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Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 498–504
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Fourier transform mid-infrared (MIR) and near-infrared (NIR) spectroscopy for rapid quality assessment of Chinese medicine preparation Honghua Oil

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Received 2 September 2007; received in revised form 9 November 2007; accepted 13 November 2007
Available online 22 November 2007

Abstract

Honghua Oil (HHO), a traditional Chinese medicine (TCM) oil preparation, is a mixture of several plant essential oils. In this text, the extended ranges of Fourier transform mid-infrared (FT-MIR) and near infrared (FT-NIR) were recorded for 48 commercially available HHOs of different batches from nine manufacturers. The qualitative and quantitative analysis of three marker components, α-pinene, methyl salicylate and eugenol, in different HHO products were performed rapidly by the two vibrational spectroscopic methods, i.e. MIR with horizontal attenuated total reflection (HATR) accessory and NIR with direct sampling technique, followed by partial least squares (PLS) regression treatment of the set of spectra obtained. The results indicated that it was successful to identify α-pinene, methyl salicylate and eugenol in all of the samples by simple inspection of the MIR-HATR spectra. Both PLS models established with MIR-HATR and NIR spectral data using gas chromatography (GC) peak areas as calibration reference showed a good linear correlation for each of all three target substances in HHO samples. The above spectroscopic techniques may be the promising methods for the rapid quality assessment/quality control (QA/QC) of TCM oil preparations.

Keywords: Essential oils; Honghua Oil; Fourier transform mid-infrared spectrometry; Near-infrared spectrometry; Partial least square regression; Quality assessment (QA); Quality control (QC)

1. Introduction

The quality control of traditional Chinese medicine (TCM) is an important issue, affecting TCM herbs, formulations, preparations and proprietary medicines of different dosage forms. One of the common dosage forms is medicinal oil. During the quality assessment of TCM medicinal oils in official drug, gas chromatography (GC) is often employed. Generally, a number of target or marker components in the medicinal oils have been identified. They were separated with GC, and the area under the peaks was used for quantitative determination of these markers [1]. The use of those chromatographic methods invariably necessitates the use of some sample preparation procedure that may be tedious, and the chromatographic separation usually takes tens of minutes to hours [2,3]. In addition, chromatographic methodology consumes solvents and/or gases as well as standards for external calibration, spiking, or internal standardization in every determination [4]. However, infrared spectroscopic calibration is needed only in the training cycle, and hence is more economical and environment-friendly in the long run. Therefore, the Fourier transform mid-infrared (FT-MIR) and near-infrared (FT-NIR) spectroscopic methods have shown the potentials for specific tasks such as long-term quality assessment/quality control (QA/QC) in production line, stability monitoring and random inspection of medicinal oil products [5–9]. This study aims to establish a FT-IR model using the partial least square (PLS) chemometric technique for quantifying marker components in the mixture of several plant essential oils, which can serve as a rapid quality evaluation tool to complement, and/or as a more cost-effective and environment-friendly tool instead of, the current chromatographic techniques.

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doi:10.1016/j.jpba.2007.11.021

Unlike most of other medicinal oils, TCM medicinal oil is a mixture of several plant essential oils. Each of these components is present in significant proportion and has pharmacological activities. These plant essential oils are generally extracted by steam-distillation or, more recently, supercritical fluid extraction from the dried plant materials, which are usually the complex mixtures of terpenoids, aromatic substances, aldehydes, ketones, alcohols and esters [10–15]. Similar with all naturally existing compounds in plant, the ingredients in essential oils are susceptible to evaporation loss, oxidation, and/or various degradation making the composition of the oils more complicated and variable than what they would be initially. In turn, such changes will alter the efficacy of the medicinal oils, leading to a stability problem needed to be continually monitored.

