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Direct and simultaneous detection of organic and inorganic ingredients in herbal powder preparations by Fourier transform infrared microspectroscopic imaging

Jian-bo Chen^a, Su-qin Sun^a, Xu-dong Tang^b, Jing-zhao Zhang^b, Qun Zhou^{a,*}^a Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084, China^b Key Laboratory for New drugs Research of Traditional Chinese Medicine (TCM), Research Institute of Tsinghua University in Shenzhen, Shenzhen 518057, China

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ABSTRACT

Herbal powder preparation is a kind of widely-used herbal product in the form of powder mixture of herbal ingredients. Identification of herbal ingredients is the first and foremost step in assuring the quality, safety and efficacy of herbal powder preparations. In this research, Fourier transform infrared (FT-IR) microspectroscopic identification method is proposed for the direct and simultaneous recognition of multiple organic and inorganic ingredients in herbal powder preparations. First, the reference spectrum of characteristic particles of each herbal ingredient is assigned according to FT-IR results and other available information. Next, a statistical correlation threshold is determined as the lower limit of correlation coefficients between the reference spectrum and a larger number of calibration characteristic particles. After validation, the reference spectrum and correlation threshold can be used to identify herbal ingredient in mixture preparations. A herbal ingredient is supposed to be present if correlation coefficients between the reference spectrum and some sample particles are above the threshold. Using this method, all kinds of herbal materials in powder preparation *Kouqiang Kuiyang San* are identified successfully. This research shows the potential of FT-IR microspectroscopic identification method for the accurate and quick identification of ingredients in herbal powder preparations.

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1. Introduction

In the form of discrete fine particles, herbal powder preparation is a kind of medicinals which can be taken internally or applied topically [1]. For a long time, herbal powder preparations have been widely used owing to the easy production and application. Herbal powder preparation is usually the powder mixture of several kinds of herbal ingredients. These ingredients may be the powder of herbal materials, extracts, and purified active substances. Herbal ingredients of botanic, animal, and mineral origins can be used [2]. Therefore, identification of various herbal ingredients is the first thing to assure the quality and safety of herbal powder preparations. At present, microscopic identification and chemical identification are primary methods.

Microscopic identification is a traditional method to identify herbal materials according to their biological characteristics, including the shape and color of tissues, cells, and ergastic substances [3]. This method is useful to identify herbal materials in powder preparations. However, inherent uncertainties of biological characteristics make the recognition criteria quite fuzzy. As a result, microscopic identification is vulnerable to the personal knowledge of operators. Besides, this method is

unavailable for the identification of extracts and purified active substances in herbal powder preparations.

Chemical identification of marker compounds is a common method for herbal ingredients in different forms. However, the separation and determination procedure for each marker compound is usually very specific. In other words, it is necessary to detect multiple marker compounds one by one to identify all herbal ingredients in a powder preparation. Table 1 shows the prescribed chemical identification procedures of *Kouqiang Kuiyang San* (KKS), which is a traditional Chinese medicinal for oral ulcers [4]. To identify all herbal materials in KKS, there are three organic compounds and three inorganic ions to be detected. At least three separation processes and five determination tests are needed. This costs a lot of time, labor, and money, in addition to chemical waste and pollution. Therefore, simpler, quicker, cheaper, and greener methods are needed for the chemical identification of herbal powder preparations.

Has been playing a more and more important role in quality control and studies of herbal medicinals [5–10], Fourier transform infrared (FT-IR) spectroscopy is one of the most promising techniques to establish the chemical fingerprints of herbal medicinals [11,12]. FT-IR spectra of most herbal medicinals can be measured directly without any separation or derivatization. These spectra are rich of information about the structures and contents of organic and inorganic substances in herbal medicinals. Therefore, identifying herbal medicinals by FT-IR spectroscopy can save a lot of time, labor, money, and chemicals.

* Corresponding author.

E-mail address: zhouqun@tsinghua.edu.cn (Q. Zhou).

Table 1
Herbal materials and chemical identification procedures of *Kouqiang Kuiyang San* in Chinese Pharmacopeia [4].

Herb material	Content (w/w)	Marker substance	Separation procedures	Determination procedures
Indigo Naturalis	10/21	Indigo Indirubin	Dissolve 40 mg sample in 5 ml chloroform and keep the filtrate for test.	thin-layer chromatography developing solvent: chloroform - ethanol (9:1, v/v) reference: indigo, indirubin
Calcined Alumen	10/21	Al ³⁺	Dissolve 0.5 g sample in 10 ml water and keep the filtrate for test.	a) Precipitation by NaOH, and the white precipitate dissolves in excess NaOH. b) Precipitation by NH ₃ ·H ₂ O, and the white precipitate become cherry red when sodium alizarin sulfonate is added.
		K ⁺	Dissolve 0.5 g sample in 10 ml water and keep the filtrate for test.	a) Purple flame reaction.
		SO ₄ ²⁻	Dissolve 0.5 g sample in 10 ml water and keep the filtrate for test.	b) Precipitation by 0.1% sodium tetraphenylboron and acetic acid. a) Precipitation by BaCl ₂ , and the white precipitate is insoluble in HCl or HNO ₃ . b) Precipitation by lead acetate, and the precipitate is soluble in ammonium acetate or NaOH. c) No precipitation by HCl.
Synthetic Borneolum	1/21	Borneol	Dissolve 0.5 g sample in 10 ml ethyl ether and filter. Volatilize the ether in the filtrate and dissolve the solute in 1 ml ethanol.	thin-layer chromatography developing solvent: cyclohexane - ethyl acetate (17:3, v/v) derivatization reagent: 5% vanillin sulfuric acid solution reference: borneol

