separations

MDPI

Article

Quality Standardization and Chemical Consistency Assessment of Chijasi-tang (Zhizichi Decoction) via Simultaneous Determination of Five Marker Compounds and Chemical Fingerprinting by UPLC-PDA

Hyun-Je Park [1], Jiha Jeong [2], Hee Jin Wang [2,*], Do-Eun Lee [3] and Hyung Won Kang [2,3,4,*]

[1] Yuhan Care R&D Center, Yuhan Care Co., Ltd., Hwaseong-si 18469, Gyeonggi-do, Republic of Korea; hjpark@yuhancare.com
[2] Department of Integrative Medicine, Division of Biomedical and Health Service Convergence, Graduate School of Jeonbuk Advanced Bio-Convergence Academy (JABA), Wonkwang University, Iksan 54538, Jeonbuk, Republic of Korea; wlgk0000@gmail.com
[3] Department of Korean Neuropsychiatry Medicine, College of Korean Medicine, Wonkwang University, Iksan 54538, Jeonbuk, Republic of Korea; dolee0210@gmail.com
[4] Integrative Medicine Innovation Center, Wonkwang University, Iksan 54538, Jeonbuk, Republic of Korea
* Correspondence: tuzi522@naver.com (H.J.W.); dskhw@wku.ac.kr (H.W.K.); Tel.: +82-10-2251-9786 (H.J.W.); +82-10-2532-0884 (H.W.K.)

Abstract

Chijasi-tang (Zhizichi decoction), a classical two-herb formula used for neuropsychiatric symptoms, combines an iridoid-rich fruit with a fermented and steamed soybean preparation, and no validated single-run assay covering the markers of both herbs, or data on the variability in commercial raw materials, has been reported. Geniposide (from *Gardenia jasminoides*) and four isoflavones (daidzin, genistin, daidzein, and genistein; from fermented *Glycine max*) were selected as markers and identified by Q-TOF MS and UV spectra. A UPLC-PDA method (254 nm) using an HSS C18 column (1.8 µm, 2.1 × 100 mm) separated the five reference standards within 12 min (resolution between adjacent markers ≥ 12.37; 25-min total run time) and was validated according to ICH Q2(R2); in the extracts, the closest marker-to-matrix pair was daidzin and an adjacent matrix peak (retention-time difference, 0.17 min). Six commercial raw materials were analyzed, and water, 30% ethanol, and 70% ethanol extracts (one per Gardeniae Fructus lot; three per solvent) were characterized by marker content and a 14-peak fingerprint. The method showed $r^2 \geq 0.9995$, mean spike recoveries of 85.54–109.30%, intra- and inter-day precision RSD ≤ 4.18%, and test-solution stability for 156 h. One Korean Gardeniae Fructus lot contained 2.40% geniposide, below the Korean Pharmacopoeia limit (≥ 3.0%), and no isoflavones were detected in one Chinese Dandouchi lot. Provisional marker specifications were proposed for each solvent. Fingerprint correlations were r ≥ 0.954 within each solvent system but did not reflect lot-level differences in geniposide content. The method is suitable for the routine quality control of Chijasi-tang, and the results indicate that raw material qualification, fingerprint-based identity testing, and quantitative marker assays should be combined. These exploratory, laboratory-scale data may inform the future CMC development of Chijasi-tang preparations.

Keywords: Chijasi-tang; quality standardization; geniposide; isoflavones; UPLC-PDA; chemical markers; chemical fingerprint; CMC (chemistry, manufacturing and controls); ICH validation

Check for updates

Academic Editor: Marcello Locatelli

Received: 13 October 2026
Revised: 2 November 2026
Accepted: 3 November 2026
Published: 6 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Chijasi-tang (known as Zhizichi decoction in Chinese), composed of *Gardenia jasminoides* (*G. jasminoides*) and fermented *Glycine max* (*G. max*), was first documented in the classical medical treatise *Shanghan Zabing Lun* (Treatise on Febrile and Miscellaneous Diseases) during the Han Dynasty [1] and has since been extensively prescribed in East Asian traditional medicine, including the *Donguibogam* (Mirror of Eastern Medicine) [2], for relieving restlessness, severe insomnia, and depressive mood [3]. In contemporary clinical practice, Chijasi-tang is used for the management of chronic stress-induced neuropsychiatric conditions, such as autonomic dysfunction, major depressive disorder, generalized anxiety disorder, and sleep disturbances [3,4]. Recent preclinical studies in rodent models have reported antidepressant-like effects of the formula, associated with modulation of PACAP/VIP signaling in the prefrontal cortex [5] and with stabilization of the hypothalamic–pituitary–adrenal (HPA) axis and catecholamine (norepinephrine and epinephrine) homeostasis under stress conditions [6].

To translate such traditional multi-herb formulations into standardized modern pharmaceuticals (e.g., botanical drug products and standardized herbal medicines), non-clinical and clinical evaluation must be supported by a Chemistry, Manufacturing, and Controls (CMC) framework that ensures batch-to-batch physicochemical consistency from raw botanical materials to finished dosage forms [7–9]. Consequently, quantitative standardization of representative chemical markers has been widely pursued for complex herbal preparations, such as LMK02 for dementia [10] and GCSB-5 for osteoarthritis [11]. However, multi-herb matrices inherently display pronounced qualitative and quantitative variations in secondary metabolite profiles due to geographic origin, environmental factors, harvesting seasons, and post-harvest processing or fermentation procedures [12,13]. To mitigate this intrinsic variability, major regulatory authorities—including the US Food and Drug Administration (FDA) [8], the European Medicines Agency (EMA) [14–16], and the Korean Ministry of Food and Drug Safety (MFDS) [7,9,17]—recommend a dual-tiered quality control approach: (1) quantitative assay of bioactive and marker compounds using validated analytical methods, and (2) chromatographic or spectroscopic chemical fingerprinting that captures the multi-component profile [17,18]. This approach is likewise reflected in the World Health Organization (WHO) Guidelines on Good Agricultural and Collection Practices (GACP) and quality control of herbal materials [19,20].

Regarding the botanical components of Chijasi-tang, *G. jasminoides* is rich in iridoid glycosides (e.g., geniposide and genipin) and carotenoid pigments (e.g., crocin and crocetin), which exhibit choleretic [21], glutathione S-transferase (GST)-inducing [22], antithrombotic [23], antioxidant, antitumor [24,25], and monoamine oxidase (MAO)-inhibiting antidepressant activities [26]. The companion herb, Hyangsi (or Dandouchi; the fermented mature seed of *G. max*) [21,27], contains substantial amounts of dietary isoflavones, including daidzin, genistin, daidzein, and genistein. These isoflavonoids have been reported to exert anticancer [28,29], hypolipidemic, antithrombotic [30], and neuroendocrine-regulatory effects [31].

Recently, Guo et al. (2023) investigated the multi-component profile of Chijasi-tang using liquid chromatography–high-resolution mass spectrometry (LC-HRMS), elucidating the herbal compatibility mechanism—traditionally described as attenuation and synergy—by tracking chemical transitions, solubility alterations of potentially toxic constituents, and enhanced dissolution of active ingredients during co-decoction [32]. That study was designed for mechanistic characterization rather than routine quality control, and several questions relevant to quality control therefore remain open. First, LC-MS and LC-MS/MS methods are widely and successfully used for quantitative quality control, including in regulated manufacturing environments. However, when the markers carry

strong UV chromophores and are present at μg/mL levels in the test solution, as in the present case, LC with photodiode array (PDA) detection provides adequate sensitivity at lower instrument and operating cost, is the technique used in the relevant pharmacopoeial assays (e.g., the geniposide assay of the Korean Pharmacopoeia [33]), and is available in most herbal medicine manufacturing and testing laboratories. Second, a quantitative procedure intended for batch release must be validated for its intended purpose according to the International Council for Harmonisation (ICH) Q2(R2) guideline [34]; Guo et al. [32] focused on qualitative identification and dissolution shifts. Third, that study used laboratory-prepared samples, so the variability in commercial raw materials—in particular of the fermented and repeatedly steamed soybean preparation (Dandouchi), whose isoflavone content may be altered by processing—was not addressed.

From an analytical standpoint, Chijasi-tang poses three specific problems for a single quality control assay: (i) The markers span a wide polarity range, from a highly polar iridoid glycoside (geniposide) to isoflavone glycosides and the comparatively hydrophobic aglycones daidzein and genistein. (ii) Their contents in the finished extracts differ by up to two orders of magnitude (approximately 1.5–4.7% geniposide versus 0.01–0.48% for the individual isoflavones; see Section 3.3), so a single injection must quantify a dominant peak and minor peaks within calibrated ranges. (iii) The fermented soybean matrix contains isoflavone conjugates and other co-extracted constituents that elute in the same retention window as the isoflavone glycosides, creating a risk of marker-to-matrix co-elution that is not apparent when only reference standards are examined. Previously reported chromatographic methods addressed each herb separately: geniposide and related iridoids in Gardeniae Fructus have been determined by HPLC-DAD-ESI-MS [35], and soy isoflavones by reversed-phase HPLC [36], frequently with run times of 45–70 min on conventional 5 μm columns or with separate detection wavelengths [35,36]. Applying two single-herb procedures to one formula doubles the analytical workload and does not yield a common chromatographic profile that can be used for identity and consistency testing of the combined extract. Moreover, to our knowledge, it has not been examined whether the commonly used fingerprint similarity criterion ($r \geq 0.95$) is sensitive enough to detect a quantitative deficiency in an individual marker of such a formula, and commercial raw materials used for Chijasi-tang have not been assessed with a validated assay.

To address these gaps, the present study had four objectives: (1) to develop and validate, according to ICH Q2(R2) [34], a single-run UPLC-PDA assay for geniposide (*G. jasminoides*) and four isoflavones (*G. max*), whose identities were confirmed by high-resolution Q-TOF MS and UV spectra, with a separate evaluation of the resolution between the reference standards (standard-to-standard) and between each marker and adjacent matrix peaks in the extracts (marker-to-matrix); (2) to apply the assay to commercial raw materials of both herbs to assess their compendial compliance; (3) to characterize water, 30% EtOH, and 70% EtOH extracts and derive provisional marker specifications; and (4) to evaluate a 14-peak chromatographic fingerprint as an identity and consistency test and to examine experimentally whether fingerprint similarity can substitute for marker quantification. The contribution of this work is therefore not a new separation principle but a validated, fit-for-purpose quality assessment strategy for a formula for which none has been reported, together with experimental evidence on how the marker assay and the fingerprint complement each other (Section 4.7). These exploratory, laboratory-scale data are intended to inform, rather than constitute, the CMC documentation of Chijasi-tang preparations.

2. Materials and Methods

2.1. Plant Materials, Reference Standards, and Reagents

Three batches of *G. jasminoides* were procured: a domestic cultivated sample from Wando, Jeonnam, Republic of Korea (Omniherb Co., Ltd., Yeongcheon, Republic of Korea; Lot No. Gardenia #1), and wild (Lot No. Gardenia #2) and cultivated (Lot No. Gardenia #3) samples originating from Henan Province, China (Dongkyung Corp., Seoul, Republic of Korea) [33,37]. Three commercial preparations of fermented *G. max* (Hyangsi/Dandouchi; Sojae Semen Praeparatum in pharmacopeial nomenclature) were acquired: domestic fermented soybean from Gyeongbuk, Korea (Bareun Herb Co., Ltd., Daegu, Republic of Korea; Lot No. Glycine #1); fermented Dandouchi from Henan Province, China (Dongkyung Corp.; Lot No. Glycine #2); and commercial Cheonggukjang pills (Hansalim Co., Ltd., Seoul, Republic of Korea; Lot No. Glycine #3) [21,27]. The Gardeniae Fructus samples were authenticated via morphological inspection by a pharmacognosist according to the identification criteria of the Korean Pharmacopoeia (KP XII) and the Korean Herbal Pharmacopoeia (KHP) [27,33]. To evaluate raw material variability and to test whether the analytical and chemical fingerprinting procedures could detect sub-compendial botanical materials, Gardenia #1 (which contained 2.40% geniposide, below the KP compendial limit of $\geq$ 3.0%) was intentionally included in the extraction series alongside the compendial-conforming Chinese lots. Because the fermented soybean preparations are processed materials for which morphological authentication is limited, their botanical identity and geographical origin are as stated by the suppliers, and no independent identification test was performed on these materials. Cheonggukjang pills (Glycine #3), a popular Korean fermented whole-soybean food preparation that is not covered by the KHP monograph, were included as a comparative commercial fermented soybean reference to examine whether traditional culinary fermentation profiles parallel pharmacopoeial medicinal Dandouchi.

