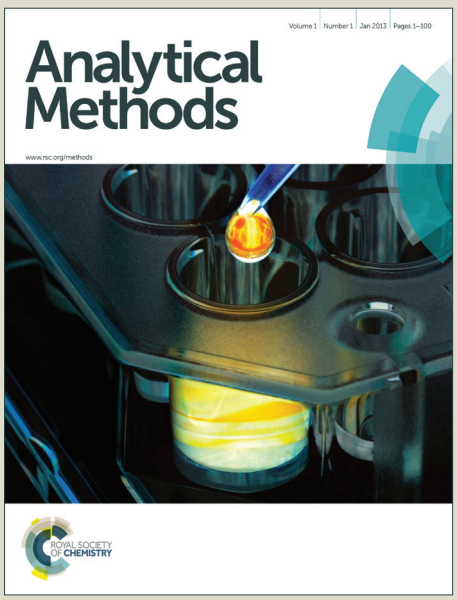
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Analysis of eight isoflavones in Radix Puerariae by capillary zone electrophoresis with ionic liquid as additive

Wen Xiao, Feng-Qin Wang, Chun-Hong Li, Qian Zhang, Zhi-Ning Xia, Feng-Qing Yang*

School of Chemistry and Chemical Engineering, Chongqing University, Chongqing 400030, China

*Author to whom correspondence should be addressed:

Asso. Prof. Feng-Qing Yang

Tel/Fax: +86-23-6510-6615; E-mail: fengqingyang@cqu.edu.cn

Abstract: Isoflavones are the main active components in Radix Puerariae (RP), the dry root of *Pueraria lobata* or *P. thomsonii*. In the present study, a simple capillary zone electrophoresis (CZE) method with ionic liquid (IL) as additive was developed for the simultaneously determination of eight isoflavones include ononin, daidzin, genistin, biochanin A, formononetin, puerarin, genistein and daizein in RP. Experimental conditions including sodium tetraborate concentration, pH, types and concentration of ILs, applied voltage and capillary temperature were intensively investigated. Finally, the eight analytes (detection wavelength of 260 nm) were well separated within 9 min by using the running buffer composed of 30 mM sodium tetraborate and 50 mM 1-butyl-3-methylimidazolium tetrafluoroborate ($BMImBF_4$) as additive at pH 9.5, with applied voltage of 18 kV and capillary temperature of 25 °C. The developed method was fully validated [LOD (1.72 - 4.92 μg/mL), LOQ (3.64 - 9.84 μg/mL), intra- (1.1% - 4.7% RSD) and inter-day (2.1% - 6.6% RSD) precision and recovery (93.1% - 107.5% with 4.0% - 5.9% RSD)] and was successfully applied to quantification of the eight analytes in three RP samples. The results indicated that *P. lobata* contains much abundance of isoflavones than *P. thomsonii*. Furthermore, CZE with IL as additive should be a promising method for the analysis of natural products.

Keywords: ionic liquid; capillary zone electrophoresis; isoflavones; Radix Puerariae

Introduction

Radix Puerariae (RP), which is the dry root of *Pueraria lobata* (Willd.) Ohwi or *P. thomsonii* Benth [1], contains large amounts of isoflavones such as puerarin, daidzin, genistein, biochanin A and so on [2-5]. Those isoflavones have beneficial effects on cardiovascular diseases [6, 7] and diabetes [7], and were reported to have antithrombosis and antiallergy [8], antimutagenic [9, 10], antioxidant [11] and estrogenic activities [12]. There were qualitative and quantitative methods reported for the analysis of isoflavones in RP, include high-performance liquid chromatography (HPLC) [2-4] and capillary electrophoresis (CE) (but only limited numbers of analytes were determined) [5, 13-15].

To date, considerable numbers of CE analysis for natural products were published because of its rapidness and high efficiency, as well as small amount of sample and solution required [16, 17]. Organic solvents such as methanol and acetonitrile were usually used as additive in capillary zone electrophoresis (CZE) analysis, but the total analysis time usually increased due to the decrease of electroosmotic flow (EOF) when organic solvents were applied. In reality, ionic liquids (ILs) have strong dissolution ability for most organic and inorganic compounds, with very low volatility and high thermal stability, and have been shown to be more environmentally friendly than organic solvents [18, 19]. Furthermore, because of their special characteristics such as good conductivity, almost zero volatile and hydrophobic interaction

of ions or electrons group [20, 21], ILs play a significant role in CZE [22-24], non-aqueous capillary electrophoresis (NACE) [25, 26], micellar electrokinetic chromatography (MEKC) [27, 28] and microemulsion electrokinetic capillary chromatography (MEEKC) [29-31]. However, there were no reports for the analysis of isoflavone by CZE with IL as additive.

