is transfer of molecules from the mobile phase to the stationary phase and back to the mobile phase and so on back and forth down the length of the column, that's how the separation occurs. The distance along the column that it takes to make one of these transfers between phases and re-equilibrate is called the theoretical plate height (H). If it takes less distance, that means the plates are narrow and you have more of them in the column, which means more transfer between phases can takes place, and the column is more efficient. If it takes comparatively greater distance for one phase transfer event, the plates are wider and there are fewer of them, so your efficiency and resolution is not as good. N characterizes the broadening of chromatographic peaks. The larger N, the narrower the peak.

The column plate no. N is calculated through examination of the chromatogram by using retention time and peak width,

$$N=16(t_R/w)^2 \text{ or } N = 5.54 (t_R/w_{1/2})^2$$

Where, t_R: band retention time, **W**: peak width, $w_{1/2}$: bandwidth at half height.

The following equation can be used to estimate column plate number for small molecules under the optimum conditions-low viscosity mobile phase and a flow rate of 0.5 to 2.0mL/min.

$$N \approx 3500L \text{ (cm)}/ d_p \text{ (µm)}$$

Where L=Column length, d_p =particle diameter

Column plate number increases with several factors:
1. Well packed columns
2. Longer columns
3. Lower flow rates (but not too low)
4. Smaller column packing particles
5. Lower mobile phase viscosity and higher temperature.
6. Smaller sample molecules
7. Minimum extracolumn effects
8. Gradient elution is one way to increase the N.

Plate number is also dependent on the flow rate of the mobile phase .There is a certain velocity, the so called optimum flow, at which the plate number is highest (and H is lowest).A lower or a higher flow rate provides less plates (higher H).In routine HPLC columns are always operated at velocities above the optimum.[2]

Stationary phase particle size is one of the most important factor. For a given column length, the plate number (N) is inversely related to the particle size of the column packing. The smaller the particles, the higher the plate number and the separation power.

Number of theoretical plates should be more than **2000. (N>2000)**

Table: Plate number for well packed HPLC columns under optimized test condition

Particle diameter (µm)	Column length (cm)	Plate number (N)
10	15	6000-7000
10	25	8000-10000
5	10	7000-9000
5	15	10000-12000
5	25	17000-20000
3	5	6000-7000
3	7.5	9000-11000
3	10	12000-14000
3	15	17000-20000

Height Equivalent to Theoretical Plates (N)
The height of one theoretical plate is referred to as the 'Height Equivalent of a Theoretical Plate'.HETP is the length, in which the chromatographic equilibrium between mobile phase and stationary phase is established. Since a large no. of theoretical plates is desired, 'H' should be as small as possible. The value of 'H' is a criterion for the quality of a column; values

depend on the particle size, the flow velocity, the mobile phase viscosity and especially on the quality of packing.

If the length of the column is L, then the HETP is
HETP = L/N
Where, H= Height equivalent to theoretical plate (HETP)
L =Length of the column
N = Number of theoretical plates

HETP is given by **Van Deemeter equation** which describes the various contributions to plate height (H) and the relation of the height of a theoretical plate H and the average linear velocity of the mobile phase.[8] In this equation the parameters that influence the overall peak width are expressed in three terms:
H = A+ B/u+ Cu

Where,
H=HETP (plate height)
A=Eddy diffusion term or multiple path diffusion which arises due to the packing of the column. This can be minimized by uniformity of packing.
B=Longitudinal diffusion term or molecular diffusion.
C=Effect of mass transfer
u=flow rate or velocity of the mobile phase.
If HETP is less, the column is more efficient (N>2000).
If HETP is more, the column is less efficient (N<2000).

