THE APPLICATION OF INFRARED FINGERPRINTING TECHNOLOGY

What is infrared fingerprinting?

Throughout the history and development of Chinese herbal medicine, concerns have always been raised about the authenticity and quality assessment of the materials used. Initially, identification of medicinal herbs was based mainly on personal experience. Later, this developed into four major identification methods – identification by origin, identification by characteristics, microscopic identification, and identification by physico-chemical analysis.

Spectroscopy is now commonly used in the identification of Chinese materia medica. Infrared (IR) spectroscopy involves shining a range of infrared frequencies through a sample of an organic compound and mapping the absorption of these frequencies on a graph. Any sample material that will interact with infrared light produces a spectrum and the technique can be used to examine samples in liquid, solid or gas phase.

IR spectroscopy is an analytical method that uses information relating to the comparative vibration of atoms within a molecule and the rotational movement of the molecules themselves, thereby identifying the structure of the molecule and differentiating the chemical compounds it contains. Organic molecules exist in a state of continuous vibration. When the vibration frequency of certain elements within an organic molecule is identical to the wave frequency emitted by a continuous wave of infrared light, a proportion of the light is absorbed by the molecule at that wave frequency. Some frequencies will pass through a substance with almost no loss through absorption, other frequencies are strongly absorbed. This produces a graph showing the degree of absorption (A) on the vertical axis and the wavenumber (defined as the reciprocal of the wavelength in cm, i.e. cm⁻¹) on the horizontal axis (see the example of *he shou wu* in Figure 1). Each peak in the graph denotes the energy being absorbed at the particular infrared frequency.

The region on the right side of the graph (from about 1500 to 500 cm⁻¹) contains a very complicated series of absorptions, mainly due to different types of rotational vibrations. This area is known as the fingerprint region. The importance of this region is that each compound produces its own unique pattern of peaks and troughs, a pattern specific to that compound in the same way as a human fingerprint is specific to a particular person. The IR spectrum possesses high specificity and every single compound has its own characteristic IR fingerprint spectrum, reflecting the complete information relating to that compound. The fingerprint region, with its features of uniqueness, stability and completeness, is therefore ideally suited to characterise a herbal material, where the entire range of constituents is not always known.

Figure 1 A typical infrared spectrum showing the fingerprint region from 1500 to 500cm⁻¹
Fingerprint analysis focuses on accurate identification of similar peaks, and not on precise calculation, making it an excellent tool for testing whether a particular material corresponds to a standard sample of that material, thus confirming its authenticity. Within the scanned wavenumber range, it is sufficient to compare the wavenumbers of the absorption peaks, the peak shape and peak intensity of the same wavenumber, and the differences in the appearance of the fingerprint region. Data analysis allows the comparison and calculation of fingerprint peaks, thus determining the overall degree of similarity between fingerprints.

IR fingerprinting therefore possesses two key advantages in the analysis of herbal materials – specificity (the uniqueness of the material) and reproducibility (batch-to-batch similarity). In addition, it can be used for analysis at all stages of the production process – with raw herbs, prepared herbs (yin pian), processed herbs (pao zhi), and final products (such as granules, powders and injections) – as well as for differentiating between genuine and fake Chinese materia medica, determining origin and period of cultivation, research into authentic (dao di) and processed (pao zhi) materia medica, and the quality control of proprietary herbal medicines.

An IR fingerprint reflects the overall nature of a Chinese medicinal herb, thereby raising the quality control target from analysis of a single component to analysis of all the materials in a herb. In recent years, fingerprinting technology has become an important means for variant identification and quality assessment of Chinese materia medica. The establishment of a Chinese herb fingerprint database provides a reference and guideline for routine testing and analytical work and serves as a solid foundation for quality standards.

IR fingerprinting as an information tool

Fingerprint identification is an effective solution to monitoring the quality and ensuring the batch-to-batch stability of Chinese medicinal herbs with their complex multiple chemical constituents that are difficult to identify and analyse. In the same way as Chinese medicine treats the person as a whole, so it can be said that understanding the whole picture of the chemical composition of a herb is an application of Chinese medicine theory to analysis of the materials used. This “macro-regulation”[1] of Chinese materia medica is a suitable way forward for research and quality control. Infrared fingerprinting takes advantage of the infrared spectrum to reflect the whole organic and inorganic ingredients of a compound in one graph providing all the information on that compound. It is an excellent analytical method for translating the traditional evaluation system of Chinese materia medica into objective, quantitative criteria.[2]

