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Review

Advanced Sample Preparation Techniques for Multi-Residue Analysis in Food and Health Research

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Abstract: The simultaneous determination of multiple organic compounds in complex food and biological matrices is increasingly essential for ensuring food safety, understanding bioactive nutrient dynamics, and assessing chronic exposure to contaminants. This review provides a comprehensive evaluation of modern sample preparation strategies, including Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS); Solid-Phase Extraction (SPE); Dispersive Solid-Phase Extraction (d-SPE); Solid-Phase Microextraction (SPME); and Liquid–Liquid Extraction (LLE), for their applicability in extracting diverse classes of organic compounds ranging from pesticides, mycotoxins, to phytochemicals and environmental residues. Each technique was assessed for recovery efficiency, selectivity, matrix effect minimization, and integration with Gas Chromatography–Tandem Mass Spectrometry (GC-MS/MS) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS), which are widely applied in food safety, environmental, and biomedical monitoring. Comparative analysis highlights that QuEChERS-based approaches offer rapid, solvent-saving workflows suitable for fruits, vegetables, and grain-based samples, whereas SPE and SPME exhibit superior performance in trace-level analysis of biofluids and lipid-rich matrices. The review further emphasizes the critical role of tailored sample preparation in improving data reliability and supporting downstream applications in dietary exposure assessment, functional food development, and food-medicine homology research. These findings reinforce the need for robust, matrix-adaptable, and automation-compatible preparation protocols to advance the analytical precision in food as medicine studies and contribute to public health strategies based on nutritional therapeutics.

Keywords: multi-residue analysis; sample preparation; QuEChERS; solid-phase extraction

1. Introduction

The increasing prevalence of chronic diseases such as obesity, diabetes, cardiovascular disorders, and certain cancers has underscored the urgent need for preventive strategies rooted in everyday exposures and dietary intake. Among these exposures, food-derived contaminants and xenobiotics, whether introduced through environmental pollution or food processing, have become a growing concern in public health. In particular, a subset of chemicals known as Contaminants of Emerging Concern (CECs) has garnered attention due to their potential roles in disrupting metabolic pathways, altering gut microbiota, or contributing to low-grade inflammation. Globally, over

350,000 commercial chemicals are currently registered, with approximately 2000 new compounds entering the market each year [1]. Notably, CECs are estimated to represent up to 15.4% of the known chemical space, while nearly 28.4% of registered substances remain poorly characterized [2]. This evolving chemical burden raises concerns about unintended dietary exposure and its implications for long-term health outcomes.

Traditionally, environmental pollutants have been classified by chemical name or functional category (e.g., antibiotics, PFAS, flame retardants). However, the increasing structural diversity and complex biological interactions of these compounds challenge conventional classification approaches. To address this complexity, recent efforts have turned to advanced simultaneous analysis methods capable of detecting a wide range of organic compounds in a single analytical run. As shown in Figure 1, the number of studies focusing on simultaneous determination has steadily increased in recent years [3]. These methods have been successfully applied to multiple chemical groups, including pharmaceuticals [4], pesticides [5,6], PFAS [7], and plant-derived regulators [8–10], across diverse matrices such as biological tissues, food, and environmental samples.

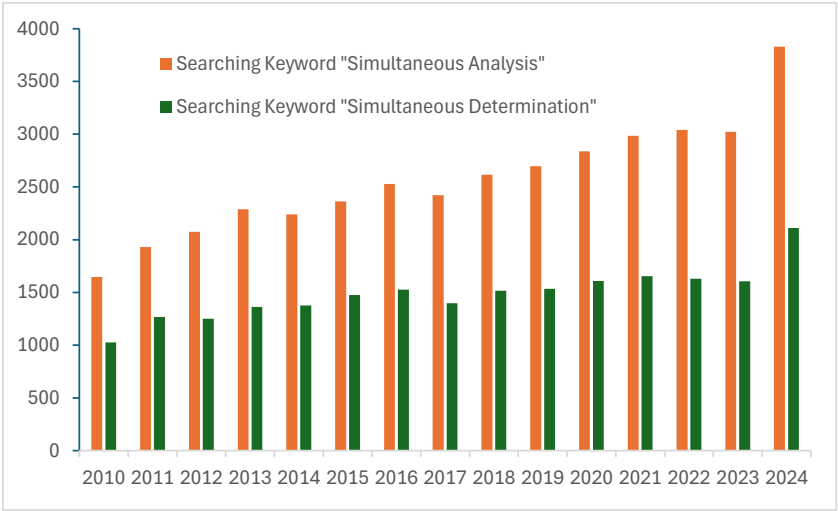

Figure 1. Increase in simultaneous analysis studies over time. (Data collected from Elsevier's platform using the keywords "simultaneous analysis" and "simultaneous determination"; accessible at https://www.scopus.com).

While recent advances in sample preparation and chromatographic technologies have expanded the analytical capabilities for detecting organic compounds in complex matrices, substantial gaps still exist in achieving comprehensive and integrated coverage-particularly in the context of real-world food systems. These systems are often characterized by complex and heterogeneous matrices that require both selective and sensitive analytical strategies. As outlined in previous works, targeted approaches remain essential for routine surveillance of known contaminants [11,12], while non-targeted and hybrid strategies have proven valuable for capturing emerging compounds and unidentified residues [13–17]. Traditional chromatographic systems typically resolve up to 1500 peaks in a single run [18], yet recent innovations in multidimensional chromatography now enable separation of tens of thousands of analytes, expanding the resolution and scope of chemical fingerprinting [19,20]. However, despite this potential, these advanced platforms remain underutilized in food-related research linked to chronic disease risk, environmental exposure, and cumulative dietary intake. Table 1 provides a representative overview of studies that employed simultaneous analysis of more than 15 compounds across diverse matrices, ranging from food products (e.g., cereal-based foods, pork, and chicken liver) to environmental samples (e.g., surface water, floodwater, sewage) and biological fluids (e.g., plasma, amniotic fluid, serum). The number of compounds detected varies widely, from 17 perfluoroalkyl substances (PFAS) in human serum [21] and 19 bromophenols in environmental matrices [22], to over 1150 micro-pollutants detected in surface water using LC-TOF-MS and GC-MS platforms [23]. Such variability underscores the necessity for flexible sample preparation techniques such as QuEChERS, SPE, LLE, and μEluting plate methods to efficiently handle diverse compound classes including pesticides, antibiotics, endocrine-disrupting chemicals, and semi-volatile organic compounds. Moreover, the inclusion of biological matrices in Table 1 such as plasma, amniotic fluid, and liver reflects their utility as biomarkers of cumulative dietary and environmental exposure rather than direct dietary components. As such, they bridge the gap between food intake and internal chemical burden. To move toward more holistic and health-relevant assessments, future analytical strategies must aim for broader integration across sample types and analytical platforms, ensuring robust characterization of the exposome from food and environmental sources alike. Table 1 summarizes representative studies utilizing chromatographic techniques for the simultaneous

determination of more than 15 compounds across diverse sample matrices. These include biological fluids, environmental samples, animal-derived foods, and plant-based products. The table demonstrates the complexity of sample matrices and the variation in both extraction techniques and instrumental methods.

