

Review

Advances in Analytical Methods for Quality Control and Authentication of Nutraceuticals: A Comprehensive Review

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Abstract

Nutraceuticals are food-based products that provide health benefits beyond basic nutrition and play an increasingly important role in preventive healthcare. Ensuring their quality, safety, and efficacy is critical as the global market expands. A systematic literature search was conducted in Scopus, Web of Science, and PubMed using keywords such as ‘nutraceuticals,’ ‘functional foods,’ and ‘quality control,’ with studies selected based on their focus on methods for standardization, characterization, and quality assessment. This review summarizes current analytical approaches, including spectroscopic, chromatographic, and techniques for elemental analysis, highlighting their applications in compound identification, quantification, detection of adulterants, and overall quality control. Emerging challenges and future trends, such as the integration of chemometrics and real-time analytical strategies, are also discussed, providing a comprehensive perspective on the evolving field of nutraceutical analysis.

Keywords: nutraceuticals; analytical methods; chromatography; spectroscopy

1. Introduction

Nutraceuticals are food-based products that provide health benefits beyond basic nutrition, including the prevention or management of disease. They encompass a wide range of products, from concentrated nutrients and dietary supplements to herbal preparations, and functional foods [1,2].

Nutraceuticals are often used in the prevention and treatment of chronic diseases [1]. They typically contain bioactive components, usually natural compounds known to support health or aid in disease prevention [1–4]. The term itself merges the words “nutrient” and “pharmaceutical” and was first introduced by Dr. Stephen De Felice in 1989 to describe food-derived agents with potential therapeutic effects [2].

Functional foods like vegetables, fruits, fermented foods with live probiotic cultures and vitamin-enriched products are also categorized as nutraceuticals [3–5]. These fortified food products (foods to which one or more essential nutrients have been added [6]) serve as a bridge between the fields of food science and medicine because they are typically sold in pharmaceutical dosage forms such as pills, injectables, powders, or capsules [7–9].

Herbal extracts, vitamin and mineral supplements, therapeutic beverages, and specially formulated medical foods are also classified as nutraceuticals [6,7]. Their role in supporting health has attracted growing interest across nutrition, pharmaceutical, and clinical research fields [4,7,8].



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Nutraceuticals include a wide range of products and that makes their classification complicated and diverse. Even so, they can be categorized depending on food source, chemical nature, regulatory definition and efficacy [1–9] (see Table 1).

Table 1. Classification of nutraceuticals by food source, chemical nature, regulatory definition, and efficacy [1–9].

Classification	Class	Examples
Food source	probiotics	<i>Lactobacillus d. bulgaricus</i> , <i>Streptococcus thermophilus</i> , <i>Bifidobacterium bifidum</i>
	prebiotics	inulin, fructo-oligosaccharides
	dietary fibers	cellulose, beta-glucans, pectin
	spices and herbs	turmeric, ginger, garlic
	antioxidants	vitamin C, E, carotenoids
Chemical nature	polyunsaturated fatty acids	omega-3, omega-6
	polyphenols	phenolic acid, flavonoids, stilbenes, lignans
	saponins	triterpenoid saponins, steroidal saponins
	phytoestrogen	isoflavones, coumestans, lignans
	carotenoids	lycopene, beta-carotene, lutein, zeaxanthin, astaxanthin
	isothiocyanate	allyl isothiocyanate, benzyl isothiocyanate, sulforaphane
	minerals	calcium, iron, zinc, chromium
* DSHEA-defined	vitamins	multivitamins or individual vitamins like vitamin D and biotin, A, B-complex, C, D, E, K
	amino acids	tryptophan, glutamine
	botanicals or herbs	echinacea, ginger, ashwagandha caffeine, curcumin
Efficacy	live microbials	probiotics
	established nutraceuticals	omega-3, probiotic yogurt, vitamin D supplements
	potential nutraceuticals	novel polyphenols, lesser-known herbs

* “DSHEA-defined” refers to the legal definition of “dietary supplement” as outlined in the Dietary Supplement Health and Education Act of 1994 (DSHEA) [7].

With the growing popularity of nutraceuticals among aging and wellness-focused consumers, it has become essential to ensure product quality, safety, and efficacy through reliable analytical methods [8].

2. Analytical Techniques in Nutraceutical and Functional Food Analysis

Globally, regulatory agencies such as the U.S. Food and Drug Administration (FDA), European Food Safety Authority (EFSA), and World Health Organization (WHO) enforce standards for nutraceutical quality [7–9]. Analytical methods must fulfill pharmacopeial standards (United States Pharmacopeia (USP), European Pharmacopoeia (EP), Japanese Pharmacopoeia (JP) and follow validated protocols for accuracy, specificity, and reproducibility [8,9].

Robust analytical methodologies are essential at every stage, from the discovery of novel bioactive compounds to maintaining batch-to-batch consistency of the finished products. They must guarantee no adulteration or contamination in the final products [10–13].

The complexity of natural products and the diversity of nutraceutical formulations—ranging from capsules and powders to functional foods—demand a comprehensive and multidisciplinary analytical approach. This is critical for ensuring safety, efficacy, and reproducibility [11,12].

Although the target analytes are very different by nature, the key components of analytical evaluation include identification and quantification of active ingredients, detection of impurities, confirmation of botanical identity, stability testing, and batch consistency of the final product [11–14].

Figure 1 illustrates the process of Analytical Characterization (AC), a critical step in evaluating nutraceutical products. AC is a comprehensive process for evaluating nutraceutical products. Its three main objectives are as follows: identification of active compounds, quantification of bioactive ingredients, and quality control to ensure product safety and efficacy. These objectives are achieved through several key steps, including detecting contaminants, confirming botanical identity, assessing stability, and verifying batch-to-batch consistency. Together, these steps ensure that nutraceutical products are safe, effective, and reliable [10].

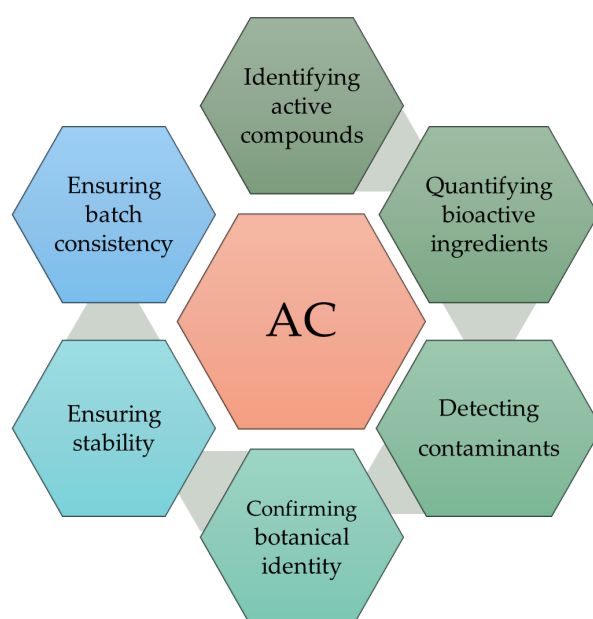


Figure 1. Analytical assessment of nutraceuticals. AC (analytical characterization) refers to the systematic evaluation of nutraceutical products.

The initial step in nutraceutical analysis is the accurate identification of active constituents, which serves as a marker of both authenticity and potency. For example, standardization of *Ginkgo biloba* extracts typically involves quantification of ginkgolides and bilobalide [15]. Routinely used for this purpose is high-performance liquid chromatography (HPLC), often coupled with photodiode array (PDA) or mass spectrometry (MS) [15].

Once identified, precise quantification of active ingredients such as vitamins, polyphenols, and omega-3 fatty acids is critical for dosage standardization and ensuring product efficacy. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is frequently employed for this task due to its sensitivity and specificity [15–17].

Ensuring product safety also requires detection and quantification of harmful contaminants like heavy metals (arsenic, cadmium, lead, mercury), pesticide residues, mycotoxins (in plant-based ingredients), and residual solvents (from extraction) [16].

Inductively coupled plasma mass spectrometry (ICP-MS) is usually the method of choice for trace elemental analysis, while gas chromatography–mass spectrometry (GC-MS) is used for volatile organic contaminants [15–17].

Authentication of botanical ingredients is equally important to prevent adulteration and ensure that the correct plant species and parts are used in the nutraceuticals. This analytical task is carried out by molecular techniques such as DNA barcoding [18].

Shelf life of the products and optimal storage conditions are established by stability studies; this testing is usually carried out by following the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines [10].

3. Broader Analytical Landscape

The complexity and diversity of nutraceutical products, which include plant-based bioactive substances [4,15], minerals [7], vitamins [19], and functional ingredients [18], require a wide range of analytical tools [20–29] to ensure quality, safety, and efficacy. The versatility of analytical techniques and methodologies in nutraceutical research and analysis stems from the need to detect, quantify, and characterize a variety of bioactive compounds in often complex and variable matrices. Although there are many advanced analytical techniques like proton nuclear magnetic resonance spectroscopy ($^1\text{H-NMR}$ [20]), HPLC [22], GC-MS [23], thin-layer chromatography (TLC) [24], spectroscopic techniques such as ultraviolet–visible spectroscopy (UV-Vis) [21] and Fourier-transform infrared (FTIR) [25] remain irreplaceable for rapid and cost-effective assessment of antioxidant compounds and functional groups, particularly in initial screenings or total content evaluations (e.g., phenolic compounds, flavonoids).

High-resolution molecular and elemental analyses are made possible by sophisticated instrumental techniques like nuclear magnetic resonance (NMR) [26], atomic absorption spectroscopy (AAS) [27], and MS—including both ICP-MS and optical emission spectrometry (ICP-OES) [28].

Reviews of biosensor technologies have examined the extent to which biosensors have advanced in analytical capabilities, enabling quick, on-site identification of pollutants and nutrients with high specificity and little sample preparation [30–35].

To authenticate botanical substances and identify adulteration in herbal supplements genomic tools like DNA barcoding and PCR-based assay can be useful but only in combination with appropriate hyphenated chemical methods for quality control [36,37].

The application of chemometric techniques like principal component analysis (PCA) and partial least squares–discriminant analysis (PLS-DA) have improved quality control and product classification by making the interpretation of complex datasets much easier [38].

Recent advances in spectrophotometric and fingerprinting techniques have significantly contributed to the quantification and quality control of nutraceuticals, particularly in the assessment of antioxidants [39], flavonoids [40], and *vitro* and *in vivo* assays for antioxidant evaluation [41–45].

In their critical review, Ignat et al. [46] made a comprehensive evaluation of analytical methods used for characterization of polyphenolic compounds in fruits and vegetables. The authors emphasize that no single method is universally optimal, but they recommend the use of HPLC—particularly coupled with PDA or MS—as the most reliable and widely applicable technique for qualitative and quantitative analysis of individual polyphenols.

HPLC and Ultra HPLC (UPLC) offer significant advantages over other techniques in nutraceutical analysis, including high resolution, sensitivity, and versatility for profiling phenolic compounds [47], enabling validated quantification of phenolic acids in plant-based supplements [48], analysis of antioxidant-rich extracts [49], targeted separation of flavonoid aglycones post-hydrolysis [50], structural elucidation of complex polyphenols [51], and comprehensive identification of anthocyanins and ellagitannins in fruit-based products [52]. HPLC is also effective in tracking carotenoid retention in processed foods [53], assessing fat-soluble vitamins [54], detection of glucosinolates [55] and vitamin C in plant materials [56,57].

HPLC-based profiling remains a cornerstone for comprehensive nutraceutical characterization and quality control in herbal and functional food products; it also has established validation guidelines, ensuring analytical robustness [57,58].

Complementary metabolomics [59] and gas chromatography methods [60] further enhance the detection and quantification of diverse bioactive compounds, including methylsulfonylmethane [61] and other small molecules, while spectroscopic and chromatographic hyphenated techniques provide detailed structural insights of plant phenolics [62,63].

Gas chromatography (GC) with different detection methods (MS, FID) is used for fatty acids [64], essential oils [65], sterols [66], and analysis of tocopherols [67]. Together with advanced chemometric fingerprinting, GC is used for authentication purposes [68–71] and it is the technique of choice for solvent residue analysis [72], ensuring product safety.

Methods like FTIR [73] and Raman spectroscopy [74], combined with chemometric techniques such as PCA and Hierarchical Cluster Analysis (HCA) [75], have greatly enhanced the rapid [76], non-destructive authentication [77] and quantification of bioactive compounds, adulterants [78,79], and phenols in foods [79], herbal medicines, and nutraceuticals [80], while quantitative ^1H NMR provides precise profiling and absolute quantification of complex phytochemicals, supporting robust quality control [81–85].

In the food, pharmaceutical, and agricultural industries, metabolomic techniques, usually in tandem with another analytical tool, are starting to have broad applications—from sample preparation to geographic authentication of herbal medicines [86–88].

Other highly effective analytical tools for quality evaluation and authentication are chemometric methods. They are usually combined with near-infrared (NIR) and infrared (IR) spectroscopy. Their applications expand from non-destructive fruit and vegetable quality assessment [89] to dried herb classification [90], adulterant detection [91], process monitoring, PAT implementation [92], and chemometrics-assisted classification [92–96].