In this text, Honghua Oil (HHO) was used as an example. It is a popular household remedy in China, especially in the Southeast China, as well as Southeast Asian countries with Overseas Chinese communities. HHO is considered to have therapeutic effects on rheumatism, other joint problems, injuries and bruise, which is also often used for relieving aches and pains of muscles and tendons by applying externally to the affected area [16,17]. According to formula provided by manufacturers, HHO is composed of some plant essential oils such as wintergreen oil, turpentine oil, clove oil and cassia leaf oil. However, there are dozens of different HHO products with same trade name but from different manufacturers. Each individual product is prepared with different proportion of essential oils, and is in turn from a different source and of different purity. This situation makes it difficult for consumers to tell which product is of higher quality even by its composition, which dose not mention its efficacy for what is stated in the label may be very inconsistent with what it contains. In fact, as to the regulatory monitoring of HHO according to the current standard of the Ministry of Health of the P.R.C., α-pinene, methyl salicylate and eugenol should be determined by GC with flame ionization (FID) or mass spectrometric (MS) detection [18,19]. However, each GC run takes more than 30 min in addition to weighing, dilution, sample preparation, not including the time to return to the initial state after temperature program cycle, as well as the time to warm up and calibrate the instrument with the proper standards. Therefore, a fast, simple, environment-friendly spectrometric method in combination with chemometrics data treatment for multi-component quantitation becomes more and more attractive in the long run for medicinal oils and other TCM with similar properties. However, the calibration with conventional chromatographic method is still needed in the training stage. Therefore, once the model is properly established, subsequent measurements are straightforward and take only a few minutes.

2. Material and methods

2.1. Sample collection

A total of 48 HHO samples were purchased from drugstores in Beijing and Hong Kong. The HHO sample collected contained the different batches from nine manufactures (or brand name) in 2005 and 2006 (Table 1). The constituent oils of HHO, i.e. wintergreen oil (containing 96–99% methyl salicylate), turpentine oil (containing 75–78% α-pinene) and clove oil (containing 95–99% eugenol), were provided by the Ling-nam Medicine Factory (Hong Kong) Limited. Pure standard substances (α-pinene, methyl salicylate and eugenol) and the internal standard

Table 1
GC determination of the three components of HHO samples

HHO samples (brand name)	Lot. no.	Componential compositions (%, w/w)		
		α-Pinene	Methyl salicylate	Eugenol
Axe brand	R050842	19.9	71.2	2.9
	R050945	18.4	70.4	2.9
	R051158	20.1	70.7	2.8
	R051158	20.0	71.0	2.9
	R050945	**18.9**	**71.0**	**2.9**
	R051158	**20.3**	**72.0**	**2.8**
Imada	200506	0	63.7	7.7
	200601	0	62.7	7.8
	200601	0	66.7	8.0
	300601	0	65.2	7.8
	300601	0	68.5	8.0
	300601	2.1	65.7	7.7
	300601	2.0	66.3	7.9
	200506	**0**	**67.9**	**8.0**
	300601	**0.2**	**66.5**	**8.1**
Yonglong	051009	21.0	54.9	0.5
	051009	22.8	56.7	0.5
	051009	23.1	57.0	0.4
	051211	19.4	58.9	0
	061120	23.3	55.4	0.5
	061120	22.6	54.4	0.5
	051009	**21.2**	**57.9**	**0.2**
	061120	**22.1**	**54.0**	**0.5**
Zhenlong	20060101	30.2	39.6	0.4
	20050703	25.6	47.3	0.5
	20050703	26.8	45.2	0.4
	20060302	28.5	41.0	0.2
	20060302	27.9	41.2	0.2
	20060302	**27.9**	**41.3**	**0.2**
Huafei	060701	4.6	43.2	39.7
	060701	5.2	44.9	44.2
	060901	4.7	44.6	44.0
	060901	4.5	44.1	44.0
	060701	**4.5**	**44.5**	**44.4**
Linam	3300029	19.1	73.2	1.4
	3300029	18.9	72.4	1.6
	3300029	19.4	73.4	1.6
	3300029	19.1	73.3	1.6
	3300029	**19.0**	**73.0**	**1.6**
Cmalo	20060223	0	57.0	24.0
	20060223	0	57.7	24.0
	20060223	0	57.1	24.2
	20060223	**0**	**57.7**	**23.7**
Linglong	051102	27.9	54.8	0
	051102	**28.1**	**55.0**	**0**
Ganchang	20060301	32.7	21.9	0.1.
	20060301	32.4	22.0	0
	20060301	**32.6**	**22.0**	**0**

