Rapid analysis of *Scrophulariae* radix using near-infrared spectroscopy

D.Y. Lee,[a] E.J. Choi,[b] S.H. Kim,[b] H.J. Kim,[c] S.U. Yi,[d] Y.C. Kim[a] and S.H. Sung[a,*]

[a]*College of Pharmacy, Seoul National University, Seoul 151-742, Korea*
[b]*Elcom Science Co., Ltd., Seoul 152-050, Korea*
[c]*College of Pharmacy, Dongduk Women's University, Wolgok-Dong, Sungbuk-Gu, Seoul 136-714, Korea*
[d]*Bruker Optics Korea Co.,Ltd., Banpo-Dong, Seocho-Gu, Seoul 137-802, Korea*

Introduction

The dried roots of *Scrophularia* spp. have long been used in Oriental medicine **as a** treatment for fever, swelling, constipation, pharyngitis, neuritis, and laryngitis.[1] Recently, the standardised ethanol extract of *S. buergeriana* is undergoing Phase II clinical trials for Alzheimer's disease in Korea. In the Korean herbal market, there are three types of commercially prevalent *Scrophularia* spp., However, there is no international standard or classification system for *Scrophularia* spp., although the species and its geographical origin can affect the chemical and biological characteristics.[2] Quality control of *Scrophulariae Radix* is very important. In the present study, we examined the possibility of developing discrimination methods based on near infrared (NIR) spectroscopy, combined with multivariate analysis. We also evaluated the role of NIR spectroscopy in determination of two active constituents [8-*O*-(E-*p*-methoxycinnamoyl)-harpagide (HG) and *p*-methoxycinnamic acid (MCA)] of *Scrophulariae Radix*.

Methods

Samples from Andong, Uisung and China were purchased from Kyungdong traditional herb market (Seoul, Korea) and authenticated by Dr. Jong Hee Park, professor of Pusan National University. To avoid the effect of particle size, samples were pulverised with a grinder, and screened through a 200-mesh (75 μm) sieve. All the samples were dried for 10 hours in an oven at 60°C.

The HPLC system consisted of a chromatographic pump (P680, Dionex, Germany) and an injector (7725i, Rheodyne, USA) equipped with a photodiode array (UVD 340U, Dionex, Germany). The chromatographic column was Waters XTerraTM RP18(150 mm × 4.6 mm i.d., 5 μm). HG and MCA were separated using a mobile phase composed of acetonitrile and water

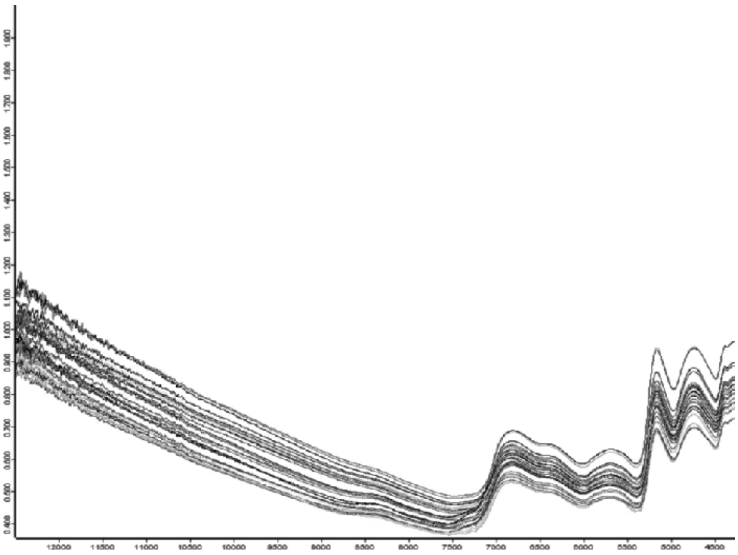

Figure 1. NIR spectra of *Scrophulariae Radix* (13500–3600 cm^{-1}).

with 0.1% acetic acid, at a flowrate of 1.0 mL min^{-1}, and measured at 280 nm. The elution was run by a 20-min isocratic elution with 23% acetonitrile.

NIR measurement was performed by using a FT-NIR spectrometer (MPA, BrukerOptics, Ettlingen, Germany). The NIR absorbance spectra of *Scrophulariae radix* powder were acquired over the wavelength range 12800–3600 cm^{-1} (resolution: 8 cm^{-1}; scanner velocity: 10 kHz; Detector:

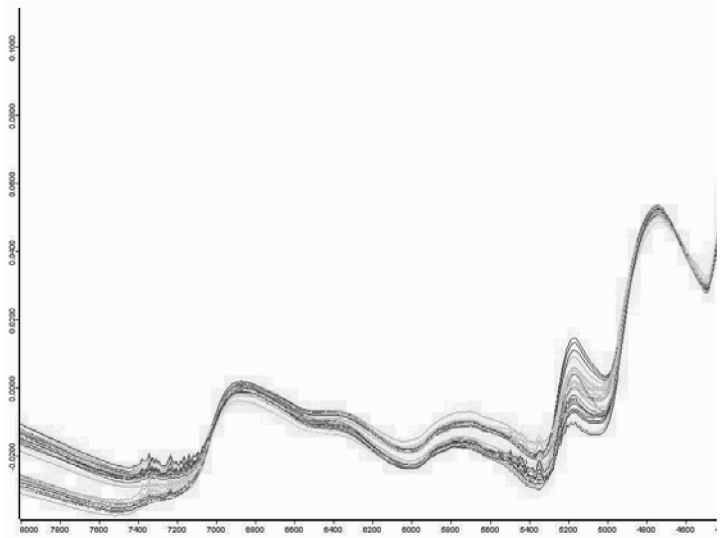

Figure 2. NIR spectra of *Scrophulariae Radix* with vector normalisation pretreatment between 8000 cm^{-1} and 4000cm^{-1}.

