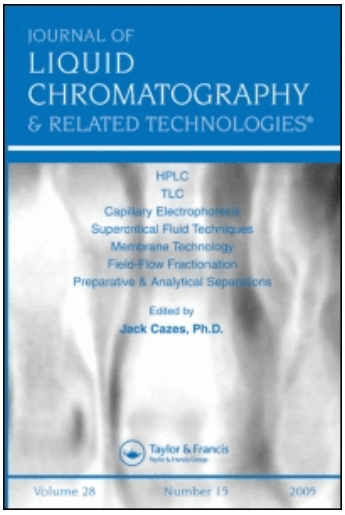

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Abstract: A simple, rapid and accurate high performance liquid chromatographic (HPLC) technique was developed for the simultaneous determination of luteolin and quercetin in the capsule of *Lamiophlomis rotata (Benth.) Kudo*. Separation of luteolin and quercetin was achieved within 20 min using a gradient of water/acetic acid (100/1, v/v) and methanol. By using weighted least squares linear regression (WLSLR), we could obtained better linear relationships for luteolin and quercetin in the range of 0.06∼6.00 μg and 0.064∼6.40 μg, respectively, compared with the results obtained by using least squares linear regression (LSLR). The detection limits for luteolin and quercetin were 7.5 ng and 16.0 ng, at a signal-to-noise ratio of 3, respectively. The mean recovery for luteolin and quercetin was 96.8% and 92.6%, respectively, and RSD was less than 2.0% (n = 3).

Keywords: *Lamiophlomis rotata (Benth.) Kudo*, Capsule, Luteolin, Quercetin, HPLC

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INTRODUCTION

Lamiophlomis rotata (Benth.) Kudo is a labiate plant, which is usually used by the People of Zang, Meng, and Naxi, in China.[1] The capsule of *Lamiophlomis rotata (Benth.) Kudo* is a Chinese medicine based on *Lamiophlomis rotata (Benth.) Kudo*. It has the abilities of hemostasia and acesodyne.[2] The main chemical components comprise flavonoids, iridoid glycosides, and phenylethanoid glycosides. Luteolin and quercetin are flavonoids and they are generally considered to be two of the main effective components.[3–4] Therefore, a method for the determination of luteolin and quercetin is necessary and useful for the quality evaluation of the capsule of *Lamiophlomis rotata (Benth.) Kudo*.

In this paper, a simple, rapid, and accurate HPLC method was described for the analysis of luteolin and quercetin in the extract of the capsule of *Lamiophlomis rotata (Benth.) Kudo*. The optimum conditions of the extraction and analytical method were investigated for the highest sensitivity of detection and best resolution, and weighted least squares linear regression (WLSLR) was adopted to calculate the data. Then, the proposed methodology was applied for determining luteolin and quercetin in the capsule of *Lamiophlomis rotata (Benth.) Kudo*.

EXPERIMENTAL

Instrumentation and Reagents

An Agilent 1100 series HPLC system (USA) including a quaternary pump, a diode array detector, a vacuum degasser, a manual injector, a column oven, and a data system (Agilent Chem Station) was used. Methanol and acetonitrile were of chromatographic grade and acetic acid was of analytical grade. They were all purchased from Tianjing Chemical Reagent Co. (China). Distilled and deionized water was used for the preparation of all the samples and solutions.

Reference Compounds

Luteolin and quercetin were purchased from Aldrich.

HPLC Procedure

The chromatographic separation of the analytes was performed on an ODS column (5 μm, 250 × 4.6 mm I.D.) (Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences; Lanzhou, China) using a gradient of water/acetic acid (100/1, v/v; A) and methanol (B). The gradient

condition was as follows: 0 min, 40%B; 20 min, 90%B. The detection wavelength and column temperature were set at 350 nm and 20°C, respectively. The flow rate was 1.0 mL/min and the loading volume was 20 μL.