Reference standards of geniposide (CAS No. 24512-63-8; Lot No. BCBV1845; purity $\geq$ 98.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Daidzin (CAS No. 552-66-9; Lot No. BCBW9214; purity 98.7%), genistin (CAS No. 529-59-9; Lot No. BCBZ4321; purity 98.8%), daidzein (CAS No. 486-66-8; Lot No. BCBT5567; purity 98.1%), and genistein (CAS No. 446-72-0; Lot No. BCBX8899; purity 97.2%) were obtained from Fluka Chemie GmbH (Buchs, Switzerland). HPLC-grade acetonitrile and methanol were obtained from Burdick & Jackson (Muskegon, MI, USA). Formic acid (MS-grade, $\geq$ 98.0%) was supplied by Sigma-Aldrich (St. Louis, MO, USA). Ultrapure deionized water (resistivity $\geq$ 18.2 MΩ·cm) was produced using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Preparation of Prescriptions, Standard Extracts, and Test Solutions

The formulation ratio of Chijasi-tang was standardized based on clinical practice guidelines in the Illustrated Compendium of Korean Herbal Formulas [4], consisting of 20 g of Gardeniae Fructus and 100 g of fermented *G. max* (dry weight ratio 1:5; total 120 g per single prescription dose). To compare different solvent systems, three types of dry extracts (distilled water, 30% *v*/*v* aqueous ethanol, and 70% *v*/*v* aqueous ethanol) were prepared under controlled laboratory conditions: (1) Pulverized Gardeniae Fructus (20 g) was placed in a reflux extractor with 2.6 L of each respective solvent (approximately 21.7 mL per g of the total 120 g of raw material, i.e., a solvent-to-material ratio of approximately 21.7:1, *v*/*w*) and pre-decocted under reflux for 2 h at the actual boiling temperature of each solvent (98–100 °C for water, 86–88 °C for 30% *v*/*v* aqueous ethanol, and 80–82 °C for 70% *v*/*v* aqueous ethanol). (2) Subsequently, 100 g of fermented *G. max* was introduced into the boiling mixture and further extracted under reflux for 40 min (total extraction time: 2 h 40 min), following the sequential decoction order (Gardeniae Fructus first, fermented

soybean added later) described in the *Shanghan Zabing Lun* [1]. Domestic fermented soybean (Glycine #1) was consistently employed as the fermented *G. max* component across all 9 extract preparations (3 Gardenia lots × 3 extraction solvent systems = 9 batches), ensuring that chemical variations in the finished extracts directly reflected the differing characteristics of the Gardeniae Fructus lots and extraction solvents. One extract was prepared from each Gardeniae Fructus lot with each solvent system (n = 3 lots per solvent; nine extracts in total). (3) The extract was vacuum-filtered through qualitative filter paper (pore size 25 μm), concentrated under reduced pressure using a rotary evaporator (EYELA, Tokyo, Japan) at 50 °C, and lyophilized using a freeze dryer (FD-8512, IlShin BioBase, Yangju, Republic of Korea) to yield dry extract powders. These process conditions were fixed a priori and were not optimized.

The mean final yields (w/w, relative to 120 g raw material) of the freeze-dried extracts across the three Gardenia lots were 9.0 ± 0.1% (10.8 g; Lot 1: 9.1%, Lot 2: 9.0%, Lot 3: 8.9%) for water, 8.2 ± 0.1% (9.8 g; Lot 1: 8.3%, Lot 2: 8.2%, Lot 3: 8.1%) for 30% ethanol, and 7.1 ± 0.1% (8.5 g; Lot 1: 7.2%, Lot 2: 7.1%, Lot 3: 7.0%) for 70% ethanol. For extract test solution preparation, 100 mg of each dry extract powder was accurately weighed, suspended in 40 mL of 50% aqueous methanol, and ultrasonically extracted (Power Sonic 520, Hwashin, Seoul, Republic of Korea; 40 kHz, 300 W) at ambient temperature for 60 min. The suspension was quantitatively transferred to a 50 mL volumetric flask, brought to volume with 50% aqueous methanol (final concentration: 2.0 mg/mL), and thoroughly homogenized. The solution was filtered through a 0.45 μm PTFE syringe filter (Millipore), with the initial 2 mL discarded prior to autosampler injection. The corresponding standard solution contained approximately 40 μg/mL geniposide and 20 μg/mL of each isoflavone in methanol. Marker contents were calculated by the external standard method from the ratio of the peak areas of the test and standard solutions, corrected for the purity and weight of the reference standard and for the weight and dilution of the sample.

Marker contents of the extracts and raw materials are expressed as % (w/w) on an as-is basis; loss on drying was not determined, and no correction for residual moisture was applied. Because the KP limit for geniposide in Gardeniae Fructus (≥3.0%) applies to the dried material [33], the as-is values for the raw materials represent lower-bound estimates of the dried-basis content. For Gardenia #1 (2.40%), the dried-basis value would reach the limit only if the moisture content were at least 20% (2.40%/[1 − 0.20] = 3.0%).

For the analysis of raw botanical materials (Section 3.1), pulverized Gardeniae Fructus (1.0 g) was extracted with 70 mL of methanol under reflux on a water bath for 1 h; after cooling, the extract was filtered and made up to 100 mL with methanol, and 5 mL of this solution was diluted to 50 mL with methanol. Pulverized fermented soybean (2.0 g) was extracted with 70 mL of water/methanol (20:80, v/v) under reflux for 1 h, filtered, and made up to 100 mL with the same solvent. Geniposide and the four isoflavones were quantified against standard solutions of geniposide (40 μg/mL) and of the four isoflavones (10 μg/mL each) in methanol, respectively, using the same calculation. A single extraction was performed, and completeness of extraction was not verified by the re-extraction of the residue.

2.3. UPLC-PDA Chromatographic Conditions

Chromatographic separation was performed on a Waters Acquity UPLC® system (Waters Corp., Milford, MA, USA) equipped with a quaternary solvent manager, a sample manager-FTN, and a photodiode array (PDA) detector. Separation was performed on a Waters Acquity UPLC HSS C18 column (1.8 μm, 2.1 × 100 mm) protected by a Vanguard pre-column (1.8 μm, 2.1 × 5 mm) maintained at 30 °C. The mobile phase comprised 0.1%

(*v*/*v*) formic acid in water/acetonitrile (99:1, *v*/*v*; Mobile Phase A) and 0.1% (*v*/*v*) formic acid in acetonitrile (Mobile Phase B).

The linear gradient elution program was as follows: 0–3 min, 0–10% B; 3–15 min, 10–50% B; 15–20 min, 50–90% B; 20–21 min, 90–0% B; and 21–25 min, with re-equilibration at 0% B (total run time, 25 min; 0% B corresponds to 1% acetonitrile). The mobile phase flow rate was set at 0.25 mL/min, and the sample injection volume was 2.0 µL. UV absorption spectra were acquired over the wavelength range of 200–500 nm. Simultaneous quantitative determination and chromatographic fingerprinting of finished extracts were performed at a monitoring wavelength of 254 nm, where all five target markers display adequate absorbance. For Gardeniae Fructus raw materials (Section 3.1), geniposide was quantified at 240 nm (its λmax), in line with the Korean Pharmacopoeia monograph, using a calibration curve constructed at the same wavelength.

The chromatographic conditions were selected by comparing three sub-2-µm reversed-phase columns (Acquity UPLC BEH C18, 1.7 µm; HSS T3, 1.8 µm; and HSS C18, 1.8 µm; all 2.1 × 100 mm), using water/acetonitrile containing formic acid as the mobile phase under isocratic and gradient elution. The HSS C18 column with gradient elution was selected because it provided sufficient retention of the highly polar constituents, including geniposide, and the best resolution among the five markers. A systematic optimization of column selectivity and mobile-phase composition (e.g., by experimental design) was not performed; the term "selected conditions" is therefore used throughout.

2.4. Q-TOF MS Parameters for Identification

Chemical identification of the five marker compounds was performed using a Q-TOF Premier mass spectrometer (Waters/Micromass, Manchester, UK) with an electrospray ionization (ESI) interface, coupled to an Acquity UPLC system. For the LC-MS analyses, separation was performed on an Acquity UPLC BEH C18 column (1.7 µm, 2.1 × 100 mm) at 30 °C with the same mobile phases at 0.25 mL/min (injection volume, 2 µL) using the gradient 0–3 min, 0–10% B; 3–13 min, 10–50% B; 13–18 min, 50–90% B; 18–19 min, 90–0% B; and 19–23 min, 0% B; retention times in the LC-MS runs therefore differ from those of the UPLC-PDA method. Positive ionization mode (ESI+) was operated with the following parameters: capillary voltage, 3.0 kV; sampling cone voltage, 35 V; extraction cone voltage, 4.0 V; source temperature, 100 °C; desolvation temperature, 250 °C; desolvation gas flow, 550 L/h; scan time, 0.3 s; mass range, *m*/*z* 100–500. Spectra were acquired in two functions (function 1, low collision energy; function 2, elevated collision energy); the fragment ions reported here were observed in function 1 as in-source fragments, and no precursor-selected MS/MS experiments were performed. Lock-mass correction was not applied; the observed *m*/*z* values therefore showed a consistent positive deviation (+23 to +38 ppm; Table S1), and identification was based on agreement of mass spectra, retention times, and UV spectra with those of authentic reference standards [35,36]. Reference standard solutions (geniposide, 10 µg/mL; isoflavones, 5 µg/mL each, in methanol) and test solutions of the raw materials and of the Chijasi-tang 30% ethanol extract were analyzed.

2.5. Method Validation per ICH Guidelines

The developed UPLC-PDA analytical procedure was validated following the International Council for Harmonisation (ICH) Q2(R2) guidelines [34] and MFDS validation regulations [38]. System suitability was assessed by analyzing six consecutive replicates of the mixed reference standard solution to determine the relative standard deviation (%RSD) of retention times (t_R), peak areas, resolution (R_s), tailing factors (T), and theoretical plate numbers (N). Theoretical plate numbers (N) were calculated according to the USP half-height formula, $N = 5.54 \times (t_R/W_{0.5})^2$, where t_R is retention time and $W_{0.5}$ is

peak width at half-height. Resolution was calculated with the USP half-height formula, $R_s = 1.18 \times (t_{R,2} - t_{R,1})/(W_{0.5,1} + W_{0.5,2})$, where $W_{0.5}$ is the peak width at half-height, and was assessed at two levels: between adjacent marker peaks in the mixed reference standard solution (standard-to-standard resolution) and between each marker and, in the extract test solutions, as the retention-time difference between each marker and the nearest adjacent matrix peak (marker-to-matrix separation).

