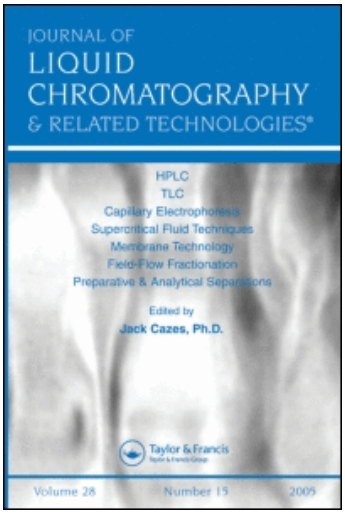

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A VALIDATED HPLC-DAD-MS METHOD FOR IDENTIFYING AND DETERMINING THE BIOACTIVE COMPONENTS OF TWO KINDS OF *LUOBUMA*

Junyou Shi[ab]; Guoliang Li[ab]; Rui Zhang[ab]; Jie Zheng[ab]; Yourui Suo[a]; Jinmao You[a]; Yong-jun Liu[a]
[a] Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China [b] Graduate School of the Chinese Academy of Sciences, Beijing, China

Online publication date: 26 March 2011

To cite this Article Shi, Junyou , Li, Guoliang , Zhang, Rui , Zheng, Jie , Suo, Yourui , You, Jinmao and Liu, Yong-jun(2011) 'A VALIDATED HPLC-DAD-MS METHOD FOR IDENTIFYING AND DETERMINING THE BIOACTIVE COMPONENTS OF TWO KINDS OF *LUOBUMA*', Journal of Liquid Chromatography & Related Technologies, 34: 7, 537 — 547
To link to this Article: DOI: 10.1080/10826076.2011.546173
URL: http://dx.doi.org/10.1080/10826076.2011.546173

Journal of Liquid Chromatography & Related Technologies, 34:537–547, 2011

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2011.546173

Taylor & Francis
Taylor & Francis Group

A VALIDATED HPLC-DAD-MS METHOD FOR IDENTIFYING AND DETERMINING THE BIOACTIVE COMPONENTS OF TWO KINDS OF *LUOBUMA*

Junyou Shi,[1,2] Guoliang Li,[1,2] Rui Zhang,[1,2] Jie Zheng,[1,2] Yourui Suo,[1] Jinmao You,[1] and Yong-jun Liu[1]

[1]*Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China*
[2]*Graduate School of the Chinese Academy of Sciences, Beijing, China*

□ *A rapid and effective HPLC-DAD-MS method for quantitatively identifying the flavonoids of* Apoacynum venetum *L and* Poacynum hendersonii *has been developed. With this method, all the main components were well separated. In* Apoacynum venetum *L, the main components are chlorogenic acid, hyperoside, isoquercitrin, and 6″-O-malonylhyperoside, while those of* Poacynum hendersonii *are quercetin3-O-sophoroside, isoquercitrin and 6″-O-malonylhyperoside. The experimental results showed that the developed approach is accurate, reproducible, and applicable for quantifying the bioactive components of* Apoacynum venetum *L and* Poacynum hendersonii.

Keywords *Apoacynum venetum* L, flavonoids, liquid chromatography-mass spectrometry, *Poacynum hendersonii*

INTRODUCTION

Flavonoids are very common and widespread secondary plant metabolites in herbal medicine. Due to the differences in number and the position of hydroxyl, methoxyl, glycosyl, and other substitutes, they make up of over 9000 compounds comprising 12 subclasses including flavonoids, flavanones, flavanols, and so on[1,2] In recent years, flavonoids have received considerable attention because of their protective role against inflammation, allergies, viruses, cancer, and other ailments.[3–5] Among them, an important compound is quercetin, which shows high anti-inflammatory and anticancer activities.[6]

To learn the varieties and amounts of flavonoids in medicinal plants, the separation of flavonoids has been previously achieved by thin-layer

Address correspondence to Yong-jun Liu, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, China. E-mail: yongjunliu_1@sdu.edu.cn

chromatography (TLC) or paper chromatography (PC),[7,8] which are tedious processes and usually inefficient. The alternative solutions are gas chromatography in combination with mass spectrometry (GC–MS)[9–11] and capillary zone electrophoresis (CZE),[12] but they also have some disadvantages. The GC–MS needs a derivatization step making it time-consuming, while the capillary zone electrophoresis only permits the use of a small amount of solvent and provides relative lower resolution and sensitivity.[13] Compared with the aforementioned methods, high-performance liquid chromatography (HPLC) has been proven to be a more powerful analytical separation method, and over the past decade its applications have dramatically expanded into almost every area of chemical and biochemical research. With the advent of numerous ionization sources such as fast atom bombardment (FAB), atmospheric pressure chemical ionization (APCI), and electrospray ionization (ESI), MS combined with high-performance liquid chromatography techniques has become a powerful approach in the identification, quantification, and structural confirmation of flavonoids.[14–19]

