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# A REVIEW ON GREEN CHEMISTRY APPROACHES IN ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

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## ABSTRACT

The concept of green analytical chemistry has become a revolutionary paradigm in combining environmental sustainability with analytical excellence in the analysis of the pharmaceuticals. This is a review paper that evaluates principles, assessment instrumentation and practice in developing and validating analytical methods using green approaches. The twelve principles of green analytical chemistry offer systematic guidelines on the design of environmentally benign procedures and quantitative measures such as Analytical Eco-Scale, GAPI, and AGREE allow objective analysis of the greenness of a method. There have been significant developments in the miniaturised sample preparation procedures, chromatographic alterations with the use of UHPLC and other solvents, direct spectroscopic analyses, and optimisation of chemometrics, which show that 50-90% cuts in the quantity of solvents that are used to achieve analytical performance are possible. Interactions among quality goals and sustainability aim at providing synergies due to the use of Quality by Design and Process Analytical Technology. Although proven to be beneficial in terms of environmental and economic advantages, issues such as technical aspects, regulatory issues and internal organizational issues influence the adoption rate. The next generation of thinking focuses on the use of artificial intelligence, nanotechnology, portable devices, and the concept of Industry 4.0 that moves towards really sustainable analytical laboratories. The development of green analytical chemistry should be expedited through collaborative activity of interested parties and effective changes in the regulatory environment where the environmental aspects are explicitly considered and pharmaceutical analysis is regarded as the active participant of the global sustainability agenda without compromising high-quality criteria.

**KEYWORDS:** Analytical method validation; Chemometrics; Green analytical chemistry; Pharmaceutical analysis; Sample preparation; Sustainable chromatography.

## INTRODUCTION

The development of analytical chemistry has been characterized by the incessant improvements in instrumentation, methodology and scope of application. Nevertheless, the ecological footprint of laboratory centers in the past decades has become one of the major issues of concern. Although the traditional analytical methods are effective in producing accurate and precise results, they usually consume a lot of toxic organic solvents, produce a lot of chemical waste, and require a lot of energy.[1] Millions of liters of solvent waste are produced every year by pharmaceutical industry alone with the help of regular testing in the laboratory, the quality control processes and the developing of the methods. This ecological challenge, which has been enhanced by the rising pressure of regulations and the rising consciousness of sustainable practices, has triggered the development of the Green Analytical Chemistry (GAC) as a new paradigm in the discipline.[2] Sustainable chemical practices were founded on the concept, which was proposed by Anastas and Warner in 1998 with their original twelve principles of green chemistry. Analytical chemistry adaptation Analytical chemistry Analytical chemistry Adaptation of these principles to analytical chemistry was first pursued by researchers in the early 2000s, who noted that despite the volume of quantities often touched by synthetic operations often being larger, the contribution of analytical laboratories to chemical use and waste production is significant.[3] The International Union of Pure and Applied Chemistry (IUPAC) has significantly been active in spreading GAC by several means: conferences, publications and initiatives that highlight the necessity of environmentally benign analytical procedures without restricting the performance of the analysis. The transition of the traditional to the green model of analytical practices is not only a change in the technical aspect but a complete paradigm shift in the sense that the environmental impact assessment becomes a part of the methodological construction, which is equal in significance to the traditional measurements of performance.[4]

Green methods of analysis are important in the analysis of pharmaceutical and chemical not just due to environmental stewardship. The economic factors are also important since the decreased consumption of solvents, decreased cost of disposal of waste, and enhanced efficiency of energy all lead to the cost reduction of the analytical laboratories.[5]

Furthermore, green strategies can have other benefits like; providing more safety to lab workers, easing the procedure by means of miniaturization, and also increasing the speed of analysis which is beneficial in improving throughput of the laboratory.[6] Their increased significance in business sustainability policies and governmental regulations has also been enhanced by the fact that green analytical practices are now in line with major environmental pledges adopted by the world such as the United Nations Sustainable Development Goals and the Paris Agreement. In this comprehensive review, the principles, tools, techniques as well as the applications of green chemistry approaches to the development and validation of analytical methods, with specific focus on pharmaceutical applications, are discussed.[7] The areas include green methods of sample preparation, adaptations to chromatographic and spectroscopic methods, chemometric methods of obtaining more optimization as well as the incorporation of sustainability factors in method validation procedures.[8] This review will critically explore the existing practices, regulatory views, and promising trends to offer practical insights to analytical scientists, creators of methods, and quality assurance personnel to adopt green methods of analysis that would not only address the environmental goals but also maintain high-quality standards.

## PRINCIPLES AND METRICS OF GREEN ANALYTICAL CHEMISTRY

Green Analytical Chemistry is based on twelve principles that give the original ideas of green chemistry its context in the field of analytical practice. These principles offer the guidelines on designing, developing and implementing

analysis techniques that would have minimal environmental effect yet remain fit in purpose.[9] The first one focuses on the immediate methods of analysis where sample treatment is removed or kept to a minimum, thus saving time and resources. The second principle encourages low sample size and sample number, not only will lead to the reduction of reagent use, but will also decrease the waste amount and the strain on sample collection.[10] The third and fourth principles deal with the paramount problem of solvent use, which encourages the rationalization of hazardous reagents with less hazardous ones, and energy efficiency during the analytical reaction.[11] The fifth principle is integration of analytical processes, which promotes the integration of several analytical operations into lean workflows that minimize resource consumption. The 6th principle encourages building of automated and miniaturized processes that increase precision and decrease the exposure of humans to dangerous substances.[12] The seventh principle, which is concerned with real-time analysis, does not require sample storage, preservation and transportation, which conserves energy and maintains sample integrity. The eighth principle promotes multi-parameter and multi-analyte approaches that should be able to provide maximum information at each analysis to enhance efficiency and avoid the use of many separate determinations.[13] The ninth principle focuses on renewable and bio-based substances, especially when it comes to products used as extraction solvents or as chromatographic phases. The tenth principle deals with the waste production and treatment, which encourages waste minimization at the point of production and proper treatment or recycling of the unavoidable waste. The eleventh principle promotes simplification and soundness of the methods and makes it easier to avoid repetitive analysis and the troubleshooting of methods. Lastly, the twelfth principle is that of operator safety by exposing them to less hazardous chemicals and safer working conditions.[14]

The greenness of the analytical methods needs to be assessed with the help of quantitative tools which can objectively consider the environmental impact and optimise the methods.[15] The Analytical Eco-Scale created by Van Aken et al. is a simple scoring system that gives an initial score of 100 points and imposes penalty points on the different aspects of the analytical process such as amounts of reagents, hazard of the procedure, use of energy and generation of waste. Scores that exceed 75 are termed as excellent green methods, scores between 50-75 are acceptable green methods and scores below 50 are likely to indicate that a lot is required to be done.[16] The simplicity of the Eco-Scale would allow it to be used on a routine basis although it has limitations in reflecting all the elements of the environmental impact, nor is it capable of making enough distinction of methods with similar scores.[17] A more detailed pictorial evaluation is provided by the Green Analytical Procedure Index (GAPI) devised by Plotka-Wasylka, which consists of five pentagrams illustrating different steps of the given analytical procedure: sample collection, sample preparation, reagents and solvents, instrumentation and final analytical procedure.[18] The pentagram section is color-coded (green (low impact), yellow (medium impact), red (high impact)) according to the particular criteria of risks to the environment and health. The GAPI pictogram has an easy visualization that makes it easy to compare other methods and extract individual areas that need to be improved. Nevertheless, color coding, which is qualitative and the absence of one numerical score can make ranking methods a challenging task.[19]

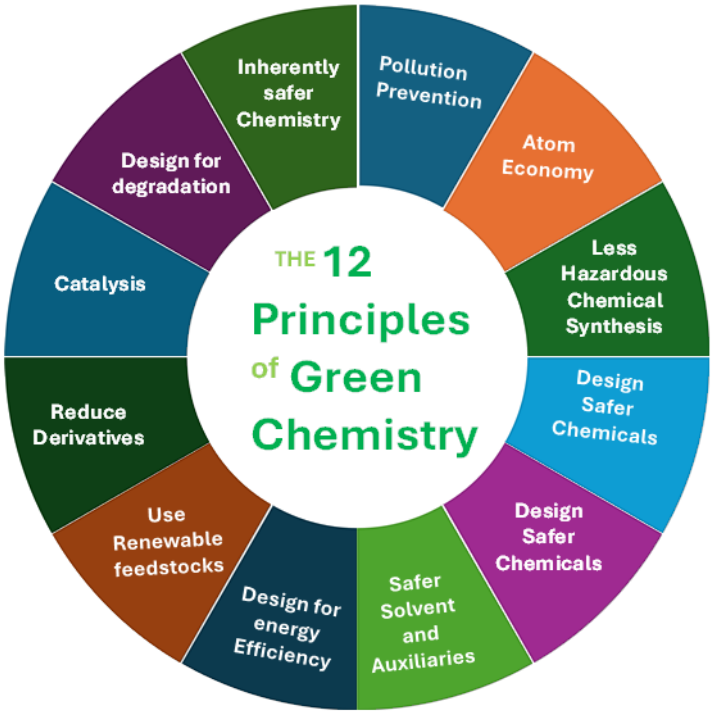


**Figure 1: Twelve principals of green chemistry.**

The Analytical GREEnness (AGREE) metric represents the most recent and comprehensive assessment tool, incorporating all twelve principles of GAC into a single circular pictogram. Each principle is scored on a scale from 0 to 1, with scores displayed as colored segments around a circle, culminating in a central overall score.[20] The AGREE tool employs a sophisticated scoring algorithm that weights different aspects of the analytical procedure and provides detailed rationale for scores assigned. The comprehensive nature of AGREE makes it particularly suitable for thorough method evaluation, though the complexity of scoring may require more time and expertise compared to simpler tools. Recent developments have introduced ComplexGAPI and RGB algorithms that attempt to combine advantages of multiple assessment approaches while addressing their individual limitations.[21]

The choice of a proper green assessment tool is based on a particular situation and the aims of the evaluation. The Analytical Eco-Scale is simple and fast when compared to most other routine quality control laboratories that require quick assessments.[22] Development teams in the method development that need in-depth outlooks on certain areas of improvement might use the stage-by-stage assessment in GAPI. Detailed reports of method validation that are to be presented to the regulators or published could be extended with the detailed evaluation of AGREE of all GAC principles.[23] Increasingly, the researchers are using a combination of tools simultaneously in order to have a complementary view on the greenness of the method, as they realize that the various tools focus on various dimensions of environmental impact. The development of these metrics will represent the maturation of green analytical chemistry as a conceptual framework to a measurable and working discipline that has practical instruments to use in its implementation and perpetual enhancement.[24]

## GREEN SAMPLE PREPARATION TECHNIQUES

Sample preparation is one of the most resource intensive and time consuming processes of analytical procedures and can usually contribute most of the solvent used and waste generated during analytical processes in analytical labs.[25]

The paradigm shift to green sample preparation is oriented to the miniaturization, decreasing or even omitting the use of solvents, and the use of the effective extraction technologies that do not deteriorate or even deteriorate the performance of the analysis and significantly decrease the impact on the environment.[26] Microextraction methods are an example of this method by taking milliliters of sample and milliliters of solvent down to microliters of each, producing concentrations factors that are as large or larger than those of more traditional techniques and producing minimal waste.[27] Liquid-phase microextraction (SPME), first introduced by Pawliszyn, is a solventless method of sampling, that is, it combines the process of sampling, extraction, concentration and sample introduction into a single step.[28] The SPME fiber is loaded with an adequate sample phase through coating with relevant stationary phase, and then subjected to the sample matrix in which the analytes equilibrate their physicochemical characteristics between the sample and the coating. When equilibrium or stipulated time of extraction has been attained, the fiber is pierced out and immersed directly to an analytical device of thermal or solvent desorption. In pharmaceutical analysis, environmental monitoring and food safety, SPME has been extensively used and has been found to have the benefit of being simple, requiring automation, and consuming insignificant amounts of samples. The latest trend in SPME technology is the use of new coating substances, arrow-SPME with increased loading capacity and in-tube SPME designs to handle liquid samples.[29]

