Chinese Journal of Natural Medicines

Composition analysis of compound Shenhua Tablet, a seven-herb Chinese medicine for IgA nephropathy: evaluation of analyte-capacity of the assays

Haiyan ZHANG, Qiuyue WANG, Jianan WANG, Sichao ZHANG, Weiwei JIA, Ning HE, Xiaoyan XIA, Ting WANG, Liyu LAI, Jiaying LI, Jing DU, Olajide E. OLALEYE, Xiangmei CHEN, Junling YANG, Chuan LI

Citation: Haiyan ZHANG, Qiuyue WANG, Jianan WANG, Sichao ZHANG, Weiwei JIA, Ning HE, Xiaoyan XIA, Ting WANG, Liyu LAI, Jiaying LI, Jing DU, Olajide E. OLALEYE, Xiangmei CHEN, Junling YANG, Chuan LI, Composition analysis of compound Shenhua Tablet, a seven-herb Chinese medicine for IgA nephropathy: evaluation of analyte-capacity of the assays, Chinese Journal of Natural Medicines, 2024, 22(2), 1-15. doi: 10.1016/S1875-5364(24)60553-4.

View online: https://doi.org/10.1016/S1875-5364(24)60553-4

Wechat

Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2024, **22**(2): 178-192
doi: 10.1016/S1875-5364(24)60553-4
Chinese Journal of Natural Medicines

•Original article•

Composition analysis of Compound Shenhua Tablet, a seven-herb Chinese medicine for IgA nephropathy: evaluation of analyte-capacity of the assays

ZHANG Haiyan[1, 2Δ], WANG Qiuyue[1, 2Δ], WANG Jianan[2, 3Δ], ZHANG Sichao[1, 2], JIA Weiwei[2], HE Ning[2], XIA Xiaoyan[2], WANG Ting[2], LAI Liyu[2, 3], LI Jiaying[2], DU Jing[2], OLALEYE Olajide E.[2], CHEN Xiangmei[4*], YANG Junling[1, 2, 3*], LI Chuan[1, 2, 3, 5, 6*]

[1] *Graduate School, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China;*
[2] *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China;*
[3] *School of Pharmacy, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing 100049, China;*
[4] *Department of Nephrology, First Medical Center of Chinese PLA General Hospital, Nephrology Institute of the Chinese People's Liberation Army, National Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing Key Laboratory of Kidney Disease Research, Beijing 100853, China;*
[5] *Zhongshan Institute for Drug Discovery, Zhongshan 528400, China;*
[6] *Haihe Laboratory of Modern Chinese Medicine, Tianjin 301617, China*

Available online 20 Feb., 2024

[ABSTRACT] Compound Shenhua Tablet, a medicine comprising seven herbs, is employed in treating IgA nephropathy. This study aimed to meticulously analyze its chemical composition. Based on a list of candidate compounds, identified through extensive literature review pertinent to the tablet's herbal components, the composition analysis entailed the systematic identification, characterization, and quantification of the constituents. The analyte-capacity of LC/ESI-MS-based and GC/EI-MS-based assays was evaluated. The identified and characterized constituents were quantified to determine their content levels and were ranked based on the constituents' daily doses. A total of 283 constituents, classified into 12 distinct categories, were identified and characterized in the Compound Shenhua Tablet. These constituents exhibited content levels of 1−10 982 μg·g^{-1}, with daily doses of 0.01−395 μmol·d^{-1}. The predominant constituents, with daily doses of ≥ 10 μmol·d^{-1}, include nine organic acids (citric acid, quinic acid, chlorogenic acid, cryptochlorogenic acid, gallic acid, neochlorogenic acid, isochlorogenic acid C, isochlorogenic acid B, and linoleic acid), five iridoids (specnuezhenide, nuezhenoside G13, nuezhenidic acid, secoxyloganin, and secologanoside), two monoterpene glycosides (paeoniflorin and albiflorin), a sesquiterpenoid (curzerenone), a triterpenoid (oleanolic acid), and a phenylethanoid (salidroside). Additionally, there were 83, 126, and 55 constituents detected in the medicine with daily doses of 1–10, 0.1–1, and 0.01–0.1 μmol·d^{-1}, respectively. The combination of the LC/ESI-MS-based and GC/EI-MS-based assays demonstrated a complementary relationship in their analyte-capacity for detecting the constituents present in the medicine. This comprehensive composition analysis establishes a solid foundation for further pharmacological research on Compound Shenhua Tablet and facilitates the quality evaluation of this complex herbal medicine.

[KEY WORDS] Compound Shenhua Tablet; Composition analysis; Analyte-capacity
[CLC Number] R917 **[Document code]** A **[Article ID]** 2095-6975(2024)02-0178-15

[Received on] 13-Oct.-2023
[Research funding] This work was supported by the National Natural Science Foundation of China (Nos. 82192912 and 82074273), the Innovation Team and Talents Cultivation Program of National Administration of Traditional Chinese Medicine (No. ZYYCXTD-C-202009), and the Program of State Key Laboratory of Drug Research (No. SIMM2103ZZ-06).
[*]**Corresponding author]** E-mails: xmchen301@126.com (CHEN Xiangmei); yangjl@simm.ac.cn (YANG Junling); chli@simm.ac.cn (LI Chuan)
[Δ]These authors contributed equally to this work.
These authors have no conflict of interest to declare.

Introduction

In China, herbal medicines, renowned for their multifaceted therapeutic applications, are predominantly used in the treatment and prevention of complex diseases. These medicines are typically prepared from multi-herb combinations, leading to intricate chemical compositions. The identification of pharmacologically active constituents within these mixtures is crucial for guiding their therapeutic use in clinical settings. However, pinpointing specific compounds for pharmacodynamic studies from such complex herbal mixtures

presents a significant challenge in the identification process. To address this challenge, researchers have developed a multi-compound pharmacokinetic methodology [1-3]. This methodology is designed to identify bioavailable compounds, in both unchanged and metabolized forms, at targeted action sites post-administration. It examines their exposure levels, disposition, and pharmacokinetic profiles, which are essential for understanding their pharmacodynamic effects. Comprehensive and precise composition analysis of herbal medicine is crucial to ensure that the multi-compound pharmacokinetic investigation complies with the principle of "precision-without-omission" [2, 3]. This principle involves identifying the potentially important compounds and accurately characterizing their disposition and pharmacokinetics, ensuring that no such compound is overlooked. Furthermore, a thorough understanding of the chemical composition of herbal medicine is essential for developing a multi-analyte assay that can be used for the quality evaluation of the medicine [4]. This process is important for selecting marker constituents and minimizing interference from co-eluting non-target constituents during marker measurements. Additionally, the comparative analysis of composition profiles serves as an effective method for authenticating herbal materials and distinguishing between closely related herb species, such as differentiating *Panax ginseng* roots and *P. notoginseng* roots based on their saponin constituents [5-7]. For a comprehensive understanding of the chemical composition of a complex herbal medicine, high "analyte-capacity" is an essential performance characteristic of the assay [2, 3]. Analyte-capacity refers to the ability of the assay to detect a broad spectrum of analytes and their classes, and to effectively resolve these from coexisting compounds in the sample using MS or chromatography in a single analytical run.

Immunoglobulin A nephropathy (IgAN) is the most prevalent form of primary glomerulonephritis, characterized by an autoimmune response [8]. This condition often progresses to end-stage renal disease in 30%−40% of patients within 20−30 years after diagnosis. Currently, there are no therapies designed specifically for IgAN, with clinical management primarily focusing on controlling blood pressure and preserving renal function. In this context, Compound Shenhua Tablet, a traditional Chinese herbal medicine, has gained attention for its application in managing IgAN. This medicine is formulated from a combination of seven herbs: *Astragalus membranaceous* roots (Huangqi in Chinese; Astragali Radix), *Ligustrum lucidum* fruits (Nvzhenzi; Ligustri Lucidi Fructus), *Curcuma phaeocaulis* rhizomes (Ezhu; Curcumae Rhizoma), *Atractylodes macrocephala* rhizomes (Baizhu; Atractylodis Macrocephalae Rhizoma), *Paeonia lactiflora* roots (Baishao; Paeoniae Radix Alba), *Sparganium stoloniferum* rhizomes (Sanleng; Sparganii Rhizoma), and *Lonicera japonica* flower buds (Jinyinhua; Lonicerae Japonicae Flos). The efficacy of Compound Shenhua Tablet was evaluated in a double-blind, randomized controlled clinical trial involving 131 IgAN patients. The trial, conducted over 12 weeks, demonstrated a significant decrease in proteinuria ($P < 0.01$) and a low incidence of adverse reactions [9]. Furthermore, pharmacological studies have indicated that the herbal medicine demonstrates efficacy in mitigating renal ischemia-reperfusion injury and inhibiting the proliferation of mesangial cells in rats with chronic anti-Thy-1 nephritis [10, 11]. The purpose of this investigation was to analyze the composition of Compound Shenhua Tablet. The analyte-capacity of the assays was evaluated to ensure an accurate and comprehensive composition analysis, which serves as the basis for subsequent pharmacological studies on the medicine and contributes to the evaluation of its quality.

Materials and Methods

Establishment of herb libraries for the composition analysis of Compound Shenhua Tablet

Herb libraries were established or updated by using literature-mined information for the component herbs of Compound Shenhua Tablet. These libraries comprise extensive lists of constituents, featuring their names, chemical abstracts service (CAS) numbers, structural details, molecular formulas, accurate molecular masses, electrospray ionization profiles, collision-induced fragmentation profiles, and the availability of reference compounds. To facilitate the construction of the libraries, we conducted literature mining, which involved the retrieval, extraction, and review of information. This process aimed to achieve three key objectives: (i) compiling a comprehensive list of all known constituents present in the herbs used to prepare the medicine; (ii) identifying potential chemical transformations that may occur during the manufacturing process of the medicine; (iii) exploring the pharmacological activities and effects of the compounds directly related to the medicine, including any potential toxic effects. To this end, research and review articles were sourced from the Embase, Scifinder, and China National Knowledge Infrastructure databases, encompassing all available publications up to March 2023. The search strategy employed specific terms related to the constituent herbs of Compound Shenhua Tablet. For type-(i) information, "*Astragalus membranaceus*", "Huangqi", "*Ligustrum lucidum*", "Nvzhenzi", "*Curcuma phaeocaulis*", "Ezhu", "*Atractylodes macrocephala*", "Baizhu", "*Paeonia lactiflora*", "Baishao", "*Sparganium stoloniferum*", "Sanleng", "*Lonicera japonica*", "Jinyinhua", "Compound Shenhua Tablet", and "Shenhua Pian" combined with "constituent", "chemical composition", "chemical component", and "chemical ingredient" were utilized. For type-(ii) information, combinations of the names of the component herbs with terms like "conversion", "changes", and "instability" were employed. For type-(iii) information, "Compound Shenhua Tablet", "Shenhua Pian", and the names of constituents of the medicine were used in conjunction with "immunoglobulin A nephropathy", "IgAN", "proteinuria", "renal function", "anti-inflammation", "anti-proliferation", and "mitigation of renal injury". Furthermore, we conducted a thorough screening of the reference lists in key

articles to identify any additional relevant articles for information extraction. To ensure the accuracy and reliability of the information extracted, four authors independently reviewed the titles and abstracts of the retrieved articles for relevance. The screening results were then cross-verified by another author. The relevant information from each article was independently extracted by the authors, with a final verification step by two authors to confirm the precision and correctness of the extracted information.