Sample Solution Preparation

An accurately weighed dried sample granule (0.6 g) was ultrasonically extracted with 20 mL of 80% methanol for 30 min. The supernatant was then filtered through a 0.46 μm filter membrane to be ready for analysis.

Standard Solution Preparation

Standard stock solutions of the two analytes with concentrations of 0.30 mg/mL and 0.32 mg/mL, respectively, were prepared in methanol. Then standard solutions with various concentrations were prepared by appropriate dilution of the stock solutions with methanol when needed.

Data Analysis

WLSLR and LSLR were calculated using the *SPSS 11.5* software.

RESULTS AND DISCUSSION

Optimization of HPLC Condition

To achieve a good chromatogram, the condition of the separation must be optimized. We tried different elution conditions with methanol-water, acetonitrile-water, and various concentration acids in liquid phase. Through trial and error, the methanol and water/acetic acid (100/1, v/v) system was chosen for the separation. Luteolin and quercetin have maximal absorbance peaks at the wavelengths of 210 nm, 275 nm, and 350 nm, respectively. However, some unknown compounds disturbed the detection of the analytes at the wavelengths of 210 nm and 275 nm, so 350 nm was selected as the detection wavelength.

Optimization of Extraction Conditions

To extract the analytes more efficiently, we adopted $L_9 3^4$ orthogonal design. The three factors comprise A: the concentration of methanol (100%, 80%, and 60%, respectively), B: the volume of solvent (10 mL, 20 mL, and

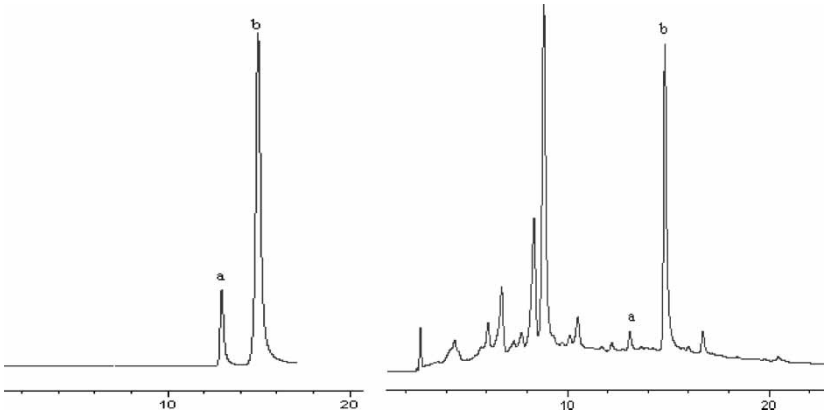

Figure 1. Chromatogram of standard mixture (left) and the extract of the capsule of *Lamiophlomis rotata (Benth.) Kudo* (right), where "a" denotes quercetin and "b" denotes luteolin.

30 mL, respectively), and C: the time of extraction (10 min, 20 min, and 30 min, respectively). The results showed that samples ultrasonically extracted with 20 mL of 80% methanol for 30 min gave the highest amount of the analytes. Several additional tests showed that the extraction time of more than 30 min could not give higher results of the analytes. Therefore, we adopted the following extraction process: the sample was ultrasonically extracted with 20 mL of 80% methanol for 30 min.

Determination of Luteolin and Quercetin

The interested components in analytical samples were identified by comparing both the retention times and the UV spectra of standards to those in actual samples. Figure 1 illustrates the chromatograms of luteolin and quercetin, along with the extract of the capsule of *Lamiophlomis rotata (Benth.) Kudo*.