Liquid-phase microextraction (LPME) covers the following methods such as single-drop microextraction (SDME), hollow-fiber liquid-phase microextraction (HF-LPME), and dispersive liquid-liquid microextraction (DLLME). In SDME the extracting solvent suspended in a microsyringe microdrop is exposed to the sample and the droplet containing the analysed compounds will partition into the droplet which is then injected into the analysis apparatus. HF-LPME utilizes the use of porous hollow fiber with extracting solvent which offers stability and keeps the organic phase free of interference with the sample matrix.[30] DLLME is one of the most commonly used microextraction methods that entails a quick injection of mixture of extraction and disperser solvents into the water sample, which forms a cloudy solution with massive surface area of mass transfer. The settled extractant phase is collected after the centrifugation and subjected to analysis. The factors that have led to the wide use of DLLME are high enrichment factors, versatility, and speed, and research continues on green disperser solvents and disperser-free methods.[31] The substitution of traditional organic solvents with ecologically friendly ones is one of the pillars of green sample preparation. Deep eutectic solvents (DES), which are a blend of hydrogen bond acceptors and donors in a particular proportion, have become prospective green substitutions, having similar properties to ionic liquids but with the benefit of easier synthesis, reduced cost, and lower toxicity.[32] Natural deep eutectic solvents (NADES) are made of natural substances (sugars, organic acids, amino acids, and choline chloride) and have a high polar solubility and have shown specific application in the extraction of natural products. The ability to make DES properties tunable by choosing the components so that they can be optimized towards a given application and the biodegradability and the low vapor pressure of the solvents handles important environmental challenges of traditional solvents.[33]

Ionic liquids (ILs) are homogeneous mixtures of ions (i.e. all composed of ions), and have distinctive solvent characteristics, such as zero vapor pressure, high thermal stability, and adjustable polarity.[34] Although early-generation ILs had raised toxicity and biodegradability concerns, some advances in biomass-based and biodegradable ionic liquids have been made to retain the beneficial extraction characteristics and tackle the issues of the environment. Supercritical fluid extraction (SFE), especially with carbon dioxide as the supercritical fluid has the benefits in that it provides solvent-free or low solvent extraction along with the benefits of selectivity, fast extraction and direct interface

to analytical devices. Low critical temperature and pressure, and GRAS (Generally Recognized As Safe) status of $CO_2$, ensure that extraction of supercritical $CO_2$ is especially appealing to pharmaceutical and food applications. Small percentages of ethanol and the like can be added, which increases the range of polarity of the supercritical $CO_2$ to extract other polar polarizers.[35,36]

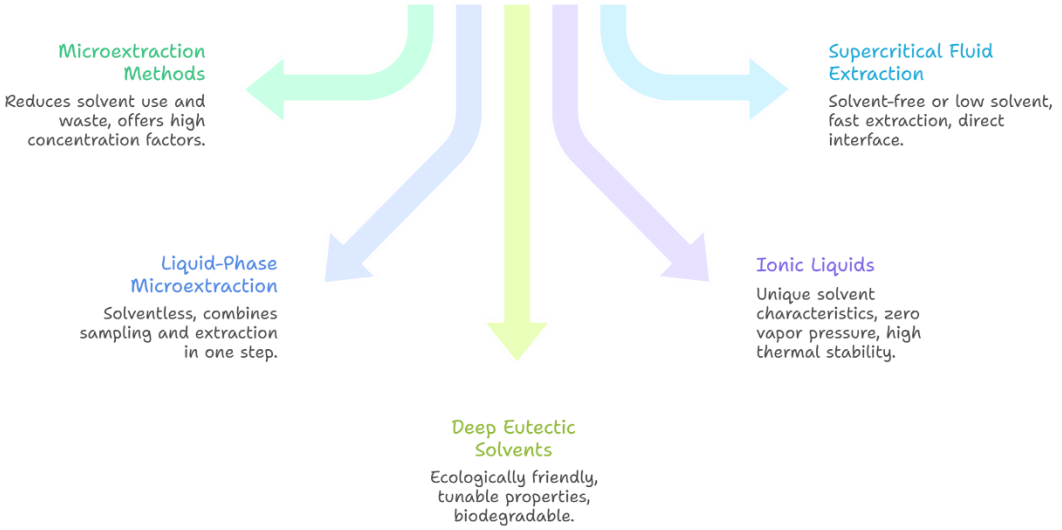


**Figure 2: Green sample preparation techniques.**

**Table 1: Comparison of Green Extraction Solvents - Properties and Applications.**

| Solvent Type | Key Advantages | Typical Applications | Environmental Considerations |
|---|---|---|---|
| Deep Eutectic Solvents | High extraction efficiency, tunable properties, low cost | Natural products, polar pharmaceuticals, metal extraction | Biodegradable, low toxicity, renewable components |
| Ionic Liquids | Negligible vapor pressure, thermal stability, selectivity | Non-polar to polar compounds, high-temperature extractions | Varies by composition; bio-based ILs preferred |
| Supercritical $CO_2$ | Solvent-free, rapid extraction, GRAS status | Essential oils, lipids, thermolabile compounds | Non-toxic, recyclable, low environmental impact |
| Bio-based Solvents | Renewable, biocompatible, readily available | General pharmaceutical analysis, natural products | Biodegradable, sustainable sourcing |

The advanced green extraction technologies utilize sources of energy like ultrasound and microwaves to increase the extraction efficiency and decrease the amount of solvent used as well as the extraction time.[37] Ultrasound-assisted extraction (UAE) uses acoustic cavitation to destabilise cell structures and increase the transfer of masses so that it can be efficiently extracted using less solvent and a shorter extraction time than traditional maceration or Soxhlet extraction.[38] The mechanical impacts of ultrasound such as micro-jets and shock waves formed during cavitation bubble collapse, help in solvent penetration in sample matrices and release of analytes.[39] In micro wave-assisted extraction (MAE) microwave energy is used to warm both the solvent and sample matrix in a short time, enhancing extraction efficiency by promoting rapid warming of both the solvent and sample and disrupting the matrix. The polarization of microwaves towards polar solvents and sample components can offer benefits of extraction selectivity and closed-vessel operation of MAE leads to minimal solvent use and loss of volatile analytes.[40] Pressurized liquid extraction (or accelerated solvent extraction) (PLE) which is also referred to as accelerated solvent extraction, uses a high temperature of pressure to increase the efficiency of the extraction without boiling the solvent out. The enhanced solubility of the analytes at the higher temperatures, and the enhanced mass transfer kinetics and reduced viscosity of the solvent allow one to extract the analytes fast and efficiently with low consumption of solvents as compared to

conventional methods. PLE instruments are automated and thus enable large volumes of sample processing with a minimum operator intervention, which makes them especially appealing to routine lab analytical facilities.[41] An example of green sample preparation using simplification and miniaturization is the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method that was designed to analyze pesticide residues.[42] The procedure is a hybrid of salting-out assisted liquid-liquid extraction and dispersive solid-phase extraction cleanup, requiring little solvents and basic equipment and offering high recovery and clean extracts, which can be analyzed using advanced analytical methods.[43]

## GREEN CHROMATOGRAPHIC METHODS

The most common techniques in analytical chemistry are chromatographic methods, which are used in the pharmaceutical field to separate complex mixtures, which are then crucial in quality, impurity profiling, and stability chemistry. The high level of solvents used in the routine chromatographic analysis has placed this technique in the center stage of the green chemistry efforts. Although HPLC is a powerful device that implies high-performance liquid chromatography with high separation efficiency and flexibility, it is traditionally highly dependent on organic solvents, including acetonitrile and methanol, which pose both environmental and economic risks.[43] Greening of HPLC is centered on various approaches such as mobile phase change, optimization of column technology, and operational parameter change in order to attain radical solvent reduction without affecting the performance of analysis. The most direct way of decreasing the environmental impact of liquid chromatography is mobile phase modification.[44] The use of ethanol or isopropanol, rather than the toxic solvent acetonitrile, which has a pronounced environmental impact and high price, has important green advantages and is still sufficient to achieve good chromatographic performance in many applications.[45] Ethanol which is also a renewable resource offers benefits of being less toxic, biodegradable and less expensive than acetonitrile. But, the viscosity of ethanol is greater and can influence the backpressure in the column and may necessitate a change in flow rates or column diameter.[46] Isoprolanol is not as toxic as acetonitrile but is more viscous and needs to be optimally adjusted to separation conditions. Mobile phases containing a high concentration of water which is made possible by using polar-embedded or polar-endcapped stationary phases consume much less organic solvent and find more and more uses in the pharmaceutical analysis. Specialty phases such as pentafluorophenyl (PFP) and other phases have allowed the use of aqueous mobile phases on a broader scale than reversed-phase separations.[47]

The history of column technology has transformed the efficacy of chromatography and the environmental friendliness. The use of particles less than 2-microns in diameter, called ultra-high-performance liquid chromatography (UHPLC), has a significantly enhanced separation efficiency, resolution and speed in comparison with conventional HPLC.[48] According to the theory, the number of plates produced in one unit length is much higher when the size of the particle is smaller, and short columns can be used that saves 70-90% of analysis time and solvent usage in comparison with the conventional methods.[49] Nevertheless, UHPLC needs a specialized equipment that is able to sustain a pressure greater than 400 bar; which is a considerable capital investment. Core-shell or superficially porous particles provide a tradeoff between the more traditional fully porous particles and sub-2-micron particles, and are more efficient at moderate pressure levels compatible with conventional HPLC equipment.[50] The high diffusion path length of the solid core in a thin porous shell minimizes the diffusion path length, which enhances kinetics of mass transfer and makes the columns shorter and analysis faster without having to make significant changes to the instrument. The monolithic columns are made of single continuous porous rod instead of packed particles, have low backpressure properties, offer high flow

rates and high speed of analysis.[51] The bimodal pore structure of monolithic columns, macropores and mesopores, is effective in convective flow and reaction with analytes respectively, so that the mass transfer is effective at rather high flow rates. Monoliths of organic polymers and silica have been created to be used in different applications with organic monoliths showing merits of stability in pH aspects and silica monoliths possessing more merits of efficiency. The adoption of shorter columns, either traditional, core-shell or monolithic is a simple solution to minimizing the use of solvents and shortening the time of analysis. Development of methods with the basis on necessary resolution, as opposed to optimum resolution, permits the optimization of column length to provide fit-to-purpose separations with minimum use of resources.[52]

