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Multispectral Imaging for Authenticity Identification and Quality Evaluation of *Flos carthami*[*]

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ABSTRACT

The identification and quality evaluation of *Flos carthami* were studied using tunable liquid spectral imaging instrument, to discuss the application range and advantages of spectral imaging technology in Chinese medicine identification and quality control field. The *Flos carthami* was indentified by extracting the normalized characteristic spectral curves of *Flos carthami*, *Crocus sativus* and *Dendranthema morifolium*, which were standard samples supplied by National Institute for Drug Control. The qualities of *Flos carthamies* collecting from different pharmacies were evaluated by extracting their normalized characteristic spectral curves. The imaging spectrum testing system was designed independently. The spectral resolution was 5 nm, and the spectral range was from 400 nm to 680 nm. The results showed that the normalized characteristic spectral curve of *Flos carthami* was significantly different from those of *Crocus sativus*' and *Dendranthema morifolium*'*s*, and the fluorescence intensity of *Flos carthami* from different commercial sources were different. Spectral imaging technology could be used to identify and evaluate *Flos carthami*, and operation method was rapid, convenient and non-destructive.

Keywords: *Flos carthami*; Spectral Imaging; Rapid Identification; Quality Evaluation

1. Introduction

Flos carthami is the dried flower of *Carthamus tinctorius* L. It is commonly used in traditional Chinese medicine. It contains a variety of ingredients such as flavonoids compounds, phenolic acids, fatty acids, volatile oils, polyacetylene, adenosine, and so on. The main effects of *Flos carthami* are promoting blood circulation, dilation of blood vessels, improving microcirculation, eliminating free radicals, anti-inflammatory and other functions. The quality of *Flos carthami* is closely related with its efficacy. High-performance liquid chromatography (HPLC) can identify *Flos carthami* and evaluate its quality by detecting the content of the main component. However, HPLC methods can not be real-time detection, and it is time-consuming.

Multispectral imaging is an emerging technology that integrates conventional imaging and spectroscopy to attain both spatial and spectral information from a sample. Multispectral imaging was originally developed for remote sensing applications [1] but has since found application in diverse fields such as environment, telemetry, agriculture and other fields [2-6]. A series of exploratory studies have been conducted about different kinds of Chinese herbal medicines by our research group [7-11].

In this paper, The *Flos carthami* was indentified, and the qualities of *Flos carthamies* collecting from different pharmacies were evaluated using the multispectral imaging technology. The result shows that multispectral imaging technology provides an objective, time-saving, real-time detection, non-destructive and simple method for the identification and quality evaluation of *Flos carthami*.

2. Materials and Methods

2.1. Samples Preparation

The standard sample(SS)s of *Flos carthami* (*FC*), *Crocus*

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sativus (*CS*) and *Dendranthema morifolium* (*DM*) were supplied by Guangzhou Institute for Drug Control (GZIDC) on December 8, 2010 and April 27, 2011, respectively. The other *Flos carthami* samples collected from Guangzhou Tong Ren Tang pharmacy(GZTRTP), Guangzhou Bao Jian Tang pharmacy(GZBJTP), Guangzhou Er Tian Tang pharmacy(GZETTP), Guangzhou Bao Zhi Lin pharmacy(GZBZLP), Guangzhou Oriental pharmacy(GZOP), respectively. The sample details are listed in **Table 1**.

2.2. Liquid Crystal Multispectral Imaging System

The testing instrument was self-designed multispectral spectrum measurement system [12]. It is composed of the light source, a light source filter, a liquid crystal tunable filter (LCTF), the controller of LCTF, lens, CMOS sensor, data acquisition card and data processing software. The ray path of the testing system is shown in **Figure 1**.

Table 1. Information of samples.

Sample name	Source	Collecting time
SS of *FC* (batch number:	GDIDC 120907-200609)	2010.12.08
SS of *CS* (batch number:	GZIDC 1009-200101)	2010.12.08
SS of *DM* (batch number:	GZIDC 120995-200704)	2011.04.27
FC sample 1	GZTRTP	2010.12.12
FC sample 2	GZBJTP	2010.12.12
FC sample 3	GZETTP	2010.12.12
FC sample 4	GZBZLP	2010.12.12
FC sample 5	GZOP	2010.12.12

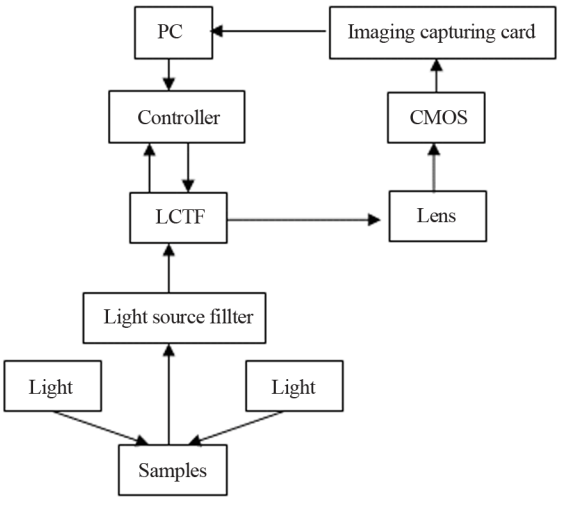

Figure 1. The ray path of the system.