Therefore, in the present study, IL used as additive in CZE for the analysis of eight isoflavones (shown in Fig. 1) was studied. Experimental conditions including sodium tetraborate concentration, pH, types and concentration of ILs, applied voltage and capillary temperature were intensively investigated. The developed method is fully validated and applied in the simultaneously determination of eight isoflavones in three RP samples.

Materials and methods

Chemicals and reagents

The ILs of tetramethylammonium tetrafluoroborate (TMA-BF_4) and 1-butyl-3-methylimidazolium tetrafluoroborate (BMImBF_4) are products of Shanghai Chengjie Chemical Co., Ltd. (Shanghai, China). N-butylmethylpyrrolidinium bromide (BMPyBr) was obtained from Beijing HWRK (Huawei-Ruike) Chem. Co., Ltd. (Beijing, China), and 1-octyl-3-methylimidazolium chloride (OMImCl) was purchased from Lanzhou Institute of Chemical Physics (Lanzhou, China). Sodium tetraborate and sodium hydroxide of analytical grade were obtained from Chengdu Kelong Chemical Works (Chengdu, China). Methanol for liquid

chromatography was purchased from InnoChem (Beijing InnoChem Science & Technology Co. Ltd, China). The acetone used as EOF marker was purchased from Chongqing Xi'nan Chemical Reagent Co., Ltd. (Chongqing, China).

Puerarin, daidzin and daidzein were obtained from Chengdu Must Biotechnology Co., Ltd & Chengdu Institute of Biology (Chengdu, China). Ononin, genistin, biochanin A, formononetin and genistein were purchased from Chengdu Preferred Biotechnology Co., Ltd (Chengdu, China). Their purity are all higher than 99% (determined by HPLC), and the chemical structures are shown in Fig. 1.

The materials of *P. thomsonii* were obtained from Heping Pharmacy (Guangxi, China), and the two different origins of *P. lobata* were purchased from Anguo Rush's medicine LLC (Guangxi, China) and Bozhou Northern Pharmaceutical Co., Ltd. (Hubei, China), respectively. The species of *R. Pueraria* were identified by the corresponding author and they were deposited at the Department of Pharmaceutics, School of Chemistry and Chemical Engineering, Chongqing University, Chongqing, China.

Apparatus and procedures

All CE separations were performed on an Agilent 7100 3D CE system (Agilent Technologies, Palo Alto, CA, USA), equipped with a DAD and an Agilent ChemStation software, and the uncoated fused-silica capillary (Hebei Yongnian Ruifeng Chromatographic Implements, Hebei, China) with 50 μm

i.d. and 48.0 cm of total length (40.5 cm effective length) was used throughout this study. The KQ-100B ultrasonic cleaner (Kunshan ultrasonic instruments Co., Ltd.) was applied for the preparation of buffer and samples. A Delta 320 pH meter (Mettler-Toledo Instruments, Shanghai) was used for measuring the pH of buffer. Reverse osmosis (RO) was prepared by AKWL-IV-16 water purification system (Chengdu Tang's Kangning Science and Technology Development Co., Ltd., Chengdu, China).

The standard stock solutions were prepared by accurately weighted of 8 analytes and dissolved in methanol (about 500 μg/mL) respectively, and stored at 4 °C refrigerator before use. A desired amount (1.0 g) of RP sample powder was extracted with 25 mL methanol in 25 mL flask by ultrasonication for 30 min according to reported method [13, 14]. Then the extract was cooled down to room temperature (about 25 °C), and made up the lost weight with methanol, and after that filtered through a 0.45 mm nylon membrane (Auto science instrument Co., Ltd., Tianjing, China). Finally, the extract was diluted according to the desired concentration with running buffer before injection.

Before first use, the capillary was conditioned by flushing with 1 M NaOH, 0.1 M NaOH and RO-water each for 10 min. And between two runs, the capillary was successive rinsed with 0.1 M NaOH, water and running buffer each for 2 min. The running buffer was refreshed every analyses. The operating conditions were: pressure injection was 35 mbar for 3 s, and the detection wavelength was set at 260 nm.

