Review

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Equivalence in the modernization of Chinese medicine: A multi-dimensional analysis of active components, processing methods, and clinical outcomes in formula granules

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SUMMARY: The feasibility of substituting traditional decoction pieces with Chinese herbal formula granules fundamentally hinges on the equivalence of their active components. Formula granules face complex compositional changes during manufacturing: volatile oils suffer substantial losses (retention rates below 30% in aromatic herbs), thermolabile glycosides undergo degradation during high-temperature processing, and Maillard reactions generate novel compounds whose pharmacological contributions remain unclear. Although advanced analytical technologies such as ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) can identify thousands of components, existing quality control standards still rely on 1-3 pharmacopeial markers, and fingerprint similarity criteria lack uniformity (0.80-0.95), inadequately reflecting the true quality of multi-component systems. Bioequivalence studies demonstrate that formula granules generally exhibit 60-90% performance of traditional decoctions *in vitro*, with comparable area under the curve (AUC) and Cmax values for certain components but significant discrepancies for others. Critically, high chemical similarity cannot guarantee clinical therapeutic equivalence—the logical chain from"component equivalence" to "therapeutic equivalence" remains unestablished. Clinical research is sparse, with high-quality randomized controlled trials (RCTs) representing less than 5% of studies and head-to-head comparisons particularly scarce. Future research must develop volatile component preservation technologies, establish comprehensive synergistic effect evaluation methodologies, and most importantly, conduct large-scale clinical trials. Without these efforts, the scientific credibility and international acceptance of formula granules will remain questionable.

Keywords: formula granules, traditional decoction pieces, active components, manufacturing process, component synergy

1. Introduction

As a modernized dosage form of Traditional Chinese Medicine (TCM) decoction pieces, Chinese herbal formula granules achieve clinical advantages of convenient dispensing and immediate preparation through standardized processes of aqueous extraction, concentration, drying, and granulation of individual herbs. The production of formula granules employs highly controlled processing workflows that ensure stability of active components and inter-batch consistency through precise regulation of parameters such as extraction temperature, solvent concentration, and drying time (*1*). In 2023, the market revenue for Chinese herbal formula granules reached 56.71 billion yuan, with a compound annual growth rate of 27.34% from 2012 to 2023 (*2*), and the market scale of China's formula granule industry reached 63.84 billion yuan in 2024 (*3*). However, the fundamental prerequisite for formula granules to truly substitute traditional decoction pieces lies in their equivalence, particularly quality equivalence at the level of active components. Current quality standards predominantly rely on content control of single or limited marker compounds listed in the Chinese Pharmacopoeia, but whether this simplified evaluation model can guarantee overall pharmacological equivalence of complex multi-component systems has become a critical scientific issue constraining the scientific development and international recognition of formula granules. The formula granule field still faces practical challenges including contradictions between fixed equivalence ratios and raw material variability,

relatively limited production processes, insufficient national standards with fragmented provincial standards (*3*)—all of these issues fundamentally point to one core proposition: can chemical similarity truly ensure clinical therapeutic equivalence?

From the perspective of chemical material basis, systematic differences exist in the component profiles between formula granules and traditional decoction pieces. Following aqueous extraction, the chemical composition of formula granules inevitably differs substantially from that of crude herbs and decoction pieces (*4*). Breakthroughs in modern analytical technologies have enabled precise characterization of these differences: high-resolution mass spectrometry techniques such as ultra-high-performance liquid chromatography coupled with Q-Exactive Orbitrap mass spectrometry (UHPLC-Q-Exactive Orbitrap-MS) can identify hundreds to thousands of chemical components, while metabolomics approaches can capture degradation of thermolabile components, loss of volatile oils, and newly generated constituents arising from chemical transformation processes such as Maillard reactions during formula granule preparation. Studies have demonstrated that formula granules differ from equivalent-dose traditional decoctions in antihypertensive effects, with granule preparations exhibiting efficacy similar to high-dose decoctions but superior to equivalent-dose decoctions (*5*). This phenomenon reveals the complexity of relationships between component transformation and pharmacological effects: certain components preserved during traditional decoction may be lost during granulation, while newly formed transformation products may possess unknown pharmacological contributions or synergistic effects. The Special Regulations on TCM Standards Management explicitly emphasizes (*6*) that research and formulation of formula granule standards should focus on consistency of fundamental quality attributes between formula granules and traditional decoctions, yet how to scientifically define and quantify this "consistency" currently lacks a unified methodological framework and assessment criteria.

The complexity of equivalence evaluation stems not only from the diversity of chemical components but also from the incomplete evidence chain between "component equivalence" and "therapeutic equivalence". In recent years, integrated application of multi-omics technologies has provided new insights for addressing this challenge: metabolomics enables panoramic scanning of component changes *in vitro* and *in vivo*, network pharmacology can construct "component-target-pathway" association networks, and pharmacokinetic studies reveal differences in systemic behavior between absorbed components and parent compounds. Advanced analytical technologies such as UHPLC-Q-Exactive Orbitrap-MS, with unparalleled resolution and mass accuracy, can precisely identify and quantify complex TCM components and their metabolites (*7*). However, current research still faces critical knowledge gaps: the pharmacological contributions of formula granule-specific transformation products remain unclear, mechanisms of multi-component synergistic actions lack systematic elucidation, and *in vitro-in vivo* correlations (IVIVC) are difficult to establish. Furthermore, the heterogeneity of raw material quality in traditional decoction pieces, variations in processing parameters across different manufacturers, and individualized differences in decoction methods all make it challenging to standardize benchmarks for equivalence comparative studies (*8*). Therefore, this review focuses on the core scientific question of active component equivalence between formula granules and traditional decoction pieces, systematically summarizing recent research data on chemical equivalence and bioequivalence evaluation methods, analyzing methodological limitations and cognitive bottlenecks in current research, and prospecting future directions for establishing multi-dimensional equivalence evaluation systems, with the aim of providing theoretical support for the scientization and internationalization of quality standards for formula granules (Figure 1).

