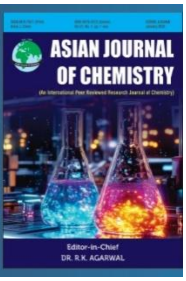
Asian Journal of Chemistry; Vol. 37, No. 11 (2025), 2631-2640
ASIAN JOURNAL OF CHEMISTRY
https://doi.org/10.14233/ajchem.2025.34677



REVIEW

AQbD-Driven Method Development for Magnetic Nanoparticle Extraction and HPLC Analysis: A Comprehensive Review

RANEEM DARRAJ[1,*], MOHAMMAD HAROUN[1], AYAT ABBOD[1] and IBRAHIM ALGHORAIBI[2]

[1]Department of Pharmaceutical Chemistry and Quality Control of Drugs Faculty of Pharmacy, Latakia University, Latakia, Syria
[2]Department of Physics, Faculty of Science, Damascus University, Damascus, Syria

*Corresponding author: E-mail: kkraneeem91@gmail.com

Received: 7 September 2025 *Accepted*: 18 October 2025 *Published online*: 27 October 2025 AJC-22149

The integration of analytical quality by design (AQbD) principles into analytical method development has revolutionized precision, robustness and regulatory compliance in the pharmaceutical and environmental sciences. This review explores the AQbD-driven optimization of magnetic nanoparticle (MNP) extraction coupled with high-performance liquid chromatography (HPLC) for enhanced analyte separation and quantification. The study outlines the systematic application of AQbD, including risk assessment, design of experiments (DoE) and control strategies, to optimize MNP extraction efficiency and chromatographic performance. Key aspects such as critical material attributes (CMAs), critical process parameters (CPPs) and critical quality attributes (CQAs) are identified to ensure method robustness and reproducibility. Furthermore, the article highlights the broad applicability of AQbD-based MNP-HPLC methods in pharmaceutical quality control, environmental monitoring and food safety analysis, especially after the advancement in magnetic nanoparticles application in different fields. Challenges associated with AQbD implementation in nanoparticle-based analytical techniques are also examined in pharmaceutical and environmental applications, alongside emerging trends such as computational modeling, artificial intelligence (AI)-driven optimization which is the most impactful for MNP-HPLC and automated method development. This review presents basic information from the 2008 UpToDate on the use of magnetic nanoparticles, while the application of AQbD in this context is relatively recent, having become widespread primarily after 2020.

Keywords: Magnetic nanoparticles extraction, Design of experiments, Pharmaceutical analysis, Environmental monitoring.

INTRODUCTION

Analytical method development is a fundamental aspect of pharmaceutical and environmental sciences, ensuring the accurate detection, quantification and characterization of analytes in complex matrices. The reliability and efficiency of an analytical method significantly impact regulatory compliance, product quality and safety assessments [1]. Traditional method development approaches have relied heavily on trial-and-error procedures, which are often time-consuming, resource-intensive and susceptible to variability [2]. These limitations have necessitated the adoption of a more structured, science-based framework–analytical quality by design (AQbD), which offers a systematic, risk-based approach to method development and validation [3]. AQbD enhances method robustness, reliability and regulatory acceptance by incorporating critical components such as risk assessment, design of experiments (DoE), multivariate optimization and continuous monitoring [3].

Magnetic nanoparticles (MNPs) have emerged as powerful tools in modern analytical chemistry due to their unique physico-chemical properties, including superparamagnetic, high surface-to-volume ratio and ease of surface modification [4]. These properties make MNPs highly efficient in sample preparation, separation and preconcentration of target analytes, offering distinct advantages over conventional extraction methods such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) [5]. The integration of MNPs with high performance liquid chromatography (HPLC) has further enhanced analytical workflows, enabling highly sensitive and selective detection of trace-level contaminants in pharmaceuticals, environmental samples and food matrices [6,7].

Despite the numerous advantages of MNP-assisted HPLC, several challenges remain, particularly concerning method reproducibility, robustness and standardization [8]. The variability in nanoparticle synthesis, functionalization and extraction

---

efficiency often impacts the consistency of analytical results [9]. To address these limitations, the AQbD framework provides a structured approach to optimize MNP-based extraction and HPLC methods by defining critical quality attributes (CQAs), critical material attributes (CMAs) and critical process parameters (CPPs) [10]. AQbD-based method development ensures the selection of optimal experimental conditions, enhances method robustness and improves the overall efficiency of the analytical process [3].

The implementation of AQbD principles in MNP-HPLC workflows involves the systematic risk assessment, rigorous experimental design and multivariate optimization strategies. By applying design of experiments (DoE), response surface methodology (RSM) and multivariate analysis, researchers can fine-tune extraction efficiency, nanoparticle surface functionalization and chromatographic parameters to achieve maximum selectivity and sensitivity [3]. In addition, qualification of methods streamlined *via* quality by design (QbD) ensures compliance with the regulatory guidelines set by organizations like the International Council for Harmonization (ICH), the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [11].

This review explores the integration of AQbD principles into MNP-based extraction and HPLC analysis, emphasizing the role of systematic risk assessment and experimental design in developing high-precision analytical methods. The discussion covers fundamental principles, validation strategies, key optimization parameters and emerging applications in pharmaceutical quality control, environmental monitoring and food safety. Furthermore, challenges in AQbD implementation and future perspectives on AI-driven method development are explored, highlighting the potential of computational modelling, automation and machine learning in advancing AQbD-based analytical science [12].

Some reviews have discussed application of magnetic solid phase extraction combined with analytical methods in pharmaceutical fields [13] and other studied the use of AQbD in HPLC method, but until this time, there aren't any review focused on using AQbD approach to use magnetic nanoparticles in extraction and HPLC method. PubMed, ResearchGate, Google scholar and National Institute of Health (NIH), provide the main information for this review from 2008 UpToDate in field of magnetic nanoparticles, optimization of HPLC method and analytical quality by design approach.

AQbD-driven optimization for magnetic nanoparticle extraction

AQbD in magnetic nanoparticle extraction: Analytical quality by design (AQbD) is a systematic approach that ensures method robustness and reliability by defining quality attributes, identifying critical parameters and implementing risk-based assessments [14]. The AQbD framework aligns with the regulatory guidelines and enhances method efficiency, reproducibility and robustness [11]. When applied to magnetic nanoparticle (MNP) extraction, AQbD provides a structured methodology for optimizing extraction efficiency, reducing variability and improving the selectivity in sample preparation workflows [3].

Magnetic nanoparticles have become essential in solid-phase extraction (SPE) and dispersive solid-phase extraction (DSPE) due to their rapid separation capabilities, tunable surface chemistry and high adsorption capacity [15]. The extraction efficiency of MNPs is significantly influenced by multiple factors, including pH, ionic strength, extraction time, nanoparticle size, surface functionalization and desorption conditions [8]. AQbD-driven optimization enables the identification and control of these factors, ensuring the development of an efficient and reproducible MNP-based extraction process.