Table 1. Selected previous studies on the simultaneous analysis of dozens of compounds using chromatographic techniques.

Compounds	Matrix	Extraction Methods	Instrument	Number Detection	Ref.
PFAS	human serum and semen	QuEChERS	UPLC-MS/MS	17	[21]
Bromophenols	environment		GC-MS	19	[22]
Antibiotic	Plasma	Oasis HLB μEluting Plate	HPLC–MS/MS	50	[23]
Endocrine disrupting chemicals	human amniotic fluid	SPE	LC-MS/MS	59	[24]
Pesticides and pharmaceuticals	soil, orange leaves and fruits	QuEChERS	LC-MS/MS	33	[25]
Lipid-soluble pesticides and metabolites	chicken liver and pork	QuEChERS	HPLC-MS/MS	24	[26]
Alkaloids	cereal-based food	QuEChERS	LC-MS/MS	42	[27]
Illegal drugs	Sewage	SPE	SPE-ISTD-UHPLC-MS/MS	28	[28]
Sulfonamide	Livestock	QuEChERS	LC/MS	31	[29]
Micro-pollutants	Surface water	LLE	GC-MS and GC-MS-MS	950	[15]
Semi-volatile organic compounds	Floodwater	LLE	GC-MS	940	[30]
Micro-pollutants	Surface water	SPE	LC-TOF-MS and GC-MS	1153	[31]
Pesticides	Medicines	QuEChERS or SPE	GC-MS-MS	147	[32]
Solvents	Drug	SLE	GC-MS	50	[33]
SVOCs	indoor air	SLE	GC-MS	73	[34]

Representative studies demonstrating simultaneous analysis of multiple compounds using chromatographic techniques across different matrices, including food, environmental, and biological samples.

Given the complexity of food matrices and the diversity of target analytes, sample preparation remains a key bottleneck in achieving reliable multi-compound detection. An optimal sample preparation strategy must accommodate diverse physicochemical properties of analytes, reduce matrix interferences, and enable accurate quantification, all while maintaining high throughput. Recovery rates between 70–120% are generally accepted depending on analytical guidelines [35–38]. In food-health research, where low-dose chronic exposure to multiple bioactive or harmful compounds can influence disease trajectories, methodological rigor in sample handling is essential for reproducible, sensitive, and selective outcomes. As dietary exposure plays a central role in both the prevention and etiology of chronic disease, improved chemical surveillance in food systems is needed to inform regulatory policy and guide nutrition-based interventions.

The objective of this review is to provide a comprehensive overview of current sample preparation strategies designed for the simultaneous determination of multiple organic compounds in food-related matrices, using chromatographic techniques. While the primary focus is on plant-based foods, fermented products, and animal-derived samples, the review also discusses representative studies involving environmental and biological samples where relevant, particularly as they relate to dietary exposure or food contamination pathways. Special emphasis is placed on approaches compatible with complex food systems and real-world matrices. This review highlights how sample preparation strategies influence analytical performance in terms of sensitivity, selectivity, and reproducibility, which are essential for translating chemical measurements into meaningful insights about food-health relationships.

This review presents a comprehensive examination of recent advancements in sample preparation and chromatographic techniques, such as solid-phase extraction (SPE), QuEChERS, liquid-liquid extraction (LLE), and solid-liquid extraction (SLE) in conjunction with high-performance detection systems including LC-MS/MS, GC-MS, and LC-TOF-MS. Both targeted and non-targeted analytical workflows are discussed, with illustrative examples covering high-throughput analyses across food-related, environmental, and biological matrices relevant to dietary exposure. By critically evaluating current methodologies and limitations, the review underscores the analytical suitability of these approaches for investigating the health implications of chemical mixtures in food systems. Unlike prior reviews that focus primarily on technical comparisons, this paper uniquely integrates sample

preparation strategies with their translational application in nutritional science, emphasizing the role of analytical chemistry in addressing real-world challenges such as chronic disease risk and cumulative dietary exposure. In doing so, it bridges food chemistry with human health research, supporting the development of functional foods, nutraceuticals, and preventive interventions, and contributes meaningfully to the evolving field of food as medicine.

2. Classification of Organic Compounds in Multi-Residue Analysis

Multi-residue analysis (MRA) plays a vital role in modern food and environmental safety assessment, enabling the simultaneous detection and quantification of a wide array of organic compounds. These include not only dietary components but also trace-level contaminants with potential implications for human health. The growing complexity of food systems and the expansion of synthetic chemical usage present significant analytical challenges due to the diverse physicochemical properties of these substances. Accurate classification is therefore essential for selecting appropriate sample preparation strategies and optimizing chromatographic conditions, particularly in research linking food contaminants to chronic disease risk.

2.1. Based on Functional Use or Source

Classification of organic compounds by functional use or source provides a practical framework for selecting appropriate sample preparation and analytical methods in complex food and biological matrices. Pesticide residues including organochlorines [39], organophosphates [6,40], carbamates [41], neonicotinoids [42], and pyrethroids [43]-vary widely in polarity and volatility, necessitating tailored extraction such as QuEChERS and detection via LC-MS/MS [5,44–46] or GC-MS/MS [47]. Similarly, antibiotics like tetracyclines, macrolides, and fluoroquinolones in animal-derived foods require selective cleanup (e.g., SPE) and sensitive LC-ESI-MS/MS analysis [48]. Mycotoxins in grains, pharmaceuticals and personal care products (PPCPs) like triclosan [49], endocrine disruptors (e.g., bisphenol A, phthalates) [50,51], and persistent pollutants including PAHs [9], PCBs [52], dioxins [53,54], flame retardants [55], and PFASs [7] demand high-resolution, matrix-adapted approaches. Such classification supports targeted monitoring, enhances exposure assessment, and strengthens food-health risk evaluations.