2. Evaluation methods and research status of chemical component equivalence

2.1. Application of analytical technologies

2.1.1. Quantitative comparison of marker components

As of January 2024, national standards for Chinese herbal formula granules in China have been established for over 300 species (*1*), with quality standards for each species relying on content control of specific marker components. In recent years, extensive quantitative comparative studies on marker components between formula granules and traditional decoction pieces have been conducted, primarily focusing on content determination of characteristic constituents such as flavonoids, saponins, and alkaloids listed in the Chinese Pharmacopoeia. These studies typically employ high-performance liquid chromatography (HPLC) or ultra-high-performance liquid chromatography (UPLC) to measure absolute contents of single or multiple marker components through establishment of standard curves, and calculate transfer rates of formula granules relative to decoction piece decoctions (*9*). The quantitative analysis of multi-components by single marker (QAMS) method has been widely applied in TCM quality control, which not only addresses challenges of obtaining reference standards or their high costs but also substantially reduces detection costs and time. However, this evaluation approach based on single or limited marker components possesses inherent deficiencies: inadequate representativeness constitutes its core problem. As complex multi-component systems, the pharmacological effects of Chinese herbal formula granules often result from synergistic actions of dozens or

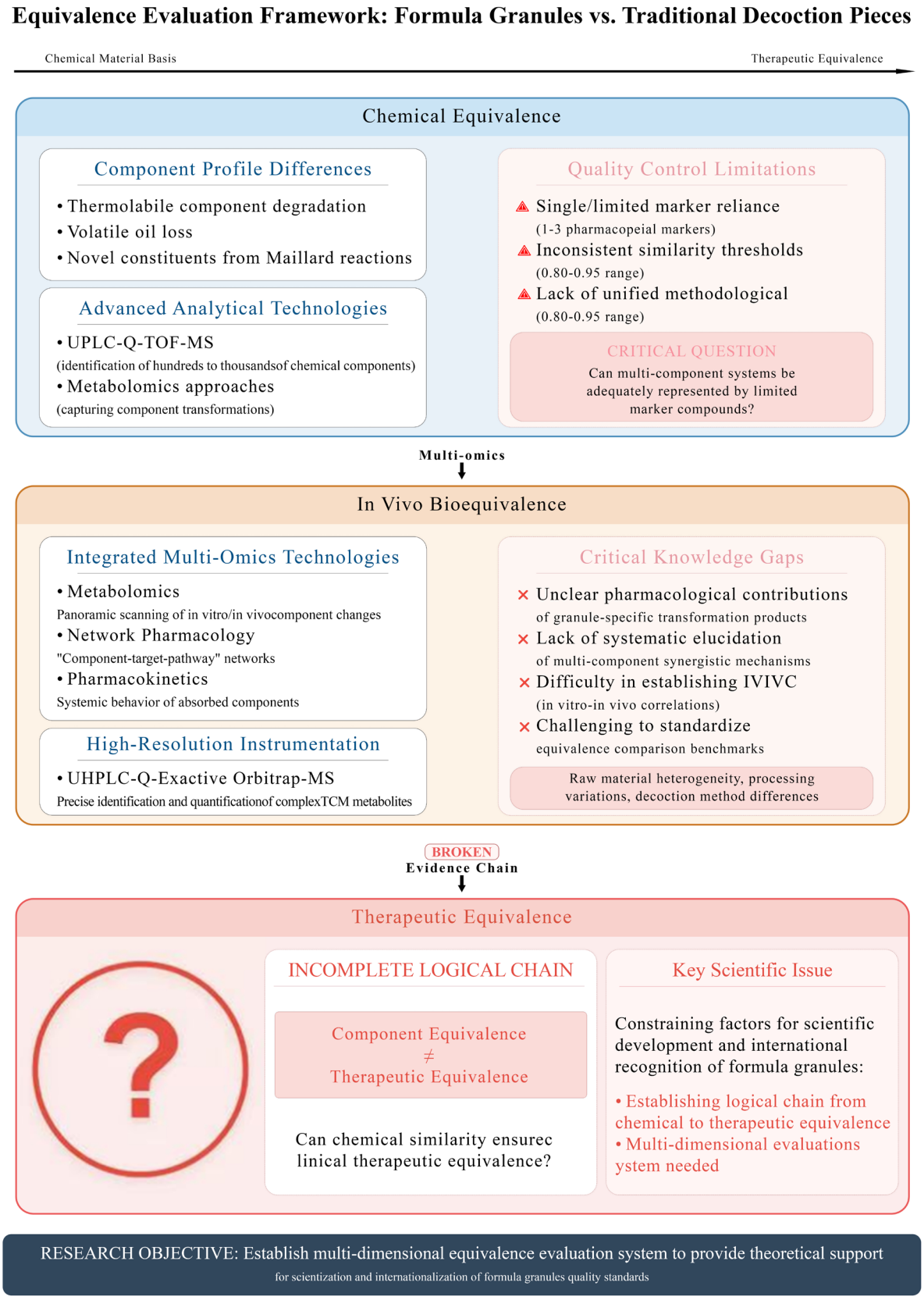

Figure 1. Multi-dimensional research framework and critical knowledge gaps in establishing equivalence between Chinese herbal formula granules and traditional decoctions. The framework illustrates three progressive levels of equivalence assessment: chemical component profile differences addressed by advanced analytical technologies, *in vivo* bioequivalence evaluated through multi-omics approaches, and therapeutic equivalence validated by clinical outcomes. Red boxes highlight current quality control limitations and critical knowledge gaps at each level, emphasizing the incomplete logical chain from component equivalence to therapeutic equivalence.

herbs, decoction pieces, and formula granule production processes, which, while ensuring consistency of industry quality standards, substantially increase production costs of formula granules (*10*). Although such stringent marker component control enhances product stability, it neglects potential pharmacological contributions of non-marker components, potentially leading to risks of "compliant components but inequivalent efficacy".