Compound Shenhua Tablet, herb materials, and chemicals

Compound Shenhua Tablet [Nanjing Suzhong Pharmacy Research (Nanjing, China)], is standardized to contain a minimum of 0.13 mg of astragaloside IV from Huangqi (*Astragalus membranaceus* roots), 3.0 mg of nuezhenide from Nvzhenzi (*Ligustrum lucidum* fruits), and 1.5 mg of paeoniflorin from Baishao (*Paeonia lactiflora* roots). For this study, samples from six distinct batches of compound Shenhua Tablet were obtained. Additionally, samples of the individual component herbs used in the formulation were collected, including Huangqi, Nvzhenzi, Ezhu (*Curcuma phaeocaulis* rhizomes), Baizhu (*Atractylodes macrocephala* rhizomes), Baishao, Sanleng (*Sparganium stoloniferum* rhizomes), and Jinyinhua (*Lonicera japonica* flower buds). Essential oil samples from Baizhu and Ezhu, all sourced from Nanjing Suzhong Pharmacy Research, were also included in the study.

Reference standards for the compounds reported for the component herbs of Compound Shenhua Tablet were obtained from Shanghai Standard Technology (Shanghai, China), Chroma-Biotechnology (Chengdu, China), Alfa Biotechnology (Chengdu, China), Yuanye Biotechnology (Shanghai, China), and Biopurify Phytochemicals (Chengdu, China) (Table S1); their purity was ≥ 98%. High-performance liquid chromatography (HPLC)-grade methanol, formic acid, and dimethyl sulfoxide were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade water was prepared in-house using a Millipore Milli-Q Integral 3 cabinet water purifying system (Bedford, MA, USA).

Conditions and sample preparation for liquid chromatography/mass spectrometry-based composition analysis of Compound Shenhua Tablet

To analyze the nonvolatile polar constituents in Compound Shenhua Tablet, we employed a Waters Synapt G2 high-definition time-of-flight mass spectrometer (Manchester, UK), interfaced with a Waters Acquity ultraperformance liquid chromatography (UPLC) separation module (Milford, MA, USA) *via* a LockSpray electrospray ionization (ESI) source. This mass spectrometer setup was selected based on literature-mined data, compiled in the herb libraries' compound lists. Before composition analysis, 100 mg samples of pulverized compound Shenhua Tablet and each component herb were subjected to ultrasound-assisted extraction three times using 6 mL of 50% methanol for 30 min. Following centrifugation, the supernatants from each sample were combined and made up to 25 mL with 50% methanol. These solutions were further diluted at 1-, 10-, and 100-fold with 50% methanol for composition analysis.

Liquid chromatographic (LC) separation was achieved on a Waters Acquity HSS T3 column (100 mm × 2.1 mm i.d., 1.7 μm; Dublin, Ireland) with a mobile phase, consisting of solvents A (water/methanol, 99 : 1, *V/V*; containing 20 mmol·L^{-1} formic acid) and B (water/methanol, 1 : 99, *V/V*; containing 20 mmol·L^{-1} formic acid). The mobile phase was delivered at 0.3 mL·min^{-1}, using a 42-min gradient program that consisted of 0−2 min at 2% solvent B (gradient curve, 6), 2−32 min from 2% to 98% solvent B (6), 32−37 min at 98% solvent B (6), and 37−42 min at 2% solvent B (1). The ESI source functioned in the positive (capillary, 3.0 kV) and negative ion modes (−2.5 kV) at 120 °C, with the sampling cone at 40 V (positive mode) and −25 V (negative mode) and the extraction cone at 4.0 V. The mass spectrometer, operated in sensitivity mode, achieved a resolution > 10 000 and was externally calibrated using sodium formate (5 mmol·L^{-1}; 10 μL·min^{-1}) over an *m/z* range of 50−1500. Mass shifts during acquisition were corrected with leucine encephalin (*m/z* 554.2615 in the negative ion mode, *m/z* 556.2771 in the positive ion mode). MS^{E} data acquisition (in centroid; *m/z* 50−1500) was achieved using low collision energy (trap collision energy, 4 V; transfer collision energy, off) and high collision energy (ramp trap collision energy, 30−50 V; ramp transfer collision energy, off) with a scan time of 0.3 s. MS^{E} data acquisition time was set over a retention time range of 0.5−40 min. For LC/ESI-MS-based composition analysis, peak intensity thresholds were set at ≥ 500 counts for low energy and ≥ 100 counts for high energy, with a mass accuracy tolerance for elemental composition analysis ranging from −10−10 ppm. To identify the constituents of Compound Shenhua Tablet, we subtracted the chromatograms obtained by ESI-MS from those of a 50% methanol blank, prepared and analyzed under the same conditions but without the addition of pulverized medicine.

We characterized constituents with established names/structures and available reference standards by comparing their chromatographic retention times, accurate molecular masses, ionization profiles, and fragmentation profiles against those of the standards. For constituents with known names/structures but without reference standards, characterization was based on literature-mined data, which included the chromatographic elution orders and MS-associated ionization and fragmentation data of previously characterized type A constituents.

Conditions and sample preparation for gas chromatography/mass spectrometry-based composition analysis of Compound Shenhua Tablet

For the volatile nonpolar constituent analysis of Compound Shenhua Tablet, specifically those originating from Ezhu and Baizhu, we employed a Trace GC Ultra Gas Chromatograph with a programmable temperature vaporizer injector (Thermo Fisher Scientific, Milan, Italy). This system was coupled to an electron ionization (EI)-based Trace DSQ II single quadrupole mass spectrometer (Thermo Fisher Sci-

entific, Austin, TX, USA). In preparation for the composition analysis, 100 mg of each pulverized medicine sample was initially mixed with 1 mL of water. This mixture was then subjected to ultrasonic-assisted extraction three times, each with 3 mL of *n*-hexane. Following centrifugation, the supernatants from these extractions were combined and made up to 10 mL with *n*-hexane. For some compounds, 10- or 100-fold dilutions were prepared in *n*-hexane for subsequent analysis.

Gas chromatographic (GC) separation was conducted on a TR-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Thermo Fischer Scientific, Cheshire, UK). Helium gas, with a purity of 99.999%, served as the carrier, flowing at a rate of 1.5 mL·min^{-1}. The GC temperature program spanned 42 min, starting with an initial 2 min at 60 °C, 2−37 min from 60 to 165 °C (3 °C·min^{-1}), 37−40 min from 165 to 240 °C (25 °C·min^{-1}), and 40−42 min at 240 °C. The mass spectrometer was operated in electron impact mode at 70 eV. The temperatures of the transfer line and ion source were both set at 250 °C. The chromatograms of the medicine detected by EI-mass spectrometry (EI-MS) were subtracted by that of an *n*-hexane blank to identify the peaks of volatile constituents of the medicine. The *n*-hexane blank sample was prepared and analyzed under conditions similar to those for the medicine samples, except that no pulverized medicine sample was added. Additionally, for the analysis of the medicine's volatile nonpolar constituents, diluted essential oil samples from Ezhu and Baizhu (1000-fold dilution with *n*-hexane) were also examined as reference samples.

Constituents with known names/structures and available reference standards were characterized by comparing them to their respective standards. This involved evaluating their chromatographic retention time and electron ionization profile. For those constituents known by name/structure but lacking reference standards, characterization involved matching their EI mass spectra with those in the MS database provided by the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA).

Simulated compound conversion studies

To elucidate the variations in isoflavonoid profiles between Compound Shenhua Tablet and its constituent herb, Huangqi (*Astragalus membranaceus* roots), we conducted a simulated compound conversion study, in which pulverized herb (30 g) was subjected to reflux extraction using 60% ethanol (300 mL) at 90 °C for 24 h. Post-extraction, three distinct samples were prepared for comparative analysis: the original pulverized Huangqi herb, its 60% ethanol extract, and the Compound Shenhua Tablet. These samples were subsequently analyzed for their isoflavonoid content using liquid chromatography coupled with LC/ESI-MS.

Data processing

To ensure the adequacy of analyte-capacity for the comprehensive composition analysis of Compound Shenhua Tablet, we employed a detailed data processing strategy. This strategy involved *in silico* calculations of molecular descriptors for all compounds identified in the candidate compound lists. These lists not only included compounds reported in the component herbs of the medicine but also those potentially chemically converted during the medicine's manufacturing process. Key molecular descriptors critical to the effectiveness of various analytical stages were calculated. These descriptors included molecular mass (MW), distribution coefficient (Log *D*) at pH 7, aqueous solubility (Log *S*) at pH 7, the sum of hydrogen bond donors and hydrogen bond acceptors (HBD + HBA), dissociation constant (pK_a), and boiling point (BP). These parameters play a pivotal role in determining the efficiency of analyte recovery during sample preparation, chromatographic retention and separation, and detection and resolution by MS. For this purpose, the ACD/Percepta software (ACD/Labs 2012 release; Toronto, ON, Canada) was employed. The retrospective assessment of the LC/ESI-MS-based assay's analyte-capacity involved examining compounds detected in assays of other herbal medicines, including those that underwent chemical transformations during their manufacturing [6, 7, 12-18]. This analysis also incorporated the constituents and chemically converted compounds identified in Compound Shenhua Tablet. Similarly, the GC/EI-MS-based assay's analyte-capacity was evaluated based on the constituents detected in Compound Shenhua Tablet. The critical stage of this process was the comparative analysis of "assay analyte-capacity" and "required analyte-capacity" datasets. This comparison was crucial to confirm whether the combined LC/ESI-MS and GC/EI-MS assays provided an analyte-capacity sufficient for a thorough composition analysis of Compound Shenhua Tablet.

Each detected and characterized constituent is assigned a unique three-digit ID number, displayed in bold, which encodes crucial information about its source and classification. Source herb: The first digit of the ID reflects the specific component herb from which the constituent is derived. The numbering scheme is as follows: '**1**', '**2**', '**3** and **4**', '**5**', '**6**', '**7**', and '**8**' represent Huangqi, Nvzhenzi, Ezhu, Baizhu, Baishao, Sanleng, and Jinyinhua, respectively. Compound class: the second and third digits in the ID number categorize the compound into its respective classes: **01**– **49**, **51**– **79**, and **81**– **99** are designated for terpenoids, phenols and organic acids, and other compound classes, respectively.

In the composition analysis of Compound Shenhua Tablet, information regarding both the content level and associated daily dose of identified constituents was provided. The constituent daily dose was calculated by multiplying the content level of each constituent by the recommended daily intake of the medicine (15 tablets·d^{-1}). This metric is particularly advantageous for comparative analysis across various herbal medicines, especially those containing identical constituents. Its significance lies in its immunity to variations in formulation excipients and dosage regimens that are often different across medicinal products. Furthermore, the constituent daily dose plays a significant role in determining the systemic exposure levels of constituents upon administering

the medicine. This exposure encompasses both the unchanged and metabolically altered forms of these constituents. In this analysis, all detected and characterized constituents within Compound Shenhua Tablet were ranked based on their constituent daily doses in a descending order and graded into 100–1000, 10–100, 1–10, and 0.01–1 μmol·d^{-1}. Constituents with a daily dose < 0.01 μmol·d^{-1} were excluded from subsequent analysis. This decision was made based on the understanding that such compounds are unlikely to achieve pharmacologically significant exposure in the body, whether in their original form or as metabolites, post-administration [2, 3].