Flame atomic absorption spectrometry (FAAS) remains a widely applied technique for the determination of micro- and macro-elements [97,98], and it has been successfully employed in the elemental analysis of traditional Ayurvedic formulations [99].

Table 2 summarizes most common analytical methods used for evaluation of nutraceuticals, detailing their common analytes and key advantages.

Table 2. Overview of analytical techniques applied in nutraceutical analysis.

Method	Common Analytes	Advantages	References
UV-Vis	polyphenols, antioxidants, vitamins, fingerprinting, total phenolic content	simple, cost-effective, fast, low-cost	[21,41–45]
LC	polyphenols, flavonoids, vitamins, alkaloids, amino acids, carotenoids	separating, identifying, and quantifying compounds in complex matrices, high sensitivity	[15,17,22,47–58]
GC	volatile and semi-volatile compounds (essential oils, aromatic compounds, lipid derivatives)	enhances analytical capability for compound identification	[23,60–65,67–71]
TLC	polyphenols, alkaloids, vitamins	simple, cost-effective, rapid screening, visual detection	[24]
FTIR	polyphenols, vitamins, lipids, proteins, adulterant detection	functional group information, minimal sample preparation	[75,77–80,95]
NIR	proteins, lipids, carbohydrates, polyphenols	rapid, non-destructive, requires no chemical reagents	[25,89–94]
NMR	polyphenols, amino acids, sugars, lipids, Vitamin B6	non-destructive, structure-rich data for compound authentication	[20,25,81–85,88]
AAS	quantifying trace metals	high specificity, relatively low cost	[27,73,74,97–99]

Table 2. Cont.

Method	Common Analytes	Advantages	References
ICP-MS	multi-elemental analysis of trace metals	ultra-low detection limits, wide dynamic range, and isotope ratio capabilities	[28]
ICP-OES	multi-element analysis essential and toxic metals	minimal matrix interference, wide linear range, and relatively fast analysis	[28,29]
Biosensors	glucose, amino acids, vitamins, polyphenols, microbial toxins	high sensitivity, portability, minimal sample preparation, high specificity, rapid analysis	[30–35]
Molecular and Genomic Tools	detect adulteration, trace genetic identity	high specificity, sensitivity	[18,36,37]
Chemometrics	complex data interpretation	enhanced pattern recognition	[45,74,78,92,95]

This extensive toolkit of analytical techniques supports the changing regulatory and customer needs in nutraceutical field. The choice of an appropriate analytical technique in nutraceutical and functional food analysis may look complicated but it largely depends on the chemical nature of the target compounds (see Figure 2).

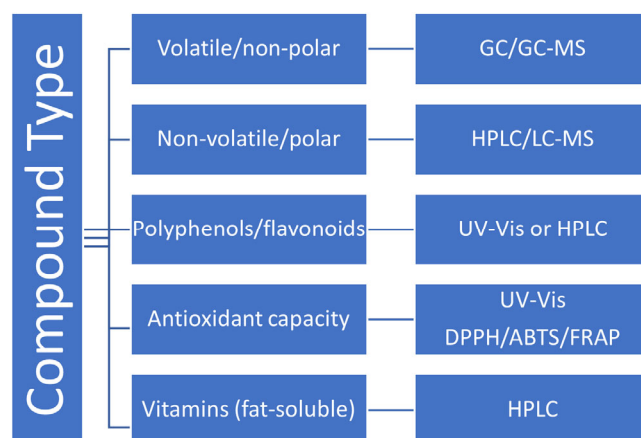


Figure 2. Decision pathway for selecting analytical method in nutraceutical analysis.

As can be seen (Figure 2), GC-MS is ideal for analysis of essential oils and aromatic compounds [23].

Non-volatile/polar compounds such as amino acids, sugars, and certain phenolic acids are usually analyzed with HPLC or LC-MS because these techniques provide accurate separation and quantification, particularly for thermally unstable or polar molecules [22].

Colorimetric assays like 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and Ferric Reducing Antioxidant Power (FRAP) are among the most widely used to estimate the antioxidant capacity [39].

Due to their lipophilicity, fat-soluble vitamins like A, D, E, and K are best quantified with HPLC, which guarantees accuracy and resolution in complex herbal or supplement matrices [54].

After selecting the method for analysis of the key nutraceutical ingredient, determination of shelf life and storage conditions of the nutraceutical is carried out by stability studies or stability testing. These testings adhere to ICH guidelines [10].

The ICH [10] guidelines are important to follow as they ensure that analytical methods used are reliable, reproducible, and scientifically sound. By specifying crucial factors like

accuracy, precision, specificity, linearity, range, detection limit, quantitation limit, robustness, and system appropriateness, the ICH guideline offers a standardized framework for certifying analytical processes. These validation concepts, which were initially created for pharmaceutical industries, are also applicable to nutraceuticals.

According to the ICH guidelines, HPLC methods used in analysis of nutraceutical must be validated [10]. Specificity, linearity, accuracy, precision, LOD (limit of detection), and LOQ (limit of quantification) should be determined, as precision is typically reported as %RSD (relative standard deviation), and recovery studies are performed by spiking known standards into the sample matrix.

A method validation workflow based on ICH guidelines [10] is shown in Figure 3.

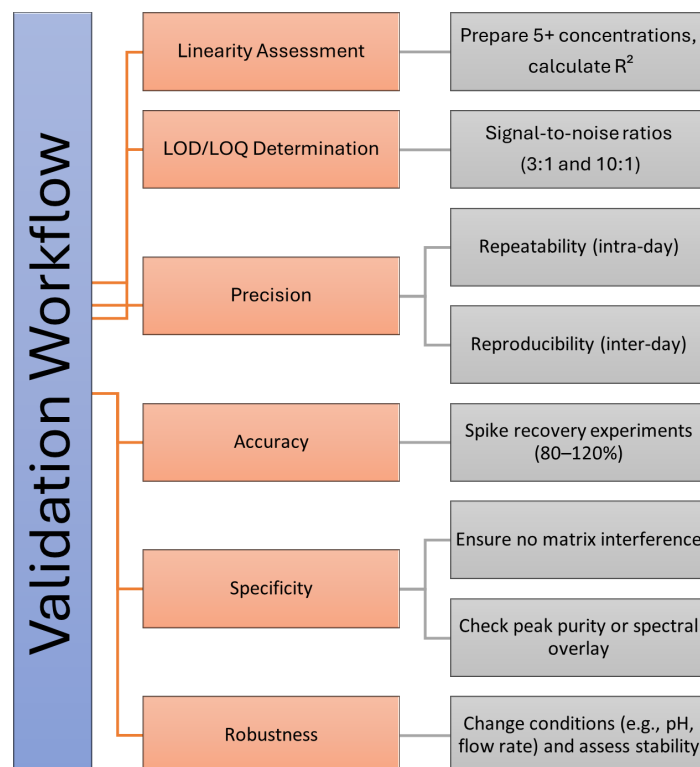


Figure 3. Validation workflow for analytical method assessment. The figure outlines key steps in the validation process, including linearity assessment, LOD/LOQ determination, precision (repeatability and reproducibility), accuracy (spike recovery experiments), specificity (matrix interference checks), and robustness (stability under varying conditions). Each step ensures the reliability and robustness of the analytical method.

Existing reviews in the literature are predominantly focused either on individual analytical techniques [12,17,18,22–39,42,58–60] or on specific classes of bioactive compounds, such as phenols and polyphenols [11,21,47,49,62,63], carotenoids [10–13], antioxidants [41], total flavonoids [44,50] and sterols [66], etc. To date, a comprehensive review systematically integrating spectroscopic, chromatographic, and elemental analysis techniques for the characterization of multi-class bioactive compounds is still lacking. Such an integrated synthesis is essential to support robust quality control, safety assessment, authentication, and regulatory oversight, particularly for complex nutraceutical matrices and multi-ingredient formulations.

Accordingly, the scope of the present review is to provide a systematic overview of established, widely applied, and emerging analytical methodologies for nutraceutical analysis, beginning with spectroscopic techniques (UV–Vis), followed by chromatographic approaches (LC and GC), advanced vibrational and magnetic resonance spectroscopies

(Raman, FTIR, NIR, NMR), and elemental profiling methods. The applicability, strengths, and limitations of each technique are critically discussed with respect to nutraceutical authentication and quality control, while emerging analytical trends and integrative strategies in nutraceutical analysis are also highlighted.

4. Sample Preparation and Pre-Treatment for Quality Control and Authentication of Nutraceuticals

Appropriate sample preparation and pre-treatment are critical for the reliable quality control and authentication of nutraceuticals, as these steps directly influence analytical accuracy, repeatability, and method robustness. Given the chemical complexity of nutraceutical matrices, preparation strategies are selected based on the target analytes, analytical technique, and regulatory requirements [10,19,49].

Solid nutraceutical samples, including tablets, capsules, powders, and botanical materials, are commonly subjected to homogenization, grinding, or milling to ensure sample uniformity. Cryogenic milling is frequently employed to prevent thermal degradation and oxidation of thermolabile constituents, including polyphenols, vitamins, and volatile compounds [62,63]. For spectroscopic fingerprinting approaches such as FTIR and NIR, minimal physical preparation (grinding and homogenization) is preferred to preserve holistic chemical signatures [73,80].

Solvent extraction remains the most widely applied preparation step for chromatographic analysis of nutraceuticals. Hydroalcoholic solvent systems are commonly used for extracting phenolic compounds and flavonoids, whereas non-polar solvents are applied for lipophilic constituents such as fatty acids, carotenoids, and sterols [46,49,63]. Cleanup procedures, including solid-phase extraction, are frequently employed to reduce matrix interferences and enhance method selectivity prior to LC-MS or GC-MS analysis [22,58].

For gas chromatographic analysis, derivatization techniques such as methylation or silylation are often required to improve volatility and thermal stability of analytes, particularly fatty acids and sterols [60,64,70].

The determination of trace elements and heavy metals in nutraceuticals requires rigorous digestion procedures to ensure complete matrix decomposition. Acid digestion, commonly assisted by microwave energy, is widely applied prior to analysis by atomic absorption spectroscopy or inductively coupled plasma-based techniques [27,29]. These pre-treatment steps are essential for accurate quantification and compliance with regulatory contaminant limits [5,19].

Spectroscopic techniques such as FTIR, NIR, Raman, and UV-VIS fingerprinting often rely on minimal sample preparation to maintain the integrity of global chemical profiles used for authentication and adulteration detection [42,73,80]. Typical pre-treatment steps include drying, grinding, and homogenization, followed by direct spectral acquisition. When combined with chemometric analysis, these approaches enable rapid, non-destructive quality assessment [38,73].

DNA-based authentication methods require specific pre-treatment steps to extract amplifiable genetic material from highly processed nutraceutical matrices. Sample preparation typically involves mechanical disruption, chemical lysis, and purification steps designed to remove PCR inhibitors commonly present in herbal extracts and dietary supplements [18,36,37]. Optimized DNA extraction protocols are critical for successful DNA barcoding, metabarcoding, and qPCR-based authentication [37].

Sample preparation procedures used for quality control and authentication must be compatible with validated analytical methods. Validation parameters, including precision, accuracy, specificity, and robustness, are assessed in accordance with ICH Q2(R1) guidelines [10]. Pharmacopeial standards and regulatory guidance from the FDA, EFSA,

WHO, and USP further define acceptable preparation practices, contaminant thresholds, and quality specifications for nutraceutical products [1,5,7–9,19].

5. UV–Vis Spectroscopy

Although we have advanced analytical techniques like HPLC and GC-MS, UV–Vis spectroscopy continues to serve as an accessible, cost-effective, and robust analytical technique. UV-Vis methods are especially valuable for quantifying phenolic compounds [39], flavonoids [40], vitamins [43], and other bioactive compounds in complex matrices [39–43].

Recent developments in UV-Vis analysis have introduced more refined protocols, improved sensitivity, and kinetic-based detection strategies suitable for quality control and standardization of nutraceutical formulations [41].

The assessment of antioxidant and flavonoid content in nutraceuticals is a key component in evaluating their health-promoting properties. Numerous *in vitro* methods have been developed to quantify antioxidant capacity, each based on distinct physicochemical principles and reaction mechanisms. These include electron transfer-based assays such as the Folin–Ciocalteu (FC) assay [21], DPPH, ABTS, FRAP, and cupric reducing antioxidant capacity (CUPRAC), as well as hydrogen atom transfer techniques [39,41].

The review of Apak et al. [39] provide a comprehensive classification of antioxidant capacity assays such as CUPRAC, FRAP, ABTS, and DPPH. These assays exploit electron transfer mechanisms and can be adapted to various matrices, including foods, supplements, and beverages. They typically operate within a wavelength range of 450–750 nm, with distinct absorbance peaks for each assay type. Antioxidant activity assays may be performed using both *in vitro* and *in vivo* models to assess radical scavenging capacity, reducing power, or inhibition of lipid peroxidation. Each assay has its own strengths and limitations, and combining multiple methods can provide a more comprehensive antioxidant profile [39].

Flavonoids, as major contributors to antioxidant activity, are commonly determined via spectrophotometric methods. An optimized and validated method [40] based on the complexation of flavonoids with iron(III) ions allows sensitive quantification with a 200-fold reduction in quercetin concentration during UV–Vis calibration. Such approaches are particularly valuable for routine analysis of herbal extracts and nutraceutical formulations.