Synthesis of MNPs: There are different ways to synthesis of MNPs *viz.* physical, chemical and biological methods. Mechanical milling, laser evaporation, wire explosion and gas-phase synthesis are the physical methods, which make the control of nanoparticles size easier, but have low scalability with high cost precipitation, coprecipitation, hydrothermal and sol-gel methods are included in chemical types of synthesis which are better in control of nanoparticles size with high scalability [16,17], although they have environmental hazardousness with high cost [18,19]. In contrast with the biological methods, which are eco-friendly with low cost, but have difficulty in control the shape and size of formed nanoparticles [20-22]. The integration of QbD principles into MNPs synthesis represents a paradigm shift from empirical optimization to systematic, risk-based development. Desired nanoparticle characteristics (*e.g.* size, magnetism and biocompatibility) can be defined [23].

Analytical target profile (ATP) for MNP extraction: ICH Q14 implication for AQbD based MNPs extraction creates a robust framework of the process. The analytical target profile (ATP) defines the desired performance characteristics of the MNP-based extraction method, serving as the foundation for method optimization. The ATP outlines key attributes, including: high extraction efficiency (> 90%) for target analytes, minimal matrix interferences to ensure selectivity, rapid separation time (< 5 min) using an external magnetic field, reproducibility (RSD < 5%) across multiple extractions, compatibility with downstream HPLC analysis without interference from nanoparticle residues. By establishing a well-defined ATP, researchers can systematically design experiments to optimize MNP extraction while ensuring compliance with regulatory requirements [10].

Identification of critical material attributes (CMAs) and critical process parameters (CPPs): In AQbD, critical material attributes (CMAs) and critical process parameters (CPPs) are essential for optimizing method performance [24]. Table-1 provides an overview of key CMAs and CPPs for the MNP extraction [25,26].

Design of experiments (DoE) for optimization: According to ICH Q14, design of experiments (DoE) is a statistical approach used in AQbD to systematically evaluate the influence of multiple factors on MNP extraction efficiency [8]. A 2^3 factorial design can be used for preliminary screening of key parameters (pH, extraction time and nanoparticle size). The response variable (extraction efficiency) is evaluated at high and low levels of each parameter to identify significant factors. The Box-Behnken design (BBD) or Central Composite Design (CCD) is commonly employed to assess interactions among CMAs and CPPs [12,34]. Table-2 summarizes common DOE

TABLE-1
CMAs AND CPPs FOR AQbD-DRIVEN MNP EXTRACTION

Parameter	Category	impact on extraction efficacy	Optimization strategy	Ref.
Nanoparticle size (nm)	CMA	Affects surface and adsorption	Control during synthesis	[27]
Surface functionalization	CMA	Enhances selectivity of analyte	Use of coating agent (*e.g.* polymer or silica)	[28]
Ph of the extraction medium	CPP	Affect ionization of target analyte	Optimize pH using DOE	[29]
Ionic strength (mM)	CPP	Influences aggregation and dispersion	Optimize salt concentration	[30]
Extraction time (min)	CPP	Determines efficiency of adsorption	Minimize (<10 min) using RSM	[31]
Temperature (ºC)	CPP	Affect diffusion rate of analyte	Maintain at optimal (25-40) C˚	[32]
kind of solvent	CPP	Impacts analyte recovery and purity	Optimize for good choice solvent by DOE	[33]

TABLE-2
LIST OF SOME COMMON DESIGN OF EXPERIMENTS (DoE) PROGRAMS

Design	Goal of applying	Advantage	Disadvantage	Ref.
Box-Behnken design	Avoids extreme factor levels to optimize the process and model nonlinear relationships	Proffered when the exact design point design points are within the design region	Limited flexibility with categorical variables and suboptimal for ratability	[35,36]
Placket-Burman technique	Identifying some factors from large variables	Need a minimal number of runs for an extensive set of variables	Cofounding of interactions and linearity assumption	[34,37]
Full factorial design	Evaluating every single variable and interactions	Deciding the primary and second any effects, while avoiding failure, efficient and more informative than one-factor-at-a-time (OFATE) designs	Trial runs depend on the number of variables, the analysis and interpretation of the results can be complex	[38,39]
Fractional factorial design	Screening and optimization for factors	fewer experimental runs are necessary, more feasible, economical and creative	Sensitive and depend of the assumption and judgments of the experimenter	[38,39]

programs highlighting their specific goals, advantages and limitations, aiding in selecting the most suitable design based on experimental needs. Each design balances efficiency and complexity differently, with trade-offs in flexibility, run numbers and interpretation.

Risk assessment and mitigation strategies: Risk assessment in AQbD involves the identification and mitigation of potential factors that may negatively impact extraction performance. To mitigate these risks, robust nanoparticle synthesis protocols are employed to ensure reproducibility. Statistical modeling techniques, such as Response Surface Methodology (RSM), are used to model the relationship between input variables (*e.g.* temperature, reaction time) and output responses (*e.g.* extraction efficiency). Moreover, Principal Component Analysis (PCA), a dimensionality reduction tool that transforms correlated variables into fewer uncorrelated principal components while retaining maximum data variance, is utilized to optimize conditions. Furthermore, real-time monitoring of process parameters helps prevent variability during synthesis. The first phase of risk analysis involves identifying and prioritizing potential risks, including instrument operation methods, reagent characteristics and cycle time crucial to establish a backup plan if the primary strategy collapses. Risk evaluation is the second phase of risk analysis with the Fishbone diagram may called the Ishikawa approach to categorize risk factors into high-risk, noise and experimental categories [40]. Semiquantitative tools for risk ranking include failure mode and effects analysis (FMEA/FMECA) (*e.g.* MNPs aggregation during extraction), relative ranking and failure mode effects and criticality analysis. In ICH Q9, various kinds of tools for risk assessment are mentioned, including failure mode and effect analysis (FMEA), comparison matrix (CM), risk estimation matrix (REM), hazard operability analysis (HAZOP) and hazard analysis critical control points (HACCP), REM and FMEA are the methods most frequently used in product development. While, FMEA uses scoring on a scale of 1-10 for risk ranking based on severity, occurrence and detectability, REM uses different risk levels, *i.e.* low, medium and high-risk ratings depending on their severity and occurrence [41].

Representation of optimization results: Method operable design range (MODR) is the design space in which the analytical method is expected to meet the defined quality target method profile (QTMP). It helps in identifying critical method variables and their optimal ranges, where a robust region for the critical method parameters could be obtained. MODR should always be broader than normal operating ranges (NOR) to ensure the robustness of the method [42]. For example, the 3D responses surfaces of some variables on the antioxidant activity (Fig. 1) [43].

Experimental validation and robustness testing: After optimizing extraction conditions, method robustness and reproducibility must be validated. Validation involves: Precision ($RSD < 5\%$) across multiple extractions, linearity ($R^2 > 0.99$) between analyte concentration and extraction efficiency, recovery studies (80-120%) for method accuracy, comparison with conventional extraction methods to demonstrate the AQbD-driven improvements.

