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RP-HPLC 法同时测定藏药珠芽蓼中牡荆素、槲皮苷和槲皮素的含量

续艳丽^{1,2}, 董琦¹, 胡凤祖^{1*}¹中国科学院西北高原生物研究所, 西宁 810008; ²中国科学院研究生院, 北京 100049

摘要: 采用 RP-HPLC 法建立了同时测定珠芽蓼中牡荆素、槲皮苷、槲皮素三种黄酮的方法, 色谱柱为 kromasil C₁₈ (250 mm × 4.6 mm 5 μm), 以甲醇-水(含 0.25% 磷酸)为流动相梯度洗脱, 流速 1 mL/min, 检测波长 360 nm。结果表明, 牡荆素、槲皮苷和槲皮素质量浓度在 8~40、5~50 μg/mL 和 5.33~52 μg/mL 范围内与色谱峰面积均呈良好的线性关系, 平均回收率分别为 100.3%、98.9% 和 100.4%, 日内稳定性偏差分别为 0.78%、0.51% 和 0.38%, 日间稳定性偏差为 2.6%、1.9% 和 2.1%。该方法简单、准确, 可为珠芽蓼的质量控制提供科学依据。

关键词: 珠芽蓼; 牡荆素; 槲皮苷; 槲皮素; RP-HPLC

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Simultaneous Quantitative Determination of Viterxin, Quercetin and Quercitrin in *Polygonum viviparum* in Tibet Plateau by RP-HPLC

XU Yan-li^{1,2}, DONG Qi¹, HU Feng-zu^{1*}¹Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, China;²Graduate University of Chinese Academy of Sciences, Beijing 100049, China

Abstract: To develop a sensitive and specific reversed-phase high performance liquid chromatography (RP-HPLC) method for simultaneous determination of three flavonoids in *Polygonum viviparum*, the HPLC separation was performed on a Kromasil C₁₈ column (250 mm × 4.6 mm 5 μm) and detected with DAD at 360 nm. The gradient elution was carried out with methanol-H₂O (containing 0.25% phosphoric acid) as the mobile phase at a flow rate of 1 mL/min. The results indicated that the calibration curves for viterxin, quercetin and quercitrin were linear over the ranges of 8-40 μg/mL, 5-50 μg/mL and 5.33-52 μg/mL, their assay recoveries were 100.3%, 98.9% and 100.4%, their intraday repeatability were 0.78%, 0.51% and 0.38%, and interday repeatability were 2.6%, 1.9% and 2.1%, respectively. This method was simple, accurate and sensitive, so it could be used for the quality control of *P. viviparum*.

Key words: *Polygonum viviparum*; viterxin; quercetin; quercitrin; RP-HPLC

Introduction

Polygonum viviparum, a widespread perennial forb, is widely distributed in Qinghai province. The root stock of *P. viviparum* which is well known as "ranbu", possess many activities and frequently used in checking diarrhea and activating blood circulation to dissipate blood stasis in folk traditional medicine^[1], especially in Tibetan medicine. Previous studies show that *P. viviparum* contains β-Sitosterol, daucosterol, sucrose,

gallic acid, flavonoids compounds^[2,3], has been found to possess an antibacterial^[4], antioxidant^[5], and anti-tumor activities^[6] and so on. In addition, *P. viviparum* grown at two different altitudes had different contents of ultraviolet-absorbing compounds, peroxidase and ascorbic acid^[7].

To this day, there is no reported method for the determination of the flavonoid compounds in *P. viviparum*. The present study developed and validated an RP-HPLC method for the simultaneous determination of viterxin, quercetin, and quercitrin in *P. viviparum*, which well collected from different areas of Qinghai province in Tibet Plateau. The method was successfully applied

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* Corresponding author. Tel: 86-971-6132750; E-mail: hufz@nwipb.ac.cn

to determine and compare with the contents of *P. viviparum* collected in different areas for the first time. This analytical method was validated to be simple, accurate and reliable, and can be used for the quality control of medicinal *Polygonum* plants.

Experimental

Materials and reagents

P. viviparum was collected from different areas of Qinghai Province. viterxin (purity > 97%), quercetin (purity > 99%) and quercitrin (purity > 99%) were all purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol was chromatographic grade, other sol-

vents were all analytical grade.

Chromatographic system

The HPLC instrument employed in the present study was Waters 515 chromatography system equipped with DAD ultraviolet-visible detector and Empower chromatography workstation. HPLC analysis was performed using a Kromasil C₁₈ column (416 mm × 300 mm, 5 μm) eluted with a mobile phase consisting of methanol (A) and 0.25% phosphoric acid H₂O (B) at a flow rate of 1.0 mL/min. The following gradient was used: 0–30 min 25%–35% A; 30–40 min 35%–60% A; 40–45 min 60%–95% A; 45–50 min 95%–25% A. The column temperature was 30 °C.

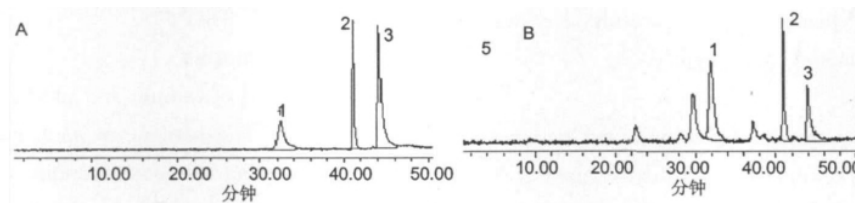


Fig. 1 RP-HPLC chromatogram of reference standards (A) and sample (B).

