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Green Analytical Chemistry (GAC) For The Simultaneous Development And Validation Of Hplc Methods For Polyherbal Formulations

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Abstract:

Less waste is produced and fewer potentially harmful chemicals and reagents are used in green analytical chemistry. Recent advances in analytical technique development include the creation of solvent-free or solvent-minimized extraction methods, the adoption of safer solvents, and the miniaturisation of sample preparation tools. The twelve GAC principles give an important set of criteria for incorporating sustainability into analytical methods. Unfortunately, no matter how strictly one adheres to these guidelines, unpleasant actions may still occur. It is important to think about how eco-friendly analytical methods are so that we can assess their effects on the environment and on employees and, if possible, seek to mitigate such effects. Analytical procedures' impact on the environment may be measured in a number of ways. The Analytical Eco-Scale, the Green Analytical Procedure Index, and the Analytical Greenness Metric are three important instruments for measuring the environmental friendliness of analytical techniques. By considering many features of the analytical process, each of these metrics determines the green index of the technique. In this overview, we looked at these metrics, the theories behind them, and some real-world applications to different types of analysis. The benefits and drawbacks of these indices are examined from the perspective of a typical user or customer. We anticipate that this research will spark a plethora of new insights and developments in the field.

Keywords: Analytical eco-scale, Analytical greenness metric, Environmental impact, Green analytical chemistry, Green analytical procedure index and Miniaturization.

Introduction:

India is a stunning nation rich with culture and history as well as flora and animals. For more than four thousand years, people have relied on Ayurveda, an ancient Indian medicinal discipline. There is still a sizable population that seeks treatment from herbal or alternative practitioners. Studies of a product's stability aim to show that it retains its original composition, efficacy, quality, and integrity within a range of acceptable values. Long-term impacts of environmental factors including temperature, humidity, light, and pH (Acidic, Basic or Neutral) changes may

also be determined with the use of stability study findings. One may learn about the interactions between two or more drugs and if they are compatible under different environmental circumstances, as well as about the degradation products formed under different settings, with the use of a stability study (1, 2).

Drug-substance interaction information is also available. The word "stability" is used to describe how long and how precisely a medicine maintains its original qualities. In order to carry out a stability programme that is suitable, rigorous, repeatable, and compliant, we must closely adhere to a number of regulatory standards. The majority of the time, the rules established by the International Conference on Harmonisation (ICH) are also followed. This ICH guideline addresses the "Stability Testing of New Drug Substances and Products." The quality, safety, and effectiveness of medications are all factors that are considered while assessing the encoders that produce them. Green chemistry is an idea spawned by the push for environmental sustainability. Historically, chemists' efforts towards sustainability have focused on industrial processes and products, as stated by the most widely accepted definition of green chemistry established by Anastas. In the first stages of green chemistry, the pharmaceutical industry, among others, focused on green organic synthesis (3).

Green Chemistry:

Anastas (1999) defines "Green Chemistry" as the use of methods and procedures that aim to reduce or eliminate the use of substances that are hazardous to the environment or human health. This decreases both refuse generation and toxicity. It is a well-established field of study that has primarily focused on innovations in organic synthesis. Developing environmentally responsible practises is a trend in analytical chemistry, which has employed similar concepts. Several initiatives have reduced or eliminated the production of hazardous residues in chemical analysis without compromising analytical performance. *Armenta et al.* (2008) coined the term "Green Analytical Chemistry" (GAC) to characterise analytical techniques that generate less waste, fewer toxic residues, or both. *Armenta et al.* (2008), *Gauszka et al.* (2013), and *Turner* (2013) all present compelling arguments concerning the evolution of GAC and the associated techniques. The first method employs reagentless techniques, but this is often impractical, necessitating sample preparation or analyte derivatization. Since then, the use of non-hazardous chemical substitutes, waste production reduction, recycling, and chemical reuse have been implemented. In the end, trash must be addressed and treated appropriately. By minimising chemical quantities and employing moderate reaction conditions, GAC techniques are frequently safer and less expensive. Furthermore, the costs associated with waste management and treatment are substantially lower (4-6). In 2000, green chemistry established its own subfield, green analytical chemistry