2.2. Based on Polarity and Solubility

Polarity and solubility are key physicochemical parameters that dictate extraction efficiency, clean-up strategy, and chromatographic performance in multi-residue analysis. Highly polar compounds such as glyphosate, formic acid, and streptomycin require aqueous extractions (e.g., methanol, acetonitrile, buffered solutions) and often polar SPE or ion-exchange materials, especially for water-based matrices like fruits or juices [56]. Non-polar contaminants PAHs, OCPs, phthalates, PCBs dissolve in non-polar solvents (hexane, dichloromethane) and are best extracted from lipid-rich samples for GC-based detection. Moderately polar compounds such as sulfonamides, triazines, and parabens benefit from mixed solvents like acetonitrile–water [12]. Lipophilic compounds (e.g., dioxins, PBDEs) accumulate in fatty tissues, while hydrophilic analytes like beta-lactams dominate in aqueous matrices. These properties also guide chromatographic choices C18 columns for non-polar to mid-polarity analytes, and HILIC for polar species where mobile phase pH and ionic strength are pivotal. Understanding these solubility-polarity dynamics is essential for designing targeted, matrix-specific analytical protocols in food-health research.

3. Overview of Sample Matrices

In multi-residue analysis of organic compounds related to food safety and health, the selection of sample matrix profoundly influences each step of the analytical process, from sample preparation to compound detection. Given the diverse nature of organic contaminants and bioactive compounds in food systems, matrix composition determines the choice of extraction technique, cleanup strategy, and chromatographic conditions. Commonly studied matrices include food (e.g., vegetables, fruits, meat, fish, milk, cereals) [5,7,55–60], water (e.g., drinking water, surface water, wastewater) [11], soil [61,62], sediment [7,15,18,53], air [9,37,63,64], and biological specimens (e.g., plasma, tissues, urine) [37,65]. Each matrix presents unique physicochemical properties, such as water content, fat composition, or organic matter load, that impact analyte recovery, signal suppression, and method sensitivity.

In the context of food and health, plant-derived matrices, such as fruits and vegetables, are frequently analyzed due to their potential to accumulate pesticide residues [5], plant growth regulators [10,66–70], and environmental pollutants [60,61,71]. These matrices are typically high in water, sugars, pigments, and organic acids, which can introduce matrix effects and interfere with mass spectrometric detection. Effective sample preparation for such matrices often involves QuEChERS-based protocols coupled with SPE or dispersive cleanup

to remove polar interferences. In animal-based foods, particularly fish and seafood raised in contaminated environments, lipophilic contaminants such as chlorinated pesticides, PCBs, and industrial dyes (e.g., malachite green) tend to concentrate in fatty tissues and liver. These matrices require specialized extraction and cleanup techniques, including protein precipitation, lipid removal, SPE, or gel permeation chromatography (GPC) [59,72–75], to ensure high specificity and sensitivity in compound quantification.

Water matrices, including surface water, ground water, and wastewater, present lower matrix complexity compared to food or biological samples but often contain contaminants at ultra-trace concentrations. Consequently, large sample volumes must be processed using enrichment techniques such as liquid-liquid extraction (LLE), solid-phase extraction (SPE), or solid-phase microextraction (SPME). These approaches enable reliable detection of a wide range of organic pollutants with environmental and dietary relevance. Soil and sediment, in contrast, are solid matrices with complex textures and high levels of natural organic matter, which can hinder analyte recovery. Efficient extraction from these matrices typically requires ultrasonic extraction, pressurized liquid extraction (PLE), or microwave-assisted extraction (MAE) to disrupt matrix structures and enhance analyte release.

Air and dust samples are critical for assessing inhalation and dermal exposure pathways, especially in indoor environments or areas near food production and processing facilities. These matrices are typically collected using glass fiber filters or polyurethane foam (PUF) and processed with non-polar solvents such as toluene or dichloromethane to recover semi-volatile organic compounds [76]. In biomedical and toxicological contexts, biological samples, including blood, urine, liver, and animal tissues, are essential for evaluating absorption, distribution, metabolism, and excretion of contaminants. However, the presence of proteins, lipids, and endogenous metabolites can compromise analytical performance. Therefore, sample preparation often incorporates protein precipitation, selective extraction, and matrix purification steps using SPE, GPC, or freeze filtration [77,78].

A detailed understanding of matrix composition and its interaction with target analytes is fundamental to developing accurate and reproducible analytical methods. Moreover, given the range of exposure pathways through diet, environment, and metabolism, the ability to apply a unified sample preparation framework across diverse matrices is essential. This supports integrated food–health research by enabling the assessment of cumulative chemical exposures and their implications for chronic disease risk, in alignment with the important role of food in health maintenance and disease prevention.

4. Technical Requirements in Sample Preparation for the Simultaneous Determination of Organic Compounds

Sample preparation is a decisive step in ensuring the reliability, reproducibility, and accuracy of chromatographic analyses, particularly when targeting multiple organic compounds of diverse physicochemical nature. In the context of food, environmental, and biological research, a robust preparation method must not only enable broad-spectrum detection but also maintain compound integrity across varying matrices. This section outlines five technical requirements critical for developing sample preparation protocols compatible with the scope of multi-residue analysis in food systems and their relevance to health outcomes:

4.1. High and Uniform Recovery Efficiency across Compound Groups

High and uniform recovery of analytes is a fundamental requirement in the simultaneous analysis of organic compounds with diverse polarity, volatility, and thermal stability [79,80]. Recovery refers to the percentage of analyte successfully transferred from the original sample into the final analytical solution without degradation or loss. Achieving this uniformly across multiple compound classes ensures analytical validity, especially when interpreting trace-level contaminants or health-relevant bioactives in complex food matrices.

The key challenge lies in the heterogeneity of target analytes. Some compounds are highly polar (e.g., carbamates, organic acids), while others are non-polar (e.g., PAHs, pyrethroids). Some tolerate GC-based thermal conditions; others require LC-MS due to thermal lability. This variability necessitates extraction strategies that are broad-spectrum and minimally selective. Overly specific solvents or sorbents may selectively extract one group while excluding others [78,80,81]. Optimized approaches include adjusting solvent pH to target both neutral and ionizable compounds, combining sorbents (e.g., PSA, C18, GCB) in dSPE to address matrix diversity, and using isotopically labeled internal standards to correct for losses. Techniques such as QuEChERS have demonstrated excellent performance for multi-class recovery in food and environmental matrices, but method validation remains essential to ensure uniformity across compound groups.

4.2. Matrix Effects

Matrix effects are among the most prominent analytical challenges in chromatographic detection, especially when utilizing highly sensitive mass spectrometry systems [48,82]. These effects arise when endogenous sample constituents interfere with analyte ionization or detection, leading to signal suppression or enhancement. In food and biological samples, compounds such as lipids, proteins, pigments, and inorganic salts are known to interfere with MS/MS detection, introducing quantification errors and affecting method sensitivity.