Although gas chromatography (GC) is typically more environmentally friendly than liquid chromatography because carrier gases are used, where liquid mobile phases would be used, this technique also has been modified to be green. Substitution of helium carrier gas that is constrained and experiencing higher costs with hydrogen has its benefits of enhanced efficiency, rapid analysis, and decreased environmental effects.[53] Hydrogen has a superior optimal velocity than helium and thus analysis can be carried out more rapidly without the loss of efficiency and sustainability is considered by the fact that hydrogen is naturally abundant and can be produced with relative ease.[54] Nevertheless, such aspects of safety as the possibility of explosion and the necessity of suitable detectors and leakage detecting systems are to be implemented attentively.[55] Vacuum outlet GC and low thermal mass GC are new techniques of lowering energy use and throughput. Vacuum outlet GC does not rely on carrier gas, but rather utilizes vacuum to draw mobile phase through the column, and low thermal mass GC utilizes resistive heating of the analytical column covered by a metal sleeve, which allows rapid temperature programming with a small energy requirement and short analysis time.[56] One of the greenest chromatographic methods is supercritical fluid chromatography (SFC) that uses carbon dioxide as the first mobile phase. Organic solvents are very harmful to the environment; the solvent of $CO_2$ is non-toxic, non-flammable, and abundant fluid, which drastically lowers the cost to the environment in comparison to solvents.[57] In present-day SFC, small percentages of organic modifiers (usually methanol or ethanol) are used to expand the polarity range and enhance the shape of the peaks of polar analytes. Its low viscosity and high diffusivity of supercritical $CO_2$ result in quicker analyses and increased efficiency compared to standard HPLC. SFC has been used specifically in chiral separations, normal-phase type separations, and in the analysis of non-polar to moderately polar compounds, and is increasingly being used in the pharmaceutical development and quality control process. The convenience with which the solvent is removed by depressurization enables fractioning to be collected used in preparative applications and allows coupling directly to mass spectrometry.[58]

**Table 2: Green Chromatographic Modifications - Comparative Benefits.**

| Modification | Solvent Reduction | Analysis Time | Equipment Cost | Applicability |
|---|---|---|---|---|
| UHPLC (sub-2µm) | 70-90% | 5-10x faster | High | Broad, requires new instrumentation |
| Core-shell particles | 50-70% | 3-5x faster | Moderate | Broad, compatible with existing HPLC |
| Short columns | 40-60% | 2-3x faster | Minimal | Method-dependent, may affect resolution |
| Ethanol mobile phase | Variable | Similar | Minimal | Moderate polarity compounds |
| SFC | 90-95% | 2-4x faster | Moderate-High | Non-polar to moderately polar compounds |

TLC and its more recent development, high-performance thin-layer chromatography (HPTLC) have natural green benefits in that little solvent is used and about several samples can be run at once on one plate. The new features of HPTLC have improved the power of planar chromatography by increasing the plate quality, size of particles, automated practice and development as well as advanced detection and quantification. The amount of mobile phase required with HPTLC is orders of magnitude less than the solvent required with HPLC, in terms of milliliters to microliters.[59,60] The method has been re-examined in pharmaceutical analysis, especially in screening mode, herbal medicine analysis and in quality control analysis in resource constrained environments such as where instrumentation access can be limited. Recent advances with forced-flow planar chromatography and overpressured layer chromatography provide only minor improvements in the performance of TLC based methods, without sacrificing the inherent environmental benefits of the techniques.[61]

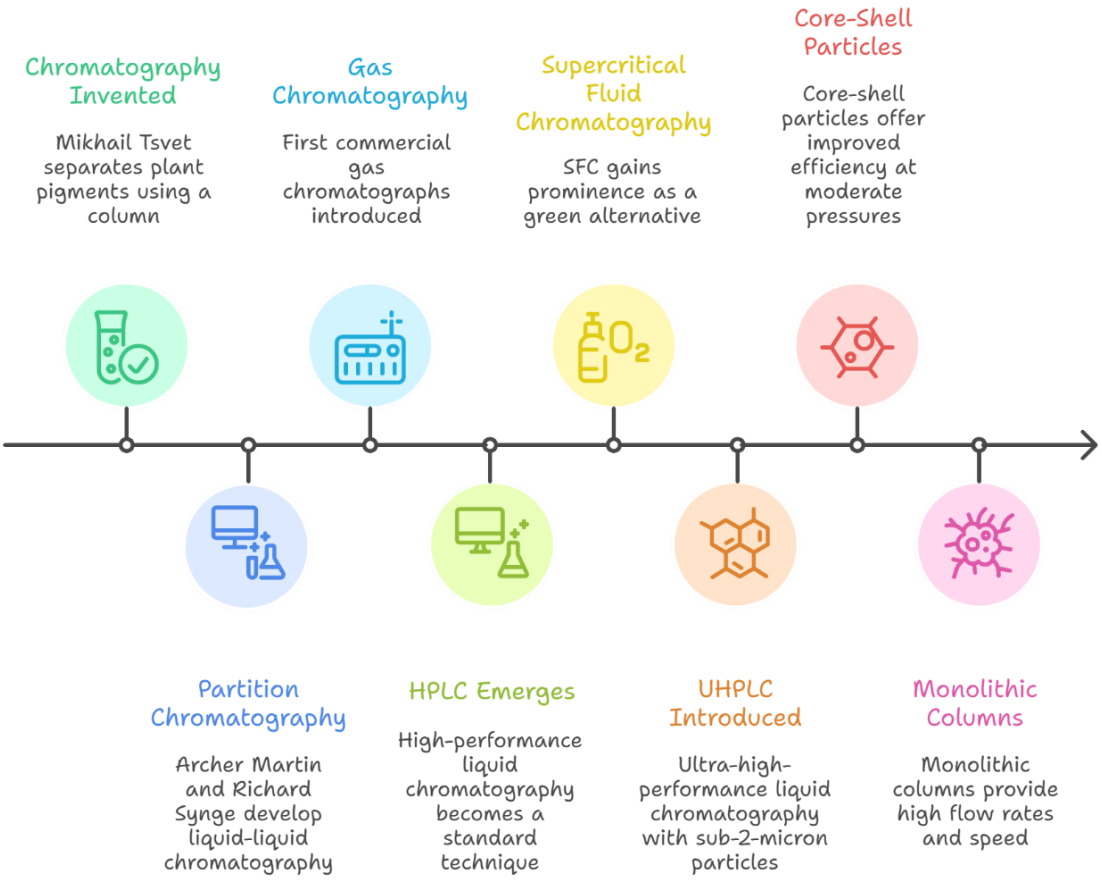


**Figure 3: Green chromatographic methods.**

## GREEN SPECTROSCOPIC AND SPECTROMETRIC TECHNIQUES

Spectroscopic techniques have an intrinsic benefit of green analytical chemistry in that they require little or no sample preparation, non-destructive analysis, and they can be fast and real-time. The near-infrared (NIR) spectroscopy is a typical example of green spectroscopy, where the sample is analyzed without chemical reagents, solvents, and significant sample preparation. The NIR radiation with the wavelengths between 780 and 2500nm detects overtone and combination of molecular vibrations, especially N-H, O-H, C-H bonds common in pharmaceutical molecules.[62] Quantitative analysis of complex mixtures using multivariate calibration models generated by methods like partial least squares (PLS) regression allows the analysis of complex mixtures using NIR spectra without the need to separate the

mixtures by chromatography and therefore without the consumption of solvents.[63] New uses of NIR spectroscopy in pharmaceutical analysis have grown exponentially with Process Analytical Technology (PAT) programs advocated by regulatory bodies. NIR instruments may be set to at-line, on-line, or in-line measurements and this allows real-time monitoring of pharmaceutical manufacturing processes without necessarily taking samples, preparing them, or disposing them.[64] The method has been effectively used to identify raw materials, measure the uniformity of blends, measure the moisture contents, quantify active pharmaceutical ingredients and measure the coating thickness. Portable and handheld NIR instruments have also increased the applications of the method to the field and decentralized quality control. The NIR analysis is non-destructive hence enables recovery and reuse of samples and the short time of measurement improves laboratory throughput. Nevertheless, the necessity of the strong multivariate models that are being constructed based on representative calibration sets is one of the issues that should be carefully developed and validated.[65]

Raman spectroscopy is a technique which uses inelastic scattering of monochromatic light to complement infrared spectroscopy, but it has other green benefits. Raman spectra may be obtained using glass or plastic cells and hence can be analyzed non-invasively without the handling or disposal of samples.[66] The low affinity of water towards Raman spectroscopy enables its analysis of water within an aqueous solution without interference as in the case of infrared where water absorption prevails over spectra. Raman microscopy is capable of spatial resolution to the micrometer scale, heterogeneous samples, contaminants and layers of coating can be analyzed with minimal or no sample preparation.[67] The invention of portable and handheld Raman devices has made it possible to deploy Raman uses to the field by application in the checking of raw materials, counterfeit drugs, and at-line processes. Surface-enhanced Raman spectroscopy (SERS) is a Raman amplification technique that interacts with metallic nanostructures to allow measurement of samples at extremely low concentrations with low amounts of sample. The signal amplification, which can be greater than six orders of magnitude, makes SERS an ultrasensitive detection method that can easily be used to analyze traces with a minute sample and reagent consumption. Pharmaceutical Applications Pharmaceutical API (application) Pharmaceutical API can be used to measure low concentrations of APIs, impurities, and biological samples. Nonetheless, the issues of reproducibility connected to the preparation of SERS substrates and the necessity to closely optimise the conditions of measurements must be considered when developing a method and validating it.[68]

One of the most developed methods of analysis, UV-visible spectrophotometry has been improved with green modifications to increase its scope and minimize resource use. Calculation of first, second, or higher-order derivatives of absorption spectra in derivative spectroscopy allows the individual resolution of overlapping peaks and analysis of multi-component mixtures without separating them chromatographically.[69] The mathematical transformation improves spectral characteristics and minimizes interference due to the baseline drift and broad background absorption and simplifies the analysis of pharmaceutical preparations and biological samples directly. Chemometric data such as the classical least squares (CLS), principal component regression (PCR), and partial least squares (PLS) have metamorphosed the UV-visible spectrophotometry, a method that involves a lot of sample preparation and separation method, into a potent instrument of multi-component examination of complex matrices.[70] Miniaturization of spectrophotometers has promoted the green agenda by minimizing the volume of samples and energy used. Microvolume spectrophotometers have a minimum sample volume of 0.5-2 microliters absorbance which has lowered the amount of sample that is required by hundreds of times, meaning that expensive or limited samples can be analyzed. Flow-through probes and fiber-optic cuvettes allow the in-situ measurement without a significant amount of

samples lost in the process streams. The lights sources created using LEDs instead of the normal deuterium and tungsten-halogen lamps lessen the energy consumption and increase the life of the instruments although they still offer satisfactory performance in the normal applications. Nevertheless, when choosing spectrophotometric methods, restrictions in the sensitivity and selectivity in comparison with chromatographic or mass spectrometric methods should be taken into account in a particular application.[71]

The elimination of chromatographic separation by direct mass spectrometry techniques is a paradigm shift to provide high throughput and rapid analysis with minimum sample preparation.[71] The ambient ionization techniques such as direct analysis in real-time (DART), desorption electrospray ionization (DESI), paper spray ionization, and atmospheric solids analysis probe (ASAP) allow the mass spectrometry of samples in their native condition with minimal or no preparation. DART, which relies on the excited-state helium or nitrogen to desorb and ionize surface analytes, has been used in the pharmaceutical quality control of API quantification, counterfeit drug detection, reaction monitoring.[72]

DESI uses droplets of charged solvents to desorb and ionize surface-bound analyte, allowing intact tablets, biological tissues, and TLC plates to be analyzed in the mass spectrometer. These characteristics of removing chromatographic separation, and minimum sample preparation are capable of reducing the time of analysis by tens of minutes to seconds and producing little waste. Paper spray ionization is a new green method, which integrates the procedure of sample collection, extraction, and ionization into a sheet.[73] The paper substrate is exposed to a small amount of a sample, which is extracted and ionized by supplying them with a high voltage and delivery of solvents into the mass spectrometer. It uses solvent in microliters and allows the analysis of dried blood spots, which opens the possibilities of minimally invasive sampling and easier bioanalytical processing. The paper substrate is disposable and thus does not raise the issue of carryover and allows potentially infectious samples to be handled safely. Although the direct mass spectrometry techniques have significant green advantages, it is important that method validation must be done to guarantee the accuracy and precision of the results similar to conventional methods especially in the cases of quantitative use.[74]