Apoacynum venetum L (AV) and *Poacynum hendersonii* (PH), also known as "luobuma,"[20,21] belong to the family of *Apoacynum* and *Poacynum Baill in* Apocynaceae, respectively, and grow widely in the middle to north western regions of China. Their leaves have been used as tea for daily drinking in Xinjiang province of China, and have gained increasing popularity among the Chinese population. According to the Pharmacopeia of People's Republic of China (2005), the soup made from the leaves of AV can be used to treat cardiac disease, hypertension, nephritis, neurasthenia, and other diseases.[22] Modern research on AV show that the ethanol extraction of AV leaves exhibits a series of pharmacological activities, such as anti-oxidation,[23–25] anti-hypertensive,[26,27] and anti-depressant effects,[28–30] cholesterol-lowering,[31] and AGEs-inhibitory activity.[32] The main active components are flavones and flavan-3-ols.[34–36] The ethanol extraction of PH also shows similar pharmacological effects such as anti-hypertensive, lipid-lowering, anti-oxidative, anti-aging, antidepressant, and diuresis effects;[34–36] the main components being isolated are flavonoids, most of which are quercetin glycosides.[37–40] As both of AV and PH are called "luobuma" by local people, though the two species are morphologically distinguishable, they have been used indiscriminately for many years regardless of their potential interspecific differences in components. According to the Pharmacopoeia of China, AV used as medicine should contain no less than 0.8% of quercetin after hydrolyzation in HCI, but this standard cannot differentiate AV from PH, as the content of quercetin in the leaves of PH is higher than that of AV.[41]

To the best of our knowledge, no comparative study on these leaves has been reported. In this paper, a HPLC-DAD-ESI-MS/MS-based analysis on

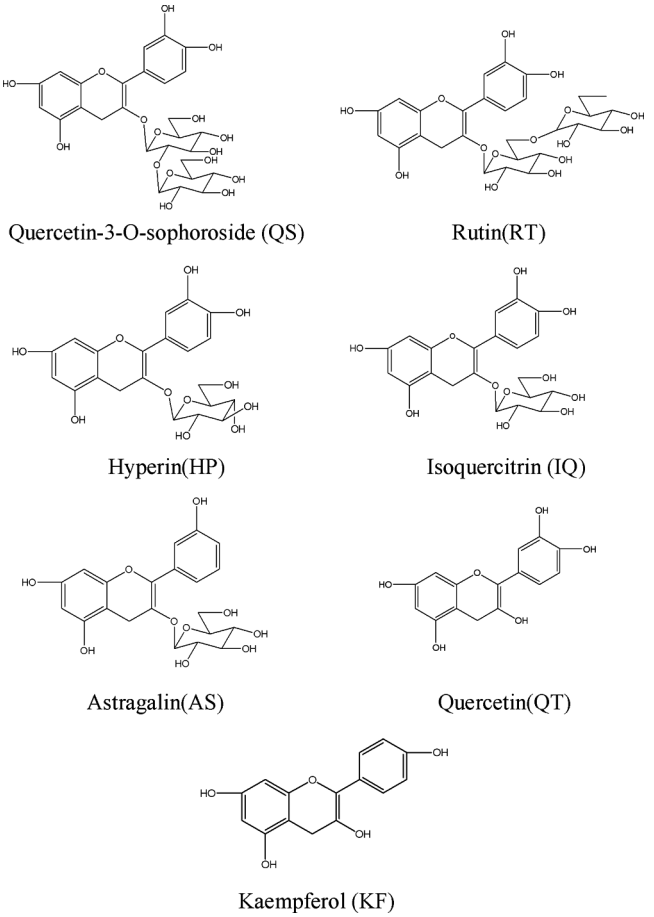

FIGURE 1 The chemical structures of the seven detected flavonoids.

the flavonoids of AV and PH was described, and seven flavonoids isolated from these two plants were determined quantitatively so that a rapid and sensitive technique to distinguish these two leave samples can be set up. The chemical structures of the seven flavonoids are shown in Figure 1.

EXPERIMENTAL

Chemicals and Materials

The leaves of *Poacynum hendersonii* (PH) were collected from Ge Er Mu, Qinghai province, in August, 2008, and that of *Apoacynum venetum* L (AV) was purchased from the Xining herb market. Quercetin, rutin, and kaempferol standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Isoquercitrin,

astragalin, and hyperin were purchased from Shanghai Shunbo Biotechnology Company LTD (Shanghai, China). Quercetin-3-O-sophoroside was isolated in our laboratory (over 95% purity by HPLC). Acetonitrile used for HPLC was of chromatographic grade (Yucheng Chemical plant, Yucheng, China). Methanol and formic acid were of analytical grade from Shanghai Chemical Reagent Co. Ultra-pure water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). Before use, all solutions were filtered through a 0.2 μm Nylon membrane (Alltech, Deerfield, IL, USA) and degassed.