The application of UV-visible spectroscopy as a fingerprinting tool in nutraceutical quality control has gained significant attention. Full-spectrum absorbance profiling combined with chemometric tools like PCA, PLS-DA, and cluster analysis enables differentiation among botanical sources, authentication of ingredients, and monitoring of compounds' stability [42]. UV fingerprinting (between 200 and 400 nm) allows the characterization of phenolic profiles without requiring compound-specific standards. When coupled with chemometric analysis, the UV-Vis method enhances authenticity verification and quality control of functional food products.

Farag and colleagues [42] demonstrate that UV fingerprinting paired with multivariate methods (PCA, PLS, HCA) provides a rapid, low-cost, non-targeted approach to authenticate botanical sources, detect adulteration, track phenolic variability across cultivars and regions, and assess sample stability. These strategies offer acceptable accuracy for screening compared to more expensive spectral tools, especially when quantitative results are not required.

Newly emerging kinetic spectrophotometric methods also allow the precise determination of water-soluble vitamins like vitamin C in complex cosmetic or nutraceutical matrices. Lavilla et al. [43] developed such an assay for determination of vitamin C even in complex emulsions like cosmetics and liquid nutraceuticals. The quantification of ascorbic acid is based on the methylene blue photoreduction method. The assay, operating at

664 nm, demonstrated high sensitivity (LOD: 0.04 µg/mL) and was successfully applied to commercial formulations, including emulsions and beverages [43].

Together, these spectrophotometric and kinetic assays serve as indispensable tools in the phytochemical analysis of nutraceuticals, enabling both targeted and untargeted quality control strategies [100].

Despite the widespread use of total flavonoid content (TFC) assays, there are limitations associated with older methods. Recent reviews advocate for revising protocols and minimizing interference from non-target compounds, thus ensuring more accurate phytochemical profiling [44].

In the review by Nicolescu et al. [44] critically revisit the determination of TFC, highlighting the evolution, standardization issues, and limitations of colorimetric assays—especially the aluminum chloride (AlCl₃) method, which remains the most widely used. The authors emphasized on the lack of methodological consistency and challenges in expressing the results due to differing standards (quercetin, catechin) and suggested future directions like hyphenated techniques (LC-MS) and improved validation protocols for more accurate TFC assessment in complex plant matrices.

Another critical mini-review [101] of spectrophotometric methods used for phenolic compounds' determination in nutraceuticals and food products points out some limitations in these approaches—such as a lack of specificity, interference from co-extracts, and calibration challenges. The authors emphasize on the need for complementary analytical techniques to verify the results.

Knez et al. [102] also made a critical evaluation of widely used spectrophotometric assays for measuring antioxidant capacity in plant-based food samples. The authors categorize assays into two categories—single-electron transfer and hydrogen atom transfer methods [102]. As a conclusion, they recommend the combined use of multiple assays. CUPRAC and oxygen radical absorbance capacity (ORAC) demonstrate greater repeatability and chemical stability and according to the authors, they better reflect physiological conditions over traditional methods like ABTS⁺, DPPH, FRAP, and FC that may produce inaccurate results due to interference from amino acids or other food constituents [102].

Despite the shortcomings and the disadvantages that spectrophotometry has, it remains among the most widely used analytical tools in the evaluation of nutraceuticals due to their simplicity, cost effectiveness, and suitability for routine analysis.

Table 3 summarizes seven representative antioxidant capacity assessment methods.

Table 3. Antioxidant capacity assignment methods [21,39,102].

Assay Type	Analytical Reaction	$\lambda_{\max}$, nm
FC	oxidation of phenol compounds in alkaline solution with a molybdotungstophosphate heteropolyanion reagent, (3H ₂ O–P ₂ O ₅ –13WO ₃ –5MoO ₃ –10H ₂ O)	750–765
FRAP	the reaction involves the reduction of Fe(III)—*** TPTZ to Fe(II)—TPTZ	590–595
CUPRAC	Cu(II)–Cu(I) reduction in the presence of a selective Cu(I)-stabilizing ligand neocuproine (2,9-dimethyl-1,10-phenanthroline)	450
Ferric–ferricyanide assay	reduction of ferricyanide (Fe(CN) ₆ ^{3−}) to ferrocyanide (Fe(CN) ₆ ^{4−}) by the antioxidant, followed by the reaction of ferrocyanide with Fe(III) to form Prussian blue	700–750
Ce(IV), Cr(VI), and Mn(VII)	strong oxidizing agents such as Ce(IV), Cr(VI), and Mn(VII) may be used as chromogenic TAC reagents	320 540 535

Table 3. Cont.

Assay Type	Analytical Reaction	$\lambda_{\max}$, nm
DPPH• Radical Scavenging	involves the reaction of DPPH with antioxidant	515
** ABTS/* TEAC	ABTS•+ accepts hydrogen atoms or electrons supplied by antioxidants and it is converted to ABTS (colorless)	734

* TEAC (Trolox Equivalent Antioxidant Capacity) assay. ** ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). *** TPTZ (iron[III]-2,4,6-tripyridyl-S-triazine).

6. Chromatographic Techniques

Liquid chromatography (LC) is a separation technique where components of a mixture are partitioned between a stationary phase and a mobile liquid phase. Its high-performance variant HPLC is a powerful separation technique enabling high-resolution and analysis of complex mixtures. Its flexibility and scalability (from analytical assays to preparative bioprocessing) make HPLC indispensable for nutraceutical quality control and compound isolation [47].

6.1. High-Performance Liquid Chromatography (HPLC)

HPLC is an advanced form of LC that employs high-pressure pumps to push solvents through tightly packed columns, enabling faster, more efficient separations with higher resolution. It is considered the gold standard for determination of nutraceutical constituents due to its sensitivity, reproducibility, and ability to handle complex matrices. HPLC can be equipped with various detectors [46].

Detection of separated analytes is commonly achieved using UV-Vis, PDA or MS detectors, allowing precise qualitative and quantitative analysis. HPLC is especially valuable for thermolabile and non-volatile compounds like polyphenols, vitamins, and alkaloids [22,46–50].

In nutraceutical and functional food analysis, HPLC is frequently used for the quantification of flavonoids via diode-array detection DAD, for vitamin profiling via UV or MS detection [45], and for fingerprinting and quality control when coupled with chemometrics [48].

HPLC is a cornerstone analytical technique in the field of nutraceutical analysis due to its high sensitivity, precision and when it is coupled with detectors such as UV-Vis, DAD, or MS, HPLC offers high sensitivity and specificity [22]. It has become one of the most robust and widely used analytical techniques for the separation, identification, and quantification of phenolic compounds in nutraceuticals and functional foods. Owing to its versatility, sensitivity, and reproducibility, HPLC is the preferred choice in analysis of complex matrices such as plant extracts, dietary supplements, and fortified products [47–52].

Nutraceuticals often contain bioactive compounds derived from plants, animals, or microbial sources [3,7]. These matrices are typically complex, and the target compounds may be present in trace amounts, making accurate detection challenging. HPLC offers robust separation and detection of these analytes without the need for extensive sample preparation, making it a preferred method for routine quality control and research applications [58].

The sample preparation for HPLC analysis typically begins with drying and homogenizing the material to ensure uniformity, followed by extraction. The extract is then clarified by centrifugation or filtration. If needed, the extract is concentrated by evaporating the solvent under reduced pressure and further purified using SPE or LLE to reduce matrix interferences. Finally, the solution is filtered through a membrane to prevent column clogging and is injected into the HPLC system under validated chromatographic conditions [47,48].

Most HPLC methods for analysis of nutraceuticals usually utilize RP columns due to their broad applicability in polar and non-polar compound separation. Gradient elution with mixtures of aqueous buffers (usually acidified water with acetic or formic acid) and organic solvents (such as methanol or acetonitrile) enables the resolution of diverse compound classes. Detection methods include UV-Vis, PDA, fluorescence, and, increasingly, LC-MS for structural elucidation and increased selectivity [45,48,52].

Phenolic compounds are identified by comparing retention times and UV spectra to those of analytical standards. Quantification is performed using external calibration with standard solutions of known concentrations. When reference standards are unavailable, semi-quantitative estimations can be made using structurally similar compounds [47].

Several studies [46–48] demonstrated that RP C₁₈ columns combined with water–acetonitrile or methanol mobile phases acidified with formic or acetic acid provide effective separation of diverse polyphenolic compounds. Detection methods ranged from DAD to MS, enabling both qualitative and quantitative analyses. These methods show high sensitivity and specificity, crucial for characterizing complex plant matrices. HPLC methods for phenolic acids commonly employ acidified water and methanol gradients with C₁₈ stationary phases, achieving efficient resolution and reliable quantification [47–49,58].

Filipiak-Szok et al. [48] used RP-HPLC with PDA for simultaneous quantification of seven biologically active phenolic acids from *Vitis vinifera* dietary supplements; the method proved precise (RSD < 5%; inter- and intra-day variations < 8%), rapid, simple, selective, and specific—successfully analyzing five commercially available Polish grapevine supplements without interference from other components.

Suciu et al. [103] analyzed the polyphenolic compounds in *Lysimachia nummularia* L. At the beginning of the analysis, they used three methods of extraction: Soxhlet, maceration, and ultrasonic-assisted extraction with 70% ethanol. TPC was measured via the FC method; antioxidant activity was assessed using DPPH, ABTS, FRAP, and CUPRAC assays [103]. The HPLC analysis of polyphenolic compounds (gallic acid, 3-O-methylgallic acid, caffeic acid, ferulic acid, chlorogenic acid, p-coumaric acid, and trans-resveratrol) was performed using a reverse-phase HPLC with diode-array detection (RP-HPLC-DAD).

Mizzi et al. [104] used HPLC with a UV-Vis DAD detector, equipped with RP C₁₈ column for analysis of phenolic compounds and flavonoids with overlapping peaks. Despite the overlapping peaks (vanillic and caffeic acid at 21.0 min and ferulic and p-coumaric acid at 24.5 min), the unique absorption spectra of each phenolic compound allow quantification in the mixture by applying compound-specific standardization constants and total absorbance measurements at different wavelengths. UV detection remains standard, but for structural confirmation or overlapping spectra, MS is increasingly used.

Two novel chromatographic methods employing sub-2 µm particle columns—RP HPLC system with a C₁₈ silica-based column and a U-HPLC system using an ethylene-bridged hybrid C₁₈ column—were developed for sequential analysis of 34 phenolic compounds and validated through system suitability metrics before application to real samples like wines and tea [45].

HPLC coupled with Quadrupole Time-of-Flight Mass Spectrometry (qTOF-MS) with a core C₁₈ column was used for two determinations of anthocyanins and other phenolic compounds (phenolic acids, ellagitannins, and flavonoids) in pomegranate juices without a previous hydrolysis of the samples [46].

Carotenoid analysis typically utilizes C₃₀ or C₁₈ columns with non-polar mobile phases such as methanol, methyl tert-butyl ether (MTBE), or acetonitrile [53]. These methods rely on UV-Vis or DAD detection within the 450–480 nm range, matching carotenoids' characteristic absorbance, and occasionally employ MS for detailed profiling.

Both fat-soluble (A, D, E, K) and water-soluble (B-complex, C) vitamins were effectively analyzed using HPLC. Methodologies typically involved buffered aqueous–organic solvent systems with UV or DAD detection (DAD detector, set to 329 nm for vitamin A and 294 nm for vitamin E [54,56,57]). Ion–pair chromatography and derivatization were applied for water-soluble vitamins to enhance detection sensitivity.

Figure 4 illustrates representative chromatographic profiles obtained during the analysis of fat-soluble vitamins in rice cereal baby foods, comparing conventional HPLC-DAD and UHPLC-APCI-MS/MS [57]. The signal intensity (mAU) is plotted as a function of retention time (min), highlighting differences in separation efficiency, sensitivity, and selectivity between the applied analytical approaches.

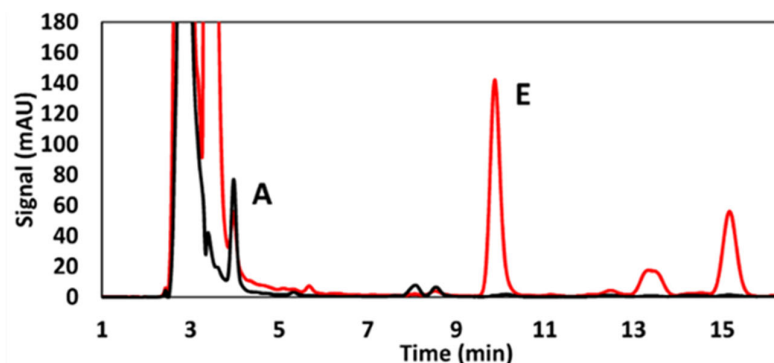


Figure 4. Representative chromatographic profiles obtained during the analysis of fat-soluble vitamins, reproduced from Ref. [57]. Licensed under CC BY 4.0.

For glucosinolates, RP HPLC with acidified mobile phases enabled robust separation. UV detection post-derivatization was commonly employed [55].

The analysis of alkaloids and amino acids often required pre-column derivatization to improve chromatographic behavior and detection, particularly when using UV or fluorescence detection [20,57]. These approaches ensured sensitive quantification in complex matrices such as medicinal plants and protein supplements.