Note: The common-faced data were separated to training set and the boldface data were separated to test set.

Table 2
References data ranges of the HHO samples

Components (w/w)	*n*	Mean	Minimum	Maximum
α-Pinene (%)	48	15.4	0	32.7
Methyl salicylate (%)	48	56.6	21.9	73.4
Eugenol (%)	48	8.6	0	44.4

Note: *n*, the total number of samples in calibration and validation set.

(Dodecane) were bought from Peking Chemical Reagents Company (P.R.C.).

2.2. Reference analysis

All of the HHO samples were analyzed by gas chromatography/flame ionization detector (GC/FID) using a Hewlett-Packard HP6890 series plus G1530A, equipped with an HP-1 column (30 m × 0.25 mm i.d. and a film thickness of 0.25 mm). Detector and injector temperatures were set at 280 °C and 300 °C, respectively. The oven temperature program was as follows: 5 min at 50 °C, from 50 °C to 90 °C at 5 °C/min, 5 min at 90 °C, then 10 °C/min up to 280 °C. Carrier gas was nitrogen with a constant flow rate of 1 mL/min (split 1:10). The determination of α-pinene, methyl salicylate and eugenol were done using correction factors on areas under their respective peaks against peak area of the internal standard, dodecane. All samples were analyzed in triplicate and averaged to give the final results. Tables 1 and 2 showed the analysis results of the three main components in the investigated HHO products by GC.

2.3. Vibrational spectroscopic measurements

The MIR spectra of all 48 HHO samples were recorded by Spectrum GX FT-IR spectrometer with a deuterated triglycine sulfate (DTGS) detector and a horizontal attenuated total reflection (HATR) accessory. This standard accessory from PerkinElmer came with a trapezoid shape ZnSe crystal bottomed trough that was 80 mm in length, 10 mm in width and 4 mm in depth. The crystal provided an angle of incidence of 45° and was enclosed in a stainless-steel cuvette. Spectra were recorded in the region of 4000–650 cm^{-1} by an average of 32 scans at a resolution of 4 cm^{-1}. Each sample was recorded three times. The crystal trough was cleaned with petroleum ether and ethanol, and then purged by air to dryness between samples. The background was collected before every sample was measured. All the acquired spectra were converted to absorbance graph and then pre-processed with ATR correction algorithm of the Spectrum software v3.02 that came with the instrument.

All the NIR spectra of the 48 HHO products were recorded with a PerkinElemer Spectrum 100N FT-NIR spectrometer interfaced to the PerkinElemer Spectrum 100 software for treatment. Each oil sample was filled into a 2 mm path length quartz micro-cell and its NIR spectrum was obtained by direct transmission without any sampling treatment. A reference spectrum was recorded before each sample measurement on an empty tube and all the spectra were performed at 4 cm^{-1} resolution between 4000 and 10,000 cm^{-1} by an average of 64 scans. All the original NIR spectra were required without data pretreatment after transmittance to absorbance using Spectrum software v3.02 (PerkinElmer).