(GAC). Green analytical chemistry is a relatively young topic of green chemistry that focuses on how analytical chemists may help labs reduce their environmental impact. Efforts are being made to lessen the environmental impact of chemical analyses and make it possible for analytical laboratories to adopt sustainable development principles in addition to developing the necessary instrumentation and methodologies to improve the quality of chemical analyses. In this light, GAC deserves credit for being a driving force behind the development of analytical chemistry. The biggest difficulty facing the field in the future is striking a balance between improving the quality of the results and improving the environmental sustainability of the analytical procedures. Guidelines for defining the structure of the GAC are required to overcome this problem (7, 8).

Principles of GAC:

Our 12 GAC concepts include the following:

1. To prevent sample manipulation, direct analytical procedures should be utilised.
2. The objectives are minimal sample size and sample count.
3. It is necessary to conduct measurements on-site.
4. Integrated analytical procedures and processes reduce reagent consumption and conserve energy.
5. Miniaturised and automated methods should be selected.
6. It is preferable to avoid derivatization.
7. It is essential to prevent the production of excessive amounts of analytical refuse and to provide suitable remediation for this waste (9).
8. Methods employing multiple analyses or parameters are preferred over those employing a single analyte.
9. Energy consumption must be kept to a minimum.
10. Use reagents derived from renewable sources whenever possible.
11. It is recommended to remove or replace toxic reagents.
12. The safety of the operator should be enhanced.

The Key Components Of Green Analysis:

Various methods and instruments available in contemporary analytical chemistry (10–12) allow for the identification of a specific analyte in various samples. In terms of greening analytical methods, the following are the primary goals to be achieved:

(1) elimination or reduction of the use of chemical substances (solvents, reagents, preservatives, pH adjustment additives, and others);

(2) minimization of energy consumption;

(3) appropriate management of analytical residue; and

(4) increased operator safety.

Reductions are usually necessary to address most of these problems, which includes things like sample size, reagent

use, energy consumption, waste creation, hazard, and peril. Finding a happy medium between performance indicators and GAC criteria is one of the challenges of greening laboratory methods. Performance indicators including precision, sensitivity, and accuracy may drop if analytical chemists follow the majority of our twelve suggestions. Analytical processes' validity is readily challenged when sample sizes are reduced, direct methods are used, and instruments are shrunk down to a more manageable size. Rapid technological advancement, together with a heightened awareness of current challenges, will, nevertheless, lead to a rise in the adoption of green analytical procedures. It is possible that these problems may be quickly and easily fixed (13, 14).

Medicinal Plants For Polyhedral Formulations:

Ayurvedic formulations are divided into three categories, namely herbal, mineral and animal based upon their material of origin. Among these, presently herbal formulation has gained abundance significance and rising universally. This development is evident as in the recent few years there has been major gain in the trade of herbal formulations usage in the developed countries. India is well-known as reservoir of herbs with the vast biodiversity centres with about 45,000 plant species. Among which 7000-7500 plants are recorded that therapeutically effective for various ailments and nearly 600-700 types of herbs are listed in its Ayurvedic system of medicine. The practice of such herbs is stated in the ancient text such as Charaka and Sushruta Samhitas. The different part/parts of an herb (leaves, flowers, seeds, roots, barks, stems etc.) are used in traditional Ayurvedic preparations. All drugs for internal or external use or for the diagnosis, treatment, mitigation, or prevention of disease or disorders in humans or animals, and all drugs manufactured exclusively according to the formula described in the authoritative books of the Ayurvedic system of medicine listed in the first schedule of the Drugs and Cosmetics Act of 1940, are considered Ayurvedic medicine under the law. The many chemicals found in medicinal plants and herbal remedies may be analysed qualitatively and quantitatively using state-of-the-art chromatographic separation methods. The use of formulations containing curcumin and piperine has been shown to potentially mitigate symptoms associated with a common cold, such as an itchy throat or continuous wheezing (15).