## GREEN ANALYTICAL METHOD VALIDATION

The validation of analytical methodology guarantees the suitability of the procedure to the intended use giving reliable, accurate and repeatable results during the lifecycle of the procedure. Green chemistry implementation into method validation has created opportunities and challenges where it needs to be demonstrated that environmental considerations do not affect analytical performance with the possibility that they may well introduce new validation considerations that are unique to green methods. The validation parameters described by the International Conference on Harmonisation (ICH) guideline Q2(R1), are specificity, linearity, range, accuracy, precision, detection limit, quantification limit, and robustness. These are the basic requirements that cannot be negotiated by green methods, but the plans of proving compliance can be streamlined in order to minimize the use of resources during validation studies. Specificity, the capability to determine the analyte in an unequivocal manner in the presence of other elements that may be anticipated to be available, is a significant validation parameter of the green methods, especially those methods making use of direct analysis or minimum sample preparation.[75] Specificity must be shown to be evaluated by ensuring that an analytical signal of the analyte of interest is obtained with no interference by placebo, degradation products, impurities, or matrix components. Green methods of specificity evaluation involve deliberate choice of exemplary test samples which exercise selectivity of methods without necessarily having to test all possible

interferents. Variations in spectroscopic techniques using multivariate analysis should pay special attention to specificity validation so that the calibration models should not take into consideration the irrelevant spectral variation that can result in inaccurate predictions of future samples. Orthogonal confirmation using alternative methods gives confidence to specificity but without the development of a lengthy method development of the confirmatory process.[76]

Linearity and range validation proves to establish proportionate relationship between analyte concentration and detector response within the indicated concentration range. Linearity assessment take green strategies that aim at designing statistical levels of calibration, making use of the best spacing that maximizes information and minimum calibration points. Linear regression on weights takes into consideration heteroscedastic variance structure of instrumental responses, making the model more accurate over large concentration ranges and does not need extensive replication on every level. When needed in techniques which are sensitive to matrix effects, the preparation of matrix-matched calibration standards can be optimized by pooled matrix preparation, which reduces the amount of blank matrix needed. The practice of using multi-point calibration curves as opposed to single-point calibration or bracket calibration is a green practice that improves quality but may also help reduce the frequency of repeated validation or troubleshooting of methods with demonstrated linearity over large ranges.[77] The proximity of the measured value to the true value is called accuracy which is normally determined using recovery studies using spiked samples or examination of reference materials. It is also possible to use green methods of accuracy validation such as using strategic choice of levels of spiking which are bracketed with the expected range and have fewer replicates than those that are statistically significant. Assessment of traceable accuracy with a small amount of experimental burden is offered by the use of certified reference materials where possible. Standard addition method, the known analyte quantities are added to the sample matrix and the increase in the signal is measured, can be used to assess the accuracy of techniques with matrix effects without the need to prepare large quantities of matrix-matched calibration standards. Nevertheless, standard addition involves delicate experimental design to make sure the added concentrations are rightfully selected and it is possible to make a series of additions to achieve a firm accuracy determination.[78]

Precision validation illustrates the level of dispersion between independent test results that are gathered under the conditions stipulated. The determination of repeatability (intra-day precision), intermediate precision (inter-day, analyst, or equipment variation), and reproducibility (inter-laboratory precision) involves replicate per analysis which consumes reagents, standards, and samples. Green methods of making precision judgments encompass rational specification of the number of replicates to make, basing on statistical power calculations, as opposed to arbitrary specification, and sufficient statistical confidence with minimum replication. The formal validation studies may be supplemented with the help of control charts and moving range calculations made on the basis of regular quality control data, which can give continued precision evaluation without special validation tests. In the case of automated strategies that have proven stability and consistency, replication can be reduced in the validation process and validated by risk evaluation and the use of augmented control measures in routine practice.[79] The detection limit (LOD) and quantitation limit (LOQ) are sensibility limits of the analytical methods where LOD is the lowest concentration of analyte that can accurately be detected and LOQ is the lowest concentration of the analyte that can be quantitatively measured with reasonable precision and accuracy. Limits can be determined using the signal-to-noise method in which the signal of the analyte is compared to the noise of the baseline and limit determination, which uses minimal experimentation. Limits calculated using the statistics of a calibration curve are estimated using the standard deviation of response and slope method which does not require the sample to be prepared at limiting concentrations. In the case

of green methods utilizing preconcentration or extraction, sufficient sensitivity with less sample volumes or extraction solvent volumes has to be proven by proving that concentration factors and recoveries are sufficient to achieve desired limits of detection and quantification.[80]

Robustness testing determines the tolerance of the method to intentional changes in method parameters to determine which factors need to be tight, and which can be relaxed without affecting performance. Factorial designs used in robustness testing have been found to meet the specifications of green chemistry appropriately since they effectively test many factors with a low rate of experimentation. Plackett-Burman design has been able to screen up to seven factors with just eight experiments and allow statistical analysis of the factor importance. AQbD design space approach establishes reasonable operating limits of the parameters of the method considering their effects on the performance of the method, eliminating the extensive robustness testing that would otherwise be necessary to determine those limits. Parameters with less significance to critical method parameters may be acceptable over broader ranges making methods more flexible and less prone to out-of-specification outcomes that require re-analyses. The stability testing of analytical solutions such as sample preparations, standard solutions, and mobile phases use high resources during both validation and life cycle of the method. Green strategies to stability evaluation are faster stability evaluation at high temperature with suitable extrapolation to storage conditions, isometric stability evaluation with lesser time and refrigerator area needed to carry out long-term studies. Laboratory stability testing of stock solutions can be eliminated or minimized by the use of commercially available certified reference standards that have set expiry dates. It should be shown that standards of stability of working procedures and sample preparations are stable under conditions that contain real practice of the laboratory and not consider worst-case conditions that tend to overestimate stability and result in too many preparations. Where unstable analytes or solutions are utilized, the stabilizing conditions or dried standard format increases the quality of the analysis and decreases the amount of wasted solution by developing stable conditions or dried standard forms.[81]

**Table 3: Green Strategies for Method Validation Parameters.**

| Validation Parameter | Traditional Approach | Green Strategy | Resource Savings |
|---|---|---|---|
| Accuracy (Recovery) | Multiple spiking levels with extensive replication | Optimized spike levels with justified replication | 30-50% reduction in standards and samples |
| Precision | Fixed high replicate numbers | Statistical power-based replication | 20-40% reduction in analyses |
| Linearity | Many calibration levels with replication | Optimized spacing with weighted regression | 25-35% reduction in standard preparations |
| Robustness | One-factor-at-time testing | Factorial designs with minimal experiments | 60-80% reduction in experimental burden |
| Stability | Long-term real-time testing | Accelerated testing with predictive models | Reduced storage and time requirements |

Precision validation shows the amount of scattering of the results of independent tests, which were obtained under the conditions stipulated. Repeatability (intra-day precision), intermediate precision (inter-day, analyst, or equipment variation), and reproducibility (inter-laboratory precision) evaluation involve replicate analysis which involves reagents, standards and samples. Green principles in making precision assessments are rational determination of the number of replicates based on statistical power estimates, instead of arbitrary criteria, adequate statistic confidence but no unnecessary replication.[82] Informal validation studies can be used alongside formal validation studies, with the use of control charts and moving range calculations drawn out of regular quality control data, and give continuous assessment of precision without specific validation experiments. In the case of automated techniques that have been

proven, in terms of stability and consistency, less replication in the validation can be supported by risk assessment and increased control measures in routine operation. Sensitivity limits Detection limit (LOD) and quantitation limit (LOQ) define the lowest concentrations of analyte that can be reliably detected and quantified respectively, respectively. Signal to noise (ratio between the signal of the analyte and the noise of the baseline) is an easy-to-implement method of determining limits and needs little experimentation. The standard deviation of response and slope method obtains the limits based on the statistics of a calibration curve, and makes estimates without subjecting the samples to specific limiting concentrations. In the case of green methods using preconcentration or extraction, to evaluate the use of a sufficient sensitivity using less sample volumes or less extraction solvent volumes, the validation must have shown that factors of concentration and recoveries can be used to achieve desirable limits of detection and quantification.[83]

The method validation documentation and waste management is one of the most neglected green improvements opportunities. The use of electronic laboratory notebooks, digital data acquisition systems, and electronic signatures help in saving paper and also increase data integrity and traceability. Use of electronic common technical document (eCTD) format in submitting regulatory documents also does away with bulky paperwork. Practices that include segregation of waste, i.e. halogenated and non-halogenated solvents, aqueous and organic wastes, and recyclable wastes, are used to treat the waste properly and recover the solvents as much as possible. Other laboratories have already adopted solvent recovery systems where HPLC solvents are purified and reused which is quite economical in terms of cost and environmental impact. Green metrics, that track solvent use, waste production, and analysis per-use of energy are established to provide awareness and perceive a continuous improvement in the sustainability of an analysis of analytical laboratories.[84]

## PHARMACEUTICAL APPLICATIONS OF GREEN ANALYTICAL METHODS

Pharmaceutical industry is one of the biggest consumers of analytical services where quality control, quality assurance, research and development as well as regulatory compliance, create colossal analytical load. The use of the green analytical techniques throughout the pharmaceutical lifecycle, including the testing of raw materials to stability tests and post-market surveillance, has immense environmental and economic advantages and does not compromise the high quality standards needed to ensure patient safety. Raw material identification and qualification, the initial stage of quality control in the manufacture of pharmaceuticals has also been taking up the green spectroscopic methods as a replacement of the old wet chemistry methods. Near-infrared spectroscopy offers non-destructive and fast analysis of incoming active pharmaceuticals and excipients, and multivariate models of classification permit the analysis to be positive without chemical reagents or waste.[85] A similar complementary capability is available with Raman spectroscopy, especially in analysis using transparent containers, since there is no need to remove any sample or contaminate the analysis. The active pharmaceutical ingredients in raw materials have been quantitatively analyzed by either titrations or chromatographic techniques, which have traditionally involved dissolution of the sample followed by dilution and the formation of chemical waste. Green methods involve quantitative NIR spectroscopy using chemometric calibration model, UV-visible spectrophotometry using derivative methods to resolve closely related substances and direct mass spectrometry which provides rapid quantification without extensive sample preparation. Handheld or portable instruments allow at-warehouse testing to be done to save time and money that would otherwise be incurred in centralized laboratory tests as well as the release decision that is made instantaneously and enhances efficiency of the chain of supply. Nonetheless, the construction and maintenance of strong multivariate models need an

upfront investment in the development of the methods and constant monitoring of the model so that the accuracy can be maintained.[86]

Green analytical methods that are in line with Process Analytical Technology initiatives have allowed in- process control testing to be carried out in pharmaceutical manufacturing. Blend uniformity, which normally involves sampling, preparation of samples and chromatographic or spectroscopic analysis, can be monitored using NIR chemical imaging, which is able to image API across blend surfaces or inside containers. The real time feedback allows the process to be adjusted before uniformity variation of the batch leads to loss of quality of the batch preventing the risk of creating batch failure and the wastage. Tablet coating processes utilize NIR or Raman spectroscopy as the real-time coating thickness and uniformity monitor instead of the destructive testing techniques that use tablets and need some sample preparation. Using the statistical process control on real-time PAT data allows much tighter process management using less frequent sampling frequency than the old at-line tests. Analysis of content uniformity, dissolution and impurity profile Finished product testing majorly represents a significant analytical load in the pharmaceutical quality control. The green changes to compendial techniques with miniaturization of columns, replacement of solvents with greener ones and optimization of methods have a lower impact on the environment and are equivalent to the processes that were in place before. The reagent consumption and burden on analysts are minimised because of the development of dissolution methods that use biorelevant media and automated sampling systems. The ultra-high-performance liquid chromatography helps to profile impurities faster than traditional techniques, consuming a fraction of the solvent needed by other techniques, and provides higher sample throughput to support quality through testing and less impact on the environment. High-throughput screening followed by confirmatory testing by traditional methods can only be considered when necessary because the application of multivariate spectroscopic methodology to determine content uniformity and direct assay using multivariate spectroscopic techniques is validated by chromatographic reference methods.[87]