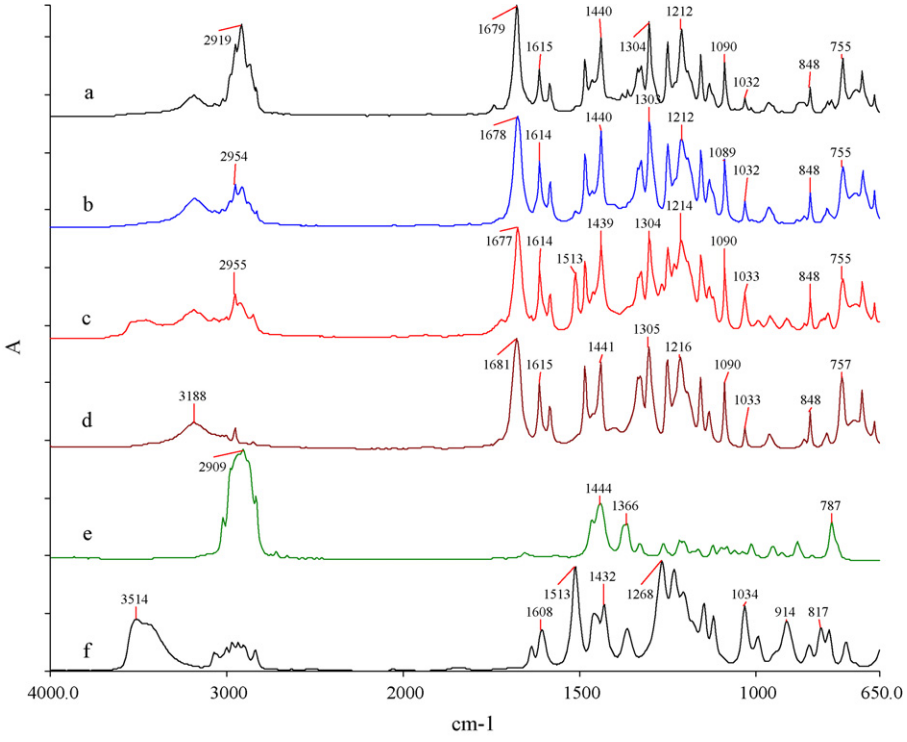

Fig. 1. HATR-IR spectra obtained from different commercial HHO products and their component standards: (a) Zhenglong HHO; (b) Linam HHO; (c) Cmalo HHO; (d) methyl salicylate; (e) α-pinene and (f) eugenol.

2.4. Chemometrics

Chemometric analyses of the HATR-MIR and NIR spectra were performed using the software (IR spectroscopy-Spectrum Quant+, PerkinElmer Inc., U.K.). The calibration models were developed using partial least square (PLS), which was then used to quantitatively predict the three main components of the validating samples on the basis of spectral information in the whole spectral range each time. All the HATR-MIR and NIR spectra of the 48 HHO samples were respectively divided into two groups: the training set of 36 samples of different manufacturers, used only in the calibration step; and the test set of 12 samples, employed exclusively in the validation step (Table 1). No outliers were removed from the analyzed spectra. The calibration models were established with 36 MIR and NIR spectra in the whole spectral region over reference values. The quality of the models was evaluated by correlation coefficient (R^2), standard error of calibration (SEC) and the standard error of prediction (SEP), respectively. Then, the respective PLS-models were used to determine the respective samples representing the nine manufacturers, and the R^2, SEC and SEP were calculated.

3. Results and discussion

3.1. Spectral feature of MIR

Fig. 1 presents the MIR-HATR spectra obtained from the HHO products from three different manufacturers and their main components (i.e. α-pinene, methyl salicylate and eugenol). All the upper three spectra of HHO preparations show the visible characteristics of methyl salicylate in the region of 1800–650 cm^{-1}. This is due to high contents of methyl salicylate in all three samples and its strong infrared absorption in the region of 1800–650 cm^{-1} dominating the MIR spectra. Therefore, qualitative analysis of HHO is possible by paying our attention to methyl salicylate as the major components. Characteristic key bands of methyl salicylate occur at ∼1681, 1615, 1441, 1305, ∼1216 and 1090 cm^{-1}, whereas absorptions characteristic of α-pinene vibrations around 2909 cm^{-1} and feature peak of eugenol at 1513 cm^{-1} can be viewed as fine features superimposing upon the general background of the methyl salicylate spectrum.