HPLC–DAD-ESI/MS

The analysis was performed with an Agilent 1100 Series LC–MSD-Trap-SL liquid chromatograph–mass spectrometer which consists of: a quaternary pump (G1311A); an auto sampler (G1329A); a degasser (G1322A; a thermostatted column compartment (G1316A); a DAD detector (G1315B); and controlled by HP Chemstation software.

The chromatographic separation of seven flavonoids was achieved by using the analytical column Water Sunfire C18 (4.8 mm × 150 mm, i.d. particle size 5 μm, Waters Corp., Milford, MA, USA). The column oven temperature was maintained at 30°C. The mobile phase consisted of 0.1% aqueous formic acid (solvent A) and acetonitrile (solvent B), which was filtered through a 0.2 μm Nylon membrane filter (Alltech, Deerfield, IL, USA). Elution was performed at a flow rate of 1 mL/min in a binary gradient mode. The initial composition of solvent B was 15% and was linearly increased to 20% at 25 min, to 70% at 35 min, and maintained at this condition up to 40 min.

The DAD was detected at 254, 280, and 360 nm wavelength with full spectral scanning from 200 to 425 nm and 0.5 nm resolution using a semi-micro flow cell.

The mass spectrometer, from Bruker Daltonik (Bremen, Germany), was equipped with an ESI source and controlled by software (version 4.1). The ESI source was operated in positive-ion mode with a full scan mass range from m/z 100 to 1000 under the following conditions: capillary potential 3,500 V; cone voltage 40 V; ESI vap temperature 450°C, and corona current is 4,000 nA (pos). Nitrogen was used as the nebulizer gas, and the pressure and flow rate of dry gas were set at 65 p.s.i and 11 L/min, respectively.

Preparation of Standard Mixture Solutions and Sample Solutions

Stock solutions of quercetin-3-O-sophoroside (0.98 mg/mL), rutin (0.63 mg/mL), hyperin (0.5 mg/mL), isoquercitrin (0.49 mg/mL), astragalin (0.46 mg/mL), quercetin (0.38 mg/mL), and kaempferol (0.30 mg/mL) were prepared in methanol. Then, a set of standard solutions were

prepared by appropriate dilution of the stock solution with methanol, containing 0.00438 to 0.140 mg/mL of quercetin-3-O-sophoroside, 0.00281 to 0.090 mg/mL of rutin, 0.00223 to 0.0714 mg/mL of hyperin, 0.00219 to 0.0070 mg/mL of isoquercitrin, 0.00205 to 0.0657 mg/mL of astragalin, 0.00170 to 0.0543 of quercetin, and 0.00134 to 0.0429 mg/mL of kaempferol. All solutions were stored at 4°C before analysis.

The air-dried powder of PH leaves (1 g) and AV (1 g) was extracted with 20 ml methanol at 60°C in an ultrasound bath for 1 hr, then the extract process was repeated two times. The combined extraction was evaporated to dryness under vacuum, and the residue was reconstituted in a 10 mL volumetric flask with methanol. A volume of 1 mL of the solution was filtered through the membrane filter (Nylon) into a HPLC sample vial before analysis.

RESULTS AND DISCUSSIONS

Optimization of Flavone Standard Separation

Figure 2 shows the UV spectra of the seven flavonoids. The absorption wavelength was obtained with the online scanning range from 190 to 400 nm. All the maximum ultraviolet absorptions of the seven compounds are around 254 nm; therefore, the ultraviolet detection was selected at 254 nm.

To satisfactorily separate the seven components in the shortest time while the co-existing ingredients in the leaves of PH and AV do not interfere in the determination of seven flavonoids, several mobile phase systems of methanol-water and acetonitrile-water with or without formic acid have been tried. When methanol was used as the mobile phase, the separation time was long and the resolution was poor and the isomers of hyperin and isoquercitrin only form one peak. When acetonitrile was used, these compounds were eluted with severe tailing. Considering these cases, formic acid was added into water to improve the peak trailing. Finally, it was found that an acetonitrile-water containing 0.4% formic acid system gave the best separation. The linear gradient was as follows: Eluent A was H_2O-HCOOH (99.6:0.4) and Eluent B was 100% acetonitrile. The percentage of mobile phase was changed as follows after injection: (1) from 0 to 25 min Eluent A changed from 85 to 80% and Eluent B from 15 to 20%; (2) from 5 to 35 min Eluent A changed from 80 to 30% and Eluent B from 20 to 70%; and (3) from 35 to 40 min the mobile phase was 30% Eluent A and 70% Eluent B.