Addressing matrix effects begins with an effective sample cleanup strategy. Solid-phase extraction (SPE) and dispersive SPE (dSPE) using sorbents like PSA, C18, and GCB are widely employed to selectively eliminate interfering substances while preserving analytes. Adjusting solvent polarity and pH can also enhance separation between matrix components and target compounds. Internal standards, particularly isotopically labeled analogs, are essential for compensating for matrix-related variability. Additionally, physical removal of particulates and macromolecules through centrifugation and filtration enhances method robustness. Emerging technologies utilizing nanomaterials and dual-phase separation show promise in matrix cleanup. Efficient mitigation of matrix effects not only improves quantification accuracy but also protects chromatographic systems from fouling, reducing downtime and operational costs.

4.3. High Repeatability and Accuracy

Repeatability and accuracy are critical performance metrics in multi-residue analysis. Repeatability ensures consistent results under identical conditions, while accuracy quantifies the closeness of measured values to true concentrations [83,84]. These parameters are particularly crucial in food safety, pharmaceutical surveillance, and environmental toxicology, where analytical data directly inform risk assessments and regulatory decisions.

Multiple factors influence these outcomes, including consistency in sample handling, solvent purity, and the stability of both analytes and extraction materials. Procedures must be standardized in terms of timing, mixing, temperature, and solvent volume to reduce operator-induced variability. Internal standards correct for signal drift and processing loss, while robust calibration curves anchor quantification accuracy. Automation of sample preparation enhances repeatability, minimizing manual errors. Additionally, QC measures such as system suitability testing, recovery verification, and inclusion of matrix-matched standards bolster reliability. In food and medicine research, where exposure-response relationships are dose-dependent, achieving repeatable and accurate results is essential for drawing valid health-related conclusions.

4.4. Selectivity

Selectivity in sample preparation is key to enriching target compounds while minimizing co-extraction of unwanted matrix components [85,86]. This is particularly relevant when analytes are present at trace levels in complex matrices, such as dietary fiber-rich vegetables, fatty meats, or metabolite-laden plasma. Selective extraction maximizes analytical clarity, reduces background noise, and prevents competition for ionization in MS detection.

Sorbent choice is central to achieving selectivity. PSA is effective for removing organic acids and sugars; C18 targets lipids; GCB eliminates pigments and chlorophyll. Combining these in dSPE allows for broad yet selective cleanup. Further, controlling pH, solvent polarity, and extraction time refines the balance between analyte retention and matrix elimination. While increasing selectivity can improve signal quality, overly aggressive conditions may result in analyte loss, especially for labile or amphiphilic compounds. Therefore, a well-calibrated approach must balance recovery and purity. Recent advances in functionalized materials, such as molecularly imprinted polymers and nanocomposite sorbents, offer enhanced selectivity toward specific analyte classes, aligning well with the growing demand for precision in food contaminant monitoring and functional compound quantification.

4.5. Integration Capability with Analytical Systems

A technically sound sample preparation protocol must ensure seamless compatibility with downstream chromatographic and detection systems [87–89]. Efficient integration minimizes manual intervention, reduces sample loss, and improves overall throughput, critical in high-demand analytical laboratories focused on food quality and health-related exposure assessments.

Integration begins with solvent compatibility. For LC-MS/MS, polar solvents such as acetonitrile and methanol are preferred due to their volatility and miscibility with common mobile phases. For GC-MS, solvents like hexane and ethyl acetate are favored, and water must be excluded. Pre-injection filtration and centrifugation remove residual particulates, proteins, and lipids, preserving instrument integrity. Sample volume and concentration must be tuned to match the dynamic range of the detector. Automated systems, such as on-line SPE-

LC-MS/MS or SPME-GC-MS setups, minimize human error and streamline the transition from extraction to analysis. Moreover, automation supports consistent time intervals between preparation and injection, reducing variability due to analyte degradation. This level of integration is especially relevant in translational food-health research, where large sample sets and multi-target panels are common

5. Common Sample Preparation Techniques in Simultaneous Analytical Methods

Given the physicochemical diversity of these analytes, the sample preparation technique plays a pivotal role in ensuring method robustness, analytical reliability, and bio-relevance of outcomes [74,90,91]. Table 2 outlines five widely used sample preparation methods for multi-residue analysis. Each method varies in extraction principle, complexity, and compatibility with downstream detection platforms, making their selection critical for specific sample types and analytical objectives.

Table 2. Common Sample Preparation Techniques in Simultaneous Analysis Methods.

Method	Advantages	Applications
QuEChERS	Fast, low-cost, suitable for multi-residue analysis (pesticides, pharmaceuticals)	Food samples, water, plasma
SPE (Solid Phase Extraction)	Good cleanup, flexible with separation phases	Environmental samples, wastewater, biological samples
SPME (Solid Phase Microextraction)	Solvent-free, ideal for volatile compound analysis	Air, water, food
LLE (Liquid-Liquid Extraction)	Widely used, easy to implement	Water samples, biological samples
dSPE (Dispersive SPE)	Enhanced matrix cleanup, commonly used in QuEChERS	Combined with complex sample matrices

Overview of common sample preparation techniques applied in simultaneous determination workflows, highlighting core principles, advantages, and limitations.

The choice of a sample preparation method must be based on its ability to (i) maintain compound integrity, (ii) reduce matrix interferences, and (iii) integrate seamlessly with chromatographic detection platforms such as LC-MS/MS and GC-MS/MS. Below, five representative techniques are discussed in detail, each offering unique benefits tailored for simultaneous multi-compound analysis.

5.1. QuEChERS

The QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) has revolutionized sample preparation in multi-residue analysis, especially in the context of food safety and pharmacokinetics [92,93]. Its robust design enables rapid extraction and cleanup of a wide array of organic compounds, including pesticides, veterinary drugs, endocrine disruptors, and antibiotics from diverse matrices such as fruits, vegetables, milk, and plasma. Figure 2 illustrates the standard workflow of the QuEChERS method, which is widely adopted for food and biological matrices due to its simplicity, cost-effectiveness, and compatibility with LC-MS and GC-MS platforms.

As illustrated in Figure 2, the QuEChERS workflow begins with solvent extraction using acetonitrile, followed by the addition of salt mixtures (e.g., NaCl, $MgSO_4$) to induce liquid–liquid partitioning. This is succeeded by a dispersive solid-phase extraction (dSPE) step involving selective sorbents like PSA, C18, and GCB to remove matrix interferences. The final extract, often clean and concentrated, is then ready for instrumental analysis via GC-MS/MS or LC-MS/MS. The method is highly compatible with automation, cost-effective, and environmentally sustainable due to reduced solvent use.