Specificity was demonstrated by comparing chromatograms of the blank diluent (50% aqueous methanol), mixed reference standard solution, and Chijasi-tang extract solutions to confirm the absence of interfering peaks at the retention windows of target analytes. Peak purity was evaluated across the PDA spectral range (200–500 nm) using Empower 3 software (Waters). In this algorithm, the purity angle is the weighted mean of the spectral contrast angles between the spectrum at the peak apex and every other spectrum acquired across the peak, and the purity threshold is the angle that can be attributed to baseline noise and solvent absorbance alone. A purity angle smaller than the purity threshold therefore indicates that the spectral variation across the peak does not exceed that expected from noise, i.e., there is no spectral evidence of a co-eluting compound. Because a co-eluting compound with a very similar UV spectrum or present at a very low relative concentration may escape this test, peak purity was used together with resolution and spike-recovery data rather than as sole proof of specificity. For all five marker peaks in the standard and extract solutions, the purity angle was smaller than the purity threshold. Linearity was evaluated at six concentration levels (1%, 10%, 25%, 50%, 100%, and 150% of the target concentration) for each analyte. The target (100%) concentrations were 40 μg/mL for geniposide and 20 μg/mL for each isoflavone, corresponding to the standard solution used for quantification, and the calibration ranges were 0.4–60 μg/mL for geniposide and 0.2–30 μg/mL for the four isoflavones. The geniposide concentrations in some extract test solutions (up to approximately 95 μg/mL) exceeded the upper calibration level; this is addressed in Section 4.6. Calibration curves were generated by plotting peak area (y) against nominal concentration (x, μg/mL). LOD and LOQ were defined as the concentrations giving signal-to-noise ratios of 3:1 and 10:1, respectively, and were estimated from a two-fold dilution of the lowest calibration standard.

Accuracy was evaluated by spike recovery: the 30% ethanol extract (20 mg) was spiked with the mixed standard stock solution at three levels corresponding to 50%, 100%, and 150% of the target concentration (approximately 20, 40, and 60 μg/mL for geniposide and 10, 20, and 30 μg/mL for each isoflavone), made up to 10 mL as described for the test solutions, and quantified against the external standard; recovery was calculated after subtraction of the native content of the unspiked extract. Three spiked solutions were prepared independently at each level ($n = 3$), and the procedure was repeated on a second day. Repeatability was expressed as the %RSD of the recoveries at each level ($n = 3$), and intermediate precision was expressed as the %RSD of the six level-mean recoveries obtained on the two days (three levels × two days). Predefined acceptance criteria were a mean recovery of 85.0–115.0% and a %RSD $\leq$ 5.0% [38]. Solution stability was evaluated using the mixed standard solution and the 30% ethanol extract test solution stored at room temperature and analyzed at 0, 1, 29, 64, 83, and 156 h; the peak areas were compared with those of the initial injection (t = 0 h; acceptance criterion, within ±5%).

Because quantification was based on external calibration with standards prepared in solvent, the spike recovery experiment in the 30% ethanol extract was used to assess whether co-extracted matrix components biased quantification; a separate comparison of calibration slopes in solvent and matrix was not performed.

Method robustness was evaluated by deliberately introducing minor, controlled variations in flow rate (0.25 ± 0.02 mL/min), column temperature (30 ± 2 °C), and mobile

phase formic acid content (0.10 ± 0.01% *v*/*v*). Acceptance criteria were retention time %RSD < 1.5%, resolution between adjacent marker peaks ≥ 1.5, and recovery within 98.0–102.0% of the values obtained under nominal conditions.

2.6. Chemical Fingerprinting and Similarity Assessment

The fingerprint was designed to serve two purposes: (i) identity confirmation of the combined extract, i.e., verification that both constituent herbs are present and that neither has been omitted or substituted, and (ii) assessment of the batch-to-batch consistency of the overall chromatographic profile. The botanical origin of each characteristic peak was assigned by comparison of retention times and UV spectra with the chromatograms of the individual raw materials (see Section 3.1): Peaks 2–4 and geniposide were attributed to Gardeniae Fructus, and other peaks were assigned as follows: Peak 1, daidzin; Peak 5, genistin; Peak 6 and Peak 9, daidzein and genistein to fermented soybean. Peaks 7 and 8 could not be unambiguously assigned to a single herb, because minor peaks were observed in the corresponding retention window in the chromatograms of both raw materials. Fourteen representative characteristic peaks (five marker components and nine common matrix peaks) displaying adequate signal intensity and reproducible retention (Peaks 7 and 8 eluted closely together, Δt_R 0.10 min, and Peak 5 eluted immediately after daidzin, Δt_R 0.17 min) were designated for chromatographic fingerprinting [17]. Geniposide was designated as the internal reference peak (S) due to its high abundance and stable retention behavior. Relative retention times (RRTs) and relative peak areas (RPAs) of the remaining 13 peaks were calculated using the equations $RRT_i = t_{R,i}/t_{R,S}$ and $RPA_i = A_i/A_S$, respectively. For identity, a sample was considered to conform when all 14 characteristic peaks were detected within ±0.02 of their mean RRT values. Within the two closely eluting pairs (daidzin/Peak 5 and Peaks 7/8), whose RRT windows overlap, peaks were assigned by their elution order and UV spectra.

Nine laboratory-scale extracts (three Gardeniae Fructus lots × three solvent systems; n = 3 per solvent system) were evaluated, and each extract was analyzed in triplicate. For each solvent system, two reference profiles were constructed from the 14-peak areas: the mean-based reference, obtained as the element-wise arithmetic mean, and the median-based reference, obtained as the element-wise median of the nine injections (three extracts × triplicate injections) [17,18]. Each extract was represented by the 14-peak area vector averaged over its triplicate injections. Because all 14 peaks were individually integrated and matched by RRT, no retention-time alignment, weighting, or scaling was applied. Calculations were performed with Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA). Because each reference profile includes the sample being compared, the correlation coefficients describe consistency within this set of extracts and are expected to be optimistic. Batch-to-batch chemical consistency was evaluated by calculating the Pearson correlation coefficient (r) using Equation (1):

$$r = \frac{\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \overline{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \overline{y})^2}} \quad (1)$$

where x_i and y_i represent the characteristic peak areas of individual batch samples and the corresponding reference profile, respectively, and $\overline{x}$ and $\overline{y}$ denote their respective arithmetic means. Batches exhibiting r ≥ 0.95 were considered chemically consistent, in accordance with regulatory guidance for botanical extract fingerprinting (MFDS Chemical Profiling Guideline [17]; Liang et al. [13]). In addition, because raw peak areas are dominated by geniposide, the Pearson correlation of the RPA vectors (RPA-normalized similarity) and the cosine similarity (congruence coefficient, $\cos\theta = \Sigma x_i y_i / (\Sigma x_i^2\, \Sigma y_i^2)^{1/2}$) were also calculated from the same 14-peak area data; these normalized indices were used for comparison only,

and no acceptance criterion was applied to them. The method precision of the fingerprint (RT, RRT, PA, and RPA) was evaluated by six replicate injections of the water extract test solution and across three consecutive days (n = 9).

3. Results

3.1. Structural Identification of Marker Compounds and Quality Evaluation of Raw Botanical Materials

The chemical structures of the five analytical markers were confirmed by comparing their accurate masses ($[M + H]^+$ or $[M + Na]^+$), fragment ions, and UV absorption maxima (λmax) with those of authenticated reference standards (Figure 1 and Table S1). Geniposide derived from Gardeniae Fructus showed the same retention time and mass spectrum as the reference standard, with a sodium adduct ion at m/z 411.1367 $[M + Na]^+$ (calculated for $C_{17}H_{24}O_{10}Na^+$, 411.1262), a weak protonated molecule at m/z 389.1591 $[M + H]^+$, and an aglycone ion at m/z 227.0984 ($[M + H-C_6H_{10}O_5]^+$, protonated genipin, formed by loss of the glucosyl residue), accompanied by its dehydrated ion at m/z 209.0874 ($[M + H-C_6H_{10}O_5-H_2O]^+$, base peak) and further fragment ions at m/z 177.0587, 149.0645, and 121.0677; the characteristic UV λmax was 240 nm. These fragment ions were observed in the full-scan spectrum as in-source fragments. The four isoflavones derived from fermented *Glycine max* were confirmed: daidzin (m/z 417.1180 $[M + H]^+$, λmax = 249 nm), genistin (m/z 433.1128 $[M + H]^+$, λmax = 260 nm), daidzein (m/z 255.0652 $[M + H]^+$, λmax = 250 nm), and genistein (m/z 271.0601 $[M + H]^+$, λmax = 260 nm). The high-resolution mass spectra, in-source fragmentation behavior, and UV profiles were identical to those of reference standard substances (Supplementary Figure S1 and Table S1).

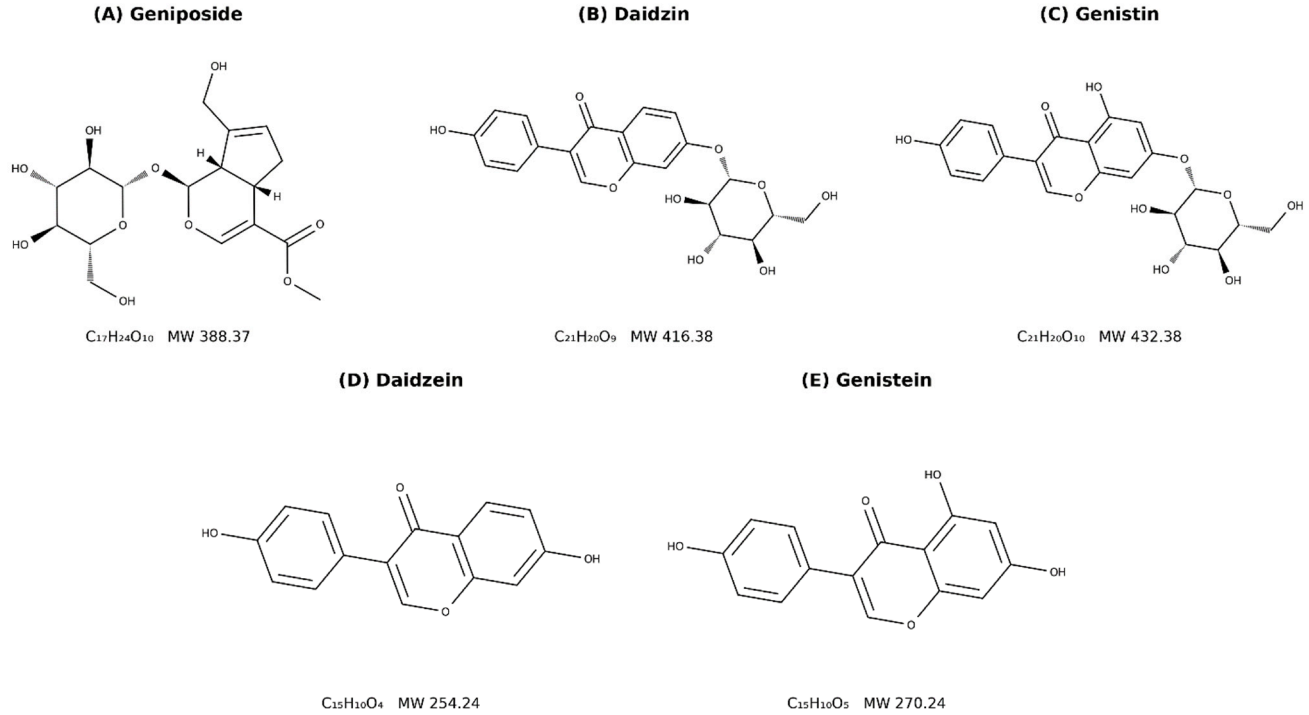


Figure 1. Chemical structures of the five analytical marker compounds in Chijasi-tang: (**A**) Geniposide from *Gardenia jasminoides* Ellis; (**B**) Daidzin, (**C**) Genistin, (**D**) Daidzein, and (**E**) Genistein from *Glycine max* Merrill. Structures were drawn with uniform bond lengths and angles; stereochemistry is shown for geniposide and the β-D-glucopyranosyl moieties.