One of the most active consumers of analytical services is the pharmaceutical industry wherein quality control, quality assurance, research and development, as well as compliance with regulations produce mammoth-sized workloads of analytical work. Green analytical techniques in the pharmaceutical lifecycle, including testing of raw materials and stability experiments, as well as post-market surveillance, have significant environmental and economic advantages without compromising on the high quality standards necessary in patient safety. The first quality control point in manufacture of pharmaceuticals is the identification and qualification of raw materials and with the advent of green spectroscopic methods, the wet chemistry methods have been slowly being replaced. Near-infrared spectroscopy offers fast and non-destructive analysis of incoming active pharmaceutical ingredients and excipients and multivariate classification models allow positive identification without the use of chemical reagents or the production of waste. Raman spectroscopy has a complementary ability, having specific benefit in analysis using transparent containers, where the sample does not have to be removed and there is less risk of contamination. Quantitative analysis of active pharmaceutical ingredients in raw materials has long been based upon titrations or chromatographic methods, both of which need the solution of a sample, their dilution and creation of a waste product. Green methods include quantitative NIR spectroscopy using chemometric calibration models, UV-visible spectrophotometry with derivative methods to resolve similar compounds, and direct mass spectrometry to determine small quantities of sample without long sample preparation. Through handheld or portable instrumentation, it is also possible to test at the warehouse and thus it saves time and cost that are incurred through the centralized laboratory testing and it also provides the immediate release

decisions that enhance the efficiency of the supply chain. Nevertheless, the output of high quality multivariate models demand an initial investment in the development of methods and subsequent monitoring into models to guarantee sustained accuracy.[88]

The study of impurity profiling and degradation has some specific complications because sensitive determination of low-level impurities and overall description of the degradation pathways are required. Environmentally-friendly methods of impurity analysis such as core-shell columns that support high performance separation under moderate pressure have been utilized, making them easier to apply to existing HPLC plumbing. The creation of techniques that separate all possible impurities, process-related materials, and degradation products in one chromatographic operation leads to fewer analyses and other resources used in comparison to individual specific methods of different impurity types. High-resolution mass spectrometry can give structural data on unknown impurities and thus avoids the possibility of isolation and spectroscopic characterization of degradation products. The type of interpretation of the mass spectrometric data and the fact that this requires specific expertise are however some complications to the everyday application of it. The extreme sensitivity and stringent specificity are necessitated by the analysis of the genotoxic impurities at very low levels as specified by ICH M7 guidelines. Green methods based on miniaturized sample preparation methods such as solid-phase microextraction or microextraction by packed sorbent (MEPS) can offer concentration factors of hundreds and thousands with little solvent usage. Small sample sizes can be coupled to sensitive mass spectrometric detector methods that allow the achievement of nanogram-per-gram detection limits during green extraction methods. Nevertheless, there is a need to be careful when validating at this low concentration in terms of blank contamination and specificity, and there is a long history of demonstration that signals are produced by the analyte and not by artifacts and other interferences.[89]

The green methods of bioanalytical application in pharmaceutical development can be challenged in special terms associated with multi-layered biological matrices, regulatory demands of cross-validation, and a requirement to have a high-throughput capability to fuel clinical trials. Volumetric absorptive microsampling (VAMS) and dried blood spots are types of microsampling methods that require less blood volume and, therefore, can be used to sample with less invasiveness especially when working with pediatric patients. The dried sample format offers stability benefits and it is easier to ship and store than frozen plasma or serum. Green techniques of extraction include protein precipitation with low levels of organic solvent or supported liquid extraction that offer clean extracts that can undergo LC-MS/MS analysis with less negative environmental impacts than the traditional liquid-liquid extraction. Nonetheless, novel regulating bodies demand proving of parity among new methods of sampling or sample preparation and the conventional ones, which augment validation stress on method development.[90]

Herbal medicine and natural product analysis spells opportunities of implementing green method, as it has complex matrices and faces the challenge of standardizing products that are not inherently standardized. This is due to the fact that development of chromatographic fingerprinting techniques that use pattern recognition and multivariate analysis allow quality evaluation and/or assessment to be made based on the overall chemical profile and not on the quantification of marker compounds alone. The extraction of bioactive compounds using deep eutectic solvents that are assisted by ultrasound or microwave and green extraction of plant materials have proven to be efficient and economical in extraction of bioactive compounds without compromising environmental benignity. High-performance thin-layer chromatography offers easy and cost-effective quality control with resources that are limited and are frequent in areas

where traditional medicines are mostly utilized. DNA barcoding as a botanical identification method is a novel addition to chemical analysis that allows one to verify a species with the use of a small amount of samples and zero chemical wastage.[91]

**REGULATORY PERSPECTIVE AND INDUSTRY ADOPTION**

The regulatory environment of analytical techniques of pharmaceutical industry is determined mainly by International Conference on Harmonisation (ICH) guidelines, regional pharmacopoeias, and regulatory agency guidance documents such as FDA, EMA, and so on. The ICH Q2(R1) principle of validation of analytical procedures sets performance criteria of analytical procedures, but does not say much about the greenness of methods, concentrating on the appropriateness to the purpose, but does not address environmental influence. This regulation provides potential and obstacles to the adoption of green analytical methods. There is no particular ban on green methods, which gives leeway to method developers to adopt methods that are environmentally harmless as long as they can perform as well as or better than conventional methods.[92] But the absence of direct support or recommendation of green practices leads to uncertainty and prima facie of regulatory risk which can discourage a take up. The pharmaceutical quality system guidelines, especially ICH Q8-Q11 that deals with pharmaceutical development, manufacturing and analytical procedures, brings in the Quality by Design aspect which is quite in line with the goals of green chemistry. The promotion of the design space technique, understanding of processes, and lifecycle management offer possibilities to show that green analytical methods achieve solid performance within specified operating limits. The analytical procedure lifecycle management concept with its focus on the constant improvement and knowledge management promotes the gradual adoption of greener alternatives as technology becomes more advanced. Nevertheless, the efforts of translating these conceptual frameworks into practical guidelines of green method validation and submission of regulations have not been fully completed.[93]

The regulation bodies in the region are starting to focus on pharmaceutical sustainability more directly, but more on manufacturing operations than on analytical ones. The European Medicines Agency has also taken up more interest in environmental risk assessment of drugs and sustainable manufacturing. New technology program offered by the FDA allows it to engage in early discussions on new practices, such as new analytical technologies that can be beneficial to the green world. Nonetheless, the inflexibility of the pharmaceutical regulation as dictated by patient safety concerns and risk aversion, poses an obstacle to the changes in the previously used analytical methods in case of green alternatives that prove to be of similar performance. The large cost of new methods validation and possible necessity of cross-validation with current procedures causes economic obstacles that can more than offset environmental advantages in the decision-making of pharmaceutical companies. The adoption of green analytical techniques by industries across different pharmaceutical firms is diverse and depends on the corporate sustainability pledge, economic assertiveness, level of regulatory comfort and technical expertise. Huge multinational pharmaceutical firms that have large sustainability programs and resources to develop methodologies have done better in the actual implementation of green methods than the small companies or contract research organization with strict cost limits. The implementation process is usually pragmatic, with special attention to the high-volume routine analyses in which the environmental and economic merits are the most significant and in which experience gained diminishes the risk perception. New product development offers chances of applying green practices at the beginning as opposed to modifying existing techniques of marketed products.[94]

The analytical method regulations within the pharmaceutical sector are laid down mainly via the International Conference on harmonisation (ICH) guidelines, regional pharmacopoeias, and other regulatory agencies such as FDA, EMA and other guidance documents. The ICH Q2(R1) guideline on validation of analytical procedures defines performance criteria on analytical methods but does not specifically address the aspect of the greenness of methods, addressing only its suitability to intended use and not how it affects the environment. Such regulatory framework generates the opportunities and challenges of the green analytical methods adoption. The fact that certain restrictions are not imposed specifically on green methods leaves room to allow method developers to adopt environmentally benign methods, as long as they can show the same or better performance than their conventional counterparts. Nevertheless, the absence of direct support or recommendations on green practices puts it in question and poses the perceived regulatory risk that can prevent its adoption. Quality by Design principles are presented by the pharmaceutical quality system guidelines, especially by the ICH Q8-Q11 that deals with the pharmaceutical development, manufacturing, and analytical processes, but they are close to the green chemistry goals. Promotion of design space strategies, process insight and lifecycle control opens up possibilities of proving that the green analytical tools are strong in terms of performance over specified operating conditions. Analytical procedure lifecycle management, which focuses on constant improvement and knowledge management, considers the presentation of a greener alternative as time progresses, with the advent of technology. Nevertheless, there is still no translation of these conceptual frameworks into the practical guidance regarding the validation of green methods and submission of regulations.[95]

Green methods most frequently embraced in the pharmaceutical industry are the replacement of acetonitrile by ethanols in the reversed-phase HPLC, the use of UHPLC in regular testing, the use of NIR spectroscopy to identify the raw materials, and sample preparation procedures were optimized to minimize solvent use. These alterations entail quite small adjustments to practice and have proved scientific and regulatory acceptability. More radical techniques like direct mass spectrometry, an alternative to chromatography using multivariate spectroscopic techniques, or seeking fundamental modifications to pharmacopoeial processes have fewer barriers to adoption because of technical difficulties, validation overhead, and regulatory ambiguity. The creation of successful case studies where the implementation was successful, accepted by regulations, and reported benefits is a valuable precedent that will make it easier to apply broadly. The adoption of green method is unique to contract research organizations (CROs) and contract manufacturing organizations (CMOs). These organizations are normally run by client specifications and might lack the authority to change the already set analytical processes without the approval of the client. The business approach that focuses on quick transfer of methods, high through-put and less validation load is predisposed towards conservative methods that adopt tried and tested methods as opposed to the adoption of new green options. The CROs and CMOs that actively build green method competencies and portray value propositions to customers as cost reduction, quicker turnaround, and sustainable environment practices can potentially achieve competitive edge, however. The fact that sustainability metrics are included in the request for proposal evaluation of certain pharmaceutical companies is the business incentive to invest in green capabilities of analytical services providers.[96]

Accreditation of laboratories in ISO/IEC 17025 as well as to adhere to the Good Laboratory Practice (GLP) regulations impose further considerations of the implementation of green methods. The accreditation bodies consider the fit and appropriate validation of the methods, yet there is no accreditation standard that deals with the impact on the environment. The ISO 14001 elements of environmental management system and the ISO/IEC 17025 elements of

quality management systems offer a framework on the systematic consideration of the laboratory environmental impact. There are a few accreditation organizations that have started to introduce sustainability factors into their evaluation requirements, but it is not unanimous across organizations and states. GLP regulations are less concerned with the approaches of being green and are more concerned with the integrity of data, protocol compliance, and quality assurance as long as scientific soundness and compliance with regulations are upheld. Economic business case of green analytical methods covers many factors other than direct solvent cost saving. Economic benefits are also obtained through the reduction of the cost of waste disposal, especially that of hazardous waste which needs special disposal. The safety of laboratories in terms of fewer exposures to toxic solvents lowers the risks of occupation-related trauma and expenses. Green technologies also provide business value to the business by enabling faster analysis results which leads to better laboratory throughput and shorter turnaround time, which can be of greater value than direct cost savings. Saving of energy in terms of less HPLC run time, decreased instrument heating needs, and more efficient equipment helps in lowering the costs and reducing the carbon footprint. The intangible benefits that are hard to measure and which are becoming more and more valuable are the improved employee recruitment and retention which are linked to the improved corporate reputation and the strong sustainability programs.[97]