4) Capacity Factor
Capacity factor is symbolized by **k′** (USP terminology) or **k** (IUPAC /ASTM terminology).Capacity factor also called as mass distribution ratio **k′**.It is the measure of the position of a sample peak in the chromatogram, being specific for a given compound, a parameter that specifies the extent of the retention of substances to be separated. A high **k** value indicates that the sample is highly retained and has spent a significant amount of time interacting with the stationary phase. Capacity (retention) factor is equal to the ratio of retention time of the analyte on the column to the retention time of a non-retained compound. The non-retained compound has no affinity for the stationary phase and elutes with the solvent front at a time $\mathbf{t_0}$, which is also known as the 'dead time'. Capacity factor is independent of some key variable factors including small flow rate variations and column geometry.[3] Therefore, it is a useful parameter when comparing retention of chromatographic peaks obtained using different HPLC systems.

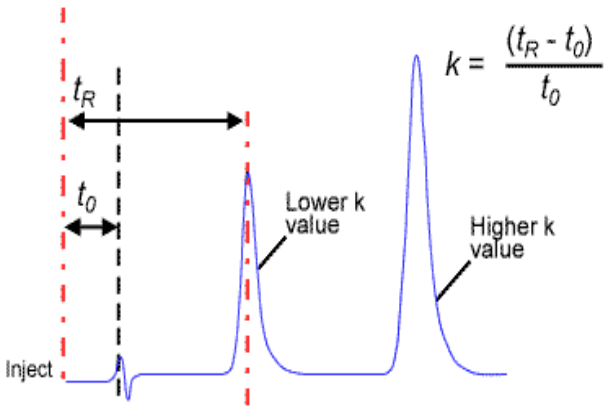


Fig: Determination of capacity factor.

$K = (t_R - t_0)/t_0$
Chromatographers like to keep 'k' values between 1 and 10 for good separations.
Capacity factor should be greater than **2.**

5) Symmetry factor/Tailing factor
A useful and practical measurement of peak shape is the peak asymmetry factor, $\mathbf{A_S}$ .Peak asymmetry is measured at 10% of full peak height. Good columns produce peaks with $\mathbf{A_S}$ values of 0.95 to 1.1.[2]It is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced.
Peak asymmetry factor, $\mathbf{A_S}$ calculated as
$A_S = B/A$

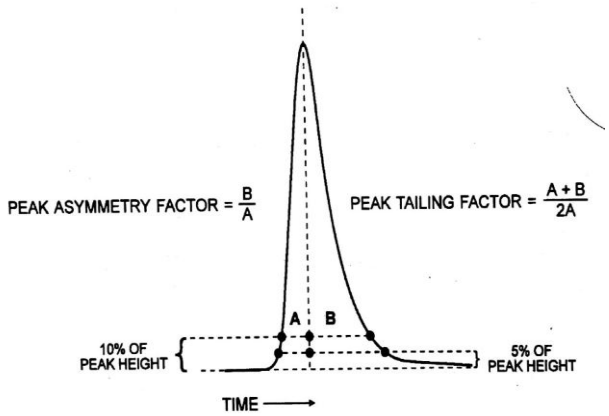


Fig: Determining peak asymmetry and peak tailing factor.

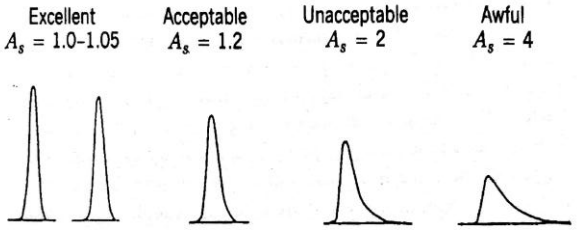


Fig: Peak shape for different asymmetry factor value.

Another useful way to define peak shape is by the peak tailing factor. The tailing factor is defined by the USP as the distance from the front edge of the peak to the back

edge, divided by the distance from the front edge to the centerline, will all distances measured at 5% of the maximum peak height. Peak asymmetry and the peak tailing are easily interconverted. peak tailing can be expressed as:

$$T = A+B/2A$$

Causes of asymmetrical (tailing) peaks: Bad column, plugged frit or void, sample overloaded, extra-column effects, inadequate buffering system, wrong solvent for sample.