Advanced techniques such as HPLC coupled with tandem MS (HPLC-MS/MS) have emerged as powerful tools in targeted metabolomic profiling, particularly in complex herbal supplements and polyherbal formulations, enabling the simultaneous detection of structurally similar and trace-level compounds with high selectivity [57].

The study by Turova et al. [58] presents a unified strategy for quality control of herbal products, integrating fast ultrasound-assisted extraction, chromatographic separation, and mass spectrometric quantification of bioactive compounds. This approach enables comprehensive evaluation of chemical composition in botanicals, offering enhanced sensitivity (1–50 ng/mL) compared to traditional HPLC–UV methods [58].

Overall, these HPLC methods demonstrate excellent applicability for quality control, compositional analysis, and bioactive profiling in nutraceutical research. The adaptability of mobile phases, stationary phases, and detection systems allows tailored approaches for the diverse chemical nature of nutraceutical compounds (Table 4).

HPLC offers excellent resolution for the separation of structurally similar phenolics, including flavonoids (quercetin, kaempferol) and phenolic acids (gallic acid, ferulic acid). However, co-elution and matrix effects may interfere with accurate quantification. For enhanced selectivity, coupling HPLC with mass spectrometry (LC-MS) has become increasingly popular, especially for targeted metabolomics in nutraceutical research. HPLC is also the method of choice in analysis of carotenoids, vitamins, alkaloids and glucosinolates in plant-based nutraceuticals [53–58].

Table 4. Representative compound classes, food matrices, and chromatographic analytical platforms commonly employed in nutraceutical and food bioactive compound analysis.

Compound Class	Food Matrix	Analytical Platform	References
Polyphenols	Grapes, fruits, vegetables, teas, herbs	HPLC-PDA	[47,48,63,64]
	Grapes, coffee, cereals	UHPLC HPLC-DAD-ESI-MS	[47,48,52]
Flavonoids	Citrus, soy, grape skins	HPLC-UV	[50]
Anthocyanins	Grape skins, berries, red cabbage, purple corn	HPLC-DAD-ESI-MS HPLC-DAD	[52]
Carotenoids	Carrots, tomatoes, leafy greens	HPLC	[53]
Fat-soluble vitamins (A, D, E, K)	Fortified foods, multivitamin supplements	HPLC-DAD, UHPLC-APCI-MS/MS	[54,57]
Water-soluble vitamins (B, C)	Juices, cereals, energy drinks	FIA/HPLC-ED	[56]
Glucosinolates	Broccoli, cabbage, mustard greens, kale	HPLC-MS	[55,58]
Alkaloids	Tea, cocoa, medicinal herbs	HPLC-MS/MS	[53,58]
Amino acids	Protein supplements, legumes, dairy	HPLC-MS/MS	[58]

As a conclusion, we can say that HPLC is a widely employed chromatographic technique for the quantification and identification of phenolic compounds, vitamins, and flavonoids in nutraceuticals. RP-HPLC with C18 columns is the most prevalent configuration, often coupled with UV, PDA, or MS detectors. Gradient elution techniques and careful solvent optimization are essential for achieving adequate separation in complex matrices. HPLC offers high resolution, reproducibility, and sensitivity. Recent advances include UHPLC and green solvent approaches for faster and more eco-friendly separations.

HPLC methods can be time-consuming and costly due to the need for high-purity solvents, standard reference compounds, and regular maintenance of instrumentation; in addition, method development and optimization may be complex, especially for multi-component herbal matrices with potential interference and variability in phytochemical composition. The reviewed literature consistently highlights the versatility and robustness of HPLC as a preferred analytical tool for a broad spectrum of nutraceutical compounds [45–58].

6.2. Gas Chromatography (GC)

Gas chromatography is an analytical technique used to separate and analyze volatile and semi-volatile compounds within complex mixtures, particularly effective for analyzing essential oils, fatty acids, aroma compounds, and other volatile nutraceutical components. Detection is often performed using flame ionization detectors (FIDs) or MS, providing high sensitivity and resolution [23]. A representative chromatogram of GC-FID is shown in Figure 5.

While GC is less frequently employed than HPLC for polar, thermolabile compounds, it plays a critical role in the analysis of essential oils, fatty acids, terpenes, sterols, and organic acids—many of which contribute to the bioactivity of nutraceutical products [59–65]. Table 5 compares sample preparation steps for HPLC and GC analysis of nutraceuticals, highlighting differences related to solvent polarity, derivatization, and preconcentration requirements.

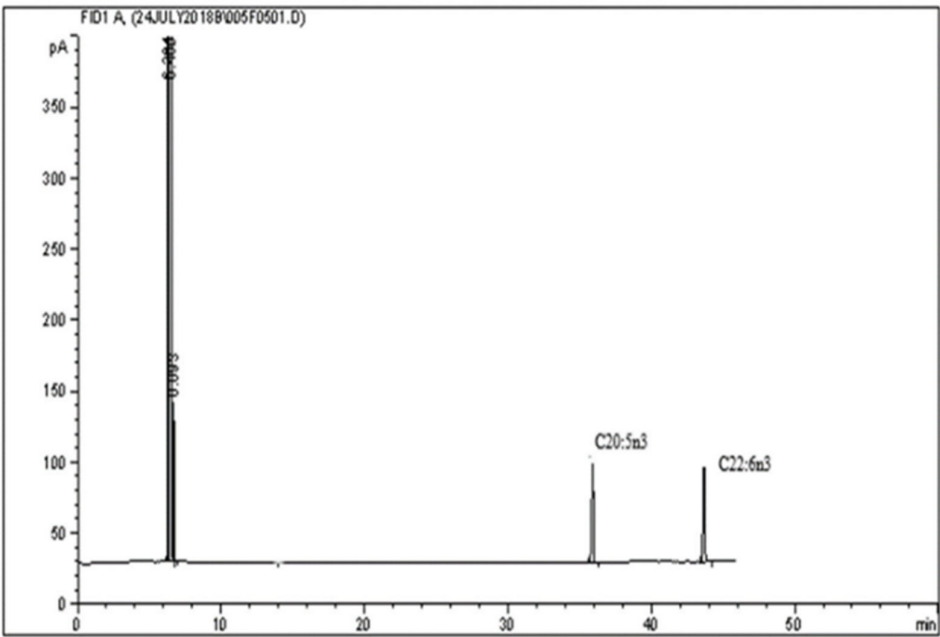


Figure 5. Representative chromatographic profile of fatty acid methyl esters (FAMES) obtained from the analyzed sample [64]. The chromatogram shows a dominant early-eluting peak at approximately 5 min and two resolved peaks corresponding to long-chain polyunsaturated fatty acids at approximately 38.5 min (C20:5n3) and 44.0 min (C22:6n3). Peak intensities are expressed in picoamperes (pA). Reproduced from Ref. [64]. Licensed under CC BY 4.0.

Table 5. Comparison between HPLC and GC in sample preparation.

Step	HPLC Preparation	GC Preparation
Homogenization	sample is homogenized to ensure uniformity	same approach to ensure representative extraction
Solvent Extraction	usually uses polar solvents (methanol, ethanol, water)	typically employs non-polar solvents (hexane, dichloromethane)
Clean-Up	often includes SPE or LLE to reduce matrix effects	used for complex matrices, especially essential oils
Preconcentration	sometimes needed	frequently applied prior to derivatization to improve sensitivity
Derivatization	not generally required	required for non-volatile analytes
Drying (Na ₂ SO ₄)	optional step in aqueous systems	essential to remove residual water before GC analysis
Final Filtration	filtration through 0.22–0.45 µm membrane filters before HPLC injection	Same—to prevent injector and column clogging

GC operates on the principle of separating volatile components based on their partitioning between a stationary phase (usually a capillary column coated with polysiloxane or polyethylene glycol derivatives) and a mobile gas phase (typically helium or nitrogen). Samples often require derivatization—commonly silylation, methylation, or acylation—to enhance volatility and thermal stability, especially for fatty acids [62–65]. These volatile and lipophilic compounds are often critical markers for quality, stability, or efficacy in nutraceutical formulations derived from plants, marine oils, or probiotic-fermented matrices [64].

GC is frequently coupled with flame ionization detection (GC-FID) or MS (Table 6).

Table 6. GC analytes, sample preparation step and detection method.

Target Analytes	Sample Preparation/ Derivatization	Detection Method	References
Essential oils	steam distillation or solvent extraction	FID, MS	[23,65,105,106]
Fatty acids	transesterification to methyl esters	FID, MS	[65,107]
Sterols	saponification and silylation	MS	[66,108]
Aroma compounds	headspace sampling or solvent extraction	MS, FID	[67,70,109]
Fat-soluble vitamins	no	MS	[64,110,111]

GC methods require rigorous validation, especially when applied to regulatory and commercial nutraceutical products. One major challenge is the thermal instability of some bioactive compounds, which can decompose during GC analysis unless properly derivatized or extracted under mild conditions [65,68–70].

Advanced GC technologies such as GC–MS/MS and two-dimensional GC (GC × GC) are emerging in the characterization of complex plant matrices and metabolic profiling. GC–MS is also essential in authenticity testing and contaminant analysis, such as detection of residual solvents, pesticides, or oxidation products [71,72].

GC coupled with flame ionization detection (FID) or MS is widely used for profiling essential oils and aroma-active compounds in herbal extracts and functional foods [105,106]. The method employs non-polar or slightly polar capillary columns with temperature programming to achieve efficient separation of complex volatile mixtures. GC–MS provides structural elucidation and high sensitivity for trace-level compounds [67]. This review of Cagliero et al. [106] highlights the emergence of new GC stationary phases—especially ionic liquids—and advancements like fast and miniaturized (micro-GC) systems as transformative tools for faster, more selective, and energy-efficient essential oil analysis.

Fatty acid profiling, including saturated, monounsaturated, and polyunsaturated fatty acids, is commonly performed by GC following derivatization to fatty acid methyl esters (FAMES) [107]. This derivatization step improves volatility and thermal stability.

GC–FID is the detection method of choice for quantitative analysis due to its robustness and linear response over a wide concentration range. Seppänen-Laakso et al. [107] found that capillary GC with high-quality stationary phases enables sensitive, reproducible profiling of fatty acids, including complex geometric isomers—in human tissues, offering critical insights into dietary fat metabolism and the balance of essential polyunsaturated fatty acids in health.

Schlag et al. [108] proposed a novel gas chromatography with MS selected ion monitoring (GC/MS–SIM) method for the comprehensive analysis of sterols after saponification and silylation. In the proposed method, the vegetable oils were saponified, the unsaponifiable fraction were silylated and analyzed using GC–EI–MS with selected-ion monitoring, using pyrrolidide as an internal standard. This method enabled the precise profiling of 30 sterols and triterpenes [108].

The study of Park et al. [109] developed and validated a sensitive GC–MS/MS analytical method for quantifying 8 priority polycyclic aromatic hydrocarbons (PAHs) across 51 different herbal medicine matrices—employing tailored extraction and purification procedures to ensure accurate detection in diverse sample types.

For the analysis of vitamins D and K, derivatization of the sample is also required, as GC can be used to separate cis/trans isomers of vitamin K [110,111].

The complexity of nutraceutical matrices needs sample pre-treatment steps such as extraction, concentration, and chemical derivatization to enhance volatility and detectability of target compounds. Method validation data reported in the literature indicates excellent

repeatability, sensitivity, and specificity, supporting GC as a reliable technique for routine quality control and research purposes [106–111].

In summary, GC methods provide essential capabilities for the characterization and quantification of volatile and semi-volatile nutraceutical compounds, complementing liquid-chromatography-based approaches. The combination of GC with MS detection broadens the analytical scope, enabling extensive profiling of bioactive components responsible for flavor, aroma, and nutritional value.

Among the new techniques being developed, it is believed that fast-GC/MS will have the most impact in the next decade for food analysis applications [60,61].

Among GC-based approaches, GC–MS is particularly valuable for nutraceutical analysis, as it enables both qualitative identification through spectral libraries and sensitive quantification of volatile bioactive compounds.

6.3. Mass-Spectrometry-Based Detection in Chromatographic Analysis

Mass spectrometry (MS) coupled with chromatographic separation (LC–MS, LC–MS/MS or GC–MS) is another highly employed technique of nutraceutical analysis. Schematic overview of the analytical workflow for analysis of nutraceutical by GC or LC is shown in Figure 6.

