

检索报告

根据委托人郭宇航的委托,通过网络检索,郭宇航发表的 2 篇论文被《科学引文索引》扩展版 (SCI-Expanded) 数据库收录。数据库具体检索结果如下:

1.标题: Compact Fabry-Perot microcavity based Fiber-optic humidity sensor constructed by hollow Core Fiber and chitosan film

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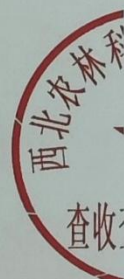
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Compact Fabry-Perot microcavity based Fiber-optic humidity sensor constructed by hollow Core Fiber and chitosan film

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ABSTRACT

Fabry-Perot interferometer (FPI) microcavity humidity sensor based on chitosan encapsulated hollow core fiber. The sensor's humidity response characteristics have been experimentally demonstrated with the average humidity sensitivity of 0.846 nm/%RH within the humidity range of 35 % ~ 65 % RH, with sub-range average sensitivity of 0.956 nm/%RH specifically in the 41 % ~ 65 % RH interval. The chitosan-FPI based optical fiber humidity sensor offers the advantages of the simplified fabrication processes, compact structural configuration, cost-effectiveness, high sensitivity, and superior humidity response performance. These attributes indicate its substantial application prospects in the development of wearable biosensing systems, particularly for real-time humidity monitoring applications.

1. Introduction

High-performance optical fiber humidity sensors can be developed by combining the specially designed optical fiber structures with the humidity sensitive materials, and monitoring the changing rules of optical wavelength, intensity, phase, and other parameter information in response to environmental humidity [1]. Among the different optical fiber structures, the manufacturing process of fiber gratings are relatively complex [2], requiring specialized and costly equipment to engrave the grating patterns on the optical fiber [3]. Based on the optical evanescent field on the surface of microfiber structures [4] and the surface plasmon resonance (SPR) effect of metal micro/nano-structures [5], the information exchange efficiency between optical signals and environmental humidity changes has been effectively enhanced, significantly improving the sensitivity of humidity response. However, most sensors based on the evanescent field mechanism require the special treatment of optical fiber structure, including tapering (micro fiber cones or micro nano fibers) [6], side polishing (D-type structured fibers) [7], and micro-bending (U-shaped structured fibers) [8]. The reproducibility, mechanical properties and stability of these fiber structures are relatively poor [9]. It is necessary to fix and package these fiber structures in advance to eliminate the impact from other

environmental factors; The humidity sensor based on SPR effect relies on the corresponding metal nanostructured thin films [10], with a more complex manufacturing process.

The humidity sensor based on optical fiber interferometer structure has small size, simple fabrication, high reproducibility, good stability, and generally high sensitivity, especially for the Mach-Zehnder and Fabry-Perot interferometer structures, which have good performance and great practical application potential. The latter, as a reflective structure [11], is more convenient for application in narrow spaces. To develop an optical fiber Fabry-Perot humidity sensor, it is required to cover the humidity sensitive materials in the form of the thin film on the fiber end-face to construct Fabry-Perot microcavity [12], or to fill the liquid status polymers into hollow core fiber (HCF) to construct Fabry-Perot microcavities [13]. Wang et al. achieved the rapid response to environmental humidity by inserting a Polyvinyl Alcohol (PVA) film into an optical fiber structure (1.5 s) [14], with a sensitivity of 36.71 pm/% RH. This sensitivity is low due to the limited thickness of the film layer inserted between the optical fiber structures. Meng et al. connected two single-mode fibers (SMF) with polymethyl methacrylate (PMMA) sol to construct a humidity sensor [15], which increased the sensitivity to 417.2 pm/% RH. Zhang et al. mixed a suitable ratio of hyaluronic acid (HA) and PVA sol to construct a humidity sensitive film on the end face

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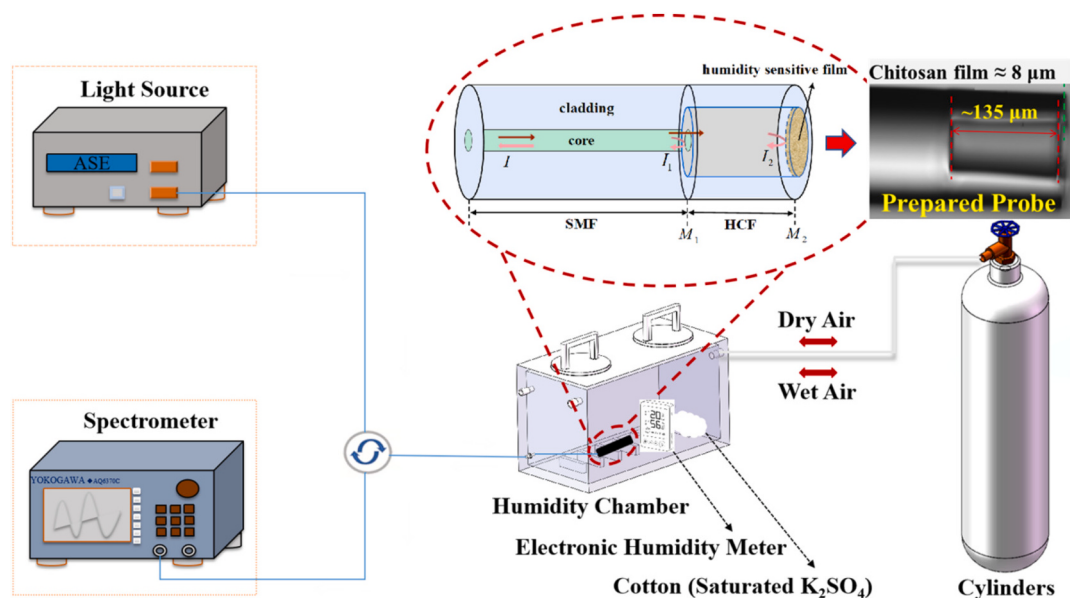


Fig. 1. Experimental system schematic diagram of chitosan-FPI fiber humidity sensor.

of the HCF [16]. At the same time, micro-pores were machined on the side wall of the HCF to enhance the humidity sensing efficiency, achieving a sensitivity of $-525 \text{ pm}/\% \text{ RH}$. In addition, many new materials have been played as the humidity sensitive materials to construct the thin film Fabry Perot microcavities, such as polydimethylsiloxane (PDMS) [17], metal-organic framework (MOF) [18], graphene (G) or graphene-oxide (GO) [19], and Chitosan [20], etc. Chitosan contains the abundant amino and hydroxyl functional groups, and hydroxyl groups, which can form hydrogen bonds with water molecules, thereby promoting reversible adsorption of water molecules. Therefore, it has attractive reversible water absorption ability and good swelling effect [21]. Due to its strong hygroscopicity, excellent film-forming ability, and stable chemical properties, chitosan has become a promising fiber optic humidity sensing material.

Based on the inherent advantages of FPI structures and the favorable humidity sensing characteristics of chitosan, this study presents a chitosan-FPI humidity sensor based on HCF and SMF spliced structure, and demonstrates its sensitivity, minimal temperature crosstalk, and superior repeatability and stability to address the stringent requirements for precision humidity sensing.

2. Hollow-core fiber structure and sensing mechanism

2.1. Working mechanism

FPIs impose the rigorous demands on the interface flatness and medium reflectivity. Depending on the intracavity sensitive materials, they can be categorized into intrinsic FPI (IFPI) and extrinsic FPI (EFPI). IFPI sensors modulate the equivalent cavity length through refractive index variations of encapsulated materials, whereas EFPI sensors typically employ air-gap microcavities or humidity-induced volumetric changes in sensitive materials for detecting humidity. To achieve the high-performance humidity sensing, conventional approaches integrate polymer materials with FPIs through the methods of outer-surface coating, end-face diaphragm forming, or air-cavity filling. These implementations effectively leverage the humidity-dependent alterations in material thickness and refractive index to directly or indirectly modify the Fabry-Perot (FP) cavity length, demonstrating a significant operational efficacy.

As illustrated in the inset diagram of Fig. 1, the proposed fiber humidity sensing architecture features with an FPI microcavity constructed

from HCF segment. Incident light undergoes dual reflections within this FPI structure, where the two reflective interfaces of FPI are respectively formed by the SMF-HCF connecting interface (M_1), and the humidity-sensitive material film (M_2). The interfering phase change ($\Delta\varphi$) of FPI is dependent on the length change (ΔL) of FP cavity by

$$\Delta\varphi = 2\pi \frac{\Delta L}{\lambda} \quad (1)$$

Here, λ is the center wavelength of interference light. When chitosan absorbs water molecules, it will produce a swelling effect, which will cause two parameter increases, namely the thickness of the chitosan film and its equivalent refractive index. In this work, as M_2 is played by the interface between the HCF air core and the chitosan film, the FP cavity starts from the SMF-HCF interface to the inside surface of polymer film, without considering the change in the equivalent refractive index of chitosan film. Therefore, the sensitivity (S_L) of the HCF-FPI fiber optic humidity sensor designed in this experiment is mainly determined by the effective length change (ΔL) of Fabry-Perot (FP) cavity, which only depends on the thickness variation (Δl) of the chitosan film. Its contribution to the change in the original cavity length (L) is $\Delta l/2$, due to the uniform expansion of the chitosan film on both sides.

$$S_L \propto \frac{\Delta L}{L} = \frac{\Delta l}{2} \frac{1}{L} \quad (2)$$

In addition, the FP cavity with different length also produce the variable interference period, named free spectra range (FSR) of FPI. Which can be expressed by

$$FSR = \frac{\lambda^2}{2nL} \quad (3)$$

As the length decreasing for the FP cavity (i.e., HCF length), FSR will be enlarged, resulting in a wider interference dip and low resolution. While the longer FP cavity has a finer interference spectrum and a limited dynamic response range. The relative change of this length under the humidity environment has the monotonic positive correlation with the sensitivity of the proposed humidity sensor. In the experiment, balancing the limitations of the manufacturing process and the sensing performance, the HCF length was selected to be approximately $135 \mu\text{m}$, as shown by the inserted microscope photo in Fig. 1.

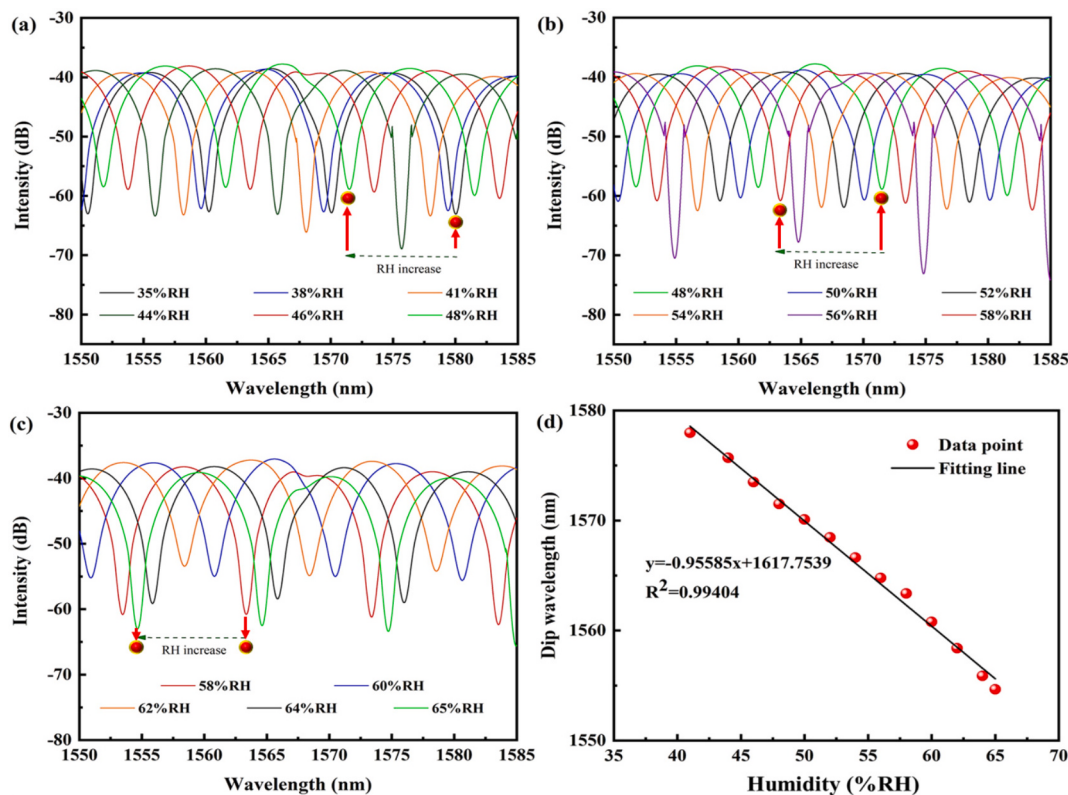


Fig. 2. Spectrum of chitosan-FPI humidity sensor during (a) 35 % ~ 48 %RH, (b) 48 % ~ 58 %RH, (c) 58 % ~ 65 %RH; and (d) sensing characteristic curve between relative humidity and dynamically tracking wavelength of interference dip.