The centre wavelength of the light source is 254 nm. The LCTF is an important component of the system. It is a splitter component based on electrically controlled birefringence of the liquid crystal. It is used to divide the light coming from the samples in two dimensions. The working wavelength of LCTF is from 400 nm to 1100 nm, which is controlled by the controller of LCTF. The wavebands from 400 nm to 680 nm are chosen in our experiment.

The samples partially reflect the light coming from the light source after the interaction of light and the sample. The light passes through LCTF carrying the information of the samples. The LCTF divides the light and focus it on the image sensor (CMOS). The images are captured by the image acquisition card and saved on the host computer, as JPG format. The two-dimensional spectrum data can be processed and the results are displayed on the monitor of the computer.

2.3. Methods

The excitation light sources are two mercury lamps with the center wavelength of 254 nm in our research. The bandwidth of each lamp is 30 nm, and its optical power is 6 w. Single channel, continuous spectrum scan was used in the detection process. The spectral scan range was from 400 nm to 680 nm, controlled by the controller of LCTF. The two adjacent frames interval is 5 nm. The spectral resolution is up to 0.5 nm. The CMOS imaging camera was adjusted so that the focal plane coincided with the surface of the test samples at 550 nm waveband. The CMOS camera was set to continuous mode with the exposure time of 1000 ms, which was synchronized with spectral scanning time, and then the fluorescence images of the test sample were acquired at the whole wavebands. The captured images were stored in the computer with jpg format. A spectral cube of the test sample, formed by 57 frame spectral images, can be obtained in one test.

The detected sample was placed on the substrate without any pre-treatment, and the two-dimension images of it at a number of narrow wavebands can be obtained. Removed noise in the images with a bandpass filter. Selected the same area in every image to calculate the average light intensity of the corresponding pixel and to normalize them according to Equation (1), then the characteristic spectral curve of the sample was obtained. **Figure 2** shows the normalized spectral curve of *Flos carthami*

$$\overline{I}_i(\lambda_i) = \frac{\sum_{n=1}^{N} I_{in}(\lambda_i)}{N}, \quad I_{iNormalized} = \frac{\overline{I}_i(\lambda_i)}{\overline{I}_{i\max}} \tag{1}$$

where, N is the number of the pixel, $\overline{I}_i(\lambda_i)$ is average light intensity of the i^{th} image, $i = 1, 2, \cdots, 57$, $\overline{I}_{i\max}$ is the biggest light intensity among the 57 frame images.

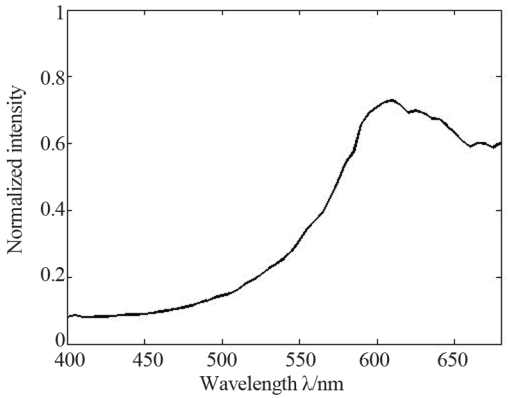

Figure 2. Normalized characteristic spectral curve of Flos carthami.

2.4. Methodological Study

1) *Stability test*

The same sample is tested five times under the same condition according to part C in the section II, and the interval is 24-hour between two times. Extract the characteristic spectral curves from the 5 times and compare them. The similarity of the 5 curves peak shape (measured by its covariance) is greater than 0.95, and the positions of characteristic peak remain unchanged. The uncertainty of characteristic peaks fluctuations in light intensity is less than 1.86% of the measurements. It shows that the samples have good stability in the detection.

2) *Precision test*

Repeat measuring the same sample five times according to part C in the section II under the same condition. Extract the characteristic spectral curves from the 5 times and compare them. The similarity of the 5 curve peak shape is greater than 0.95, and the positions of characteristic peak remain unchanged. The uncertainty of characteristic peaks fluctuations in light intensity is less than 1.86% of the measurements. It shows that the imaging system has good precision.

