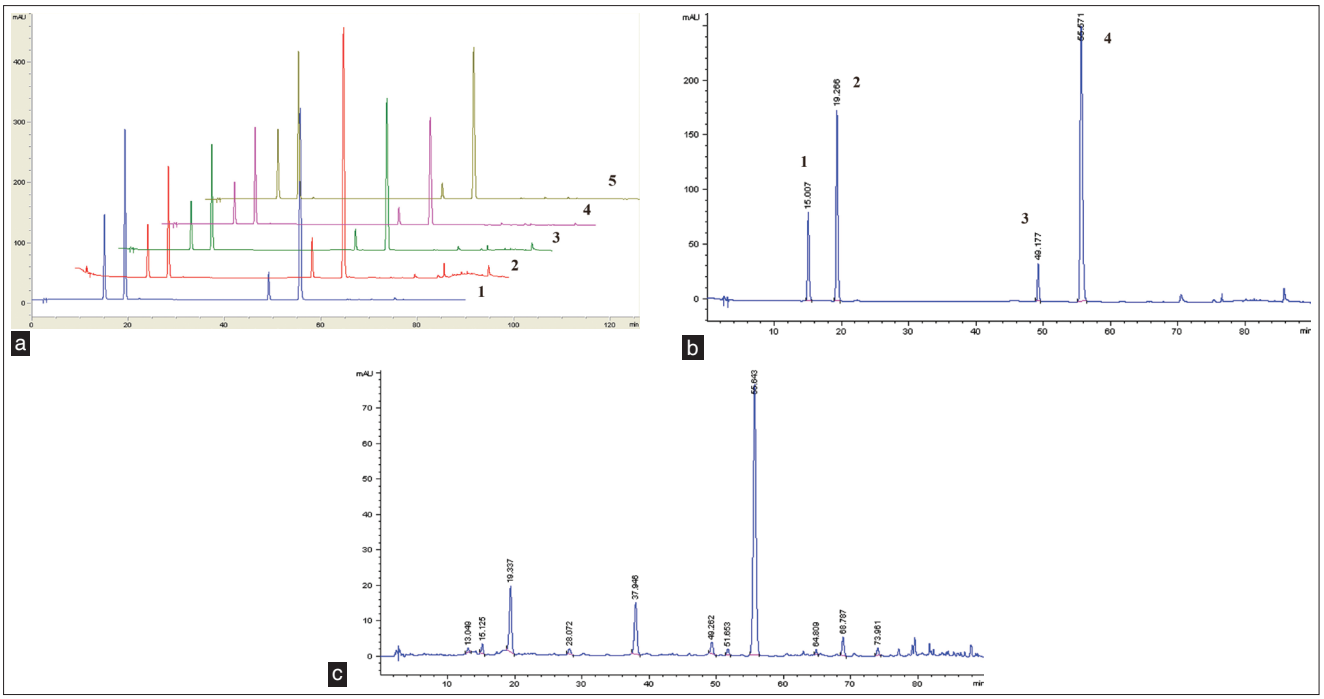

Figure 2: (a) Typical chromatograms of reference standards in different detection wavelengths: (1) 325 nm, (2) 210 nm, (3) 230 nm, (4) 280 nm, (5) 300 nm; (b) Typical chromatograms of reference standards in optimized wavelength: (1) chlorogenic acid; (2) caffeic acid; (3) apigenin-7-O-glucoside; (4) rosmarinic acid; (c) Typical chromatograms of *G. longituba* samples

Table 1: Calibration plots, LOD and LOQ of the four compounds in *G. longituba* extract

Compound	Calibration equation	r	Linearity range (mg/ml)	LOD (μg/ml)	LOQ (μg/ml)
Chlorogenic acid	Y = 23.46X + 0.98	0.9999	1.64 ~ 164.00	1.2	6.0
Caffeic acid	Y = 48.61X + 41.59	0.9998	0.176 ~ 880.00	1.3	4.2
Apigenin-7-O- glucoside	Y = 3.15X - 2.39	0.9999	0.174 ~ 580.00	1.4	5.1
Rosmarinic acid	Y = 3.02X - 26.43	0.9999	370.50 ~ 2470.00	1.8	6.5

listed in [Table 1] clearly indicated that the analytical method was acceptable with excellent sensitivity.

Precision, repeatability and stability

Intraday and interday variability were investigated by determining each marker compound at one concentration level in six replicates during a single day and by replicating the experiments on three consecutive days, variations were expressed by relative standard deviations (RSD) and remained <1.74% for all the marker compounds. Six independently prepared sample solutions with the same amount were calculated for evaluation of repeatability, RSD were less than 2.89%. The processes of the treatments were in parallel. One of the samples was injected into the instruments at 0 h, 2 h, 4 h, 6 h, 8 h, 12 h and 24 h to evaluate the stability of the solution. RSD values of the peak area were less than 1.39% for all the compounds studied. These results suggested that it was feasible to analyze samples within 1 day.