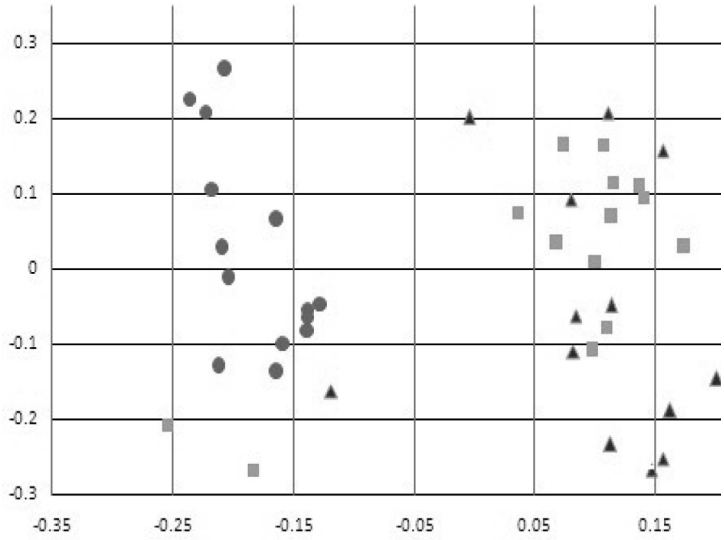

Figure 3. Scores and plots form PCA for *Scrophulariae Radix*.

RT-PbS; Background: 32 scans, Sample: 32 scans). OPUS software (v. 6.0 Bruker Optics, Ettlingen, Germany) was used for spectra pretreatment, chemometrics analysis and instrumental control.

Data and discussion

NIR spectra of 44 *Scrophulariae Radix* samples were collected (Figure 1).

In order to improve the spectral features, the vector normalisation (SNV) algorithm was utilised in 8000-4000 cm^{-1} (Figure 2).

In order to classify the samples by geographical region, principal component analysis (PCA) was utilised in this study. The best classification among these samples was found with a PCA plot of using the PC1(98.75%) and PC2(1.13%) in a two-dimension plot (Figure 3).

Samples from "Andong" were nicely separated from the others by negative scores on PC1. However, Uisung and China samples were partially overlapped. Partial least squares (PLS) regression was used to determine the two active constituents (HG and MCA). Different pretreatments were carried out in various spectral regions. Because the number of samples is only 44, all samples were used. Validation was performed by leave-one-out full cross validation.

Table 1. Performance PLS regression optimisation for HG and MCA.

	Pre-treatments	*RMSECV*	*RPD*	Bias	*R*	Region (cm^{-1})
HG	First derivatives	0.0639	3.22	0.0019	0.9486	6102–4246.7
MCA	First derivatives + straight line subtraction	0.0247	3.16	0.00106	0.9505	6102–4246.7

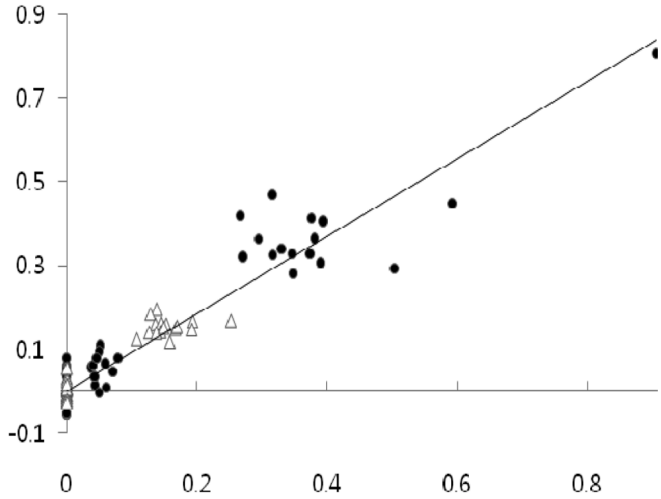

Figure 4. The plot of predicted (NIR) versus determined (HPLC) contents of HG (circle) and MCA (triangle) by using PLS Regression method.

Performance evaluation. The best validation equations were selected based on the lowest *RMSECV* and highest R^2. Excellent validation equations were obtained for HG and MCA (Table 1).

The regression graphs between the reference HPLC data and NIR prediction value for HG and MCA are depicted in Figure 4.

The results suggested that the quantitative NIR systems developed could be used in the quality control of *Scophulariae Radix*.

Conclusion

The applications of NIR spectroscopy to classify by geographical region, and determine the HG and MCA in *Scrophulariae Radix* were established successfully. These studies showed the feasibility of NIR spectroscopy for determination of active constituents, as well as the possibility of using NIR spectroscopy for quality control of herbal medicines based on geographical authentication.

References

1. S.U. Park, Y.A. Chae, P.J. Facchini, *Plant Cell Rep.* **21,** 1194 (2003).
2. S.H. Kim *et al*. *Microchem. J.* **94,** 118 (2010).