**Table 4: Green Method Adoption - Drivers and Barriers.**

| Factor | Drivers for Adoption | Barriers to Adoption | Strategies to Overcome Barriers |
|---|---|---|---|
| **Economic** | Reduced solvent costs, lower waste disposal, improved throughput | Initial validation costs, equipment investment, perceived risk | Calculate total cost of ownership, pilot in high-volume applications |
| **Regulatory** | Alignment with QbD principles, lifecycle management | Conservative attitudes, lack of specific guidance, cross-validation burden | Early regulatory engagement, robust validation, peer-reviewed publications |
| **Technical** | Improved performance, faster analysis, enhanced safety | Method development challenges, need for new expertise, limited applicability | Training programs, vendor partnerships, phased implementation |
| **Corporate** | Sustainability goals, reputation enhancement, employee engagement | Competing priorities, resource constraints, organizational inertia | Executive sponsorship, success metrics, recognition programs |

## 9. CHALLENGES AND LIMITATIONS

The pharmaceutical sector has regulatory frameworks designed to manage the analytical approaches in their operations that are mainly by guidelines on International Conference on Harmonisation (ICH), regional pharmacopoeias, regulatory agency guidance documents like FDA, EMA and others. The ICH Q2(R1) guideline on validation of the analytical procedures sets performance criteria of analytical procedures but does not say much about the greenness of the methods, merely stating that they fit the purpose of intended use, not necessarily impacting the environment. Such a regulatory framework offers both opportunities and challenges to the adoption of green methodological approaches to analysis. The lack of particular restrictions in green methods gives room to developers of methods in which they can adopt environmentally benign methods so long as they prove to be as effective or even more so than the traditional methods. Nonetheless, the absence of a direct approval or support of the green solutions makes it uncertain and raises perceived regulatory risk that might restrain adoption. The pharmaceutical quality system guidelines, specifically, ICH Q8-Q11 dealing with the pharmaceutical development, manufacturing, and analytical processes, present Quality by Design principles that are quite consistent with the goals of green chemistry. The promotion of design space techniques, process knowledge and lifecycle management sets the position to prove that green analytical techniques can offer strong performances within a set operating range. The idea of lifecycle management of analytical procedures, with

the focus on the constant improvement and knowledge management, will help to implement the greener alternatives progressively as the technology improves. Nevertheless, these conceptual frames are not entirely translated into specifications on how green methods should be approved and submitted to authorities.[98]

The green strategies that have been most widely implemented within the pharmaceutical industry are the use of ethanol, instead of acetonitrile, in reversed-phase HPLC, the use of UHPLC to test regularly, the use of NIR spectroscopy in identifying the raw material, and also optimization of the sample preparation procedures in order to cut down on solvent usage. Such modifications involve comparatively small adjustments in the existing practices and have shown scientific and regulatory approval. Other more radical solutions like direct mass spectrometry, multivariate spectroscopic methods as alternatives to chromatography or paradigm shifts in pharmacopoeial processes are more difficult to adopt because of technical problems, heavy use of validation, and uncertainty about regulation. Building case studies of successful implementation, regulatory acceptance and quantifiable benefits is good precedent which can be used to promote wider use. CROs and CMOs have special issues when it comes to green method implementation. The organizations usually work on the specifications of the clients and may not have the power to alter the laid out analytical procedures without clients consent. The business model that focuses on the transfer of methods quickly, high throughput, and low validity burden encumbers conservative methods that adopt the known ways over adopting the new green ways. Nevertheless, CROs and CMOs that will actively build a set of green method tools and explain their value propositions to the customers in terms of cost reduction, high-speed turnaround, and environmental friendliness could have some competitive advantages. The incorporation of sustainability measures in the considerations of requests to propose by certain pharmaceutical firms generates business pressure on analytical service providers to invest in green capacities.[99]

Green analytical chemistry is an implementation that is experiencing complex challenges that necessitate systematic mitigation measures. Technical constraints come due to the fact that traditional techniques which have been optimized decades to achieve optimality of sensitivity and selectivity might be undermined by green changes. Small scale extraction methods, although consuming less solvent by far, can have low recovery and high deviations. Pharmaceutical applications that need trace impurities to be detected are also a special concern as the issues of sensitivity are exacerbated by the fact that reduced injection volumes in UHPLC or shorter columns would reduce the absolute amounts of analyte reaching detectors. Selectivity issues arise with little sample preparation as the unremoved components of the matrix can disrupt the detection, and that requires a complex instrument, such as high-resolution mass spectrometry. The acceptance of regulations is also not easy because of the lack of knowledge of new technologies and the risk-aversion factor. This leaves lack of pharmacopoeial green methods in doubt and much needs to be done to prove the equivalence to the traditional methods. Economic obstacles entail large sums of money required in capital to obtain sophisticated instrumentation, cost of validation, and cost of opportunity in method development. Particular constraints are on smaller pharmaceutical companies and contract laboratories. Knowledge gaps are a very serious issue in organizations since analytical chemists who are used to the conventional methods might not be convinced by green methods. No specialized knowledge in chemometrics and multivariate analysis might exist and hence training investments. A culture of precedent within an organization causes resistance to change despite it being technically and economically justified to use green methods.[100]

## FUTURE PERSPECTIVES AND EMERGING TRENDS

The future of green analytical chemistry is focused on integration of artificial intelligence, automation, miniaturization and real-time monitoring. Machine learning systems are changing the development of methods away towards an empiric style method development towards predictive, knowledge-based design and can screen thousands of potential methods in-silico with impressive accuracy. Deep learning has been shown to improve spectroscopic pattern recognition, and may allow a direct analysis of multi-component without chromatography separation. Closed-loop optimization systems integrating robotics and AI automatically plan the experiment, perform measurements, and optimize the conditions iteratively, finding optimal conditions through the efficient exploration of multidimensional parameter space towards performance and environmental goals. Applications of nanotechnology provide better performance using magnetic nanoparticles to extract with high speed and nanomaterial sensors that have high sensitivity on low sample volumes. Portable and point of care devices make the analysis capacity a democratic right, and smartphone-based systems and lab-on-a-chip technologies only require nanoliter volumes. The integration of Industry 4.0 allows networked devices that send data to cloud-computers to be used to conduct a more complex analysis and predictive maintenance. Sustainable laboratory development involves energy positive infrastructure, solvent recovery systems, and solvent recovery systems, and full green chemistry training. The creation of sustainability metrics and reporting frameworks allows the tracking of progression, whereas the inclusion in the corporate ESG reporting establishes accountability in the stakeholders. The movement of regulations toward more explicit considerations of the environment such as explicit guidance as well as incentive systems would initiate more rapid adoption without compromising quality that is critical to patient safety.[101,102]

## 12. CONCLUSION

Green analytical chemistry has developed as an idea to a demonstrably useful field with tools, methods and applications that show that environmental sustainability and analytical performance are complementary goals. The objective evaluation is made possible by the development of quantitative assessment tools such as Analytical Eco-Scale, G API as well as AGREE, and significant advances have been made in the green sample preparation, chromatographic adjustments, and the spectroscopic techniques that have brought 50-90 percent reduction in the solvent usage with greater safety and economic gain. Although these are made, there are still some issues such as technical barriers, uncertainty in regulations, economical barriers and gaps in knowledge that demand collaborative efforts between the analytical scientists, pharmaceutical corporations, regulatory bodies, and the scholars. The convergence of green practices with the Quality by Design principles and Process Analytical Technology forms synergies between quality improvement and sustainability and makes analytical chemistry an active participant in environmental solutions and global commitments such as Sustainable Development Goals. Artificial intelligence incorporation, miniaturization, rules and regulations that clearly consider the environmental aspect, and reinforced economic motivations will define future development. The pharmaceutical analytical community is at a place of turning point where environmental sustainability is a marginal issue to center-stage objective alongside accuracy and precision. The process of achieving sustainable analytical chemistry is a process of continually improving itself through small steps as well as breakthroughs, a process that must include the inclusion of analytical quality and environmental sustainability as two complementary values that can serve the needs of the society and at the same time ensure that the quality of the environment is maintained and preserved by the generation to come.

**Authors Contribution**

All authors Contributed Equally to this study

**Conflict of Interest**

Authors declare no conflict of interest regarding this study

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NA

**REFERENCES**

1. Raccary B, Loubet P, Peres C, Sonnemann G. Evaluating the environmental impacts of analytical chemistry methods: From a critical review towards a proposal using a life cycle approach. TrAC Trends Anal Chem, 2022 Feb 1; 147: 116525.
2. S k M, M p G, P M, Paul K, P r HV, Abdo Mohsen MM, et al. Development of a green GC-FID method for residual solvent analysis in paliperidone nanocrystal formulations: Validation and greenness assessment. Talanta Open, 2025 Dec 1; 12: 100520.
3. Kurul F, Doruk B, Topkaya SN. Principles of green chemistry: building a sustainable future. Discov Chem, 2025 Apr 7; 2(1): 68.
4. Maxim L. The Birth of Green Chemistry: A Political History. Sci Technol Hum Values [Internet], 2023 Oct 12 [cited 2026 Jan 5]; Available from: https://journals.sagepub.com/doi/abs/10.1177/01622439231203063
5. Ogbuagu OO, Mbata AO, Oladapo O, Ojo OO, Muonde M. Sustainable Pharmaceutical Supply Chains: Green Chemistry Approaches to Drug Production and Distribution, 2024; 8(4).
6. Ražić S, Arsenijević J, Mračević SĐ, Mušović J, Trtić-Petrović T. Greener chemistry in analytical sciences: from green solvents to applications in complex matrices. Current challenges and future perspectives: a critical review. Analyst, 2023 July 10; 148(14): 3130–52.
7. Kokilambigai KS, Lakshmi KS. Analytical quality by design assisted RP-HPLC method for quantifying atorvastatin with green analytical chemistry perspective. J Chromatogr Open, 2022 Nov 1; 2: 100052.
8. Castiello C, Junghanns P, Mergel A, Jacob C, Ducho C, Valente S, et al. GreenMedChem: the challenge in the next decade toward eco-friendly compounds and processes in drug design, 2023 Mar 20 [cited 2026 Jan 5]; Available from: https://pubs.rsc.org/en/content/articlehtml/2023/gc/d2gc03772f
9. Martínez J, Cortés JF, Miranda R. Green Chemistry Metrics, A Review. Processes, 2022 July; 10(7): 1274.
10. Sajid M, Płotka-Wasylka J. Green analytical chemistry metrics: A review. Talanta, 2022 Feb 1; 238: 123046.
11. Mateusz Nowak P. What does it mean that “something is green”? The fundamentals of a Unified Greenness Theory. 2023 June 19 [cited 2026 Jan 5]; Available from: https://pubs.rsc.org/en/content/articlehtml/2023/gc/d3gc00800b
12. Wojnowski W, Tobiszewski M, Pena-Pereira F, Psillakis E. AGREEprep – Analytical greenness metric for sample preparation. TrAC Trends Anal Chem, 2022 Apr 1; 149: 116553.
13. Manousi N, Wojnowski W, Płotka-Wasylka J, Samanidou V. Blue applicability grade index (BAGI) and software: a new tool for the evaluation of method practicality. Green Chem, 2023; 25(19): 7598–604.
14. Fantozzi N, Volle JN, Porcheddu A, Virieux D, García F, Colacino E. Green metrics in mechanochemistry. Chem Soc Rev, 2023; 52(19): 6680–714.