3.2. Spectral feature of NIR

Fig. 2 illustrates the NIR spectra of above-mentioned three HHO products and their main constituent oils (i.e. wintergreen oil containing 96–99% methyl salicylate, turpentine oil containing 75–78% α-pinene and clove oil containing 95–99% eugenol). The spectra were offset to allow visual comparison. The similarity between the spectra of HHO and wintergreen oil indicated methyl salicylate is the main components of all the HHO preparations, which was consistent with the analysis results by GC. In general, the absorption patterns of the different spectra are less specific due to overlapping over the overtones of the different constituent oils. The absorption bands of α-pinene was clearly observed around 8338 cm^{-1} together with absorption bands of eugenol at 6842 cm^{-1}. Bands around 8338 cm^{-1} arose from the second overtones of C–H stretching vibrations, while those at 6842 cm^{-1} were attributable to the first overtones of O–H stretching vibrations. Fig. 2 showed that NIR spectra of the three HHO products have no noticeable difference other than those around 8351 cm^{-1} in spectrum a and those around 6922 cm^{-1} in spectrum c, where those stronger peaks in these spectra are indicative of a higher proportions of α-pinene and eugenol in both HHO samples.

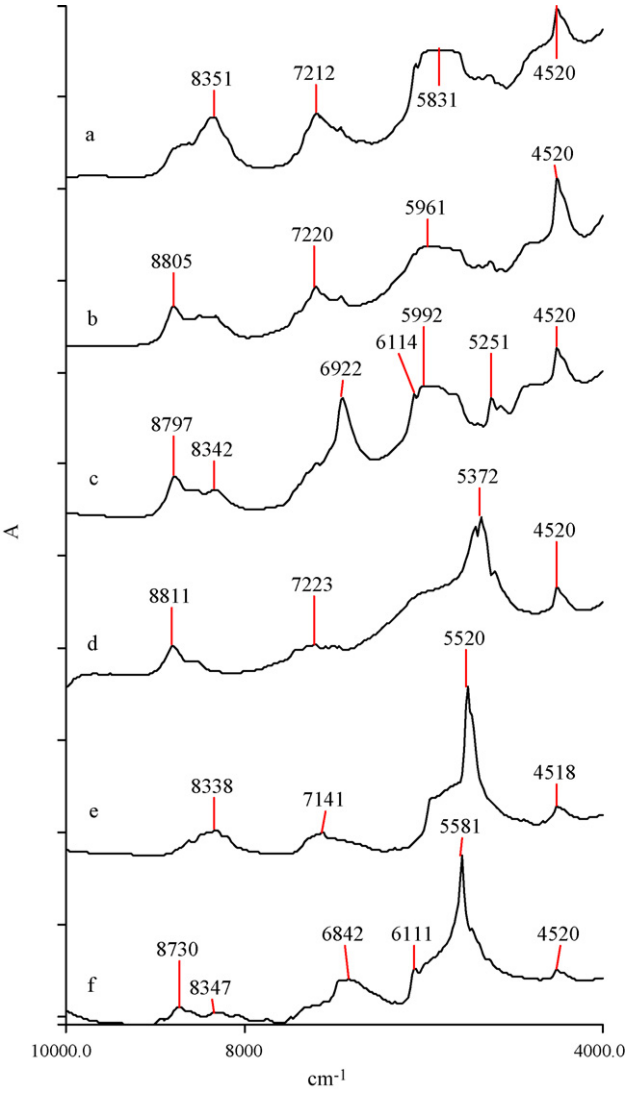

Fig. 2. NIR spectra obtained from different HHO products and their component standards: (a) Zhenglong HHO; (b) Linam HHO; (c) Cmalo HHO; (d) methyl salicylate; (e) α-pinene and (f) eugenol.

