Proceedings of ICBioMed 2026 Symposium: Biomedical Engineering and Smart Medicine
DOI: 10.54254/2753-8818/2026.HZ35863

An Analytical Study on Quality Control Techniques for the Chemical Constituents of Angelica Sinensis Based on Solubility Classification

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Abstract. Angelica sinensis (Oliv.) Diels is one of the most widely used traditional Chinese medicinal materials in clinical practice in China. Its components include lipophilic volatile oils and phthalides, water-soluble phenolic acids and polysaccharides, as well as insoluble cellulose and inorganic salts. Owing to significant differences in solubility among these constituents, separate quality control technologies must be systematically established according to solubility. This paper categorizes and summarizes quality control techniques for these three types of constituents based on their solubility: lipophilic constituents, represented by the thermally unstable Z-ligustilide, are primarily analyzed using GC-MS and QAMS; water-soluble constituents, represented by ferulic acid and polysaccharides, are analyzed using well-established methods such as HPLC and the phenol-sulfuric acid method; insoluble constituents remain a weak link, with NIR and ICP-MS offering new avenues for analysis. Furthermore, the future direction of quality marker (Q-Marker)-guided screening is discussed, aiming to provide a reference for refining the quality standards of *Angelica sinensis*.

Keywords: *Angelica sinensis*, Quality control, Z-ligustilide, Ferulic acid, *Angelica sinensis* polysaccharides

1. Introduction

Angelica sinensis(Oliv.) Diels, the dried root of *Angelica sinensis*(Oliv.) Diels (Apiaceae), is traditionally used to enrich blood, activate blood circulation, regulate menstruation, relieve pain, and moisten the intestines to relieve constipation. It is commonly said that nine out of ten traditional Chinese medicine (TCM) prescriptions contain Danggui [1, 2], and its quality is directly related to the safety and efficacy of clinical medication.

The diversity of chemical constituents in *Angelica sinensis* poses challenges for quality control. To date, nearly 200 chemical constituents have been isolated and identified from *Angelica sinensis*; Based on their solubility, these constituents can be broadly classified into three categories: lipophilic constituents, which mainly include volatile oils and phthalide compounds—represented by Z-ligustilide, butylidenephthalide and senkyunolide A—and form the chemical basis for the efficacy in promoting blood circulation and dispelling blood stasis; water-soluble constituents, comprising

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mainly phenolic acids such as ferulic acid and chlorogenic acid, and *Angelica sinensis* polysaccharide; ferulic acid is a quality control indicator specified in the *Chinese Pharmacopoeia*; insoluble constituents, comprising mainly macromolecular insolubles such as cellulose, inorganic precipitates such as calcium oxalate crystals, and heavy metals and harmful elements such as lead, cadmium, arsenic and mercury. These three categories of constituents differ in terms of their physicochemical properties, stability, extraction methods and analytical techniques; consequently, comprehensive quality control cannot be achieved using a single technical platform. The current *Chinese Pharmacopoeia* only prescribes content determination for volatile oils and ferulic acid, while polysaccharides are merely identified without quantitative criteria, and the examination of insoluble matter is limited to total ash and acid-insoluble ash, which fails to comprehensively reflect the intrinsic quality of *Angelica sinensis* [1, 3].

This paper uses solubility as a classification criterion to review advances in quality control techniques for lipophilic, water-soluble and insoluble components, analyze the advantages and limitations of various methods, and explore future directions for the quality control of *Angelica sinensis*.

2. Quality control techniques for the lipophilic constituents of *Angelica sinensis*

The fat-soluble components consist primarily of phthalides in the volatile oil, with Z-ligustilide serving as the key quality control indicator; however, its instability and fluctuations in content pose the main challenges for quality control. This section reviews methodological advances in both pre-extraction sample preparation and analytical quantification techniques.

2.1. Types of lipophilic constituents and stability challenges

The lipophilic constituents of *Angelica sinensis* consist primarily of volatile oils. Among these, phthalide compounds are the key pharmacologically active constituents of the volatile oils, with Z-ligustilide being the most abundant marker compound; other constituents include butylidenephthalide and senkyunolide A.