Every coin has two sides. Measuring herbal samples without separation also leads to the bottleneck of FT-IR spectroscopy. It is usually difficult to find out absorption bands of specific substances in the signal-overlapped spectra of herbal samples which are complex mixtures. Herbal powder preparations are mixtures of powdered herbal ingredients, while each herbal ingredient is usually also a mixture of many kinds of organic and inorganic substances. In this sense, it seems impossible to find out absorption bands of marker compounds in the signal-overlapped FT-IR spectra to identify the ingredients in herbal powder preparations.

As mentioned previously, a herbal powder preparation is composed of discrete fine particles of multiple origins. The bulk spectrum collected by a conventional FT-IR instrument is the cumulative spectral signals of various particles, which make it difficult to discriminate the absorption bands of different herbal ingredient particles. If every particle of the preparation is measured separately, the resultant spectra should almost be free of interferences. In other words, absorption signals of different herbal ingredients can be resolved. If a herbal ingredient contains some characteristic particles with particular spectral signals, powder preparations consisting of this ingredient should also contain these characteristic particles. In this way, FT-IR microspectroscopy can be used to recognize these characteristic particles by their particular spectral signals, thereby to identify the ingredients in herbal powder preparations [6].

Using KKS powder preparation as an example, this research explores the feasibility of FT-IR microspectroscopic identification method for the direct and simultaneous recognition of multiple organic and inorganic ingredients in herbal powder preparations.

2. Material and methods

2.1. Materials

All herbal materials (Indigo Naturalis, Alumen, and Synthetic Borneolum) and the commercial KKS preparation (No. 3100023) were purchased from Tongrentang Pharmacy in Tsinghua University (Beijing, China). Calcined Alumen was obtained by heating Alumen in a muffle furnace at 300 °C for 30 min [13]. The model mixture was obtained by mixing fine powder of Indigo Naturalis (1 g), Calcined Alumen (1 g), and Synthetic Borneolum (0.1 g) homogeneously.

Analytical reagents, including indigo, CaCO₃ (calcite), and KAl(SO₄)₂·12H₂O, were purchased from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China). All reagents were used directly without any further purification. KAl(SO₄)₂ was obtained by heating KAl(SO₄)₂·12H₂O in a muffle furnace at 300 °C for 30 min.

All materials were powdered by a ceramic mortar and screened with a 100-mesh sieve. The fine powder was used for tests without any further processing.

2.2. Thermogravimetric analysis (TGA)

Thermogravimetric curves of Alumen and KAl(SO₄)₂·12H₂O were tested by Pyris 1 TGA (PerkinElmer, Waltham, MA, USA). The furnace was purged by nitrogen at a flow rate of 40 ml/min, while the balance was purged by purified nitrogen at a flow rate of 20 ml/min. Sample powder (~10 mg) was transferred into the platinum crucible, then heated from 50 °C to 850 °C at a constant rate of 20 °C/min. Pyris software (PerkinElmer, Waltham, MA, USA) was used to manage TGA instrument and data.

2.3. Macroscopic ATR-FTIR spectroscopy

Bulk infrared spectra of all samples were collected using a Frontier FT-IR/NIR spectrometer with a universal ATR sampling accessory (PerkinElmer, Waltham, MA, USA). The internal reflection element (IRE) was a diamond/ZnSe composite crystal and the angle of incidence was 45°. Sample was placed on the surface of IRE crystal and pressed by a lever to ensure a tight and constant contact during the measurement. Each spectrum was the average of 32 scans in the range of 4000–650 cm⁻¹ with a spectral resolution of 4 cm⁻¹. The influence of H₂O and CO₂ was subtracted automatically. Spectral ordinate was transformed into absorbance and baseline was corrected automatically by Spectrum v6.0 (PerkinElmer, Waltham, MA, USA).

2.4. ATR-FTIR microspectroscopic imaging

FTIR imaging system was composed of the above Frontier FT-IR/NIR spectrometer and a Spotlight 400 FT-IR microscope (PerkinElmer, Waltham, MA, USA). Samples were measured by an ATR imaging accessory with a germanium IRE crystal (PerkinElmer, Waltham, MA, USA). A linear array detector of narrow-band MCT was used to collect the microscopic spectra.

Sample powder was supported by a piece of aluminum foil and fixed into the ATR imaging accessory on a motorized microscope stage. Thereby, the accessory and sample were moving synchronously. Microscopic spectra were collected by altering the point of incidence of IR beam into the IRE crystal [14]. Each sample was measured in a region of 500 × 500 μm with a nominal pixel size of 6.25 × 6.25 μm. Microscopic spectra in the range of 4000–750 cm⁻¹ were collected with a spectral resolution of 8 cm⁻¹, while each pixel spectrum was the average of 8 scans. SpectrumIMAGE v1.7 (PerkinElmer, Waltham, MA, USA) was used to control the imaging system and optimize the raw spectra. ATR correction with a zero contact factor, atmospheric correction, and baseline-offset were performed by default. Then, correlation images

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