Identification of the Components in AV and PH

Figure 3 shows the gradient separation of flavonoid standards at 254 nm. For comparing the retention times and UV-vis spectra of the seven

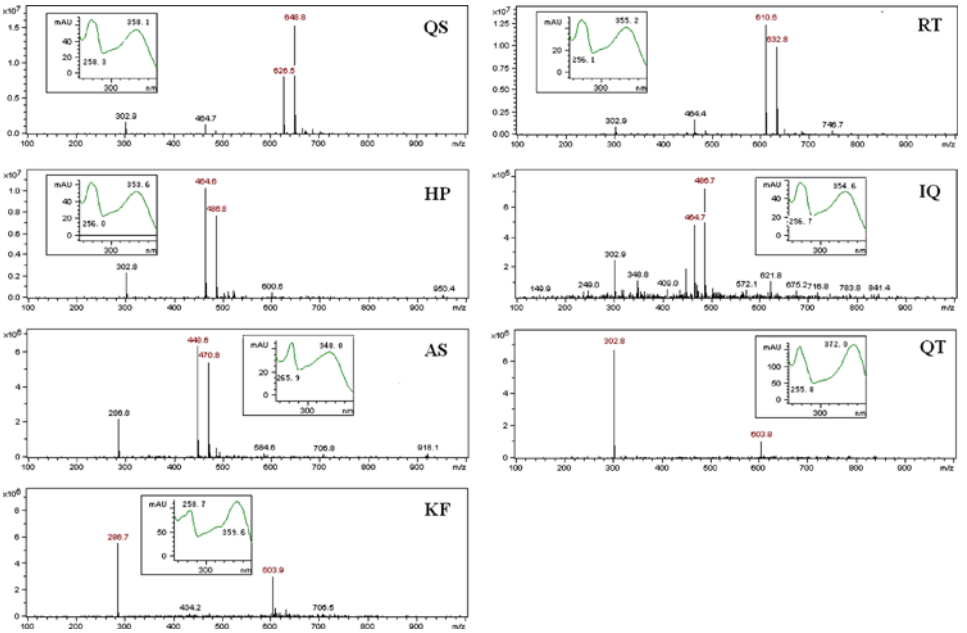

FIGURE 2 The UV and positive mode ESI–MS spectrum for the seven detected flavonoids. (Figure available in color online.)

standards at the same chromatographic conditions, the elution order was fixed. The two flavonoids with disaccharide aglycone, quercetin-3-*O*-sophoroside, and rutin, were firstly eluted followed by the three flavonoids with one monosaccharide aglycone, hyperoside, isoquercetin, and astragalin. Finally, the two flavonoids, quercetin and kaempferol were eluted out. The ionization and fragmentation of the seven components agree well with our conclusion. In the MS spectra of quercetin3-*O*-sophoroside, the characteristic fragment ions [M−H-162] (m/z 464.7) and [M−H-324] (m/z 302) of flavone-*O*-glucosides were observed, implying that the glucoside and sophoroside have split up. Similarly, in the MS spectra of rutin, the fragment ions [M−H-146] and [M−H-308] were observed. As for hyperoside, isoquercetin, and astragalin, the characteristic fragment ions [M−H-162] (m/z 302.8, 302.8 and 286.8) were observed indicating the monosaccharide has split up. For quercetin and kaempferol, the characteristic fragment ions were [M−H] and the m/z of their molecular ions were 302.8 and 286.7, which were consistent with these two compounds.

According to the m/z values and compounds that have been recognized from the leaves of AV and PH, we gave the prediction of the peaks in Figure 2, which are shown in Table 2. There are some isomers among these compounds, and we have identified them successfully. Peaks 10, 12, and 13 belong to the isomers with the same m/z *value* 464.4. As hyperoside and