2.1.2. Holistic chemical fingerprinting

To overcome limitations of single marker component evaluation, chemical fingerprinting technology has become an important tool for equivalence assessment of formula granules. With advances in TCM quality control technologies, combined application of chemical fingerprints and physical fingerprints has become a trend, enabling comprehensive evaluation of formula granule quality from both chemical composition and physical property dimensions (*11*). This technology records the holistic chromatographic profile of samples through multi-wavelength detection (diode array detector (DAD), evaporative light scattering detector (ELSD), *etc*.), generating a "chemical identity card" containing dozens of characteristic peaks, and then employs similarity evaluation algorithms (such as cosine angle method and correlation coefficient method) to calculate the degree of similarity between fingerprints of formula granules and traditional decoction pieces. HPLC-DAD technology, through diode array detectors, can simultaneously acquire multi-wavelength chromatographic data, significantly improving detection sensitivity and accuracy for trace components compared to single-wavelength detection (*12*). Similarity and hierarchical cluster analysis have been applied to identify Xiao Chai Hu granules produced by different manufacturers, with peak pattern matching used to evaluate preparation processes (*13*). Fingerprint similarity evaluation is widely applied in formula granule quality control.

For instance, Chen Shengjun *et al*. demonstrated that similarities of different processed products of Dahuang (Rhubarb) formula granules were 0.85, 0.90, and 0.90 respectively (*14*); Wang Yingjun *et al*. showed fingerprint similarities ranging from 0.824 to 0.989 in studies of 35 batches of Danggui (Angelica sinensis) samples (*15*). However, similarity threshold settings lack unified standards: different species in national standards adopt 0.85 or 0.90 as acceptance criteria (*14*), while academic research also employs various thresholds such as 0.80 and 0.95 (*16*), and this inconsistency in standards undermines comparability of evaluation results. Although the fingerprinting method has been approved by WHO, US FDA, European Medicines Agency, and Chin's National Medical Products Administration, it has limitations in evaluating TCM quality, as characteristic common peaks may not reflect representative information of TCM quality (*17*).

2.1.3. Untargeted metabolomics for comprehensive component profiling

The introduction of untargeted metabolomics technology has brought revolutionary breakthroughs to chemical equivalence evaluation of formula granules. Metabolomics analytical methods based on UPLC-Q-TOF-MS/MS have been established to evaluate chemical consistency before and after compatibility of Zhenwu Decoction (*5*). This technology, without presetting target compounds, can identify hundreds to thousands of chemical components in a single analysis, precisely determine molecular formulas through high-resolution mass spectrometry (resolution exceeding 50,000), and infer compound structures by combining secondary mass spectral fragmentation information. UHPLC-Q-TOF-MS has been established as a powerful and reliable analytical technology due to its exceptional sensitivity, superior resolution, and accurate mass measurement, holding significant importance in analyzing complex compounds in TCM formulae (*18*).

At the data processing level, principal component analysis (PCA) can intuitively display clustering relationships among samples, orthogonal partial least squares- discriminant analysis (OPLS-DA) can screen chemical markers with significant differences, and S-plots are used to locate key components causing differences. Chemical differences have been identified between traditional decoctions and formula granule decoctions of Taohong Siwu Decoction, with paeoniflorin, albiflorin, gallic acid, amygdalin, and safflor yellow A identified as components undergoing significant changes during decoction (*19*). The formula granule preparation process involves high-temperature concentration and drying procedures, which theoretically may induce Maillard reactions (non-enzymatic browning between sugars and amino acids), generating new components such as pyrazines and furans (*13*), while certain volatile components and thermolabile glycosides may degrade or dissipate. However, systematic identification of formula granule-specific components and quantitative comparative studies with traditional decoction pieces remain insufficient, requiring further in-depth investigation using modern analytical technologies such as UPLC-Q-TOF-MS (*20*). These findings provide molecular-level evidence for understanding chemical differences between formula granules and decoction pieces, but simultaneously raise new scientific questions: what are the contributions of these differential components to overall pharmacological effects?

2.2. Component changes induced by processing

2.2.1. Loss of thermolabile components

High-temperature treatment during formula granule preparation (extraction temperatures typically 60-100°C,

concentration and drying temperatures up to 80-120°C) inevitably leads to degradation or dissipation of certain thermolabile components. Volatile oil constituents represent the most severely affected category. Thermal degradation of certain phytochemicals in specific aromatic crops may result in reduced levels of key aroma substances or formation of new volatile compounds, potentially negatively impacting sensory characteristics of final products (*21*).

During traditional decoction piece decoction, volatile oils partially dissipate with water vapor but a considerable proportion remains in the decoction, whereas during concentration and drying stages of formula granules, volatile oils are almost completely lost. Taking aromatic herbs such as Bohe (Mentha) and Huoxiang (Pogostemon) as examples, retention rates of volatile components like menthol and patchouli alcohol in formula granules are generally below 30%. Although steam distillation technology is faster and suitable for large-scale production, higher temperatures may lead to degradation of thermosensitive compounds (*22*). Thermal stability of alkaloid components varies significantly: tropane alkaloids (such as atropine and scopolamine) undergo significant degradation above 270°C, as tropane alkaloids atropine and scopolamine possess thermal instability and may be overlooked due to degradation at high temperatures (*23*); whereas berberine-type alkaloids are relatively stable. Heat treatment leads to increased levels of pyrrolizidine alkaloids while simultaneously decreasing their N-oxide forms, indicating concurrent transformation (*24*). Glycoside components such as ginsenosides and flavonoid glycosides may undergo hydrolysis reactions under prolonged heating conditions, generating corresponding aglycones. Studies have demonstrated that rare ginsenosides and aglycones generated from hydrolysis of orally administered ginsenosides in the intestine represent the primary forms absorbed into blood and exerting pharmacological effects (*25*), with intestinal microbiota-mediated stepwise deglycosylation reactions playing important roles in their *in vivo* metabolism and pharmacokinetic behavior. During formula granule preparation, retention of different component types exhibits significant differences: volatile oil components are lost substantially due to high-temperature treatment, alkaloid components are relatively stable but certain thermosensitive structures may degrade, and glycoside components may be accompanied by generation of hydrolysis products — all these changes may influence final pharmacological performance (*13*).