Results

Literature-mined information facilitating composition analysis of Compound Shenhua Tablet

The composition analysis of Compound Shenhua Tablet was significantly enhanced by literature reviews focusing on the chemical constituents of its component herbs. Below is a summary of the insights gained from these reviews: Huangqi: reviews by CHU *et al.*, SU *et al.*, and CHANG *et al.* detailed the chemical constituents present in *Astragalus membranaceus* var. *mongholicus* roots and *A. membranaceus* roots [19–21], leading to the identification of 159 candidate compounds across three classes for Huangqi. Nvzhenzi: comprehensive analyses by LI *et al.*, SHANG *et al.*, and CAO *et al.* provided insights into the constituents of *Ligustrum lucidum* fruits [22–24], resulting in a list of 146 candidate compounds spanning six classes for Nvzhenzi. Ezhu: research by CHANG *et al.*, LI *et al.*, and WU *et al.* covered *Curcuma phaeocaulis* rhizomes, *C. Kwangsiensis* rhizomes, and *C. wenyujin* rhizomes [25–27], identifying 190 candidate compounds across five classes for Ezhu. Baizhu: ZHU *et al.* and YANG *et al.* focused on *Atractylodes macrocephala* rhizomes [28, 29], compiling a list of 96 candidate compounds in seven classes for Baizhu. Baishao: investigations by WU *et al.* and YANG *et al.* into *Paeonia lactilora* roots [30, 31] led to the identification of 120 candidate compounds across five classes for Baishao. Sanleng: studies by JIA *et al.* and LU *et al.* explored *Sparganium stoloniferum* rhizomes [32, 33], resulting in 83 candidate compounds in eight classes for Sanleng. Jinyinhua: reviews by LI *et al.,* ZHENG *et al.,* and PAN *et al.* highlighted the constituents of *Lonicera japonica* flower buds [34–36], culminating in a list of 183 candidate compounds across five classes for Jinyinhua. After literature mining, terpenoids, phenols, and organic acids were identified as important constituents for Compound Shenhua Tablet due to their pharmacological or toxicological activities. This process led to the creation of targeted candidate compound lists for each of the seven herbs, facilitating a comprehensive composition analysis of the medicine.

Analyte-capacity of LC/MS-based and GC/MS-based assays for composition analysis of Compound Shenhua Tablet

The analyte-capacity required for the composition analysis of Compound Shenhua Tablet is depicted in Fig. 1A, detailed the molecular descriptors, MW, Log D_{pH7}, Log S_{pH7}, HBD + HBA, pK_a, and BP. These parameters were calculated for all compounds listed as potential constituents in the candidate compound lists of the medicine's component herbs. Meanwhile, the retrospective assessment of the analyte-capacity of the LC/ESI-MS-based assay was conducted using data from constituents previously detected in other herbal medicines and those identified in Compound Shenhua Tablet.

The LC/ESI-MS-based assay's analyte-capacity was retrospectively evaluated with constituents previously detected and characterized in other herbal medicines (Fig. 1B), as well as those in Compound Shenhua Tablet (Fig. 1C). This evaluation facilitated a comparison between the analyte-capacity required and that provided by the assay. This comparison revealed certain limitations in the LC/ESI-MS-based assay, particularly in detecting compounds with a Log S_{pH7} value less than −6, an HBD + HBA value ranging from 0 to 1, or a BP lower than 300 ℃. Conversely, as shown in Fig. 1D, the GC/EI-MS-based assay could be a complement to the LC/ESI-MS-based assay for detecting compounds that were challenging for LC/ESI-MS, especially those with a Log S_{pH7} of −8.5−−6, an HBD + HBA of 0−1, or BP of 150−300 ℃. Additionally, the GC/EI-MS-based assay enhanced the detection of compounds with a MW < 220 Da or Log D_{pH7} of 4.0−6.5 or without pK_a. The data revealed a positive correlation between MW and HBD + HBA and BP. The detected constituents with MW < 220 Da normally had no HBD and 0−2 HBA, and these compounds generally exhibited a BP of < 300 ℃. Fig. 1 also elucidates the the overlapping detection capabilities of the LC/ESI-MS-based and GC/EI-MS-based assays in the comprehensive composition analysis of Compound Shenhua Tablet.

Constituents originating from Huangqi (Astragalus membranaceus roots) in Compound Shenhua Tablet

The analysis of Compound Shenhua Tablet *via* LC/ESI-MS identified 24 constituents (daily dose ≥ 0.01 μmol·d^{-1}) originating from Huangqi, i.e., ten cycloartane triterpene saponins (**101**–**110**), 13 isoflavonoids (**151**–**163**; comprising four aglycones with their respective glycosides), and an organic acid (**171**, citric acid) (Table S2 and Fig. 2A). Generally, the saponins predominantly ionized as sodiated molecules ([M + Na]$^+$) in the positive ion mode (used for collision-induced dissociation), and, to a less extent, as formate adducts ([M + HCOO]$^-$) in the negative ion mode. The triterpene saponins generated characteristic fragment ions including the aglycone residues at *m/z* 473.3631 and ions of its dehydrated species at *m/z* 455.3525, 437.3420, and 419.3314. The isoflavonoids predominantly ionized as [M + Na]$^+$ and, in some cases, as protonated molecules ([M + H]$^+$) in the positive ion mode (used for collision-induced dissociation) and as formate adducts ([M + HCOO]$^-$) in the negative ion mode. They displayed characteristic fragment ions such as aglycone residues at *m/z* 285.0763, 269.0841, and 303.1269, along with

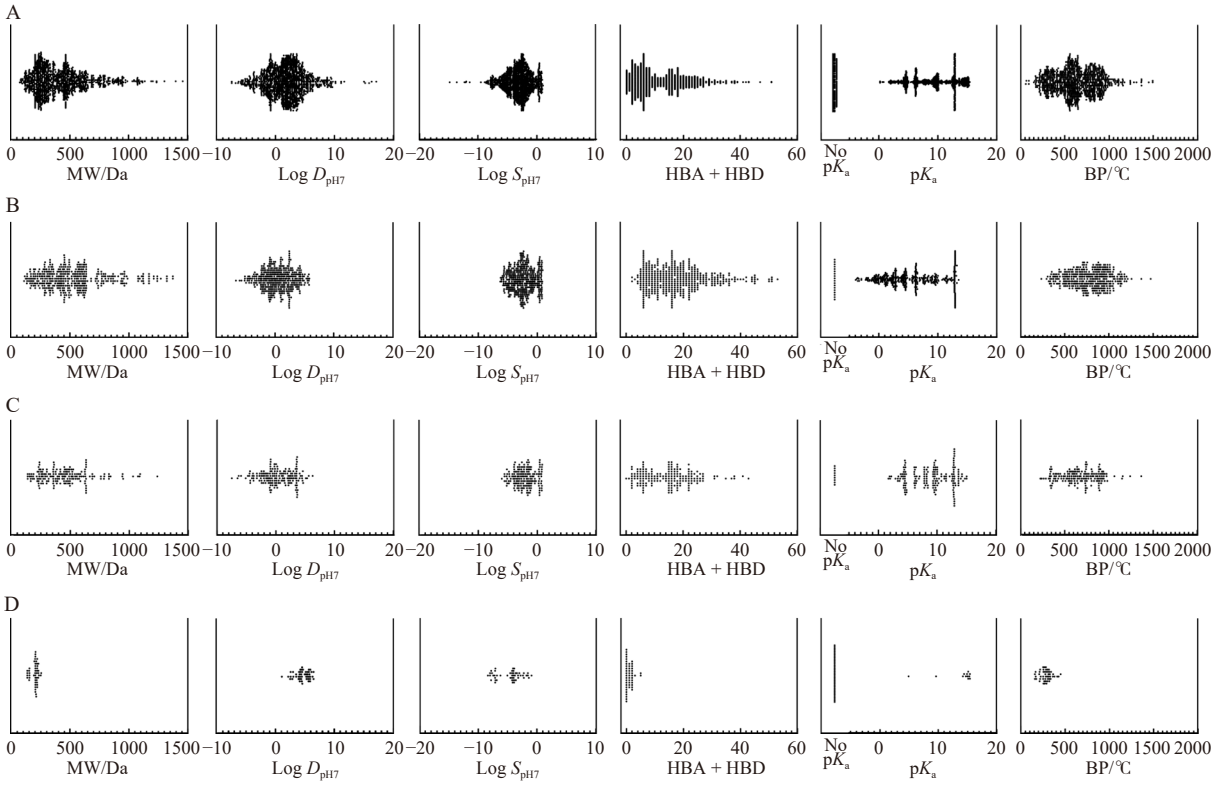

Fig. 1 Comparison of the analyte-capacity required for composition analysis of Compound Shenhua Tablet with that of the LC/ESI-MS-based and the GC/EI-MS-based assays. (A) The required analyte-capacity was evaluated with all compounds in the candidate compound lists of the medicine's component herbs. (B) LC/ESI-MS-based assay's analyte-capacity was retrospectively evaluated with compounds previously detected in other herbal medicines. (C) LC/ESI-MS-based assay's analyte-capacity was evaluated with constituents detected in Compound Shenhua Tablet. (D) GC/EI-MS-based assay's analyte-capacity was evaluated with constituents detected in Compound Shenhua Tablet. MW, molecular mass; Log *D*, distribution coefficient; Log *S*, aqueous solubility; HBD + HBA, hydrogen bond donors plus hydrogen bond acceptors; pK_a, dissociation constant; and BP, boiling point.

losses of CH_3, CH_3OH, and/or CO. Citric acid ionized as $[M + Na]^+$ and deprotonated molecules ($[M - H]^-$ in the positive and negative ion mode, respectively. Astragaloside I (**101**), astragaloside II (**102**), isoastragaloside I (**103**), calycosin-7-*O*-*β*-D-glucoside (**151**), calycosin (**152**), formononetin (**153**), isomucronulatol-7-*O*-*β*-D-glucoside (**154**), and methylnissolin-3-*O*-*β*-D-glucoside (**155**) were the major constituents originating from Huangqi, each with a daily dose of 1–10 μmol·d^{-1} in Compound Shenhua Tablet. Their respective content levels in the medicine are also shown in Table S2 and Fig. 3A. Citric acid, with a constituent daily dose of 395 μmol·d^{-1} in Compound Shenhua Tablet, was found to originate not only from Huangqi but also from the other six component herbs. Fig. 4 shows the chemical structures of these compounds. Significant differences in the compound profile were observed for several isoflavonoids, i.e., calycosin-7-*O*-*β*-D-glucoside-6"-*O*-malonate, isomucronulatol-7-*O*-*β*-D-glucoside-6"-*O*-malonate, methylnissolin-3-*O*-*β*-D-glucoside-6"-*O*-malonate, and formononetin-7-*O*-*β*-D-glucoside-6"-*O*-malonate, significantly present in *A. membranaceus* var. *mongholicus* roots, but limitedly in the medicine (Fig. 5). In 60% ethanol, these isoflavonoids could be converted, at 90 ℃ in a time-dependent manner, into **151**, **154**, **155**, and **158**, respectively; these findings were consistent with those by LIU *et al.* who reported conversion of formononetin-7-*O*-*β*-D-glucoside-6"-*O*-malonate into **158** [37]. Although astragaloside IV (**105**) is a minor constituent in Compound Shenhua Tablet, the medicine's quality specification that stipulates a lower limit for the content level of this compound was based on the deacetylated species of **101**, **102**, **103**, and isoastragaloside II (**104**) under basic conditions for sample preparation of assay [38, 39]. Based on the content levels of **101**–**104** in Compound Shenhua Tablet (Table S2), the mean content level of the artificial astragaloside IV was calculated to be 0.49 ± 0.01 mg per tablet, which well meets the required level (≥ 0.13 mg per tablet) for the compound in the medicine.