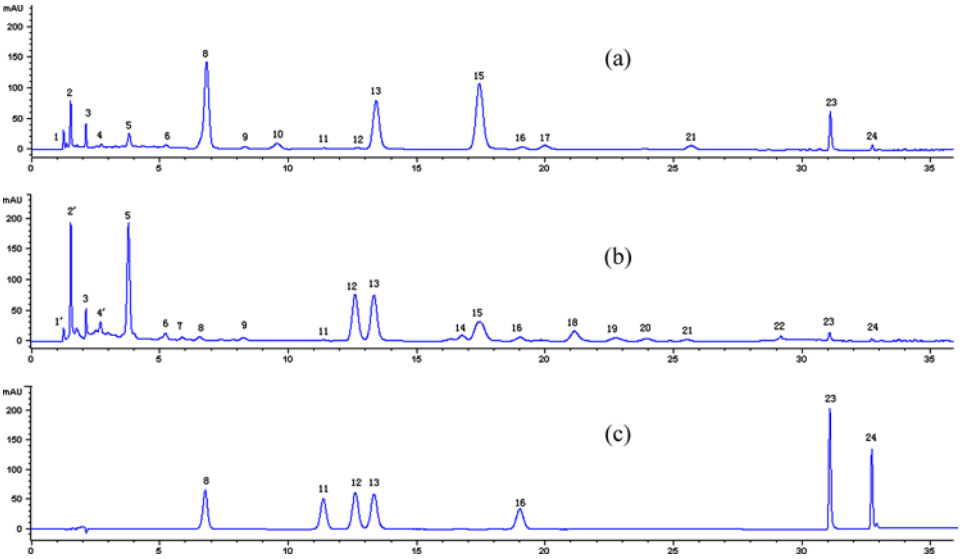

FIGURE 3 HPLC chromatograms of PH (a), AV (b) and standards mixture (c), DAD detection at 254 nm. (Figure available in color online.)

isoquercetin have been identified by comparing with the standards, and eriodictyol-7-*O*-glucoside has been isolated from PH but not AV, we deduced that peak 10 belongs to eriodictyol-7-*O*-glucoside. As for the isomers of trifolin and astragalin, since astragalin has been identified and trifolin has been isolated from AV, we confirm peak 14 is trifolin. Furthermore, we predict that peaks 15 and 17 belong to 6″-*O*-malonylhyperoside and 6″-*O*-malonylisoquercitrin, respectively, because their polarity are similar to hyperoside and isoquercetin. From Table 1, it can be seen that AV and PH share 12 peaks, i.e., 5, 6, 8, 9, 11, 12, 13, 15, 16, 21, 23, and 24, which have the same retention time and ion species both in AV and PH. For PH, the main ingredients are quercetin3-*O*-sophoroside (peak 8), isoquercetin (peak 13), and 6″-*O*-malonylhyperoside (peak15). But, for AV, the main ingredients are chlorogenic acid, hyperoside, isoquercetin, and 6″-*O*-malonylhyperoside. The contents of chlorogenic acid and hyperoside in AV are much higher than those of PH. But, the contents of quercetin3-*O*-sophoroside and 6″-*O*-malonylhyperoside are much lower than that of PH.

Quantification of Seven Flavone-*O*-glycosides with HPLC–MS/MS System

Quercetin 3-*O*-sophoroside, rutin, hyperoside, isoquercetin, astragalin, quercetin, and kaempferol in AV and PH samples have been quantitatively determined by using the developed method.

TABLE 1 HPLC–ESI/MS Data for Ethanolic AV and PH Extracts

Peaks	$[M–H]^+$	Predicted Name	HPLC Retain Time
1/1′	554.2/620.9	unknown	1.241
2/2′	626.4/364.6	unknown	1.522
3	609.4	unknown	2.112
4	428.8/354.4	unknown	2.676
5	354.6	chlorogenic acid	3.764
6	348.8	unknown	5.208
7	486.5	unknown	5.851
8	626.4	quercetin3-*O*-sophoroside	6.806
9	479.8	isorhamnetin (3-*O*-glucoside)	8.295
10	464.4	eriodictyol 7-*O*-glucoside	9.546
11	609.7	rutin	11.330
12	464.4	hyperoside	12.588
13	464.4	isoquercitrin	13.319
14	448.3	trifolin	16.752
15	550.2	6″-*O*-malonylhyperoside	17.427
16	448.3	astragalin	18.997
17	550.2	6″-*O*-malonylisoquercitrin	19.974
18	506.2	isoquercetin 6-*O*-acetate	21.127
19	534.3	malonated trifolin/malonated astragalin	22.730
20	490.3	kaempferol 6-*O*-acetate	23.942
21	316.4	isorhamnetin	25.663
22	348.6	malonated isorhamnetin	29.157
23	302.8	quercetin	31.060
24	286.8	kaempferol	32.704

A series of standard mixture solutions were tested to determine the linearity between concentration and peak areas. As described in Table 2, all the calibration curves exhibit good linear regressions with correlation coefficients larger than 0.999, and the ranges of curves are adequate for analyzing the seven bioactive compounds.