The primary challenge facing the quality control of lipophilic constituents is stability. Z-ligustilide is unstable and is prone to conformational changes or degradation when exposed to factors such as temperature, light, oxygen, pH and solvent type [4, 5]. Furthermore, the total content of volatile oils in *Angelica sinensis* varies considerably between batches, which further complicates quality control [3].

2.2. Extraction and pretreatment

The extraction of lipophilic constituents is typically carried out by steam distillation to obtain volatile oils, or by ultrasonic extraction using organic solvents, followed by subsequent analysis. Steam distillation, the method specified in the *Chinese Pharmacopoeia*, is easy to operate; however, the extraction yield is highly dependent on variables including the particle size of the medicinal material and the extraction duration. High-speed counter-current chromatography (HSCCC) is capable of efficiently isolating Z-ligustilide from the volatile oil of *Angelica sinensis*. Supercritical fluid extraction (SFE) also serves as an effective technique for the recovery of lipophilic components.

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2.3. Detection and quantification techniques

There are a variety of methods for detecting lipophilic constituents.

Gas chromatography (GC-FID/GC-MS) is the traditional method for analyzing the constituents of volatile oils. GC-FID offers high resolution and good linearity, making it suitable for determining the total content of volatile oils and for the quantitative analysis of major constituents. GC-MS, on the other hand, provides mass spectrometric information and can be used for the structural identification of unknown constituents and comparative fingerprint analysis [1, 3]. However, phthalide compounds such as Z-ligustilide are prone to thermal degradation or isomerization at high temperatures in the injection port; therefore, the injection port temperature and temperature-programmed conditions must be optimized to minimize thermal losses [4].

High-performance liquid chromatography (HPLC-UV) overcomes the limitations of gas chromatography in the analysis of thermally unstable components and is commonly used for the determination of phthalate monomers. An HPLC-UV method for the determination of Z-ligustilide in *Angelica sinensis* has been established using a Hypersil ODS2 column, with acetonitrile-water (80:20, v/v) as the mobile phase and a detection wavelength of 350 nm. Under the described conditions, Z-ligustilide achieved baseline separation from the neighboring peaks.

The quantitative analysis of multi-components by a single-marker (QAMS) is a significant advancement in the quality control of lipophilic components in recent years. However, the application of the external standard method is limited by the instability and high cost of the Z-ligustilide reference standard. The QAMS method utilizes butylphthalide, which has relatively stable chemical properties, as an internal standard. By establishing relative correction factors, it enables the simultaneous determination of multiple phthalides, such as senkyunolide A and Z-ligustilide, using only a single reference standard. The results show no significant difference from those obtained by the external standard method, effectively resolving the issue of reference standard availability, reducing testing costs, and making it suitable for routine batch testing [6].

LC-MS/MS offers higher sensitivity and selectivity, and its multiple reaction monitoring mode can effectively eliminate matrix interference, making it suitable for the accurate quantification of trace amounts of trace-level phthalides or degradation products. Nevertheless, its use in routine quality control is constrained by the high equipment cost [3].

These four technical methods complement one another, forming a relatively comprehensive quantitative analytical system. However, current analytical approaches are largely confined to a limited number of phthalide monomers, and the monitoring of phthalide interconversion as well as their degradation products is still inadequate, highlighting a critical area for future methodological advancement [5].

Each of the four methods mentioned above has its own advantages: the GC series is suitable for volatile profile analysis; HPLC-UV is the standard method for phthalate quantification; QAMS strikes a balance between cost and accuracy; and LC-MS/MS is used for trace analysis. In practice, the choice of method should be made flexibly based on the detection objectives and sample matrix.

3. Quality control techniques for water-soluble constituents of *Angelica sinensis*

Having previously discussed quality control methods for fat-soluble components, this section focuses on water-soluble components, which primarily include two categories: phenolic acids and polysaccharides. While analytical methods for the former are well-established, quality control for the latter lags behind due to their macromolecular nature. The following sections outline their extraction and detection methods, respectively, and highlight the shortcomings of current standards.

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3.1. Types of water-soluble constituents and their significance for quality control

The water-soluble constituents of *Angelica sinensis* mainly comprise two major categories: phenolic acids and polysaccharides. Phenolic acids are represented by ferulic acid and chlorogenic acid, and ferulic acid is the quality control marker specified in the *Chinese Pharmacopoeia* [1, 2]. Polysaccharides in *Angelica sinensis* account for approximately 8.5 per cent of the dried crude drug and consist mainly of heteropolysaccharides composed of glucose, galactose, arabinose and other sugars. However, the qualitative and quantitative analysis of polysaccharides is far more complex than that of small-molecule compounds, which is the primary reason for the long-standing lag in their quality control [7].