Constituents originating from Nvzhenzi (Ligustrum lucidum fruits) component of Compound Shenhua Tablet

A total of 47 constituents (compound dose, ≥ 0.01 μmol·day^{-1}) originating from Nvzhenzi, i.e., 27 iridoids (**201**–**227**; comprising secoiridoids and cyclopentane iridoids), five triterpenes (**231**–**235**), ten phenylethanoids (**251**–**260**), and five flavonoids (**271**–**275**), were detected in Compound Shenhua Tablet by LC/ESI-MS (Table S2 and Fig. 2B). Generally, the iridoids predominantly ionized as $[M + Na]^+$ in the positive ion mode and as $[M - H]^-$ in the

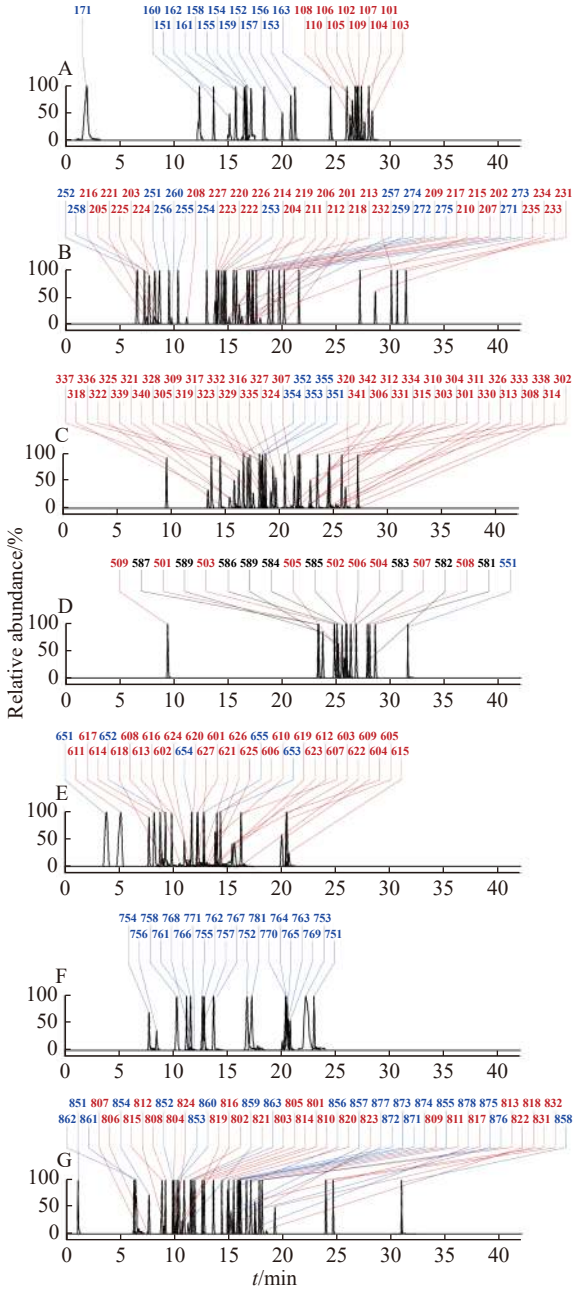

Fig. 2 Stacked liquid chromatograms of nonvolatile constituents detected by ESI-MS in a typical sample of Compound Shenhua Tablet. Each constituent has an ID number and its name is shown in Table S2. The constituents with IDs marked in red, blue, and black represent terpenoids, phenols/organic acids, and others, respectively. (A) The constituents originating from Huangqi (*A. membranaceus* roots); (B) The constituents from Nvzhenzi (*L. lucidum* fruits); (C) The constituents from Ezhu (*C. phaeocaulis* rhizomes); (D) The constituents from Baizhu (*A. macrocephala* rhizomes); (E) The constituents from Baishao (*P. lactilora* roots); (F) The constituents from Sanleng (*S. stoloniferum* rhizomes); (G) The constituents from Jinyinhua (*L. japonica* flower buds).

negative ion mode (used for collision-induced dissociation), a pattern also observed in triterpenes, phenylethanoids and flavonoids. The iridoids are characterized by an iridoid backbone, a glycosyl group, and a phenylethanoid moiety, producing characteristic fragment ions of the iridoid aglycone residue at *m/z* 223.0606 and of phenylethanoid glycoside residue at *m/z* 299.1131. Phenylethanoids typically generated characteristic fragment ions of a phenylethanol group at *m/z* 119.0497 and a caffeoyl group (if present) at *m/z* 161.0317. Specnuezhenide (**201**), nuezhenoside G13 (**202**), nuezhenidic acid (**203**), oleanolic acid (**231**), and salidroside (**251**) were the major constituents originating from Nvzhenzi, each with a daily dose of 10−125 μmol·d^{-1} from Compound Shenhua Tablet, while there were also 16 iridoids, four triterpenes, and seven phenylethanoids (each with a daily dose of 1−10 μmol·d^{-1}). The content levels of these constituents in the medicine are shown in Table S2 and Fig. 3B, and their chemical structures are shown in Fig. 4. The quality specification of the medicine stipulates a lower limit for the content level of specnuezhenide, i.e., ≥ 3.0 mg per tablet. Using the current assay for composition analysis of the medicine, the mean content level of **201** was determined to be 5.7 ± 0.1 mg per tablet.

Constituents originating from Ezhu (Curcuma phaeocaulis rhizomes) in Compound Shenhua Tablet

The LC/ESI-MS analysis of Compound Shenhua Tablet revealed a total of 47 constituents (compound dose, ≥ 0.01 μmol·d^{-1}) derived from Ezhu, i.e., 42 sesquiterpenes (**301**–**342**), and five diarylheptanoids (**351**–**355**) (Table S2 and Fig. 2C). Generally, the sesquiterpenes primarily ionized as $[M + Na]^+$ and $[M + H]^+$ (often to less extent) in the positive ion mode (with collision-induced dissociation) while showing minimal ionization in the negative ion mode. The diarylheptanoids predominantly ionized as $[M + Na]^+$ and protonated molecules ($[M + H]^+$) in the positive ion mode and as $[M - H]^-$ in the negative ion mode (for acquiring collision-induced dissociation data). Considering that Ezhu undergoes steam distillation to extract essential oils during the manufacturing process of Compound Shenhua Tablet, GC/EI-MS was also applied to analyze the volatile constituents from this herb. This analysis identified 33 volatile constituents (compound dose, ≥ 0.01 μmol·d^{-1}) from Ezhu, i.e., 21 sesquiterpenes (**301**, **302**, **401**–**419**), ten monoterpenes (**421**–**430**), and two other compounds (**441**, **442**) (Table S3 and Fig. 6A). Curzerenone (**301**) was identified as the primary constituent from Ezhu, with a daily dose of 24 μmol·d^{-1} in the tablet. Moreover, 15 other sesquiterpenes and two monoterpenes, each with a daily dose of 1−10 μmol·d^{-1}. Notably, **302** and **303** were constituents originating from both Ezhu and Baizhu (*Atractylodes macrocephala* rhizomes).

Constituents originating from Baizhu (Atractylodes macrocephala rhizomes) in Compound Shenhua Tablet

A total of 19 constituents (compound dose, ≥ 0.01 μmol·day^{-1}) originating from Baizhu, i.e., nine sesquiterpenes (**501**–**509**), an organic acid (**551**, linoleic acid), and nine polyacetylenes (**581**–**589**) were detected in Compound Shenhua Tablet by LC/ESI-MS (Table S2 and Fig. 2D). Gen-

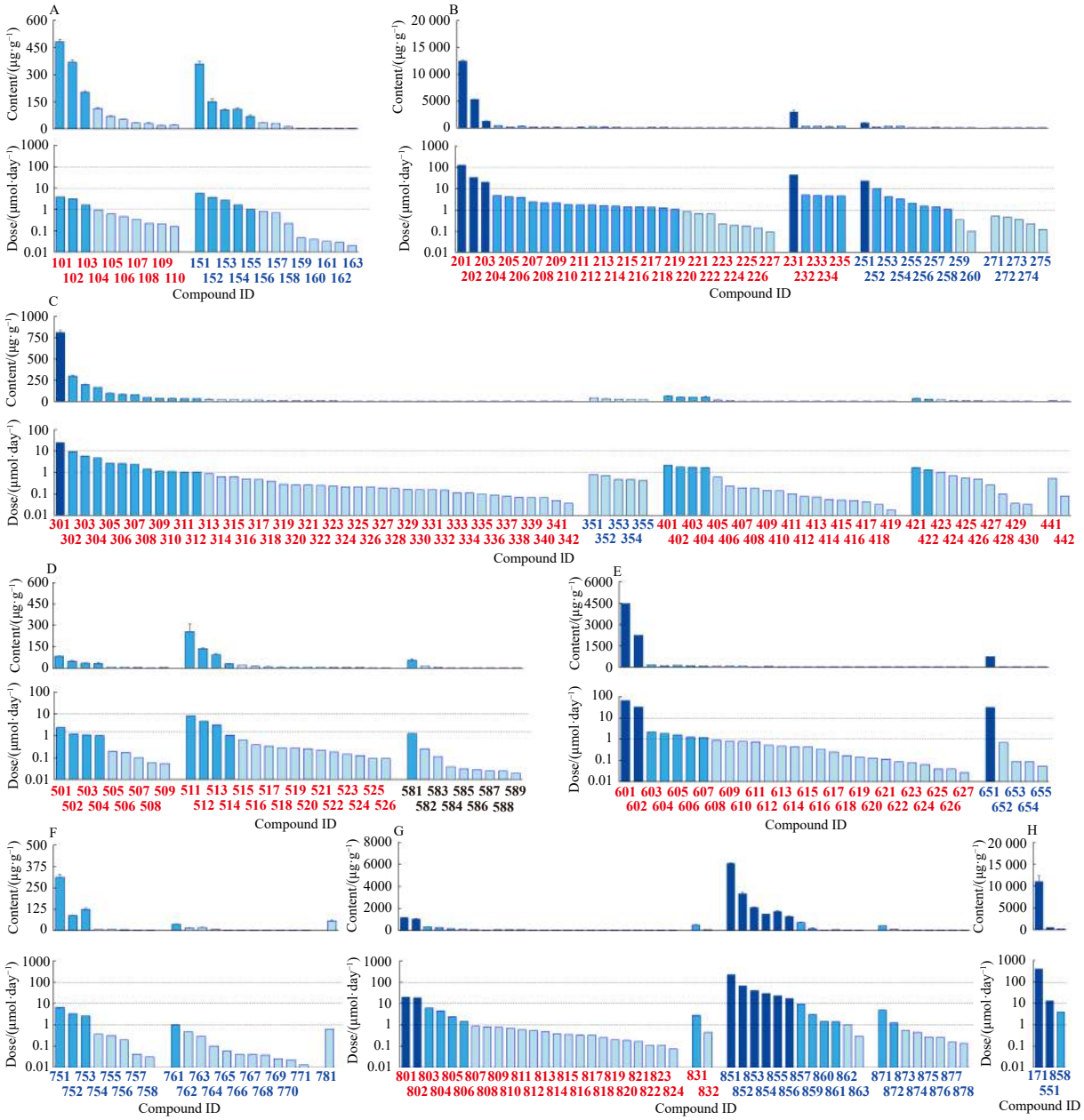

Fig. 3 Content levels and daily doses of constituents detected in Compound Shenhua Tablet. Each constituent has an ID number and its name is shown in Tables S2 and S3. The constituents with bold IDs marked in red, blue, and black represent terpenoids, phenols/organic acids, and other classes, respectively. (A) Data of constituents originating from Huangqi (*A. membranaceus* roots); (B) Data of constituents from Nvzhenzi (*L. lucidum* fruits); (C) Data of constituents from Ezhu (*C. phaeocaulis* roots and rhizomes); (D) Data of constituents from Baizhu (*A. macrocephala* roots and rhizomes); (E) Data of constituents from Baishao (*P. lactilora* roots); (F) Data of constituents from Sanleng (*S. stoloniferum* rhizomes); (G) Data of constituents from Jinyinhua (*L. japonica* flower buds); (H) Data of the constituents citric acid (171), linoleic acid (551), and linolenic acid (858) from all the seven component herbs.