2.2.2. Generation of new components

Formula granule preparation is not only a process of component loss but also one of new component generation. The Maillard reaction represents one of the most important chemical transformation pathways, occurring between reducing sugars and amino acids, proteins, or other nitrogen-containing compounds, generating a series of brown pigments and flavor substances under heating conditions (*26*). During the concentration and drying stage of formula granules (80-120°C), Maillard reactions occur significantly, with generated products including pyrazines (such as 2,5-dimethylpyrazine and 2,3,5-trimethylpyrazine), furans (such as 5-hydroxymethylfu rfural, 5-HMF), pyrroles, and other heterocyclic compounds (*26,27*).

Studies have shown that 11 volatile Maillard reaction products were identified during steaming of Heshouwu (Polygonum multiflorum), including 4 furanones, 2 furans, 2 nitrogen-containing compounds, 1 pyran, 1 alcohol, and 1 sulfur compound. These newly generated components significantly enhanced DPPH free radical scavenging activity of the processed product (processed Heshouwu) compared to the raw material, with IC_{50} values decreasing from 2.9 mg/mL to 0.43 mg/mL (*27*). During the steaming process, DDMP (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran- 4-one) and 5-HMF gradually form, while 16 amino acids (especially lysine and arginine) undergo significant consumption (*28*). These newly generated components possess characteristic roasted and nutty aromas, and some compounds have been demonstrated to possess biological activities including antioxidant, anti-inflammatory, hepatoprote ctive, and immunomodulatory effects, though they may also contain trace amounts of potentially harmful components such as acrylamide and advanced glycation end products (*26,29*).

Glycation products and oxidation products constitute two other important categories of newly generated components. Under high-sugar environments and heating conditions, certain polyphenolic and flavonoid compounds can undergo non-enzymatic glycation reactions with sugars, forming new glycation derivatives. These melanoidins possess high molecular weights and complex structures, potentially improving solubility and stability of active components (*29,30*). Unsaturated fatty acids and terpenoid compounds readily undergo autoxidation under heating and air exposure conditions, generating degradation products such as peroxides, aldehydes (*e.g.*, hexanal, heptanal), and ketones. These lipid oxidation products (LOPs) increase significantly after heating at 200°C, potentially leading to quality loss and reduced pharmacological properties (*31*).

The critical question lies in: the pharmacological effects of newly generated components remain unclear. Existing research mostly remains at the component identification level, lacking systematic pharmacodynamic evaluation of these transformation products. Do newly generated components compensate for pharmacological effects of partially lost components, or do they introduce potential safety risks? For example, 5-HMF, as a common Maillard reaction product, although possessing antioxidant activity in steamed Heshouwu products (*27*),

may also produce cytotoxicity and neurotoxicity at high doses (*26*). Answers to these questions are crucial for scientifically evaluating equivalence between formula granules and traditional decoction pieces (Figure 2).

2.3. Controversies in chemical equivalence assessment criteria

Formulation of chemical equivalence assessment criteria for formula granules represents a controversial scientific issue, with core disagreements concentrated in three dimensions. First, single marker *vs.* multi-marker control. Traditional quality standards tend to select 1-3 pharmacopoeia-listed marker components for content control; this simplified approach is convenient to implement but scientifically insufficient. According to Academician Zhang Boli's 2024 Two Sessions proposal, inconsistent provincial review standards result in different standards for the same species across provinces, including variations in content and specifications, and recommended abolishing provincial-level standard formulation for formula granules to avoid duplicate research and waste of social resources (*32,33*). Multi-marker control can more comprehensively reflect chemical composition, but marker number selection (5, 10, or 20?), weight allocation, and acceptable range settings all lack recognized scientific foundations. The Special Regulations on TCM Standards Management explicitly emphasizes that research and formulation of formula granule standards should focus on consistency of fundamental quality attributes between formula granules and traditional decoctions (*32*), but how to scientifically define and quantify this "consistency" currently lacks a unified methodological framework. Second, content equivalence *vs.* ratio equivalence. Content equivalence requires that absolute contents of marker components in formula granules approximate those in decoction piece decoctions (typically allowing 80-120% deviation), but this ignores quality fluctuations in raw decoction piece materials across different batches. Ratio equivalence emphasizes that relative proportional relationships among components within formula granules should be consistent with decoction pieces; this approach better aligns with TCM's "holistic" philosophy but presents greater technical implementation challenges. National standards adopt a clinical application-oriented approach examining formula granules as an integrated whole, emphasizing water-soluble properties consistent with decoctions (*34,35*), which is relatively comprehensive and rational. However, current research predominantly employs content equivalence standards, while evaluation methodologies for ratio equivalence remain immature, and differences in enterprise production processes and equipment, along with lack of reasonable evaluation standards, pose challenges for product consistency evaluation (*34*). Third, significant domestic and international standard differences impact the internationalization process of formula granules. Chinese standards emphasize marker component content control combined with fingerprint similarity evaluation, establishing quantity transmission data tables and characteristic spectrum control indicators based on "standard decoctions" as benchmarks (*35*). Japanese Kampo preparation standards focus on overall quality consistency: the 17th edition of the Japanese Pharmacopoeia includes 176 crude drugs and 35 Kampo extracts, with official definitions of sources, descriptions, limits, and detection methods (*36*), achieving quality control through standardized extraction processes (over 95% being extract preparations) (*37*). The European Pharmacopoeia requires detailed phytochemical data and biological activity data, with all limits formulated by referencing multiple national pharmacopoeias (Chinese Pharmacopoeia, British Pharmacopoeia, European Pharmacopoeia, Japanese Pharmacopoeia, and United States Pharmacopeia) (*36*). This lack of standardization uniformity results in the same formula granule species facing different entry thresholds in different countries/regions, constraining international circulation of products and academic exchange. Establishing scientific, unified, and internationally recognized chemical equivalence assessment criteria requires integration of multidimensional evidence from chemical analysis, pharmacodynamic validation, and clinical evaluation—a direction urgently requiring breakthroughs in current research.