15. Semysim FA, Hussain BK, Hussien MA, Azooz EA, Snigur D. Assessing the Greenness and Environmental Friendliness of Analytical Methods: Modern Approaches and Recent Computational Programs. Crit Rev Anal Chem, 2025 May 19; 55(4): 670–83.
16. Cetinkaya A, Kaya SI, Ozkan SA. An overview of the current progress in green analytical chemistry by evaluating recent studies using greenness assessment tools. TrAC Trends Anal Chem, 2023 Nov 1; 168: 117330.
17. Ferreira SS, Brito TA, Santana APR, Guimarães TGS, Lamarca RS, Ferreira KC, et al. Greenness of procedures using NADES in the preparation of vegetal samples: Comparison of five green metrics. Talanta Open, 2022 Dec 1; 6: 100131.
18. Mahgoub SM, Mahmoud MR, Binsaleh AY, Almalki MA, Mohamed MA, Nassar HF. Analytical assessment of a novel RP-HPLC method for the concurrent quantification of selected pharmaceutical drugs levodopa and carbidopa using eight greenness metrics comparing to the lean six sigma approach. Sustain Chem Pharm, 2023 Dec 1; 36: 101291.
19. Mansour FR, Płotka-Wasylka J, Locatelli M. Modified GAPI (MoGAPI) Tool and Software for the Assessment of Method Greenness: Case Studies and Applications. Analytica, 2024 Sept; 5(3): 451–7.
20. Hammad SF, Hamid MAA, Adly L, Elagamy SH. Comprehensive review of greenness, whiteness, and blueness assessments of analytical methods. Green Anal Chem, 2025 Mar 1; 12: 100209.
21. Haque SM. Comparative Studies of GAC Metric Tools AGREE, Analytical Eco-Scale, BAGI and WAC RGB Model to Determine Atorvastatin in Pharmaceuticals. Crit Rev Anal Chem, 2025 May 24; 0(0): 1–15.
22. Rizk M, El-Alamin MMA, Elkhalek OA, Shallan AI. Development and validation of a novel Spectrofluorimetric method of oral anticoagulant Edoxaban via derivatization with 9-fluorenyl methyl chloroformate: green assessment of the method by Eco-Scale and ComplexGAPI. BMC Chem, 2022 Nov 23; 16(1): 102.
23. Sinzervinch A, Torres IMS, Kogawa AC. Tools to Evaluate the Eco-efficiency of Analytical Methods in the Context of Green and White Analytical Chemistry: A Review. Curr Pharm Des., 2023 Sept 1; 29(31): 2442–9.
24. Sakur A alhaj, Karman M. Eco-Friendly and Smart Spectrophotometric Approaches for Quality Control Analysis of One of B-Blockers in Their Dosage [Internet]. Rochester, NY: Social Science Research Network; 2023 [cited 2026 Jan 5]. Available from: https://papers.ssrn.com/abstract=4627698
25. López-Lorente ÁI, Pena-Pereira F, Pedersen-Bjergaard S, Zuin VG, Ozkan SA, Psillakis E. The ten principles of green sample preparation. TrAC Trends Anal Chem, 2022 Mar 1; 148: 116530.
26. Câmara JS, Perestrelo R, Berenguer CV, Andrade CFP, Gomes TM, Olayanju B, et al. Green Extraction Techniques as Advanced Sample Preparation Approaches in Biological, Food, and Environmental Matrices: A Review. Molecules, 2022 Jan; 27(9): 2953.
27. González-Martín R, Gutiérrez-Serpa A, Pino V, Sajid M. A tool to assess analytical sample preparation procedures: Sample preparation metric of sustainability. J Chromatogr A, 2023 Sept 27; 1707: 464291.
28. Pena-Pereira F, Tobiszewski M, Wojnowski W, Psillakis E. A Tutorial on AGREEprep an Analytical Greenness Metric for Sample Preparation. Adv Sample Prep, 2022 Aug 1; 3: 100025.
29. Krumplewski W, Rykowska I. New Materials for Thin-Film Solid-Phase Microextraction (TF-SPME) and Their Use for Isolation and Preconcentration of Selected Compounds from Aqueous, Biological and Food Matrices. Molecules, 2024 Jan; 29(21): 5025.
30. Sahragard A, Varanusupakul P, Miró M. Interfacing liquid-phase microextraction with electrochemical detection: A critical review. TrAC Trends Anal Chem, 2022 Dec 1; 157: 116749.

31. Notardonato I, Avino P. Dispersive Liquid–Liquid Micro Extraction: An Analytical Technique Undergoing Continuous Evolution and Development—A Review of the Last 5 Years. Separations, 2024 July; 11(7): 203.
32. Ijardar SP, Singh V, Gardas RL. Revisiting the Physicochemical Properties and Applications of Deep Eutectic Solvents. Molecules, 2022 Jan; 27(4): 1368.
33. López-Flores FJ, Ramírez-Márquez C, González-Campos JB, Ponce-Ortega JM. Machine Learning for Predicting and Optimizing Physicochemical Properties of Deep Eutectic Solvents: Review and Perspectives. Ind Eng Chem Res., 2025 Feb 12; 64(6): 3103–17.
34. Hu Y, Xing Y, Yue H, Chen T, Diao Y, Wei W, et al. Ionic liquids revolutionizing biomedicine: recent advances and emerging opportunities. Chem Soc Rev, 2023; 52(20): 7262–93.
35. Herzyk F, Piłakowska-Pietras D, Korzeniowska M. Supercritical Extraction Techniques for Obtaining Biologically Active Substances from a Variety of Plant Byproducts. Foods, 2024 Jan; 13(11): 1713.
36. Milovanovic S, Lukic I, Horvat G, Novak Z, Frerich S, Petermann M, et al. Green Processing of Neat Poly(lactic acid) Using Carbon Dioxide under Elevated Pressure for Preparation of Advanced Materials: A Review (2012–2022). Polymers, 2023 Jan; 15(4): 860.
37. Mgoma ST, Basitere M, Mshayisa VV, De Jager D. A Systematic Review on Sustainable Extraction, Preservation, and Enhancement in Food Processing: The Advancement from Conventional to Green Technology Through Ultrasound. Processes, 2025 Apr; 13(4): 965.
38. Guo Y, Nan S, Qiu C, Song C, Wu B, Tang Y, et al. Ultrasound-assisted enzymatic extraction of jujube (*Ziziphus jujuba* Mill.) polysaccharides: Extraction efficiency, antioxidant activity, and structure features. Ultrason Sonochem, 2024 Dec 1; 111: 107088.
39. Martins R, Barbosa A, Advinha B, Sales H, Pontes R, Nunes J. Green Extraction Techniques of Bioactive Compounds: A State-of-the-Art Review. Processes, 2023 Aug; 11(8): 2255.
40. Koina IM, Sarigiannis Y, Hapeshi E. Green Extraction Techniques for the Determination of Active Ingredients in Tea: Current State, Challenges, and Future Perspectives. Separations, 2023 Feb; 10(2): 121.
41. Barp L, Višnjevec AM, Moret S. Pressurized Liquid Extraction: A Powerful Tool to Implement Extraction and Purification of Food Contaminants. Foods, 2023 Jan; 12(10): 2017.
42. Martínez-Pérez-Cejuela H, Gionfriddo E. Evolution of Green Sample Preparation: Fostering a Sustainable Tomorrow in Analytical Sciences. Anal Chem, 2024 May 21; 96(20): 7840–63.
43. González-Curbelo MÁ, Varela-Martínez DA, Riaño-Herrera DA. Pesticide-Residue Analysis in Soils by the QuEChERS Method: A Review. Molecules, 2022 Jan; 27(13): 4323.
44. Noureldeen DAM, Boushra JM, Lashien AS, Hakiem AFA, Attia TZ. Novel environment friendly TLC-densitometric method for the determination of anti-coronavirus drugs “Remdesivir and Favipiravir”: Green assessment with application to pharmaceutical formulations and human plasma. Microchem J., 2022 Mar 1; 174: 107101.
45. Imam MS, Batubara AS, Gamal M, Abdelazim AH, Almrasy AA, Ramzy S. Adjusted green HPLC determination of nirmatrelvir and ritonavir in the new FDA approved co-packaged pharmaceutical dosage using supported computational calculations. Sci Rep, 2023 Jan 4; 13(1): 137.
46. Moema D, Makwakwa TA, Gebreyohannes BE, Dube S, Nindi MM. Hollow fiber liquid phase microextraction of fluoroquinolones in chicken livers followed by high pressure liquid chromatography: Greenness assessment using

National Environmental Methods Index Label (NEMI), green analytical procedure index (GAPI), Analytical GREEnness metric (AGREE), and Eco Scale. J Food Compos Anal, 2023 Apr 1; 117: 105131.

47. Dembek M, Bocian S. Stationary Phases for Green Liquid Chromatography. Materials, 2022 Jan; 15(2): 419.
48. Guarducci MA. Development of materials with increasing structural complexity: from chiral stationary phases to covalent organic polymers and metal-organic frameworks. 2024 Dec 20 [cited 2026 Jan 5]; Available from: https://iris.uniroma1.it/handle/11573/1730128
49. Handlovic TT, Armstrong DW. Strategies and considerations to green analytical separations: a review. Environ Chem Lett, 2024 Dec 1; 22(6): 2753–75.
50. [No title found]. J Polym Compos.
51. Mahdi G, Alamdary A, Kaghazian H, Ahmadi N, Mardani R, Gholami A. Biopharmaceutical Analysis by HPLC: Practices and Challenges. Chonnam Med J., 2025 Jan; 61(1): 19–31.
52. Rasheed MA, Perveen S, Qamar S. Theoretical Investigation of the Nonlinear General Rate Model with the Bi-Langmuir Adsorption Isotherm Using Core–Shell Adsorbents. ACS Omega, 2023 Nov 21; 8(46): 43964–77.
53. Maji SR, Roy C, Sinha SK. Gas chromatography–mass spectrometry (GC-MS): a comprehensive review of synergistic combinations and their applications in the past two decades. J Anal Sci Appl Biotechnol, 2023 Dec 30; 5(2): 72–85.
54. Westphal K, Dudzik D, Waszczuk-Jankowska M, Graff B, Narkiewicz K, Markuszewski MJ. Common Strategies and Factors Affecting Off-Line Breath Sampling and Volatile Organic Compounds Analysis Using Thermal Desorption-Gas Chromatography-Mass Spectrometry (TD-GC-MS). Metabolites, 2023 Jan; 13(1): 8.
55. Taylor CW, Bowden SA. What about nitrogen? Using nitrogen as a carrier gas during the analysis of petroleum biomarkers by gas chromatography mass spectrometry. J Chromatogr A., 2023 May 24; 1697: 463989.
56. Pastor K, Ilić M, Vujić D, Ačanski M, Kravić S, Stojanović Z, et al. Gas Chromatography and Mass Spectrometry: The Technique. In: Pastor K, editor. Emerging Food Authentication Methodologies Using GC/MS [Internet]. Cham: Springer International Publishing; 2023 [cited 2026 Jan 5]. p. 3–31. Available from: https://doi.org/10.1007/978-3-031-30288-6_1
57. Chen Y, Qiu J, Xu K, Zhu H, Zhang S, Lu X, et al. Development of a portable gas chromatograph–mass spectrometer embedded with a low-temperature adsorption thermal desorption module for enhanced detection of volatile organic compounds. Analyst, 2025 Jan 27; 150(3): 470–80.
58. Kaplitz AS, Schug KA. Gas chromatography—vacuum ultraviolet spectroscopy in petroleum and fuel analysis. Anal Sci Adv, 2023; 4(5–6): 220–31.
59. Kowalska T, Sajewicz M. Thin-Layer Chromatography (TLC) in the Screening of Botanicals–Its Versatile Potential and Selected Applications. Molecules, 2022 Jan; 27(19): 6607.
60. Singh S, Khan N, Sawant T, Raheja R. Green High-Performance Thin-Layer Chromatography: A Step Towards Eco-Friendly Analysis. Chromatographia, 2025 Apr 1; 88(4): 287–302.
61. Agatonovic-Kustrin S, Wong S, Dolzhenko AV, Gegechkori V, Ku H, Tucci J, et al. Evaluation of bioactive compounds from *Ficus carica* L. leaf extracts via high-performance thin-layer chromatography combined with effect-directed analysis. J Chromatogr A., 2023 Sept 13; 1706: 464241.
62. Spangenberg B, Seigel A, Brämer R. Screening of orange peel waste on valuable compounds by gradient multiple development diode-array high-performance thin-layer chromatography. JPC – J Planar Chromatogr – Mod TLC, 2022 June 1; 35(3): 313–30.