3.3. Quantification

Quantification of the main components in the HHO samples was performed using PLS algorithm. According to the above analysis, α-pinene, methyl salicylate, eugenol have their respective characteristic absorption bands in MIR and NIR spectral region, which was considered to be unnecessary for any spectral preprocessing. As stated above, for chemometric evaluation,

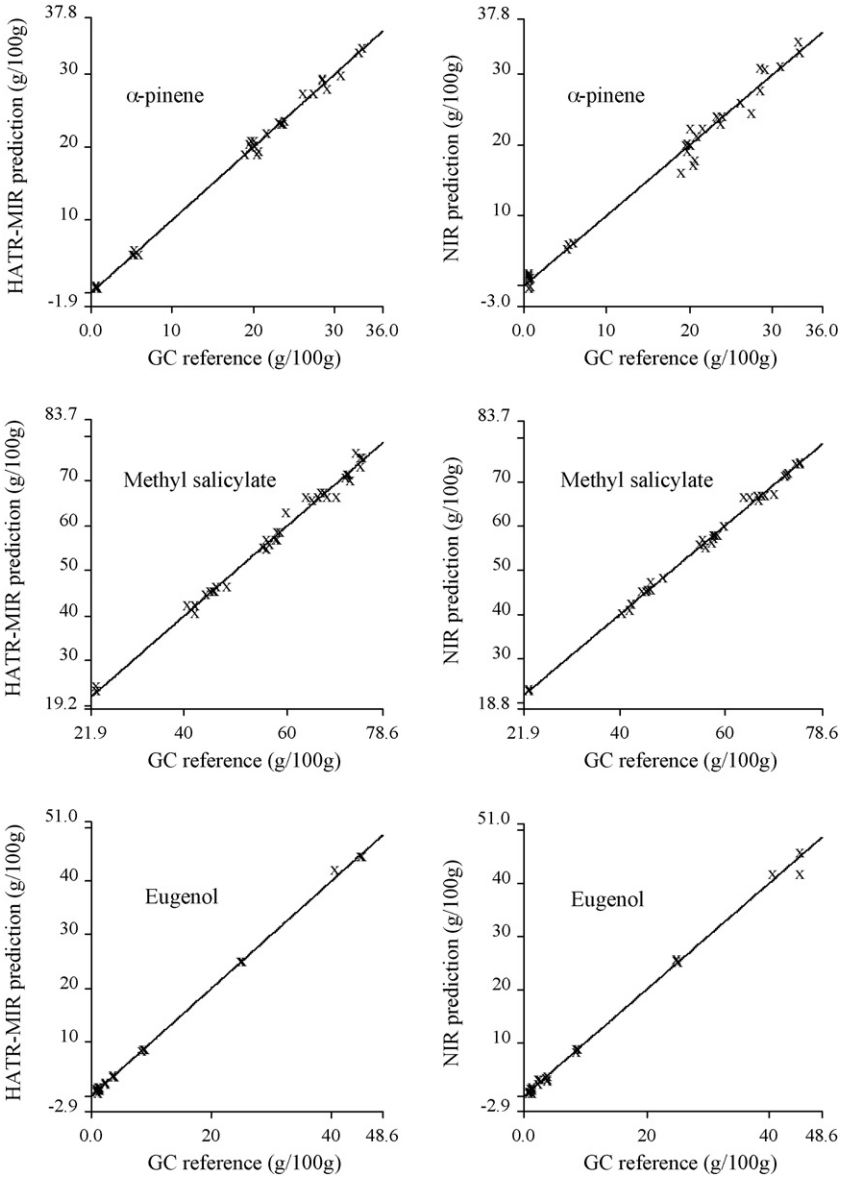

Fig. 3. Calibration plots by PLS regression.