The AQbD-driven approach to MNP-based extraction offers a structured and efficient method development strategy. By defining the analytical target profile (ATP), optimizing CMAs and CPPs and utilizing DoE for systematic optimization, this framework ensures robust, reproducible and regulatory compliant extraction methods. Future advancements in AI-driven optimization, machine learning and real-time monitoring will further enhance AQbD implementation, leading to

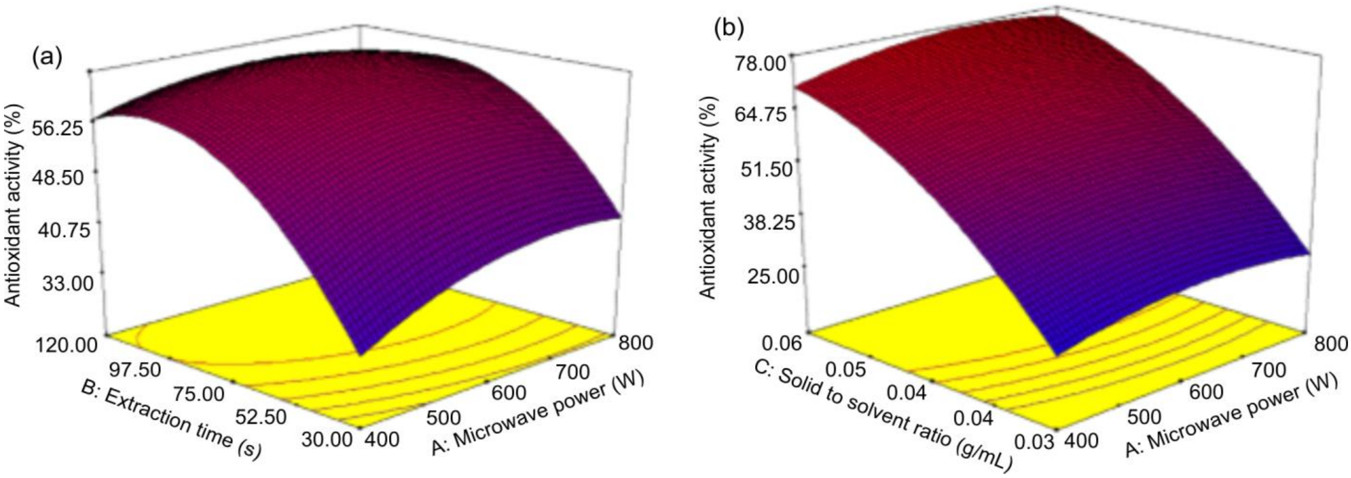


Fig. 1. The 3D responses surfaces of (a) extraction time and microwave power, (b) solid to solvent ratio and microwave power and (c) solid to solvent ratio and extraction time on the antioxidant activity [Ref. 45]

improved analytical precision in pharmaceutical, environmental and food safety applications [12].

Performance evaluation and analytical validation

Analytical validation in AQbD: Analytical validation is a crucial step in analytical quality by design (AQbD)-driven method development, ensuring that the method is accurate, precise, robust and suitable for its intended application [11]. The validation process establishes the method's reliability and reproducibility by assessing key performance parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability. In context of MNP extraction coupled with HPLC, validation confirms that the optimized method effectively isolates and quantifies target analytes with minimal interference. The AQbD framework ensures that validation parameters are statistically optimized and aligned with regulatory standards [13].

Validation parameters and performance criteria: The International Council for Harmonization (ICH) guidelines (ICH Q2(R1)) define several key validation parameters for analytical methods [11]. Table-3 summarizes the performance criteria for each parameter in the context of MNP-based HPLC analysis.

Specificity and selectivity evaluation: Specificity is evaluated by analyzing blank samples, spiked samples and real samples to determine whether the method can selectively detect the target analyte without interference from excipients, degradation products or nanoparticle residues. A chromatogram comparison is performed for blank sample (without analyte or MNPs), analyte standard solution (pure compound), spiked sample with MNPs (analyte extracted using magnetic nanoparticles) [11].

Linearity and calibration curve assessment: Linearity is determined by preparing six concentration levels of the analyte and measuring the HPLC peak area response for each

TABLE-3
ANALYTICAL VALIDATION PARAMETERS AND PERFORMANCE CRITERIA

Parameter	Definition	Acceptance criteria
Specificity	Distinguish analyte from interface	No interferences observed
Linearity	Proportional response to analyte concentration	Correlation > 99%
Accuracy	Agreement with true value	Recovery (80-120)%
Precision	Reproducibility	RSD < 5
Limit of detection (LOD)	Limit of the lowest analyte concentration at which detection is feasible	Signal to noise ratio (S/N) ratio = 3/1 (In instrumental techniques, the noise is often measured from the baseline of a blank sample or chromatogram) This means that the signal from the analyte needs to be at least 3 times (LOD) stronger than the background noise for reliable detection [44]
Limit of quantitation (LOQ)	Limit of lowest concentration at which the analyte can not only be reliably detected but at witch some predefined goals for bias and imprecision are met. The LOQ may be equivalent to the LOD or it could be at a much higher concentration.	S/N ratio = 10/1 This means that the signal from the analyte needs to be at least 10 times (LOQ) stronger than the background noise for reliable quantification [44]
Robustness	Stability under small variation	No significant change in results With potential of reusability of MNPs for many cycles without reducing the efficiency (*e.g.* extraction efficiency did not drop more than 15% after 5 cycles [45]
System suitability	Ensures proper HPLC function	Meet predefined criteria

concentration. A straight-line relationship confirms linearity, with the correlation coefficient greater than 99% indicating strong proportionality [11].

Accuracy and recovery studies: Accuracy is evaluated through recovery studies, in which a known quantity of analyte is added to the sample matrix and subsequently extracted using MNPs. The amount of analyte recovered is then compared to the known added amount to calculate the percentage recovery. Since matrix components can influence both the detection and quantification of the analyte, assessing recovery in the actual sample matrix is essential. Accuracy is considered acceptable when the recovery percentage falls within the range of 80% to 120% of true value, reflecting the method's reliability despite potential matrix effects [11].

Precision and reproducibility: Precision is assessed by analyzing six replicate samples under identical conditions to ensure consistency. The variability among these replicates is quantified by calculating the relative standard deviation (RSD). Precision is considered acceptable when the RSD is less than 5%, indicating reliable reproducibility of the method [13].

Detection and quantification limits (LOD and LOQ): LOD and LOQ are determined using the signal-to-noise (S/N) ratio method. The LOD is the lowest concentration where the signal is three times the noise level, while the LOQ is where the signal is ten times the noise level. A low LOD ensures the method can detect trace levels of analyte, while a low LOQ ensures accurate quantification at minimal concentrations.