3) *Reproducibility test*

The same sample is divided into 5 parts. Every part is tested using the same system under the same condition according to part C in the Section II. Five characteristics spectra curves can be obtained and compared. The similarity of the 5 curve peak shape is greater than 0.95, and the positions of characteristic peak remain unchanged. The uncertainty of characteristic peaks fluctuations in light intensity is less than 1.86% of the measurements. It shows that this method has good reproducibility.

3. Result and Discussion

3.1. Characteristic Spectral Curves of FC, CS and DM

The standard samples of *FC*, *CS* and *DM* were detected according to Sections 2.3 and 2.4. **Figure 3** shows their normalized characteristic spectral curves. It indicates that the normalized characteristic spectral curves are significant differences each other. The general trends and characteristics peaks are completely different. The characteristic spectral curve of *FC* has partial peak structure, and the peak position is at 610 nm with peak height of 0.73. The characteristic spectral curve of *DM is* close to the normal distribution in the 450 - 650 nm wavebands, and the peak position is at 530 nm with peak height of 0.64. The fluorescence intensity of *CS* increased slowly in the detection wavebands, there was no peak. *FC* and *DM* are both composites, *FC* and *CS* in effect has similarities. However, the characteristic spectral curves of the three traditional Chinese medicines are significantly different. The result indicates that spectral imaging method can distinguish between different types of flower herbs.

3.2. Quality Evaluation of the *Flos carthami* Samples

The *FC* samples listed in **Table 1** were tested according to Sections 2.3 and 2.4. **Figure 4** shows their normalized characteristic spectral curves. As can be seen from **Figure 4**, the general trends and peak positions of the characteristic spectral curves are exactly the same, therefore, they are all genuine *Flos carthami*. The changes in fluorescence intensity reflect freshness and quality differences between the FC samples. These results are consistent with the results of physical and chemical identification under double-blind conditions. The purity levels of No.1-No.4 samples are similar; however, No.5 sample is impurity. In addition, from the fluorescence intensity of view, the intensity of No.1 sample is highest, No.5 sample's is lowest, and No.2 to No.4 samples' is close. These are also consistent with the results of experience identification, that is, No.1 sample is the freshest one, No.5

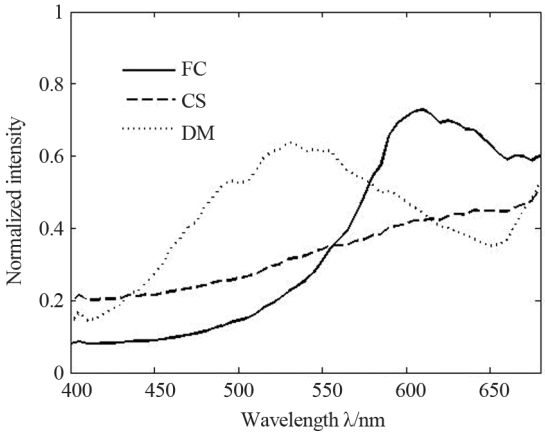

Figure 3. Normalized characteristic spectral curves of FC, CS and DM.

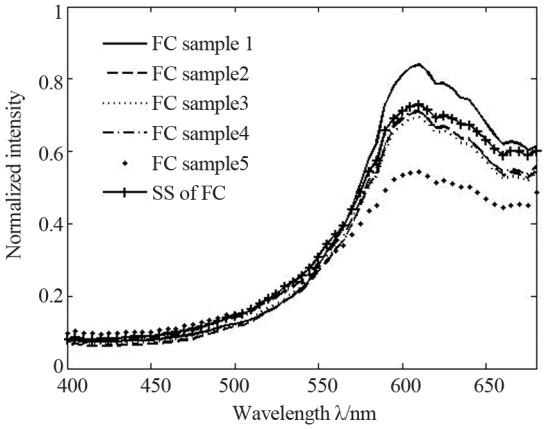

Figure 4. Normalized characteristic spectral curves of FC, samples.

sample is the least fresh one, and the freshness of No.2 to No.4 sample is in the middle.

4. Conclusion

The multispectral imaging method is developed to identify authenticity and evaluate quality of *Flos carthami* in this paper. Compared to chemical methods, the multispectral imaging method has more advantages: rapidity, simplicity, safety, low operational costs and samples being tested directly using the system without any pretreatment. The measuring process is time-saving and the results are steady, precise and repeatable. The result shows that the multispectral imaging method is an effective, nondestructive technique, and can be used to identify and evaluate the traditional Chinese herbal medicine powders.

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