Quality assessment of commercial raw materials showed differences among the lots examined (one lot per source; Figure 2). For Gardeniae Fructus (*G. jasminoides*), the do-

mestic sample from Wando, Korea (Gardenia #1) contained 2.40% geniposide (%RSD 0.20, $n = 3$; as-is basis, determined by the present method), failing to satisfy the minimum Korean Pharmacopoeia (KP) compendial requirement ($\geq 3.0\%$). Conversely, both wild (Gardenia #2) and cultivated (Gardenia #3) samples from Henan, China, conformed to the standard, yielding 3.35% (%RSD 0.20) and 4.47% (%RSD 0.20) geniposide, respectively [33,39].

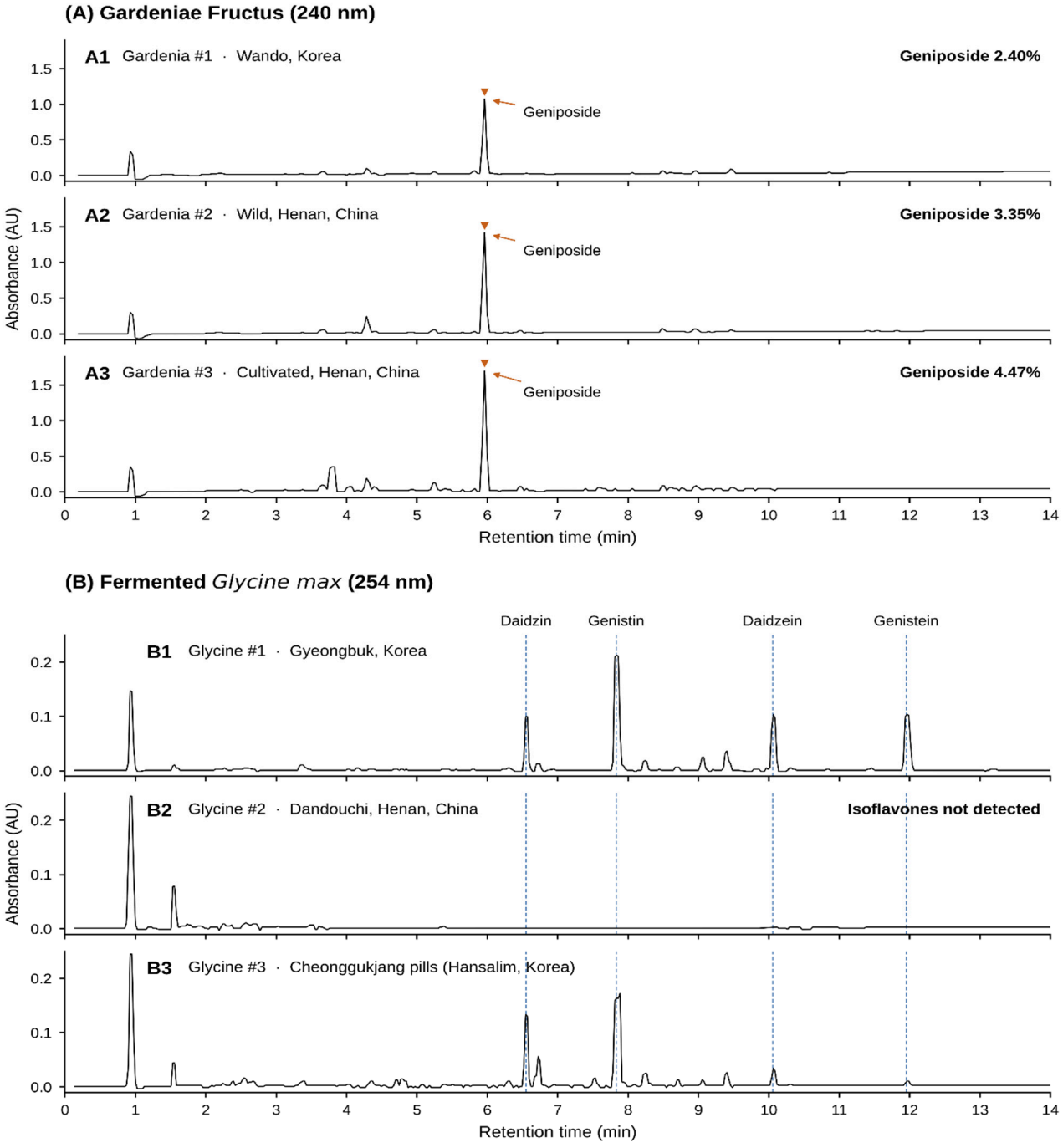


Figure 2. UPLC chromatograms of the raw materials. (**A**) Gardeniae Fructus at 240 nm: (**A1**) Wando, Korea; (**A2**) wild, Henan, China; (**A3**) cultivated, Henan, China. (**B**) Fermented *Glycine max* at 254 nm: (**B1**) Gyeongbuk, Korea; (**B2**) Dandouchi, Henan, China; (**B3**) Cheonggukjang pills (Hansalim, Korea). The geniposide content of each Gardeniae Fructus lot is indicated in (**A**); dashed lines in (**B**) mark the retention times of daidzin, genistin, daidzein, and genistein. Chromatograms are shown for 0–14 min on a common absorbance scale within each panel set.

The evaluation of commercial fermented *G. max* samples showed marked differences among the lots. While the four target isoflavones were consistently quantified in domestic Gyeongbuk fermented soybeans (Glycine #1: daidzin 0.029%, genistin 0.063%, daidzein 0.029%, genistein 0.034%) and commercial Cheonggukjang pills (Glycine #3: 0.040%, 0.048%, 0.008%, 0.003%), none of the four isoflavones were detectable (not detected, N.D.) in commercial Dandouchi sourced from Henan, China (Glycine #2). The chromatogram of this sample showed no peaks at the retention times of the four isoflavones, and no other soy-derived peaks were observed at 254 nm (Figure 2(B2)) or at the other wavelengths examined (280 and 300 nm). Because the same extraction and analytical procedure quantified all four isoflavones in Glycine #1 and Glycine #3, a failure of the procedure specific to Glycine #2 appears unlikely; however, recovery in this matrix was not tested, so an extraction or matrix effect cannot be completely excluded. Possible causes include extensive thermal degradation or leaching during processing, or a material that does not correspond to authentic fermented soybean, and these could not be distinguished in this study.

3.2. Validation of the UPLC-PDA Simultaneous Determination Method

Under the selected UPLC conditions, the five marker compounds were separated within 12 min (25-min total run time), with no interfering peaks at their retention times in the blank (Figure 3). The retention times were 5.96 min for geniposide, 6.55 min for daidzin, 7.82 min for genistin, 10.05 min for daidzein, and 11.95 min for genistein. In system suitability testing, theoretical plate numbers (USP half-height method; apparent values under gradient elution) exceeded 2000 for all markers (260,018 for geniposide and 286,916 for daidzin), tailing factors were 0.98–1.10, resolution between adjacent marker peaks in the standard-to-standard solution was 12.37–35.26, and system precision of peak areas (%RSD, n = 6) was 0.54–0.91%. These values met the criteria of N $\geq$ 2000, T $\leq$ 2.0, Rs $\geq$ 1.5, and RSD $\leq$ 1.5% [38]. Marker-to-matrix separation was evaluated separately in the extract test solutions on the basis of the retention-time differences (Δt_R) between each marker and the nearest designated fingerprint peak (Table S3). Geniposide, genistin, daidzein, and genistein were separated from the nearest matrix peaks by $\Delta t_R \geq 0.40$ min (lowest for genistin/Peak 6). Daidzin eluted closest to a minor matrix peak (Peak 5; Δt_R 0.17 min). The two peaks were integrated separately with reproducible areas (%RSD 0.44% for daidzin and 1.10% for Peak 5, n = 6), and the recoveries of daidzin were 100.8–105.4% (Table S2); its quantitation was therefore considered acceptable. Because the resolution of this critical pair was not determined numerically, it should be determined and included as a system suitability parameter before routine use.

Specificity was confirmed by comparing chromatograms of blank diluent, mixed standard solutions, and Chijasi-tang extracts: no interfering peaks were observed in the blank at the retention times of the markers. All five markers exhibited excellent linearity over the tested ranges (1–150% of the target concentration), with coefficients of determination (r^2) $\geq$ 0.9995 (Table 1). The limits of detection (LOD) and quantitation (LOQ) ranged from 0.0058 to 0.022 μg/mL and from 0.019 to 0.074 μg/mL, respectively, which are well below the lowest marker concentrations in the test solutions.

In accuracy and precision evaluations, mean recoveries across three spike levels ranged from 85.54% to 109.30% with %RSD $\leq$ 2.12%, meeting the predefined acceptance criterion (85.0–115.0%). The lowest mean recovery was observed for daidzein at the 50% spike level (85.54% $\pm$ 1.82%), while geniposide, daidzin, genistin, and genistein displayed recoveries between 91.20% and 109.30% (Table S2). Intra-day precision (n = 6) yielded %RSD values of 0.65–1.88%, and inter-day precision over three consecutive days (n = 9) yielded %RSD values between 1.67% and 4.18%, fully meeting acceptance criteria (%RSD $\leq$ 5.0%) [34,38]. Furthermore, in the solution stability test at room temperature, the peak areas of the five

markers varied by 0.20–1.98% RSD over 156 h (mixed standard solution, 0.20–0.47%; 30% ethanol extract test solution, 0.56–1.98%). The 30% ethanol extract test solution was stable for at least 83 h, with all marker peak areas within ±3.2% of the initial values; at 156 h, the peak area of genistin had increased by 5.2%, slightly exceeding the acceptance limit of ±5%, and test solutions should therefore be analyzed within 83 h of preparation. Under deliberate variations in flow rate (±0.02 mL/min), column temperature (±2 °C), and formic acid content (±0.01%), retention time %RSD was 0.12–0.85%, resolution between adjacent markers was ≥1.8, and recoveries were 98.4–101.5%. Matrix effects were not evaluated separately; however, the spike recoveries obtained in the 30% ethanol extract with external solvent calibration (85.54–109.30%; Table S2) met the acceptance criterion, indicating that matrix effects did not compromise quantification in this extract. Matrix effects in the water and 70% ethanol extracts were not assessed and should be confirmed before the method is applied to these extracts in routine use.