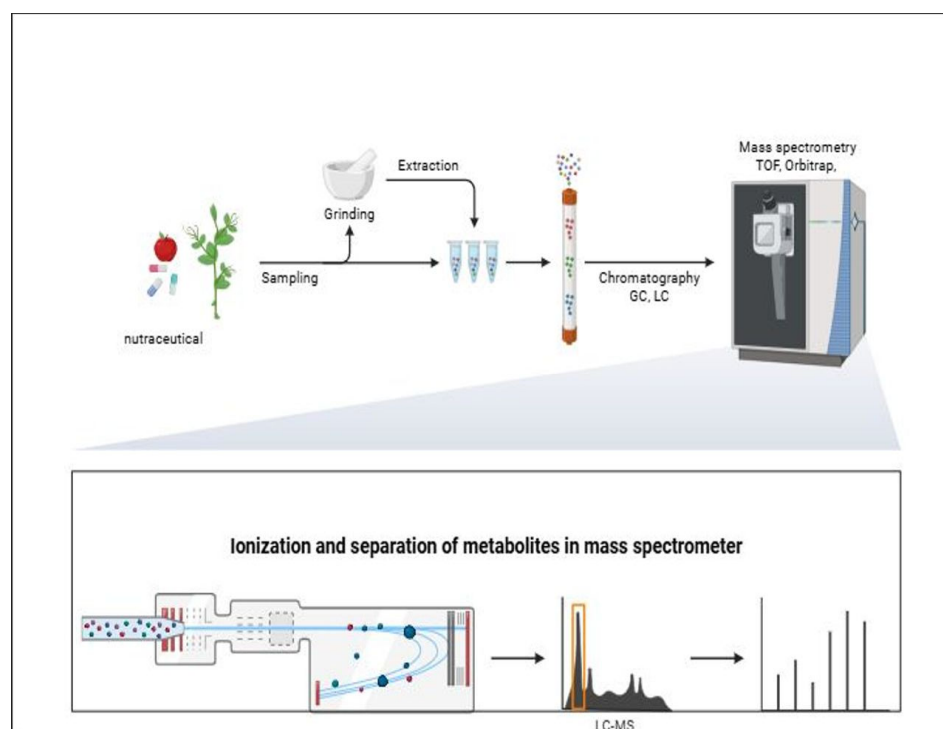


Figure 6. Schematic overview of the analytical workflow for analysis of nutraceutical samples, including sampling, grinding, extraction, chromatographic separation, ionization, and mass spectrometric detection (TOF, Orbitrap). The lower panel illustrates ionization and separation of analytes within the mass spectrometer and subsequent signal detection. Created with BioRender.com.

MS provides superior selectivity and sensitivity compared to optical detectors, enabling confident identification, structural confirmation, and trace-level quantification in complex matrices. LC–MS is ideal for non-volatile and thermolabile compounds [15,17,52,57,58,87], whereas GC–MS is suited for volatile and semi-volatile constituents [67,68,105,108,109], including essential oils, fatty acids, sterols, and aroma compounds.

High-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry has become a powerful analytical platform for the comprehensive char-

acterization of bioactive compounds in complex food and nutraceutical matrices [87]. In this hyphenated technique, chromatographic separation minimizes matrix interferences, while the quadrupole enables precursor ion selection prior to high-resolution mass analysis by the time-of-flight detector. The QTOF analyzer provides accurate mass measurements with high resolving power, allowing reliable elemental composition assignment and structural elucidation of unknown or closely related compounds [87].

High-resolution chromatographic–mass spectrometric platforms such as UPLC–QTOF-MS have been successfully applied in untargeted metabolomics and chemometrics studies of complex herbal matrices, enabling comprehensive profiling and differentiation of phytochemical composition across environmental factors and geographical origins. For example, UPLC-QTOF-MS was employed to profile flavonoids and other metabolites in *Acer truncatum* leaves used as traditional herbal tea, demonstrating its utility for detailed metabolite characterization in a complex plant matrix [87].

7. Raman Fourier-Transform Infrared Spectroscopy (FTIR) Near-Infrared Spectroscopy (NIR)

Raman spectroscopy is an analytical technique that uses scattered light to provide information about the molecular vibrations, structure and chemical composition of a sample. This technique is non-destructive, and the sample can often be analyzed without additional sample preparation steps [112–115].

A rapid, non-destructive, and cost-effective method using Raman microspectroscopy combined with chemometrics (PCA and HCA) to detect L-Cysteine and L-Cystine adulteration in wheat flour was developed by Chebi et al. [74]. They achieved a detection limit of 0.125% (*w/w*) and successfully differentiated authentic from adulterated samples with 100% accuracy within the 500–2000 cm^{-1} fingerprint spectral range.

The quality of essential oils is usually controlled via GC, although with limitations when adulterants are non-volatile substances. Jentzsch et al. [116] demonstrated that Raman spectroscopy, combined with chemometric analysis, PCA can reliably detect non-volatile adulterants in essential oils, offering a rapid, non-destructive method for quality control. They [116] examined the essential oils of *Lavandula angustifolia* Mill., *Mentha piperita* L. and *Pogostemon cablin* Benth, and their adulterated versions were measured by GC coupled with flame ionization detectors (GC-FID) and Raman spectroscopy.

Raman spectroscopy has several advantages over FTIR and NIR, as shown in Table 7.

Table 7. Comparison table between Raman, FTIR and NIR.

	Raman Spectroscopy	FTIR	NIR
Principle	inelastic scattering of light (Raman effect)	absorption of mid-infrared light	absorption of near-infrared light
Spectral Region	532–1064 nm	4000–400 cm^{-1}	780–2500 nm
Target Analytes	polyphenols, flavonoids, pigments, structural compounds	protein, fat, carbohydrates	protein, fat, carbohydrates
Sample Types	powders, tablets, plant tissue, extracts	dried powders, extracts, tablets, raw materials	powders, capsules, whole plant parts, liquids
Sample Preparation	minimal	minimal	minimal
Typical Applications	authentication, adulteration detection, component mapping	authentication, composition analysis, quality control	quantification, quality control

Table 7. Cont.

	Raman Spectroscopy	FTIR	NIR
Strengths	non-destructive, water-insensitive, sharp peaks	rich functional group info, well-established technique	fast, robust for bulk quantification, non-destructive
Limitations	fluorescence interference, weaker signals in some samples	sensitive to moisture	broad peaks, overlapping bands

Each molecule has a unique Raman spectrum; unlike infrared (IR) spectroscopy, Raman can work well in aqueous environments, there is minimal water interference, and the method works for solid, liquid and gaseous samples.

Infrared (IR) techniques such as FTIR and NIR have a pivotal role in rapid, non-destructive screening for food authentication and detection of adulteration [73]. Portable infrared devices combined with multivariate statistical tools (PCA, PLS,) enable real-time, field-based authenticity assessments in food matrices ranging from oils to syrups and dairy [75]. Spectral fingerprinting, focusing on specific absorption bands, allows for the classification of authentic samples vs. adulterated ones with high accuracy—ensuring product integrity and addressing fraud. There is growing integration of machine learning and chemometric approaches with IR data to enhance sensitivity and selectivity in detecting subtle compositional differences among food samples.

FTIR spectroscopy is increasingly used as a rapid, non-destructive, and reagent-free technique for the identification and authentication of nutraceutical products. By measuring characteristic molecular vibrations, FTIR enables qualitative and semi-quantitative analysis of functional groups present in plant-based and formulated products, offering a powerful tool for quality control (QC), adulteration detection, and product standardization [25,73]. Its minimal sample preparation requirements, often just drying or forming a thin film, make it especially useful for routine quality control and authenticity verification of plant-based materials [73–77].

FTIR provides rapid fingerprinting of nutraceuticals and is effective in detecting functional groups such as –OH, –NH, and C=O, identifying key functional groups characteristic of polyphenols, flavonoids, lipids, proteins, and polysaccharides in complex botanical matrices [76]. ATR-FTIR is particularly favored due to its ease of use and capability to analyze solid and semi-solid samples without sample preparation [75,77].

The “fingerprint region” (600–1500 cm^{-1}) in FTIR spectra is particularly useful for differentiating complex matrices without the need for separation or derivatization steps. For example, bioactive components in herbal supplements can be rapidly profiled by comparing their infrared spectra to authenticated references [75,76].

Typical applications include fingerprinting for species authentication, monitoring lipid oxidation states in oils, assessing protein secondary structures, and characterizing carbohydrate compositions [73,76–78].

Recent applications have demonstrated FTIR capability in detecting adulteration in nutraceuticals, such as dilution with starch, synthetic fillers, or substitution with less expensive plant materials [75].

Coupling FTIR with chemometric methods such as PCA and PLS enhances sensitivity and allows for robust classification and quantification [77]. The study of Cahya et al. [77] showed that FTIR-ATR spectroscopy combined with multivariate calibration (PLSR and PCR) as well as discriminant analysis (DA) enables accurate detection and quantification of

synthetic drug adulterants prednisone and metamizole in complex ternary herbal medicine mixtures, providing a rapid, non-destructive, and cost-effective authentication approach.

Another study demonstrated that FTIR spectroscopy combined with chemometric analysis and micro-FTIR imaging can effectively detect and spatially map adulterants in turmeric, confirming that FTIR is a powerful tool for food authentication and quality control [78].

Ensemene et al. [79] established that PLS-FTIR spectroscopy enables rapid, accurate, and solvent-efficient quantification of phenolic acids in medicinal plant extracts, offering a reliable alternative to conventional chromatographic methods.

The study by Choi et al. [95] demonstrates that FT-IR spectroscopy, combined with multivariate analysis, can rapidly and effectively distinguish *Panax ginseng* powder from various root plant adulterants. PCA revealed distinct clusters between pure and adulterated ginseng samples, while PLS models achieved high prediction accuracy for key ginsenosides and aromatic compounds, indicating the feasibility of FT-IR as a tool for quality control in ginseng products [95].

Çebi et al. [112] developed a rapid ATR-FTIR spectroscopic method combined with chemometric techniques (HCA and PCA) to detect illicit adulteration of sibutramine in green tea, green coffee, and mixed herbal tea samples. Using the spectral window from 2746 to 2656 cm^{-1} , the method achieved 100% accuracy in distinguishing between adulterated and unadulterated samples—with no false positives or negatives [112].

In their review, Bunaciu and Aboul-Enein [113] analyze the application of FTIR spectroscopy in detecting adulteration in both chemical and herbal pharmaceutical products. It highlights FTIR's effectiveness in identifying counterfeit drugs, assessing quality control, and monitoring modifications in drug formulations. Coupling FTIR with chemometric methods such as PCA and Cluster Analysis (CA) enhances the accuracy and reliability of adulteration detection.

The FTIR spectra of Figure 7 shows distinct differences between the non-fermented and fermented olive leaf extracts [117]. Key changes in the absorbance peaks indicate alterations in functional groups, which may be attributed to the fermentation process. For example, shifts in the 3283 cm^{-1} and 2924 cm^{-1} peaks in the EtOH extract suggest modifications in hydroxyl and methylene groups, while similar trends can be observed in the EtOAc extracts at 2925 cm^{-1} . These spectral variations highlight the impact of fermentation on the chemical composition of olive leaf extracts [117].

In summary, FTIR spectroscopy is a rapid, non-destructive, and cost-effective method for identifying and quantifying active pharmaceutical ingredients and contaminants/adulterants in both synthetic and herbal drugs. It also proves to be a powerful complementary technique in the multi-analytical toolkit for ensuring the quality, safety, and efficacy of nutraceutical products (Table 8).

Table 8. Target analytes and key information obtained from FTIR analysis.

Target Analytes	Key Information Obtained	References
polyphenols, flavonoids	functional group identification, fingerprinting for authenticity and quality	[79,117]
lipids, fatty acids	degree of saturation, oxidation state, lipid class profiling	[75,118]
proteins, peptides	secondary structure analysis, protein content estimation	[119]
polysaccharides, fibers	characterization of carbohydrate functional groups	[76–78,95]

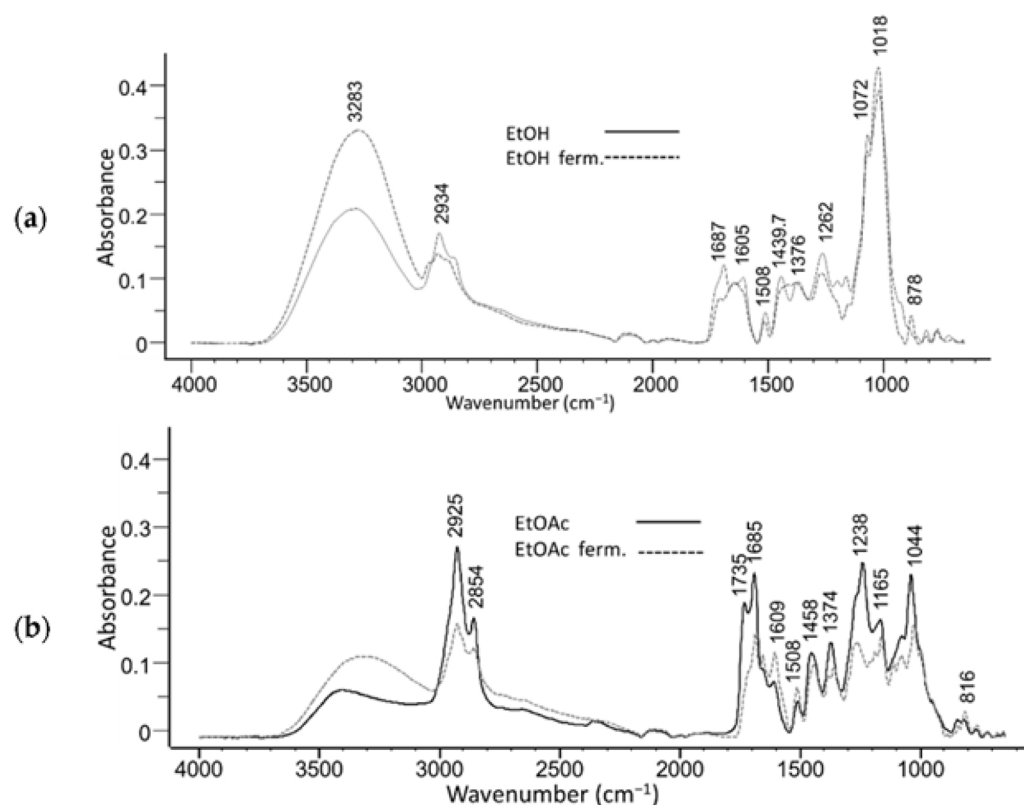


Figure 7. FTIR spectra of non-fermented and fermented olive leaf extracts prepared using ethanol (EtOH) (a) and ethyl acetate (EtOAc) (b). Spectral changes indicate modifications in key functional groups due to fermentation. Reproduced from [117] under CC BY 4.0.