3. Methods and research progress in bioequivalence evaluation

3.1. *In vitro* biological activity comparison

3.1.1. Antioxidant activity testing

Antioxidant activity serves as one of the fundamental indicators for evaluating the bioequivalence between formula granules and traditional decoction pieces. Commonly employed methods include the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and the ABTS [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] free radical scavenging assay. The DPPH method, owing to its rapidity, simplicity, and cost-effectiveness, has been widely applied to assess the potential of substances as hydrogen donors or free radical scavengers (*38*). This method is based on the principle that DPPH free radicals exhibit purple absorption at 517 nm wavelength, which undergoes decolorization upon reaction with antioxidant substances. The free radical scavenging rate is calculated by measuring changes in absorbance (*39*).

Antioxidant activity values determined by different methods exhibit variations: DPPH and RP methods yield higher antioxidant activity values compared to ABTS and FRAP methods. Consequently, methodological

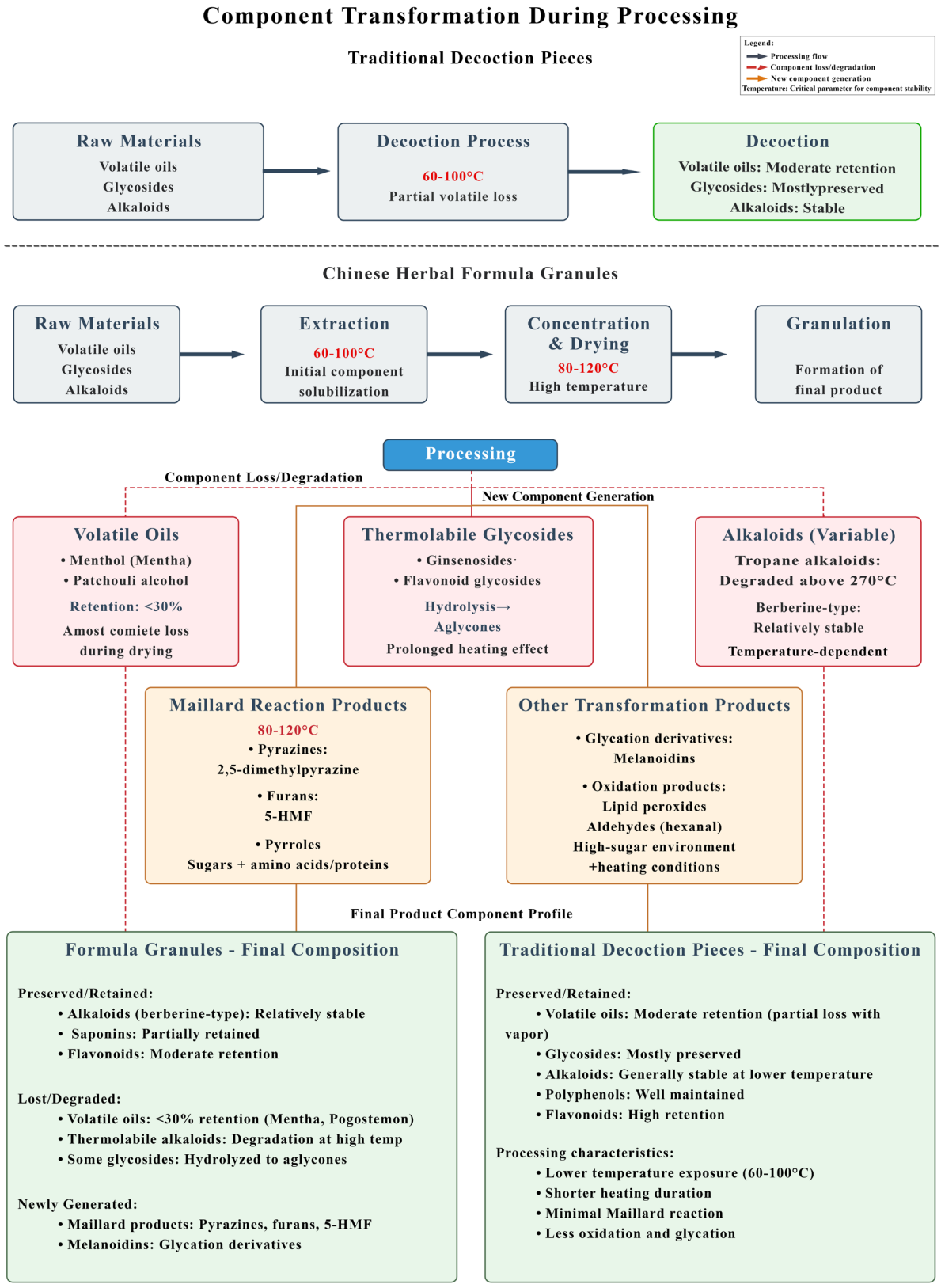

Figure 2. Component transformation pathways during the processing of traditional decoction pieces and formula granules, and their impact on final product composition. The diagram illustrates how thermal processing conditions (60-100°C for extraction, 80-120°C for concentration and drying) induce differential component changes in both preparation methods. Key transformation mechanisms include: (1) volatile oil partial loss and component degradation, (2) thermolabile glycoside hydrolysis and aglycone formation, (3) alkaloid temperature-dependent degradation, (4) Maillard reaction products generation from reducing sugars and amino acids/proteins (80-120°C), and (5) oxidation-induced derivative formation. The comparison reveals that formula granules experience higher temperature exposure during concentration and spray drying, leading to more pronounced component losses and transformation compared to traditional decoctions, ultimately resulting in compositional differences between the two preparation forms.