Table 1 :List of Ayurvedic Herbals

Sr. No.	Botanical Name	Common Name/Part used
1.	*Emblica officinalis*	Amla (Fruit)
2.	*Saraca asoca*	Ashok (Bark)
3.	*Withania somnifera*	Ashwagandha (Root)

4.	*Aconitum Heterophyllum*	Atees (Rhizome)
5.	*Aegle marmelos*	Bael (Fruit/Bark)
6.	*Phyllanthus amarus (P. niruri)*	Bhumi amlaki (Whole Plant)
7.	*Bacopa monnieri*	Brahmi (Whole Plant)
8.	*Santalum album*	Safed Chandan (Heart Wood)
9.	*Swartia chirata*	Chirata (Whole Plant)
10.	*Tinospora cordifolia*	Giloe (Stem)
11.	*Gymnema sylvestre*	Gudmar (Leaf)
12.	*Commiphora wightii*	Guggal (Extract)
13.	*Barberis aristata*	Indian Berberry (Root/Stem)
14.	*Plantago ovata*	Isabgol (Husk/Seed)

Curcumin:

Curcumin, the primary component of Haldi, possesses anti-inflammatory, antibacterial, anti-allergic, and anti-cancer properties. Curcumin may have an anti-inflammatory effect by binding to its own receptor, which then stimulates signalling pathways that result in the up-regulation of PPAR- and the reduction of inflammatory cytokine production (16).

Figure 1. Curcumin Plant

Piperine:

Piperine, the primary component of piper, has neurodepressant, anti-inflammatory, antipyretic, analgesic, antioxidant, and bio-enhancer properties. Piperine increases gastrointestinal absorption and decreases medication metabolism in the stomach via multiple mechanisms. Consequently, piper is used in numerous Ayurvedic formulations. Piperine enhances the bioavailability of curcumin and Gallic acid by inhibiting the cytochrome p450 enzymes (17).

Figure 2. Piperine Plant

Need For Standardization Of Polyherbal Formulations:

Due to its efficacy in treating a vast array of medical conditions, Ayurveda is still practised today. Ayurvedic formulations have numerous applications, including solid (*Vati, Churna*), semisolid (*Avaleha, Ghrita*), and liquid (*Asava, Arishta*) dose forms. By influencing the balance of the three doshas in the body, they effect or help restore homeostasis, which begins in the digestive system and progresses to the tissues. In response to the growing interest and acceptance of Ayurveda, the appropriate standardisation and validation procedures for certifying Ayurvedic medications are being developed. It is impossible to exaggerate the importance of standardisation, and it merits serious consideration. Evolution is a

dynamic process that can alter the identity and structure of organic substances. Now, accurate identification and distribution of primary materials are required to bring them to market. Other vegetation species have also sadly become extinct. In addition, it is not uncommon for the compounds purportedly responsible for the therapeutic effect to be unknown or inadequately understood (18).

When multiple herbs are used in a singular preparation, referred to as a Polyherbal formulation, ensuring quality consistency is a complex and time consuming process. Existing information on *Ayurveda* and indigenous treatments is being validated in accordance with modern manufacturing, quality control, and standardisation requirements. Government health sectors recognise the value of traditional remedies and have therefore developed a variety of techniques, norms, and standards to guarantee their quality. Standardisation is the process of establishing technical standards. A standard is a document that codifies common requirements, norms, criteria, techniques, and processes in engineering and other technical fields. Ayurvedic medicine presents substantial challenges when it comes to standardisation due to its many distinctive qualities. In Ayurvedic medicine, "standardisation" refers to the provision of processed plant material containing a known marker component in a predetermined quantity. This is essential because many Ayurvedic treatments rely on plant components, whole plants, or plant extracts as active constituents. The quality of market-sourced primary materials is typically inconsistent (19).

