METHOD DEVELOPMENT AND VALIDATION BY RP – HPLC METHOD

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Abstract: High performance Liquid chromatography (HPLC) is a one of the most important Chromatographic technique is used for the separate a mixture of different compounds in various fields like Analytical chemistry, bio chemistry and so many industries purpose also. One of the main advantages by using this HPLC technique is for identifying, purifying and quantifying the individual components of the mixture.

Keywords: Method Development, Method Validation, RP-HPLC, ICH.

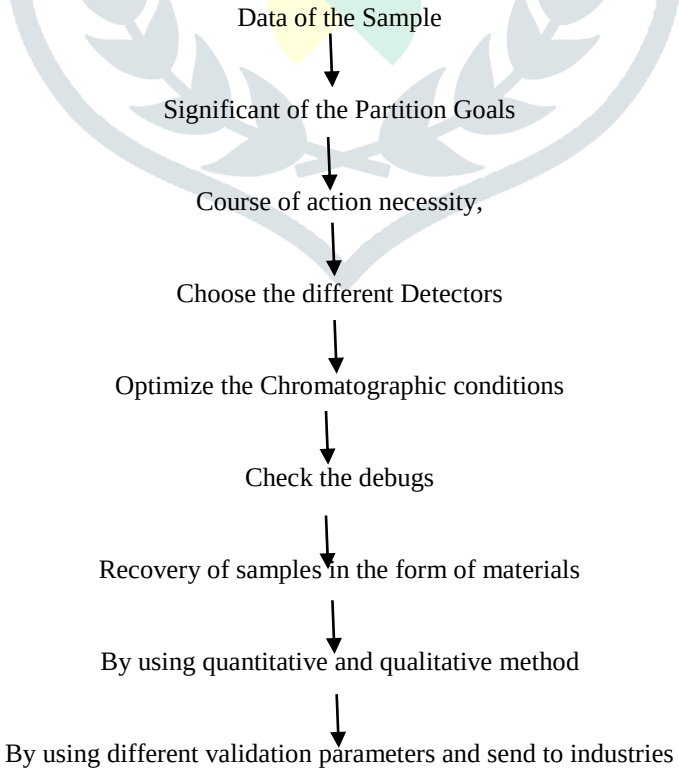
Introduction

Analytical method development and validation play a very important role in the innovation, expansion and fabricate of pharmaceuticals. The official test method that results from these process are used by quality control industries to ensure the distinctiveness, limpidness, effectiveness and concert of drug product 'quality', necessary for drug safety and efficacy. In pharma and biochemical industries, a current major issue is the high cost of research introduction of new drugs. In essence, it takes several hundred million dollars to discover, develop and gain regulatory approval. There is a need for high throughput in order to maximize exclusive rights lifetime and as a result, generate the profits to sustain the research and to increase the swiftness with which is the product that can be deliver into the souk. **[1,2]**

Method development and validation

A variety of prototype has been used to build up investigative separation. The conversation here is limited to conformist chromatographic approach. These looms are advances and often based on observant verdict and the data of the chromatographer. Whereas personage may exhibit considerable variety, method development often follows the series of steps summarized below. **[3,4,5].**

Different Steps involved in Method development and validation



Before starting the method development, it is require to appraisal what is known about the sample. The aim of method development should also define at partition stage. The type of sample-related in sequence that can be vital that are nature of the sample, number of compound current, chemical structure and molecular weight of the analytes, Pka values of the compounds, sample solubility and sample type. The preference of the mode of HPLC method should be made primarily from the properties of the sample that has been determined concerning molecular weight. On the basis of the solubility of the sample in polar or non polar solvents, sort of HPLC can be select as per below the diagram.

The parameters that are consider for fine chromatographic condition and being optimized are

1. Resolution (R)
2. Theoretical plates (N)
3. Tailing factor (T)
4. Capacity factor (k)
5. Selectivity (α)

Resolution(R)

Resolution express the partition of two components in a mixture that can be explained by the following formula.

$$R = \frac{2(t_2 - t_1)}{W_2 + W_1}$$

Where by t_2 and t_1 is the retention times of the two components and W_2 and W_1 are corresponding widths at the bases of the peaks obtained by extrapolating the comparatively straight sides of the peaks to the baseline.

Where electronic integrators are used, it may be suitable to resolve the resolution, R by the following formula.

$$R = \frac{2(t_2 - t_1)}{1.70(W_{\frac{2h}{2}} + W_{\frac{1h}{2}})}$$

Where by $W_{1h/2}$ and $W_{2h/2}$ are the widths at half-height of corresponding peaks. For better partition, the ideal value of R is 1.5.

Theoretical Plates(N)

The number of theoretical plates is a measure of column efficiency. It is articulated by following formula.

$$N = 16(\frac{t}{W})^2 = 5.54(\frac{t}{W_{\frac{h}{2}}})^2$$

Where t is retention time of the peak and W is the width for the peak. $W_{h/2}$ is width at half-height of the peak. Value of theoretical plates higher than 4000 is representative good column performance.

Tailing Factor (T)

The tailing factor (T) is a evaluate of peak symmetry. It is unity for perfectly proportioned peak to value of 1.0 and its value increases as tailing becomes more pronounced. It is determined by following formula:

$$T = \frac{W_{0.05}}{2f}$$

Where by W0.05 is width of the peak at 5% height and f is distance from the peak maximum to the leading edge of the peak which being measured at a point of 5% of the peak height from the base line. In general, value of tailing factor should be less than 2.0.