3.2. Extraction and pretreatment

The extraction of phenolic acids is typically carried out using alcohol solvents, ultrasonication or heated reflux, and the procedure is relatively straightforward. For the extraction of Angelica polysaccharides, taking advantage of their reduced solubility in ethanol, the water-boiling followed by ethanol precipitation method is commonly employed: after extraction with hot water and subsequent concentration, ethanol is added to a final concentration of approximately 80% to precipitate the polysaccharides. Different extraction and processing methods have a significant impact on the polysaccharide content of *Angelica sinensis*; studies have shown that there are considerable differences in the levels of neutral and acidic polysaccharides in *Angelica sinensis* samples prepared using different processing methods [7].

3.3. Analytical and quantitative techniques

Chromatographic determination of phenolic acids: HPLC is the mainstream method for the determination of phenolic acid constituents. A high-performance liquid chromatography (HPLC) method has been reported for the simultaneous determination of eight constituents in *Angelica sinensis*, i.e., tryptophan, chlorogenic acid, ferulic acid, senkyunolide I, senkyunolide H, imperatorin, Z-ligustilide, and butylidenephthalide [8]. In addition, an HPLC method coupled with a photodiode array detector (PDA) has been established for the simultaneous quantification of six bioactive compounds, including ferulic acid, senkyunolide I, senkyunolide H, coniferyl ferulate, Z/E-ligustilide, and Z/E-butylidenephthalide [9]. The method combining HPLC fingerprint profiles with chemometric analysis to identify characteristic chromatographic peaks is now also widely used in the quality assessment of *Angelica sinensis* [8].

Methods for the determination of Angelica polysaccharides: Angelica polysaccharides are mixtures of macromolecules, it is not possible to quantify individual monomers directly using chromatography. Currently, the total sugar content is primarily determined by UV-visible spectrophotometry following color development with phenol and sulfuric acid [7]. Furthermore, the m-hydroxybiphenyl method is used to determine uronic acid content, whilst the Coomassie Brilliant Blue method is employed to determine protein content. Pre-column derivatization HPLC (PMP HPLC) can be used to analyze the monosaccharide composition of Angelica polysaccharides [10], providing more in-depth chemical information for the quality control of these polysaccharides.

Strategy for Simultaneous Determination of Multiple Components: In the quantitative analysis of multi-components by a single-marker (QAMS) method, with ferulic acid as the single internal standard, eight active constituents, namely ferulic acid, senkyunolide I, senkyunolide H, coniferyl ferulate, *E*-ligustilide, *E*-butylidenephthalide, Z-ligustilide, and Z-butylidenephthalide, can be

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simultaneously quantified using relative correction factors [6]. This method is simple, rapid, and cost-effective, and has been validated on 44 batches of *Angelica sinensis* samples from different origins and 45 batches processed using different preparation methods [8]. Two-dimensional liquid chromatography (2D-LC) technology offers new possibilities for the simultaneous separation and analysis of phenolic acids and polysaccharides.

It is noteworthy that, despite the extensive confirmation of the pharmacological activities of Angelica polysaccharides, the current standards of the *Chinese Pharmacopoeia* prescribe only identification tests for polysaccharides, with no quantitative requirements. The absence of standardized quality control for polysaccharides constitutes a significant deficiency in the quality standard system of *Angelica sinensis*.

Methods for detecting phenolic acids are now relatively well-established, with HPLC and QAMS capable of simultaneously analyzing multiple components; however, the analysis of *Angelica sinensis* polysaccharides still primarily relies on total sugar content determination, and there is a lack of precise quantitative methods targeting specific bioactive fragments.

4. Quality control techniques for insoluble components in *Angelica sinensis*

While relatively mature quality control methods already exist for the first two categories of components, insoluble components, including cellulose, calcium oxalate crystals and heavy metals, represent the weakest link in the quality control system. This section introduces the classification of these components and the corresponding characterization and detection techniques.