consistency must be maintained when comparing formula granules with decoction pieces (*40*). DPPH measurements correlate only with other methods, suggesting that due to different reaction mechanisms, it reflects distinct antioxidant properties. Total antioxidant capacity (T-AOC) determination evaluates the comprehensive antioxidant potential of samples through methods such as FRAP (ferric reducing antioxidant power) (*41*). Recent comparative data on antioxidant activity between formula granules and decoction pieces demonstrate that the DPPH scavenging capacity of most formula granules ranges from 70-95% of traditional decoction pieces, though significant variations exist among different varieties. For instance, formula granules of species rich in polyphenolic components, such as Salvia miltiorrhiza and Scutellaria baicalensis, retain high antioxidant activity (> 85%), whereas those containing volatile oil components, such as Mentha haplocalyx and Pogostemon cablin, show significant reduction (< 60%) (*41*). This variability suggests that antioxidant activity cannot serve as the sole criterion for equivalence evaluation and must be comprehensively assessed in conjunction with the chemical characteristics of specific varieties.

3.1.2. Cell model validation

Cell model-based biological activity validation provides more direct functional evidence for equivalence evaluation of formula granules. Anti-inflammatory activity evaluation commonly employs lipopolysaccharide (LPS)-induced RAW264.7 macrophage inflammation models, assessing anti-inflammatory effects by detecting the release of inflammatory factors such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), as well as the phosphorylation levels of key proteins in the nuclear factor-κB (NF-κB) signaling pathway (*42*). Polyphyllin can block the NF-κB signaling pathway in bone marrow-derived macrophages, inhibiting the phosphorylation of IKKα/β and p65, and significantly reducing the production of key pro-inflammatory cytokines IL-1β, TNF-α, and IL-6. Oral administration of Yangqing Chenfei Formula can alleviate pathological changes, reduce inflammatory cell infiltration, inhibit collagen deposition, and decrease inflammatory factor levels. This combined *in vitro* and *in vivo* research model provides a paradigm for equivalence evaluation of formula granules (*43*). Protective effect evaluation involves multiple target cell models: HepG2 hepatocytes for assessing hepatoprotective activity (by detecting ALT and AST release and cell viability), PC12 neuronal cells for evaluating neuroprotective effects (by detecting apoptosis rate and axonal growth), and H9c2 cardiomyocytes for assessing cardioprotective effects (*44*). Recent research cases indicate that the protective effects of formula granules in cell models are generally slightly lower than equivalent doses of traditional decoction liquids (activity retention 60-90%), but the regulatory trends of key signaling pathways remain consistent. For example, both Salvia miltiorrhiza formula granules and decoction pieces exert their effects by activating the Nrf2/HO-1 pathway when inhibiting oxidative damage in H9c2 cardiomyocytes. Although the EC50 value of formula granules is slightly higher (1.2-1.5 times), the shape of the dose-response curve is similar, suggesting equivalence in the mechanism of action between the two (*45*).

3.1.3. *In vitro* dissolution/release studies

In vitro dissolution/release studies draw upon classic methods from chemical drug bioequivalence evaluation, though their application in the field of Chinese medicine formula granules remains in the exploratory stage. Dissolution curve comparison methods typically employ the paddle method (Chinese Pharmacopoeia general rule 0931) or basket method, measuring the cumulative dissolution percentage of indicator components in various media such as simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8), and plotting time-dissolution curves. The similarity factor (f2) calculation is an FDA-recommended method for comparing dissolution curves, with the formula: $f2 = 50\times\log\{[1+(1/n)\Sigma(Rt-Tt)^2]^{(-0.5)}\times100\}$, where Rt and Tt represent the average dissolution at time t for the reference and test formulations respectively, and n is the number of sampling points. When the f2 value falls between 50-100, the two dissolution curves are considered similar (*46*). However, dissolution studies of formula granules face particular challenges: traditional decoction liquids are in liquid form and can be directly absorbed without dissolution, whereas formula granules require steps such as dissolution upon administration and gastrointestinal disintegration. Therefore, the f2 similarity factor is more suitable for evaluating quality consistency among different batches of formula granules rather than determining equivalence between formula granules and decoction pieces. The lack of established *in vitro-in vivo* correlation (IVIVC) represents a major limitation of current research (*47*). Mature IVIVC models (A/B/C level correlations) in the chemical drug field require clear quantitative relationships between pharmacokinetic parameters (such as AUC, Cmax) and *in vitro* dissolution parameters. However, the multi-component nature of Chinese medicine formula granules makes it difficult for IVIVC of a single component to represent overall efficacy, while establishing multi-component combined IVIVC models faces tremendous technical challenges and data requirements.