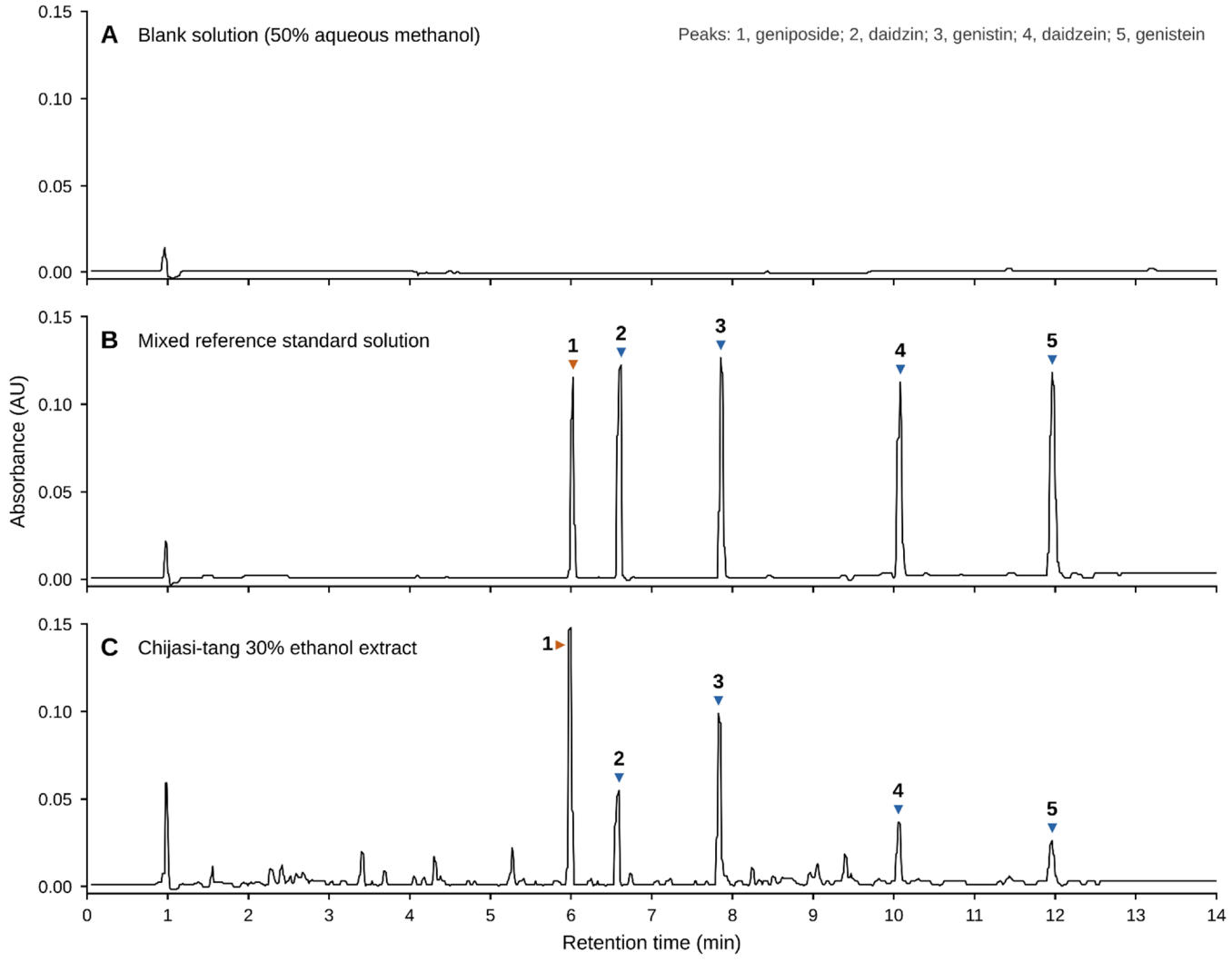


Figure 3. Representative UPLC chromatograms for specificity validation at 254 nm: (**A**) Blank solution (50% aqueous methanol); (**B**) mixed reference standard solution; (**C**) sample solution of the Chijasi-tang 30% ethanol extract. Peaks: 1, geniposide; 2, daidzin; 3, genistin; 4, daidzein; 5, genistein. Orange and blue symbols denote the Gardeniae Fructus- and fermented soybean-derived markers, respectively.

Table 1. Retention time, detection wavelength, calibration data, LOD, and LOQ of the five marker compounds.

Marker	RT (min)	λ (nm)	Linear Range (μg/mL)	Regression Equation (y = ax + b)	r^2	LOD (μg/mL)	LOQ (μg/mL)
Geniposide	5.96	254	0.50–100.0	y = 6964x − 987	0.9999	0.022	0.074
Daidzin	6.55	254	0.10–30.0	y = 29,615x − 1583	0.9999	0.0059	0.019
Genistin	7.82	254	0.10–30.0	y = 36,627x − 2668	0.9999	0.0058	0.020
Daidzein	10.05	254	0.10–30.0	y = 48,795x − 6566	0.9995	0.0064	0.021
Genistein	11.95	254	0.10–30.0	y = 49,790x − 167	0.9999	0.0085	0.028

Linear regression equations are expressed as y = ax + b, where y is the peak area and x is the concentration of the marker compound (μg/mL). LOD, limit of detection (S/N = 3); LOQ, limit of quantitation (S/N = 10). Linear ranges were evaluated across six concentration levels in triplicate.

3.3. Marker Component Contents and Criteria by Extraction Solvent

Quantitative determination of the five markers in the dry extracts prepared with water, 30% ethanol, and 70% ethanol (three extracts per solvent; Table 2) revealed solvent-dependent profiles. Increasing the ethanol proportion increased the contents of the isoflavone aglycones: daidzein increased from 0.03% (water) to 0.07% (30% EtOH) and 0.17% (70% EtOH), and genistein increased from 0.02% to 0.04% and 0.17%, respectively. Geniposide, a hydrophilic iridoid glycoside, showed its highest mean content in the 70% ethanol extract (3.97%) and its lowest in the 30% ethanol extract (2.03%). Extract yields decreased with increasing ethanol concentration (water 9.0 ± 0.1% > 30% EtOH 8.2 ± 0.1% > 70% EtOH 7.1 ± 0.1%). These values describe the marker contents of extracts obtained under the defined process conditions (Section 2.2), not the total marker contents of the raw materials; differences between solvents therefore reflect extraction selectivity and extract yield under these conditions.

As an initial benchmark, lower limits were calculated as 90% of the mean of the three extracts for each solvent [7,9] (Table 2). However, several extracts fell below these benchmarks: geniposide in the 30% and 70% ethanol extracts of Lot 3 (1.51% and 2.81%), daidzein and genistein in the 70% ethanol extract of Lot 3 (0.11% and 0.07%), and genistein in the water extract of Lot 1 (0.01%). A 90-of-mean rule derived from three extracts therefore does not accommodate the variability in the raw materials. Provisional lower limits based on the minimum observed values are proposed instead for pilot-stage testing: water extract (geniposide ≥ 2.80%, daidzin ≥ 0.10%, genistin ≥ 0.14%, daidzein ≥ 0.025%, genistein ≥ 0.010%); 30% ethanol extract (geniposide ≥ 1.45%, daidzin ≥ 0.13%, genistin ≥ 0.22%, daidzein ≥ 0.055%, genistein ≥ 0.035%); and 70% ethanol extract (geniposide ≥ 2.70%, daidzin ≥ 0.24%, genistin ≥ 0.40%, daidzein ≥ 0.10%, genistein ≥ 0.065%). Notably, the extracts prepared from the sub-potent Gardenia #1 met both sets of limits, indicating that extract specifications alone cannot detect a non-compliant raw material and that the compendial raw material assay remains the critical control point. These limits are provisional and should be replaced by statistically derived limits (e.g., tolerance intervals) once data from at least ten production-scale batches are available.

Table 2. Marker content of Chijasi-tang dry extracts categorized by extraction solvent and proposed provisional specifications (%, *w*/*w*).

Extraction Solvent	Lot	Geniposide	Daidzin	Genistin	Daidzein	Genistein
Water	Lot 1 (Korea, Wando)	2.89	0.11	0.15	0.03	0.01
	Lot 2 (China, wild)	3.78	0.12	0.16	0.03	0.02
	Lot 3 (China, cultivated)	2.95	0.11	0.15	0.03	0.02
	Mean ± SD	3.21 ± 0.50	0.11 ± 0.01	0.15 ± 0.01	0.03 ± 0.00	0.02 ± 0.01
	Benchmark (90% of mean)	≥2.89	≥0.10	≥0.14	≥0.03	≥0.02
	Provisional limit	≥2.80	≥0.10	≥0.14	≥0.025	≥0.010
30% EtOH	Lot 1 (Korea, Wando)	2.34	0.17	0.28	0.07	0.04
	Lot 2 (China, wild)	2.25	0.15	0.25	0.07	0.05
	Lot 3 (China, cultivated)	1.51 [a]	0.14	0.24	0.06	0.04
	Mean ± SD	2.03 ± 0.46	0.15 ± 0.02	0.26 ± 0.02	0.07 ± 0.01	0.04 ± 0.01
	Benchmark (90% of mean)	≥1.83	≥0.14	≥0.23	≥0.06	≥0.04
	Provisional limit	≥1.45	≥0.13	≥0.22	≥0.055	≥0.035
70% EtOH	Lot 1 (Korea, Wando)	4.35	0.27	0.48	0.21	0.22
	Lot 2 (China, wild)	4.74	0.28	0.47	0.20	0.21
	Lot 3 (China, cultivated)	2.81 [a]	0.26	0.43	0.11 [a]	0.07 [a]
	Mean ± SD	3.97 ± 1.02	0.27 ± 0.01	0.46 ± 0.03	0.17 ± 0.06	0.17 ± 0.08
	Benchmark (90% of mean)	≥3.57	≥0.24	≥0.41	≥0.16	≥0.15
	Provisional limit	≥2.70	≥0.24	≥0.40	≥0.10	≥0.065

Marker contents and proposed provisional specifications of Chijasi-tang dry extracts according to extraction solvent (unit: %, *w*/*w* of freeze-dried extract, as-is basis). Values are the mean of triplicate injections determined by UPLC-PDA. Lots 1, 2, and 3 correspond to extracts prepared from the Wando (Korea), wild Henan (China), and cultivated Henan (China) Gardeniae Fructus lots (Gardenia #1, #2, and #3), respectively, each co-decocted with Glycine #1. Benchmark = 90% of the mean of the three extracts. Provisional limits were set at or slightly below the minimum observed values and are intended for pilot-stage testing only (see Section 3.3). [a] Value is below the 90-of-mean benchmark.

3.4. Method Validation of 14-Peak Chromatographic Fingerprinting

Fourteen characteristic peaks (Peaks 1–9 and the five marker compounds) consistently resolved across the Chijasi-tang chromatographic profile were designated for fingerprinting (Figure 4). The characteristic UV absorption maxima (λmax) were as follows: Peak 1 (272 nm), Peak 2 (236 nm), Peak 3 (238 nm), Peak 4 (240 nm), geniposide (240 nm), daidzin (249 nm), Peak 5 (260 nm), genistin (260 nm), Peak 6 (250 nm), Peak 7 (220 nm), Peak 8 (229 nm), Peak 9 (260 nm), daidzein (250 nm), and genistein (260 nm). The UV maxima of Peaks 2–4 (236–240 nm) are consistent with iridoid glycosides of Gardeniae Fructus, and those of Peaks 5, 6, and 9 (250–260 nm) are consistent with isoflavone conjugates of soybean; Peak 1 (272 nm) was attributed to fermented soybean on the basis of the single-herb chromatograms, whereas Peaks 7 and 8 (220 and 229 nm) could not be assigned to a single herb (Section 2.6; Table S3). MS data were not acquired for Peaks 1–9, and these peaks were not structurally identified. Peaks 7 and 8 (8.93 and 9.03 min) form a closely eluting pair, and Peak 5 (6.71 min) elutes immediately after daidzin (6.54 min); within these pairs, peaks are assigned by elution order and UV spectra. Method precision for the 14 characteristic peaks in replicate analyses of the water extract (n = 6) yielded %RSD values of 0.01–0.04% for retention time (RT), 0.01–0.04% for relative retention time (RRT), 0.18–1.91% for peak area (PA), and 0.27–1.91% for relative peak area (RPA; excluding the reference peak), all within the acceptance criterion (≤ 5.0%). Inter-day precision over three days yielded %RSD values of 0.08–0.14% for RT, 0.01–0.18% for RRT, 0.22–3.53% for PA, and 0.10–3.70% for RPA. All 14 characteristic peaks were detected and integrated in all nine extracts.