erally, the sesquiterpenes predominantly ionized as $[M + Na]^+$ and $[M + H]^+$ (often to less extent) in the positive ion mode (with collision-induced dissociation) while exhibiting poor ionization in the negative ion mode. Similar to Ezhu, Baizhu undergoes steam distillation to extract essential oils. These oils are then integrated with a combined herbal extract in the production of Compound Shenhua Tablet. Accordingly, GC/EI-MS was also employed to analyze the volatile constituents from Baizhu. This analysis revealed the presence of 16 volatile constituents (compound dose, ≥ 0.01 μmol·d^{-1}) originating from Baizhu, i.e., 16 sesquiterpenes (**511**–**526**) (Table S3 and Fig. 6B). Linoleic acid (**551**) demonstrated a constituent daily dose of 13 μmol·d^{-1} in Compound Shenhua Tablet, indicating its derivation not only from Baizhu but also

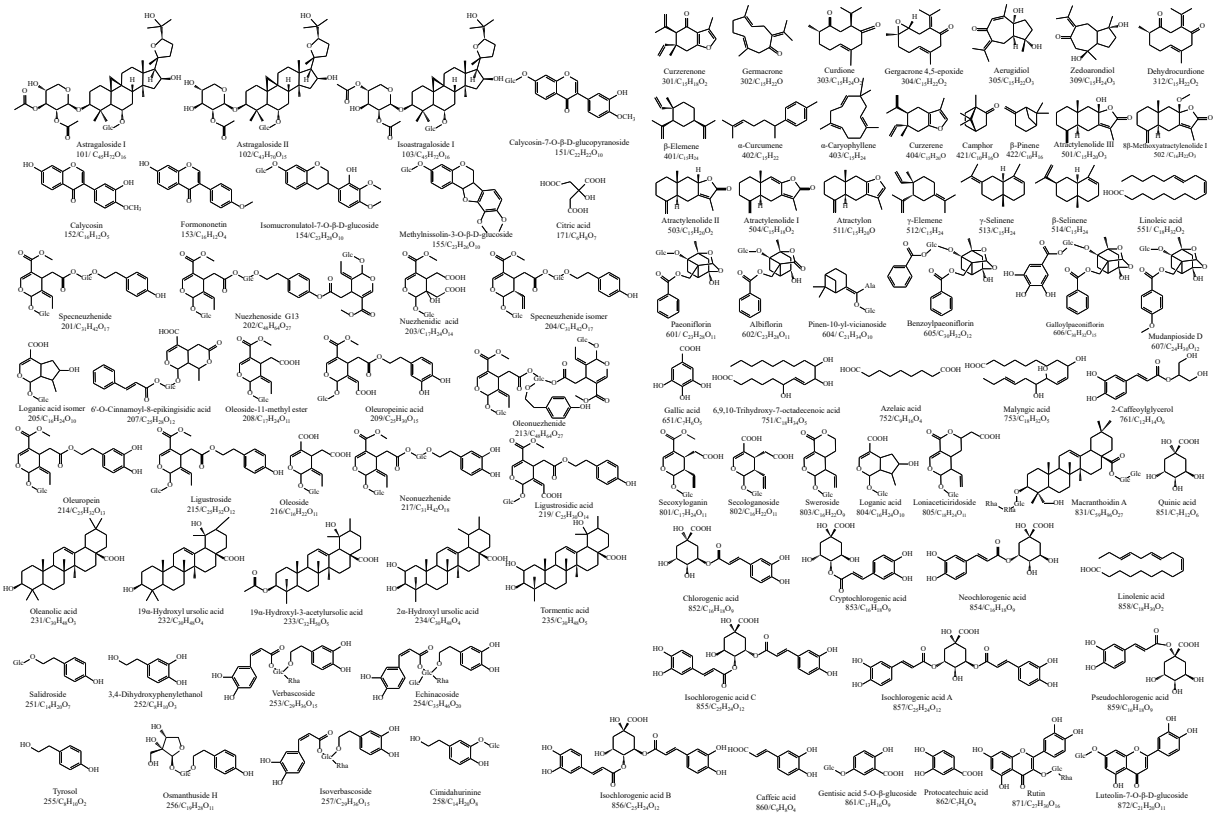

Fig. 4 Chemical structures of constituents with a daily dose of ≥ 1 μmol·d^{-1} in Compound Shenhua Tablet. Glc, glucopyranosyl; Rha, rhamnopyranosyl.

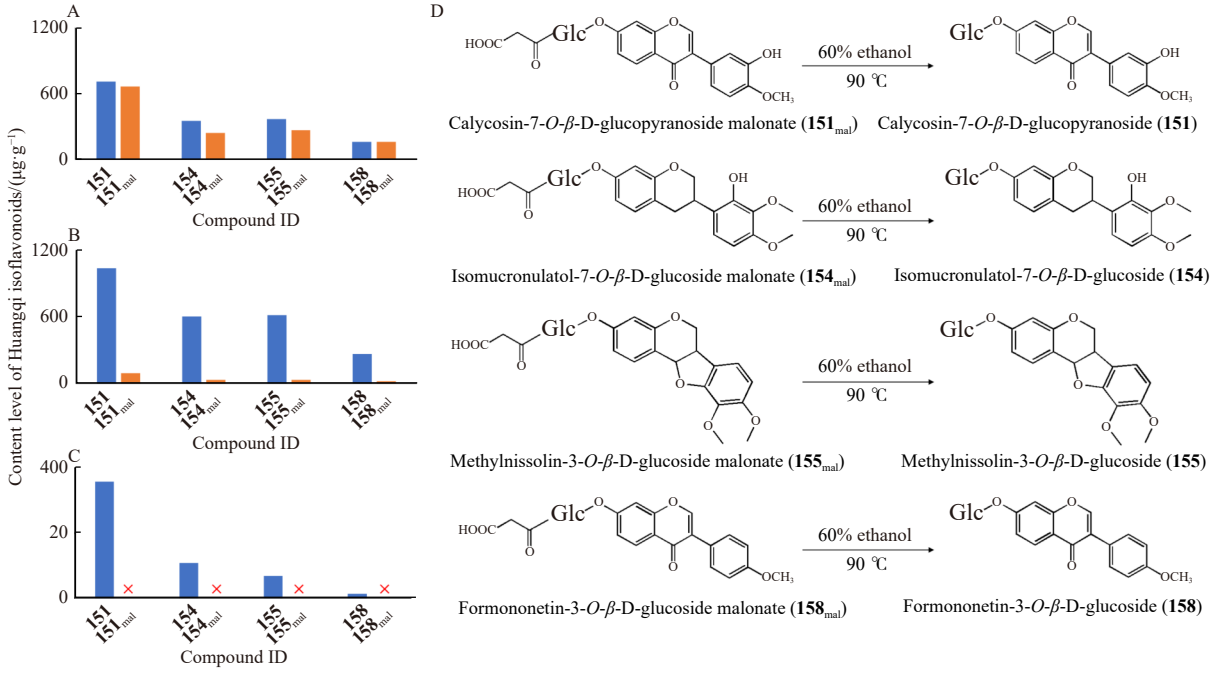

Fig. 5 Proposed chemical conversion of Huangqi isoflavonoids in the manufacturing process of Compound Shenhua Tablet. (A) Isoflavonoids detected in pulverized Huangqi (*A. membranaceus* roots); (B) Isoflavonoids detected in extract of Huangqi after reflux extraction with 60% ethanol at 90 °C for 24 h; (C) Huangqi isoflavonoids detected in Compound Shenhua Tablet; (D) Proposed hydrolysis reactions of calycosin-7-*O*-*β*-D-glucoside-6''-*O*-malonate into calycosin-7-*O*-*β*-D-glucoside (151), isomucronulatol-7-*O*-*β*-D-glucoside-6''-*O*-malonate into isomucronulatol-7-*O*-*β*-D-glucoside (154), methylnissolin-3-*O*-*β*-D-glucoside-6 ″-*O*-malonate into methylnissolin-3-*O*-*β*-D-glucoside (155), and formononetin-7-*O*-*β*-D-glucoside-6″-*O*-malonate into formononetin-7-*O*-*β*-D-glucoside (158).

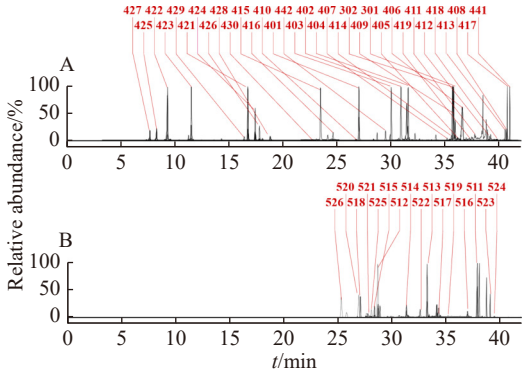

Fig. 6 Stacked gas chromatograms of volatile constituents detected by EI-MS in a typical sample of Compound Shenhua Tablet. Each constituent has an ID number and its name is shown in Table S3. The constituents with IDs marked in red represent terpenoids. (A) The constituents originating from Ezhu (*C. phaeocaulis* rhizomes); (B) The constituents from Baizhu (*A. macrocephala* rhizomes).

from the other six component herbs. Atractylenolide III (**501**), 8*β*-methoxyatractylenolide (**502**), atractylenolide II (**503**), atractylenolide I (**504**), atractylon (**511**), *γ*-elemene (**512**), *γ*-selinene (**513**), *β*-selinene (**514**), and 14*β*-methylbutyryltetradeca-2*E*,8*Z*,10*E*-trien-4,6-diyne-1-ol (**581**) were the major constituents, originating from Baizhu, each with a daily dose of 1−10 μmol·d^{-1} in Compound Shenhua Tablet. Their respective content levels in the medicine are provided in Tables S2 and S3 and Fig. 3D.

Constituents originating from Baishao (Paeonia tactilora roots) in Compound Shenhua Tablet

A total of 32 constituents (compound dose, ≥ 0.01 μmol·day^{-1}) originating from Baishao, i.e., 27 monoterpene glycosides (**601**–**627**) and five tannins (**651**–**655**), were detected in Compound Shenhua Tablet by LC/ESI-MS (Table S2 and Fig. 2E). Generally, the monoterpene glycosides predominantly ionized as $[M + Na]^+$ in the positive ion mode and as $[M + HCOO]^-$ and $[M - H]^-$ in the negative ion mode (used for collision-induced dissociation). The tannins exhibited a similar ionization pattern, predominantly ionizing as $[M + Na]^+$ and $[M + H]^+$ in the positive ion mode and as $[M - H]^-$ in the negative ion mode, the latter being essential for collision-induced dissociation. A notable feature of the monoterpene glycosides was the common fragment ion at *m/z* 121.0290 ([benzoic acid − H]$^-$). Additionally, galloylated monoterpene glycosides were identified by a characteristic fragment ion at *m/z* 169.0137 ([gallic acid − H]$^-$). Among these constituents, paeoniflorin (**601**), albiflorin (**602**), and gallic acid (**651**) were prominent, each presenting a daily dose of 30−64 μmol·d^{-1} in Compound Shenhua Table. Furthermore, five additional monoterpene glycosides were detected, each with a daily dose of 1−10 μmol·d^{-1}. The quality specification of the medicine stipulates a lower limit for the content level of paeoniflorin, i.e., ≥ 1.5 mg per tablet. Using the current assay for composition analysis of the medicine, the mean content level of **601** was determined to be 4.3 ± 0.0003 mg per tablet.