Capacity Factor(K)

Capacity factor is the ratio of the condensed retention volume to the dead volume. Capacity factor is a compute of how well the sample molecule is retained by a column during an isocratic separation. It is resolute by using following formula.

$$K' = \frac{t_1 - t_0}{t_0}$$

Where t_0 is the void volume of the column and t_1 is the retention time of the corresponding peak. The ideal value of k ranges from 2-10.

Selectivity(∞)

The selectivity is a evaluate of relative retention of two components in a mixture. Selectivity is the ratio of the capacity factors of both consequent peaks. It can be calculated by following formula.

$$\propto = \frac{t_2 - t_1}{t_1 - t_0}$$

Where t_0 is the void volume of the column and t_1 and t_2 are the retention times of the corresponding peak. The ideal value of ∞ should not be less than 1.

Analytical Method Validation [6, 7, 8, 9, 10, 11]

The developed analytical system used to gauge the quality of pharma products. It is necessary to comfort that the performance uniqueness of the industrial analytical procedure meet the requirements for the planned analytical application

Policy for the Validation of Methods

Method development and validation are an involving iteration process. The persuade of operating parameters on the performance of the method can be assessed at the validation stage. **Parameters for Method Validation**

The parameters for the method validation comprise in different functioning groups of national and international committee and are described in the literature. An effort at harmonization was made for pharma applications through the ICH (International Council for Harmonization). The distinct validation parameters by the ICH are summarized below.

A. Specificity
B. Linearity and Range
C. Limit of Detection (LOD) and Limit of Quantification (LOQ)
D. Precision
E. Accuracy
F. Robustness
G. Solution stability
H. System suitability.

A. Specificity

Specificity of an analytical method is its capability to measure perfectly an analyte in the presence of intervention, such as synthetic precursor, excipients, enantiomers and known degradation products that may be expected to be present in the sample matrix.

B.Linearity and Range

The linearity of an analytical method is its aptitude to bring forth test results that are directly proportional to the concentration of analyte in samples within a given range. Linearity may be verified directly on the test material and or by using part weights of synthetic mixtures of the test product components using the projected procedure.

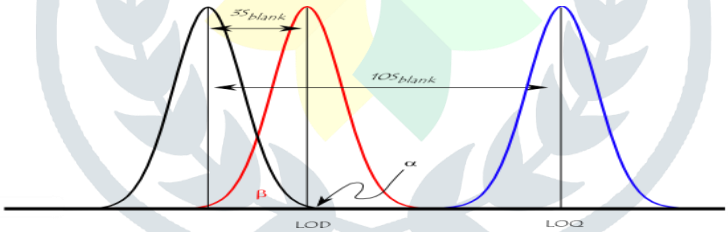
to evaluate correlation coefficient. In addition, y-intercept slope of the regression line and residual sum of squares should also calculate.

C. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The detection limit of an analytical method is the lowly amount of analyte in a sample which can be detected but not essentially quantities as an exact value.

LOD can be deliberate by another three different methods; (i) Visual scrutiny (ii) Standard variation of the blank reaction (iii) Standard variation of the reaction based on the slope of the calibration curve.

The quantification limit of an analytical method is the lowly amount analyte in a taster which can be quantities with suitable precision and accuracy.



D. Precision

The precision of an analytical method state the clones of concord between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions.

Reproducibility

Reproducibility expresses the precision sandwiched between laboratories. The reproducibility of an analytical method is resolute by analyzing aliquots from same standardized lots. Intermediate precision: Intermediate precision express within-laboratories variations; different days, different analysts, different equipments.

E. Accuracy

The accuracy of the analytical method is the proximity of agreement between the value which is accepted either as a predictable true value or an accepted reference value and the value found.

F. Robustness

The robustness of an analytical method is a measure of its aptitude to remain impassive by small, but conscious, various in method parameters and provides an indication of its reliability during normal usage

G. Solution Stability

Many solutes readily fester prior to chromatographic investigations, for example during the preparation of the sample solutions, extraction, cleanup, phase transfer or storage of prepared vials.

H. System suitability

System suitability should be determined by imitate analysis of the standard or before solution. System suitability is considered proper RSD, theoretical plates, tailing factor and resolution parameters.

References

[1] Sharma BK. Instrumental Methods of Chemical Analysis, 24th Edition, Pg: 68-110.

[2] Beckett AH, Stenlake JB. Practical Pharmaceutical chemistry, University of London ., Volume I,I 4th Edition., 1962 .,

[3] Gurdeep R Chatwal, Sham Anand. Instrumental Methods of Chemical Analysis, pg:185-190.

[4] James W Munson. Pharmaceutical Analysis Modern Method. pg:15-154.

[5] Snyder LR, Kirkland JJ and Glajch JL, Practical HPLC Method development., 2nd Edition., John Wiley and Sons., Newyork,1997,165.

[6] Willard HL, Merritt Ll, Dean JA and Settle FA. Instrumental methods of Analysis., 7th Edition, Wadsworth Publishing Company,California.,1988,196.

[7] Ranjit Singh. HPLC method development and validation –an overview. J Pharm Educ Res. 2013;4(1).

[8] International Conference on Harmonization of technical Requirements for Registration of Pharmaceuticals for Human use, ICH Harmonized Tripartite guideline-Validation of Analytical procedures: Text and methodology Q2 (R1), Current step 4 version., London 2005.

[9] Harshal A. Pawar, Lalitha K.G., "Development and validation of a novel RP-HPLC method for Estimation of losartan potassium in Dissolution samples of Immediate and sustained release tablets., Chromatography Research International. 2014.

[10] Anjaneyalu Y, Chandrasekhar K, ValliManickam. Text book of Analytical Chemistry, pg:273-278., 2006.

[11] Text book of Pharmaceutical analysis by Dr.S. Ravi Sankar, Rx Publications,Tirunelveli.