QuEChERS has demonstrated high recovery rates (often >80%) across compound classes with distinct polarity and stability profiles, and is easily adaptable via pH modulation or sorbent combinations to accommodate specific analytical targets [94,95]. This versatility renders QuEChERS highly suitable for food-as-medicine research, especially in screening bioactive contaminants and verifying nutritional quality in processed and raw food matrices.

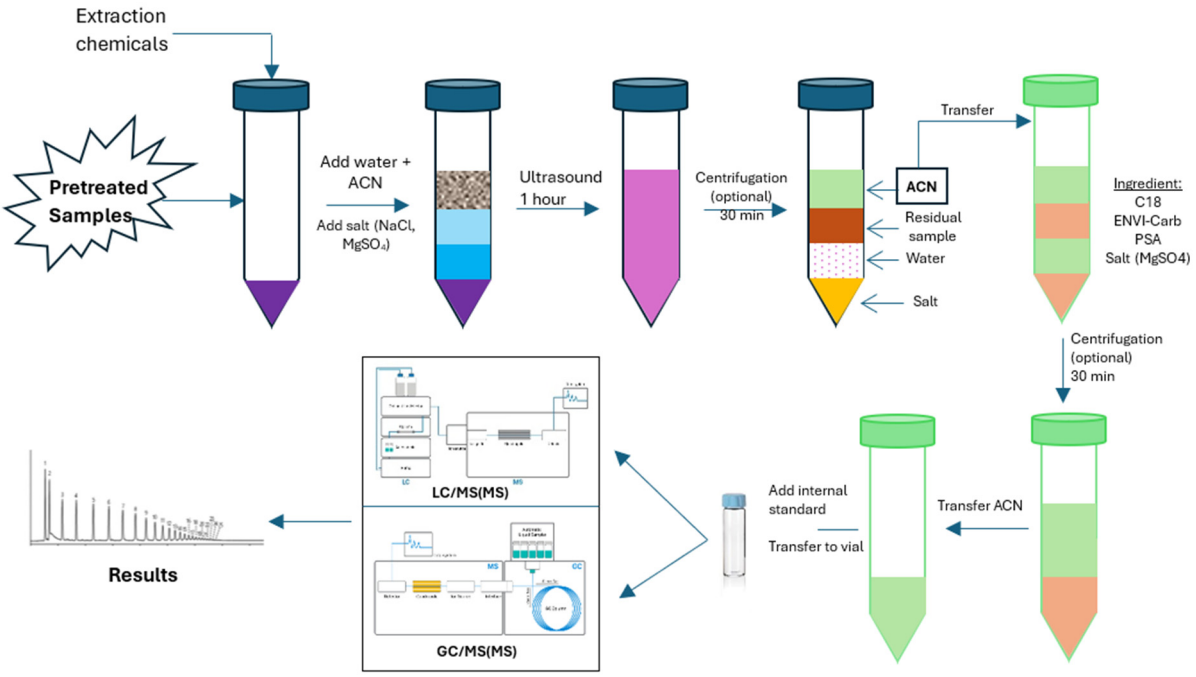

Figure 2. QuEChERS sample preparation process (Schematic workflow of the QuEChERS extraction procedure highlighting its key steps from sample homogenization to extract filtration).

5.2. Solid Phase Extraction

Solid Phase Extraction (SPE) remains a cornerstone in sample cleanup and pre-concentration due to its modular architecture and ability to selectively retain or exclude compounds based on sorbent chemistry [96–98]. SPE cartridges packed with phases such as C18, HLB, or ion-exchange materials enable targeted isolation of analytes, especially in complex matrices like herbal infusions, dairy, or blood plasma.

Figure 3 schematically illustrates the stepwise workflow of solid-phase extraction (SPE), comprising conditioning, sample loading, washing, and elution. This structured approach enables selective retention and recovery of target analytes by preparing the sorbent, facilitating analyte binding, removing non-specific interferences, and subsequently releasing the compounds of interest. The method's modularity allows precise optimization through sorbent choice and elution conditions, supporting high reproducibility and reduced matrix effects in complex sample analyses.

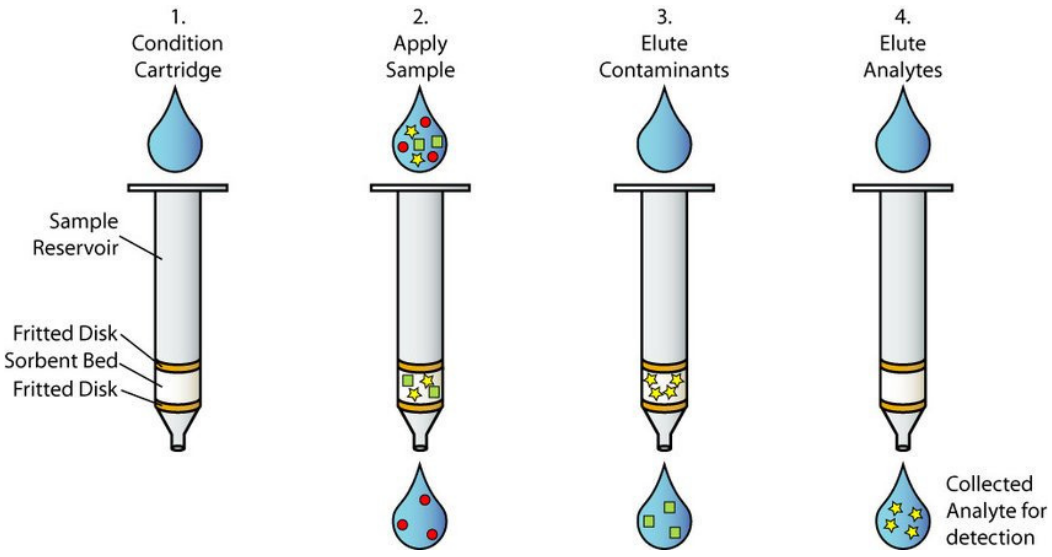

Figure 3. SPE process workflow for selective multi-compound analysis (Workflow of SPE for multi-residue extraction, demonstrating conditioning, loading, washing, and elution phases).