the samples of all the HHO samples were divided randomly into a validation set. The calibration set consisted of 36 spectra while the validation set consisted of 12 spectra (Table 1). Considering the discrepancy of component contents in various commercially available HHO products, the both sets was spanned the full range of oil substance contents. The analysis results illustrated the information concerning the reference and the prediction value obtained through calibration (Fig. 3). The

Table 3
MIR calibration and validation statistics of α-pinene, methyl salicylate and eugenol contents in HHO samples (calibration, $n = 36$; validation, $n = 12$)

Components (w/w)	Calibration		Validation		
	SEC[a]	R^{2b}	SEP[a]	R^2	Prediction bias[c]
α-Pinene (%)	0.659	0.997	0.793	0.995	−0.0291
Methyl salicylate (%)	1.273	0.991	1.667	0.987	−0.0825
Eugenol (%)	0.342	0.999	0.360	0.999	−0.143

[a] SEC: standard error of calibration, SEP: standard error of prediction.
[b] Correlation coefficient.
[c] Prediction bias: mean difference between predicted and reference values.

Table 4
NIR calibration and validation statistics of α-pinene, methyl salicylate and eugenol contents in HHO samples (calibration, $n = 36$; validation, $n = 12$)

Components (w/w)	Calibration		Validation		
	SEC[a]	R^{2b}	SEP[a]	R^2	Prediction bias[c]
α-Pinene (%)	1.355	0.986	1.554	0.981	0.178
Methyl salicylate (%)	0.944	0.995	0.957	0.996	−0.423
Eugenol (%)	0.705	0.997	0.389	0.999	0.0385

[a] SEC: standard error of calibration, SEP: standard error of prediction.
[b] Correlation coefficient.
[c] Prediction bias: mean difference between predicted and reference values.