2.2. Fabrication process

HCF is a circular waveguide with three-layer dielectric structure: core, cladding, and coating. Distinct from conventional SMF, the HCF's core is played by an air cavity ($n \approx 1$), resulting in minimal optical absorption. For sensing applications, its coating layer will be removed, simplifying the HCF similar to a silica capillary. The FPI-based fiber optic humidity sensor was prepared by splicing its one end to SMF (core refractive index ~ 1.457), and encapsulating its other end with the hygroscopic polymer, named chitosan.

To splice one cut of HCF with SMF, the manual mode of a FITEC S179 fusion splicer was used and the splicing parameters were carefully optimized with the discharging intensity of ~ 15.6 mA and duration time of 200 ms. Too high intensity or duration time for the arc discharge will destroy the air cavity of HCF near the splicing point, where the air tube may collapse in the arc heating with high temperature. To obtain the micro-thickness of chitosan film on the open-end of HCF, Chitosan is a natural alkaline polysaccharide polymer that appears as a light-yellow powder, slightly soluble in water, and soluble in dilute acids with a $\text{pH} < 6.5$. Chitosan surface contains abundant and active hydroxyl functional groups, which are easy to bind with water molecules, making it one of the most promising humidity sensing materials in the field of fiber optic humidity sensing. 0.5 g chitosan powder was dissolved in 50 ml acetic acid solution and stirred at 300 rpm/min for 20 min at a constant temperature of 120°C to obtain a chitosan solution with a mass concentration of 1 %. Subsequently, the HCF end face was dipped in chitosan solution using a coating machine and slowly pulled (speed: 0.5 mm/s). After multiple repeated operations, the desired thickness of polymer film could be achieved. Finally, after 24 h of natural air drying, the moisture sensitive fiber optic probe with cured film in the thickness of ~ 8 μm was obtained.

3. Humidity sensing experiment

The humidity sensing test system is shown in Fig. 1, consists of an ASE broadband light source (1528–1603 nm), an optical spectrum analyzer (AQ6370D), and a sealed chamber, where the FPI sensing probe, cotton balls, and an electronic hygrometer were placed for measurement in this controlled humidity environment.

The chitosan-FPI fiber sensor was subjected to humidity gradient detection within a controlled humidity range of 35 % ~ 65 %RH supplied by the water molecules volatilizing from a saturated K_2SO_4 cotton, while maintaining a constant ambient temperature ($\pm 0.5^\circ\text{C}$) near the sensing probe. Post-processing of spectrometer data yielded the spectral profiles depicted in Fig. 2.

By selecting the interference dip near 1580 nm under 35 %RH to dynamically track the wavelength change, a consistent blue-shift (toward shorter wavelengths) was observed with the increasing humidity. Notably, the wavelength shifts intensified significantly when the humidity value exceeded 41 %RH. At 48 %RH, the spectral shift reached 8.532 nm, approaching the aliasing threshold for spectral wavelength. To systematically evaluate the humidity sensing performance, the experimental data acquisition was conducted at 2 %RH increments in three distinct humidity ranges in Figs. 2(a-c). Further analysis revealed the continued regularity for wavelength blue-shift during both 48 % ~ 58 %RH (~ 8.14 nm) and 58 % ~ 65 %RH (~ 8.704 nm) ranges, respectively, accompanied by wavelength aliasing phenomena. Meanwhile, the humidity-dependent light intensity variations were detected as well, because that the swelling effect of chitosan film induced the length alterations for FPI cavity and its effective refractive index become different after absorption of water molecules. However, due to the irregular and comparatively subtle nature of intensity fluctuations relative to wavelength shifts, the investigation only focused on the wavelength demodulation for humidity sensing analysis.

When the environmental humidity increasing from 35 %RH to 65 %

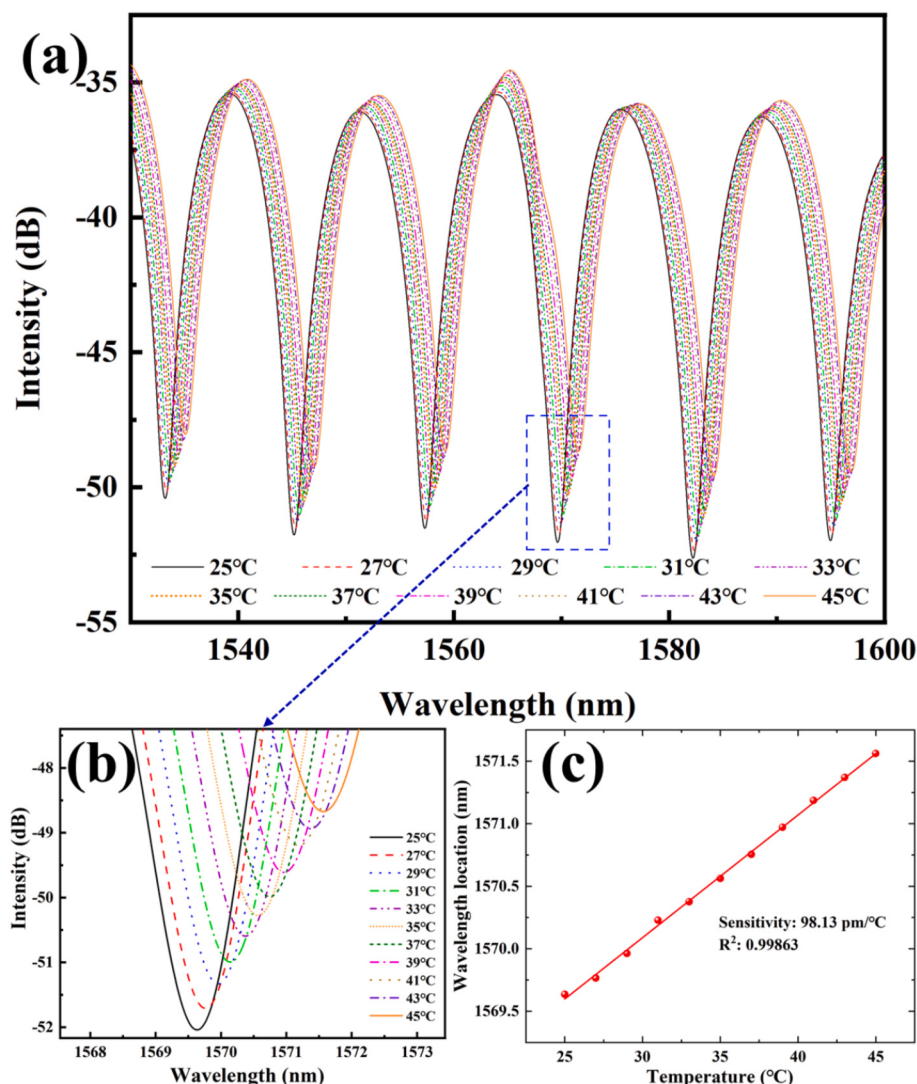


Fig. 3. Temperature cross-talk performance of HCF-FPI humidity sensor. (a) Whole spectrum and (b) enlarged dip range of chitosan-FPI humidity sensor under different temperature; (c) depending relationship between temperature and interference dip wavelength near 1570 nm.

RH, the recorded spectral dip demonstrated with a total blue-shift of 25.376 nm, from 1580.036 nm to 1554.66 nm. During the whole humidity test range, the average sensitivity can be calculated through dividing the total wavelength shift by the humidity range, that is the wavelength shift corresponding to each unit change in humidity. The average humidity sensitivity of 0.846 nm/%RH was calculated within the humidity range of 35 % ~ 65 % RH. However, it is seen from Fig. 2 (a) that the wavelength shift for the first three dips is not uniform, as the humidity value increases from 35 % RH to 41 % RH at 2 % RH intervals. Therefore, we did not consider these three points and only fitted the sensing curves during 41 % ~ 65 %RH range in Fig. 2(d). The sensing characteristic curve between the pronounced wavelength shifts as a function of humidity change reveals a sensitivity of 0.956 nm/%RH. This quantitative relationship confirms the enhanced humidity responsiveness of the chitosan-functionalized FPI fiber architecture.

4. Temperature crosstalk effect analysis

To explore the temperature cross-sensitivity during humidity sensing experiment, the experimental temperature was maintained at 20.0 °C (± 0.5 °C). However, practical deployment scenarios inherently involve the environmental temperature fluctuations, the temperature crosstalk effect should be concerned for every sensor used in the open

environment. Consequently, temperature gradient experiments were conducted to characterize the thermal response of the chitosan-FPI humidity sensor.

A humidity-controlled chamber (fixed at 37 %RH) was integrated with the experimental setup to isolate the temperature crosstalk effect. A calibrated heating stage was used to elevate the chamber temperature from 25 °C to 45 °C with a step of 2 °C, where the spectra were recorded at each interval. Fig. 3 illustrates the correlation between interference spectra and temperature variations. Analysis of Fig. 3(a) reveals temperature-dependent spectral sensitivity, attributed to thermal expansion of the chitosan polymer film. Elevated temperatures increase atmospheric water vapor saturation, prompting hygroscopic swelling of the chitosan layer. This induces microscale alterations in the FPI cavity length, consequently modulating both wavelength and intensity of interference spectra.

As shown in Fig. 3(b), the spectral dip near 1570 nm exhibits a systematic red-shift (longer wavelengths) with temperature changing, accumulating a total displacement of 1.896 nm across the 25–45 °C range. Quantitative analysis in Fig. 3(c) demonstrates a linear temperature-wavelength relationship ($R^2 = 0.99863$) with a sensitivity coefficient of 98.13 pm/°C. Comparative evaluation indicates the minimal thermal crosstalk in humidity measurements, yielding a temperature-induced humidity error of 0.1027 %RH/°C. This negligible

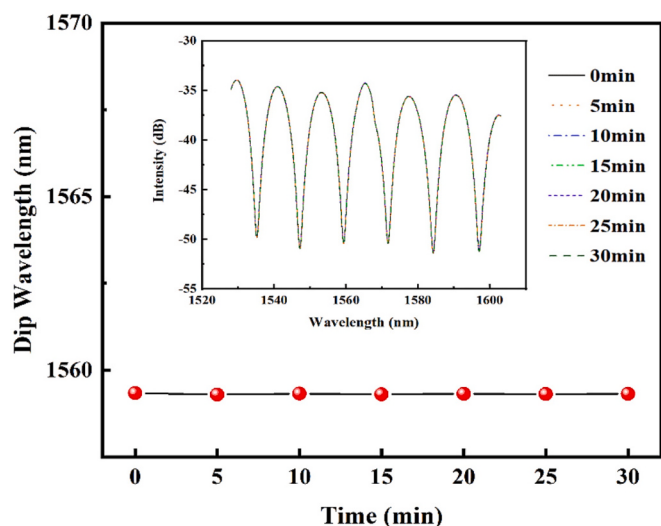


Fig. 4. Stability performance based on monitoring wavelength location for different times.

thermal influence confirms the sensor's robust performance against ambient temperature variations, validating its suitability for environments requiring simultaneous temperature-humidity discrimination.

In summary, the proposed sensor requires no thermal compensation design in humidity measurement scenarios due to the minimal temperature variations. However, if the environment exhibits the significant thermal fluctuations, the temperature-induced interference must be considered and compensated by introducing a separate temperature sensor in the humidity sensing system.

5. Stability and repeatability evaluation of humidity sensing performance

To quantitatively evaluate the responding stability, the sensing probe was subjected to controlled experiment within a sealed chamber with the constant temperature and humidity ($20.0\text{ }^{\circ}\text{C} \pm 0.1\text{ }^{\circ}\text{C}$, $51\text{ }\% \text{RH} \pm 0.3\text{ }\% \text{RH}$). The corresponding interference spectra were timely recorded at 5-min intervals over 30 min, yielding seven consecutive measurements. The temporal evolution of the spectra was illustrated in Fig. 4, reveals a wavelength shift of 0.38 nm (equivalent to $0.37\text{ }\% \text{RH}$ variation), empirically validating the sensor's operational stability under sustained hygroscopic exposure.

The experimental data demonstrate the stable spectral wavelength retention within a 30-min monitoring window, exhibiting maximum

fluctuations of $\pm 21\text{ pm}$. These minor wavelength variations at constant humidity levels may originate from two technical factors: (1) the inherent limitations in hygostat control precision ($\pm 0.3\text{ }\% \text{RH}$), and (2) the micro-perturbations induced by humidity modulation (via dry/humid air mixing), which may concurrently generate localized thermal gradients ($< 0.1\text{ }^{\circ}\text{C}$) within the chamber. Relative to the calibrated humidity sensitivity of $0.846\text{ nm}/\% \text{RH}$, the observed wavelength instability translates to a negligible humidity measurement deviation of $\pm 0.0248\text{ }\% \text{RH}$ ($\pm 21\text{ pm}/0.846\text{ nm}/\% \text{RH}$). Such sub-percent-level fluctuations fall within acceptable margins for most practical applications, confirming the exceptional operational stability of chitosan-FPI fiber humidity sensor. Slight fluctuations in temperature will not significantly affect the performance of chitosan humidity sensors. However, like other polymers, long-term high-temperature work can lead to the degradation of their mechanical properties. Other polymers or functional materials need to be added to chitosan to increase its service life [22].