Infrared fingerprinting highlights the multi-layer structure of the constituents of Chinese medicinal herbs, with analysis progressing from the whole to the parts, determining the characteristics first and then the quantities.[3] Some of the data can be broadly understood, other parts must be analysed in detail. The complete information reflected in the infrared fingerprint allows us to examine the major ingredients which contribute significantly to the effectiveness of a particular materia medica. We can even analyse micro amounts of active ingredient, while broadly understanding the main ingredients. For instance, if the main ingredient is protein, we only need to understand the amount of protein contained rather than all the different types of protein; at the same time, we need to analyse the micro active ingredients closely, both in terms of type and quantity. Using information on the overall ingredients and micro ingredients allows multi-level determination of the characteristics of the herb being analysed and the quantities of a particular ingredient can be determined on the basis of these characteristics.

Chromatography and spectroscopy

Nowadays chromatography and spectroscopy are the technologies most commonly applied in the analysis of Chinese materia medica. Chromatography involves a separation process that is very useful in identifying specific constituents found in a particular substance. Spectroscopy is less suitable for identifying individual constituents, but is very effective in facilitating comparisons between samples because it allows the whole chemical nature of a substance to be seen at a glance. They both have a role to play in relation to Chinese materia medica.

The efficacy of a medicinal herb depends on how various chemical components work together. Therefore the practice of evaluating traditional Chinese materia medica based on the quantitative and qualitative analysis of a single chemical component is gradually being questioned in terms of its effectiveness and accuracy. This is because it is difficult effectively to evaluate the quality and authenticity of a Chinese herb based on any single active ingredient, especially so if the ingredient analysed is a common ingredient in many herbs.
Chromatography can separate out each individual chemical ingredient within a mixture (including a medicinal herb extract). The visual output (the chromatogram) clearly presents the chemical information and the peak can be identified easily. By comparing the output of a sample material against a standard output under the same conditions, chromatography can accurately determine the characteristics and quantity of a target ingredient and whether the sample conforms or not to the standard. This limits its use in analysing Chinese materia medica to those herbs whose active ingredients or other constituents are known. Chromatography is most useful in ensuring a herb contains the required quantity of a target ingredient, for instance as specified in the Chinese Pharmacopoeia, but it cannot provide the whole picture. In addition, separation is a relatively time-consuming process and the solvents used are not environmentally friendly.

IR fingerprint spectroscopy focuses on the complete qualitative information related to a particular materia medica rather than on specific target ingredients. It presents a complete and accurate image of the individual chemical constituents of a medicinal herb. The specific properties of these chemical constituents permit the identification of the herb. In addition, this technique allows identification of the origin of a herb, thereby ensuring its authenticity (dao di). It has the advantages of accuracy, speed and low cost and it is environmentally friendly since no separation material is required. The equipment fits comfortably on a laboratory desktop (Figure 2).

Nevertheless, since similar functional groups of chemical compounds can exist in different herbs, classification, differentiation and interpretation of wave peaks is comparatively difficult, especially where they are overlapping. This disadvantage can be overcome through the application of stoichiometry to establish pattern recognition in the identification of Chinese herbs. b

IR fingerprint spectrum technology for herbal medicines is the only method that can represent a complete set of information for a particular materia medica (including locality, species, conformity to processing requirements, and length of cultivation), thereby enabling fake materia medica to be detected and rejected. Since, unlike chromatography, this technology can be used on all types of material, it enables quality to be controlled throughout the production process – solid materials at the raw herb and prepared herb (yin pian) stage, extraction liquids during the extraction process, powders during the spray drying stage and granules produced from herb extracts at the finished product stage. This ensures a consistently high, stable level of product quality from batch to batch. Kang Ren Tang, the manufacturer of FCG granules, has documented more than 100,000 standard fingerprints to date and has established a comprehensive database for quality control.

**Application of infrared fingerprinting technology in the production of FCG granules**

**Quality control of raw herbs**

Every materia medica has a different fingerprint depending on its locality and species and the methods employed in its processing. The quality of a raw Chinese herb directly affects the quality of FCG granules produced from it. The good or bad quality of raw herbs is determined by a variety of factors involved in the chain from collection to processing – including location, period of growth, harvesting season and drying conditions. Current national standards in China rely on thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) to control the active ingredient content, but this does not take location, harvesting and processing into account. After research covering the range of commonly used Chinese herbs, we found that infrared spectroscopy technology provides unique differentiation criteria for factors affecting herb quality. It has helped to set standards for the control of authentic (dao di) Chinese herbs and to safeguard their authenticity. IR fingerprinting allows every single batch to be compared against the established standard.