Peak 1: viterxin; Peak 2: quercetin; Peak 3: quercitrin.

Standard solution preparation

A stock solution of mixed standards including viterxin (40 μg/mL), quercetin (50 μg/mL), and quercitrin (53.3 μg/mL) was prepared by dissolving the accurately weighed standard substances in methanol. 1.0, 2.0, 4.0, 6.0, 8.0 mL and 10.0 mL of the stock solutions were each diluted to 10 mL with methanol for calibration curves and stored away from light at 4 °C.

Sample solution preparation

A 1.0 g pulverized *P. viviparum* was refluxed for 2 h with 20 mL trichloromethane, after removing the trichloromethane under reduced pressure, the residue was refluxed with 40 mL ethanol for 4 h. The extracted solutions were condensed under reduced pressure and then the residue was refluxed with 25 mL aqueous solution containing 80% methanol (v/v) and 2.4 M hydrochloric acid for 2 h in 80 °C water bath. The solution was filtered into a 100 mL volumetric flask. The stock solutions were filtered through a 0.45 μm filter membrane and kept at 4 °C before use. The filtrate (10 μL) was injected into the chromatographic system for

analysis.

Results and Discussion

Optimization of HPLC system

In the experiment, various mobile phase systems acetonitrile–water, acetonitrile–phosphoric acid water, methanol–water, and methanol–phosphoric acid water were investigated. Using acetonitrile–water as the mobile phase systems, the components were not completely separated although the retention time was shortened. With methanol–water as the mobile phase, these components were completely separated within 45 min, but the peak was not well. So we choose methanol–H₂O (containing 0.25% phosphoric acid) as the mobile phase to obtain a better analysis system.

Calibration curves and linearity

The mixed standard solutions at various concentrations were analyzed to construct the calibration curves. The chromatographic peak areas versus the injection concentrations were in good linearity. The detailed description of the regression curves are listed in Table 1.

Table 1 Standard work curves of three markers

Mark components	Regression equation	<i>r</i>	Linear range
Viterxin	$y = 18105x + 480$	0.9998	8-40 $\mu\text{g/mL}$
Quercetin	$y = 18750x + 21.721$	0.9999	5-50 $\mu\text{g/mL}$
Quercitrin	$y = 28351x + 9945$	0.9998	5.33-52 $\mu\text{g/mL}$

Precision

The precision of the method was evaluated through intra-day and inter-day repeated analysis. The *P. viviparum* sample in six replicates were analyzed repeatedly in one day or five successive days and the RSD values of viterxin ,quercetin ,quercitrin were 0.78% ,0.51% and 0.38% for intra-day ,2.6% ,1.9% and 2.1% for inter-day analysis ,respectively. These results showed that precision of the method was high.

Stability

The sample solutions were used for stability evaluation. The contents of three components were determined at 0 h ,4 h ,8 h ,12 h ,24 h and 48 h. The result showed that the solution was rather stable. The RSD values were all

lower than 2.6% .

Recovery

The recovery was evaluated by analyzing samples spiked with known concentrations of standard solution prior to extraction. These spiked samples were processed and analyzed as normal samples. The determined recoveries (%) are listed in Table 2.

Table 2 Recoveries of three substances from *Polygonum viviparum* (*n* = 3)

Compound	Contained (mg)	Added (mg)	Found (mg)	Recovery (%)	RSD (%)
Viterxin	0.168	0.812	0.983	100.3	1.6
Quercetin	0.901	0.630	1.514	98.9	2.1
Quercitrin	0.564	0.297	0.863	100.4	1.9

Analysis of the samples

The chromatograms of sample and mixed standards are shown in Fig. 1. The content of each component was calculated according the corresponding calibration equation. The determined contents of flavonoids in *P. viviparum* are shown in Table 3.

Table 3 Contents of the three components in the extract of *Polygonum viviparum*

Sample No.	Lacation	Altitude (m)	Viterxint (mg/g)	Quercetin (mg/g)	Quercitrin (mg/g)	Total content (mg/g)
1	Laji hill	3100	1.426	1.044	0.583	3.054
2	Henan city	3800	1.264	1.816	1.034	4.114
3	Huzhu city	3000	0.953	1.14	0.365	2.457
4	Qilian city	2900	0.21	1.126	0.705	2.041
5	Qilian hill	3800	1.06	1.88	1.261	4.201

The results (table 3) indicated that *P. viviparum* contained viterxin ,quercetin ,and quercitrin ,and quercetin was found to be the most abundant component in the samples ,accounting for 1.044-1.880 mg/g. The results also showed that the total contents of flavonoids varied markedly between samples growing at different altitude ,and *P. viviparum* growing at 3800 m altitude has apparently higher contents of flavonoids than at 2900 m altitude. The early research report that plants growing at higher altitude had apparently higher contents of ultra-violet-absorbing compounds and ascorbic acid and significantly higher activities of superoxide dismutase ,peroxidase and ascorbic peroxidase [7]. The activities of

antioxidation systems with the contents of flavonoids in *P. viviparum* which grown at different altitude areas need further investigation.

Conclusions

In conclusion ,a rapid and validated analytical method had been developed for the simultaneous quantification of three flavonoids components in *P. viviparum*. The established method provided an accurate and simple procedure for both qualitative and quantitative analyses of the biological active components in *P. viviparum*. This method is simple ,accurate and sensitive ,so it can be used for the quality control of *P. viviparum*.

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