The repeatability performance for the proposed HCF-FPI humidity sensor has been studied in Fig. 5 by recording and comparing the wavelength locations in the interference spectrum when the humidity sensing experiment was repeated for 4 cycles (Fig. 5(a)) and the humidity values underwent a round trip between $35\text{ }\% \text{RH}$ and $65\text{ }\% \text{RH}$ (Fig. 5(b)).

It is indicated that the wavelength location blue-shifts with the similar curves, where the biggest difference of 0.792 nm was observed at $50\text{ }\% \text{RH}$ humidity. In one round trip testing process, the difference of 0.16 nm was calculated when the humidity decreased back to $35\text{ }\% \text{RH}$. The humidity sensing performance of the chitosan related fiber optic sensors have been compared in Table 1.

By integrated an IFPI and EFPI cascaded structure in silica capillary based on chitosan film [23], the Vernier effect has been excited and resulted in a high sensitivity of $7.15\text{ nm}/\% \text{RH}$. This sensitivity has been further improved to $-83.77\text{ nm}/\% \text{RH}$ by the same group [24]. However, the modulation process and fabrication must be precisely controlled for the microscale manipulation for the chitosan film in the capillary. Instead, the chitosan film can be elaborated on the out-surface of the

Table 1

Sensitivity and working range comparison of chitosan-based fiber humidity sensors.

Structure or effect	Sensitivity	Range	Refs.
Composite IFPI+EFPI fiber	$7.15\text{ nm}/\% \text{RH}$	$40\text{--}92\text{ }\% \text{RH}$	[23]
	$-83.77\text{ nm}/\% \text{RH}$	$40\text{--}90\text{ }\% \text{RH}$	[24]
Tapered MZI	$119.6\text{ pm}/\% \text{RH}$	$10\text{--}90\text{ }\% \text{RH}$	[25]
Microstructured optical fiber	$81.05\text{ pm}/\% \text{RH}$	$90\text{--}95\text{ }\% \text{RH}$	[26]
SMF-HCF end	$0.846\text{ nm}/\% \text{RH}$	$35\text{--}65\text{ }\% \text{RH}$	This work

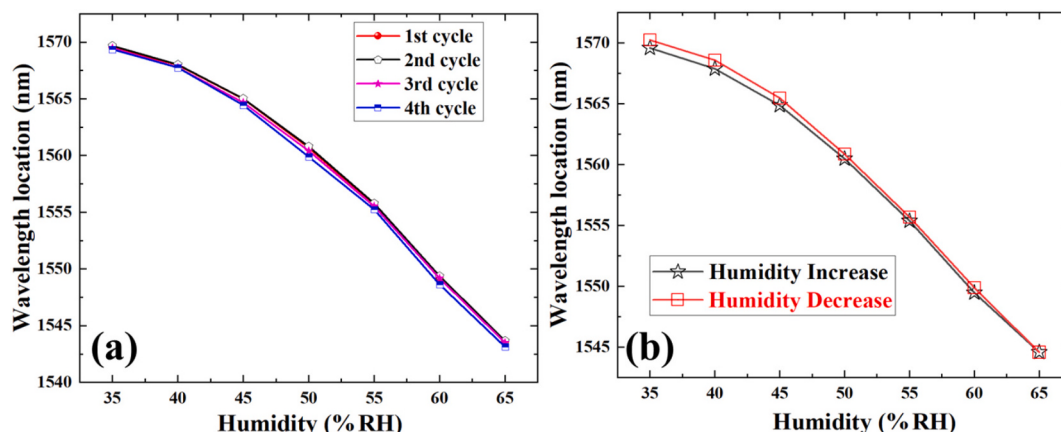


Fig. 5. Repeatability performance based on monitoring wavelength location for different humidity cycles (a) and increase-decrease process (b).

cascaded fiber structures [25] or the end-face of optical fiber to develop the low-cost and easy-fabrication humidity fiber probes. When the air tube of optical fibers is too small, the exchange efficiency of water molecules will become worse, resulting in the low sensitivity and limited working range [26]. In summary, fiber optic humidity sensors need to be designed in humidity sensitive materials and sensing actuator structures, balancing sensitivity, stability, repeatability, dynamic response capability, and manufacturing cost to meet different application scenarios. By adopting different HCF based structures [27], introducing different functional materials [28], and advanced fusion data processing methods [29], its sensing performance can be effectively improved.

6. Conclusions

Capitalizing on the unique air-core microstructure of hollow-core fibers, we developed a novel fiber humidity sensing architecture through disparate-diameter splicing of SMF and HCF, followed by precision length trimming. The chitosan layer was integrated via dip-coating technique, establishing a functional FPI humidity transducer. The systematic evaluations revealed its remarkable performance metrics: average humidity sensitivity of 0.846 nm/%RH across 35–65 %RH; and average sensitivity of 0.956 nm/%RH ($R^2 = 0.994$) within 41 % ~ 65 %RH. This chitosan-FPI sensor exhibits some advantages, such as high sensitivity exceeding conventional polymer-based sensors, compact footprint (core diameter: 125 μm), and stability. Experimental results demonstrate that this architecture successfully addresses the critical requirements for next-generation humidity sensors (miniaturization, operational convenience, and smart-system compatibility) while maintaining industrial-grade reliability under diverse environmental conditions.

CRedit authorship contribution statement

Xian-ge Xue: Writing – original draft, Investigation, Formal analysis, Data curation. **Yuhang Guo:** Writing – original draft, Investigation. **Fengxin Yan:** Funding acquisition. **Naimov Alisher:** Writing – original draft. **Jin Li:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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Review Article

Advances in analytical techniques for bioactive compound quantification in medicinal Plants: Innovations, Challenges, and pharmaceutical applications

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ABSTRACT

Medicinal plants (MPs), as natural sources of structurally diverse bioactive components such as flavonoids and alkaloids, combine pharmacological activity with functional food properties. However, their complex matrices pose significant technical barriers to accurate quantification. This review summarizes the current status of existing methods and applications for the detection of active ingredients in MPs, and systematically compares the advantages of traditional chromatographic techniques (e.g., HPLC, UHPLC, GC, et al.), spectroscopic methods (e.g., MS, NMR, IR, et al.), and modern nano-sensing technologies (e.g., colorimetric sensor array, fiber optic sensor) for breakthrough applications in the field. It further in-depth analyzes critical challenges, including data collection and standardization, the demand for rapid, non-destructive, and cost-effective detection technologies, accuracy in detection data, and the integration of multimodal detection data. Research identifies intelligentization, precision, rapidity, and environmental sustainability as the key developmental priorities for future Chinese herbal medicine detection. This review provides a methodological framework for efficient bioactive compound analysis in MPs, offering practical significance in enhancing quality control standards for pharmaceuticals and functional food products.

1. Introduction

Within the time-honored pharmacopeia of Traditional Chinese Medicine and global ethnomedical systems, medicinal plants (MPs) have maintained an irreplaceable therapeutic role, their pharmacotherapeutic properties etching an enduring legacy in the annals of human healthcare. MPs constitute over 85 % of essential botanical materia medica in traditional medical systems across 88 WHO-member nations

[1]. The global botanical medicine sector currently exceeds US\$100 billion in market valuation, representing 20 % of total pharmaceutical commerce [2]. As herbal product utilization escalates globally, critical parameters including pharmacological efficacy, toxicological profiles, and phytochemical consistency have become focal points for regulatory bodies and healthcare consumers alike. The vertically integrated regulatory framework governing botanical raw materials ensures therapeutic reproducibility through batch-to-batch standardization, functioning as

Abbreviations: MPs, medicinal plants; HPLC, High performance liquid chromatography; AOC, amino columns; SC, sugar columns; AEC, amide columns; HC, hydrophilic columns; ANEC, anion exchange columns; UVD, ultraviolet detection; RID, refractive index detection; FLD, fluorescence detection; CAD, charged aerosol detection; ELSD, evaporative light scattering detection; MS, mass spectrometric; TCM, Traditional Chinese Medicine; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; DAD, diode array detection; UHPLC, ultra-performance liquid chromatography; GC, gas chromatography; EEO, essential oil; CE, Capillary electrophoresis; CZE, Capillary zone electrophoresis; MEKC, Micellar electrokinetic chromatography; MEEKC, Microemulsion electrokinetic chromatography; CGE, Capillary gel electrophoresis; CIEF, capillary isoelectric focusing; ITP, isotachopheresis; CEC, Capillary electrochromatography; CE-LIF, CE-laser induced fluorescence; CE-PDA, capillary electrophoresis-photodiode array detection; AQbD, Analytical Quality by Design; TLC, Thin layer chromatography; ELISA, enzyme-linked immunosorbent assay; NIRS, near-infrared spectroscopy; MBSD, moving block standard deviation; ADSD, absolute distance standard deviation; RS, Raman spectroscopy; SERS, surface-enhanced Raman spectroscopy; HSI, hyperspectral imaging; PLSR, Partial Least Squares Regression; LASSO, Least Absolute Shrinkage and Selection Operator; AM, Attention Mechanism; CNN, Convolutional Neural Network; LSTM, Long Short-Term Memory; DAE, Deep Autoencoder; LS-SVM, Least Squares-Support Vector Machine; SVM, Support Vector Machine; PLS-DA, Partial Least Squares Discriminant Analysis; NMR, Nuclear Magnetic Resonance spectroscopy; FLS, Fluorescence; THz, Terahertz; TRTS, time-resolved terahertz spectroscopy; TES, terahertz emission spectroscopy; SPR, surface plasmon resonance; WDM, wavelength division multiplexing.

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the critical quality control nexus in phytopharmaceutical manufacturing protocols. This paradigm is fundamentally interwoven with the structural reformation of modern medical frameworks and the evolution of global healthcare governance paradigms.

Herbal medicines are primarily derived from botanical sources and contain diverse bioactive constituents with complex structural configurations and distinctive pharmacological properties. The phytochemical components identified in analytical specimens, including *alkaloids*, *glycosides*, and *phenolic compounds*, exhibit significant therapeutic potential for metabolic disorders (type II diabetes, chronic kidney disease), neurophysiological conditions (pain sensitization), and immune-related pathologies (asthma, systemic inflammation) through their multi-target modulatory effects. The pharmacological efficacy of MPs demonstrates strong correlations with the profile and concentration of their characteristic phytochemical constituents. Given the intricate chemical composition inherent to traditional Chinese medicinal materials, the spatial distribution of bioactive compounds is governed by multiple regulatory factors including genetic variations among species, tissue-specific biosynthesis patterns, and organ differentiation within plant structures. These biological determinants contribute to substantial chemical variability across specimens from different geographical origins. To optimize therapeutic outcomes and ensure standardized clinical application, precise characterization of phytochemical disparities among plant species and medicinal organs becomes imperative [3]. However, comprehensive phytochemical profiling of MPs presents considerable analytical challenges. First, individual medicinal materials may contain hundreds of chemically distinct components. Second, substantial concentration variations occur due to multiple influencing parameters such as geographical origin, harvest chronology, and post-collection processing techniques. Consequently, the qualitative and quantitative analysis of MPs' bioactive constituents represents a sophisticated analytical undertaking requiring meticulous experimental design.

Traditional methodologies for MPs identification primarily encompass empirical evaluation and physicochemical characterization [4]. Empirical identification depends on practitioners' accumulated expertise, a process inherently characterized by subjective interpretation and demanding substantial botanical proficiency. Physicochemical approaches employ analytical chemistry principles and instrumental techniques for material authentication. The chemical identification paradigm involves extracting characteristic phytochemical markers from MPs substrates followed by modern instrumental analysis for component qualification and quantification, thereby enabling both species authentication and quality standardization. Core chemical methodologies including chromatographic separation, mass spectrometric detection, and spectroscopic analysis each offer distinct analytical advantages and limitations when applied to phytochemical profiling of MPs. Quality control standards for medicinal plants in national pharmacopoeias are also being improved. Currently, more than 21,000 plants with medicinal value have been documented, but only about 3,000 of them have been studied for their chemical composition[5]. Taking triterpene saponins as an example, the review study by Frolova et al. [6] indicates that although traditional thin-layer chromatography (TLC) and spectrophotometric methods exhibit limitations in sensitivity, resolution, and specificity, they remain widely used due to their high throughput, operational simplicity, and rapid analysis. In contrast, high-performance liquid chromatography (HPLC) and mass spectrometry techniques, particularly HPLC-MS/MS, enable comprehensive detection of triterpene saponins in matrices, achieving rapid identification and simultaneous quantification of trace and multiple saponins. These advanced techniques can also determine the types, sequences, and absolute configurations of monosaccharides in sugar chains, as well as structural types of certain saponin. From a broader perspective, the study conducts comparative analyses on the standardization of triterpene saponin-containing herbs in pharmacopoeias including the Eurasian Economic Union, European, American, and Japanese

pharmacopoeias. The investigated herbs encompass horse chestnut seeds, *Aralia elata* roots, licorice roots, among others. Regarding analytical methods, the review covers (HP)TLC, (U)HPLC, spectrophotometry, and potentiometric titration, highlighting discrepancies among pharmacopoeias in selecting active components, analytical approaches, and quality standards. This work provides valuable references for harmonizing herbal standardization methodologies. Nevertheless, the inherent complexity of phytochemical matrices necessitates advanced instrumentation, standardized operational protocols, and complex data interpretation processes. This requirement elevates analytical costs while limiting broader implementation feasibility.