First, let’s take zhi shi as an example. We procured batches of zhi shi from three different provinces – Jiangxi, Sichuan and Hunan. The synephrine contained in the batches from all three locations meets the requirement of the Chinese Pharmacopoeia (>0.3%). So, can we consider all three batches as authentic? When we tested them through IR fingerprinting, we found differences of more than 50% compared with the standard fingerprint (see Figure 2).

Further study led to the finding that an important active
ingredient known as naringin is only contained in zhi shi from the authentic location of Xingan county in Jiangxi Province, but not in the herb sourced from Sichuan and Hunan provinces (see Figure 4). Interestingly, the earliest documented centrally-organised Chinese pharmacopoeia, Ben Cao Tu Jing (1061), states that zhi shi “is found in Jingxi and Jianghu counties, but the best comes from Shang county.” Shang county in the Song Dynasty corresponds to present-day Xingan county.

Fig 3 Zhi Shi fingerprints from 3 different locations

Figure 4. HPLC graphs of Zhi Shi from different locations

Fig 4. HPLC graphs of Zhi Shi from different locations

Gan cao is another example. The three major species of gan cao in the market are Glycyrrhiza uralensis Fisch., Glycyrrhiza inflata Bat. and Glycyrrhiza glabra. We analysed the constituents of Glycyrrhiza uralensis and Glycyrrhiza inflata and found that Glycyrrhiza uralensis regularly contains high amounts of both glycyrrhizic acid and liquiritin, two major ingredients. We also found that, although Glycyrrhiza inflata has a high glycyrrhizic acid content, its liquiritin content is very low. In recent years Glycyrrhiza inflata from Xinjiang province has been widely used in clinical treatment, especially in gan cao exported to Western countries. This can be explained by the use of glycyrrhizic acid extract in the USA as an additive to food supplements and cigarettes, thus outweighing the application of gan cao as a herbal medicine. However, the liquiritin contained in gan cao is considered by materia medica researchers as the major active ingredient in clinical treatment, which means that Glycyrrhiza uralensis should therefore be considered as the standard species.

The comparison between Glycyrrhiza uralensis and Glycyrrhiza inflata is depicted in Figure 5, which shows a clear difference around 1000cm⁻¹.

Figure 5. IR standard spectra of two different species of gan cao (graphs superimposed)
To establish a standard fingerprint criterion for the raw material, we analysed the infrared spectrum of 30 batches of dao di gan cao and compare their spectra to obtain a standard spectrum (see Figure 6). On this basis, we can quickly identify the origin of a batch of gan cao before we accept it for the production stage of FCG granules.

**Quality control of processed Chinese materia medica (pao zhi)**

Chinese materia medica must be processed before being used for medicinal purposes. Processing affects the medicinal properties of a herb, for example its ascending or descending properties, the channels entered, toxicity, etc. Traditionally, processing relied mainly on the skill and experience of practised materia medica technicians rather than on scientific criteria or standards. In our research into the optimum production methods for FCG granules, we found that application of infrared spectroscopy can monitor the chemical changes of the processed materia medica throughout the procedure. It also makes up for the disadvantage of HPLC, which can only control single ingredients, as well as avoiding the risk of leaving quality control to manual methods.

Two examples illustrate the usefulness of IR fingerprinting in controlling processing quality. The first example is relatively straightforward and shows the difference between sheng di huang and shu di huang. Shu di huang is produced by steaming sheng di huang in yellow rice wine for 24 hours, after which its polysaccharides break down into monosaccharides. This is reflected in the IR fingerprint spectrum (see Figure 7) where a single peak breaks down into a double peak (circled in red); insufficiently-cooked di huang retains its single peak (shu di huang 1 in the figure). This indicates that the IR fingerprint spectrum technique can be used to differentiate between those materia medica that have been processed correctly and those processed incorrectly.