Constituents originating from Sanleng (Sparganium stoloniferum rhizomes) in Compound Shenhua Tablet

In the LC/ESI-MS analysis of Compound Shenhua Tablet, 20 constituents originating from Sanleng were identified, each with a compound dose of ≥ 0.01 μmol·d^{-1}, i.e., eight organic acids (**751**–**758**), 11 phenylpropanoids (**761**–**771**), and a flavonoid (**781**) (Table S2 and Fig. 2F). These constituents predominantly ionized as $[M - H]^-$ in the negative ion mode (with collision-induced dissociation) but showed poor ionization in the positive ion mode. The coumaroyl phenylpropanoids presented a common fragment ion at *m/z* 163.0395 ([coumaric acid − H]$^-$). 6,9,10-Trihydroxy-7-octadecenoic acid (**751**), azelaic acid (**752**), malyngic acid (**753**), and 2-caffeoylglycerol (**761**) were the major constituents originating from Sanleng, each with a daily dose of 1−10 μmol·d^{-1} in Compound Shenhua tablet. Their respective content levels in the medicine are also shown in Table S2 and Fig. 3F.

Constituents originating from Jinyinhua (Lonicera japonica flower buds) in Compound Shenhua Tablet

In the LC/ESI-MS analysis of Compound Shenhua Tablet, a total of 47 constituents originating from Jinyinhua were identified, each with a compound dose of ≥ 0.01 μmol·d^{-1}), i.e., 24 iridoids (**801**–**824**), two triterpene saponins (**831**, **832**), 13 organic acids (**851**–**863**), and eight flavonoids (**871**–**878**) (Table S2 and Fig. 2G). Generally, the iridoids primarily ionized as $[M + Na]^+$ in the positive ion mode and $[M - H]^-$ (to less extent) in the negative ion mode (used for collision-induced dissociation). Secoxyloganin (**801**), secologanoside (**802**), quinic acid (**851**), chlorogenic acid (**852**), cryptochlorogenic acid (**853**), neochlorogenic acid (**854**), isochlorogenic acid C (**855**), and isochlorogenic acid B (**856**) were the major constituents originating from Jinyinhua, each with a daily dose of 10−218 μmol·d^{-1} in Compound Shenhua Tablet. Additionally, four iridoids, one triterpene, six organic acids, and two flavonoids were detected, each with a daily dose of 1−10 μmol·d^{-1}. The content levels of these constituents in the medicine are shown in Table S2 and Fig. 3G, and their chemical structures are depicted in Fig. 4.

Discussion

Gaining a comprehensive understanding of the chemical composition of herbal medicine is essential as it serves as the foundation for multi-compound pharmacokinetic investigations and the development of quality evaluation assays. Chemical constituents present in herbal medicines are categorized into three types: type A, B, and C. Type A constituents are those with known names/structures and reference standards readily available. Type B constituents are also identifiable by their names and structures; however, they lack corresponding reference standards. Type C constituents present the greatest challenge, as they are compounds with unknown names or structures, rendering the acquisition of reference standards impossible. In the current investigation of Compound Shenhua Tablet, a candidate-compound-list-guided ap-

proach was employed for the development of compositional assays. This approach involved the compilation of a candidate compound list through extensive literature mining, encompassing constituents reported in each of the medicine's component herbs. Additionally, the lists included compounds that are generated through chemical conversions of certain constituents in the manufacturing process of the medicine. All the compounds included in these lists fall under types A and B. The success of this candidate-compound-list-guided approach relies on the wealth of existing phytochemical and analytical research associated with the component herbs of the medicine. This aspect can be determined by examining the volume of related publications and the availability of commercially sourced or in-house preparable reference standards. The composition analysis of the medicine involved the detection of its constituents, followed by the characterization of the detected constituents. The subsequent quantification is essential for ranking and grading the characterized constituents. In the analysis of Compound Shenhua Tablet, the detection of types A and B constituents was guided by pre-analysis information, including names, molecular formulas, molecular masses, and structures, as outlined in the candidate compound lists. The results obtained from this analysis were then verified by conducting similar analyses on the component herbs. Substantial disparities in constituent occurrence between the medicine and its component herb indicated possible occurrence of chemical conversions. These suspected conversions were then investigated through simulated compound conversion studies. Type C constituents were found by subtracting the chromatograms of the associated blank sample from those of the medicine and its component herbs, while excluding type A and B constituents. Only major type C constituents were highlighted and applied to further characterization and quantification. Type A constituents were characterized through direct comparisons with their respective reference standards, focusing on chromatographic retention time, accurate molecular mass, ionization profile, and fragmentation profile. Type B constituents, on the other hand, were characterized by comparing them with literature-sourced data, which included their chromatographic elution order in relation to one or two characterized type A constituents, as well as MS-associated ionization and fragmentation information. Quantification strategies varied based on the type of constituent. Type A constituents were quantified using calibration curves derived from reference compounds, while type B constituents were quantified using calibration methods involving structurally similar type A compounds. In instances where a type B or C constituent displayed either a high constituent daily dose (e.g., > 10 μmol·d^{-1}) or a significant detection response, additional steps were taken to enhance the composition analysis, which involved isolating the phytochemical from the component herb and conducting in-depth characterization, including nuclear magnetic resonance spectrometry-based analysis, to transition the compound into a type A constituent.

Analyte-capacity is a crucial characteristic of assays used for analyzing complex samples, such as multi-herb medicines. It refers to the total number of analytes and their classes that can be detected by mass spectrometry (MS) and distinguished from coexisting compounds in the sample, either by MS or chromatography, within a single analytical run. For an assay to be effective in the context of multi-herb medicines, its three tandem components–sample preparation, chromatographic separation, and MS detection–must exhibit matching analyte-capacity. In our study, we developed and employed a retrospective method to evaluate the assay's analyte-capacity. This evaluation involved a comparison between the assay's actual capability and the required analyte-capacity, which was determined based on all compounds included in the candidate compound lists of the medicine's component herbs. The assessment of analyte-capacity utilized several molecular descriptors: MW, Log D_{pH7}, Log S_{pH7}, HBD + HBA, pK_a, and BP. These descriptors are associated with the analyte recovery by sample preparation, retention and separation by chromatography, and/or detection and resolution by MS. The current development of the concept of analyte-capacity allows us to define a complementary relationship between the LC/ESI-MS-based and GC/EI-MS-based assays for composition analysis of Compound Shenhua Tablet. Successful assay development for the composition analysis of complex herbal medicines is centered on effectively detecting, accurately characterizing, and precisely quantifying the major and potentially important constituents. This approach prioritizes a comprehensive analysis of key compounds over merely tallying the total number of detected constituents. In this context, the concept of analyte-capacity serves as a crucial performance metric. It aids in enhancing assay development, facilitating more thorough and precise analyses by incorporating advanced techniques, including multidimensional chromatographic separation. The analyte-capacity is different from the peak capacity, a concept proposed by Giddings [40]. Peak capacity relates to the maximum number of ideally spaced peaks a chromatographic system can resolve under optimal conditions and is a measure of the quality of chromatographic separation, especially in gradient mode. This aspect of chromatographic separation has been extensively reviewed, focusing on unidimensional separations by Neue [41] and on multidimensional separations by Stoll *et al.* [42]. In traditional chromatography combined with ultraviolet or other universal detection methods, co-elution occurs when the number of compounds in a sample exceeds the system's peak capacity. However, in chromatography/MS-based measurements, the selective nature of MS detection typically resolves most co-eluted compounds. Chromatographic separation in this context is primarily needed for resolving isobaric compounds. For chromatography/MS-based composition analysis of a complex herbal medicine, the analyte-capacity offers significant advantages over peak capacity as a performance measure. Unlike peak capacity, which focuses solely on chromatographic separation, analyte capacity provides a more

comprehensive assessment. It takes into account the synergy between sample preparation, chromatographic separation, and MS detection.

In Compound Shenhua Tablet, a total of 283 constituents were detected, with daily doses ranging from 0.01 to 395 μmol·d^{-1}. The results reflect the entirety of the medicine's formula, as each component herb, which is believed to contribute to the therapeutic action of the medicine, had its constituents detected. Among the 12 classes of constituents detected, the most significant classes in terms of their total constituent daily doses in the medicine are organic acids, iridoids, monoterpene glycosides, and sesquiterpenoids (Fig. 7). Organic acids, including chlorogenic acid (**852**) and gallic acid (**651**), garnered attention for their anti-inflammatory and antioxidant activities. Research has also highlighted their ability to enhance renal function in rats, notably by reducing proteinuria [43–46]. Iridoids, such as specnuezhenide (**201**), have shown protective effects against renal injury in rats with diabetic nephropathy [47]. Monoterpene glycosides, such as paeoniflorin (**601**) and albiflorin (**602**), have been found to inhibit rat mesangial cell proliferation and inflammatory response [48, 49]. Sesquiterpenes, such as germacrone (**302**), curdione (**303**), and *β*-elemene (**401**), have been shown to possess anti-tumor activities against various cancer cells [50]. IgAN is a multifactorial disease. The therapeutic action of Compound Shenhua Tablet is likely to be multifaceted and is attributed to multiple active constituents. Understanding how this intricate herbal formulation delivers therapeutic benefits necessitates a transition from initial composition analysis to in-depth multi-compound pharmacokinetic investigations.