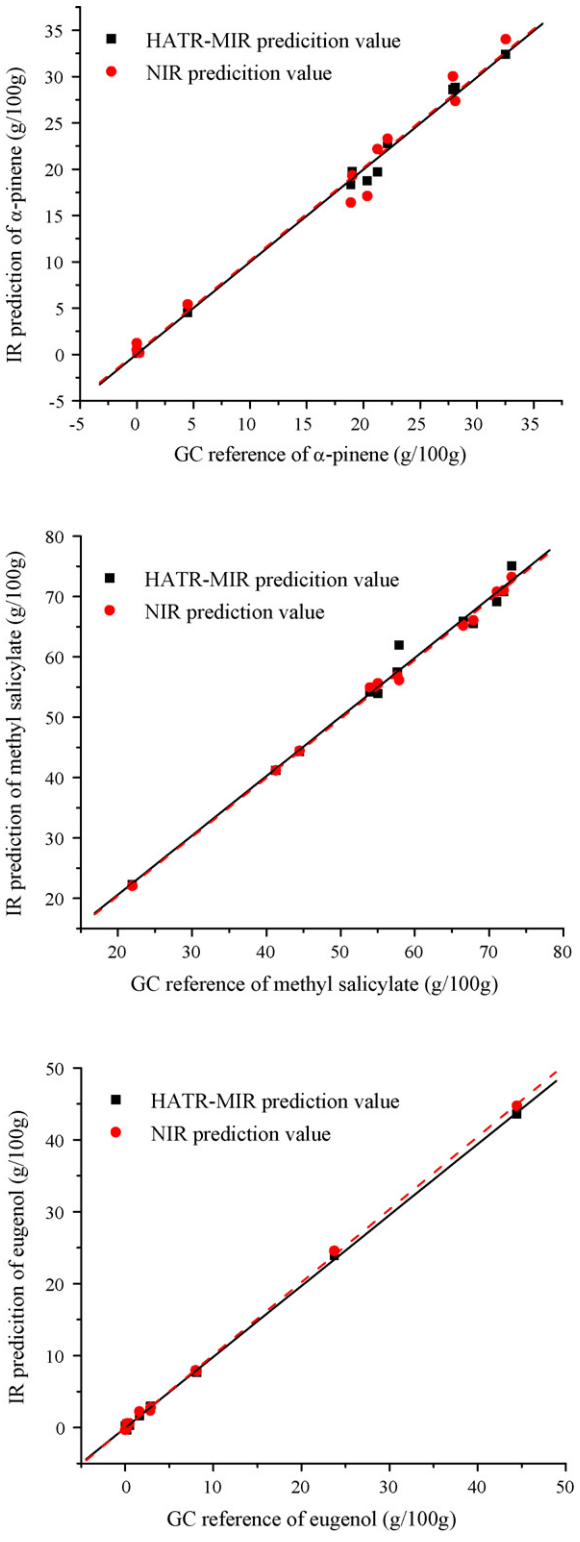

Fig. 4. Predicted values based on MIR and NIR spectra of HHO samples vs. actual concentrations of α-pinene, methyl salicylate and eugenol of the samples.

values of correlation coefficient (R^2), standard error of calibration (SEC), standard error of prediction (SEP) and prediction bias in Tables 3 and 4 indicated the precision achieved in calibration and validation. The high R^2 and low value of SEC and SEP indicated that those three oil substances (i.e. α-pinene, methyl salicylate and eugenol) could be reliably predicted by both vibration spectroscopic methods. In most cases the correlation coefficients (R^2) exceeded the values of 0.98, indicating a very high correlation with the reference data. Furthermore, the relationship of predicted values and actual concentrations of three components of HHO samples in test set were illustrated in Fig. 4.

According to the comparison of the PLS regression results between MIR and NIR spectra (Tables 3 and 4 and Fig. 4), the summary of MIR results show slightly more precise prediction than those of NIR. It may be due to MIR spectroscopy providing more specific and distinct absorption bands than NIR spectroscopy. In general, for HHO products from different manufacturers, the percentage compositions of oil components vary over a wide range. However, the compositions of different batches of individual product remain relatively constant. Collection of variety samples is important to develop PLS calibration model. Furthermore, all the component oils under analysis are present in significant quantities and possess individual absorption bands with strong enough intensity, which suffer insignificant interferences from spectral absorptions of other components. In other words, there exist sufficient stand-alone spectral features free from interferences from the other components for a satisfactorily accurate quantitative correlation to be obtained in such a complicated system.

4. Conclusion

The quality control of natural products such as a mixture of various essential oils is a challenging task in modern analysis. Compared with the current standard methods of GC and HPLC, which are resource- and time-consuming, the vibrational spectroscopy is fast, economical and environment-friendly. It can provide simultaneously qualitative characterization and quantitative monitoring of the major component oils.

In this study, we have demonstrated that both MIR and NIR spectroscopy can be applied for these purposes with the help of a chemometric software. We have also compared the MIR against the NIR range with the same set of samples. According to the results presented, it seems that both techniques should be able to identify the main components in different HHO preparations based on spectral analysis. The quantification of components in different HHO products was obtained by PLS regression without any data pretreatments. The PLS models developed with both techniques predicted the contents of α-pinene, methyl salicylate and eugenol in commercial HHO products with good accuracy and precision, although in this case, MIR is shown to be better by a narrow margin. Both spectral methods are expedient and easy to perform, avoiding problems associated with sample preparation and standard consumption, as well as possessing the advantage of determining several substances with a single measurement. The MIR and NIR techniques combined with multivariate data treatment are especially promising methods for rapid quality assurance of TCM oil preparations.

Acknowledgements

The authors gratefully acknowledge financial support from China Postdoctoral Science Foundation (20060400073), the State Administration of Traditional Chinese Medicine of the People's Republic of China (2001ZDZX01), Ministry of Science and Technology of the People's Republic of China (2002BA906A29-4), and Prof. Chen Peirong and Yang Chendui, Tsinghua University, for technical assistance in GC analysis.

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