In simultaneous determination scenarios, SPE offers exceptional cleanup flexibility, particularly for food-derived samples containing proteins, polyphenols, or lipids. It also enables offline or online coupling with LC-MS/MS platforms, facilitating trace-level detection of bioactive compounds and foodborne contaminants. When applied correctly, SPE improves sensitivity, selectivity, and throughput, key priorities in food-as-medicine workflows.

5.3. Solid Phase MicroExtraction

Solid-phase microextraction (SPME) is a solvent-free, integrated technique that combines sampling, isolation, and pre-concentration into a single step, making it particularly effective for trace-level analysis of volatile and semi-volatile organic compounds (VOCs/SVOCs) in environmental, biological, or headspace-above food matrices. As illustrated in Figure 4, a polymer-coated fiber such as PDMS or CAR/PDMS is exposed directly to the sample or its headspace, allowing selective adsorption of target analytes, which are then thermally desorbed into a gas chromatograph (GC or GC-MS) for analysis. The non-invasive nature of SPME minimizes matrix interferences, reduces sample handling, and enhances reproducibility, while eliminating the need for solvents and post-extraction cleanup. Figure 4 visually summarizes the SPME workflow, highlighting its utility as a clean, efficient, and reproducible approach for isolating VOCs and SVOCs across various application domains.

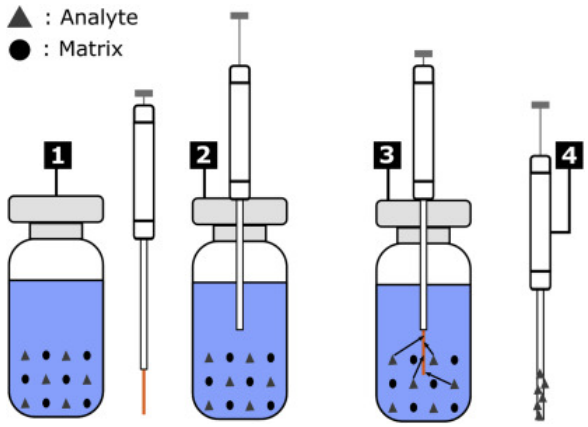

Figure 4. Schematic diagram of the Solid Phase Microextraction (SPME) process (Diagram of the SPME process showing fiber exposure, analyte absorption, and desorption for GC/LC analysis).

SPME's ability to accommodate simultaneous extraction of aroma compounds, pollutants, or biomarkers makes it highly valuable in food quality control, metabolomics, and environmental exposure assessment within dietary studies. Emerging fiber technologies, such as SPME Arrow, further extend its applicability by increasing phase volume and enhancing analyte capacity.

5.4. Liquid-Liquid Extraction

Liquid-Liquid Extraction (LLE), as shown in Figure 5, utilizes the differential solubility of compounds between immiscible aqueous and organic solvents to isolate target analytes. Despite its simplicity and broad applicability, LLE remains one of the most employed techniques for analyzing pharmaceuticals, lipophilic pollutants, and vitamins in food and biological matrices.

The procedure typically involves sample pH adjustment, mixing with an organic solvent, phase separation, and concentration of the organic layer. Common solvents include dichloromethane, hexane, and ethyl acetate, selected based on the polarity and volatility of target compounds. LLE's scalability and ability to process large sample volumes are advantageous for batch analysis in routine monitoring or population-wide nutrition studies. Figure 5 presents the liquid-liquid extraction (LLE) procedure for isolating organic compounds from aqueous matrices. The workflow emphasizes phase separation, organic solvent recovery, and downstream analysis using chromatographic platforms.

However, LLE may suffer from low selectivity, high solvent consumption, and analyte loss during phase separation or evaporation. Advanced adaptations like ultrasound-assisted LLE and dispersive LLE are being explored to enhance efficiency and sustainability. Despite these limitations, LLE remains a robust first-line method in food-as-medicine research, particularly in screening lipophilic toxins or supplement metabolites.

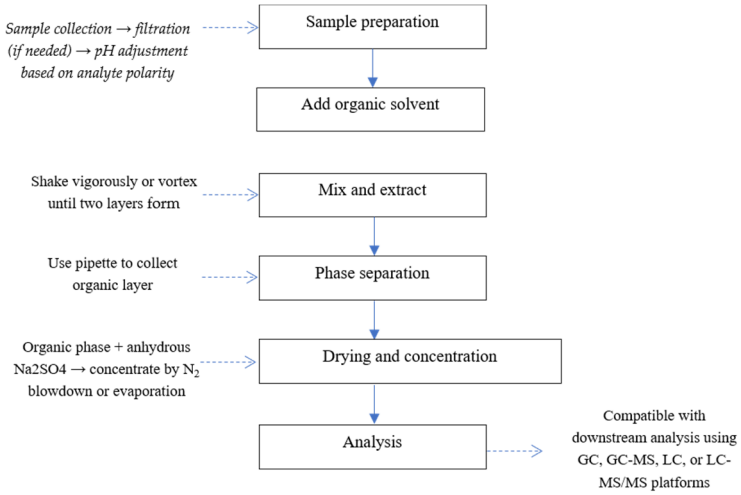

Figure 5. General Workflow of Liquid-Liquid Extraction (LLE) for Organic Compound Isolation from Aqueous Matrices (Note: LLE is widely used for extracting non-polar to moderately polar compounds from aqueous matrices in food, environmental, and biological research).

5.5. Dispersive Solid Phase Extraction

Dispersive solid-phase extraction (dSPE), commonly incorporated into QuEChERS workflows, offers a rapid and efficient clean-up strategy by directly mixing extract solutions with sorbents such as PSA, C18, or magnetic nanoparticles to adsorb matrix interferences including pigments, sugars, and lipids, without the need for column-based setups. Figure 6 illustrates the typical dSPE mechanism, highlighting both magnetic and conventional sorbents, followed by separation via centrifugation or magnetic decantation to yield a purified supernatant. As a post-extraction refinement step, dSPE enhances analyte recovery and reduces ion suppression, making it especially suitable for complex food and environmental matrices prior to LC-MS or GC-MS analysis.

Key sorbents in dSPE include:

- PSA: targets fatty acids and organic acids.
- C18: removes lipophilic materials and sterols.
- GCB: efficiently eliminates pigments and aromatic interferences.
- $MgSO_4$: aids in drying and reducing residual water.

dSPE is particularly beneficial in simultaneous analysis due to its speed, ease of operation, and adaptability to various sample types. The technique enhances signal quality in LC-MS/MS or GC-MS by significantly reducing background noise and ion suppression, while requiring minimal solvent use. It is also amenable to field-portable setups and high-throughput automation, making it ideal for decentralized testing in public health or agricultural surveillance.