Numerous techniques are utilised by manufacturers to develop their unique formulas. Additionally, preparation varies based on the type of operation employed. There are a number of issues with the product's quality because the botanical compounds used in Polyherbal formulations are complex mixtures of different secondary metabolites. As indicated in the standard Ayurvedic texts, it is necessary to standardise the Ayurvedic plants and associated techniques with acceptable standard deviations in order to ensure the therapeutic efficacy of the finalised botanical products (20).

Quality Control Methods For Traditional Herbs And Formulations:

Since its inception, the WHO has worked to ensure that all pharmaceuticals are safe and effective. The term "quality control" refers to the processes used to ensure that a product's integrity and dependability are maintained. The efficacy and safety of herbal remedies are directly proportional to the quality of their constituent ingredients. The formulation's active constituents and the conditions under which the basic materials were gathered and utilised each contribute to the quality of the final product. For this reason, regulatory bodies, academic institutions, and manufacturers have adopted traditional medicine's quality criteria. Herbs, extracts, and formulations are currently evaluated using organoleptic,

microscopic, physical, chemical, chromatographic fingerprinting, microbiological contamination, pesticide levels, and biological techniques (19-21).

Macroscopic Analysis:

Visual inquiry is used, which is the simplest and fastest way to determine the identification, purity, and maybe quality. Medicinal plant materials' size, colour, texture, fracture qualities, and cut surface appearance all contribute to its macroscopic identification.

Microscopic Evaluation:

Under a microscope, the finer details of a drug can be examined, and the histology properties of organised substances can be identified with relative ease. This procedure also includes the use of histological sections, pulverised compounds, and chemical assays administered in minute quantities. Characteristics of the cell wall and its contents, trichomes and their contents, fibre and vessel measurements, tissue dimension analysis, etc., can all be studied in great detail. Each description is accompanied by images and photographs that serve as visual mementos of the accurately described items.

Physicochemical Analysis:

Qualitative assessments of the herbs/herbal products are carried out in terms of purity which includes following parameters like total ash content, acid-insoluble ash, moisture content, specific gravity, refractive index, extractive value, pH, total solid content, alcohol content (22).

Biochemical Analysis:

This analysis of the herbs/herbal products is done to assess the appropriate specifications and potency in terms of their active principles. Chemical parameters include phytochemical analysis, limit tests, chemical assays etc. These methods are designed to set the permissive limits of tolerance for the product when it reaches patients.

Microbial Contamination:

All the herbs contain certain bacteria and molds that enters from soil and atmosphere. Analysis of the limits of E. coli and molds evidently confirms the collecting, making practices and also indicates the toxicity of the final product formed. The substance known as aflatoxin produces severe side-effects if used up along with the crude drugs.

Spectroscopic Analysis:

The capacity of individual molecules to absorb electromagnetic radiation at a particular wavelength is essential to spectroscopy. Spectroscopy may be used to identify which species are engaged in the interaction based on absorption or emission since the energy states of the interacting chemical species are unique for each species. Among the most often used methods in the analytical sector are atomic absorption spectrophotometry, visible infrared, and ultraviolet. New ingredients in herbal treatments may be discovered using spectrophotometric methods including UV, IR, and chromatography (23).

Biological Analysis:

To check the pharmacological activity, several clinical trials have been carried out on living animal either on their complete or isolated organs which indicate the strength of the formulations in order to have standard quality check of the final product. In Ayurvedic medicines the clinical experiences, observations are available but there is a need of scientific documented proof which can be possible done by "Laboratory to Clinical" study known as Reverse pharmacology.

Chromatographic Analysis:

Chromatographic technique represents the most useful and powerful technique for the separation of components of a complex mixture. Though it's a separation technique it provides identification of compounds and also the quantitative estimation about the main active constituents present in the crude drug as chemical markers in the fingerprint evaluation of herb and herbals products (21-23).