63. Gaur P, Gupta P, Tiwari N, Gupta N, Shanker K. Validation and greenness assessment of HPTLC-densitometric method for simultaneous estimation of six bioactive alkamides in *Piper longum* Linn. root. Microchem J., 2023 Aug 1; 191: 108766.
64. Mehta M, Mehta D, Mashru R. Recent application of green analytical chemistry: eco-friendly approaches for pharmaceutical analysis. Future J Pharm Sci., 2024 July 8; 10(1): 83.
65. Mattarozzi M, Laski E, Bertucci A, Giannetto M, Bianchi F, Zoani C, et al. Metrological traceability in process analytical technologies and point-of-need technologies for food safety and quality control: not a straightforward issue. Anal Bioanal Chem, 2023 Jan 1; 415(1): 119–35.
66. Deluca M, Hu H, Popov MN, Spitaler J, Dieing T. Advantages and developments of Raman spectroscopy for electroceramics. Commun Mater, 2023 Oct 9; 4(1): 78.
67. Siddhanta S, Kuzmin AN, Pliss A, Baev AS, Khare SK, Chowdhury PK, et al. Advances in Raman spectroscopy and imaging for biomedical research. Adv Opt Photonics, 2023 June 30; 15(2): 318–84.
68. Optical photothermal infrared spectroscopic applications in microplastics—comparison with Fourier transform infrared and Raman spectroscopy. In: Molecular and Laser Spectroscopy [Internet]. Elsevier; 2022 [cited 2026 Jan 5]. p. 305–36. Available from: https://www.sciencedirect.com/science/chapter/edited-volume/abs/pii/B9780323912495000077
69. Katamesh NS, Abbas AEF, Mahmoud SA. Four chemometric models enhanced by Latin hypercube sampling design for quantification of anti-COVID drugs: sustainability profiling through multiple greenness, carbon footprint, blueness, and whiteness metrics. BMC Chem, 2024 Mar 18; 18(1): 54.
70. Lackey HE, Sell RL, Nelson GL, Bryan TA, Lines AM, Bryan SA. Practical Guide to Chemometric Analysis of Optical Spectroscopic Data. J Chem Educ, 2023 July 11; 100(7): 2608–26.
71. Mostafa A, Shaaban H. Chemometric Assisted UV-Spectrophotometric Methods Using Multivariate Curve Resolution Alternating Least Squares and Partial Least Squares Regression for Determination of Beta-Antagonists in Formulated Products: Evaluation of the Ecological Impact. Molecules, 2023 Jan; 28(1): 328.
72. Rankin-Turner S, Reynolds JC, Turner MA, Heaney LM. Applications of ambient ionization mass spectrometry in 2021: An annual review. Anal Sci Adv, 2022; 3(3–4): 67–89.
73. Rankin-Turner S, Sears P, Heaney LM. Applications of ambient ionization mass spectrometry in 2022: An annual review. Anal Sci Adv, 2023; 4(5–6): 133–53.
74. Gupta S, Samal N. Application of direct analysis in real-time mass spectrometry (DART-MS) in forensic science: a comprehensive review. Egypt J Forensic Sci., 2022 Mar 17; 12(1): 17.
75. Yu S, Abbas J, Álvarez-Otero S, Cherian J. Green knowledge management: Scale development and validation. J Innov Knowl, 2022 Oct 1; 7(4): 100244.
76. Alam P, Shakeel F, Alshehri S, Alhaiti A, Alqarni MH, Foudah AI, et al. Analysis of the greenness to determine risperidone in commercial products using a green stability-indicating HPTLC method. Arab J Chem, 2024 Apr 1; 17(4): 105693.
77. Rathi R, Kaswan MS, Garza-Reyes JA, Antony J, Cross J. Green Lean Six Sigma for improving manufacturing sustainability: Framework development and validation. J Clean Prod, 2022 Apr 15; 345: 131130.
78. Janicka P, Płotka-Wasylka J, Jatkowska N, Chabowska A, Fares MY, Andruch V, et al. Trends in the new generation of green solvents in extraction processes. Curr Opin Green Sustain Chem, 2022 Oct 1; 37: 100670.

79. Kraus C, Jakubec P, Girhe S, Fellner F, Härtl M, Jaensch M, et al. Adaptation and Engine Validation of an FTIR Exhaust Gas Analysis Method for C1-Based Potential GHG-Neutral Synthetic Fuels/Gasoline-Blends Containing Dimethyl Carbonate and Methyl Formate [Internet]. SAE Technical Paper; 2022 Mar [cited 2026 Jan 5]. Report No.: 2022-01–0569. Available from: https://legacy.sae.org/publications/technical-papers/content/2022-01-0569/
80. Puška A, Božanić D, Nedeljković M, Janošević M. Green Supplier Selection in an Uncertain Environment in Agriculture Using a Hybrid MCDM Model: Z-Numbers–Fuzzy LMAW–Fuzzy CRADIS Model. Axioms, 2022 Sept; 11(9): 427.
81. Ahmed RR, Streimikiene D, Qadir H, Streimikis J. Effect of green marketing mix, green customer value, and attitude on green purchase intention: evidence from the USA. Environ Sci Pollut Res. 2023 Jan 1;30(5):11473–95.
82. Desalegn G, Tangl A. Enhancing Green Finance for Inclusive Green Growth: A Systematic Approach. Sustainability, 2022 Jan; 14(12): 7416.
83. Yin S, Dong T, Li B, Gao S. Developing a Conceptual Partner Selection Framework: Digital Green Innovation Management of Prefabricated Construction Enterprises for Sustainable Urban Development. Buildings, 2022 June; 12(6): 721.
84. Zhang Y, Berhe HM. The Impact of Green Investment and Green Marketing on Business Performance: The Mediation Role of Corporate Social Responsibility in Ethiopia's Chinese Textile Companies. Sustainability, 2022 Jan; 14(7): 3883.
85. Imam MS, Abdelrahman MM. How environmentally friendly is the analytical process? A paradigm overview of ten greenness assessment metric approaches for analytical methods. Trends Environ Anal Chem, 2023 June 1; 38: e00202.
86. da Silva RF, Carneiro CN, do C. de Sousa CB, J. V. Gomez F, Espino M, Boiteux J, et al. Sustainable extraction bioactive compounds procedures in medicinal plants based on the principles of green analytical chemistry: A review. Microchem J., 2022 Apr 1; 175: 107184.
87. Habeeb Rahuman HB, Dhandapani R, Narayanan S, Palanivel V, Paramasivam R, Subbarayalu R, et al. Medicinal plants mediated the green synthesis of silver nanoparticles and their biomedical applications. IET Nanobiotechnol, 2022; 16(4): 115–44.
88. Nair GM, Sajini T, Mathew B. Advanced green approaches for metal and metal oxide nanoparticles synthesis and their environmental applications. Talanta Open, 2022 Aug 1; 5: 100080.
89. Gedda G, Sankaranarayanan SA, Putta CL, Gudimella KK, Rengan AK, Girma WM. Green synthesis of multi-functional carbon dots from medicinal plant leaves for antimicrobial, antioxidant, and bioimaging applications. Sci Rep, 2023 Apr 19; 13(1): 6371.
90. Kirubakaran D, Wahid JBA, Karmegam N, Jeevika R, Sellapillai L, Rajkumar M, et al. A Comprehensive Review on the Green Synthesis of Nanoparticles: Advancements in Biomedical and Environmental Applications. Biomed Mater Devices, 2026 Mar 1; 4(1): 388–413.
91. Cannavacciuolo C, Pagliari S, Frigerio J, Giustra CM, Labra M, Campone L. Natural Deep Eutectic Solvents (NADESs) Combined with Sustainable Extraction Techniques: A Review of the Green Chemistry Approach in Food Analysis. Foods, 2023 Jan; 12(1): 56.
92. Ullah S, Khan FU, Ahmad N. Promoting sustainability through green innovation adoption: a case of manufacturing industry. Environ Sci Pollut Res., 2022 Mar 1; 29(14): 21119–39.

93. Thomas A, Scandurra G, Carfora A. Adoption of green innovations by SMEs: an investigation about the influence of stakeholders. Eur J Innov Manag, 2021 Mar 5; 25(6): 44–63.
94. Nazir R, Gillani S, Shafiq MN. Realizing direct and indirect impact of environmental regulations on pollution: A path analysis approach to explore the mediating role of green innovation in G7 economies. Environ Sci Pollut Res, 2023 Mar 1; 30(15): 44795–818.
95. Cheng Z, Kong S. The effect of environmental regulation on green total-factor productivity in China's industry. Environ Impact Assess Rev., 2022 May 1; 94: 106757.
96. Yin S, Zhao Y. An agent-based evolutionary system model of the transformation from building material industry (BMI) to green intelligent BMI under supply chain management. Humanit Soc Sci Commun, 2024 Apr 1; 11(1): 468.
97. Panagiotidou E, Chountalas PT, Magoutas AI, Kitsios FC. The multifaceted impact of ISO/IEC 17025 accreditation: a sector-specific analysis in civil engineering testing and calibration laboratories. TQM J., 2024 Feb 23; 37(4): 998–1035.
98. Pal K, Chakroborty S, Nath N. Limitations of nanomaterials insights in green chemistry sustainable route: Review on novel applications. Green Process Synth, 2022 Jan 1; 11(1): 951–64.
99. Lane MKM, Rudel HE, Wilson JA, Erythropel HC, Backhaus A, Gilcher EB, et al. Green chemistry as just chemistry. Nat Sustain, 2023 May; 6(5): 502–12.
100. Sheldon RA, Brady D. Green Chemistry, Biocatalysis, and the Chemical Industry of the Future. ChemSusChem, 2022; 15(9): e202102628.
101. Kim J, Kim YM, Lebaka VR, Wee YJ. Lactic Acid for Green Chemical Industry: Recent Advances in and Future Prospects for Production Technology, Recovery, and Applications. Fermentation, 2022 Nov; 8(11): 609.
102. Ncube A, Mtetwa S, Bukhari M, Fiorentino G, Passaro R. Circular Economy and Green Chemistry: The Need for Radical Innovative Approaches in the Design for New Products. Energies, 2023 Jan; 16(4): 1752.