2. Materials and methods

This study used various scientific databases (e.g., China Academic Journal Database, Scopus, PubMed, ScienceDirect, Google Scholar, Web of Science, Springer Link) for literature retrieval in the past 5 years; and refer to the official pharmacopoeia text that determines drug naming, quality requirements and detection methods: Pharmacopoeia of the People's Republic of China (ChP), European Pharmacopoeia (Ph. Eur.), US Pharmacopoeia (USP), British Pharmacopoeia (BP), Japanese Pharmacopoeia (JP), and the national pharmacopoeias of the Eurasian Economic Union (EEU) Member States.

The method of this study involves the combination of different analytical methods and chemometric analysis. The study was classified according to the analytical methods used, including chromatography (HPLC, GC, CE, TCL, Immunoassay), spectroscopy (NIRS, RS, HIS, NMR, FLS) and miniaturized sensing detection equipment (colorimetric sensor array and fiber optic sensor).

3. Current hot topics in MPs

The past decade has witnessed substantial technological innovation, stimulating transformative developments in analytical methodologies, particularly through advancements in near-infrared spectroscopy (NIRS) and hyperspectral imaging (HSI) platforms. These emerging techniques demonstrate enhanced sensitivity and analytical precision compared to conventional approaches, offering notable improvements in detection throughput and methodological reliability. While persistent challenges hinder industrial-scale implementation—including instrumentation costs, operational complexity, and standardization requirements—these cutting-edge technologies provide pivotal support for modernizing quality assessment frameworks for MPs, demonstrating superior specificity and methodological adaptability relative to traditional analytical paradigms.

In summary, the quantification of bioactive constituents in MPs represents a multifaceted scientific endeavor necessitating the strategic integration and continued advancement of multidisciplinary analytical technologies. This review summarizes the principal analytical methods for bioactive compounds in medicinal plants (MPs), reviews the current applications of chromatographic and spectroscopic techniques, and highlights emerging paradigms such as nanotechnology-based detection, biosensing platforms, and microfluidic systems that play critical roles in accelerating MPs' component analysis. Furthermore, it prospectively discusses existing technical limitations and emerging opportunities in this rapidly evolving field, aiming to provide insights for advancing high-quality development of MPs.

4. Mps active ingredients

MPs synthesize abundant secondary metabolites, with predominant bioactive constituents structurally categorized into *flavonoids*, *alkaloids*, *anthraquinones*, *terpenes*, *coumarins*, *phenylpropanoids*, *lignans*, *tannins*, and related derivatives.

4.1. Alkaloids

Alkaloids represent nitrogen-containing heterocyclic compounds characterized by oxidized nitrogen atoms within their ring architectures, renowned for distinctive physiological interactions and therapeutic applications. These phytochemicals demonstrate broad phylogenetic distribution, with significant concentrations observed in *Solanaceae*, *Ranunculaceae*, and *Berberidaceae* species [7,8]. Their structural diversity spans simple amine derivatives to complex polycyclic systems including *pyrrolidine*, *pyridine*, *isoquinoline*, and *benzylisoquinoline* frameworks, underpinning diverse bioactivity profiles. Pharmacologically, *alkaloids* exhibit multi-target therapeutic effects against malignancies, microbial infections, inflammatory processes, oxidative stress, and neurodegenerative pathologies. Notable examples include *berberine* and *matrine* for oncological applications [9], *atropine* and *scopolamine* as anticholinergic agents [10], and morphine derivatives for pain management [11]. Additional pharmacological actions encompass antihypertensive, antitussive, antifungal, and melanogenesis-inhibitory properties.

4.2. Carbohydrates

Carbohydrates constitute fundamental structural components within plant cellular matrices, gaining pharmaceutical relevance through their favorable pharmacokinetic properties and biocompatibility. Advances in *glycomics* and *glycobiology* have progressively elucidated the biofunctional roles of plant-derived *polysaccharides*, paralleled by technological innovations in carbohydrate-based drug development. *Polysaccharides* isolated from *ginseng*, *reishi* mushrooms, and *Eucommia ulmoides* exhibit not only classical immunomodulatory and anti-neoplastic activities [12,13] but also demonstrate regulatory capacities in *nucleic acid* metabolism, cell cycle modulation, and mitotic coordination [14]. Empirical studies confirm that polysaccharide bioactivity correlates with structural parameters including molecular mass, polymerization index, branching architecture, and tertiary conformational states [15].

4.3. Phenolic acids

Phenolic acids constitute a class of *aromatic carboxylic acids* defined by *phenolic hydroxyl moieties*. These phytochemicals are systematically categorized into *benzoic acid* derivatives, *cinnamic acid* derivatives, and related structural analogs based on their core aromatic frameworks [16]. Their phenolic hydroxyl groups and conjugated aromatic systems confer diverse pharmacological properties, encompassing antioxidant activity, radical neutralization, antiplatelet efficacy, antiatherogenic effects, antimicrobial action, and neoplastic growth inhibition [17,18]. Recognized for their multifunctional bioactivity, *phenolic acids* have emerged as critical compounds in therapeutic development, nutraceutical formulations, and cosmetic science. Chen et al. demonstrated *gallic acid*'s efficacy in obesity suppression and hepatoprotection [19], while Predalikit et al. validated *chlorogenic acid*'s potent anti-inflammatory capacity [20]. Notably, curcumin exemplifies multimodal antiviral mechanisms through influenza A viral replication inhibition and SARS-CoV-2 cellular entry blockade via pleiotropic pathway regulation [21]. Resveratrol further exhibits broad-spectrum antiviral activity through suppression of viral structural protein synthesis and disruption of genomic RNA biogenesis [22].

4.4. Flavonoids

Flavonoids represent a ubiquitous class of plant secondary metabolites distributed across fruits, nuts, vegetables, and medicinal herbs. Following the initial isolation of *rutin* from orange peels in 1930, over 4,000 structurally distinct *flavonoids* have been characterized [23]. These compounds feature a bicyclic phenolic architecture comprising

two *aromatic* rings (C6) interconnected by a three-carbon bridge (C3), forming the characteristic C6-C3-C6 backbone. Their structural diversity originates from modifications to the *2-phenylchromone* core, as schematically represented in Fig. 1.

Flavonoids are distinguished by redox-active moieties including *phenolic hydroxyl*, *methoxy*, *methyl*, *isopentenyl*, and *acetyl groups*, conferring robust antioxidant capacity. These compounds naturally occur as aglycones or glycosides formed through covalent linkage between aglycone cores and saccharide residues. As principal bioactive phytoconstituents, *flavonoids* demonstrate polypharmacological properties spanning antioxidant, antineoplastic, anti-inflammatory, immunoregulatory, antiviral, hepatoprotective, and antimicrobial effects, with favorable toxicological profiles enabling extensive therapeutic exploration [24].

Homoplantagin, a flavonoid glycoside isolated from *Salvia plebeia* R. Br., exhibits vascular endothelial protection through inflammatory pathway modulation [25]. *Quercetin* exemplifies multi-target functionality with well-characterized anti-inflammatory, antioxidant, and anti-cancer mechanisms, particularly its *redox-modulating* capacities that have stimulated recent mechanistic investigations [26]. *Kaempferol* demonstrates clinical potential in managing respiratory pathologies, metabolic disorders, and neurological conditions via anti-inflammatory actions [27].

4.5. Terpenoids

Terpenoids originate from mevalonate pathway derivatives, synthesized via divergent biosynthetic routes including methylation and oxidative modifications. Their structural frameworks comprise *isoprene* (C5) subunits polymerized into *diterpenes* (C20), *triterpenes* (C30), and *sesquiterpenes* (C15), with structural diversification enabling broad bioactivity [28,29]. Notable examples include *artemisinin*'s antimalarial efficacy and *paclitaxel*'s antimitotic properties. Mechanistic studies reveal *Rhodojaponin VI* from *Rhododendron molle* roots mediates analgesia through *N-ethylmaleimide*-sensitive factor interactions with Cav2.2 calcium channels [30], while *sesquiterpene dimers* from *Artemisia* species inhibit *hepatocellular carcinoma* via AKT/STAT3 pathway modulation [31].

4.6. Anthraquinone

Quinones encompass structurally distinct subclasses: *benzoquinones*, *naphthoquinones*, *phenanthraquinones*, and *anthraquinones*. *Anthraquinones* and their glycosidic derivatives constitute predominant phytochemicals in *Fabaceae*, *Liliaceae*, and *Polygonaceae* species [32,33]. Emodin exemplifies multifunctional bioactivity through antimicrobial, organoprotective, and antineoplastic mechanisms [34,35,36]. *Rhein* derivatives from *Rheum officinale* demonstrate immunosuppressive potency and anti-inflammatory effects, while *rhubarb phenols* exhibit neuroprotective and anti-aging properties in intestinal models [37]. Both *emodin* and *chrysophanol* display antifungal activity through membrane integrity disruption [38].

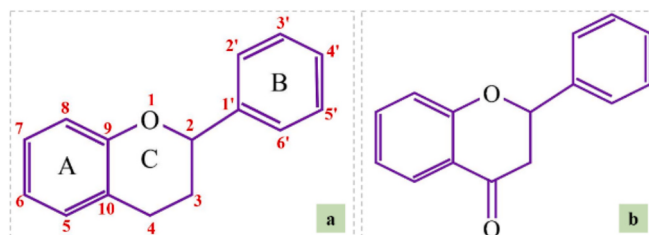


Fig. 1. a) C₆-C₃-C₆ Parent nucleus; b) 2-Phenylchromone.

5. Analytical techniques and methods for MPs components

The phytochemical comprehension of MPs has evolved progressively with advancements in modernization, industrial processing standardization, and globalization of traditional medicine systems. The expanding utilization of MPs and their bioactive derivatives has driven the establishment of standardized quality assurance protocols. Concurrently, analytical methodologies for phytoconstituent characterization have undergone systematic refinement through technological innovation. Nevertheless, the inherent phytochemical complexity of plant matrices, characterized by diverse secondary metabolite profiles, persists as a fundamental challenge in contemporary phytopharmaceutical research. Consequently, the development of integrated analytical platforms combining enhanced sensitivity, systematic compound profiling, and high-throughput capabilities remains a critical focus in MPs modernization initiatives. Recent methodological progress reveals five predominant developmental trajectories:

- (1) Instrumentation Convergence: Analytical methodologies are transitioning toward hyphenated instrumental platforms with automated workflows, enabling rapid micro-scale analysis.
- (2) Comprehensive Profiling Paradigms: Modern approaches emphasize multiplexed constituent monitoring through holistic chemometric evaluation.
- (3) Target-Specific Methodologies: Enhanced emphasis on developing compound-specific detection protocols with improved molecular recognition capabilities.
- (4) Subcellular Resolution Analysis: Technological advancements facilitate in-depth investigation of compartmentalized phytochemical biosynthesis and spatial distribution.

- (5) Process Analytical Technology Integration: Implementation of real-time monitoring systems for continuous manufacturing quality assurance.

5.1. Common analytical techniques and methods

5.1.1. High performance liquid chromatography (HPLC)

HPLC evolved from conventional liquid chromatography through the incorporation of high-pressure infusion systems, microparticulate stationary phases (3–5 μm particle size), and integrated detection technologies, establishing itself as a foundational analytical platform following its development in the 1960 s [39]. As demonstrated in Fig. 2a, this methodology circumvents analytical constraints imposed by analyte volatility and molecular mass, achieving efficient separation of thermally unstable compounds, nonvolatile substances, and ionic analytes. Its analytical selectivity is augmented through mobile phase compositional adjustments, enabling discrimination of structural homologs via differential partitioning coefficients. Key technical advantages encompass: Photostability preservation for light-sensitive phytoconstituents; Baseline resolution of isomeric compounds; Strict adherence to secondary metabolite stability criteria. The ongoing system miniaturization and enhanced hydraulic precision have solidified HPLC's position as the benchmark technique for generating standardized phytochemical fingerprints in medicinal plant analysis [40].