S/N ratio is different from matrix to another. The matrix itself can contribute to the overall background noise in the measurement. This can make it harder to distinguish the analyte signal from the background, further reducing the S/N ratio. In biological samples, the component of proteins can affect on analyte signals. Also, the organic and inorganic compound in soils could affect the analysis. It is important when analyzing drugs similarly to take into consideration the drug formulation and metabolites, which may reduce the sensitivity of the analytical methods or led to inaccurate quantification of analyte. In early 1990s, can be described as the difference between the mass spectrometric response for an analyte in standard solution and the response for the same analyte in a biological matrix, such as plasma. Matrix effects result from co-eluting matrix components that affect the ionization of the target analyte, resulting either in ion suppression or in some cases, ion enhancement. Matrix effects can be highly variable and can be difficult to control or predict. An alternative approach to increasing S/N is to improve the quality of chromatography purification. This decreases matrix effects and makes possible the use of comparatively less expensive single quadrupole instruments while still achieving high analytical performance [46]. Techniques used to combat MEs while recovering analytes, particularly in the analysis of complex matrices such as biological samples (*e.g.*, tissues, biofluids), food samples and environmental samples (including air, water, plants and soil), include dilute-and-shoot, quick, easy, cheap, effective, rugged and safe (QuEChERS), liquid-liquid extraction (LLE), solid-phase extraction (SPE) and solid-phase microextraction (SPME), to name a few [47].

In 2011, Niina *et al.* [48] have investigated the effect of matrix within direct analysis of benzodiazepines and opioids from urine with desorption electrospray ionization-mass spectrometry (DESI-MS) and desorption atmospheric pressure photoionization-mass spectrometry (DAPPI-MS) the decrease in sensitivity due to the urine matrix was higher with DESI (typically 20-160-fold) than with DAPPI (typically 2-15-fold).

Robustness and system suitability testing: Robustness is tested by introducing small variations in method conditions (pH, extraction time, mobile phase composition) and analyzing their effect on method performance [12]. A method is considered robust if minor variations do not significantly impact results.

System suitability parameters: System suitability tests (SST) are performed by analyzing a standard solution before sample analysis to ensure HPLC performance meets predefined criteria. Key SST parameters include: retention time consistency, peak area stability and resolution between peaks.

The performance evaluation and analytical validation of the AQbD-driven MNP-HPLC method confirm its reliability, sensitivity and robustness. The validated method meets regulatory standards by demonstrating high specificity, accuracy, precision and reproducibility, ensuring suitability for pharmaceutical and environmental applications. The AQbD approach ensures method adaptability for future enhancements including automated optimizations and AI-driven real-time adjustments [13].

Life cycle management and control strategies

Method operable design region (MODR): It defines a multidimensional space where method parameters can vary without impacting performance, ensuring robustness for complex MNP matrices [47].

AQbD-driven optimization for HPLC analysis: The physico-chemical properties of analyte play important role in method development and optimization to achieve the ATP of HPLC like theoretical plates of more than 2000, retention time of no more than 5 min, tailing factor of less than 2, resolution between two peaks is more than 5 and %RSD of analyte peaks area in standard chromatogram must be more than 2% [49].

The application of the AQbD approach in HPLC method development offers a significant advantage by reducing the number of experiments needed to achieve more reliable results, thereby saving time and resources during the development stage [50]. Furthermore, using MNPs for extraction prior to HPLC analysis provides further benefits, particularly in protecting the HPLC column. Since the analyte is eluted from the MNPs directly using the mobile phase before injection, the influence of matrix components is minimized, reducing potential column contamination compared to other extraction methods [51].

Selection of chromatographic conditions

Selection of column: It depends on several parameters like hydrophobicity, steric activity, ion-exchange, silanolol activity, carbon load and hydrogen bonding. In addition, column bed dimensions, particle size, surface area and pore size, bonding type, base material, particle shape and end-capping need to be considered while choosing a column for separation of assay, impurities and degradation products [52].

Selection of chromatographic mode: The selection of isocratic or gradient mode depends on the number of active components to be resolved. An initial gradient run is performed to conclude the choice of isocratic or gradient and the ratio between total gradient time and the difference in first and last gradient time components are observed. Isocratic elution appears more preferable when the sample occupies less than 10% of the separation space off the standard gradient scouting run. Samples occupying more than 40% of the separation space certainly require gradient elution. However, for samples occupying between 10 and 40% of the separation space, either elution technique could be used but the only pragmatic reasons to avoid gradient elution is that gradient elution instrumentation is not available or the gradient baseline limits trace analysis [53].

Optimization of mobile phase:

Selection of solvents (methanol, acetonitrile, tetrahydrofuran): Choice between methanol and acetonitrile may depend on the analyte's solubility and buffer used. THF is the least polar among three solvents, responsible for large changes in selectivity and compatible with low wavelength detection. Mobile phase optimization procedures are normally based on the solvent selectivity triangle, Solvents that are located far away from each other in this triangle have the largest differences in these properties and, hence, greater differences in selectivity will be found when using them in the mobile phase. Considering miscibility, ease of use, availability and reasonable boiling point as well. The best organic solvent choices for reversed-phase HPLC are acetonitrile, methanol and tetrahydrofuran [54].

Column temperature: The column temperature has unpredictable effects on selectivity. Employing high temperature will reduce viscosity and back pressure. It may help to operate at high flow rates or to use longer columns smaller particle sizes, reduce elution time and improve method reproducibility. The target temperature 30-40 ºC range is good for reproducibility [55].

Challenges in the application of AQbD-driven MNPs extraction coupled with HPLC: The integration of AQbD principles in MNP-assisted HPLC has significantly improved method robustness, selectivity and reproducibility. However, despite these advantages, the application of AQbD-driven MNP extraction presents several technical and regulatory challenges in various fields, including pharmaceutical analysis. This section explores the key obstacles encountered in the practical implementation of AQbD-driven MNP-based HPLC methods, focusing on method optimization, regulatory compliance, sample complexity, instrumentation constraints and future improvements. Addressing these challenges is essential to ensure the widespread adoption and sustainability of AQbD in modern analytical workflows [56]. Extraction using magnetic nanoparticles (MSPE) is a highly efficient and eco-friendly method for paraben extraction. This technique uses functionalized magnetic nanoparticles as adsorbents to selectively extract methylparaben (MP) and propylparaben (PP) from complex matrices through external magnetic separation. Surface modifications such as surfactant functionalization or polymer coatings enhance the selectivity and binding capacity of MNPs. Table-4 provides the key benefits of functionalized MNPs like high selectivity, fast separation, reusability and low solvent use alongside challenges like complex functionalization, agglomeration and high initial costs. Optimizing MNP performance requires balancing these factors.

Challenges in pharmaceutical applications

Complexity of pharmaceutical matrices: Pharmaceutical samples, such as drug formulations and biological fluids, contain complex matrices that interfere with the extraction process. The main challenges include matrix effects influencing MNP binding efficiency, variability in drug formulations affecting extraction reproducibility, possible adsorption of interfering substances onto MNP surfaces [14]. To mitigate these challenges, extensive design of experiments (DoE) studies are required to optimize surface modifications of MNPs to the enhance selectivity.