NIR is another rapid, non-destructive, and eco-friendly method favored in nutraceutical quality control that requires minimal to no sample preparation [90]. It analyzes overtone and combination vibrations of C–H, N–H, and O–H bonds, making it effective for handling powders, tablets, and capsules [89,90].

When paired with chemometric modeling, NIR enables authentication and classification of bioactive compounds such as polyphenols, vitamins, and amino acids [91]. It supports the process of analytical technology in manufacturing by delivering real-time, reagent-free analysis [91–93].

A study from 2010 achieved near-perfect correlation ($R^2 > 0.98$) between NIR-PLSR quantification of total phenolics in grape seed supplements and FC assays [94].

NIR can also detect adulteration in herbs. The study of Galvin-King et al. [114] demonstrates that both NIR and FTIR spectroscopy, coupled with chemometric analysis, can effectively detect various adulterants in garlic samples. The models developed for NIR and FTIR data achieved high R^2 and Q^2 values, indicating robust predictive capabilities for identifying adulteration in garlic.

A comprehensive review by Fodor et al. [25] highlights the advancements in NIR spectroscopy over the past two decades, emphasizing its fundamental role in food quality assurance. The authors draw attention to NIR spectroscopy's capability for rapid, non-destructive analysis of various food products, including meats, dairy, baked goods, fruits, vegetables, and luxury items like coffee, tea, and chocolate. The review underscores the integration of chemometric techniques, such as PCA and HCA, which enhance the precision and reliability of NIR-based analyses. Furthermore, the study discusses the evolution of NIR instrumentation and its growing application in detecting food adulteration, determining authenticity, assessing origin, and evaluating maturity. The authors also address the challenges associated with NIR spectroscopy, such as the need for calibration with reference

methods and the variability introduced by sample heterogeneity. Despite these challenges, NIR spectroscopy continues to be a valuable tool in the food industry, offering a balance between efficiency, accuracy, and environmental sustainability.

Table 9 summarizes the major NIR applications in combination with chemometric approaches in nutraceutical analysis, highlighting target analytes, sample sources, and data-processing techniques.

Table 9. Applications of NIR in nutraceutical analysis.

Target Analytes	Source	Data Analysis	References
polyphenols, flavonoids, antioxidants	herbal extracts, powders, functional foods	chemometrics (PCA, PLSR)	[94]
moisture content	plant materials, powders, capsules	multivariate calibration models	[25,91]
authentication and adulteration detection	plant raw materials, commercial supplements	pattern recognition, classification models	[92,95,96]
QC, quantification and classification	plant products, powders	chemometric models for differentiation	[90,92,93]

8. Nuclear Magnetic Resonance (NMR)

NMR is another powerful and non-destructive analytical technique widely used for structural elucidation, quantification, and metabolomic profiling of nutraceuticals [80].

Quantitative ^1H NMR provides a precise, versatile, and non-destructive method for the accurate quantification of natural products without the need for reference standards [81–83].

Advantages such as minimal sample preparation, absolute quantification without reference standards, and insightful data on molecular environments are making NMR ideal for identifying active compounds and monitoring formulation integrity [25,81–83]. It excels in elucidating structures of polyphenols, alkaloids, carotenoids, and terpenoids. Using ^1H and ^{13}C NMR enables the characterization of curcuminoids in turmeric supplements with high specificity [83,85,89].

Quantitative NMR is increasingly trusted in regulatory and industrial applications. NMR-based metabolomics also allows fingerprinting of extracts, their geographic origin discrimination, and adulteration detection through profiles analyzed via PCA and OPLS-DA [86,87]. One study even used ^1H NMR spectra to distinguish authentic ginseng from spiked samples [88].

Figure 8 presents quantitative qHNMR spectra of *Pueraria lobata* extracts obtained by methanolic sonication (PLs) and methanolic reflux (PLr), compared with seven major reference isoflavones (1–7) [85]. Panel (a) shows expanded spectral regions corresponding to structurally diagnostic proton environments, while panel (b) displays the full spectral range, illustrating overall matrix complexity and signal distribution [85].

In the expanded spectra (Figure 8a), the downfield aromatic region (δ ~6.5–8.5 ppm) contains well-resolved signals arising from protons on the A, B, and C rings of the isoflavone skeleton. These resonances are highly characteristic and structurally specific, making them particularly suitable for both identification and quantification by qHNMR.

The reference isoflavones (1–7) exhibit sharp, well-defined singlets and doublets in these regions, reflecting their high purity and simple chemical environments. Corresponding signals are clearly observable in both PLs and PLr extracts, confirming the presence of these isoflavones in the complex botanical matrix. Notably, the PLr extract shows higher signal intensities and improved resolution in the aromatic region compared to PLs, indicat-

ing enhanced extraction efficiency under reflux conditions and reduced signal suppression by matrix components.

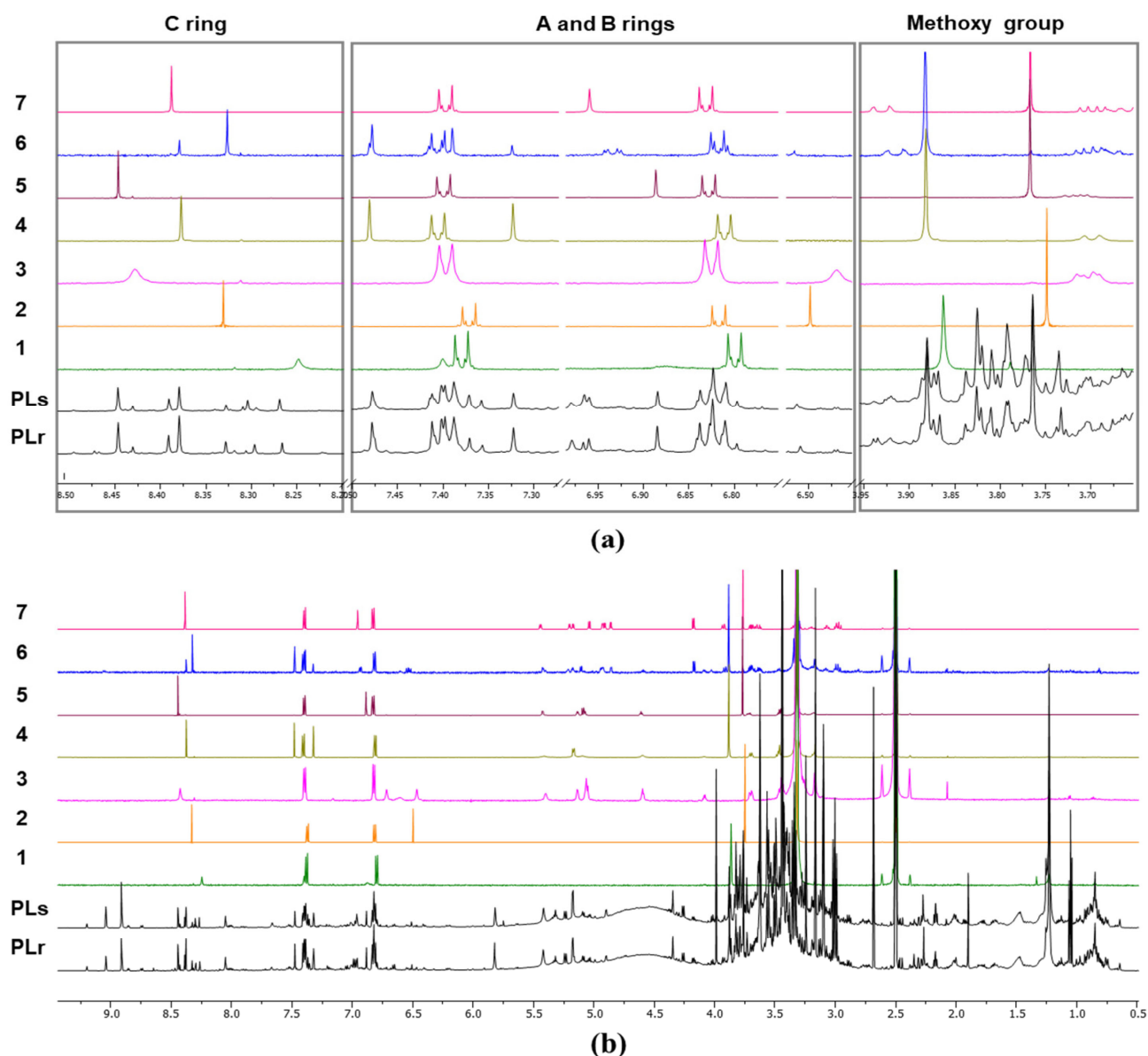


Figure 8. Quantitative qHNMR spectra of *Pueraria lobata* methanolic extracts obtained by sonication (PLs) and reflux (PLr), compared with seven major reference isoflavones (1–7). (a) Expanded spectral regions highlighting diagnostic aromatic protons of the A, B, and C rings and methoxy group signals; (b) full qHNMR spectra showing overall matrix complexity and signal distribution. Reproduced from [85] under CC BY 4.0 license.

The methoxy proton signals appear in the δ ~3.7–3.9 ppm region (Figure 8a, right panel). These resonances are diagnostic for O-methoxylated isoflavones and provide an additional level of structural confirmation. In the PLr extract, methoxy signals closely match those of the reference compounds in both chemical shift and relative intensity, supporting compound-specific authentication. In contrast, the PLs extract exhibits weaker methoxy signals and increased baseline distortion, consistent with partial extraction and greater matrix interference.

The full qHNMR spectra (Figure 8b) highlight the complexity of crude nutraceutical extracts. The aliphatic region (δ ~0.5–4.5 ppm) is dominated by overlapping signals from sugars, amino acids, organic acids, and other primary metabolites co-extracted with the

target isoflavones. These signals contribute to spectral congestion and emphasize the importance of selecting non-overlapping, structurally specific resonances—typically in the aromatic region—for reliable qHNMR integration.

Despite this complexity, qHNMR enables simultaneous qualitative and quantitative assessment by integrating selected, well-resolved proton signals that are insensitive to matrix variation. The comparison between PLs and PLr demonstrates that extraction pre-treatment strongly influences spectral quality, signal-to-noise ratio, and quantitative reliability [85].

Overall, the spectra illustrate the suitability of qHNMR as a robust tool for nutraceutical quality control and authentication. The direct comparison of crude extracts with authentic reference compounds allows the confirmation of identity, assessment of extraction efficiency, and detection of compositional differences arising from processing conditions. The superior spectral clarity observed for the reflux extract highlights the importance of standardized sample pre-treatment to ensure reproducible and regulatorily acceptable qHNMR-based analysis.

9. Elemental Profiling

9.1. Atomic Absorption Spectroscopy (AAS)

AAS is an established method for quantifying trace metals such as lead, cadmium, arsenic and essential minerals like iron, zinc, magnesium, calcium, copper in nutraceuticals [23,94]. AAS usually requires sample digestion and is compatible with tablets, capsules, and herbal matrices.

Both flame AAS (FAAS) and graphite furnace AAS (GFAAS) provide high selectivity and sensitivity at ppm to ppb levels, and are routinely used under pharmacopeial standards for elemental profiling. AAS affordability and simplicity make a practical alternative to ICP-MS and ICP-OES for routine quality control and safety assessment.

Applications include verifying fortification, quantifying essential elements, and detecting toxic metal contamination [96–98].

A study by Hina et al. [120] demonstrated that FAAS effectively quantifies both essential minerals Cu, Co, Ni, Zn, Fe, and Cr and toxic heavy metals like Pb, and Cd, which were determined in medicinal plant *J. adhatoda*, highlighting variations in elemental distribution that are critical for safety and therapeutic efficacy.

In their recent review [120], the authors highlighted that GFAAS is a highly sensitive and precise method for quantifying trace metals in medicinal plants, supporting quality control and safety assessment. The authors also noted that GFAAS allows direct analysis of dried plant samples as a slurry or pulverized solid, requiring only small, well-homogenized amounts to ensure representative and accurate measurements as an advantage over ICP-MS technique. The plant material, mostly dried, must be introduced into the graphite tube as slurry or directly in pulverized form [120].

Table 10 shows nutraceuticals analyzed by FAAS, and it highlights that in this type of analysis, GFAAS is the preferred technique.

Table 10. Determination of trace and toxic elements in plant-based nutraceuticals by AAS.