In HPLC analytical workflows, method optimization hinges on precise control of critical chromatographic system parameters to ensure analytical validity. The triad of column selection, detector configuration, and mobile phase optimization forms the methodological foundation for chromatographic method development. Using carbohydrate analysis as a representative case, the following selection criteria apply:

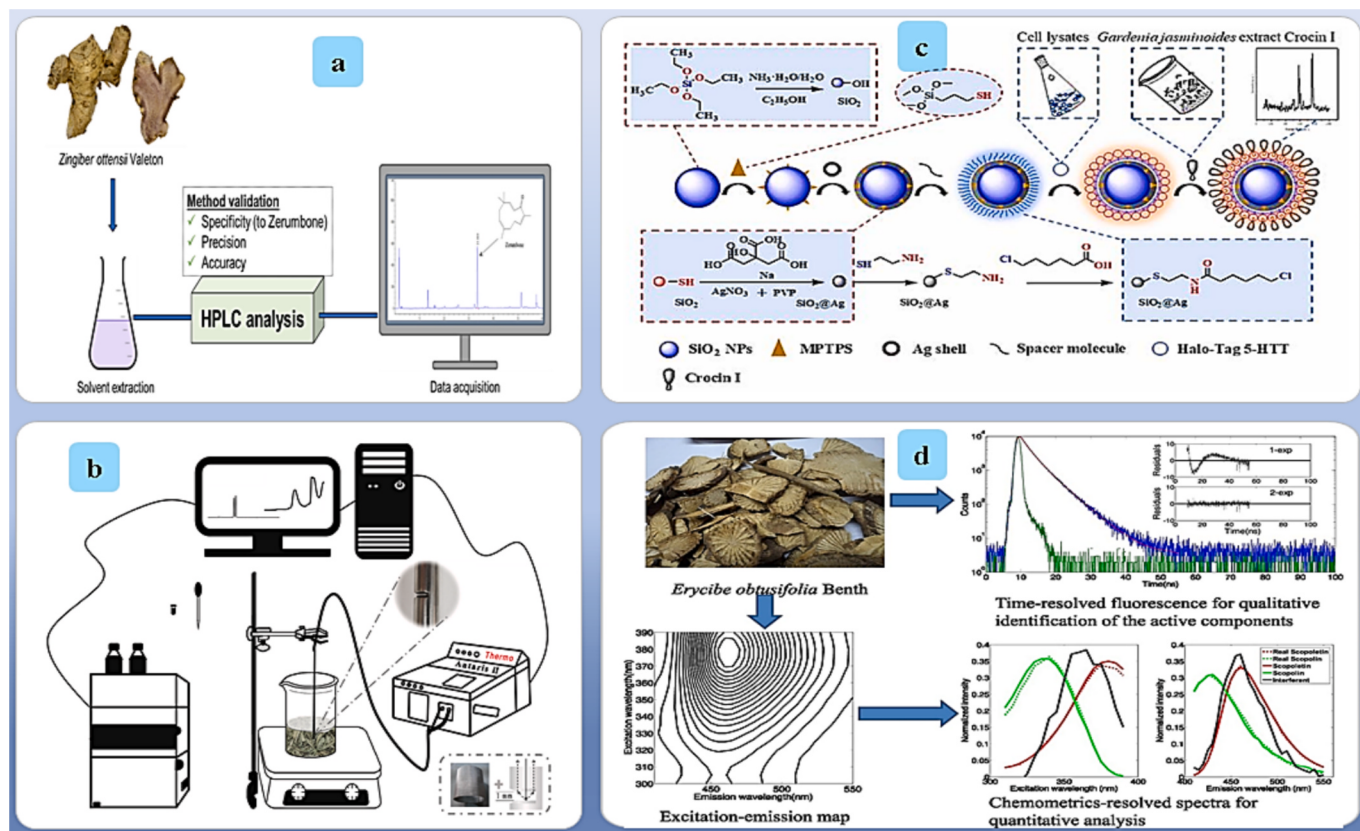


Fig. 2. a) HPLC separation system [44]; b) Application of NIRS in component detection [72]; c) diagrammatic explanation of the immediate detection of putative bioactive constituents within extracts derived from *Gardenia jasminoides* using affinity-selected SERS [77]; d) The application of fluorescence spectral imaging technology [97].

(1) Column Selection: Commercial stationary phases for carbohydrate analysis include C8/C18 silica columns for derivatized analytes, with underivatized carbohydrate separation typically employing amino columns (AOC), sugar columns (SC), or amide columns (AEC). Hydrophilic columns (HC) and anion exchange columns (ANEC) provide alternative selectivity profiles. (2) Detector Configuration: Modern HPLC systems incorporate diverse detection modalities: ultraviolet (UVD), refractive index (RID), fluorescence (FLD), charged aerosol (CAD), evaporative light scattering (ELSD), mass spectrometric (MS), pulsed electrochemical (PAD/ECD), and chemiluminescence detectors[41]. While UVD and FLD require analyte derivatization to introduce chromophores/fluorophores[42], alternative detectors enable direct carbohydrate analysis. ELSD demonstrates particular advantages as a universal detection modality, exhibiting enhanced sensitivity, reproducibility, and environmental stability compared to RID. Its operational independence from analyte volatility and elimination of derivatization requirements make it preferable for underivatized carbohydrate analysis [43].

HPLC has become a pivotal analytical platform for multicomponent quantitation in complex biological matrices. An optimized HPLC protocol was implemented for phytochemical profiling of *Zingiber ottensii* Valetton, achieving precise *zerumbone* quantitation [44]. For complex sample matrices requiring advanced data mining, the integration of HPLC with chemometric approaches significantly enhances analytical resolution and information extraction efficiency. Zhang et al. [45] established a multidimensional HPLC fingerprinting strategy combined with chemometrics to characterize polysaccharide molecular weight distributions in *Poria cocos* (PCPs). Chromatographic analysis of 16 PCP batches identified four characteristic marker peaks. Subsequent integration with Traditional Chinese Medicine (TCM) chromatographic software enabled reference fingerprint construction, revealing adulteration with low-MW *glucans* in commercial samples through principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) pattern recognition. Moratalla-López et al. [46] developed a validated HPLC-diode array detection (DAD) system for simultaneous determination of *crocholanol*, *saffranal*, *crocetin esters*, *flavonols*, and *anthocyanins* in *Crocus sativus* L., establishing a robust quality assessment framework. The HPLC-based rapid quantitation of four *isoquinoline alkaloids* (*berberine*, *palmatine*, *columbamine*, *jateorhizine*) across *Mahonia* species has been achieved, providing phytochemical evidence for their medicinal utilization [47]. Through HPLC analysis, Meena et al. conducted a comprehensive study on the chemical profile similarity between stem bark and twigs of *Cassia* plants. The results revealed that the number of chromatographic peaks in stem bark and twig samples was nearly identical, with perfect correspondence in retention times of all characteristic peaks [48]. Additionally, researchers have utilized HPLC technology to investigate the impact of drying techniques on bioactive constituents during the optimization of drying processes for *Angelica sinensis* [49] and *Lycium barbarum* [50].

However, HPLC implementation faces notable limitations including significant equipment costs that restrict accessibility in resource-constrained laboratories. The methodology also necessitates substantial volumes of environmentally hazardous solvents, raising ecological concerns regarding waste management. Technical constraints further include potential co-elution phenomena compromising analytical accuracy, inherent vulnerability of conventional silica-based columns to stationary phase degradation under extreme mobile phase conditions (pH < 2 or > 9) and elevated temperatures (>60 °C), along with minimum sample volume requirements that challenge high-throughput applications. While remaining essential for MPs phytochemical analysis, these chromatographic selectivity limitations and detection specificity challenges necessitate ongoing methodological optimization in pharmaceutical quality assessment.

5.1.2. Ultra-performance liquid chromatography (UHPLC)

With technological advancements, UHPLC has emerged as a reliable

analytical approach through hardware upgrades to conventional HPLC systems, enabling precise characterization of MPs. Technological evolution has transformed UHPLC into a robust analytical platform through instrumental enhancements of conventional HPLC infrastructure, enabling advanced MPs characterization. Operating at pressures ≤ 15,000 psi[51], UHPLC employs sub-2 μm stationary phase particles to achieve superior sensitivity and chromatographic resolution compared to standard HPLC. The reduced particle diameter (1.7–1.8 μm) optimizes theoretical plate counts while shortened column geometries (typically 50–100 mm) enable 80 % reduction in analytical runtimes. Solvent consumption decreases proportionally, with UHPLC demonstrating equivalent separation efficiency to HPLC using 1/8th the original analysis time [52].

The combination of UHPLC-mass spectrometry (UHPLC-MS) fingerprint profiling with multicomponent quantitation strategies has become a cornerstone in contemporary quality control frameworks. A UPLC-MS/MS-based method has been developed for simultaneous quantification of 11 bioactive constituents in *Ziziphus jujuba* seeds, achieving baseline separation within a 10-minute chromatographic run. The validated methodology demonstrated satisfactory precision, repeatability, and storage stability, establishing an efficient protocol for phytochemical standardization of *jujuba* seeds [53]. Simultaneous measurements were recently conducted for *protocatechuic acid*, *epicatechin*, *chlorogenic acid*, *quercetin*, *shaftoside*, and *scutellarin A* in *Gaultheria leucocarpa* var. *yunnanensis*. Their systematic evaluation of geographical and plant organ variations demonstrated method robustness through precision parameters (RSD < 2.5 %) and specificity validation. The identification of *shaftoside*, *epicatechin*, and *protocatechuic acid* as origin-specific chemical markers advances quality standardization research for this medicinal species [54].

5.1.3. Gas chromatography (GC)

GC operates through differential partitioning interactions between a gaseous mobile phase and stationary phase, enabling compound separation via inert carrier gas transport. The analytical sequence comprises three critical phases: 1) flash vaporization of samples in temperature-controlled injection ports; 2) chromatographic resolution in precision capillary columns driven by chromatography-grade carrier gas, where separation efficiency depends on compound volatility and stationary phase affinity; 3) quantitative detection through high-sensitivity detectors. While excelling in volatile compound analysis, the mandatory thermal stability requirement (typically 50–300 °C operating range) constrains its application to thermally stable organics and essential oils [55]. Non-volatile analytes require chemical derivatization to enhance volatility prior to GC analysis.

GC enables analysis of various MP components including organic acids, alkaloids, flavonoids, and phytosterols, particularly through GC-MS and GC-IR integrations that significantly expand phytochemical investigation capabilities. These hyphenated techniques facilitate comprehensive characterization of medicinal plant resources, supporting their phytochemical classification and pharmacological exploration [56]. The GC-MS coupled methodology demonstrates superior analytical performance through enhanced sensitivity, rapid detection cycles, exceptional resolution, and compound-specific identification capabilities. This technological synergy substantially improves detection accuracy for volatile constituents, proving particularly effective for trace-level small molecules and volatile organic compounds. Methodological innovations have enabled efficient quantification of terpenoids, alkaloids, carbohydrates, and fatty acid derivatives [57].

Zubaid-UI-Khazir's research team conducted pioneering GC-MS analysis of plant *essential oils*, identifying twelve characteristic *monoterpene* constituents [58]. The profile revealed *limonene* (38.9 %), *α-pinene* (36.5 %), *β-pinene* (6.9 %), and *α-phellandrene* (4.4 %) as dominant components, collectively representing 86.7 % of total composition. Systematic bioactivity assessments demonstrated significant antioxidant capacity, broad-spectrum antimicrobial efficacy, and

potential anticancer properties. *Carboxylic acids* demonstrate critical applications across pharmaceutical development, agricultural science, and food technology due to their distinctive structural features. Mykhailenko et al. conducted pioneering comparative analysis of *carboxylic acid* profiles in four *Ukrainian Iris* species using GC/MS, identifying 26 common *carboxylic acids* with preferential accumulation of short-chain derivatives (C4-C8) [59]. The substantial presence of pharmacologically active *carboxylic acids* in *Iris* specimens establishes theoretical foundations for their potential utilization in nutraceutical additives and novel drug formulations. Zhou et al. performed systematic GC-MS characterization of *essential oil* (EEO) from *Eucalyptus grandis* × *E. urophylla*, revealing monoterpenes and their oxygenated derivatives as principal constituents[60]. *α-Pinene* (17.02 ± 0.35 %) emerged as a predominant terpenoid with putative anti-inflammatory pharmacophores. Concentration-dependent radical scavenging activities against DPPH and ABTS confirmed exceptional antioxidant capacity, providing scientific basis for further exploration of this hybrid eucalyptus oil's bioactivity.

Post-GC separated components undergo sequential mass spectrometric detection to generate compound-specific mass spectra. Database matching against standardized spectral libraries enables preliminary structural identification, with literature cross-verification enhancing confirmation reliability. A critical consideration involves database limitations when analyzing novel compounds, potentially yielding multiple structural candidates from spectral similarity matching. The inherent challenge of differentiating isobaric compounds with analogous fragmentation patterns necessitates supplementary analytical techniques for definitive qualitative confirmation.

5.1.4. Capillary electrophoresis (CE)

CE, as a pivotal technique in high-resolution liquid separation systems, enables precise analyte discrimination in complex matrices through diversified electrokinetic separation mechanisms. This methodology encompasses six principal operational modes: (1) Capillary zone electrophoresis (CZE) for ionic species separation based on charge-to-mass ratio differentials; (2) Micellar electrokinetic chromatography (MEKC) utilizing surfactant-based pseudostationary phases for hydrophobic compound resolution; (3) Microemulsion electrokinetic chromatography (MEEKC) employing nanoscale oil-water emulsion systems to enhance lipophilic substance analysis; (4) Capillary gel

electrophoresis (CGE) with cross-linked polymer matrices for biomacromolecule size-based separation; (5) Focusing techniques [capillary isoelectric focusing (CIEF) and isotachopheresis (ITP)] for amphoteric compound fractionation via pH gradients; (6) Capillary electrochromatography (CEC) integrating chromatographic stationary phases with electroosmotic flow modulation. Comparative performance characteristics of these modes are systematically presented in Table 1.