The more complex example of he shou wu indicates the advantages of infrared spectroscopy in monitoring chemical changes throughout the processing period. The main ingredients of he shou wu include anthraquinones (such as emodin and chrysophanol), stilbene glycosides, phospholipids (such as lecithin), and polysaccharides. According to reports, these ingredients change dramatically after processing. The increase in the nourishing and supplementing effect of processed...
Figure 8  Infrared spectrum depicting changes in stilbene glycoside hydrolases and polysaccharides during processing of he shou wu

Figure 9  Anthraquinone changes during processing of he shou wu reflected in the spectrum

Figure 10  Quantitative changes in stilbene glycosides, anthraquinones, polysaccharides and alcohol-soluble extracts during processing of he shou wu
he shou wu may relate to a certain extent to glycoside hydrolases; some research articles indicate that the proportion of stilbene glycosides or polysaccharides varies according to the cooking (steaming) time.[4,5]

Infrared fingerprinting spectroscopy can indicate these changes for various processing times (see Figure 8). The spectrum between 1200cm⁻¹ and 1000cm⁻¹ reflects changes in sugars and glycosides; the spectrum peak of 1050cm⁻¹ disappears at 0-4 hours. This indicates that stilbene glycosides are mainly hydrolysing during processing and are transforming into glycosides and sugars. At 6-14 hours, the peak increases strongly in the 1050 to 1080 cm⁻¹ section, indicating a major accumulation of polysaccharides during that processing period. After 16 hours, this section of the peak shows no further obvious change, indicating that stilbene glycoside hydrolysis and polysaccharide accumulation has reached equilibrium. Therefore, if stilbene glycosides and polysaccharides are criteria, the optimum processing time should be 16 hours.

Anthraquinones: As the processing time of he shou wu increases, bound anthraquinones decrease and free anthraquinones increase.[6] These changes can be observed along the infrared spectrum. The change in the peaks in the 1790 to 1610cm⁻¹ range is consistent with the change in bound anthraquinones, whereas the changes in the peak in the 1580 to 1470cm⁻¹ range is consistent with the changes in free anthraquinones. The change in the ingredients is greater up to 12 hours, becomes smaller after 12 hours and there is no change after 16 hours (Figures 9 and 10).

The discussion above shows how infrared spectroscopy can be applied to monitor chemical changes during the processing period. However, it can also be applied in determining the optimum processing target. In Figure 11, the wave peaks at 1018cm⁻¹ and 1510cm⁻¹ represent glycoside and the benzene ring respectively. Changes in the ratio of the two peaks clearly show the transformation from glycoside to aglycone during processing of he shou wu, thus indicating the changing trend of bound anthraquinones hydrolysing into free anthraquinones. Calculating the absorption peak ratio gives the ratio between bound anthraquinones and total anthraquinones. Comparing this peak ratio with HPLC test results allows us to analyse the optimum processing period (see Table 1 and Figure 12).

![Table 1 Changes to zhi he shou wu target ingredient at different times during processing](image)

<table>
<thead>
<tr>
<th>Processing time (high pressure steaming)</th>
<th>1018cm⁻¹/1510cm⁻¹ peak ratio</th>
<th>Ratio of bound to total anthraquinones</th>
</tr>
</thead>
<tbody>
<tr>
<td>2h</td>
<td>2.30</td>
<td>0.96</td>
</tr>
<tr>
<td>4h</td>
<td>2.28</td>
<td>0.75</td>
</tr>
<tr>
<td>6h</td>
<td>1.60</td>
<td>0.65</td>
</tr>
<tr>
<td>8h</td>
<td>1.63</td>
<td>0.51</td>
</tr>
<tr>
<td>10h</td>
<td>1.53</td>
<td>0.30</td>
</tr>
<tr>
<td>12h</td>
<td>1.53</td>
<td>0.30</td>
</tr>
<tr>
<td>14h</td>
<td>1.32</td>
<td>0.09</td>
</tr>
<tr>
<td>16h</td>
<td>1.31</td>
<td>0.08</td>
</tr>
<tr>
<td>18h</td>
<td>1.30</td>
<td>0.03</td>
</tr>
<tr>
<td>20h</td>
<td>1.30</td>
<td>0.03</td>
</tr>
</tbody>
</table>

![Figure 11 Infrared spectrum of zhi he shou wu at different times during processing](image)

![Figure 12 Changing trend of zhi he shou wu target ingredient at different times during processing](image)
Quality control of finished products (FCG granules)

After concentration and extraction, herbal granules obviously lose the characteristic visual features of prepared raw herbs (yin pian) and so cannot be differentiated through external appearance, microscopic structure or cellular properties. Therefore, it is essential to establish an effective method for testing granules, especially for related species such as bai shao and chi shao or jin yin hua and shan yin hua. Once granulated, it is very difficult to differentiate the origin of these species from their external appearance or by testing their chemical ingredients with HPLC and TLC.