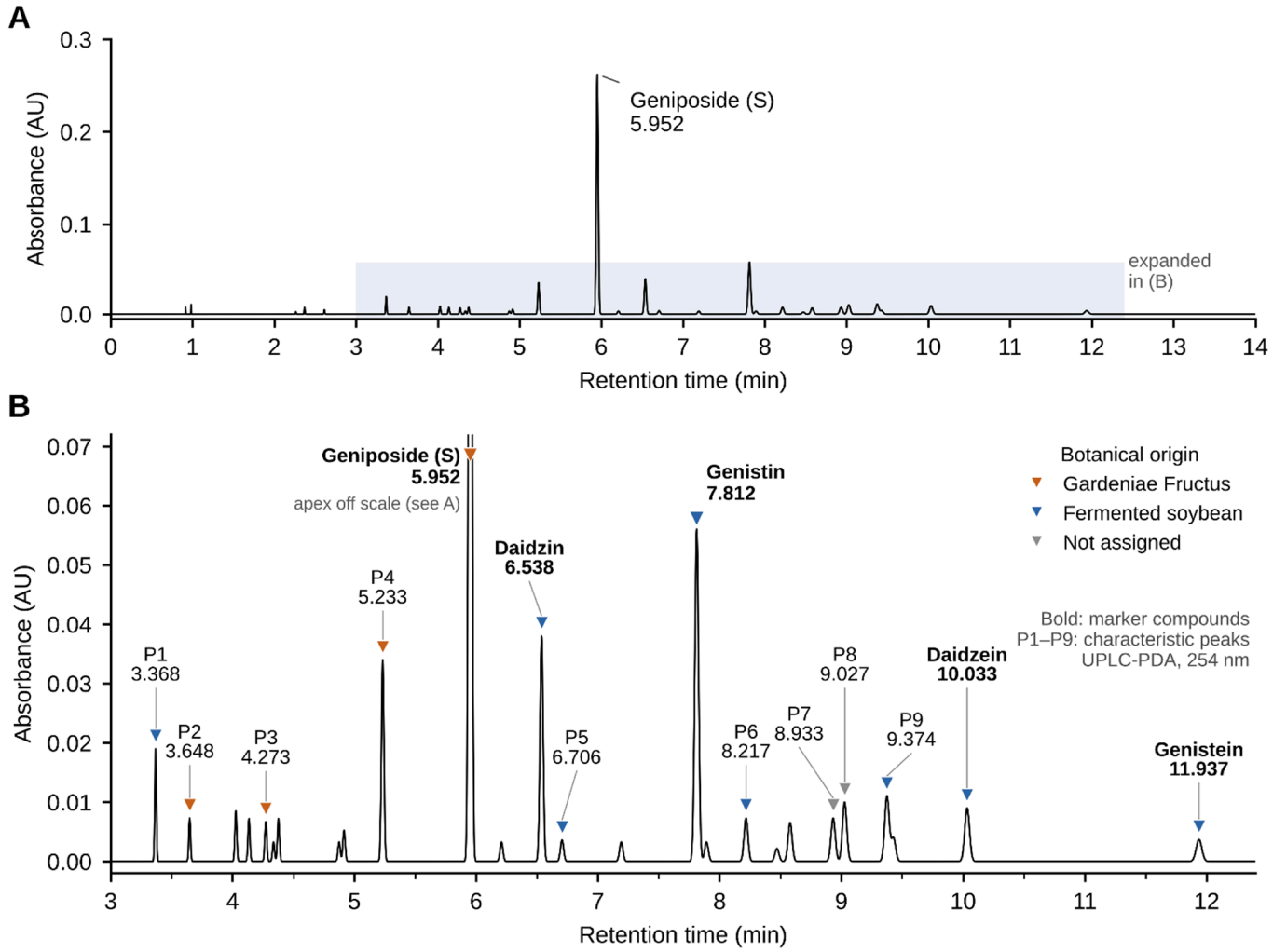


Figure 4. Representative chemical fingerprint chromatogram of the Chijasi-tang water extract (UPLC-PDA, 254 nm), showing the 14 characteristic peaks (Peaks 1–9 and the five marker compounds): (**A**) full chromatogram (0–14 min); (**B**) expanded view of the 3–12.4 min region (shaded in (**A**)), with the 14 characteristic peaks labeled by botanical origin. Geniposide (S) was used as the reference peak. Retention times are those assigned by the instrument data system.

3.5. Batch-to-Batch Chemical Consistency and Similarity Evaluation

To assess batch-to-batch consistency of the laboratory-scale extracts, the 14 characteristic peak vectors of individual batches were compared against reference profiles (the element-wise mean and median of the three extracts per solvent; Section 2.6) using Pearson correlation coefficients (r) (Table 3).

All extracts exceeded the acceptance criterion of $r \geq 0.95$: water extracts showed r = 0.997–1.000, 30% ethanol extracts r = 0.981–0.998, and 70% ethanol extracts r = 0.954–0.999 (Table 3). Extracts prepared from Gardenia #1 showed $r \geq 0.998$ in all three solvents. The 30% ethanol extract of Lot 3 also showed r = 0.981–0.989, although its geniposide content (1.51%) was 26% below the solvent mean and below the 90-of-mean benchmark. Because raw peak areas are dominated by the geniposide peak, Pearson correlation mainly reflects overall pattern congruence and is relatively insensitive to quantitative deviations in individual markers. RPA-normalized similarity and cosine similarity (SES) were 0.932–0.985 and 0.935–0.985, respectively (Table 3). These results indicate that fingerprint similarity complements, but cannot replace, quantitative marker assays, which supports the dual-tiered quality control approach.

Table 3. Correlation coefficient (r) between samples and reference profiles by extraction solvent of Chijasi-tang.

Judgment ($r \geq 0.95$)	Lot 3 (China, Cultivated)	Lot 2 (China, Wild)	Lot 1 (Korea, Wando)	Correlation Coefficient Calculation Criteria	Extraction Solvent
Pass	0.999	0.999	0.999	Mean-based reference	Water extract
Pass	1.000	0.997	1.000	Median-based reference	
Pass	0.989	0.997	0.998	Mean-based reference	30% EtOH extract
Pass	0.981	0.998	0.998	Median-based reference	
Pass	0.968	0.997	0.998	Mean-based reference	70% EtOH extract
Pass	0.954	0.999	0.999	Median-based reference	

EtOH: aqueous ethanol (*v*/*v*). Mean-based and median-based references are the reference chromatographic profiles obtained, for each solvent system, as the element-wise mean and median, respectively, of the 14-peak areas of the nine injections (three extracts × triplicate injections); each extract (Lot 1–3) is represented by the mean of its triplicate injections. Lots 1, 2, and 3 were prepared from Gardenia #1, #2, and #3, respectively, each co-decocted with Glycine #1. Pass indicates that the correlation coefficient meets or exceeds the acceptance criterion adopted in this study ($r \geq 0.95$) [13,17]. Cosine similarity (congruence coefficient) ranged from 0.973 to 1.000 across all extracts; it was calculated for comparison only, and no acceptance criterion was applied to it.

4. Discussion

The present study was designed to establish an analytical quality standardization framework (one component of CMC) for Chijasi-tang (Zhizichi decoction)—a classical formula used clinically for neuropsychiatric symptoms—as an exploratory step toward its development as a standardized herbal medicine or botanical drug product [3–8].

4.1. Choice of Detection Technique

In a recent study, Guo et al. (2023) explored the multi-component profile of Chijasi-tang using LC-HRMS to track chemical transitions between individual herb decoctions and the co-decocted prescription, thereby elucidating the traditional compatibility concept of attenuation and synergy based on solubility shifts in potentially toxic constituents (e.g., genipin) and enhanced dissolution of active ingredients [32]. While that study provided a valuable mechanistic rationale for herbal co-decoction, its objectives differ fundamentally from the quality control aims of the present work. LC-MS-based methods are widely used for quantitative quality control, offer higher selectivity and sensitivity, and would be the method of choice for low-abundance or weakly UV-absorbing constituents. The choice of PDA detection in the present study was based on fitness for purpose rather than on a limitation of mass spectrometry: all five markers have strong UV chromophores and are present well above the PDA quantitation limits (Table 1). UV detection is the technique used in the relevant pharmacopoeial assays [33]; it is available in most herbal medicine manufacturing and contract testing laboratories at lower acquisition and operating cost, and the PDA provides spectral information for peak purity assessment. The UPLC-PDA method developed herein separated the five reference standards within 12 min (25-min total run time), separated the markers from the adjacent matrix peaks in the extracts (closest pair, daidzin/Peak 5, Δt_R 0.17 min), and was validated according to ICH Q2(R2) [34] (Section 3.2). Mass spectrometry remained complementary in this work, being used for marker confirmation only. Furthermore, our evaluation of commercial raw materials revealed differences among the lots examined, including undetectable isoflavones in one commercial Dandouchi lot and sub-compendial geniposide in one domestic Gardeniae Fructus lot, which is consistent with the need for incoming raw material testing. Finally, the comparison of water, 30% EtOH, and 70% EtOH extracts and the 14-peak fingerprint provide preliminary data that may inform the future definition of specifications for a botanical drug substance (BDS) [7–9,14–17].

4.2. Chromatographic Considerations for Markers of Divergent Polarity

A common challenge in the chromatographic analysis of multi-herb preparations is resolving constituents of widely differing polarity within a single run, both from each other and from co-extracted matrix components [12,35]. The Chijasi-tang matrix is chemically complex, comprising highly polar iridoid glycosides (geniposide) from *G. jasminoides*, moderately polar isoflavone glycosides (daidzin and genistin), and hydrophobic aglycones (daidzein and genistein) from fermented *G. max* [24,28,29].

Previously reported HPLC-DAD methods for these constituents typically required lengthy run times of 45–70 min on conventional 5 µm C18 columns [35,36]. Furthermore, under highly aqueous initial mobile phase conditions, conventional C18 stationary phases can experience phase dewetting ('phase collapse'), resulting in irreproducible retention and premature elution of polar iridoids near the solvent front [40]. In this study, the Waters ACQUITY UPLC HSS C18 column (1.8 µm, 2.1 × 100 mm) demonstrated good chromatographic performance. The stationary phase incorporates high-strength silica designed for mechanical stability under ultra-high pressures. To reduce the risk of phase dewetting, 1% acetonitrile was included in Mobile Phase A (0.1% formic acid in water/acetonitrile, 99:1 *v*/*v*), and geniposide was retained well away from the void volume (t_R 5.96 min) with reproducible retention (RT %RSD $\leq$ 0.04%). The sub-2-µm particles delivered high column efficiency (N = 18,500–32,400 plates per 100 mm column; 185,000–324,000 plates/m), and the five reference standards were separated within 12 min with standard-to-standard Rs $\geq$ 12.37. In the extracts, the critical marker-to-matrix pair was daidzin/Peak 5 (Δt_R 0.17 min); the quantitation of daidzin was considered acceptable because the two peaks were integrated reproducibly and the daidzin recoveries met the acceptance criterion, but the resolution of this pair should be determined and included in system suitability testing for routine use. The addition of 0.1% (*v*/*v*) formic acid (pH approximately 2.7) keeps the phenolic hydroxyl groups of the isoflavones in the mobile phase in their neutral, non-deprotonated form and suppresses the deprotonation of residual silanol groups on the silica surface of the stationary phase, thereby minimizing secondary (ion-exchange) interactions with the column; this contributed to symmetrical peaks (T $\leq$ 1.10). Moreover, test solutions demonstrated adequate autosampler stability over 156 h at ambient temperature (peak area %RSD $\leq$ 1.98%), providing practical flexibility for batch analysis.

4.3. Chemical Heterogeneity of Commercial Raw Materials, Including Fermented and Processed Botanicals

The inherent variability in raw herbal substances can affect the quality, efficacy, and safety of finished botanical products [11,18,19]. In our evaluation of Gardeniae Fructus, the domestic sample from Wando, Korea (Gardenia #1) yielded a geniposide content of 2.40%, falling short of the Korean Pharmacopoeia specification (≥3.0%), whereas wild (3.35%) and cultivated (4.47%) samples from Henan, China, met the requirement [33,39]. These findings align with prior surveys reporting substantial market variation in geniposide content (1.20–7.17%) [39], suggesting that factors such as origin, harvest timing, and post-harvest drying may contribute to this variation. As only one lot per source was analyzed, these results cannot be generalized to the sources as a whole.

The commercial fermented *G. max* samples (Hyangsi/Dandouchi) [21,27] also differed markedly. Glycine #1 and Glycine #3 contained quantifiable levels of all four isoflavones, whereas none was detected in the commercial Dandouchi from Henan, China (Glycine #2). Dandouchi manufacturing traditionally involves soaking soybeans in decoctions of *Morus alba* leaves and *Artemisia annua*, followed by solid-state fermentation and repeated steaming [21,27]. Because the same procedure quantified the isoflavones in the other two fermented soybean materials, an analytical failure is unlikely, although recovery

in this matrix was not tested. The absence of isoflavones may reflect extensive thermal degradation or leaching during processing; however, because no other soy-derived peaks were observed, misidentification or substitution of the material cannot be excluded.