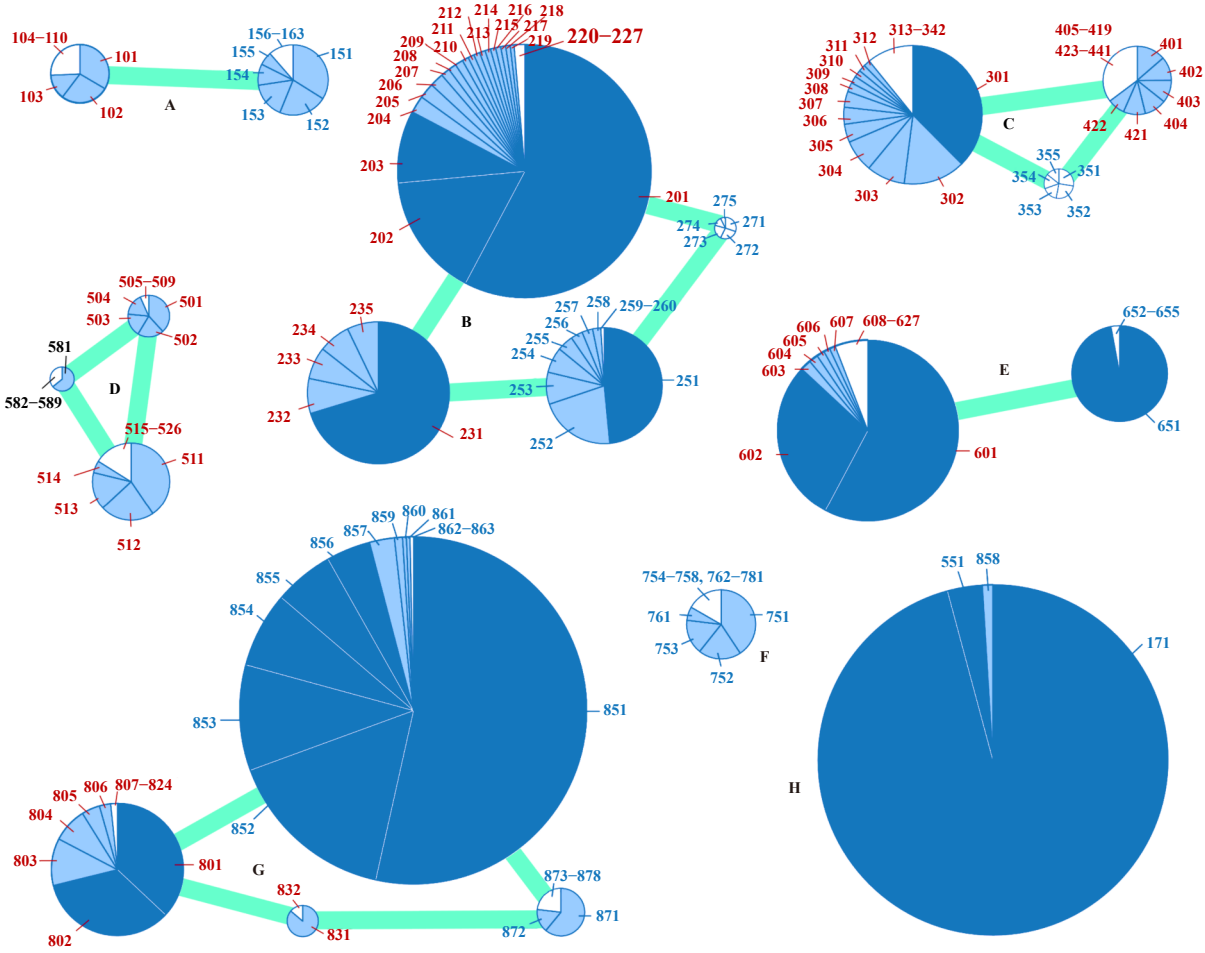

Fig. 7 Percentage daily doses of constituents in the total daily dose of all constituents of the class originating from each component herb of Compound Shenhua Tablet. Each constituent has an ID number and its name is shown in Tables S2 and S3. The constituents with bold IDs marked in red, blue, and black represent terpenoids, phenols/organic acids, and others, respectively. (A) Data of constituents originating from Huangqi (*A. membranaceus* roots); (B) Data of constituents from Nvzhenzi (*L. lucidum* fruits); (C) Data of constituents from Ezhu (*C. phaeocaulis* rhizomes); (D) Data of constituents from Baizhu (*A. macrocephala* rhizomes); (E) Data of constituents from Baishao (*P. lactilora* roots); (F) Data of constituents from Sanleng (*S. stoloniferum* rhizomes); (G) Data of constituents from Jinyinhua (*L. japonica* flower buds); (H) Data of the constituents citric acid (171), linoleic acid (551), and linolenic acid (858) from all the seven component herbs. Germacrone (302) and curdione (303) originated not only from Ezhu but from Baizhu as well, while secologanoside (802), quinic acid (851), caffeic acid (860), protocatechuic acid (862), and luteolin-7-*O*-*β*-D-glucoside (872) not only from Jinyinhua but from Nvzhenzi as well.

These investigations are pivotal for identifying and evaluating the key compounds from all the component herbs of the medicine, particularly in relation to their pharmacodynamic effects. The primary objective of these pharmacokinetic studies is to pinpoint compounds that are bioavailable at the targeted sites of action in their original or metabolized forms and that maintain significant exposure levels following the administration of the medicine. To achieve a reliable identification of potentially important compounds in the pharmacokinetic investigation, without overlooking any such compound (i.e., "precision without omission"), a comprehensive and precise composition analysis of the medicine is crucial, because the individual doses of constituents obtained through the analysis play a role in determining the levels of systemic exposure to the unchanged and/or metabolized compounds. In addition to composition analysis, the pharmacokinetic investigation integrates multiple studies and methods. These include a human pharmacokinetic study as the primary study, two types of supportive studies conducted in experimental animals and *in vitro* settings, and three groups of supportive techniques related to literature mining, sample analysis, and data processing [2, 3].

The current composition analysis has several limitations. A significant challenge in this analysis is the co-elution or inadequate chromatographic resolution of isobaric constituents, which are not distinguishable by MS alone. The strategies involving multidimensional chromatography and/or enantiomeric chromatography (for the resolution of herbal enantiomers) can provide useful solutions to this type of analytical challenge [51–54]. In addition, quantification of a type B constituent of Compound Shenhua Tablet was based on the regression equation of a structurally similar type A constituent for calibration, and the selection of the type A constituent is crucial for the accuracy of quantification of the type B constituent. However, the way to properly define a type A constituent possessing close structural similarity to the type B constituent remains to be delineated, such as correlation between the ionization response and the physicochemical properties of the analyte [55]. Furthermore, when GC/EI-MS was used in the composition analysis of Compound Shenhua Tablet, putative identification of compounds involved searching for a closely matched mass spectrum within the NIST database containing previously collected EI-MS reference spectra. A limitation arises when the database lacks a reference spectrum for a specific target compound, a situation not uncommon in the analysis of herbal medicines. Some constituents of these medicines might not be included in the database. The recent development of competitive fragmentation modeling for electron ionization presents a promising solution to this gap [56]. This innovative approach offers an alternative strategy for the identification of compounds in cases where no reference standards are available, particularly for metabolomics measurements. Its potential application in GC/EI-MS-based composition analysis of herbal medicines could significantly enhance the capability to identify and characterize previously challenging compounds due to the absence of reference spectra in standard databases.

The analysis of herbal medicine centers around obtaining comprehensive and precise information. In this investigation, we achieved a thorough and accurate understanding of the composition of the Compound Shenhua Tablet by focusing on five key stages. Pre-analysis literature mining: this initial stage involved extensive research to collect pertinent information essential for guiding the development of the analytical assay. Sample collection: this stage included the collection of diverse samples, encompassing different batches of Compound Shenhua Tablet, the component herbs, and essential oils from Baizhu and Ezhu. Additionally, samples from a simulated compound conversion study were gathered to assess changes in constituents during the manufacturing process. Analyte prediction: at this stage, we generated lists of candidate compounds. Assay development: the assay's development was a crucial stage, with a focus on fulfilling the analyte capacity requirements. Data processing: the final stage involved evaluating the assay's analyte-capacity. This step included the rigorous ranking and grading of a substantial number of herbal constituents based on their daily doses and content levels. We considered this analysis approach as the "one core and five elements" model. In summary, a total of 283 constituents with a daily dose of 0.01−395 μmol·d^{-1} (content level, 1−10 982 μg·g^{-1}) were detected in Compound Shenhua Tablet *via* LC/ESI-MS and GC/EI-MS. The detected constituents are classified into 12 classes, i.e., organic acids (originating from Huangqi, Jinyinhua, Baizhu, and Sanleng), iridoids (from Nvzhenzi and Jinyinhua), monoterpene glycosides (from Baishao), sesquiterpenes (from Ezhu and Baizhu), isoflavonoids (from Huangqi), monoterpenes (from Ezhu and Baizhu), triterpenes (from Nvzhenzi), triterpene saponins (from Nvzhenzi, Huangqi, and Jinyinhua), phenylethanoids (from Nvzhenzi), tannins (from Baishao), flavonoids (from Nvzhenzi and Jinyinhua), phenylpropanoids (from Sanleng). The major constituents (compound dose, ≥ 10 μmol·d^{-1}) were the organic acids citric acid (**171**), quinic acid (**851**), chlorogenic acid (**852**), cryptochlorogenic acid (**853**), gallic acid (**651**), neochlorogenic acid (**854**), isochlorogenic acid C (**855**), isochlorogenic acid B (**856**), and linoleic acid (**551**); the iridoids specnuezhenide (**201**), nuezhenoside G13 (**202**), secoxyloganin (**801**), and secologanoside (**802**); the monoterpene glycosides paeoniflorin (**601**) and albiflorin (**602**); the sesquiterpenoid curzerenone (**301**); the triterpene oleanolic acid (**231**); and the phenylethanoid salidroside (**251**). For precise and comprehensive composition analysis of such a complex herbal medicine, it is essential for the assay to have a high analyte-capacity. This capacity is crucial for the accurate identification and quantification of a wide spectrum of constituents in a single assay. In this study, we explored the concept of analyte-capacity within the context of composition analysis assays, introducing a method for its assessment. This method is retrospective, focusing on evaluating the assay's capacity to meet the demands of analyzing complex

herbal formulations. Though still in developmental stages, this concept and its assessment method have proven to be invaluable in evaluating the effectiveness of our assay for analyzing herbal medicine compositions. These tools also guide us in making necessary modifications to the assay for its intended use.

Supporting Information

Supporting information for this paper can be accessed by scanning the QR code or by requesting it *via* email from the corresponding authors.

References

[1] Li C. Multi-compound pharmacokinetic research on Chinese herbal medicines: approach and methodology [J]. *China J Chin Mater Med*, 2017, **42**(4): 607-617.

[2] Li C, Cheng C, Jia WW, *et al*. Multi-compound pharmacokinetic research on Chinese herbal medicines: identifying the medicines' potentially therapeutic compounds and characterizing their disposition and pharmacokinetics [J]. *Acta Pharm Sin*, 2021, **56**(9): 2426-2446.

[3] Li C, Jia WW, Yang JL, *et al*. Multi-compound and drug-combination pharmacokinetic research on Chinese herbal medicines [J]. *Acta Pharmacol Sin*, 2022, **43**(12): 3080-3095.

[4] Yu X, Niu W, Wang YY, *et al*. Novel assays for quality evaluation of Xuebijing: quality variability of a Chinese herbal injection for sepsis management [J]. *J Pharm Anal*, 2022, **12**(4): 664-682.

[5] Schaneberg BT, Sara Crockett, Bedir E, *et al*. The role of chemical fingerprinting: application to Ephedra [J]. *Phytochemistry*, 2003, **62**(6): 911-918.

[6] Olaleye OE, Niu W, Du FF, *et al*. Inhibition of hepatic OATP1Bs by circulating saponins from intravenous Shenmai: potential joint precipitants of drug interactions [J]. *Acta Pharmacol Sin*, 2019, **40**(6): 833-849.

[7] Pintusophon S, Niu W, Duan XN, *et al*. Intravenous formulation of *Panax notoginseng* root extract: human pharmacokinetics of ginsenosides and potential for perpetrating drug interactions [J]. *Acta Pharmacol Sin*, 2019, **40**(10): 1351-1363.

[8] Lai KN, Tang SC, Schena FP, *et al*. IgA nephropathy [J]. *Nat Rev Dis Primers*, 2016, **2**: 16001.

[9] Chen XM, Chen J, Chen YP, *et al*. Multicentered, randomized, controlled clinical trial on patients with IgA nephropathy of Qi-yin deficiency syndrome type [J]. *Chin J Integr Med*, 2007, **27**(2): 101-105.

[10] Li QP, Wei RB, Yang X, *et al*. Protective effects and mechanisms of Shenhua Tablet on toll-like receptors in rat model of renal ischemia-reperfusion injury [J]. *Chin J Integr Med*, 2019, **25**(1): 37-44.

[11] Geng WJ, Wei RB, Liu SW, *et al*. Shenhua Tablet inhibits mesangial cell proliferation in rats with chronic anti-Thy-1 nephritis [J]. *Biol Res*, 2016, **49**: 17.

[12] Li L, Zhao YS, Du FF, *et al*. Intestinal absorption and presystemic elimination of various chemical constituents present in GBE50 extract, a standardized extract of *Ginkgo biloba* leaves [J]. *Curr Drug Metab*, 2012, **13**(5): 494-509.