The limit of detection (LOD) was defined as the lowest concentration of compound resulting in a signal-to-noise ratio of 3:1. As shown in Table 2, the LOD of these seven compounds ranges from 2.4 to 9.5 μg/mL. The limit of quantification (LOQ) was defined as the lowest concentration of

TABLE 2 Calibration Curve Data for the Seven Detected Flavonoids

Standard Curves	Concentration (mg/mL)	Correlation	LOD (mg/mL)	LOQ (mg/mL)
1 $Y=4\times10^6\times-24828$	0.00438–0.140	0.9998	0.0095	0.0290
2 $Y=5\times10^6\times-19965$	0.00281–0.090	0.9995	0.0086	0.0258
3 $Y=8\times10^6\times-69855$	0.00223–0.0714	0.9991	0.0079	0.0241
4 $Y=8\times10^6\times-53286$	0.00219–0.0070	0.9996	0.0093	0.0283
5 $Y=6\times10^6\times-6286.4$	0.00205–0.0657	0.9998	0.0087	0.0263
6 $Y=1\times10^7\times-94165$	0.00170–0.0543	0.9999	0.0038	0.0116
7 $Y=1\times10^6\times+3988.9$	0.00134–0.0429	0.9997	0.0024	0.0094

TABLE 3 Recovery (%) of Seven Flavonoids for this Method ($n=2$)

Compounds	Amount Found (mg)	Amount Found (mg)	Recovery (%)	R.S.D. (%)
Quercetin3-*O*-sophoroside	0.110	0.106	96.4	2.2
Rutin	0.133	0.137	103.1	1.6
Hyperoside	0.126	0.124	98.4	2.0
Isoquercetin	0.140	0.143	102.2	1.5
Astragalin	0.105	0.101	96.2	4.3
Quercetin	0.126	0.125	99.2	1.9
Kaempferol	0.093	0.091	97.8	3.1

TABLE 4 The Contents of Seven Flavonoids in AV and PH (mg/g)

Analytes	AV	PH
QS	0.046	7.889
RT	0.112	0.154
HP	1.506	0.066
IQ	1.561	2.547
AS	0.162	0.186
QR	0.098	0.414
KF	0.068	0.084

compound resulting in a signal-to-noise ratio of 10:1, and the LOQ of these seven compounds ranges from 9.4 to 29.0 μg/mL. Both the LOD and LOQ of these seven standards are low enough for detecting traces of these compounds.

To examine the reliability of this method, the recoveries of the seven standards were determined by adding known amounts of standards into the methanol extraction of AV under the same conditions which are shown in Table 3. All the recoveries are in the range of 96.2–103.1%, suggesting the method is reliable.

Table 4 lists the contents of the seven flavonoids in AV and PH. One can see that the contents of flavonoids in PH are higher than those of AV except hyperoside. In the leaves of AV, the contents of hyperoside and isoquercetin are 1.506 and 1.561 mg/g, respectively, which are much higher than the other ingredients. In the leaves of PH, the contents of quercetin3-*O*-sophoroside, isoquercetin, and quercetin are very high. In particular, the content of quercetin3-*O*-sophoroside reached 7.889 mg/g.

CONCLUSION

In the present study, a useful, rapid and simple HPLC-DAD-ESI-MS/MS method has been developed for quantitatively analyzing the flavonoids in

Apoacynum venetum L and *Poacynum hendersonii*. The similar components of AV and PH leaves provide valuable information for understanding their similar pharmacological efficacy, but the contents of some flavonoids are very different between these two "luobuma."