3.2. *In vivo* pharmacokinetic equivalence

3.2.1. Blood-entering component analysis

Blood-entering component analysis serves as a critical bridge connecting chemical equivalence and bioequivalence. Liquid chromatography-tandem mass

spectrometry (LC-MS/MS) detection technology for plasma samples has become the mainstream method. Through ultra-high performance liquid chromatography-triple quadrupole mass spectrometry (UPLC-QqQ-MS/MS) in multiple reaction monitoring (MRM) mode, highly sensitive quantification of trace prototype components and metabolites in plasma can be achieved (detection limits reaching pg/mL level) (*48,49*). UPLC-Q-TOF-MS can be combined with exogenous metabolomics for qualitative analysis of prototype components and metabolites, making the data analysis process more accurate. Identification of prototype components and metabolites requires integration of *in vitro* metabolic stability experiments and *in vivo* metabolite identification (*50,51*). After oral administration, significant differences exist in the blood-entering component profiles between formula granules and decoction pieces: some macromolecular polysaccharides and tannins with high content in decoction liquids are difficult to enter the bloodstream due to poor absorption. Components that truly enter blood circulation and exert pharmacological effects are often small-molecule glycosides, alkaloids, flavonoids, and their metabolites. Comparison of pharmacokinetic parameters for typical components focuses on key indicators such as maximum plasma concentration (Cmax), time to peak (Tmax), and area under the concentration-time curve (AUC). Recent summarized research data show: ginsenoside Rg1 after administration of ginseng formula granules has a Cmax of 85-110% of the decoction group, AUC of 90-115%, with no significant difference in Tmax (*52*); salvianolic acid B in *Salvia miltiorrhiza* formula granules has a Cmax of 70-95% of the decoction group, but the AUC ratio can reach 85-105%, suggesting slightly slower absorption rate but similar total absorption (*53*); baicalin bioavailability in *Scutellaria baicalensis* formula granules is generally higher than decoction pieces (F = 110-130%), possibly related to glycoside hydrolysis to aglycone during formula granule preparation, with the latter showing better absorption. These data reveal that pharmacokinetic equivalence of a single component does not equate to overall pharmacological equivalence, requiring comprehensive consideration of multi-component synergistic pharmacokinetic characteristics.

3.2.2. Tissue distribution studies

Tissue distribution studies reveal the targeting and accumulation characteristics of active components *in vivo*. By collecting major organ tissues such as heart, liver, spleen, lung, kidney, and brain at different time points after administration, and measuring indicator component content using LC-MS/MS, tissue concentration-time curves are plotted to calculate differences in target organ accumulation (*54*). Representative studies show: ginsenoside components (such as Rg1, Rb1, Re) distribute at higher concentrations in the heart and spleen, suggesting their cardiovascular protective and immunomodulatory effects may be related to target organ accumulation; the tissue distribution pattern of formula granule groups is essentially consistent with decoction piece groups, but peak concentrations (Cmax, tissue) are generally reduced by 10-25% (*55*). Salvianolic acid components (salvianolic acid A, salvianolic acid B) are highly enriched in the liver, with liver AUC of formula granules at 80-95% of the decoction group, supporting the equivalence of hepatoprotective activity (*56*). Flavonoid components (such as quercetin, kaempferol) have relatively limited tissue distribution due to high plasma protein binding rates, but selective accumulation in inflammatory tissues can be observed. Notably, differences in brain tissue distribution between formula granules and decoction pieces warrant attention: some components with neuroprotective effects (such as ginsenoside Rg1) need to penetrate the blood-brain barrier, and transformation products generated during formula granule preparation may affect their brain delivery efficiency (*55*). Additionally, biotransformation of Chinese medicine components by gut microbiota (such as ginsenoside → rare ginsenoside, flavonoid glycoside → flavonoid aglycone) also affects tissue distribution patterns. Differences in intestinal residence time and degree of contact with microbiota between formula granules and decoction pieces may lead to changes in transformation product profiles, thereby affecting target organ exposure.

3.2.3. Pharmacokinetic equivalence determination

Bioequivalence (BE) evaluation standards are well-established in the chemical drug field. Bioavailability is typically assessed by measuring the area under the plasma concentration-time curve (AUC), which is the most reliable measurement of drug bioavailability. Bioavailability refers to the extent to which the active form of a drug reaches systemic circulation unchanged, with this definition assuming that 100% of the active drug entering systemic circulation can successfully reach the target site (*57,58*). Both Food and Drug Administration (FDA) and National Medical Products Administration (NMPA) guidelines require that the 90% confidence interval (CI) of AUC and Cmax for two formulations must fall within the equivalence boundary range of 80.00-125.00% to be determined as bioequivalent. However, application in formula granule research faces numerous difficulties. First, Chinese medicine formula granules are complex multi-component systems—which component pharmacokinetic parameters should serve as the basis for equivalence determination? Current research predominantly selects indicator components included in the pharmacopoeia, but whether these components represent the true pharmacodynamic material basis remains controversial. Second, even with selected indicator components, pharmacokinetic characteristics vary tremendously among different components: some components (such as alkaloids, saponins) can be

directly absorbed into blood (*59*), while others (such as polysaccharides, tannins) can only exert effects after gut microbiota metabolism—how should these be comprehensively evaluated? Third, reference standards between formula granules and traditional decoction pieces are not uniform: pharmacokinetic parameters of decoction pieces from different origins and under different decoction conditions exhibit considerable variation themselves — which decoction pieces should serve as the reference? Challenge: the complexity of equivalence determination in multi-component systems is the fundamental reason restricting the in-depth development of pharmacokinetic equivalence research. The ideal solution is to establish a comprehensive evaluation system based on "pharmacodynamic material basis groups": first screening 5-10 core components highly correlated with efficacy through spectrum-effect relationship studies, then measuring the pharmacokinetic parameters of these components separately, and calculating a comprehensive bioequivalence index using a weighted scoring method (assigning weights according to each component's contribution to efficacy). However, implementation of this method requires substantial preliminary research accumulation and has only been preliminarily explored in a few bulk formula granule varieties (such as ginseng, *Salvia miltiorrhiza*, and *Astragalus*).