[13] Cheng C, Du FF, Yu K, *et al*. Pharmacokinetics and disposition of circulating iridoids and organic acids in rats intravenously receiving Reduning Injection [J]. *Drug Metab Dispos*, 2016, **44**(11): 1853-1858.

[14] Li MJ, Wang FQ, Huang YH, *et al*. Systemic exposure to and disposition of catechols derived from *Salvia miltiorrhiza* roots (Danshen) after intravenous dosing Danhong Injection in human subjects, rats, and dogs [J]. *Drug Metab Dispos*, 2015, **43**(5): 679-690.

[15] Li XX, Cheng C, Wang FQ, *et al*. Pharmacokinetics of catechols in human subjects intravenously receiving Xuebijing injection, an emerging antiseptic herbal medicine [J]. *Drug Metab Pharmacokinet*, 2016, **31**(1): 95-98.

[16] Cheng C, Lin JZ, Li L, *et al*. Pharmacokinetics and disposition of monoterpene glycosides derived from *Paeonia lactiflora* roots (Chishao) after intravenous dosing of antiseptic Xuebijing Injection in human subjects and rats [J]. *Acta Pharmacol Sin*, 2016, **37**(4): 530-544.

[17] Zhang NT, Cheng C, Olaleye OE, *et al*. Pharmacokinetics-based identification of potential therapeutic phthalides from Xuebijing, a Chinese herbal injection used in sepsis management [J]. *Drug Metab Dispos*, 2018, **46**(6): 823-834.

[18] Lan XF, Olaleye OE, Lu JL, *et al*. Pharmacokinetics-based identification of pseudoaldosterogenic compounds originating from *Glycyrrhiza uralensis* roots (Gancao) after dosing Lianhua Qingwen Capsule [J]. *Acta Pharmacol Sin*, 2021, **42**(12): 2155-2172.

[19] Chu C, Qi LW, Liu EH, *et al*. Radix Astragali (Astragalus): latest advancements and trends in chemistry, analysis, pharmacology and pharmacokinetics [J]. *Curr Org Chem*, 2010, **14**(16): 1792-1807.

[20] Su HF, Shaker S, Kuang Y, *et al*. Phytochemistry and cardiovascular protective effects of Huang-Qi (Astragali Radix) [J]. *Med Res Rev*, 2021, **41**(4): 1999-2038.

[21] Chang XN, Chen XF, Guo YX, *et al*. Advances in chemical composition, extraction techniques, analytical methods, and biological activity of Astragali Radix [J]. *Molecules*, 2022, **27**(3): 1058.

[22] Li H, Yao WF, Liu Q, *et al*. Application of UHPLC-ESI-Q-TOF-MS to identify multiple constituents in processed products of the herbal medicine Ligustri Lucidi Fructus [J]. *Molecules*, 2017, **22**(5): 689.

[23] Shang ZP, Xu LL, Zhang YQ, *et al*. An integrated approach to reveal the chemical changes of Ligustri Lucidi Fructus during wine steaming processing [J]. *J Pharm Biomed Anal*, 2021, **193**: 113667.

[24] Cao M, Wu J, Peng Y, *et al*. Ligustri Lucidi Fructus, a traditional Chinese medicine: comprehensive review of botany, traditional uses, chemical composition, pharmacology, and toxicity [J]. *J Ethnopharmacol*, 2023, **301**: 115789.

[25] Chang YL, Xu GL, Wang XP, *et al*. Anti-tumor activity and linear-diarylheptanoids of herbal couple Curcumae Rhizoma-Sparganii Rhizoma and the single herbs [J]. *J Ethnopharmacol*, 2020, **250**: 112465.

[26] Li YH, Wu YC, Li YM, *et al*. Review of the traditional uses, phytochemistry, and pharmacology of *Curcuma wenyujin* Y. H. Chen et C. Ling [J]. *J Ethnopharmacol*, 2021, **269**: 113689.

[27] Wu P, Dong XM, Song GQ, *et al*. Bioactivity-guided discovery of quality control markers in rhizomes of *Curcuma wenyujin* based on spectrum-effect relationship against human lung cancer cells [J]. *Phytomedicine*, 2021, **86**: 153559.

[28] Zhu B, Zhang QL, Hua JW, *et al*. The traditional uses, phytochemistry, and pharmacology of *Atractylodes macrocephala* Koidz. : a review [J]. *J Ethnopharmacol*, 2018, **226**: 143-167.
[29] Yang L, Yu H, Hou AJ, *et al*. A review of the ethnopharmacology, phytochemistry, pharmacology, application, quality control, processing, toxicology, and pharmacokinetics of the dried rhizome of *Atractylodes macrocephala* [J]. *Front Pharmacol*, 2021, **12**: 727154.
[30] Wu LF, Wang XQ, Chen XM, *et al*. Chemical constituents and pharmacological activities of *Radix Paeoniae Alba*: research advances [J]. *J Int Pharm Res*, 2020, **47**(3): 175-187.
[31] Yang SS, Zhang XY, Dong YQ, *et al*. Cleavage rules of mass spectrometry fragments and rapid identification of chemical components of Radix Paeoniae Alba using UHPLC-Q-TOF-MS [J]. *Phytochem Anal*, 2021, **32**(5): 836-849.
[32] Jia J, Li X, Ren XY, *et al*. Sparganii Rhizoma: a review of traditional clinical application, processing, phytochemistry, pharmacology, and toxicity [J]. *J Ethnopharmacol*, 2021, **268**: 113571.
[33] Lu XN, Zheng YW, Wen F, *et al*. Study of the active ingredients and mechanism of Sparganii Rhizoma in gastric cancer based on HPLC-Q-TOF-MS/MS and network pharmacology [J]. *Sci Rep*, 2021, **11**: 1905.
[34] Li YK, Li Wen, Fu CM, *et al*. Lonicerae Japonicae Flos and Lonicerae Flos: a systematic review of ethnopharmacology, phytochemistry and pharmacology [J]. *Phytochem Rev*, 2020, **19**(1): 1-61.
[35] Zheng SW, Liu ST, Hou AJ, *et al*. Systematic review of Lonicerae Japonicae Flos: a significant food and traditional Chinese medicine [J]. *Front Pharmacol*, 2022, **13**: 1013992.
[36] Pan HQ, Zhou H, Miao S, *et al*. An integrated approach for global profiling of multi-type constituents: comprehensive chemical characterization of Lonicerae Japonicae Flos as a case study [J]. *J Chromatogr A*, 2020, **1613**: 460674.
[37] Liu Y, Chen HB, Zhao YY, *et al*. Quantification and stability studies on the flavonoids of Radix Hedysari [J]. *J Agric Food Chem*, 2006, **54**(18): 6634-6639.
[38] Chu C, Liu EH, Qi LW, *et al*. Transformation of astragalosides from Radix Astragali under acidic, neutral, and alkaline extraction conditions monitored by LC-ESI-TOF/MS [J]. *Chin J Nat Med*, 2014, **12**(4): 314-320.
[39] Mei XD, Wang YQ, Liu ZH, *et al*. The chemical transformations for Radix Astragali *via* different alkaline wash conditions by quantitative and qualitative analyses [J]. *J Pharm Biomed Anal*, 2020, **185**: 113164.
[40] Giddings JC. Maximum number of components resolvable by gel filtration and other elution chromatographic methods [J]. *Anal Chem*, 1967, **39**(8): 1027-1028.
[41] Neue UD. Peak capacity in unidimensional chromatography [J]. *J Chromatogr A*, 2008, **1184**(1-2): 107-130.
[42] Stoll DR, Li XP, Wang XL, *et al*. Fast, comprehensive two-dimensional liquid chromatography [J]. *J Chromatogr A*, 2007, **1168**(1-2): 3-43.
[43] Bao LP, Li JS, Zha DQ, *et al*. Chlorogenic acid prevents diabetic nephropathy by inhibiting oxidative stress and inflammation through modulation of the Nrf2/HO-1 and NF-κB pathways [J]. *Int Immunopharmacol*, 2018, **54**: 245-253.
[44] Ahad A, Ahsan H, Mujeeb M, *et al*. Gallic acid ameliorates renal functions by inhibiting the activation of p38 MAPK in experimentally induced type 2 diabetic rats and cultured rat proximal tubular epithelial cells [J]. *Chem Biol Interact*, 2015, **240**: 292-303.
[45] Bai JR, Zhang YS, Tang C, *et al*. Gallic acid: pharmacological activities and molecular mechanisms involved in inflammation-related diseases [J]. *Biomed Pharmacother*, 2021, **133**: 110985.
[46] Peng CC, Hsieh CL, Wang HE, *et al*. Ferulic acid is nephrodamaging while gallic acid is renal protective in long term treatment of chronic kidney disease [J]. *Clin Nutr*, 2012, **31**(3): 405-414.
[47] Yin JN, Jiang J, Wang HJ, *et al*. Protective effects of specneuzhenide on renal injury in rats with diabetic nephropathy [J]. *Open Med (Wars)*, 2019, **14**: 740-747.
[48] Liu BH, Lin J, Bai LX, *et al*. Paeoniflorin inhibits mesangial cell proliferation and inflammatory response in rats with mesangial proliferative glomerulonephritis through PI3K/AKT/GSK-3*β* pathway [J]. *Front Pharmacol*, 2019, **10**: 978.
[49] Yu HY, Wang Y, He ZD, *et al*. Albiflorin ameliorates mesangial proliferative glomerulonephritis by PI3K/AKT/NF-κB pathway [J]. *Hum Exp Toxicol*, 2023, **42**: 9603271221145386.
[50] Chen Y, Zhu ZP, Chen J, *et al*. Terpenoids from Curcumae Rhizoma : their anticancer effects and clinical uses on combination and versus drug therapies [J]. *Biomed Pharmacother*, 2021, **138**: 111350.
[51] Giddings JC. Two dimensional separations: concept and promise [J]. *Anal Chem*, 1984, **56**(12): 1258A-1270A.
[52] Qiu S, Yang WZ, Shi XJ, *et al*. A green protocol for efficient discovery of novel natural compounds: characterization of new ginsenosides from the stems and leaves of *Panax ginseng* as a case study [J]. *Anal Chim Acta*, 2015, **893**: 65-76.
[53] Zhou WJ, Guo ZM, Yu L, *et al*. On-line comprehensive two-dimensional liquid chromatography tandem mass spectrometry for the analysis of *Curcuma kwangsiensis* [J]. *Talanta*, 2018, **186**: 73-79.
[54] Wang M, Xu XY, Wang HD, *et al*. A multi-dimensional liquid chromatography/high-resolution mass spectrometry approach combined with computational data processing for the comprehensive characterization of the multicomponents from *Cuscuta chinensis* [J]. *J Chromatogr A*, 2022, **1675**: 463162.
[55] Kruve A. Strategies for drawing quantitative conclusions from nontargeted liquid chromatography-high-resolution mass spectrometry analysis [J]. *Anal Chem*, 2020, **92**(7): 4691-4699.
[56] Allen F, Pon A, Greiner R, *et al*. Computational prediction of electron ionization mass spectra to assist in GC/MS compound identification [J]. *Anal Chem*, 2016, **88**(15): 7689-7697.

Cite this article as: ZHANG Haiyan, WANG Qiuyue, WANG Jianan, ZHANG Sichao, JIA Weiwei, HE Ning, XIA Xiaoyan, WANG Ting, LAI Liyu, LI Jiaying, DU Jing, OLALEYE Olajide E., CHEN Xiangmei, YANG Junling, LI Chuan. Composition analysis of Compound Shenhua Tablet, a seven-herb Chinese medicine for IgA nephropathy: evaluation of analyte-capacity of the assays [J]. *Chin J Nat Med*, 2024, **22**(2): 178-192.