REFERENCES

1. Merken, H.; Beecher, G. Measurement of Food Flavonoids by High-Performance Liquid Chromatography: A Review. *J. Agric. Food Chem.* **2000**, *48*, 577–599.
2. Pietta, P. Evaluation of the Total Peroxyl Radical-Scavenging Capacity of Flavonoids: Structure–Activity Relationships. *J. Nat. Prod.* **2000**, *63*, 1035–1042.
3. Deschner, E.; Ruperto, J.; Wong, G.; Newmark, H. Quercetin and Rutin as Inhibitors of Azoxymethanol-Induced Colonic Neoplasia. *Carcinogenesis* **1991**, *12*, 1193.
4. Soleas, G.; Grass, L.; Josephy, P.; Goldberg, D.; Diamandis, E. Clin. A Comparison of the Anticarcinogenic Properties of Four Red Wine Polyphenols. *Biochem.* **2002**, *35*, 119–124.
5. Pereira, M.; Grubbs, C.; Barnes, L.; Li, H.; Olson, G.; Eto, I.; Juliana, M.; Whitaker, L.; Kelloff, G.; Steele, V. Effects of the Phytochemicals, Curcumin and Quercetin, upon Azoxymethane-Induced Colon Cancer and 7,12-Dimethylbenz [*a*]Anthracene-Induced Mammary Cancer in Rats. *Carcinogenesis.* **1996**, *17*, 1305–1311.
6. Ferry, D. R.; Smith, A.; Malkhandi, J.; Fyfe, D. W.; Takats, P. G.; Anderson, D.; Baker, J.; Kerr, D. J. Phase I Clinical Trial of the Flavonoid Quercetin: Pharmacokinetics and Evidence for Invivo Tyrosine Kinase Inhibition. *Clin. Cancer. Res.* **1996**, *2*, 659–668.
7. Sosa, S.; Altinier, G.; Politi, M.; Braca, A.; Morelli, I.; Della Loggia, R. Extracts and Constituents of Lavandula multifida with Topical Anti-Inflammatory Activity. *Phytomedicine* **2005**, *12*, 271–277.
8. Inbaraj, B.; Lu, H.; Kao, T.; Chen, B. Simultaneous Determination of Phenolic Acids and Flavonoids in Lycium barbarum Linnaeus by HPLC–DAD–ESI-MS. *J. Pharm. Biomed. Anal.* **2010**, *51*, 549–556.
9. Sakakibara, H.; Honda, Y.; Nakagawa, S.; Ashida, H.; Kanazawa, K. Simultaneous Determination of all Polyphenols in Vegetables, Fruits, and Teas. *J. Agric. Food Chem.* **2003**, *51*, 571–581.
10. Stevens, J.; Hart, H.; Elema, E.; Bolck, A. Flavonoid Variation in Eurasian Sedum and Sempervivum. *Phytochemistry* **1996**, *41*, 503–512.
11. Soleas, G.; Yan, J.; Goldberg, D. Ultrasensitive Assay for Three Polyphenols (Catechin, Quercetin and Resveratrol) and their Conjugates in Biological Fluids Utilizing Gas Chromatography with Mass Selective Detection. *J. Chromatogr. B* **2001**, *757*, 161–172.
12. Molnar-Perl, I.; Füzfai, Z. Chromatographic, Capillary Electrophoretic and Capillary Electrochromatographic Techniques in the Analysis of Flavonoids. *J. Chromatogr. A* **2005**, *1073*, 201–227.
13. Da Costa, C.; Horton, D.; Margolis, S. Analysis of Anthocyanins in Foods by Liquid Chromatography, Liquid Chromatography–Mass Spectrometry and Capillary Electrophoresis. *J. Chromatogr. A* **2000**, *881*, 403–410.
14. Parejo, I.; Jauregui, O.; Sanchez-Rabaneda, F.; Viladomat, F.; Bastida, J.; Codina, C. Separation and Characterization of Phenolic Compounds in Fennel (Foeniculum vulgare) Using Liquid Chromatography-Negative Electrospray Ionization Tandem Mass Spectrometry. *J. Agric. Food Chem.* **2001**, *52*, 3679–3687.
15. Prasain, J.; Jones, K.; Kirk, M.; Wilson, L.; Smith-Johnson, M.; Weaver, C.; Barnes, S. Profiling and Quantification of Isoflavonoids in Kudzu Dietary Supplements by High-Performance Liquid Chromatography and Electrospray Ionization Tandem Mass Spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 4213–4218.
16. Romani, A.; Vignolini, P.; Isolani, L.; Ieri, F.; Heimler, D. HPLC-DAD/MS Characterization of Flavonoids and Hydroxycinnamic Derivatives in Turnip Tops (Brassica rapa L. subsp. sylvestri s L.). *J. Agric. Food Chem.* **2006**, *54*, 1342–1346.
17. Weisskopf, L.; Tomasi, N.; Santelia, D.; Martinoia, E.; Langlade, N.; Tabacchi, R.; Abou-Mansour, E. Isoflavonoid Exudation from White Lupin Roots is Influenced by Phosphate Supply, Root Type and Cluster-Root Stage. *New Phytol.* **2006**, *171*, 657–668.