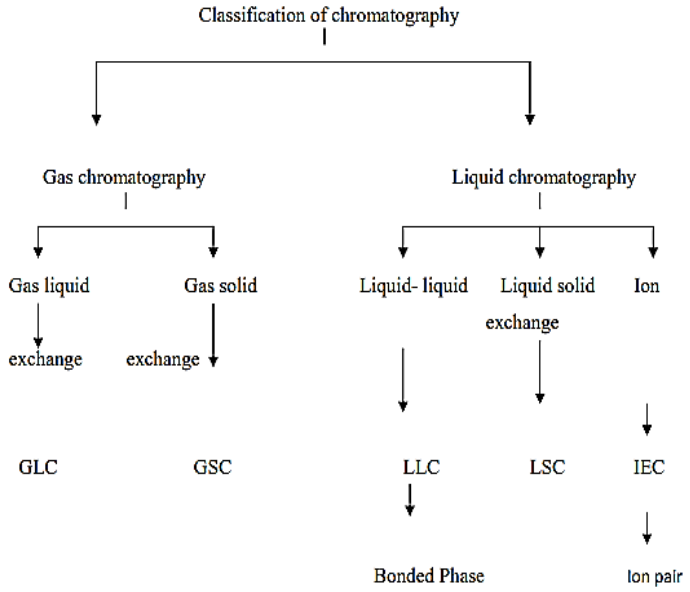

Figure 3 Classification of chromatography

Standardization Of Herbal Formulation Using Different Analytical Methods:

High Performance Liquid Chromatography:

Chromatography can only be performed successfully if one has the capacity to separate molecules into a stationary phase and a mobile phase. After a molecule has been isolated from the initial mixture, its concentration may be evaluated using the appropriate statistical methods. In the separation procedure known as liquid chromatography (LC), a liquid is used for the mobile phase of the apparatus. Column and planar chromatography are two useful alternatives to the more traditional method of liquid chromatography. High performance liquid chromatography, often known as HPLC, is a kind of modern liquid chromatography that generally

involves the application of a high pressure and the use of very minute packing particles. High-pressure liquid chromatography involves pumping a sample through a porous monolithic layer or a column comprised of particles that are either spherical or irregular in form (20-23).