For example, the level of chlorogenic acid contained in shan yin hua and jin yin hua both achieve the level required by the Chinese Pharmacopoeia standard. Therefore, based on content, the two types cannot be differentiated. However, the IR fingerprint spectrum technique can immediately identify whether a sample of jin yin hua is authentic when compared with a standard sample. This is important because according to the latest edition of the Chinese Pharmacopoeia, shan yin hua does not meet the criteria for the amount and size of flower buds; although it is cheaper, it is not as clinically effective as jin yin hua.

As another example, both huang lian and huang bai granules contain berberine, but it is very difficult to differentiate them by testing for this ingredient. We managed to resolve this issue by combining IR spectroscopy with existing physico-chemical analyses, chromatography and other analytical techniques to establish an IR fingerprint database.

Figures 12 and 13 clearly illustrate the effectiveness of IR fingerprinting in granule differentiation. Figure 12 indicates the obvious difference between jin yin hua and shan yin hua when comparing the infrared spectrum. Figure 13 represents the same target chemical ingredient of berberine contained in huang lian and huang bai and paeoniflorin contained in bai shao and chi shao.

Figure 13  Infrared spectroscopy comparison between jin yin hua and shan yin hua

Figure 14  Infrared spectroscopy comparison between huang bai and huang lian (left), bai shao and chi shao (right)
Conclusion

Infrared fingerprinting has proved to be a key element in raising the overall standard of Chinese medicinal herbs in terms of cultivation, collection, processing, production and analysis and plays a crucial role in quality control of the production of FCG herbal granules. By differentiating between genuine and false raw materia medica, it ensures the authenticity of the source of herbal granules. The unique quality of IR spectroscopy fingerprinting means it can be used to establish a specific label for each and every Chinese herbal granule and its comprehensiveness means that it can demonstrate the entire range of internal characteristics of these granules to ensure batch-to-batch consistency. It can also determine the quantity of excipients used in the production of any granules on the market since the graph peaks are highly sensitive to the addition of extracts or excipients. In this way, the excessive addition of excipients can be monitored, thus helping to ensure fair competition and patient trust. Quality control through fingerprinting will significantly increase the level of controllability of the production process and promote greater confidence in the reliability and consistency of products subject to testing by this method.

About the authors

Zhou Yong Kang graduated from Xinan University with a degree in pharmacology and is currently studying for a master’s degree at Beijing TCM University. He developed an interest in research during his university studies and subsequently joined the research institute of Kang Ren Tang’s parent company. In 2014, he was appointed a leading member of the group responsible for establishing the Beijing FCG Engineering and Enterprise Technology Centre. He is also involved in research and development of ultraviolet and near infrared wave control systems for use with herbal extracts.

Shen Jian Mei graduated from Capital Medical University with a degree in TCM pharmacology and is currently studying for a master’s degree at Beijing TCM University in the chemistry of Chinese materia medica. In 2011, she joined the research institute of Kang Ren Tang’s parent company, initially researching FCG quality standards and the production environment for cooking herbs individually or together. She is currently working on developing infrared fingerprinting technology and DNA coding of Chinese materia medica to meet the requirements of FCG granule testing and has been involved in setting IR quality standards for herbs such as jin yin hua, ge gen, dan shen and gan cao.

Zhao Zhen Guang graduated from Jilin Agricultural University with a degree in materia medica resources and is currently studying for a master’s degree at Beijing TCM University. In 2011, he joined the research institute of Kang Ren Tang’s parent company, working initially on research into materia medica sourcing and quality and the industrialisation of pao zhi procedures. He has written a report for the Chinese Ministry of Industry and Information Technology on the legacy base of Chinese pao zhi materia medica techniques. His main research interests include optimisation and local handling of material sources, differentiation between genuine and false materia medica and the development of pao zhi procedures.

Acknowledgements

The authors would like to express their gratitude to Yanping Li and Rodger Watts of Donica Health for their invaluable contribution towards the English version of this article.

Notes

a. Measurement of absorption (A) on the vertical axis runs from 0% to 100%, but where two or more herbs are shown on one graph, the vertical scale becomes relative rather than absolute because the graphs of individual herbs would overlap too much to be readable. What is important is to show the wavelengths of the peaks and their characteristics.

b. Stoichiometry is a branch of chemistry that involves using relationships between reactants and/or products in a chemical reaction to determine desired quantitative data, for example the quantitative relationship between constituents in a chemical substance or between two or more substances in processes involving physical or chemical change.

References