Tailing factor should be less than **2.0 (T_f <2.0)** and asymmetry factor should be less than **1.5**. **(As <1.5)**

6) Selectivity/Separation factor (α)

Selectivity is the ratio of the capacity factors of the both peaks or the ratio of its adjusted retention times. Selectivity represents the separation power of particular adsorbent to the mixture of these particular components.This parameter is independent of the column efficiency, it only depends on the nature of the components, eluent type, eluent composition and adsorbent surface chemistry. High **α** value indicates good separation power and a good separation between the Apexes of each peak. By definition selectivity is always greater than one-as when **α** is equal to one, the two peaks are co-eluting. The greater the selectivity value, the further apart the apices of the two peaks become.

$$\alpha = k_2/k_1 = t_{R2}-t_0 / t_{R1}-t_0$$

As the selectivity of separation is dependent upon the chemistry of the analyte, mobile, and stationary phases, all of these factors may be altered in order to change or optimize the selectivity of an HPLC separation.[3]

Some of the many factors that can be used to manipulate the selectivity of HPLC separations are shown below

Three main variables can be used in Reverse phase chromatography to change selectivity for neutral samples are, mobile phase composition, column type and temperature.

Solvent strength Selectivity-In most cases an intermediate solvent strength will be preferred so that 0.5<k<20 for all bands.[2] The primary effect of decrease in %B (organic solvent) is to increase k for every sample component. There is generally some range of %B values that provide acceptable values of k for all compounds of given samples. The selection of an optimum solvent strength (%B) can be achieved by systematic trial and error experiments.

Solvent type selectivity-A change in organic solvent type is a powerful way to change band spacing for both reversed and normal phase HPLC. The solvent-selectivity triangle is a useful guide for choosing among different solvents for the purpose of a large change in band spacing.

A change of the strong solvent (B solvent) often results in large changes in band spacing ,such that bands that were formerly overlapped are now resolved and bands that were formerly resolved are overlapped. As a result, a mixture of the two strong solvents often provides intermediate band spacing and acceptable resolution.

Column type selectivity-A change in column type can produce useful changes in selectivity. A change in selectivity by changing column type may also be advantageous if only one organic solvent can be used. A change in column type for the purpose of improving selectivity and separation should be tried after the use of solvent-strength or solvent-type selectivity has failed. Systematic and reproducible changes in selectivity are best achieved by varying column functionality (e.g. for RP-HPLC, by changing from a C18 column to a cyno or phenyl column).

Temperature selectivity

An increase in column temperature by 1^0C will usually decrease retention (k) by 1 to 2 %.A change in k can also result in changes in α, so temperature is a potentially useful parameter for changing band spacing and improving resolution.

- **Techniques for improving Selectivity**
  - Vary solvent strength
  - Change solvent type (ACN, MeOH, THF)
  - Mix different solvents.
  - Change column type.
  - Vary temperature.

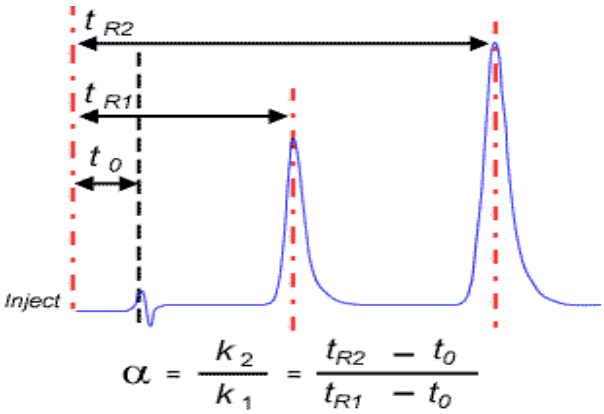


Fig: Determination of Selectivity (α)

Selectivity (separation factor) should be greater than 1.0. (α >1.0)

7) Column Efficiency

Column efficiency can also be used as system suitability requirement. It is a measure of peak sharpness, which is important for the detection of trace components. It is defined in terms of the number of theoretical plates, by the expression

$$N = 16(t_R/w)^2$$

The efficiency of a chromatographic peak is a measure of the dispersion of the analyte band as it travels through the HPLC system and column. Sharpness of the peak is an indication of an efficient column, but due to dispersion effect the peaks take on their familiar 'Guassian shape'. We can express column efficiency in terms of Plate number, Peak asymmetry, peak tailing and retention time. If the retention time is high and peak width is narrow then it shows excellent chromatogram. Peak width is an indication of peak sharpness and is dependent on a number of parameters such as column length, flow rate and particle size.