4.1. Types of insoluble components in *Angelica sinensis* and gaps in quality control

The insoluble components in *Angelica sinensis* primarily include macromolecular insolubles (cellulose), inorganic precipitates (calcium oxalate crystals), and heavy metals and harmful elements such as lead, cadmium, arsenic, and mercury. Compared to lipophilic and water-soluble components, quality control for insoluble components is the weakest. In the *Chinese Pharmacopoeia*, inorganic impurities are only indirectly indicated by total ash and acid-insoluble ash, with no targeted quantitative methods provided [1, 3]. This has led to the neglect of issues such as excessive heavy metal levels, safety risks associated with calcium oxalate, and the impact of macromolecular insolubles on formulation processes.

4.2. Characterization techniques for macromolecular insolubles

Near-infrared (NIR) spectroscopy offers a new approach for the rapid characterization of insoluble components. Researchers have used NIR to develop spectral prediction models for six parameters in *Angelica sinensis*—total ash, acid-insoluble ash, moisture, extractives, essential oil, and ferulic acid—which can be used to distinguish medicinal materials at different storage periods. Combined with chemometrics, this technique can also be used for authenticity verification and adulteration analysis. Although NIR is currently used primarily for the rapid prediction of routine parameters, it has also shown potential for the semi-quantitative analysis of insoluble substances such as cellulose and calcium oxalate.

4.3. Detection of inorganic elements and heavy metals

Inductively coupled plasma mass spectrometry (ICP-MS) is the standard method for detecting inorganic elements in *Angelica sinensis*. It features low detection limits, high sensitivity, a wide linear range, and the ability to simultaneously determine multiple elements. Previous studies have used ICP-MS to determine the concentrations of 12 essential trace elements and 5 heavy metals in *Angelica sinensis* [3]. In addition, the detection methods for the five elements—lead, cadmium, arsenic, mercury, and copper—in *Angelica sinensis*-containing preparations exhibit good linear relationships. Atomic absorption spectroscopy (AAS) serves as a supplementary method and is suitable for routine monitoring of specific elements.

ICP-MS, with its high sensitivity and ability to analyze multiple elements simultaneously, has become the preferred technique for monitoring insoluble substances; the combination of rapid NIR screening and precise ICP-MS quantification is expected to form a tiered testing strategy ranging from rapid initial screening to accurate validation.

5. Conclusion

In summary, quality control techniques for Angelica sinensis have evolved into distinct methodological systems based on solubility. Fat-soluble components are primarily analyzed using GC-MS, HPLC, and QAMS. Water-soluble components are mainly analyzed using HPLC and UV-Vis. Insoluble components are typically analyzed using NIR and ICP-MS. However, the development of these three categories of techniques is uneven, and the lack of quantitative standards for polysaccharides and insufficient risk assessment of insoluble components are the current major shortcomings.

Q-Marker-guided screening and the concept of end-to-end quality control provide new approaches for improving the quality control system. The shift from precise detection based on component classification to integrated evaluation using Q-Markers represents the core trend in the development of Angelica sinensis quality control.

Looking ahead, the key to breakthroughs in Angelica sinensis quality control lies in addressing existing shortcomings. Regarding water-soluble components, while the pharmacological activity of Angelica sinensis polysaccharides is well-established, quality control has long remained stagnant at the identification level. Future efforts should focus on establishing quality control standards for polysaccharides based on monosaccharide composition profiles and molecular weight distributions, thereby truly incorporating polysaccharides into the core indicator system for evaluating Angelica sinensis quality. In terms of safety, calcium oxalate crystals and heavy metal residues are priority risk factors among insolubles. Systematic data on the effects of calcium oxalate crystals are still lacking, and while established methods exist for heavy metal testing, these need to be integrated from the research level into routine quality control processes.

The diversity in the solubility of Angelica sinensis's chemical constituents presents both a challenge for quality control and a starting point for comprehensive quality evaluation. While the detection of fat-soluble and certain water-soluble small-molecule constituents has now reached a mature stage, future efforts should focus on the standardization of polysaccharide quality control and safety assessment of insoluble substances. At the same time, the Q-Marker concept should be transformed from academic research into practical quality standards, thereby establishing a more scientific and comprehensive quality control system for Angelica sinensis.

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DOI: 10.54254/2753-8818/2026.HZ35863

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