Since its pharmacognostic implementation in 1992, CE has evolved from initial applications in alkaloid and flavonoid glycoside analysis to comprehensive detection of *coumarins*, *cardenolides*, *saponins*, *phenolic acids*, and other bioactive constituents. This technological progression has enabled phytochemical characterization of numerous medicinal species including *Glycyrrhiza uralensis*, *Ephedra sinica*, *Phellodendron amurense*, *Coptis chinensis*, *Rheum palmatum*, *Paeonia lactiflora*, *Panax ginseng*, *Angelica sinensis*, and *Prunus armeniaca*. CE-based quality control protocols have been incorporated into multiple pharmacopeial standards, notably the Chinese Pharmacopoeia, United States Pharmacopoeia, and European Pharmacopoeia, reflecting its global regulatory acceptance.

The CE separation process employs fused-silica capillaries under high-voltage electric fields, achieving component differentiation through electrophoretic mobility variations ($\mu_e = v/E$). Method optimization involves strategic mode selection according to analyte properties including charge state, hydrophobicity, and molecular mass. With advantages such as superior separation efficiency, minimal sample consumption, and method flexibility, CE has become indispensable in biomedical research, nanopharmaceutical development, and phytochemistry modernization initiatives.

CE is recognized for its compatibility with diverse detection modalities, including ultraviolet absorption spectroscopy, mass spectrometric interfaces, evaporative light scattering detectors, and laser-induced fluorescence systems. Effective analytical performance for complex samples can be achieved through strategic detector selection and configuration. A CE-laser induced fluorescence (CE-LIF) methodology was developed by Xia et al. through in-capillary dynamic derivatization, enabling simultaneous *flavonoid* derivatization and separation. This approach was successfully applied for quantitative determination of *flavonoid* profiles in *Medicago sativa* plants and their granule formulations, with validation parameters confirming method sensitivity (LLOQ: 0.92 nmol/L; ULOQ: 35.46 nmol/L) and accuracy (spike recovery:

Table 1
Comparison of different separation modes for CE analysis technology.

Technical Name	Principle	Separation Mechanism	Application Field	Advantage	Disadvantage
CZE	Separation of charged particles driven by an electric field	Charge and size	Biomolecules (amino acids, proteins), drug analysis	Separation and detection of various substances High separation efficiency Simple operation	1. Lack of electrophoretic mobility of neutral substances 2. Certain limitations in separation of neutral compounds
MEKC	Micelles as a separation medium in an electric field	Hydrophobicity and charge	Drug and environmental sample analysis	Nonpolar compounds can be separated	1. Micelle concentration needs to be optimized 2. Separation of polar compounds is poor
MEEKC	Microemulsions as a separation medium	Partition coefficient and charge	Drug and food analysis	Efficiently separating for complex samples	1. Stability of microemulsions is poor 2. Operation is complex
CGE	Combining gel matrix and electrophoretic separation	Molecular size	Protein and nucleic acid analysis	Wide range of molecular weight separation	1. Separation speed is slow 2. Operation is complex
CIEF	Separation based on isoelectric point	Focusing charged particles in a pH gradient	Protein separation	High resolution, suitable for complex samples	1. Requires high standards 2. Sensitive to pH stability
ITP	Separation of charged particles in a constant electric field	Charge and frictional force	Biomolecules, drug analysis	1. Fast separation speed 2. High efficiency	1. Separation process needs optimization 2. Strict requirements for samples
CEC	Combining electrophoretic and chromatographic separation mechanisms	Distribution coefficient and charge separation	Analysis of complex compounds	1. Wide applicability 2. High separation efficiency	1. Operation is complex 2. Equipment costs are high

80.55–94.25 %)[61]. In parallel, the capillary electrophoresis-photodiode array detection (CE-PDA) protocol for iridoid glycoside analysis was systematically optimized using the Analytical Quality by Design (AQbD) framework by Zhang et al., achieving baseline resolution of four target markers in *Gentiana* species. Method sensitivity (LOD: 2.32–9.58 mg/L) validated the scientific rationale of AQbD-driven analytical development, establishing a novel strategy for phytopharmaceutical quality assessment[62].

5.1.5. Thin layer chromatography (TLC)

TLC separation is fundamentally governed by differential adsorption/desorption equilibria and compound-medium interactions in specified chromatographic environments. Component resolution is achieved through repeated cycles of adsorption and partitioning as the sample solution migrates through the stationary phase. This rapid analytical technique requires minimal sample volumes and exploits polarity-dependent interactions between analytes and silica-based adsorbents. In standard TLC workflows, samples are applied to predefined baseline positions on chromatographic plates using calibrated microsyringes, followed by mobile phase elution under capillary action. Differential compound migration rates are observed due to polarity variations between analytes and eluent systems. Separation efficiency is quantified through retention factor (R_f value) calculations, defined as the ratio of compound migration distance to solvent front displacement. This dimensionless parameter serves as a critical identification metric, reflecting compound-specific chromatographic behavior under standardized conditions.

The adsorption capacity of compounds is positively correlated with their polarity, where increased polarity results in stronger adsorption interactions and consequently reduced R_f values. Under standardized chromatographic conditions (adsorbent type, mobile phase composition, layer thickness), the distance ratio between analyte migration and solvent front progression remains constant. This R_f value constitutes an intrinsic physicochemical property determined exclusively by molecular structure, enabling selective compound discrimination.

Since its inception in 1938, TLC has been extensively utilized for mixture component characterization. Effective phytochemical profiling is facilitated through this technique, permitting compound identification and semi-quantitative analysis, while serving as a critical component in contemporary herbal pharmacopeial standards. Technological advancements, particularly in digital imaging systems, have enabled quantitative TLC applications. A novel TLC-spray ionization mass spectrometry platform was developed for evaluating chemical heterogeneity in *Poria cocos* specimens [63]. Additional applications include vitexin quantification in *Passiflora foetida* [64], bioactive compound assessment in *Manilkara hexandra* [65], and phytochemical profiling of *Melaleuca alternifolia* [66]. However, TLC is limited by moderate specificity and sensitivity, dependency on reference standards/visualization reagents, and rudimentary detection methodologies, rendering it primarily suitable for preliminary sample evaluations.

5.1.6. Immunoassay

Immunoassay techniques are founded upon the specific antigen-antibody binding interaction, employing labeled antibodies for signal amplification in analyte quantification. Compared to conventional analytical methods, this approach better addresses practical requirements for rapid, accurate, and user-friendly detection in industrial and clinical settings. Widespread implementation has been documented in clinical diagnostics, food safety monitoring, and environmental toxicology. Methodological advantages include technical integration, operational efficiency, and automated processing capabilities. The increasing analytical challenges posed by ultraviolet-inactive bioactive constituents in MPs have driven substantial adoption of antibody-based detection systems for phytopharmaceutical quality control. Current immunoassay variants are categorized by labeling methodology and detection principles, including enzyme-linked immunosorbent assay

(ELISA), fluorescence immunoassay, and immunochromatographic techniques. Comparative technical specifications of these methodologies are systematically summarized in Table 2.

Despite successful implementation in MPs bioactive constituent analysis, immunoassay methodologies remain underdeveloped compared to existing natural product characterization platforms. Critical technical limitations persist in this domain. Small molecular compounds are typically non-immunogenic, necessitating hapten-carrier conjugation to generate immunoreactive complexes. Throughout immunological recognition processes, precise antigen-antibody binding specificity must be maintained, as minimal cross-reactivity with structural analogs could compromise detection accuracy. Current antibody production predominantly depends on animal immunization protocols, presenting challenges in industrial-scale manufacturing and extended development timelines. Furthermore, antibody stability is frequently compromised by operational variables including environmental conditions and reagent interactions. Future research priorities should emphasize 1) optimization of synthetic antigen preparation techniques to generate high-specificity antibodies, 2) development of animal-free antibody production systems with scalable capacity, and 3) enhancement of antibody stability across diverse analytical conditions.

5.2. Non-destructive detecting methods based on spectral analysis

5.2.1. Near-infrared spectroscopy technology (NIRS)

The NIR spectral region is operationally defined as 780–2526 nm per ASTM standards, corresponding to molecular vibration overtone and combination band transitions. Absorption signatures in this range are predominantly generated by harmonic oscillations of fundamental chemical moieties [67]. Composition-dependent variations in wavelength-specific optical density responses enable qualitative and quantitative determinations through multivariate spectral interpretation. This non-destructive approach has been extensively implemented for rapid quantification of characteristic MP constituents including alkaloids [68], flavonoids [69], and polysaccharides [70].

A hybrid analytical platform integrating NIRS with spectrophotometric and HPLC was established for comprehensive MP bioactivity assessment[71]. Chemometric analysis of 16 medicinal species enabled simultaneous determination of primary metabolites and secondary bioactive compounds. Experimental validation has confirmed this method's capacity for rapid parallel quantification of carbohydrates, nitrogenous compounds, and phenolic derivatives in MP extracts. As a secondary analytical strategy, standardized reference databases must be initially constructed using validated physicochemical methods, followed by chemometric model optimization through algorithmic refinement. This calibrated system ultimately permits efficient constituent identification and concentration prediction in novel samples.

The extraction endpoint for *Stevia rebaudiana* leaves was determined through NIRS-chemometrics integration, as illustrated in Fig. 2b[72]. A qualitative analytical protocol was developed utilizing moving block standard deviation (MBS), absolute distance standard deviation (ADSD), and principal component analysis (PCA), enabling real-time extraction endpoint determination via statistical process parameter monitoring. This NIRS-MBS approach achieved equivalent accuracy to HPLC methodologies while reducing spectral acquisition time to 18 s per measurement, significantly enhancing temporal efficiency. Validation studies confirmed the system's cost-effectiveness and operational practicality for stevia extraction endpoint discrimination, providing valuable insights for MPs production optimization.

NIRS has been further implemented for simultaneous quantification of lipophilic constituents (tetracyclines IIA) and hydrophilic biomarkers (rosmarinic acid, salvianolic acid B) in *Lamiaceae* species [73], as well as rapid identification of benzoic acid derivatives (e.g., gallic acid) in *Cornus officinalis* [74]. Accurate quantification capabilities have been demonstrated for polysaccharide content and antioxidant activity assessment in *Poria cocos*, with method robustness and adaptability confirmed through

Table 2

Applications of Immunoassays in MPs.

Technology	Principle	Sensitivity	Specificity	Application	Advantages and disadvantages	Ref.
Enzyme-linked immunosorbent assay	Enzyme-labeled antibodies, the substrate is converted to generate a measurable signal	Medium to high	High	<i>Baicalin, puerarin, PapaVerine, Huperzine A, Naringin</i>	1. Simple operation 2. Low cost 3. Limited sensitivity	[111]
Fluorescence immunoassay	Fluorescence-labeled antibodies to measure fluorescence intensity	High	High	<i>Paclitaxel, Saikosaponin A</i>	1. High sensitivity 2. High instrument cost	[112]
Immunoaffinity chromatography column	Antibodies are immobilized on chromatography column to separate target molecules	High	High	<i>Hesperidin, Naringin, Ginsenoside</i>	1. Strong selectivity 2. Operation is complex	[113]
immunochromatography technology	Antibodies react with sample, to form visible lines on the membrane	Medium	Medium	<i>Dihydroartemisinin, Artemether, Asiatica</i>	Simple operation Strong portability Lower sensitivity	[114]
Immunochip	Multiplex antigen detection using microarray technology	High	High	<i>Compound Glycyrrhizin, Liquiritin, Atrazine, Nonyl Phenol, Chloramphenicol</i>	1. High throughput 2. Data processing is complex	[115]
Immunosensor	Biosensor technology for real-time detection	High	High	<i>Baicalin, Chlorogenic acid</i>	Real-time monitoring High sensitivity High technical requirements	[116]

iterative optimization cycles[75]. Despite advantages including non-destructive analysis, rapid operation, and ease of implementation, conventional NIRS instrumentation remains cost-prohibitive, while miniaturized systems are prone to instrumental parameter drift during prolonged operation. This necessitates the development of portable spectrometers balancing stability, precision, and affordability. Furthermore, infrared spectral responses are sensitive to environmental variables including temperature fluctuations, relative humidity variations, and source-sample distance alterations. Predictive models derived from environment-specific spectral datasets may consequently exhibit reduced accuracy when deployed under divergent operational conditions.

5.2.2. Raman spectroscopy (RS)

The Raman scattering phenomenon was initially documented in 1928. However, practical implementation of this detection technology was delayed for 32 years due to inherent signal intensity limitations ($<10^{-8}$ of incident radiation), until advancements in laser excitation sources enabled operational viability [76]. This spectroscopic technique employs inelastic scattering phenomena to probe molecular vibrational and rotational states, with characteristic frequency shift profiles being leveraged for critical quality assurance applications such as MPs origin verification, geographical authentication, functional group characterization, and bioactive constituent quantification. Despite its analytical utility, widespread adoption has been constrained by intrinsic Raman signal attenuation and fluorescence interference effects. Through exploitation of nanoscale metallic substrate-mediated localized surface plasmon resonance, analyte-specific vibrational/rotational spectral signatures can be amplified exponentially. This enhancement principle underpins surface-enhanced Raman spectroscopy (SERS), which has demonstrated synergistic potential when integrated with orthogonal analytical platforms for selective target identification and structural analog resolution in complex matrices.