18. March, R.; Miao, X.; Metcalfe, C.; Stobiecki, M.; Marczak, L. A Fragmentation Study of An Isoflavone Glycoside, Genistein-7-O-Glucoside, Using Electrospray Quadrupole Time-of-Flight Mass Spectrometry at High Mass Resolution. *Int. J. Mass Spectrom.* **2004**, *232*, 171–183.
19. Li, T.; Zhang, H. Identification and Comparative Determination of Rhodionin in Traditional Tibetan Medicinal Plants of Fourteen Rhodiola Species by High-Performance Liquid Chromatography-Photodiode Array Detection and Electrospray Ionization-Mass Spectrometry. *Chem. Pharm. Bull.* **2008**, *56*, 807–814.
20. Dong, Z. J. *The Comprehensive Utilization of Luobuma*, Science Press: Beijing, 1978.
21. Liu, Y.; Luo, X.; Wu, R. *Flora of China Illustrations*, Science Press: Beijing, 1996, Vol. 30.
22. Pan, Z.; Liu, J.; Yang, J. *The Pharmacopoeia Committee of the Health Ministry of the People' Republic of China, Part 1*, Chemical Technologic Publisher: Beijing, 2005.
23. Sandei, N.; Michiko, M.; Chisako, C. Flavonid Content in Apocynum and Poacnum Leaves and Lipid-Peroidation Inhibiting Efeet of Flavonoids. *Nat. Med.* **1994**, *48*, 322–323.
24. Yokozawa, T.; Kashiwada, Y.; Hattori, M.; Chung, H. Study on the Components of Luobuma with Peroxynitrite-Scavenging Activity. *Biol. Pharm. Bull.* **2002**, *25*, 748–752.
25. Shirai, M.; Kawai, Y.; Yamanishi, R.; Terao, J. Approach to Novel Functional Foods for Stress Control 5. Antioxidant Activity Profiles of Antidepressant Herbs and their Active Components. *J. Med. Invest.* **2005**, *52*, 249–251.
26. Kim, D.; Yokozawa, T.; Hattori, M.; Kadota, S.; Namba, T. Effects of Aqueous Extracts of Apocynum venetum Leaves on Spontaneously Hypertensive, Renal Hypertensive and NaCl-fed-hypertensive Rats. *J. Ethnopharmacol.* **2000**, *72*, 53–59.
27. Kagawa, T.; Nakazawa, Y.; Tagashira, E.; Takahashi, Y.; Onizuka, S.; Nishibe, S. Studies on Antihypertensive Effect of Luobuma (Apocynum venetum L.) Leaf Extract (1). *Nat. Med.* **2004**, *58*, 109–112.
28. Willner, P. The Validity of Animal Models of Depression. *Psychopharmacology* **1984**, *83*, 1–16.
29. Butterweck, V.; Simbrey, K.; Seo, S.; Sasaki, T.; Nishibe, S. Long-Term Effects of an Apocynum venetum Extract on Brain Monoamine Levels and Beta-AR Density in Rats. *Pharmacol. Biochem. Behav.* **2003**, *75*, 557.
30. Grundmann, O.; Nakajima, J.; Seo, S.; Butterweck, V. Anti-Anxiety Effects of Apocynum venetum L. in the Elevated Plus Maze Test. *J. Ethnopharmacol.* **2007**, *110*, 406–411.
31. Kim, D.; Yokozawa, T.; Hattori, M.; Kadota, S.; Namba, T. Effects of Aqueous Extracts of Apocynum venetum Leaves on Hypercholesterolaemic Rats. *Phytotherapy Res.* **1998**, *12*, 46–48.
32. Yokozawa, T.; Nakagawa, T. Inhibitory Effects of Luobuma Tea and its Components against Glucose-Mediated Protein Damage. *Food Chem. Toxicol.* **2004**, *42*, 975–981.
33. Xiong, Q.; Fan, W.; Tezuka, Y.; Adnyana, I.; Stampoulis, P.; Hattori, M.; Namba, T.; Kadota, S. Hepatoprotective effect of Apocynum venetum and its Active Constituent. *Planta Med.* **2000**, *66*, 127–133.
34. Qian, Z. N.; Gu, Z.; Jin, L.; Mao, D. The Pharmacological Research *of Poacynum hendersonii*-3 – the Effects on the Cardiovascular System. *Chin. Trad. Patent Med.* **1991**, *13*, 27–29.
35. Qian, Z. N.; Gu, Z.; Fang, J.; Chen, Y.; Wang, E. The Pharmacological Research *of Poacynum hendersonii*-2 – the Anti-Aging Effect. *Chin. Trad. Patent Med.* **1990**, *12*, 28–30.
36. Gu, Z. L.; Qian, Z.; Wang, Z. N.; Xie, M. The Study on the Pharmacological Research *of Poacynum hendersonii*-1. The Influence on the Platelet Aggregation. *Chin. Trad. Patent Med.* **1989**, *11*, 28–30.
37. Wei, J. P.; Liu, E. N. L.; Zhang, L. W.; Lin, W. H.; Li, Q. S. Studies on Chemical Constituents of Poacynum hendersonii. *Chin. Trad. Herbal Drugs* **2008**, *39*, 1304–1306.
38. Hu, B. H.; Tian, Z.; Lou, Z. C. The Isolation and Identification of the *Poacynum hendersonii's* Flavonoid Glycoside. *JIPB* **1988**, *30*, 565–568.
39. Shi, Q. M.; Turuhong, P.; Abbas, A. Determination of Total Flavonoid and Hyperoside Contents in Different Parts of Poacynum hendersonii (Hook.f.) Woodson. *Food Sci. (China)* **2009**, *30*, 263–266.
40. Zhang, Y. F.; Wei, D.; Du, N. S. Studies on Chemical Constituents of Poacynum hendersonii. *Nat. Prod. Res. Develop. (China)* **2006**, *18*, 954–957.
41. Ma, M.; Hong, C.; An, S.; Li, B. Seasonal, Spatial, and Interspecific Variation in Quercetin in Apocynum venetum and Poacynum hendersonii, Chinese Traditional Herbal Teas. *J. Agric. Food Chem.* **2003**, *51*, 2390–2393.