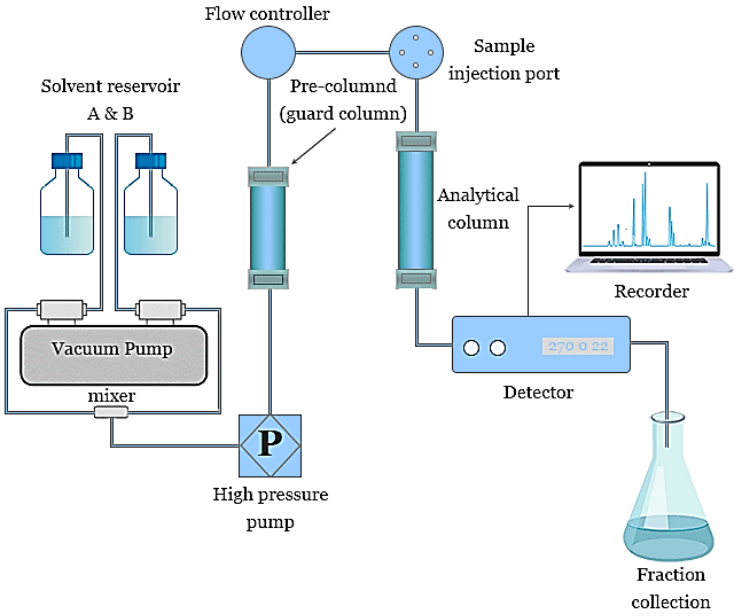

Figure 4 HPLC Instrumentations

In the past, HPLC was divided into two distinct categories based on the polarity of the mobile and stationary phases. When doing NPLC, the stationary phase is more polar than the mobile phase, but when performing RPLC, the stationary phase is more non-polar than the mobile phase. Despite "normal phase" having fewer uses, RPLC is more often used. High-performance liquid chromatography is essentially an advanced version of column chromatography. Instead, then letting a solvent slowly percolate through a column, it is pumped through at pressures of up to 400 atmospheres. This results in a significant increase in speed. The smaller particle sizes of the packing materials for the column allow for a greater surface area to be available for interactions between the stationary phase and the molecules moving through the column. This greatly facilitates disentangling the various parts of the mash-up. In addition, modern detection methods represent a major advancement over traditional column chromatography. These processes are highly mechanised and sensitive (24).

Parts of HPLC:

Solvent Reservoir:

The solvent reservoir has to have a few characteristics. Both aqueous and non-aqueous mobile phases must be impermeable to the reservoir due to its composition. The reservoir should have a capacity more than 500 ml. This quantity enables for 4 hours of operation given that the normal mobile phase velocities are 1-2 ml/min. The reservoir cover must have a hole big enough for the tubing intake line to fit through. Some aqueous and organic

solvents need to be degassed before use. Here, it's important to avoid letting gas pockets develop within the detectors. Both agitating it in a vacuum and sparing it with helium gas may be used to degas the mobile phase. In order to avoid solvent leaking in the case of a rupture, further safety measures must be performed if the reservoir is constructed of glass. Storage in plastic is advised for the reservoir (22-24).

Tubing:

The kind of cable used to transmit signals between the various parts of the system should be well thought out. Tubing must be inert, pressure-resistant, and capable of conveying an adequate amount; however, the internal diameter of the tubing prior to the injection device is not critical.

Pumps:

Modern liquid chromatography requires certain pressures and flow rates, both of which may be met by a variety of pump designs. In the early years of the LC renaissance, pneumatic amplification and the syringe pump where a large, robust syringe with a motor-driven plunger were both extensively used. In the most majority of contemporary chromatographs, pistons or diaphragms make up the reciprocating compressor.

Pneumatic Pump:

Pneumatic pumps outperform piston pumps in terms of flow rate, although their primary use is in column packing rather than in the laboratory. Although the high-pressure variants of the pneumatic pump are bulky and may waste a significant volume of compressed air at high flow rates, they are reasonably affordable and can create exceptionally high pressures.

Syringe Pump:

Similar to a real hypodermic syringe, the syringe pump works on a much greater scale. Despite being popular in the early days of the LC renaissance, this sort of compressor is seldom utilised nowadays due to its design. This is because the capacity of the pump limits the amount of mobile phase that may be used. The separation must be halted and the pump must be filled in order for the development to proceed. The only solutes that will elute are those whose retention volumes are less than or equal to the pump's capacity (25).

Sample Injecting System:

A sampling valve or hypodermic may be utilised to introduce the sample. A self-sealing elastomer septum can be used with a high-pressure injector to introduce a sample. The leaching action of the mobile phase in contact with the septum, which can result in phantom peaks, is one of the issues with septum injectors. HPLC syringe injection may be more difficult than GC syringe injection. However, stop-flow septum-less injection will completely eradicate the need for septum injectors. Commercial chromatographs utilise micro volume sampling valves more frequently than any other component at present. Multiple injections of samples into pressurised columns have no appreciable effect on the flow of the mobile phase. When the valve is in the precise position, a sample of atmospheric pressure is placed

in an external loop and injected into the mobile phase. The quantity of infused sample can be altered from 2 l to over 100 l by modifying the sample loop's capacity or by employing variable volume sample valves. There are also automatic sample injectors available, allowing the machine to operate without human intervention. For quantitative work, valve injection is preferable because it is more precise than hypodermic injection (23-25).