In the present study, we chose two analytical methods including the usually adopted least squares linear regression (LSLR) and weighed least squares linear regression (WLSLR) to deal with the data. Table 1 lists the

Table 1. Regression equations for the two analytes by WLSLR and LSLR, respectively

Analyte	Regression equation (WLSLR)	Regression equation (LSLR)
Luteolin	$Y = 1292.65x - 15.37$	$Y = 1320.20x - 46.00$
Quercetin	$Y = 770.78x + 27.09$	$Y = 768.62x + 18.35$

Table 2. The analytical result of the determination of luteolin by WLSLR and LSLR, respectively

Concentration values (μg)	1	2	3	4	5	6
Theoretical values	$6.00 \cdot 10^{-2}$	$1.20 \cdot 10^{-1}$	$2.40 \cdot 10^{-1}$	$6.00 \cdot 10^{-1}$	3.00	6.00
Estimated values by						
$Y = 1292.65x - 15.37$ (WLSLR)	$5.90 \cdot 10^{-2}$	$1.24 \cdot 10^{-1}$	$2.44 \cdot 10^{-1}$	$5.62 \cdot 10^{-1}$	2.96	6.25
$Y = 1320.20x - 46.00$ (LSLR)	$8.10 \cdot 10^{-2}$	$1.45 \cdot 10^{-1}$	$2.62 \cdot 10^{-1}$	$5.73 \cdot 10^{-1}$	2.92	6.14

Table 3. The analytical result of the determination of quercetin by WLSLR and LSLR, respectively

Concentration values (μg)	1	2	3	4	5	6
Theoretical values	$6.40 \cdot 10^{-2}$	$1.28 \cdot 10^{-1}$	$2.56 \cdot 10^{-1}$	$6.40 \cdot 10^{-1}$	3.20	6.40
Estimated values by						
$Y = 770.78x + 27.09$ (WLSLR)	$6.40 \cdot 10^{-2}$	$1.30 \cdot 10^{-1}$	$2.54 \cdot 10^{-1}$	$5.80 \cdot 10^{-1}$	3.56	6.22
$Y = 768.62x + 18.35$ (LSLR)	$7.56 \cdot 10^{-2}$	$1.42 \cdot 10^{-1}$	$2.66 \cdot 10^{-1}$	$5.93 \cdot 10^{-1}$	3.58	6.25

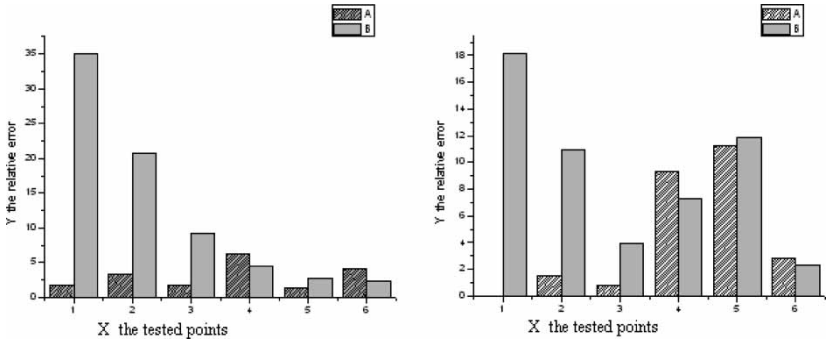

Figure 2. The column plot of the relative error of the calculated values and theoretical values. A denotes the relative error calculated by WLSLR, and B denotes the relative error calculated by LSLR. The left plot shows the data of luteolin and the right one shows the data of quercetin.

regression equations for the two analytes by WLSLR and LSLR, respectively. The detection limits for the analytes ranged from 7.5 ng to 16.0 ng at a signal-to-noise ratio of 3. Table 2 and Table 3 list the comparative results of the determination of luteolin and quercetin by WLSLR and LSLR, respectively.

The column plot of the relative error of the calculated values and theoretical values is as follows in Figure 2. Figure 2 showed that the estimated values at the lower concentrations were much more accurate calculated by WLSLR than by LSLR, while the estimated values at the higher concentrations did not show much difference. Owing to the lower concentrations of the analytes in the sample, we adopted WLSLR to deal with the data in order to achieve more accurate results.

Table 4 presents the amount of the analytes and the recoveries of standards spiked in the samples according to the regression equations of WLSLR. The recoveries of the analytes were 96.8% and 92.6% with RSDs ($n = 3$) of 1.36% and 1.95%, respectively. The method was reproducible with inter-day RSDs ($n = 5$) of 3.95% and 4.12% for luteolin and quercetin in the capsule of *Lamiophlomis rotata (Benth.) Kudo*, respectively. The results showed that the extraction method was adequate and appropriate for the analysis.