4.4. Solvent-Dependent Marker Contents and Implications for Extract Selection

Extraction solvent selection influences extract yield, marker recovery, and the overall phytochemical profile [9,17]. Increasing the ethanol proportion increased the contents of the isoflavone aglycones: daidzein increased approximately 6-fold from water (0.03%) to 70% ethanol (0.17%), and genistein approximately 8-fold (0.02% to 0.17%; based on rounded values). The isoflavone glycosides daidzin and genistin were also higher in the ethanolic extracts. Despite being a hydrophilic iridoid glycoside, geniposide reached its highest content in the 70% ethanol extract (3.97%), compared with 3.21% in water and 2.03% in 30% ethanol. These differences reflect the combined effects of extraction selectivity, extract yield, and process conditions; because exhaustive extraction of the raw materials was not performed and the extraction process was not optimized, they should not be interpreted as differences in the total marker contents of the herbs.

One possible explanation is that higher ethanol fractions reduce the co-extraction of high-molecular-weight hydrophilic constituents such as mucilage, polysaccharides, and proteins. This lowers the extract yield and concentrates lower-molecular-weight metabolites. However, this effect (a 1.27-fold difference in yield between water and 70% ethanol) cannot explain the 6- to 8-fold enrichment of the aglycones, the lowest geniposide content in the 30% ethanol extract, or the differences among Gardeniae Fructus lots. A notable mass balance anomaly was observed regarding Gardeniae Fructus lots and extraction recovery. Specifically, although Gardenia #3 raw material possessed the highest initial geniposide content (4.47%, corresponding to 0.894 g per 120-g prescription), its resulting extracts yielded unexpectedly lower geniposide concentrations in 30% EtOH (1.51%) and 70% EtOH (2.81%) compared to Lot 1 (2.34% and 4.35%, respectively; derived from raw material with only 2.40% geniposide). Consequently, geniposide recovery from Gardenia #3 was only 16–35%, whereas Gardenia #1 exhibited 49–78% recovery. Intriguingly, in the 70% ethanol extract of Lot 3, the contents of the hydrophobic aglycones daidzein (0.11%) and genistein (0.07%) were also reduced by approximately half relative to Lots 1 and 2 (~0.21% and ~0.21%), despite utilizing the identical fermented soybean lot (Glycine #1) across all formulations.

This phenomenon may be explained by macromolecular matrix interactions and physical entrapment during sequential co-decoction. Fermented *Glycine max* is abundant in storage proteins (e.g., glycinin and β-conglycinin) and soluble fibers. Under thermal reflux—and particularly in high-ethanol solvent regimes (70% EtOH) where soy proteins undergo extensive denaturation and precipitation—the substantially higher initial load of iridoids and phenolic co-constituents in Gardenia #3 may promote the formation of insoluble protein–polyphenol/iridoid aggregates. Such precipitates could co-trap both polar iridoids (geniposide) and hydrophobic isoflavone aglycones (daidzein and genistein), causing them to be retained within the filtration cake during coarse clarification (25 μm pore size). While this hypothesis could explain the observed recovery discrepancy, these observations were made with laboratory-scale single-batch decoctions (n = 1 preparation per lot) and require targeted verification through sediment analysis and replicated, larger-scale extractions.

These solvent-dependent differences may inform the choice of extraction solvent in future product development, but they do not by themselves indicate which extract is preferable. The 70% ethanol extract contained the highest amounts of all five markers per unit mass of extract, which could be practical for solid oral dosage forms (tablets or

capsules) in which a smaller unit dose is desirable. However, the present study evaluated chemical composition only; pharmacological activity, safety, bioavailability, and clinical effectiveness were not assessed, and a higher marker content cannot be taken to indicate greater therapeutic efficacy. Whether any of the extracts offers a pharmacological advantage must be established in dedicated non-clinical and clinical studies. Regulatory considerations may also influence solvent selection: under the regulatory framework of the Korean MFDS (Notification No. 2023-64, Annex 2/Article 24) [7], traditional herbal prescriptions documented in recognized historical compendia (such as the *Donguibogam* [2]) may be eligible for reduced toxicological and clinical data requirements when prepared using traditional aqueous or low-ethanol extraction processes consistent with those described in the recognized compendia, which would favor the water or 30% ethanol extract for such a pathway.

The provisional specifications proposed herein (Section 3.3; Table 2) are intended only as a starting point for future release criteria and must be re-established statistically with additional production-scale batches [7–9,15].

4.5. Chemical Fingerprinting for Identity and Batch-to-Batch Consistency

Because botanical drugs are complex multi-component mixtures whose pharmacological activity is generally attributed to multiple constituents, individual marker assays alone cannot fully ensure consistency [12,16]. In line with regulatory guidance [17], we established a 14-peak UPLC chromatographic fingerprint capturing both iridoid and isoflavonoid domains (Figure 4). The method showed acceptable repeatability and intermediate precision (tested on the water extract), with %RSD values for the relative retention time (RRT) and relative peak area (RPA) remaining well below 5.0%.

All extracts met the criterion of $r \geq 0.95$ against the reference profiles (0.997–1.000 for water, 0.981–0.998 for 30% ethanol, and 0.954–0.999 for 70% ethanol; Table 3). Because the geniposide peak was the largest single peak, accounting for 29–52% of the total area of the 14 characteristic peaks (approximately 50% in the water extract and 29–34% in the ethanolic extracts), Pearson correlation of raw peak areas was relatively insensitive to quantitative deviations in individual markers. For example, the 30% ethanol extract of Lot 3, whose geniposide content (1.51%) was 26% below the solvent mean and below the 90-of-mean benchmark, still showed $r = 0.981–0.989$. Cosine similarity (0.988–0.994 for this extract; 0.973–1.000 across all extracts) likewise did not discriminate this extract. Weighting approaches such as autoscaling or log transformation may improve the sensitivity of the similarity assessment in future work. For the same reason, a high correlation coefficient alone would not necessarily reveal the omission or substitution of the minor herb component; the identity criterion (presence of all 14 characteristic peaks, including the soybean-derived isoflavone peaks and the Gardenia-derived iridoid peaks, within their RRT windows) is therefore the more informative part of the fingerprint for authenticity, as illustrated by the Glycine #2 raw material, in which no soybean-derived peaks were detected. Because the reference profiles were constructed from the same three extracts, these correlations represent internal consistency only. For regulatory submission, an independent reference extract prepared from multiple qualified lots should be established. Fingerprinting should therefore always be combined with quantitative marker assays to prevent the release of sub-potent batches [7,8,14,17].

4.6. Limitations and Future Perspectives

Several limitations of this study should be noted. First, while the five markers were authenticated against reference standards by Q-TOF MS and UV spectra, Peaks 1–9 were assigned only to their botanical origin and, where possible, to a compound class on the basis

of UV spectra (Table S3); they were not structurally identified, and their characterization by UPLC-Q-TOF MS/MS and comparison with reference standards or isolation and NMR analysis is required. Second, the extracts were prepared under one set of process conditions per solvent, and neither the extraction process nor the analytical sample preparation was systematically optimized (e.g., by experimental design); the reported marker contents therefore describe extracts obtained under these conditions and are not representative of the total marker contents of the raw materials. Likewise, the chromatographic conditions were selected through limited scouting rather than by systematic optimization of column chemistry and mobile-phase composition. Third, only one lot per raw material source and one extract per Gardeniae Fructus lot and solvent (n = 1 decoction preparation per lot with triplicate instrument determinations) were analyzed, so the provisional specifications and the matrix-entrapment hypothesis (Section 4.4) require confirmation with replicated and production-scale batches. Fourth, the fingerprint reference profiles were derived from the same extracts that were evaluated; an independent reference extract and a larger sample set are needed before fingerprint acceptance criteria can be used for release. Fifth, the study evaluated chemical composition only; no conclusions regarding the pharmacological activity, safety, bioavailability, or clinical effectiveness of any extract can be drawn, and linking chromatographic profiles to biological activity (e.g., in chronic unpredictable mild stress models in vivo) would require dedicated studies. Finally, stability was demonstrated for test solutions only; the stability of extracts and finished products (long-term 25 °C/60% RH and accelerated 40 °C/75% RH conditions) would need to be established according to ICH Q1A(R2).

4.7. Proposed Quality Assessment Strategy

Based on these results, we propose a combined, stepwise quality assessment strategy in which marker quantification and fingerprinting have distinct and non-interchangeable roles, and all steps use the same validated UPLC-PDA method: (1) Raw material qualification: each Gardeniae Fructus lot is assayed for geniposide against the KP limit ($\geq$ 3.0%) [33], and each fermented soybean (Sojae Semen Praeparatum) lot is tested for the presence and content of the four isoflavones; lots without detectable isoflavones, such as Glycine #2, are rejected. This step is essential because extracts prepared from the sub-compendial Gardenia #1 met the provisional extract limits (Section 3.3). (2) Extract identity: the extract chromatogram must show all 14 characteristic peaks within their RRT windows, including the Gardenia-derived iridoid peaks and the soybean-derived isoflavone peaks, which confirms that both herbs are present and that neither has been omitted or substituted. (3) Extract consistency: the similarity to an established reference profile must be $r \geq 0.95$. (4) Extract assay: the five markers are quantified against the solvent-specific provisional limits (Table 2), which detects quantitative deficiencies that fingerprint similarity does not reveal (Section 3.5). A batch would be released only if all four criteria are met. Because the limits and the reference profile are provisional, this strategy should be regarded as a framework to be confirmed with production-scale batches.

5. Conclusions

In this study, a single-run UPLC-PDA method was developed and validated according to ICH Q2(R2) for the quality control of Chijasi-tang. Using a sub-2-μm HSS C18 column, five markers of differing polarity, geniposide from *G. jasminoides* and four isoflavones (daidzin, genistin, daidzein, and genistein) from fermented *G. max*, were separated as reference standards within 12 min (25-min total run time) and quantified in extracts in which the markers were separated from the adjacent matrix peaks, using UV detection that is widely available in manufacturing laboratories. Among the commercial raw materials,

one Gardeniae Fructus lot failed the compendial geniposide requirement (2.40% vs. ≥3.0%), and no isoflavones were detected in one Dandouchi lot, underscoring the need for raw material qualification by validated quantitative methods. Extraction with 70% ethanol gave the highest marker contents per unit mass of extract; this reflects chemical composition only and does not imply pharmacological superiority. Provisional specifications were proposed for each solvent, and a 14-peak fingerprint showed high within-solvent consistency ($r \geq 0.954$), although fingerprint similarity alone did not reflect quantitative differences in individual markers. Raw material qualification, fingerprint-based identity testing, and quantitative marker assays should therefore be combined. These exploratory, laboratory-scale findings may inform the future CMC development of Chijasi-tang botanical drug substances and standardized herbal medicines.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/separations13110332/s1. Figure S1: UV absorption spectra and Q-TOF mass spectra of the five marker compounds; Table S1: Accurate mass, fragment ion, and UV data of the five marker compounds; Table S2: Accuracy (recovery), intermediate precision, and solution stability data; Table S3: Retention times, relative retention times, UV maxima, relative peak areas, and botanical origin of the 14 fingerprint peaks.

Author Contributions: Conceptualization, H.-J.P. and H.W.K.; methodology, H.-J.P. and J.J.; software, J.J.; validation, H.J.W., D.-E.L. and H.W.K.; formal analysis, J.J. and H.J.W.; investigation, H.-J.P.; resources, H.-J.P. and D.-E.L.; data curation, H.-J.P., H.J.W., and H.W.K.; writing—original draft preparation, H.-J.P.; writing—review and editing, J.J. and H.J.W.; visualization, J.J., H.J.W., and D.-E.L.; supervision, H.-J.P. and H.W.K.; project administration, H.-J.P. and H.W.K.; funding acquisition, H.W.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant numbers: RS-2021-KH109310 and RS-2025-02303579).