Sample(s)	Analyte(s)	Analytical Technique	References
<i>Punica granatum</i> (pomegranate)	Cd, Co, Cr, Cu, Pb	GFAAS	[121,122]
<i>Allium cepa</i> (onions)	As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Sb, Zn	GFAAS	[123]

Table 10. Cont.

Sample(s)	Analyte(s)	Analytical Technique	References
<i>Melissa officinalis</i> (lemon balm)	Cd, Cu, Pb	GFAAS	[124]
<i>Catharanthus roseus</i> (periwinkle)	Ca, Fe, Mg, Se, Zn	GFAAS	[125]
<i>Daucus carota</i> (carrots)	As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Sb, Zn	GFAAS	[123]
<i>Dioscorea polystachya</i> (Chinese yam)	Mn, Mo, Mg	GFAAS	[126]
<i>Origanum majorana</i>	Cr, Ni	GFAAS	[127]
<i>Valeriana officinalis</i> (valerian root)	Pb, Cd	GFAAS	[128]

9.2. Inductively Coupled Plasma-Based Techniques

Inductively coupled plasma (ICP) techniques represent advanced elemental analysis approaches capable of detecting and quantifying both trace and major elements in complex nutraceutical and dietary supplement matrices. ICP-OES employs a high-temperature argon plasma to excite atoms and ions, which emit element-specific radiation measured by optical detectors. This technique offers rapid simultaneous multi-element analysis, a wide dynamic range, and good tolerance to matrix interferences, making it particularly suitable for mineral profiling in herbal products and fortified foods [28].

ICP-MS also relies on plasma for ionization but analyzes ions based on their mass-to-charge (m/z) ratios. This technique offers exceptionally low detection limits (ng/L to pg/L), high selectivity, and isotopic resolution, making it ideal for detecting heavy metals like lead, arsenic, cadmium, and mercury in nutraceuticals.

Inductively Coupled Plasma–Mass Spectrometry (ICP-MS)

Inductively coupled plasma–mass spectrometry (ICP-MS) is one of the most powerful analytical techniques for the determination of trace and ultra-trace elements in nutraceuticals, offering high sensitivity, wide linear dynamic range, and multi-element detection capability. Its application is particularly relevant for the assessment of essential minerals, trace elements, and potentially toxic metal(loid)s, which are critical parameters for safety, quality control, and regulatory compliance of dietary supplements and herbal products [27,29,97,129].

Mineral composition and heavy metal contamination are tightly regulated due to their direct implications for consumer safety. International regulatory bodies such as EFSA, FDA, WHO, and pharmacopeial organizations emphasize the monitoring of elements such as Pb, Cd, Hg, As, Ni, and Cr in nutraceuticals, alongside nutritionally relevant elements including Fe, Zn, Cu, Mn, Ca, Mg, and Se [1,5,7–9,19]. ICP-MS is widely recognized as a reference technique capable of meeting these stringent regulatory requirements due to its low detection limits and robustness [29].

Accurate ICP-MS analysis relies heavily on appropriate sample preparation to ensure complete matrix decomposition and quantitative element recovery. Microwave-assisted acid digestion is the most applied pre-treatment approach for nutraceutical matrices, including plant materials, extracts, tablets, and capsules. This procedure typically employs nitric acid, often combined with hydrogen peroxide, to achieve efficient digestion while minimizing contamination and analyte loss [27,129].

Such digestion protocols are essential for complex botanical matrices rich in organic compounds, which may otherwise cause signal suppression or spectral interferences during plasma ionization [29].

ICP-MS has been extensively applied to evaluate toxicological risks associated with heavy metal contamination in herbal medicines and dietary supplements. Studies have demonstrated its effectiveness in detecting potentially hazardous levels of Pb, Cd, Hg, As, Ni, and Cr in commercial nutraceutical products, supporting risk assessment and regulatory enforcement [97].

In addition to safety monitoring, ICP-MS is used to quantify essential and trace elements contributing to the nutritional value of supplements. Elemental profiling provides insight into geographical origin, cultivation conditions, soil-to-plant transfer, and processing effects, which are increasingly relevant for authenticity assessment and quality assurance [130].

Beyond quantification, ICP-MS elemental fingerprints can be combined with chemometric tools to discriminate nutraceuticals based on botanical origin, geographical provenance, or manufacturing process. Variations in elemental composition have been successfully used as authenticity markers, particularly when integrated with multivariate statistical analysis [38]. This approach enhances the role of ICP-MS from a purely quantitative technique to a complementary tool for nutraceutical authentication.

Despite its advantages, ICP-MS requires careful control of spectral and non-spectral interferences, often addressed through collision/reaction cell technology or internal standardization. Additionally, the technique provides elemental but not chemical speciation information, necessitating complementary methods when oxidation state or bioavailability is of interest [27,29].

Some of the nutraceuticals analyzed by AAS were also analyzed by ICP-MS or ICP OES (see Table 11).

Table 11. Trace element analysis of nutraceuticals by ICP.

Sample	Analyte(s)	Analytical Technique	References
<i>Punica granatum</i>	Mn, Cu, Zn, Fe, Li and Se	ICP-MS	[129]
<i>Allium cepa</i>	Cd, As, Pb, Se, Fe, Mn, Zn, and Cu	ICP-MS	[130]
<i>Melissa officinalis</i>	As, Cd, Co, Cu, Zn, Mn, Ni, and Pb	ICP-OES	[131]
<i>Daucus carota</i>	Al, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, Se, Sr, V and Zn	ICP-OES	[132]
<i>Dioscorea polystachya</i>	Mn, Mo, Mg	ICP-MS	[133]

These techniques for elemental methods of analysis have their advantages and limitations in quality control, regulatory compliance, and safety assurance of nutraceutical and functional food products.

As we can tell for elemental analyses of nutraceuticals, acid digestion or microwave-assisted mineralization is required to ensure complete breakdown of complex plant and formulation matrices prior to instrumental measurement. Among the studies summarized, only the analysis of *Valeriana officinalis* did not involve a dedicated sample preparation step, as the authors applied direct analysis of the herbal material [128]. In contrast, all other studies [122,123,126,129–133] employed various wet digestion protocols using con-

concentrated nitric acid, hydrogen peroxide, sulfuric acid, or perchloric acid, either alone or in combination.

The choice of digestion strategy was closely related to the analytical technique employed, with GFAAS typically using conventional wet digestion, while ICP-based techniques frequently relied on microwave-assisted digestion to achieve complete mineralization and minimize matrix effects. These sample preparation approaches are summarized in Table 12.

Table 12. Overview of sample digestion and preparation methods applied prior to elemental analysis of nutraceutical matrices by AAS or ICP.

Sample(s)	Sample Preparation	Analytical Technique	References
<i>Punica granatum</i>	digestion with 67% HNO ₃ and 65% HClO ₄ in 2 to 1 ratio at 250 °C for 3 h	GFAAS	[122]
	digestion with 4 mL of 65% HNO ₃ + 2 mL of 30% H ₂ O ₂	ICP-MS	[129]
<i>Allium cepa</i>	digestion with HNO ₃ at 150 °C for 1 h and 30% H ₂ O ₂ until bleaching occurred	GFAAS	[123]
	1 g of the powdered sample was treated with a mixture of 10 mL of 65% HNO ₃ and 5 mL of 70% H ₂ SO ₄	ICP-MS	[130]
<i>Melissa officinalis</i>	digestion with HNO ₃ , HCl, HClO ₄ , H ₂ O ₂ 10:30:1:4 until bleaching occurred	GFAAS	[124]
	1 g of powdered sample was treated with a mixture of 10 mL of 65% HNO ₃ and 5 mL of 70% H ₂ SO ₄ at ~80 °C until a transparent solution was obtained	ICP-OES	[131]
<i>Daucus carota</i>	digestion with HNO ₃ at 150 °C for 1 h, plus H ₂ O ₂ (30%) until bleaching occurred	GFAAS	[123]
	microwave digestion of 0.5 g powdered samples + 15 mL pure HNO ₃ were added. at 200 °C	ICP-OES	[132]
<i>Dioscorea polystachya</i>	digestion with 12% HNO ₃ at 100 °C for 2 h	GFAAS	[126]
	microwave digestion with 8 mL of HNO ₃ and 2 mL of H ₂ O ₂ for 1 h	ICP-MS	[133]

Overall, GFAAS is frequently favored for targeted elemental determination using conventional wet digestion, whereas ICP-based techniques, particularly ICP-MS, benefit from microwave-assisted digestion to support multi-elemental analysis with enhanced sensitivity and reduced matrix effects.

Although elemental analysis is still not routinely performed for nutraceuticals, determining the elemental composition of plants is essential—not only for monitoring toxic elements but also for accurately quantifying essential elements, whose concentrations can fluctuate over time and vary with geographical origin, environmental conditions, and cultivation practices [133]. Ginkgo seeds were analyzed for Fe, Zn, Cu content by ICP-MS [134]. The sample was mineralized with 2 mL of conc. HNO₃ and 1 mL of deionized water at 190 °C in a microwave oven (ramp for 20 min, hold for 20 min, and cool for 10 min).

While ICP-MS is primarily applied for elemental determination, mass spectrometry as a broader analytical platform extends beyond elemental profiling and plays a central role in the molecular identification, quantification, and structural characterization of nutraceutical constituents. Owing to its high sensitivity and selectivity, mass spectrometry—often cou-

pled with chromatographic separation—has become indispensable for the comprehensive analysis of complex nutraceutical matrices.

10. DNA Barcoding and PCR-Based Assays

In addition to chemical and spectroscopic methods, molecular approaches such as DNA barcoding and next-generation sequencing provide powerful tools for verifying species identity and detecting adulteration in complex food and nutraceutical matrices, supporting comprehensive authenticity assessment [135].

DNA barcoding and PCR-based assays provide accurate botanical identification, which is critical for preventing adulteration and substitution in herbal products [18].

DNA barcoding typically targets short, standardized regions of DNA, such as the chloroplast *rbcL* and *matK* genes, which are conserved within species, yet sufficiently variable to enable discrimination [18,36,37].

The process involves few sample steps: first, the DNA is extracted from the sample, second is the PCR amplification of the barcode region using specific primers, the third step is sequencing of the amplified DNA, and finally a comparison of the obtained results with a reference database (BOLD, GenBank) is carried out to confirm species identity.

PCR-based assays—including conventional PCR, real-time PCR, and multiplex PCR—enable rapid, sensitive, and specific detection of target DNA even from degraded or processed samples. These techniques are essential for species-level identification, contamination detection, and label claim verification [136].

Among DNA-based techniques, quantitative real-time PCR (qPCR) remains the gold standard for accurate and sensitive detection of extraneous/foreign matter of biological origin [136].

The study of Behr et al. [136] developed green-based real-time qPCR assays to specifically detect the five most common adulterants in six widely used spices and herbs—paprika (chili), turmeric, saffron, cumin, oregano, and black pepper.

Another study developed and validated a qPCR-based method capable of detecting and quantifying hazelnut and almond DNA in olive oil with high sensitivity, enabling reliable identification of nut-derived adulteration for food authentication and allergen risk assessment [137].

Highly sensitive qPCR-based assays are developed for detecting and quantifying species-specific DNA in milk and dairy products, enabling accurate identification of adulteration and ensuring authenticity in the dairy supply chain [138].

Eight yam landraces from two species (*D. alata* and *D. polystachya*) were analyzed with nano-liquid chromatography–tandem mass spectrometry (nanoLC-MS), protein identification, RNA isolation and qRT-PCR analysis [138]. The study revealed significant inter- and intraspecific differences in allantoin, phenol, and flavonoid contents between *D. polystachya* and *D. alata*.

The double-tube multiplex TaqMan qPCR assay, capable of simultaneously detecting eight dairy-derived components with high specificity and sensitivity, is another powerful tool for dairy authentication and adulteration control [139].

Another high-throughput method that can analyze entire genomes or specific genetic regions is NGS. NGS enhances DNA barcoding by enabling parallel sequencing of many samples at once, and offers parallel sequencing of millions of DNA fragments, providing an advanced approach for authenticating multi-ingredient or processed nutraceutical products [140].

It is a powerful tool for detecting adulterants in various matrices including food, pharmaceuticals, and other products. By analyzing the genetic material of suspected adulterants,

NGS can identify the presence, origin, and even the quantity of these substances, providing a more precise and comprehensive approach compared to traditional methods [140].

The evolution of DNA barcoding in herbal medicine analysis and application is summarized in the review of Chen et al. [141], emphasizing its expansion from standard barcodes to super- and mini-barcodes, integration with advanced molecular techniques, and the development of comprehensive reference libraries to improve species authentication and quality control.

The study of Sánchez et al. [142] combined DNA barcoding for species authentication with UHPLC–MS metabolomics to simultaneously verify the identity and characterize the chemical profiles of medicinal plant samples (*Matricaria recutita* L., *Valeriana officinalis* L., *Tilia* spp., and *Camellia sinensis* (L.)). The DNA barcoding, using *matk* as a barcode marker, revealed that CH1, sold as *Camellia sinensis* was *Blepharocalyx tweediei*, and sample TS2, sold as linden, belong to Malvales.

11. Emerging Analytical Trends in Nutraceutical Analysis

Recent advances in analytical methodologies have transformed the quality control and characterization of nutraceuticals. Among these, several emerging trends are shaping the field, as detailed below.