Superior sensitivity (single-molecule detection limits) and stereochemical discrimination capabilities position SERS as a promising methodology for online secondary metabolite profiling and bioactive marker discovery in MPs. For instance, affinity-guided SERS screening was implemented for direct identification of bioactive candidates in *Gardenia jasminoides* extracts, with workflow schematics detailed in Fig. 2c[77]. Earlier applications include SERS-based qualitative and quantitative analysis of *Coptis chinensis* and *Phellodendron amurense* phytoconstituents without prior chromatographic separation [78].

Parallel developments involved ratiometric quantification of *puerarin* in *Pueraria lobata* via characteristic Raman bands at 1628 cm^{-1} (*puerarin*) and 880 cm^{-1} (ethanol matrix), establishing concentration-dependent linear correlations that bypassed extraction requirements [79]. These studies collectively validate SERS as a rapid, precise, and non-destructive alternative to conventional phytochemical analysis. Structural differentiation capacity has been further demonstrated for homologous bioactive molecules across herbal species[80].

5.2.3. Hyperspectral imaging (HSI)

HSI technology was initially established in the 1970 s[81] as a spectral-spatial dual-data fusion analytical platform. In contrast to conventional single-point spectral techniques, this methodology enables synchronous acquisition of sample geometric spatial features and full-spectral dimensional data while supporting parallel multi-sample analysis[82]. It should be noted that acquisition artifacts including noise contamination, pixel anomalies, and spectral distortion may critically compromise feature extraction efficiency, thereby degrading predictive model accuracy. These limitations are mitigated through the implementation of advanced chemometric processing protocols that extract chemically relevant information from high-dimensional datasets, overcoming the constraints of conventional analytical approaches while enhancing detection precision and visualization capabilities. For MPs, HSI has been successfully implemented for quality characterization and geographical origin authentication.

In MP bioactive constituent quantification, Partial Least Squares Regression (PLSR) and Least Absolute Shrinkage and Selection Operator (LASSO) models were employed to predict gingerol-shogaol ratios [83]. This integrated approach enabled non-destructive rapid quantification of phytochemical compositional profiles through chemometric-spectral fusion strategies. A hybrid deep learning architecture incorporating Attention Mechanism (AM), Convolutional Neural Network (CNN), and Long Short-Term Memory (LSTM) modules was developed by Wang et al. for hyperspectral feature selection[84]. During MP traceability verification, this model achieved optimal geographical discrimination accuracy while minimizing prediction errors (mean deviation and root mean square error) in nutrient parameter estimation, confirming the synergistic potential of spectral-chemometric integration in MPs bioactive compound analysis.

Zhang et al. [50] implemented a deep regression framework with near-infrared HSI for simultaneous quantification of *anthocyanins*, *flavonoid* polymers, and *phenolic* derivatives in *Lycium ruthenicum*

matrices. A dual-mode feature extraction system combining supervised CNN and unsupervised Deep Autoencoder (DAE) architectures was integrated with PLS and Least Squares-Support Vector Machine (LS-SVM) algorithms, yielding multi-component analytical solutions for herbal materials. Experimental validation revealed $\pm 1.5\%$ prediction error convergence between deep learning and conventional algorithms, demonstrating the engineering viability of hierarchical feature extraction in MP active substance modeling.

For MP authentication and adulterant detection, a multispectral imaging system (400–1000 nm) coupled with chemometric analysis was developed, achieving simultaneous *saffron* origin identification and *exogenous* contaminant screening [85]. Kabir et al. [86] implemented HSI-CNN integration for non-destructive discrimination of 12 *Fritillaria* species, employing a tripartite evaluation matrix incorporating Support Vector Machine (SVM) and Partial Least Squares Discriminant Analysis (PLS-DA) models. This methodology demonstrated superior classification accuracy, underscoring the transformative potential of spectral-deep learning fusion in botanical material analysis. Abundant spatial-spectral information can be acquired from MPs through HSI, enabling comprehensive characterization of external morphological features, dimensional parameters, structural defects, and internal chemical constituents. Consequently, superior accuracy in non-destructive quality assessment is achieved. However, practical implementation has been constrained by significant data redundancy, computationally intensive processing requirements, prolonged analytical durations, elevated computational resource demands, and the prohibitive costs associated with hyperspectral instrumentation.

5.2.4. Nuclear magnetic resonance spectroscopy (NMR)

NMR spectroscopy is recognized as a robust analytical methodology that elucidates molecular configurations through detection of nuclear spin resonance phenomena under external magnetic fields. The chemical environment and bonding networks of atomic nuclei are revealed through characteristic spectral signatures determined by nuclear magnetic properties and local electronic surroundings. A standard NMR spectrometer consists of three core components: 1) a superconducting magnet generating stable high-field conditions, 2) radiofrequency (RF) transmitter/receiver systems for nuclear spin excitation and signal acquisition, and 3) control modules for parameter optimization. Multiple NMR modalities exist, including proton nuclear magnetic resonance (^1H NMR) for hydrogen atom chemical shift analysis and carbon-13 NMR (^{13}C NMR) for carbon skeleton characterization, which are employed to investigate hydrogen bonding environments and carbon connectivity patterns, respectively. Molecular structural determination is facilitated through interpretation of chemical shift values, coupling constants, integration ratios, and splitting patterns. NMR spectroscopy has been extensively implemented across diverse disciplines including organic chemistry, pharmaceutical research, natural product analysis, and materials science, serving as a critical technique for molecular architecture elucidation.

Within the quality management framework of herbal products, NMR technology has been established as a core analytical platform for unknown compound identification and structural origin tracing, leveraging its non-destructive nature, minimal sample preparation requirements, and broad-spectrum detection capabilities for secondary metabolites. Quantitative NMR (qNMR) was employed by Machado et al. for simultaneous multi-component analysis of anticancer *annonaceous acetogenins* in *Annonaceae* species, demonstrating rapid quantification without chromatographic separation [87]. *Brucine* and *strychnine alkaloids* in *Strychnos nux-vomica* extracts were quantitatively differentiated by Fr  d  rich et al. [88] using ^1H NMR spectroscopy, achieving accurate measurements without reference calibration curves. Rapid quantification of ginkgolide derivatives in *Ginkgo biloba* materials was demonstrated through ^1H NMR analysis, with multi-component detection completed within 5 min [89].

NMR spectroscopy is particularly instrumental in carbohydrate

structural characterization systems. Through NMR spectral analysis, *glycosidic residue* types, relative abundance, linkage positions, predominant conformational states, and substituent orientations can be determined [90]. Structural elucidation of carbohydrate compounds via NMR is fundamentally dependent on the integration of multidimensional techniques, where inter-technique correlation and complementary analytical capabilities enable comprehensive interpretation. This multi-modal approach has been validated in structural investigations of MP-derived pectic polysaccharides [91], water-soluble heteroglycans [92], and neutral oligosaccharides [93].

5.2.5. Fluorescence (FLS) spectroscopy

Fluorescence imaging methodology was first conceptualized in 1966 [94]. This technique exploits fluorescence emission characteristics for systematic chemical profiling through simultaneous acquisition of excitation and emission spectral data. The analytical platform incorporates pulsed optical signal detection modules and spatial resolution algorithms to derive functional group assignments and concentration gradients of target analytes. Key advantages include trace-level sensitivity, non-destructive operation, and real-time monitoring capabilities, as schematically illustrated in Fig. 2d.

Approximately 75 % of 315 commonly used medicinal materials have been documented to exhibit intrinsic fluorescence responses, owing to the prevalence of fluorophore-containing organic compounds in MPs. Distinct emission/excitation spectral profiles have been leveraged for species authentication and phytochemical discrimination [95]. In *Houpu* analysis, synchronous fluorescence spectroscopy was implemented with *amino acid* ionic liquid additives, achieving complete spectral resolution of overlapping *magnolol* and *honokiol* signals [96]. Parallel factor analysis combined with EEM fluorescence spectroscopy was developed for concurrent quantification of *scopoletin* and *scopolin* in *Erycibe obtusifolia*, yielding concentration predictions statistically equivalent to HPLC-fluorescence detection results [97].

Advanced FLS modalities including synchronous fluorescence, light scattering correlation, anisotropy measurement, derivative spectroscopy, and time-resolved detection have been established for investigating MPs-biomacromolecule interactions. Such approaches enable mechanistic analysis of structure–activity relationships and pharmacological efficacy through fluorescence-based binding studies. DNA interaction characteristics of quercetin were elucidated through fluorescence quenching experiments [98], while resveratrol-pepsin binding dynamics were quantified without pretreatment requirements [99]. These techniques permit direct determination of binding constants and displacement mechanisms, contingent upon the intrinsic fluorescence properties of target analytes [100].

5.2.6. Terahertz (THz) spectroscopy

THz spectroscopy can be categorized into three principal modalities: time-domain spectroscopy (THz-TDS), time-resolved terahertz spectroscopy (TRTS), and terahertz emission spectroscopy (TES), based on the operational configuration of terahertz transmission microscopy [101]. In medicinal plant (MP) quality evaluation, THz-TDS has been established as the predominant analytical methodology due to its simultaneous amplitude-phase detection capability of terahertz waveforms [102]. The technique is typically implemented through transmission or reflection configurations to acquire time-domain spectral data, with sample-specific optical parameters subsequently derived via Fourier transform. The analytical potential of THz spectroscopy for MPs constituent identification lies in its sensitivity to organic compound signatures. Molecular vibrational modes, weak intermolecular interactions, and crystalline lattice oscillations within these compounds are spectrally resolved within the terahertz frequency regime. While absorption peak positions may suffice for component discrimination in chemically simple systems, direct spectral interpretation becomes problematic for MPs containing complex mixtures. Furthermore, subtle compositional differences often result in spectrally indistinguishable

features, necessitating integration with chemometric pattern recognition algorithms for reliable discrimination.

A THz-based quantitative model for baicalin content determination was developed through PLS-SVM and PLS regression, achieving minimal RMSE and strong linear correlations [103]. A THz-TDS detection platform for *Panax notoginseng* bioactive constituents was constructed with mathematical modeling of signal-response relationships validating its quantitative accuracy [104]. Structural-activity correlation analysis of *flavonoid isomers* was performed using THz-TDS, establishing a non-destructive framework for simultaneous qualitative identification and quantitative analysis [105]. These studies confirm THz spectroscopy's capability to resolve structurally analogous phytomolecules, particularly isomer differentiation, while highlighting its underexploited potential in MPs research. Current applications have been predominantly restricted to basic spectral pattern recognition in simplified model systems, underscoring the need for advanced multivariate analysis techniques to address MP chemical complexity.

5.3. Other novel detection methods

5.3.1. Colorimetric sensor array

While conventional chromatographic, mass spectrometric, and spectroscopic techniques demonstrate notable precision and specificity, their implementation is constrained by mandatory sample pretreatment protocols and extended analytical durations, rendering them primarily suitable for centralized laboratory batch analyses. In contrast, miniaturized sensing platforms have demonstrated enhanced applicability for

active constituent detection in MPs.

Conventional sensing methodologies exhibit limitations in achieving discriminative analysis of structurally analogous compounds and engineering selective detection units for complex matrices. These challenges have been addressed through the development of multichannel sensor array-based high-throughput detection systems, which enable simultaneous acquisition of differential response profiles across multiple sensing elements, thereby facilitating multiplex target identification. As an emerging detection paradigm, colorimetric sensor arrays are fundamentally composed of stimuli-responsive chromogenic materials that undergo selective color transitions upon analyte interaction. Quantitative and qualitative characterization is achieved through computational analysis of chromatic variance patterns, with integrated machine learning algorithms enabling comprehensive interpretation of multidimensional response data. Within MPs bioactive compound analysis, this technology has shown considerable potential. A urease inhibition-based colorimetric array for *benzylisoquinoline alkaloid* discrimination was pioneered, establishing a novel phytochemical screening strategy through enzymatic activity modulation [106]. The operational principle of this urease-responsive sensing platform is schematically illustrated in Fig. 3a.