Regulatory and validation challenges: Regulatory bodies such as the USFDA and ICH Q14 guidelines, require rigorous validation of AQbD-based methods. Common hurdles include defining critical quality attributes (CQAs) in compliance with regulatory expectations, addressing method robustness and stability over time [24].

Standardization of AQbD method implementation: The absence of standardized AQbD guidelines for MNP-assisted HPLC complicates method transferability between laboratories. This limitation affects the global harmonization of analytical procedures, reproducibility of results in inter-laboratory studies, scalability of AQbD-based methods for large-scale pharmaceutical applications [67]. Table-5 outlines key challenges in MNP analysis and suggests targeted solutions including surface coating, early regulatory integration, strict quality control and collaborative regulatory efforts to enhance reliability and method transferability.

TABLE-4
COMPARATIVE EVALUATION OF THE ADVANTAGES AND LIMITATIONS OF FUNCTIONALIZED MAGNETIC NANOPARTICLES

Pros	Cons
High selectivity: Functionalized surfaces allow specific interaction with MP and PP molecules [60]. In AQbD approach, the kind of coating material is CPP to achieve the best selectivity (ATP) [10]	**Functionalization complexity:** Chemical process required to modify MNP surface requires precise control and expertise [64]
Rapid separation: External magnetic fields simplify the separation process, reduces extraction time [61]	**Particle agglomeration:** MNPs may cluster, reduce effective surface area and adsorption efficiency [65]
Reusability: Modified MNPs show high stability and can be reused multiple times [62]	**Cost of functionalization:** While operational cost is low, initial cost for functionalized MNP synthesis is high [66]
Low solvent usage: MSPE minimizes organic solvents usage, follows green chemistry principles [63]	

TABLE-5
KEY CHALLENGES IN PHARMACEUTICAL APPLICATIONS OF AQbD-DRIVEN MNP EXTRACTION COUPLED WITH HPLC

Challenge	Impact on analysis	Potential solution	Ref.
Matrix interference	Reduced extraction selectivity and accuracy	Coated surface MNPs	[68]
Regulatory compliance	Increased validation burden	Early integration of regulatory frameworks (risk assessment)	[69]
Batch variability in MNPs	Inconsistent recovery rates	Strict QC manufacturing control (DOE which minimizes trial and error)	[70]
Lack of standard AQbD guidelines	Difficult method transferability	Collaborative regulatory efforts	[71]

Challenges in environmental applications

Sample heterogeneity in environmental matrices: The environmental samples such as water, soil, and food products often exhibit high heterogeneity, which can significantly influence the extraction performance of MNPs. The variability in contaminant distribution, along with the presence of competing ions, can reduce extraction efficiency by interfering with analyte binding. Moreover, complex interactions between MNPs and organic matter present in these matrices may further hinder adsorption and recovery, posing key challenges to achieving consistent and reliable extraction results. A study on pesticide detection in agricultural runoff found that MNP-HPLC had a 15% lower recovery rate in soil samples compared to aqueous matrices, highlighting the need for optimized extraction conditions [12].

Sensitivity limitations in trace contaminant detection: While AQbD enhances method robustness, trace-level detection of heavy metals, endocrine disruptors and pharmaceuticals in wastewater remains a challenge due to insufficient MNP surface area for ultra-trace analyte binding, interference from background environmental noise, limited selectivity of functionalized MNPs for multi-analyte detection [72]. To address these challenges, computational modeling can be employed to better understand the interactions between MNPs and matrix components, thereby guiding the refinement of MNP functionalization strategies for enhanced selectivity and extraction efficiency.

Instrumentation and cost barriers: Despite the advantages of AQbD-driven MNP-HPLC methods, high costs and technical expertise are required for specialized HPLC instrumentation with magnetic separation capabilities, development of tailored MNPs with optimized properties, integration of real-time monitoring for AQbD process control [49]. A cost-benefit analysis comparing conventional HPLC with MNP-based AQbD-HPLC revealed that the initial setup cost is 40% higher, though long-term operational savings offset this expense over time. Table-6 addresses the analytical challenges and broadens the applicability of nanoparticle-based methods.

Future perspectives in AQbD-driven MNP extraction and HPLC analysis: Future perspectives in this field focus on automated AQbD-driven optimization, AI-assisted predictive modeling and the use of real-time monitoring systems for robust extraction efficiency and quality control [31]. Machine learning and AI-driven models are transforming AQbD-based method optimization by predicting ideal experimental conditions before laboratory testing. These models analyze previous experimental data and identify the most influential factors, reducing the number of required experiments [12]. By integrating AI with DoE and RSM, researchers can optimize MNP extraction processes and HPLC analysis faster and with greater accuracy.

Asahi *et al.* [75] reviewed machine learning (ML) approaches within the various staged of nanomedicine development from nanoparticle synthesis and characterization to performance prediction and disease diagnosis. ML for example, was used to predict the heat generation of MNPs with different magnetic properties under varying conditions [70]. Recently, Dawoud *et al.* [76] explored the integration of AI with QbD principles in the formulation of lecithin/chitosan nanoparticles for delivering a poorly water-soluble drug in carcinoma treatment. Silymarin is employed as a model drug due to its potential efficacy in liver cancer. A design framework was developed, yielding an optimized formulation with a lecithin-to-chitosan molar ratio of 18.33:1 and 39.25 mg of silymarin. AI demonstrated strong predictive accuracy for the drug's release rate. The optimized formulation enhanced the cytotoxic effect of silymarin [76].

AI models examine and screen historical data as pre-extraction process to determine the optimal solvent type, concentration and temperature for a specific plant waste, microbe, enzyme and target molecule. During the extraction, AI models can handle enormous amounts of data and assist in real-time monitoring and process control. Sensors continuously monitor critical parameters like pressure, temperature and solvent flow rate. AI can help to interpret extraction data and assist in decision making for additional processing, purification and quality

TABLE-6
CHALLENGES IN ENVIRONMENTAL APPLICATIONS OF AQbD-DRIVEN MNP EXTRACTION COUPLED WITH HPLC

Challenge	Impact on analysis	Potential solutions	Ref.
Sample heterogeneity	Lower recovery rates	Matrix-matched calibration standards	[73]
Trace contaminant sensitivity	Reduced detection capability	Enhanced MNP surface functionalization (correct kind of bonds between MNPs and the analyte)	[72]
Background interference	Increased noise in chromatographic	Improved sample cleanup methods (coated nanoparticles to increase selectivity of the specific analyte)	[72]
High instrumentation costs	Limited adoption in resource-limited setting	Development of cost-effective alternatives computational tools to predict the interactions)	[74]

control processes at the final phase of bioactive molecule recovery, providing data-driven decision support and processing [77].