11.1. Biosensors

Electrochemical and optical biosensors provide rapid, sensitive, and on-site alternatives to conventional techniques such as chromatography and mass spectrometry [27–30,143]. These devices employ biorecognition elements and electrode-based transduction (amperometric, potentiometric, impedance) to detect contaminants, toxins, allergens, pesticides, and bioactive compounds directly within complex matrices. For example, Kundu et al. [144] developed a flower-like α -Fe₂O₃ nanostructured electrochemical biosensor capable of detecting formaldehyde in fruit juices at nanomolar levels. Biosensors offer real-time screening, although challenges remain in terms of cost and labor-intensive fabrication [145]. Recent applications include detection of vitamins [145] and polyphenols [146].

11.2. Chemometrics

Chemometrics applies statistical and mathematical modeling techniques to extract meaningful information from complex analytical datasets. In recent years, it has become increasingly integrated with chromatographic [69], spectroscopic [74,75,77,114], and elemental analytical techniques [141,142], significantly improving data interpretation, classification, authentication, and quality assessment in food and nutraceutical analysis. Widely used chemometric approaches—including PCA, PLS, HCA, discriminant analysis, and orthogonal PLS–discriminant analysis (OPLS-DA)—enable efficient pattern recognition, detection of adulteration, authentication of raw materials, and optimization of quality control protocols [38,42,69,74,86,87,91,147].

Numerous practical applications demonstrate the effectiveness of chemometrics in food and nutraceutical quality control. For example, Raman spectroscopy combined with chemometric modeling enabled the detection of L-cysteine and L-cystine adulteration in wheat flour with 100% accuracy [74]. Similarly, femtosecond laser ablation inductively coupled plasma mass spectrometry (fsLA-ICP-MS), when coupled with chemometrics, has been successfully applied for multi-elemental profiling of garlic and onion, facilitating reliable authenticity assessment based on elemental fingerprints [148].

Chemometrics also plays a critical role in the quality control of dietary supplements, particularly those with complex formulations. A notable example includes the simultaneous analysis of structurally related bioactive amines and caffeine in weight-loss and

pre-workout supplements [149]. Conventional chromatographic techniques, although effective, often require extensive sample preparation due to matrix interferences from co-extracted phytochemicals such as flavonoids, polyphenols, and essential oils. To overcome these limitations, multivariate calibration models—including PLS-1, genetic algorithm–PLS and genetic algorithm–artificial neural networks—have been successfully employed for the simultaneous quantification of 2-phenethylamine, caffeine, p-synephrine, and p-hordenine in complex nutraceutical matrices [150]. These chemometric strategies enable rapid, non-destructive, and cost-effective analyses while enhancing classification accuracy, adulteration detection, and regulatory compliance [151]. By exploiting full spectral information from UV–Vis and other spectroscopic techniques, chemometrics provides robust and selective quantification even in highly complex matrices, underscoring its growing importance in modern nutraceutical standardization and quality assurance [152].

The integration of portable spectroscopic devices with chemometric tools further expands the applicability of these approaches. Portable infrared instruments combined with PCA and PLS models allow real-time, field-based authenticity assessments across a wide range of food matrices, including edible oils, syrups, and dairy products [74]. NIR and FTIR coupled with chemometric analysis have also demonstrated strong capabilities for detecting adulteration in herbal products. Galvin-King et al. [114] reported robust predictive performance for both NIR- and FTIR-based models in identifying adulterants in garlic samples, as reflected by high R^2 and Q^2 values.

NMR-based metabolomics represents another powerful chemometric-driven approach for nutraceutical authentication. qNMR fingerprinting combined with PCA and OPLS-DA has been successfully applied to discriminate extracts based on geographic origin and to detect adulteration [86,87]. In one study, NMR spectra enabled clear differentiation between authentic ginseng samples and those intentionally spiked with adulterants [88]. Complementary spectroscopic techniques have yielded similar outcomes: Choi et al. demonstrated that FT-IR spectroscopy combined with multivariate analysis could effectively distinguish Panax ginseng powder from various root plant adulterants. PCA revealed distinct clustering between pure and adulterated samples, while PLS models achieved high predictive accuracy for key ginsenosides and aromatic compounds, confirming the suitability of FTIR for ginseng quality control [95].

More recently, Çebi et al. [112] developed an ATR-FTIR method coupled with HCA and PCA for detecting illicit sibutramine adulteration in green tea, green coffee, and mixed herbal tea products. Using a targeted spectral window ($2746\text{--}2656\text{ cm}^{-1}$), the method achieved 100% classification accuracy, with no false positives or false negatives reported. Collectively, these studies highlight the versatility and robustness of chemometric-assisted analytical techniques in addressing the growing challenges of authenticity, adulteration detection, and quality control in food and nutraceutical products.

11.3. Lab-on-a-Chip and Miniaturized Platforms

Microfluidic lab-on-a-chip systems integrate sample preparation, separation, and detection in a single miniaturized device. These platforms enable rapid, low-volume, and cost-effective analysis, particularly suitable for high-throughput screening in nutraceutical development [153].

11.4. Portable and Hand-Held Spectrometers

Advances in portable NIR, Raman, and UV-Vis spectroscopy enable in-field analysis of raw materials, facilitating rapid quality control and authentication without the need for centralized laboratories [154].

11.5. Multi-Technique Integration and AI-Driven Analysis

The future of nutraceutical analysis lies in the combination of chromatographic, spectroscopic, and elemental techniques with chemometric modeling and AI/ML [155]. This multi-technique approach enhances reliability, selectivity, and sensitivity, enabling comprehensive detection, authentication, and standardization of nutraceuticals, functional foods, and dietary supplements. Such integration ensures product safety, efficacy, and consumer confidence in an increasingly complex global market.

12. Disadvantages of the Manuscript

While this review comprehensively covers commonly applied analytical techniques for the characterization, authentication, and quality control of nutraceuticals, several limitations should be acknowledged.

First, advanced protein structural characterization techniques—such as ion-exchange chromatography, size-exclusion chromatography, electrophoretic methods, circular dichroism, and fluorescence spectroscopy—are primarily applied for detailed molecular and conformational studies. These methods fall outside the scope of routine analytical approaches focused on multi-class bioactive compounds and were therefore not included. A dedicated review would be required to cover these specialized protein-based analyses adequately.

Second, physicochemical characterization of fats and oil-based nutraceutical products—including acid value, saponification value, iodine value, ester value, peroxide value, specific gravity, refractive index, and total ash content—is also not included [156]. These parameters are important for assessing quality, stability, and oxidative status of oil-based nutraceuticals, but they represent well-established and standardized testing procedures. Incorporating them would extend the scope beyond analytical methodologies for compositional and instrumental characterization.

By clarifying these limitations, this review maintains its focus on analytical techniques capable of addressing complex matrices, trace-level contaminants and adulteration challenges specific to modern nutraceutical products while acknowledging areas that require separate, specialized discussion.

13. Conclusions and Future Perspectives

This review presents a comprehensive overview of analytical methodologies applied to nutraceuticals, covering chromatographic, spectroscopic, and elemental analysis techniques (Figure 9). Classical methods such as HPLC, GC, AAS, and ICP-MS remain indispensable for accurate quantification, identification, and safety assessment of bioactive compounds, vitamins, polyphenols, fatty acids, and trace elements in complex matrices.

Emerging analytical trends are rapidly transforming the field. The integration of chemometrics with chromatographic, spectroscopic, and elemental data enables pattern recognition, classification, adulteration detection, and optimization of quality control protocols. Advanced biosensors, lab-on-a-chip platforms, and portable spectrometers offer rapid, non-destructive, and cost-effective alternatives, facilitating in-field screening, authentication, and real-time monitoring. These innovations not only enhance data interpretation but also strengthen reproducibility, reliability, and standardization in nutraceutical quality control.

Looking forward, the future of nutraceutical analysis lies in multi-technique integration, combining classical separation methods with advanced detection, chemometrics, and predictive modeling. Such approaches will allow comprehensive profiling of complex formulations, improve regulatory compliance, and support the development of safe, effective, and high-quality functional foods and dietary supplements. Continuous methodological

advancements and adoption of emerging technologies will be pivotal in ensuring consumer safety, confidence, and trust in the global nutraceutical market.

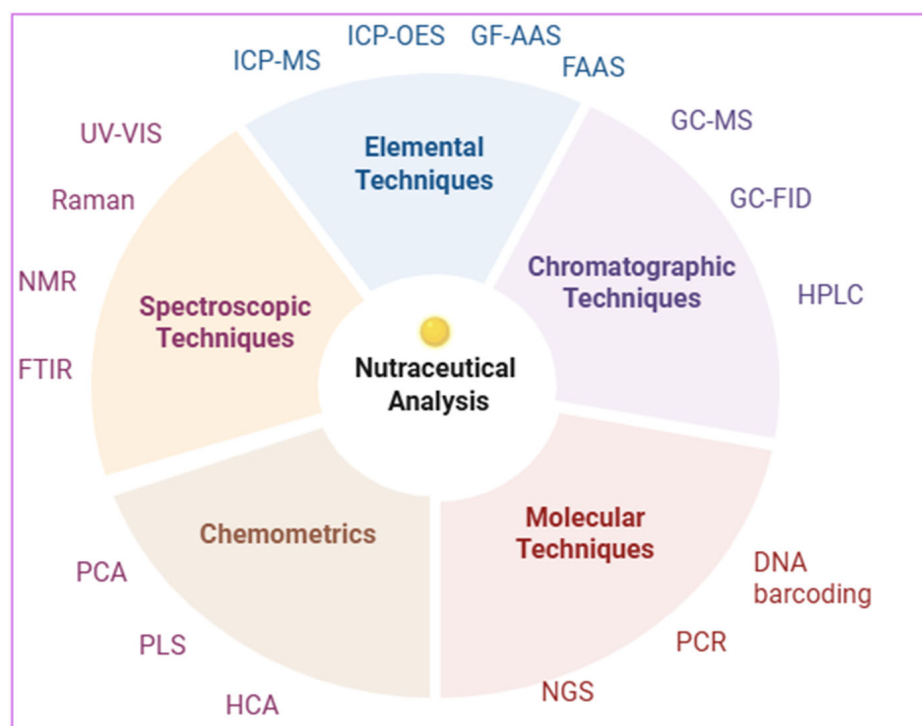


Figure 9. Overview of analytical techniques used in nutraceutical analysis, including spectroscopic, chromatographic, elemental, molecular, and chemometric methods.

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Abbreviations

The following abbreviations are used in this manuscript:

DSHEA	Dietary Supplement Health and Education Act
FDA	Food and Drug Administration
EFSA	European Food Safety Authority
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
WHO	World Health Organization
USP	United States Pharmacopeia
UV-Vis	Ultraviolet–Visible Spectroscopy
EP	European Pharmacopoeia
JP	Japanese Pharmacopoeia
LOD	Limit of Detection
LOQ	Limit of Quantification
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
PDA	Photodiode Array

MS	Mass Spectrometry
AAS	Atomic Absorption Spectroscopy
LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
ICP	Inductively Coupled Plasma
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
HPLC	High-Performance Liquid Chromatography
GC	Gas Chromatography
GC-MS	Gas Chromatography–Mass Spectrometry
FID	Flame Ionization Detector
¹ H-NMR	Proton Nuclear Magnetic Resonance Spectroscopy
TLC	Thin-Layer Chromatography
FTIR	Fourier-Transform Infrared Spectroscopy
QC	Quality Control
DAD	Diode-Array Detection
ESI-MS	Electrospray Ionization–Mass Spectrometry
RP	Reverse-Phase
RP-HPLC-DAD	Reverse-Phase High-Performance Liquid Chromatography with Diode-Array Detection
qTOF-MS	Quadrupole Time-of-Flight Mass Spectrometry
CA	Cluster Analysis
PCA	Principal Component Analysis
PLS	Partial Least Squares Regression
PLS-DA	Partial Least Squares—Discriminant Analysis
UPLC	Ultra Performance Liquid Chromatography
U-HPLC	Ultra-High-Performance Liquid Chromatography
HCA	Hierarchical Cluster Analysis
NIR	Near-Infrared
IR	Infrared
PAT	Process Analytical Technology
DPPH	1,1-diphenyl-2-picrylhydrazyl
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
TPTZ	Iron[III]-2,4,6-Tripyridyl-S-Triazine
ATR-FTIR	Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy
FRAP	Ferric Reducing Antioxidant Power
LLE	Liquid-Liquid Extraction
SPE	Solid-Phase Extraction
FC	Folin–Ciocalteu
FAME	Fatty Acid Methyl Esters
ORAC	Oxygen Radical Absorbance Capacity
CUPRAC	Cupric Reducing Antioxidant Capacity
TFC	Total Flavonoid Content
TEAC	Trolox Equivalent Antioxidant Capacity
FAAS	Flame Atomic Absorption Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
LC	Liquid Chromatography
qPCR	Quantitative Real-Time PCR
nanoLC-MS	Nano-Liquid Chromatography–Tandem Mass Spectrometry
MTBE	Methyl Tert-Butyl Ether
NGS	Next-Generation Sequencing
OPLS-DA	Orthogonal PLS–Discriminant Analysis
fsLA-ICP-MS	Femtosecond Laser Ablation Inductively Coupled Plasma Mass Spectrometry

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