3.3. Spectrum-effect relationship studies

3.3.1. Correlation analysis between chemical components and pharmacological effects

Spectrum-effect relationship studies aim to establish quantitative relationships between the chemical component spectrum of formula granules and pharmacological effects, identifying key components for equivalence. Grey relational analysis (GRA) is a commonly used mathematical tool that identifies components with the greatest contribution by calculating grey relational coefficients between chromatographic peak areas of chemical components and pharmacological indicators (such as antioxidant activity, cell protection rate) (*60,61*). The advantage of this method lies in its lack of requirement for large sample sizes, making it suitable for scenarios in Chinese medicine research with limited samples. Partial least squares regression (PLSR) is a more powerful multivariate statistical method that can simultaneously process multiple independent variables (chemical component concentrations) and dependent variables (pharmacological indicators) to establish predictive models (*62,63*). Network pharmacology analysis shows that 210 potential action targets of Qingjin Yiqi Granules for anti-fatigue are mainly enriched in signaling pathways such as PI3K-AKT, MAPK, HIF-1, and FoxO. In formula granule spectrum-effect relationship studies, the typical workflow includes: (1) preparing formula granule samples of different batches or different extraction processes ($n \geq 15$); (2) obtaining chemical fingerprints using HPLC or UPLC-MS, calibrating and quantifying major chromatographic peaks; (3) conducting *in vitro* or *in vivo* pharmacological evaluations to obtain quantitative pharmacological data; (4) establishing correlation models between chemical components and pharmacological effects using GRA or PLSR, calculating correlation coefficients or regression coefficients for each component, and identifying key pharmacodynamic components (*64,65*). Recent research cases show: the immune-enhancing effect of *Astragalus* formula granules has the highest correlation with astragaloside IV and calycosin glycoside ($r > 0.85$), while the contribution of polysaccharide components is relatively small; the antiplatelet aggregation effect of *Salvia miltiorrhiza* formula granules is mainly contributed by salvianolic acid B (PLSR coefficient 0.72), while the contribution of tanshinones is limited (coefficient 0.15) (*66*). These findings provide data support for identifying key components of equivalence: in equivalence evaluation, priority should be given to ensuring content consistency of highly correlated components rather than simply requiring proportional retention of all components.

3.3.2. Network pharmacology-assisted prediction

Network pharmacology provides a holistic "component-target-pathway" perspective for equivalence evaluation of formula granules by integrating cheminformatics, bioinformatics, and systems biology. The standard procedure for "component-target-pathway" network construction includes: (1) retrieving chemical components of formula granules through databases such as TCMSP, HERB, and PubChem, setting oral bioavailability (OB $\geq$ 30%) and drug-likeness (DL $\geq$ 0.18) to screen active components (*67*); (2) predicting action targets of active components using tools such as SwissTargetPrediction, SEA, and STITCH (*68*); (3) obtaining relevant disease targets from disease databases such as GeneCards, OMIM, and DisGeNet (*68*); (4) taking the intersection of drug targets and disease targets to construct a protein-protein interaction (PPI) network (*69*); (5) visualizing the network using Cytoscape software, calculating topological parameters such as node degree and betweenness centrality to identify core targets (*70*); (6) performing Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis through platforms such as DAVID and Metascape (*71*). Network pharmacology combined with spectrum-effect relationship analysis shows that the main anti-inflammatory substances of Wuwei Ganlu are flavonoids, which exert anti-inflammatory effects by inhibiting NLRP3 inflammasome activation (*72,73*). Virtual screening of key active components employs molecular docking technology to assess the binding affinity between chemical components and core target proteins. Binding energy < -5.0 kcal/mol is generally considered to have good binding activity, while <

-7.0 kcal/mol suggests strong binding (*74*). Validation experimental design is an indispensable component of network pharmacology research: it is necessary to confirm the authenticity of the predicted "component-target-pathway" relationships through *in vitro* enzyme inhibition experiments, cellular signaling pathway detection (Western blot detection of key protein phosphorylation), and animal model pharmacological validation (*75*). In formula granule equivalence research, the value of network pharmacology lies in: even if differences exist in the chemical component spectra between formula granules and decoction pieces, pharmacological equivalence may still be achieved as long as both regulate the same core targets and key pathways (*76*). This provides theoretical basis for establishing more scientific and systematic equivalence evaluation standards.

4. Future development directions and prospects

The future development of formula granules should focus on the synergistic advancement of technological innovation, standard refinement, and clinical translation. In terms of technology, novel extraction and stabilization approaches — such as ultrasound-microwave synergistic extraction, supercritical CO_2 extraction, nano-lipid carriers, and cyclodextrin inclusion complexes— can significantly improve the retention of volatile and thermolabile components. Moreover, the integration of AI-driven quality control models offers a transformative opportunity. Machine learning algorithms can analyze high-dimensional chemical fingerprint, metabolomic, and pharmacodynamic data to establish predictive correlations between process parameters and key quality attributes, enabling real-time monitoring and adaptive optimization of manufacturing processes. Intelligent systems based on the Internet of Things and AI can achieve precise control of critical parameters (error < 1%), fundamentally enhancing inter-batch consistency and product stability.

From a regulatory and industrial perspective, the internationalization of formula granules requires accelerated participation in international standard development and technical alignment with major pharmacopoeias. Blockchain technology could be leveraged to establish transparent, tamper-proof traceability systems for the entire supply chain — from raw material sourcing to finished product distribution — ensuring data integrity and building international trust. Clinical translation must prioritize large-sample, head-to-head randomized controlled trials (RCTs) comparing formula granules with traditional decoctions, supported by real-world evidence. Furthermore, personalized medication strategies based on genetic polymorphisms and the development of patient-centric dosage forms (*e.g.*, instant-dissolving granules) should be explored to improve therapeutic outcomes and adherence. Ultimately, through coordinated progress in science, standards, clinical validation, and industrial upgrading, formula granules can evolve from an "alternative" to a "preferred" modern TCM dosage form, offering globally accessible, quality-assured therapeutic options.

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