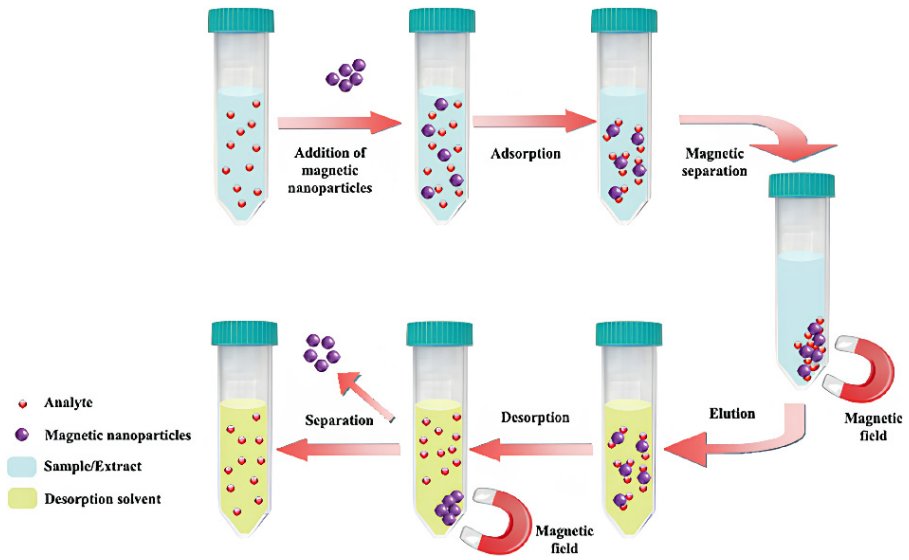

Figure 6. Step-by-Step Illustration of dSPE Sample Clean-Up Process (Step-by-step schematic of the dSPE clean-up process, including sorbent addition, vortexing, and supernatant recovery).

6. Comparison and Selection of Appropriate Sample Preparation Techniques

Selecting an optimal sample preparation method is a foundational aspect in the accurate and efficient simultaneous determination of diverse organic compounds, especially within complex biological and food matrices relevant to health and disease prevention research. The growing emphasis on the role of food-based bioactives, functional ingredients, and dietary contaminants in chronic diseases (e.g., metabolic syndrome, neurodegeneration, or cancers) necessitates rigorous and matrix-sensitive sample preparation workflows that align with the analytical goals of nutritional, epidemiological, and clinical investigations [99–102]. Each technique, be it QuEChERS, SPE, SPME, dSPE, or LLE, offers specific advantages related to matrix cleanup, analyte enrichment, processing time, and compatibility with LC-MS/MS or GC-MS systems. The selection should thus be evidence-driven, guided by the nature of both the target analytes (e.g., polarity, volatility, stability) and the food or biological matrix (e.g., lipids, pigments, proteins, water content). Table 3 compares the operational features, strengths, and limitations of major sample preparation techniques, serving as a guide for method selection in multi-analyte studies.

Table 3. Comparison of main features, advantages, and limitations of common sample preparation techniques for multi-residue analysis.

Technique	Advantages	Limitations	Typical Applications
QuEChERS	- Fast, simple, low-cost-Suitable for various analyte groups- Integrates extraction and clean-up	- May not effectively clean complex matrices-Unsuitable for highly polar compounds	Pesticide residues, contaminants in food and environmental samples
SPE	- Excellent clean-up, high reproducibility-Allows sample enrichment-Highly customizable by analyte	- Multi-step procedure requiring equipment-Costly when multiple cartridges are used	Pharmaceuticals, pollutants in water and biological matrices
dSPE	- Simple and time-saving-Easily integrated with QuEChERS-No need for specialized equipment	- Strongly dependent on sorbent selection-Unsuitable for strongly adsorptive analytes	Rapid clean-up of food extracts, environmental matrices
SPME	- Solvent-free-Combines extraction and preconcentration-Well-suited for GC and GC-MS	- Limited to analytes that can be extracted-Requires specialized fibers and equipment; high cost	VOCs and SVOCs in air, water, food headspace
LLE	- Effective for non-polar compounds-Easy to perform, no complex equipment needed	- High solvent consumption, not environmentally friendly-Poor phase separation with emulsions or complex matrices	Organic compounds in water, serum, biological samples

Comparison of main features, advantages, and limitations of common sample preparation techniques for multi-residue analysis.

Table 3 summarizes the practical characteristics of these core techniques. QuEChERS, due to its speed, cost-effectiveness, and compatibility with a wide range of compound classes, is widely adopted in food safety testing, particularly for pesticide residues or trace contaminants in fruits, vegetables, cereals, or animal-derived foods. However, its lower selectivity and reduced efficiency in removing highly complex matrices or very polar analytes (e.g., amino acids, polar pesticides) must be carefully considered. In contrast, SPE offers exceptional cleanup and high selectivity but may be cost-prohibitive for high-throughput studies due to its multi-step nature and the use of expensive cartridges. SPME stands out in volatile compound analysis without solvent usage, yet the high cost and limited analyte spectrum make it less ideal for broad-spectrum analyses. Meanwhile, LLE remains a classical technique but faces challenges in sustainability and reproducibility. Finally, dSPE, especially when integrated into QuEChERS workflows, enables quick, matrix-specific cleanup using combinations of sorbents like PSA, C18, and GCB, enhancing analyte recovery and method sensitivity. Table 4 provides a summary of performance metrics, including throughput, cost, and matrix applicability, among five widely used extraction methods.

Table 4. Summary comparison of key operational criteria among five sample preparation techniques.

Criterion	LLE	SPE	dSPE/QuEChERS	SPME	DLLME
Selectivity	Low	High	Medium	High	High
Automation	Limited	Possible	Possible	Possible	Difficult
Solvent saving	No	Moderate	Yes	Yes	Very high
Processing time	Moderate	Moderate	Fast	Moderate	Very fast

Summary comparison of key operational criteria across five sample preparation techniques for simultaneous compound analysis.

Beyond technical specifications, laboratories must also evaluate operational feasibility. Table 4 provides a comparative overview of key operational criteria. Techniques like dSPE and QuEChERS strike a balance between speed, automation potential, and solvent economy, making them highly favorable for functional food research and biomarker screening in nutritional epidemiology. SPE and SPME, while offering high selectivity and automation compatibility, require greater infrastructure and cost investment. DLLME, despite its very fast extraction and high enrichment factors, remains technically demanding for routine applications. Ultimately, hybrid approaches, such as combining LLE with dSPE or integrating online SPE with LC-MS, may provide the best analytical performance in projects assessing food-derived compounds' preventive or therapeutic effects in non-communicable diseases. Thus, the rational selection and optimization of sample preparation must be contextualized within the broader framework of food-health interactions and precision nutrition strategies.