In recent years, nanozymes have been increasingly utilized in bio-sensing applications owing to their facile synthesis, cost efficiency, and structural stability. Substantial research interest has been attracted by the advancement of nanozyme-integrated colorimetric sensor arrays. A pioneering study demonstrated the detection of bioactive constituents in *Glycyrrhizae Radix* through an iron oxide nanozyme-mediated

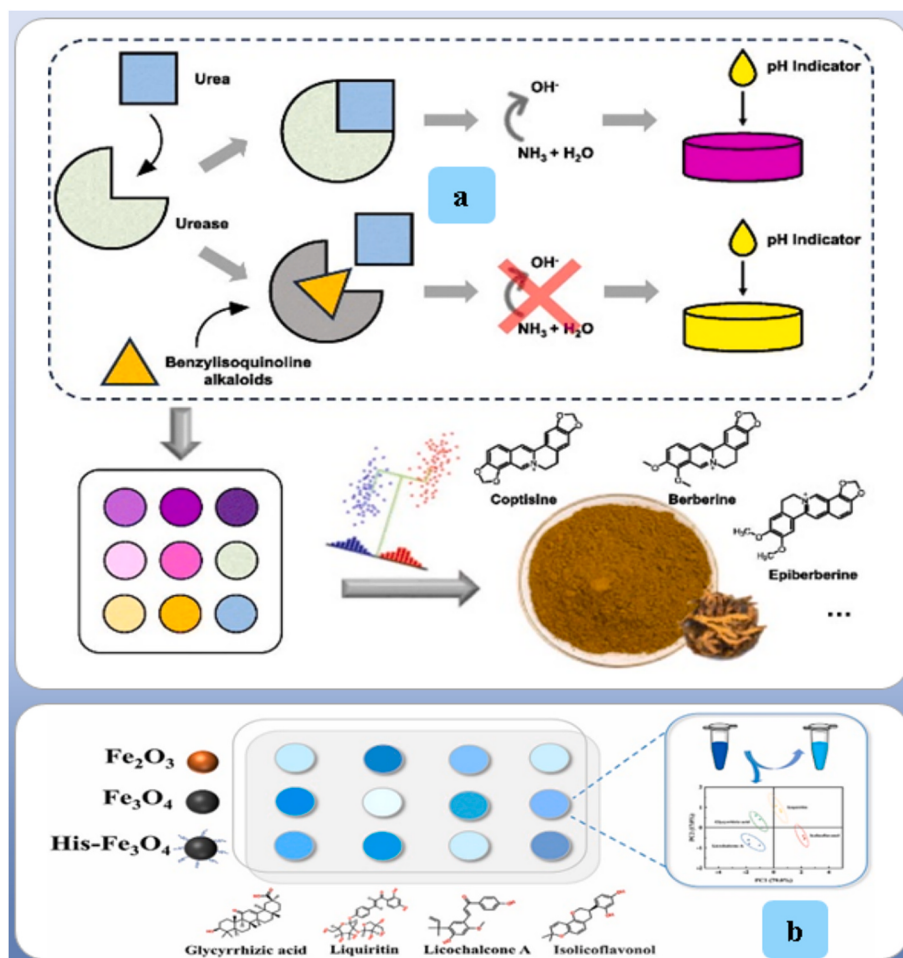


Fig. 3. a) Identification of alkaloids by colorimetric sensor array [106]; b) Identification of active substances in Chinese herbal medicines based on nanozyme-based sensor array [107].

colorimetric array [107]. This system achieved discrimination of glycyrrhizin, liquiritigenin, licochalcone A, and isoflavonol across a 1–200 μM concentration range, thereby establishing an innovative approach for licorice phytochemical profiling. The operational workflow is illustrated in Fig. 3b.

Sensor array technology is distinguished by procedural simplicity, economic viability, and high-throughput analysis, exhibiting exceptional performance in resolving complex multicomponent systems. However, existing systems are predominantly limited to known compound identification, with an urgent requirement for intelligent sensing platforms capable of structural characterization for unknowns in practical scenarios.

5.3.2. Fiber-optic biosensor

The advent of microfluidic technology has generated considerable research momentum in analytical science. Electrophoretic and chromatographic microfluidic chips have been implemented for detecting signature phytochemicals in MPs including *Magnolia officinalis* and *Agrimonia pilosa*. Nevertheless, current microfluidic platforms for MPs analysis remain constrained to centimeter-scale architectures. Miniaturization of core components to micrometer precision is critically required to advance sensor integration and mechanical robustness. Fiber-optic sensing technology was developed following rapid advancements in photonic engineering during the late 20th century, marking a paradigm shift from conventional data transmission to intelligent sensing systems. Initial research efforts were concentrated on exploiting optical transmission properties for diverse sensing configurations. Subsequent advancements have been driven by synergistic developments in photonics, nanofabrication, and material engineering, resulting in transformative enhancements in fiber-optic sensor performance.

Fiber-optic biosensors, characterized by their independence from reference electrodes or batteries and immunity to electromagnetic interference, are recognized as having substantial potential for detecting MPs bioactive constituents. Among these, surface plasmon resonance (SPR)-based fiber-optic sensing technology has been prioritized in recent research due to its label-free operation and ultrahigh sensitivity.

A miniaturized all-fiber SPR microfluidic chip was proposed, as illustrated in Fig. 4a[108]. Through synergistic enhancement via gold nanorods and molecular recognition by target proteins, a fully sealed microstructure was engineered, enabling *arctigenin* detection at trace levels. This work establishes a novel framework for dynamic analysis and quality control of MP bioactive compounds.

In a subsequent study, wavelength division multiplexing (WDM) technology was employed by Wei et al. to realize dual-parameter synchronous demodulation [109]. This innovation permitted the fiber-optic SPR biosensor to execute both specific berberine molecular recognition and ambient temperature monitoring. A dynamic coupling model correlating temperature and optical wavelength was developed, accompanied by a self-adaptive compensation algorithm to counteract temperature-induced interference during biomolecular measurements. Exceptional specificity detection performance in complex biomolecular matrices was experimentally validated.

However, the currently proposed fiber-optic SPR sensors for traditional Chinese medicine analysis still face critical challenges in limited response sensitivity. Coating optical fiber surfaces with nanomaterials or metal oxides has been proven effective in enhancing surface plasmon resonance coupling effects. Tang et al. developed a ring-core fiber-based SPR sensor[110]. By immobilizing protein tyrosine phosphatase-1B (PTP1B) on the gold film surface, they achieved specific detection of *isoquercitrin*, with quantitative results demonstrating significant concordance with HPLC measurements. The detection process is shown in Fig. 4b. With the continuous progress of nanotechnology and sensing technology, the introduction of new nanomaterials and the functional modification of biomolecules can provide new ideas for the research of optical fiber biosensors. With ongoing advancements in nanotechnology and biosensing, the integration of novel nanomaterials and biomolecular functional modifications is anticipated to drive innovation in fiber-optic biosensor research.

6. Conclusion and Outlook

The quality assessment of MPs necessitates comprehensive consideration of multiple factors and continuous optimization of analytical

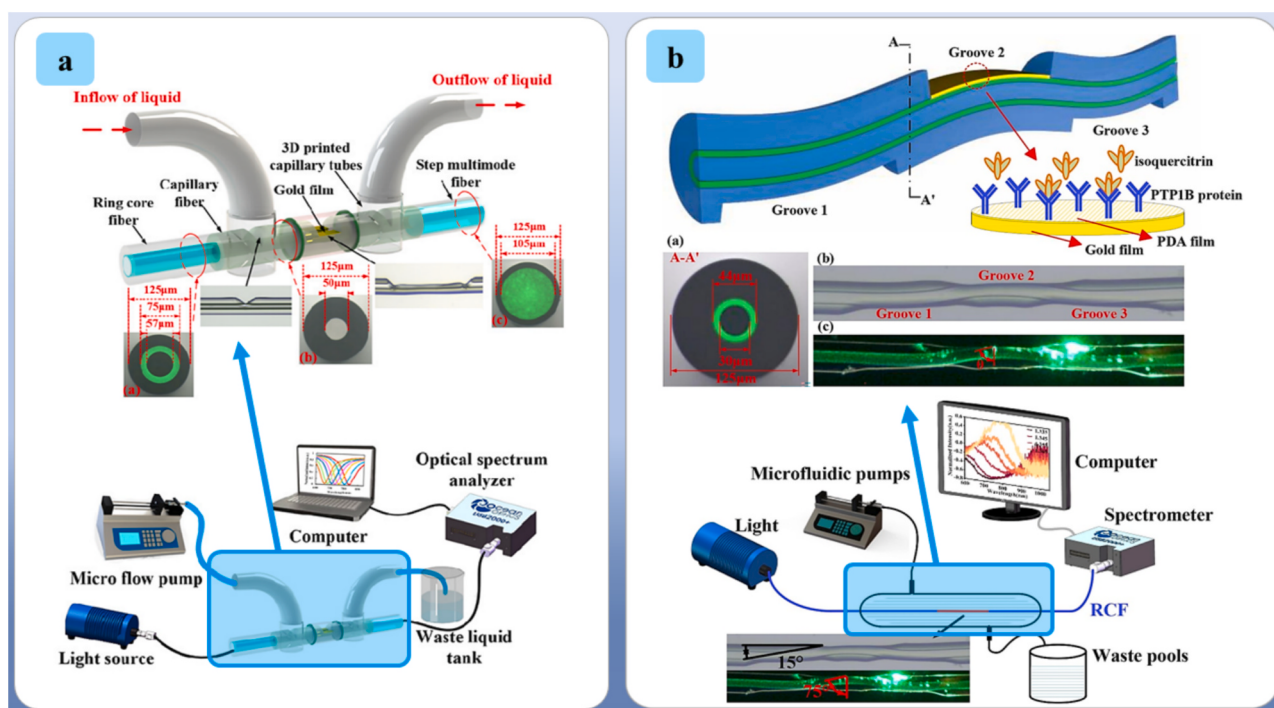


Fig. 4. Determination of a) berberine [108], b) isoquercitrin [110] by SPR sensor.

methodologies and systems. Significant discrepancies exist in quality evaluation protocols for identical MPs across different pharmacopoeias. Many compendial analytical methods exhibit limited selectivity, failing to accurately assess the quality of plant materials. Furthermore, insufficient research on the chemical composition and pharmacological activities of certain MPs in some pharmacopoeias has resulted in a lack of information regarding their primary bioactive constituents. Nevertheless, through in-depth analysis of the relationship between chemical profiles and pharmacological activities, more rational and robust quality evaluation strategies can be proposed for these botanicals.

Regarding the practicality and robustness of analytical technologies, HPLC remains the gold standard for impurity analysis and quantification due to its superior reproducibility, well-established validation criteria, and widespread adoption in quality control laboratories. High-performance thin-layer chromatography (HPTLC) and spectrophotometric methods, characterized by operational simplicity and cost-effectiveness, are suitable for routine analysis, particularly in resource-limited laboratories, though their specificity and sensitivity may be inferior to HPLC. These techniques may serve as supplementary approaches in pharmacopoeial standards.

Emerging technologies such as UHPLC-MS/MS and CE have been successfully applied to triterpenoid saponin analysis. These advanced methods demonstrate enhanced sensitivity, selectivity, and resolution for detecting trace components in complex matrices. However, their relatively high operational costs and requirement for specialized equipment and technical expertise may limit routine implementation. Despite these challenges, their potential incorporation as reference methods in pharmacopoeias warrants consideration.

The burgeoning global demand for phytopharmaceuticals and traditional medicine systems has catalyzed unprecedented advancements in analytical technologies for bioactive compound detection in MPs. This evolution is poised to transform quality control paradigms and natural product drug discovery through five strategic developmental vectors.

- (1) Technological innovation is anticipated to remain the core driver of advancements in MPs active ingredient detection. Established methodologies including high-performance HPLC, LC-MS, and NMR will continue to undergo refinement. Concurrently, emerging paradigms such as nanotechnology-enabled detection, biosensing platforms, and microfluidic systems are projected to assume critical roles in expediting MP component analysis. The convergence and modernization of these technologies are expected to enable automated, intelligent workflows, thereby elevating analytical precision and operational efficiency.
- (2) Data analytics and artificial intelligence (AI) are projected to emerge as transformative forces in detection methodologies. Through machine learning algorithms, complex phytochemical datasets will be systematically processed and interpreted, facilitating enhanced accuracy in compound identification and quantification. Furthermore, AI-driven predictive modeling of pharmacological activities may be leveraged to advance personalized therapeutics and precision drug discovery.
- (3) Multicomponent analytical technologies are poised to achieve comprehensive characterization of intricate phytochemical compositions and bioactivity profiles. While current multiplex detection systems remain constrained by selectivity and sensitivity limitations, these challenges are anticipated to be progressively resolved through sustained technological innovation.
- (4) Rapid detection and field-deployable analytical platforms are projected to significantly enhance herbal product quality assurance and supply chain oversight. The development of portable detection platforms is expected to enable real-time quality assessment, a critical advancement for ensuring therapeutic safety and efficacy.

- (5) Environmental sustainability principles will be increasingly integrated into detection technology development. Environmentally conscious analytical protocols designed to minimize chemical reagent consumption and waste generation are expected to be prioritized. Simultaneously, international standardization initiatives and collaborative frameworks will be strengthened to facilitate global trade of MPs-derived products, thereby supporting the harmonization and innovation of active ingredient detection methodologies.

These paradigm-shifting innovations collectively address critical challenges in phytopharmaceutical analysis while creating novel research trajectories. The convergence of hyperspectral imaging databases, quantum computing-enabled molecular simulations, and blockchain-enabled quality tracking systems will ultimately establish next-generation analytical ecologies for sustainable drug discovery pipelines.

CRediT authorship contribution statement

Qian Zhang: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Yuanman Yue:** Writing – original draft, Methodology, Investigation. **Xue Li:** Formal analysis, Data curation. **Chi Zhang:** Methodology, Investigation. **Yuhang Guo:** Writing – original draft. **Zi Wang:** Writing – original draft. **Jin Li:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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