Data Availability Statement: The original contributions presented in the study are included in the article and its Supplementary Materials. Further inquiries can be directed to the corresponding authors.

Acknowledgments: The authors are grateful to the institutional research core facility for technical and instrumental support.

Conflicts of Interest: Author Hyun-Je Park was employed by the company Yuhan Care Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

BDS	Botanical Drug Substance
CMC	Chemistry, Manufacturing, and Controls
DAD	Diode Array Detector
EMA	European Medicines Agency
ESI	Electrospray Ionization
EtOH	Ethanol
FDA	Food and Drug Administration
GACP	Good Agricultural and Collection Practices
HPA	Hypothalamic–Pituitary–Adrenal
HPLC	High-Performance Liquid Chromatography
HSS	High-Strength Silica

ICH	International Council for Harmonisation
KHP	Korean Herbal Pharmacopoeia
KP	Korean Pharmacopoeia
LOD	Limit of Detection
LOQ	Limit of Quantitation
MAO	Monoamine Oxidase
MFDS	Ministry of Food and Drug Safety
PA	Peak Area
PDA	Photodiode Array
PTFE	Polytetrafluoroethylene
Q-TOF MS	Quadrupole Time-of-Flight Mass Spectrometry
RPA	Relative Peak Area
RRT	Relative Retention Time
RSD	Relative Standard Deviation
RT	Retention Time
SES	Similarity Evaluation System
S/N	Signal-to-Noise Ratio
UPLC	Ultra-Performance Liquid Chromatography
USP	United States Pharmacopeia

References

1. Chae, I.S. *Interpretation of Shanghan Lun*; Gomon-sa: Seoul, Republic of Korea, 1987; p. 70. (In Korean)
2. Heo, J. *Donguibogam*; Translation Committee of Donguibogam, Translator; Bubin Publishing Co.: Seoul, Republic of Korea, 2002; pp. 1033–1034. (In Korean)
3. Han, S.Y.; Kim, D.H.; Kim, S.H. A Study on Zhizichitang Formulas in Shanghanlun. *J. Inst. Korean Med. Daejeon Univ.* **1999**, *7*, 385–397.
4. Lee, J.H. *Illustrated Compendium of Korean Herbal Formulas*; Euibang Publishing Co.: Wonju, Republic of Korea, 2007; pp. 727–728. (In Korean)
5. Ren, L.; Fan, Y.; Luo, H.; Hu, J.; Hu, J. PACAP/VIP in the Prefrontal Cortex Mediates the Rapid Antidepressant Effects of Zhizichi Decoction. *J. Ethnopharmacol.* **2024**, *335*, 118638. [CrossRef] [PubMed]
6. Wang, Y.Q.; Li, R.R.; Jiang, Y.M.; Liu, Z.Y. Antidepressant-like Effects of Zhizichi Decoction on Chronic Unpredictable Mild Stress in Rats through HPA Axis Regulation and Monoaminergic System Modulation. *Chin. J. Exp. Tradit. Med. Formulae* **2018**, *24*, 112–117.
7. Ministry of Food and Drug Safety (MFDS). *Regulations on the Approval and Notification of Herbal (Botanical) Medicinal Products*; MFDS Notification No. 2023-64; MFDS: Cheongju, Republic of Korea, 2023.
8. US Food and Drug Administration (FDA). *Botanical Drug Development: Guidance for Industry*; Center for Drug Evaluation and Research (CDER), US FDA: Silver Spring, MD, USA, 2016.
9. Ministry of Food and Drug Safety (MFDS). *Quality Guideline for Herbal (Botanical) Medicinal Products (Guidance for Industry)*; MFDS: Cheongju, Republic of Korea, 2020.
10. Kang, H.W.; Kim, S.T.; Son, H.J.; Han, P.R.; Cho, H.K.; Lee, Y.J.; Lyu, Y.S. Quality Standardization of LMK02 and Its Anti-Dementia Effects on Aβ Oligomer-Induced Rat Hippocampal H19-7 Cell Line. *Korean J. Orient. Physiol. Pathol.* **2009**, *23*, 397–404.
11. Lee, E.H.; Cha, B.C. Quantitative Analysis of Glycine Semen Nigra and Eucommiae Cortex for Standardization of GCSB-5 Preparation. *Korean J. Pharmacogn.* **2009**, *40*, 18–24.
12. Heinrich, M.; Appendino, G.; Efferth, T.; Fürst, R.; Izzo, A.A.; Kayser, O.; Pezzuto, J.M. Best Practice in Research: Overcoming Common Challenges in Phytopharmacological Research. *J. Ethnopharmacol.* **2020**, *246*, 112230. [CrossRef] [PubMed]
13. Liang, Y.Z.; Xie, P.; Chan, K. Quality Control of Herbal Medicines. *J. Chromatogr. B* **2004**, *812*, 53–70. [CrossRef] [PubMed]
14. *CPMP/QWP/2819/00 Rev. 2*; Guideline on Quality of Herbal Medicinal Products/Traditional Herbal Medicinal Products. European Medicines Agency (EMA): London, UK, 2011.
15. *CPMP/QWP/2820/00 Rev. 2*; Guideline on Specifications: Test Procedures and Acceptance Criteria for Herbal Substances, Herbal Preparations and Herbal Medicinal Products/Traditional Herbal Medicinal Products. European Medicines Agency (EMA): London, UK, 2011.
16. *EMEA/HMPC/253629/07*; Reflection Paper on Markers Used for Quantitative and Qualitative Analysis of Herbal Medicinal Products and Traditional Herbal Medicinal Products. European Medicines Agency (EMA): London, UK, 2008.

17. Ministry of Food and Drug Safety (MFDS). *Guideline on Establishing Chemical Profiles for Herbal (Botanical) Medicinal Products (Guidance for Industry)*; MFDS: Cheongju, Republic of Korea, 2015.
18. Therapeutic Goods Administration (TGA). *Guidance on Equivalence of Herbal Extracts in Complementary Medicines*; Australian TGA: Woden, ACT, Australia, 2011.
19. World Health Organization (WHO). *Quality Control Methods for Herbal Materials*; World Health Organization: Geneva, Switzerland, 2011.
20. World Health Organization (WHO). *WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants*; World Health Organization: Geneva, Switzerland, 2003.
21. Compilation Committee of Herbology, National Association of Korean Medicine Colleges. *Herbology*; Younglim-sa: Seoul, Republic of Korea, 2010; pp. 345–347.
22. Kuo, W.H.; Wang, C.J.; Young, S.C.; Sun, Y.C.; Chen, Y.J.; Chou, F.P. Differential Induction of the Expression of GST Subunits by Geniposide in Rat Hepatocytes. *Pharmacology* **2004**, *70*, 15–22. [CrossRef] [PubMed]
23. Suzuki, Y.; Kondo, K.; Ikeda, Y.; Umemura, K. Antithrombotic Effect of Geniposide and Genipin in the Mouse Thrombosis Model. *Planta Med.* **2001**, *67*, 807–810. [CrossRef] [PubMed]
24. Xiao, W.; Li, S.; Wang, S.; Ho, C.-T. Chemistry and Bioactivity of *Gardenia jasminoides*. *J. Food Drug Anal.* **2017**, *25*, 43–61. [CrossRef] [PubMed]
25. Hsu, J.D.; Chou, F.P.; Lee, M.J.; Chen, H.C.; Wang, C.J. Suppression of the TPA-Induced Expression of Nuclear Proto-Oncogenes in Mouse Epidermis by Crocetin via Antioxidant Activity. *Anticancer Res.* **1999**, *19*, 4221–4227. [PubMed]
26. Tian, J.X.; Chen, S.W.; Liu, C.M.; Zhang, X.Y.; Liu, X. Antidepressant-like Effects of Geniposide Involve the Inhibition of Monoamine Oxidase A and Activation of BDNF Signaling. *Phytomedicine* **2021**, *84*, 153518.
27. Ministry of Food and Drug Safety (MFDS). *The Korean Herbal Pharmacopoeia (KHP)*; MFDS Notification No. 2023-88; MFDS: Cheongju, Republic of Korea, 2023.
28. Zaheer, K.; Akhtar, M.H. An Updated Review of Dietary Isoflavones: Nutrition, Processing, Bioavailability and Impacts on Human Health. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 1280–1293. [CrossRef] [PubMed]
29. Křížová, L.; Dadáková, K.; Kašparovská, J.; Kašparovský, T. Isoflavones. *Molecules* **2019**, *24*, 1076. [CrossRef] [PubMed]
30. Peluso, M.R. Flavonoids Attenuate Cardiovascular Disease, Inhibit Phosphodiesterase, and Modulate Lipid Homeostasis in Adipose Tissue and Liver. *Exp. Biol. Med.* **2006**, *231*, 1287–1299. [CrossRef] [PubMed]
31. Choi, S.I.; Kim, J.E.; Hwang, I.S.; Lee, H.R.; Lee, Y.J.; Son, H.J.; Kim, D.S.; Park, K.M.; Hwang, D.Y. The Effect of Fermented Soybean (Cheonggukjang) Extracts on Growth Hormone Secretion and Its Receptor Signaling Pathway in GH3 Pituitary Cells. *J. Life Sci.* **2012**, *22*, 1243–1253. [CrossRef]
32. Guo, Y.; Wang, S.; Zhang, Y.; Li, Y.; Liu, C.; Li, P.; Wang, X. Characterization and Quantification of the Chinese Medical Formula Zhi-Zi-Chi Decoction, a Systematic Strategy for the Attenuation and Synergy of Compatibility Mechanism. *J. Pharm. Biomed. Anal.* **2023**, *223*, 115130. [CrossRef] [PubMed]
33. Ministry of Food and Drug Safety (MFDS). *The Korean Pharmacopoeia (KP)*, 12th ed.; MFDS Notification No. 2019-102 (amended by No. 2023-75); MFDS: Cheongju, Republic of Korea, 2023.
34. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). *Validation of Analytical Procedures Q2(R2)*; ICH Harmonised Guideline; ICH: Geneva, Switzerland, 2023.
35. Bergonzi, M.C.; Righeschi, C.; Isacchi, B.; Bilia, A.R. Identification and Quantitation of Constituents of *Gardenia jasminoides* Ellis (Zhizi) by HPLC-DAD-ESI-MS. *Food Chem.* **2012**, *134*, 1199–1204. [CrossRef] [PubMed]
36. Griffith, A.P.; Collison, M.W. Improved Methods for the Extraction and Analysis of Isoflavones from Soy-Containing Foods and Nutritional Supplements by Reversed-Phase High-Performance Liquid Chromatography and Liquid Chromatography-Mass Spectrometry. *J. Chromatogr. A* **2001**, *913*, 397–413. [CrossRef] [PubMed]
37. National Institute of Food and Drug Safety Evaluation (NIFDS). HerbMed Standard Herbal Medicine Specifications: Gardeniae Fructus. Available online: https://www.nifds.go.kr/herbmed/ (accessed on 1 October 2026).
38. Ministry of Food and Drug Safety (MFDS). *Guideline on Validation of Analytical Procedures for Pharmaceuticals (Guidance for Industry)*; MFDS: Cheongju, Republic of Korea, 2015.
39. Jeong, M.S.; Chae, H.B.; Choi, I.S.; Kim, Y.H.; Kang, J.S.; Bae, K.H. Establishment of Criteria for Geniposide Content in Gardeniae Fructus. *Korean J. Med. Crop Sci.* **2004**, *12*, 262–265.
40. Neue, U.D.; Cheng, Y.F.; Lu, Z. Retention and Column Stability of Reversed-Phase Packings under Highly Aqueous Mobile Phase Conditions. *J. Sep. Sci.* **2001**, *24*, 735–744.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.