Accuracy

Recovery tests were used to evaluate the accuracy of the method by analysis of spiked samples at one certain concentration level (approximately equivalent to 1.0 time to the concentration contained in the sample). The extracted solution was analyzed by the proposed HPLC method. Quantity of each compound was subsequently obtained by use of the corresponding calibration plots. The recoveries of analytes varied from 99.77% to 100.89% and RSD values were in the range of 0.20-1.75%, which demonstrated the reliability and accuracy for the measurement of these constituents.

Sample analysis

The developed method was applied to the simultaneous determination of chlorogenic acid, caffeic acid, apigenin-7-O-glucoside and rosmarinic acid in three batches of *G. longituba* samples obtained in different provinces in China. Each sample was determined in triplicate, and the peaks in chromatograms were identified by comparing the retention times and UV spectra with those of the standards. The contents were calculated and summarized ($n = 3$) in [Table 2]. According to the quantitative analysis results, we noticed that the total contents of four compounds varied in the same

Table 2: Concentrations (%) of the four compounds in three batches of *G. longituba*

Batch	Content (n = 3)			
	Chlorogenic acid	Caffeic acid	Apigenin-7-O-glucoside	Rosmarinic acid
Zhejiang	0.00225	0.0242	0.00271	0.840
Anhui	0.00225	0.0242	0.00273	0.830
Jiangsu	0.00234	0.0238	0.00313	0.896

type of samples from different provinces, which might be due to the differences of growing condition and climate in each region. Thus it is necessary to control the main active components in *G. longituba* by good agricultural practice (GAP) and the norm of Chinese medicinal materials processing.

CONCLUSIONS

This novel HPLC method for simultaneous quantitative analysis of four active components was established and validated for quality evaluation of *G. longituba*. Chlorogenic acid, caffeic acid, apigenin-7-O-glucoside and rosmarinic acid were considered as the marker components to evaluate the intrinsic quality of *G. longituba* methanol extract, and three batches of samples which collected from Zhejiang, Anhui and Jiangsu Provinces were analyzed. The newly developed method has achieved desired linearity, precision and accuracy, and has been elucidated to be a simple, sensitive, accurate and reliable procedure for quality control of *G. longituba*, which can also be further applied for the quantification of phenolic acids and flavonoids in its related processed drugs or preparations.

ACKNOWLEDGMENTS

The authors are grateful to the financial support of the Open Project of National First-Class Key Discipline for Science of Chinese Materia Medica, Nanjing University of Chinese Medicine (2011ZYX2-006), the National Natural Science Foundation of China (No.81202918, 81274056, 30873438 and 81173546), the International Science and Technology Cooperation Project of Jiangsu Province (No. BZ2011053), the Science and Technology of Chinese Medicine of Zhejiang Province (No. 2009CB008), the fund of Zhejiang Modernization of Traditional Chinese Medicine Item ([2008]436), the Chinese Medicine Research Program of Zhejiang Province (2008ZA002, 2011ZB101), and the Science Foundation of Zhejiang Chinese Medical University (7211093).

REFERENCES

1. Chinese Pharmacopoeia Commission, Pharmacopoeia of the People's Republic of China. Vol. 1. Beijing: Chemical Industry Press; 2010. p. 158.
2. Kim J, Song S, Lee I, Kim Y, Yoo I, Ryoo I, *et al*. Anti-inflammatory activity of constituents from Glechoma hederacea var. Longituba. Bioorg Med Chem Lett 2011;21:3483-7.
3. State Administration of TCM of the PRC, Chinese Medicinal Materials. Vol.7. Shanghai: Shanghai Technology Press; 1999. p. 6058.
4. Yang NY, Duan JA, Li P, Qian SH. Chemical constituents of Glechoma Longituba. Yao Xue Xue Bao 2006;41:431-4.
5. Zhu YD, Zou J, Zhao WM. Two new monoterpenoid glycosides from Glechoma longituba.J Asian Nat Prod Res 2008;10:199-204.
6. She GM, Xu C, Liu B, Shi RB. Polyphenolic acids from mint (the aerial of menthe haplocalyx Briq.) with DPPH radical scavenging activity. J Food Sci 2010;75:359-62.
7. Dorman HJ, Kosar M, Baser KH, Hiltunen R. Phenolic profile and antioxidant evaluation of mentha x piperita L. (peppermint) extracts. Nat Prod Commun 2009;4:535-42.
8. Wu ZG, Xu HY, Ma Q, Cao Y, Ma JN, Ma CM. Isolation, identification and quantification of unsaturated fatty acids, amides, phenolic compounds and glycoalkaloids from potato peel. Food Chem 2012;135: 2425-9
9. Zaabat N, Hay AE, Michalet S, Darbour N, Bayet C, Skandrani I, *et al*. Antioxidant and antigenotoxic properties of compounds isolated from Marrubium deserti de Noé. Food Chem Toxicol 2011;49:3328-35.

Cite this article as: Shan Q, Cao G, Cai H, Cai B. Simultaneous determination of four bioactive compounds in *Glechoma longituba* extracts by high performance liquid chromatography. Phcog Mag 2013;9:216-9.

Source of Support: Nil. **Conflict of Interest:** None declared.