Nezhadali *et al*. [78] developed a UV-Vis spectrophotometric method for the determination of fluoxetine in pharmaceutical formulations and human serum samples using a SPE approach based on molecularly imprinted polymers (MIPs). To optimize the extraction conditions, they employed a Plackett-Burman Design (PBD) consisting of 12 randomized experiments to evaluate the influence of various factors, including sorbent amount, initiator concentration, monomer-to-template ratio, shaking speed, extraction time, wash buffer pH, and polymerization time. Following the screening step, optimization was further refined using a combination of Central Composite Design (CCD), Artificial Neural Networks (ANN) and Genetic Algorithms (GA). The study demonstrated that integrating these statistical and computational tools provides a powerful approach for predicting optimal conditions and achieving global optimization without experimental limitations [78].

Taking into consideration of the preclinical safety evaluation of biotechnology-derived pharmaceuticals (ICH S6) guidelines recommended ensuring a rapid regulatory process and that product information includes criteria for selection of cell lines, culture media, assay reagents and how interferences with each are eliminated. The EMA has approved 3D cultures that mimic the biological environment. The standard for the assessment of *in vitro* cytotoxicity of medical devices has developed a screening method to assess the safety of nanoparticles in 3D cultures and provide guidance on the selection of cell lines in the biological evaluation of medical devices [79].

Application of MNP for extraction coupled with HPLC methods: Farajzadeh *et al.* [80] have studied $Fe_3O_4@SiO_2@C18$ efficiency of cardiovascular drugs extraction from human plasma coupled with HPLC-PDA. LOD was 0.05-0.07 µg/mL and recovery were 31-49%. Similarly, Abughrin *et al.* [81] employed magnetic nanoparticles coated with stearic acid, coupled with HPLC-DAD, for the extraction and determination of UV filters in cosmetic samples. The developed method exhibited satisfactory recovery values ranging from 81.2% to 112%, demonstrating its efficiency and reliability for trace analysis in complex cosmetic matrices. Recently, Yang *et al.* [82] have used $Fe_3O_4@mTiO_2$ nanoparticles for lipid removal in blood samples prior to HPLC-MS analysis of drugs and the recovery was 79.5-99.9%.

Conclusion

The integration of Analytical Quality by Design (AQbD) with magnetic nanoparticle (MNP)-assisted extraction and high-performance liquid chromatography (HPLC) analysis represents a transformative strategy to enhance analytical method robustness, efficiency and reproducibility. By applying AQbD principles such as risk assessment, design of experiments (DoE) and identification of critical quality attributes (CQAs) researchers can systematically develop high-precision extraction and analytical protocols with optimized performance parameters. This review has demonstrated that the AQbD-driven approach further strengthens method development by enabling systematic parameter optimization, predictive modeling and effective risk mitigation. These advancements facilitate streamlined regulatory compliance with ICH, FDA and USP guidelines, thereby making AQbD-based methodologies increasingly attractive for applications in pharmaceutical quality control, environmental monitoring and food safety analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1), Current Step 4 Version, Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005).
2. S.T. Narenderan, S.N. Meyyanathan and V.V.S.R. Karri, *Food Chem.*, **289**, 384 (2019); https://doi.org/10.1016/j.foodchem.2019.03.045
3. A. Bairagi, R. Kothrukar, H. Chikhale, S. Kosanam and L. Borse, *Future J. Pharm. Sci.*, **10**, 138 (2024); https://doi.org/10.1186/s43094-024-00706-1
4. N. Zhu, H. Ji, P. Yu, J. Niu, M.U. Farooq, M.W. Akram, I.O. Udego, H. Li and X. Niu, *Nanomaterials*, **8**, 810 (2018); https://doi.org/10.3390/nano8100810
5. S.E. Kepekci-Tekkeli and Z. Durmus, *J. Chil. Chem. Soc.*, **64**, 4448 (2019); https://doi.org/10.4067/S0717-97072019000204448
6. J. Zhang, S. Feng, Y. Xi, M. Shi, and L. Yin, *Adv. Sample Prep.*, **15**, 100208 (2025); https://doi.org/10.1016/j.sampre.2025.100208
7. T. Tatarchuk, L. Soltys and W. Macyk, *J. Mol. Liq.*, **384**, 122174 (2023); https://doi.org/10.1016/j.molliq.2023.122174
8. M. Manouchehri, S. Seidi and F.O. Abdullah, *Talanta*, **229**, 122273 (2021); https://doi.org/10.1016/j.talanta.2021.122273
9. F. Ahmad, M.M. Salem-Bekhit, F. Khan, S. Alshehri, A. Khan, M.M. Ghoneim, H.-F. Wu, E.I. Taha and I. Elbagory, *Nanomaterials*, **12**, 1333 (2022); https://doi.org/10.3390/nano12081333
10. T. Bastogne, F. Caputo, A. Prina-Mello, S. Borgos and M. Barberi-Heyob, *J. Pharm. Biomed. Anal.*, **219**, 114911 (2022); https://doi.org/10.1016/j.jpba.2022.114911
11. Guidance for Industry Q8(R2) Pharmaceutical Development. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER), ICH Revision 2, November 2009
12. C.L. Mustoe, A.J. Turner, S.J. Urwin, I. Houson, H. Feilden, D. Markl, M.M. Al Qaraghuli, M.W.S. Chong, M. Robertson, A. Nordon, B.F. Johnston, C.J. Brown, J. Robertson, C. Adjiman, H. Batchelor, B. Benyahia, M. Bresciani, C.L. Burcham, J. Cardona, C. Cottini, A.S. Dunn, D. Fradet, G.W. Halbert, M. Henson, P. Hidber, M. Langston, Y.S. Lee, W. Li, J. Mantanus, J. McGinty, B. Mehta, T. Naz, S. Ottoboni, E. Prasad, P.-O. Quist, G.K. Reynolds, C. Rielly, M. Rowland, W. Schlindwein, S.L.M. Schroeder, J. Sefcik, E. Settanni, H. Siddique, K. Smith, R. Smith, J.S. Srai, A.A. Thorat, A. Vassileiou, and A.J. Florence, *Int. J. Pharmaceut.*, **681**, 125625 (2025); https://doi.org/10.1016/j.ijpharm.2025.125625
13. S.E. Kepekci-Tekkeli and Z. Durmus, *J. Chil. Chem. Soc.*, **64**, 4448 (2019); https://doi.org/10.4067/S0717-97072019000204448
14. R. Peraman, K. Bhadraya and Y. Padmanabha Reddy, *Int. J. Anal. Chem.*, **2015**, 868727 (2015); https://doi.org/10.1155/2015/868727