7. Coupling Sample Preparation with Chromatographic Techniques

The synergy between sample preparation and chromatographic analysis is central to ensuring precise quantification of bioactive compounds, contaminants, or nutrient metabolites in functional foods and medicinal plants. In the context of food-as-medicine research, the analytical focus extends beyond classical pesticide residues to include polyphenols, flavonoids, carotenoids, alkaloids, and other phytochemicals with proven or hypothesized health benefits. Efficient sample preparation removes interfering matrix components, such as pigments, lipids, and proteins, and enriches the analytes of interest, thus facilitating sensitive and selective detection when coupled with modern chromatography-mass spectrometry platforms. This integration is particularly vital in nutritional epidemiology and pharmacognosy, where small variations in analyte concentration may correlate with significant clinical or preventive outcomes.

Depending on the matrix (e.g., fruits, vegetables, whole grains, fermented foods) and the physicochemical characteristics of the analytes, techniques such as QuEChERS-dSPE, SPE, or SPME are commonly employed. These are often followed by analytical workflows using UHPLC-MS/MS or GC-MS for precise compound separation and quantification. For instance, QuEChERS combined with gradient elution in LC-MS/MS enables simultaneous determination of polar antioxidants and hydrophobic contaminants within a single run. In contrast, volatile aroma compounds or residues in fermented foods (e.g., alcohols, esters, sulfur volatiles) are more effectively analyzed via HS-SPME coupled with GC-MS. The compatibility between sample preparation and chromatography must be ensured to avoid analyte degradation or retention losses, especially for labile compounds such as anthocyanins or sulfur-containing bioactives. This workflow harmony is essential to advancing food-based diagnostics, dietary biomarker validation, and mechanistic studies on food-microbiome-host interactions.

8. Method Validation in Multi-Residue Analysis

Rigorous method validation is fundamental to the reliability and scientific merit of food-as-medicine research, particularly when analyzing diverse bioactives and xenobiotics in complex food and biological matrices. As functional food research increasingly intersects with clinical and public health domains, validated methods ensure the generation of high-quality data that support causative inferences regarding food-derived compounds in disease prevention. Key validation parameters include selectivity, accuracy, repeatability, reproducibility, linearity, LOD, LOQ, and matrix effects. These metrics provide quantitative benchmarks to assess method performance across a wide dynamic range of analytes, from trace contaminants (e.g., mycotoxins, endocrine disruptors) to health-promoting phytochemicals (e.g., flavonoids, lignans).

For example, when analyzing antioxidants in fermented vegetables or pesticide residues in herbal supplements, method validation must confirm that matrix complexity (e.g., salt content, plant polysaccharides) does not compromise analytical accuracy. Matrix effects, particularly ion suppression or enhancement in LC-MS/MS, can be mitigated by matrix-matched calibration or isotopically labeled internal standards. Acceptable recovery rates (typically 70–120%) and RSDs (<20%) are essential for inter-laboratory reproducibility and compliance with global standards such as SANTE or Codex Alimentarius. Additionally, the stability of bioactives during sample handling and storage must be verified, particularly for thermolabile or oxidizable compounds. In translational studies linking dietary intake to biomarkers of exposure or effect, the robustness of the analytical method becomes as important as the biological hypothesis it seeks to test.

9. Perspectives

The evolving field of multi-residue analysis in food matrices offers exciting opportunities to reshape how we understand and apply nutrition in the prevention and management of chronic diseases. In alignment with the Food as Medicine paradigm, analytical chemistry is shifting from a contamination-centric model toward a health-

oriented, systems-based approach, capable of simultaneously profiling beneficial and harmful compounds within a single assay. Innovations in chromatographic resolution, detector sensitivity, and sample preparation efficiency are now being complemented by emerging technologies such as high-resolution mass spectrometry (HRMS) and non-targeted metabolomics. These advancements enable the identification of novel food bioactives or food-microbiome co-metabolites that may serve as therapeutic agents or disease-modifying factors.

Concurrently, artificial intelligence (AI) and machine learning are poised to revolutionize how data from multi-residue analyses are processed, interpreted, and translated into actionable insights. When applied to food-based matrices, AI can predict compound interactions, identify unknown peaks, and even suggest optimized extraction protocols based on matrix complexity. However, these capabilities depend critically on the consistency and quality of upstream sample preparation. In this context, sample preparation is not merely a technical prerequisite, it becomes a strategic enabler of AI-driven discovery in nutritional science, personalized nutrition, and functional food development. The future of this discipline will lie in integrated platforms that unify intelligent sample processing, real-time data analytics, and dynamic interpretation models, paving the way for evidence-based dietary interventions and personalized therapeutic nutrition grounded in robust analytical chemistry.

10. Conclusions

In the evolving landscape of food, environmental, and biomedical research, the simultaneous determination of multiple organic compounds in complex matrices has become a central analytical challenge. Owing to the wide variability in analyte chemistry and matrix composition ranging from functional foods and fermented products to biological fluids and environmental samples no single preparation method is universally applicable. QuEChERS offers operational simplicity and is ideal for food and agricultural matrices containing diverse residues. Solid-phase extraction (SPE) provides high selectivity for aqueous samples in clinical and environmental contexts, while solid-phase microextraction (SPME) enables solvent-free, trace-level detection of volatiles. Dispersive-SPE (dSPE), often integrated with QuEChERS, enhances matrix cleanup in high-complexity samples. A matrix-guided strategy aligned with analyte polarity, matrix characteristics, and chromatographic compatibility is essential for method selection and optimization.

More importantly, robust sample preparation is integral to realizing the Food as Medicine paradigm. Accurate profiling of nutrients, bioactives, and contaminants underpins dietary exposure assessment, functional food innovation, and personalized nutrition strategies. When combined with automation and AI-driven analytics, optimized workflows can facilitate high-throughput, high-fidelity data acquisition. This review uniquely positions sample preparation as a translational bridge between analytical chemistry and health sciences, supporting evidence-based dietary interventions and reinforcing the role of food in disease prevention and management.

Author Contributions

T.Q.N.: Conceptualization, Writing-original draft, Figure design, Methodology, Literature analysis. M.N.T.: Supervision, Writing—review & editing, Section coordination, Final approval. M.Q.B.: Literature collection, Table design, Data organization. B.Y.K.: Figure design, Visual editing, Layout formatting. T.T.N.: Literature collection, Reference management, Manuscript formatting. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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