Evaluation of column efficiency based on theoretical plate number:

- If the retention times are the same, the peak width is smaller for the one with the larger theoretical plate number.
- If the peak width is the same, the retention time is longer for the one with the larger theoretical plate number.

Factors affecting column efficiency

1. Column length
2. Particle size
3. Packing quality
4. Linear velocity (flow)
5. Instrument quality (dead volume)
6. Retention factor

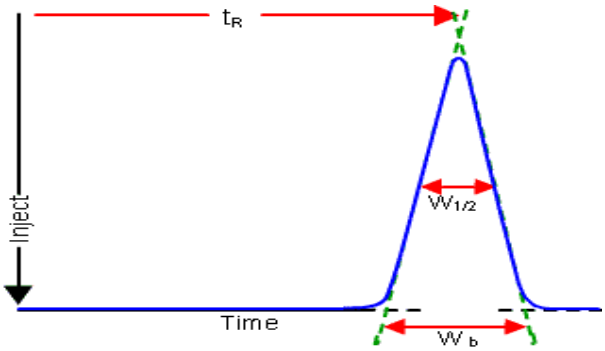


Fig: Determination of efficiency (N).

8) Relative Retention

Retention time is a measure of the time taken for a solute to pass through a chromatography column. It is calculated as the time from injection to detection. Relative retention is the ratio between the net retention time of a substance and that of a standard compound.

Relative retention, $\mathbf{r_{a/b}}$ is calculated as an estimate from the expression

$$\mathbf{r_{a/b} = t_{r,b}/t_{r,a}}$$

Where,

$\mathbf{t_{r,b}}$ = retention time of the peak of interest,

$\mathbf{t_{r,a}}$ = retention time of the reference peak.

Techniques to modify Retention in RP-HPLC

Decrease retention	Increase retention
More polar column (cyano, C_4)	Less polar column (C_8,C_{18})
Less polar mobile phase	More polar mobile phase
Higher temperature	Lower temperature

9) Relative standard deviation

Replicate injections of a standard preparation used in the assay or other standard solution are done to ascertain whether requirements for precision are met. Unless otherwise specified in the individual monograph data from five replicate injections of the standard are used to calculate the relative standard deviation, if the requirement is 2.0% or less. Data from six replicate injections are used if the relative standard deviation requirement is more than 2.0%.[6]

RSD should be less than 1.0% for five replicates.

10) Signal to noise ratio

Determination of signal to noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected/quantified.[6]

The signal to noise ratio is determined from the expression:

S/N = 2H/h

Where,

H = height of the peak corresponding to the component concerned ,in the chromatogram obtained with the prescribed reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to 20 times the width at half height.

h = Range of the background noise in a chromatogram obtained after injection of a blank , observed over a distance equal to 20 times the width at half- height of the peak in the chromatogram obtained with the prescribed reference solution and ,if possible , situated equally around the place where this peak would be found.

Table: Limits for system suitability tests.

SST	Limits
Resolution (Rs)	**>2.0**
Repeatability (RSD)	**<1.0% for five replicate**
Plate count (N)	**>2000**
Tailing factor (Tf)	**<2.0**
Separation factor (α)	**>1.0**

CONCLUSION

The accuracy and precision of HPLC data collected begin with a well behaved chromatographic system.The system suitability specifications and tests are parameters that provide assistance in achieving this purpose. This article summarizes the system suitability parameters,

which can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. With proper validation and tight chromatographic performance (SS) criteria an improvement in the reliability of the data can be obtained. In this way, all the SST parameters are explained along with regulatory requirement.

Abbreviation

SST=System suitability test
HPLC = High performance liquid chromatography
ICH = International conference on harmonization
USP = United state pharmacopoeia
IP = Indian pharmacopoeia
RSD = Relative standard deviation
e.g = Example

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