15. D. Szynkiewicz, P. Georgiev, S. Ulenberg, T. Bączek and M. Belka, *Microchem. J.*, **187**, 108367 (2023); https://doi.org/10.1016/j.microc.2022.108367
16. B.L. Cushing, V.L. Kolesnichenko and C.J. O'Connor, *Chem. Rev.*, **104**, 3893 (2004); https://doi.org/10.1021/cr030027b
17. C.J. Murphy, T.K. Sau, A.M. Gole, C.J. Orendorff, J. Gao, L. Gou, S.E. Hunyadi and T. Li, *J. Phys. Chem. B*, **109**, 13857 (2005); https://doi.org/10.1021/jp0516846.
18. S. Iravani, H. Korbekandi, S.V. Mirmohammadi and B. Zolfaghari, *Res. Pharm. Sci.*, **9**, 385 (2014).
19. J. Jeevanandam, A. Barhoum, Y.S. Chan, A. Dufresne and M.K. Danquah, *Beilstein J. Nanotechnol.*, **9**, 1050 (2018); https://doi.org/10.3762/bjnano.9.98
20. P. Mohanpuria, N.K. Rana and S.K. Yadav, *J. Nanopart. Res.*, **10**, 507 (2008); https://doi.org/10.1007/s11051-007-9275-x
21. D. Sharma, S. Kanchi and K. Bisetty, *Arab. J. Chem.*, **12**, 3576 (2019); https://doi.org/10.1016/j.arabjc.2015.11.002.
22. A.K. Mittal, Y. Chisti and U.C. Banerjee, *Biotechnol. Adv.*, **31**, 346 (2013); https://doi.org/10.1016/j.biotechadv.2013.01.003
23. S.R. Ansari, Y.C. Suárez-López, T. Thersleff, L. Häggström, T. Ericsson, I. Katsaros, M. Åhlén, M. Karlgren, P. Svedlindh, C.M. Rinaldi-Ramos and A. Teleki, *ACS Nano*, **18**, 15284 (2024); https://doi.org/10.1021/acsnano.4c04685
24. F.G. Vogt and A.S. Kord, *J. Pharm. Sci.*, **100**, 797 (2011); https://doi.org/10.1002/jps.22325
25. A.S. Lapchuk, I.V. Gorbov, A.V. Prygun, I.V. Balagura and Y.M. Morozov, *Sens. Diagn.*, **1**, 829 (2022); https://doi.org/10.1039/D2SD00078D.
26. J. Gamonchuang, Y. Santaladchaiyakit and R. Burakham, *ACS Omega*, **7**, 12202 (2022); https://doi.org/10.1021/acsomega.2c00596
27. M.A. Ibrahim, M.Z. Jaafar, M.A.M. Yusof, C.A. Shye and A.K. Idris, *Colloids Surf. A Physicochem. Eng. Asp.*, **674**, 131943 (2023); https://doi.org/10.1016/j.colsurfa.2023.131943
28. R. Keçili, F. Ghorbani-Bidkorbeh, İ. Dolak, G. Canpolat, M. Karabörk and C.M. Hussain, *Trends Analyt. Chem.*, **143**, 116380 (2021); https://doi.org/10.1016/j.trac.2021.116380
29. Y. Zhang, F. Jiang, D. Huang, S. Hou, H. Wang, M. Wang, Y. Chi and Z. Zhao, *RSC Adv.*, **8**, 31348 (2018); https://doi.org/10.1039/C8RA05781H
30. C.O. Ogunkunle, S. Oyedeji H.K. Okoro and V. Adimula, in eds.: A. Amrane, D. Mohan, A.A. Assadi, G. Yasin and T.A. Nguyen, Interaction of Nanoparticles with Soil, In: Nanomaterials for Soil Remediation, Elsevier, Chap. 6, pp. 101-132 (2021).
31. G.M. Al-Senani and N. Al-Kadhi, *Appl. Sci.*, **10**, 1 (2020); https://doi.org/10.3390/app10144840
32. S. Ferdous, M.A. Ioannidis and D.E. Henneke, *J. Nanopart. Res.*, **14**, 850 (2012); https://doi.org/10.1007/s11051-012-0850-4
33. A.L. Crăciun and G. Gutt, *Appl. Sci.*, **13**, 823 (2023); https://doi.org/10.3390/app13020823
34. AMCTB No Analytical Methods Committee, *Anal. Methods*, **5**, 1901 (2013); https://doi.org/10.1039/C3AY90020G
35. S.L.C. Ferreira, R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David, G.C. Brandão, E.G.P. da Silva, L.A. Portugal, P.S. dos Reis, A.S. Souza and W.N.L. dos Santos, *Anal. Chim. Acta*, **597**, 179 (2007); https://doi.org/10.1016/j.aca.2007.07.011
36. I. Veza, M. Spraggon, I.M.R. Fattah and M. Idris, *Results Eng.*, **18**, 1 (2023); https://doi.org/10.1016/j.rineng.2023.101213
37. K. Vanaja and R.H. Shobha Rani, *Clin. Res. Regul. Aff.*, **24**, 1 (2007); https://doi.org/10.1080/10601330701220520
38. L. Hynčík, H. Čechová, L. Havelková, M. Jansová, L. Krofta, M. Němec and V. Kališ, in eds.: F. Chinesta, E. Cueto, Y. Payan and J. Ohayon, Reduced Order Model for Prediction of a Successful Course of Vaginal Delivery, In: Reduced Order Models for the Biomechanics of Living Organs, Academic Press, Chap. 17, pp. 327-348 (2023).
39. S.R. Mandpe, V.R. Parate and J.B. Naik, *Futur. J. Pharm. Sci.*, **8**, 38 (2022); https://doi.org/10.1186/s43094-022-00426-4
40. K. Younes, O. Mouhtady, H. Chaouk, E. Obeid, R. Roufayel, A. Moghrabi, and N. Murshid, *Membranes*, **11**, 979 (2021); https://doi.org/10.3390/membranes11120979
41. ICH Guideline Q9 on Quality Risk Management, European Medicines Agency, EMA/CHMP/ICH/24235/2006, Committee for Human Medicinal Products September 2015.
42. N. Kumar and D. Sangeetha, *J. Pharm. Sci. Res.*, **12**, 1298 (2020).
43. F.M. Said, J.Y. Gan and J. Sulaiman, *Eng. Sci. Technol.*, **23**, 781 (2020); https://doi.org/10.1016/j.jestch.2019.12.005
44. D.A. Armbruster and T. Pr, *Clin. Biochem. Rev.*, **29(Suppl 1)**, S49 (2008).
45. S.M. Siddeeg, M.A. Tahoon, W. Mnif and F. Ben Rebah, *Processes*, **8**, 5 (2020); https://doi.org/10.3390/pr8010005
46. Y. Modhav, *Int. J. Pharm. Phytopharmacol. Res.*, **1**, 403 (2012).
47. M.L. Williams, A.A. Olomukoro, R.V. Emmons, N.H. Godage and E. Gionfriddo, *J. Sep. Sci.*, **46**, (2023); https://doi.org/10.1002/jssc.202300571.
48. N.M. Suni, P. Lindfors, O. Laine, P. Östman, I. Ojanperä, T. Kotiaho, T.J. Kauppila and R. Kostiainen, *Anal. Chim. Acta*, **699**, 73 (2011); https://doi.org/10.1016/j.aca.2011.05.004
49. P. Nagar, M. Garg, C. Chauhan, R. Kumar and A.K. Chaudhary, *Int. J. Pharm. Qual. Assur.*, **13**, 103 (2022).
50. A. Najmi, Z. Rehman, H.A. Alhazmi, M.M. Albratty, N.H. Majrashi, K.M. Hakami, N.A. Najmi and A.A. Mobarki, *Separations*, **10**, 346 (2023); https://doi.org/10.3390/separations10060346
51. M. Faraji, *Nanochem. Res.*, **1**, 264 (2016); https://doi.org/10.7508/ncr.2016.02.014
52. J.J. Gilroy, J.W. Dolan and L.R. Snyder, *J. Chromatogr. A*, **1000**, 757 (2003); https://doi.org/10.1016/S0021-9673(03)00512-0
53. A.P. Schellinger and P.W. Carr, *J. Chromatogr. A*, **1109**, 253 (2006); https://doi.org/10.1016/j.chroma.2006.01.047
54. M.C. Breitkreitz, I.C.S.F. Jardim and R.E. Bruns, *J. Chromatogr. A*, **1216**, 1439 (2009); https://doi.org/10.1016/j.chroma.2008.12.093
55. Y. Yang, *Anal. Chim. Acta*, **558**, 7 (2006); https://doi.org/10.1016/j.aca.2005.11.011
56. T. Verch, C. Campa, C.C. Chéry, R. Frenkel, T. Graul, N. Jaya, B. Nakhle, J. Springall, J. Starkey, J. Wypych and T. Ranheim, *AAPS J.*, **24**, 34 (2022); https://doi.org/10.1208/s12248-022-00685-2
57. Y. Bao, Y. Zhai, T. Ning, P. Chen and S. Zhu, *Se Pu*, **58**, 1005 (2022); https://doi.org/10.3724/SP.J.1123.2022.06006
58. M.M. Ariffin, N.M. Sohaimi, B.S. Yih and N.M. Saleh, *Anal. Methods*, **11**, 4126 (2019); https://doi.org/10.1039/C9AY01147A
59. R. Darraj, M. Haroun, A. Abbod and I. Al Ghoraibi, *Clin. Med. Health Res. J.*, **5**, 1145 (2025) https://doi.org/10.18535/cmhrj.v5i1.450
60. H.-W. Chen, C.-S. Chiou and S.-H. Chang, *Powder Technol.*, **311**, 426 (2017); https://doi.org/10.1016/j.powtec.2017.01.060.
61. M. Farzin, A.H. Poshtiri and R. Kouhikamali, *Proc. Inst. Mech. Eng., C J. Mech. Eng. Sci.*, **238**, 9889 (2024); https://doi.org/10.1177/09544062241259613.
62. C. Boukoufi, A. Boudier, S. Lahouari, J. Vigneron and I. Clarot, *Results Chem.*, **5**, 100979 (2023); https://doi.org/10.1016/j.rechem.2023.100979.
63. B. Hu, M. He and B. Chen, in eds.: C.F. Poole, Magnetic Nanoparticle Sorbents, In: Solid-Phase Extraction Handbook in Separation Science, Elsevier, Chap. 9, pp. 235-284 (2020).
64. N. Desai, *AAPS J.*, **14**, 282 (2012); https://doi.org/10.1208/s12248-012-9339-4
65. S. Shrestha, B. Wang and P. Dutta, *Adv. Colloid Interface Sci.*, **279**, 102162 (2020); https://doi.org/10.1016/j.cis.2020.102162
66. R. Bosetti and S.L. Jones, *Nanomedicine*, **14**, 1367 (2019); https://doi.org/10.2217/nnm-2019-0130.
67. R.A. Lionberger, S.L. Lee, L.M. Lee, A. Raw and L.X. Yu, *AAPS J.*, **10**, 268 (2008); https://doi.org/10.1208/s12248-008-9026-7
68. Y. Huang, H. Zheng, X. Hu, Y. Wu, X. Tang, Q. He and S. Peng, *J. Hazard. Mater.*, **422**, 126856 (2022); https://doi.org/10.1016/j.jhazmat.2021.126856