Column:

Because columns are like tubes, they are often measured by their internal diameter × length (4.6mm × 250mm). When doing large-scale preparative chromatography, you will come across columns with an inside width of 0.050 to 4.6 mm or even bigger, like a mass spectrometer. This is important to know because shorter columns are often cheaper and create less back pressure. When it comes to mass spectrometry, short reverse phase columns work almost as well as long ones.

Detectors:

The detector, which performs the analysis of the chemicals that have been separated by the column, is the "mind's eye" of the LC system. The chromatographer must verify that the detector selected during the technique development phase is sensitive enough to respond to changes in the concentration of all components of the sample, including trace compounds, before injecting the first sample (24).

UV Absorbance Detectors:

The mobile phase moves the radiation beam of a UV/visible photometer or spectrophotometer through a small flow cell. When a flow cell is exposed to a UV-absorbing solute, a signal is produced that is directly proportional to the concentration of the solute. Only alkenes, aromatics, and compounds with many C–O–N–S linkages are found in nature because they absorb UV light. Careful consideration must be given while selecting the mobile phase components to ensure they absorb as little radiation as possible.

Computer, Integrator or Recorder:

A data-gathering tool, such a computer, integrator, or recorder, is linked to the detector. The chromatogram shown here represents the electrical signal produced by the detector and may be evaluated by the user. The inability to store and utilise data has rendered recorders obsolete. While both computers and integrators are capable of integrating chromatogram peaks, computers have the additional benefit of storing chromatograms digitally for later examination.

Advantages of HPLC:

1. Leans towards automation and quantification (less time and less labour);
2. Precise and repeatable;
3. Analysis can be completed in less than twenty minutes.
4. Enhanced Sensitivity (various detectors are available)

5. Enhanced resolution (vast array of stationary phases)
6. Reusable columns (columns that are costly but can be used for multiple analyses);
7. Suitable for substances with low volatility.
8. Easy sample retrieval, maintenance, and manipulation.
9. Instrumentation calculations are performed by the integrator.
10. Suitable for much larger-scale preparative liquid chromatography (20-25).

Analytical Method Validation:

Validation process is confirming that the analytical procedure for the specific tests to the intended requirements. Validation simply termed as document evidence of the developing of analytical method. Method validation is done for the according to ICH guidelines (Q2R1) (24, 25).

Specificity and Selectivity:

Specificity is the capacity to evaluate the analyte unequivocally in the presence of components that are probable to be present. Typical examples include impurities, degradation products, matrix components, etc.

1. The capacity to selectively assess the analyte in a sample matrix including other components is known as selectivity.
2. When an assay is specific, the signal seen is caused by the analyte of interest and not by any confounding factors like degradation products, contaminants, or excipients.
3. The integrity and provenance of the peak will provide this information.
4. Using diode array detectors might streamline the development and validation of HPLC investigations. When recognising peaks, retention time data is supplemented with spectral information from diode array detectors. In addition, a shift in the spectrum often reflects the highest degree of purity.
5. Spectral data from the peak's leading and trailing margins are compared in depth to ascertain the peak's relative integrity. An unadulterated peak has a value of 1.5, whereas an adulterated peak has a value larger than 1.5.

Linearity:

Linearity refers to the capacity of an analytical method to provide findings that are proportionate, or can be made proportional, to the concentration of an analyte in a sample. Validity of linearity data is often assessed by calculating the correlation coefficient and y-intercept of the linear regression line for the response versus concentration plot. A correlation coefficient higher than 0.99 is considered statistically significant. The response of the analyte at the desired concentration shouldn't be more than a small fraction of the y-intercept (24).