Table 4. Amount of the analytes and recoveries of the analytes spiked in the samples

Analyte	Amount in the sample[a]	RSD (%)	Recovery[a] (%)	RSD (%)
Luteolin	1.12 mg/g	1.58	96.8	1.36
Quercetin	0.15 mg/g	2.31	92.6	1.95

[a]Values are means of triplicate determinations.

CONCLUSION

Although, some methods have been reported for analyzing luteolin or quercetin from herbal samples,[5–11] to the best of our knowledge, there are no HPLC methods to analyze them simultaneously from the extracts of the capsule of *Lamiophlomis rotata (Benth.) Kudo*. The present study successfully develops a rapid HPLC method for the determination of luteolin and quercetin in the capsule of *Lamiophlomis rotata (Benth.) Kudo*. Because of the lower concentrations of the analytes, we adopted WLSLR to calculate the data, which could give more accurate results than that calculated by LSLR. Our method can be a reference for the quality control of the capsule of *Lamiophlomis rotata (Benth.) Kudo* and for the determinations of some other compounds with much lower concentration.

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REFERENCES

1. Yang, Z.; Xuejun, C.; Zhengling, C. Advances in studies on traditional tibetan herb *Lamiophlomis rotata (Benth.) Kudo*. Chin. Trad. Herb. Drugs **2001**, *32* (12), 1141–1143.
2. Guojing, L.; Yan, H. Clinical applications of tibetan herb *Lamiophlomis rotata (Benth.) Kudo*. Chin. Trad. Pat. Medicine **2004**, *26* (10), 848–851.
3. Jinhai, Y.; Xianzhong, Y.; Zeyuan, L.; Zhichang, Z. Studies on the chemical components of the roots of tibetan herb *Lamiophlomis rotata (Benth.) Kudo*. Acta. Phar. Sinica. **1995**, *30* (3), 206–210.
4. Jinhai, Y.; Xiaoping, H.; Yan, C. Studies on iridoid glucosides of the roots of tibetan herb *Lamiophlomis rotata (Benth.) Kudo*. Acta. Phar. Sinica. **1992**, *23* (7), 382–383.
5. Kaiser, R. Quantitative analyses of flavonoids in yellow dye plant species weld (*Reseda luteola L.*) and Sawwort (*Serratula tinctoria L.*). Angewandte Botanik. **1993**, *67* (3–4), 128–131.
6. Ferreres, F.; Amparo, M.; Isabel, M. Separation of honey flavonoids by micellar electrokinetic capillary chromatography. J. Chromatogr. A. **1994**, *669* (1–2), 268–274.
7. Chunsheng, L.; Yun Seon, S.; Kejian, Z. Gas chromatographic/mass spectrometric profiling of luteolin and its metabolities in rat urine and bile. J. Pharm. and Biom. Anal. **1995**, *13* (11), 1409–1414.
8. Orska-Gawry, J.; Surowiec, I.; Kehl, J. Identification of natural dyes in archeological coptic textiles by liquid chromatography with diode array detection. J. Chromatogr. A. **2003**, *989* (2), 239–248.

9. Cristea, D.; Bareau, I.; Vilarem, G. Identification and quantitative HPLC analysis of the main flavonoids present in weld (*Reseda Daniela Cristea, Isabelle Bareau and Gerard Vilarem luteola L.*). Dyes Pigments **2003**, *57* (3), 267–272.
10. Shuping, W.; Kuojun, H. Determination of flavonoids by high performance liquid chromatography and capillary electrophoresis. J. Chromatogr. **2004**, *1032* (1–2), 273–279.
11. Fang, F.; Jingming, L.; Qiuhong, P.; Weibong, H. Determination of red wine flavonoids by HPLC and effect of aging. Food Chem. **2007**, *101* (1), 428–433.

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