69. S. Satyam and S. Patra, *Heliyon*, **10**, e29573 (2024); https://doi.org/10.1016/j.heliyon.2024.e29573
70. M.T. Luiz, J.S. Rosa Viegas, J.P. Abriata, F. Viegas, F.T.M. de Carvalho Vicentini, M.V.L. Badra Bentley, M. Chorilli, J.M. Marchetti and D.R. Tapia-Blácido, *Eur. J. Pharm. Biopharm.*, **165**, 127 (2021); https://doi.org/10.1016/j.ejpb.2021.05.011
71. V. Weisfeld and T.A. Lustig, International Regulatory Harmonization Amid Globalization of Drug Development, Workshop Summary Washington, DC: Institute of Medicine (IOM) (2013).
72. A. Pratt, *Front. Nanosci.*, **6**, 259 (2014); https://doi.org/10.1016/B978-0-08-098353-0.00007-5
73. A. Nene, S. Sadeghzade, S. Viaroli, W. Yang, U.P. Uchenna, A. Kandwal, X. Liu, P. Somani and M. Galluzzi, *Environ. Sci. Eur.*, **37**, 7 (2025); https://doi.org/10.1186/s12302-024-01044-y
74. S. Garg, N.P. Rumjit and S. Roy, *Adv. Agrochem.*, **3**, 115 (2024); https://doi.org/10.1016/j.aac.2023.11.001
75. A. Tomitaka, A. Vashist, N. Kolishetti and M. Nair, *Nanoscale Adv.*, **5**, 4354 (2023); https://doi.org/10.1039/D3NA00180F
76. M.H. Dawoud, I.S. Mannaa, A. Abdel-Daim and N.M. Sweed, *AAPS PharmSciTech*, **24**, 169 (2023); https://doi.org/10.1208/s12249-023-02609-5
77. W. Alloun and C. Calvio, *Fermentation*, **10**, 126 (2024); https://doi.org/10.3390/fermentation10030126
78. M. Ghaedi, F.N. Azad, K. Dashtian, S. Hajati, A. Goudarzi and M. Soylak, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **167**, 157 (2016); https://doi.org/10.1016/j.saa.2016.05.025
79. T.I. Ramos, C.A. Villacis-Aguirre, K.V. López-Aguilar, L. Santiago Padilla, C. Altamirano, J.R. Toledo and N. Santiago Vispo, *Pharmaceutics*, **14**, 247 (2022); https://doi.org/10.3390/pharmaceutics14020247
80. M.A. Farajzadeh, A. Yadeghari and M. Abbaspour, *Adv. Pharm. Bull.*, **9**, 138 (2019); https://doi.org/10.15171/apb.2019.017
81. S.E. Abughrin, U. Alshana and S. Bakirdere, *Int. J. Environ. Res. Public Health*, **19**, 6037 (2022); https://doi.org/10.3390/ijerph19106037
82. F. Yang, K. Ma, Y. Cao and C. Ni, *RSC Adv.*, **11**, 19874 (2021); https://doi.org/10.1039/D1RA01530C