Range:

The analytics range includes the concentrations at both ends of the spectrum. Range refers to the concentration interval between the greatest

and lowest analyte concentrations in a sample for which it has been shown that the analytical process is linear, accurate, and exact. It's important to at least think about the following minimums:

i) For the analysis, the drug substance or a finished (drug) product: typically, between 80 and 120 percent of the test concentration.

ii) For content uniformity, covering a minimum of 70-130% of the test concentration, unless a broader, more appropriate range is justifiable based on the nature of the dosage form (e.g. metered dose inhalers).

iii) For dissolution testing, it must be within 20% of the specified range; for instance, if the specifications for a controlled-release product cover a range from 20% after 1 hour, to 90%, after 24 hours, the validated range would be 0-110% of the label claim.

iv) For impurity determination, from the impurity reporting level of 1-120 percent of the specification.

v) For impurities known to be extraordinarily potent or to generate toxic or unanticipated pharmacological effects, the detection/quantitation limit should be proportional to the level at which the impurities must be controlled.

vi) If assay and purity are conducted as a single test using only a 100% standard, linearity must encompass the range from the impurity reporting level of 1% to 120% of the assay specification.

Precision:

Under certain conditions, the accuracy of an analytical technique indicates the degree to which a set of measurements made from many samples of the same homogenous material agree with one another (dispersion). It is possible to quantify it on three distinct scales: repeatability, intermediate precision, and reproducibility. It is expressed as a fraction of the standard deviation (SD) or the relative standard deviation (RSD). It should be ≤ 2.0 %.

Accuracy:

Accuracy refers to the analytical procedure used to show how near the measured value is to the actual value. The accuracy of an analytical method is measured by how closely the result matches the accepted authentic value or accepted reference value. One possible way to define something is as "authentic." Extra analyte is measured and reported as a percentage of a target value. An example of an accuracy criterion for an assay technique might be a mean recovery of 100 ± 2% for each concentration between 80% and 120% of the target concentration.

Limit of Detection:

In individual analytical procedure lowest amount of analyte which can be detected. But not quantified. Formula for LOD is,

LOD = 3.3 *δ/S……. (1)

Limit of Quantification:

In individual analytical procedure lowest amount of analyte which can be detected and quantified. Formula for LOQ is,

LOQ = 10 *δ/S……. (2)

Where, δ = standard deviation of response.
S = Mean of slopes of the calibration curves.

Robustness:

The robustness of an analytical method is measured by its ability to preserve its original form in the face of carefully manipulated modifications to its parameters. During an HPLC run, the flow rate, column temperature, sample temperature, pH, and mobile phase composition are among the process parameters that may vary.

System Suitability:

System appropriateness refers to the entirety of the analytical procedures. It involves conducting system performance tests prior to or during the unknown's analysis. Plate count, tailing factor, resolution, and retention are a few examples of acceptable system suitability criteria (25).

Detection Limit:

The detection limit of a specific analytical procedure is the smallest quantity of an analyte in a sample that can be detected, but not necessarily quantified.

Quantitation Limit:

The quantitation limit of an individual analytical procedure is the smallest quantity of analyte that can be quantified with sufficient precision and accuracy.

Summary and Conclusion:

The greening of analytical labs will be greatly aided by the guidance provided by the GAC principles. It is challenging to develop universal standards due to the wide variety of analytical approaches and the specific needs each has. In the future, quantitative assessment of particular analytical techniques and processes would need a broader approach, which is where our idea comes in. Pharmaceutical companies throughout the world are struggling to adapt to new regulations requiring them to use green analytical chemistry (GAC). High-performance liquid chromatography (HPLC) is widely utilised in the pharmaceutical business, but it also produces a large volume of organic toxic waste (25). Thus, it is crucial to include GAC ideas into Pharma analysis. Despite the growing body of literature on green chromatography methods, few HPLC methods that are gentler on the environment have found their way into the pharmaceutical business. Whether it's because of confusion among analysts or because of the time and effort required to modify standard HPLC equipment, this is often the case. In order to build eco-friendly HPLC processes for pharmaceutical analysis, this chapter gives an overview of green approaches that may be easily applied to conventional liquid chromatography (LC) gear. The goal is to persuade the pharmaceutical industry's analytical community that switching to green HPLC practises is straightforward by showing how straightforward it is to build green methodologies for pharmaceutical analysis.

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