Results and discussion

Optimization of separation conditions

Effect of sodium tetraborate concentration and buffer pH

Sodium tetraborate solution was used as background electrolyte in the present study, and the concentration of 10, 20, 30, 40, 50 and 60 mM were investigated for the separation of eight isoflavones by CZE (other conditions were: buffer pH 9.3, applied voltage was 20 kV, cassette temperature was 25 °C). The results (Fig. 2A) indicated that better separation (but with longer analysis time) for the analytes can be obtained when using higher concentration of sodium tetraborate, but biochanin A (4) and formononetin (5) cannot be separated under the investigated concentration of sodium tetraborate. In reality, lower ionic strength (lower concentration) of sodium tetraborate buffer generated relatively low current and high EOF value. Therefore, a medium concentration (30 mM) of sodium tetraborate was chosen as background electrolyte, because further increase the concentration (40, 50 and 60 mM) of sodium tetraborate didn't further improve the resolutions of peaks but increased the analysis time. Furthermore, the migration of analyte is affected by the buffer pH (solute ionization degree and EOF velocity). So different buffer pH (8.5, 8.8, 9.0, 9.3, 9.5 and 9.8) were investigated (the other conditions were: 30 mM sodium tetraborate buffer, 20 kV and 25 °C). The result (Fig. 2B) showed that the buffer pH has significant effect on the migration of analytes. The ionization of substituent phenolic hydroxyl group

on the 4′-C (R_4=H in Fig.1) is affected by the pH of buffer, so the change of migration time of compounds with 4′-C phenolic hydroxyl group (compounds 2, 3, 6, 7 and 8) are much more obvious than those without such hydroxyl group (compounds 1, 4 and 5) (Fig. 2B). The higher pH is good for the separation of investigated analytes except biochanin A (4) and formononetin (5), which have very similar chemical structures. Although there are two phenolic hydroxyl groups on biochanin A (4), its 5-C hydroxyl group will form intramolecular hydrogen bond with its 4-C keto group. Herein, the concentration of sodium tetraborate and pH of the buffer have almost no effect on the resolution of biochanin A (4) and formononetin (5). Therefore, 30 mM sodium tetraborate buffer with pH 9.5 was chosen for further optimization. In addition, organic solvents usually used as additive in CZE, methanol and acetonitrile as additive was preliminarily investigated in the present study, but no improvement on the resolution of biochanin A (4) and formononetin (5) was observed. Therefore, ILs as additive was investigated subsequently.

Effect of type and concentration of ILs

In the present study, four different ILs include TMA-BF_4, $BMImBF_4$, BMPyBr and OMImCl with different concentrations (10, 20, 30, 40, 50 and 60 mM) used as CZE additives were investigated for the separation of eight isoflavones (other conditions: 30 mM borate buffer at pH 9.5, 20 kV and 25 ^{o}C). The results (Fig. 2C) indicated that $BMImBF_4$ can improve the separation of

biochanin A and formononetin with little effect on the EOF. Therefore, 50 mM of $BMImBF_4$ as additive was chosen for further study.

Most studies believe that the mechanism of ILs as additive or electrolyte in buffer maybe involved hydrogen bonding [22], electrostatic [24], hydrophobic or ion-dipole/ ion-induced-dipole interaction between the ILs and analytes in the separation [23]. In the present study, the change of concentration of $BMImBF_4$ had little effect on the EOF, thus, the separation mechanism is most likely the hydrophobic interaction between the analytes and ILs, and/or the analytes formed complexes with the ionic liquid, which is heteroconjungation between the anions and cations in the ILs and the analytes [25, 26]. As shown in Fig. 2C, the migration time of compounds with high logP values (compounds 4, 5, 7 and 8) decreased with the increase of concentration of $BMImBF_4$, which may be associated with the hydrophobic interaction of analytes with the hydrophobic cationic imidazolium of $BMImBF_4$. Furthermore, the migration time of biochanin A (4) and genistein (7), which have higher logP values, are decreased much more than that of formononetin (5) and daidzein (8). Therefore, biochanin A (4) and genistein (7) can be separated from their adjacent peaks with higher concentration of $BMImBF_4$ (Fig. 2C). It should be mentioned that, the other three ILs include TMA-BF_4, BMPyBr and OMImCl as additive could also affected on the migration of investigated compounds, but unfortunately they didn't improve but deteriorate the resolutions of peaks in the present study.

Effect of applied voltage, temperature and injection time and pressure

The applied voltage is another important factor in the CE analysis. The applied voltages from 15 to 25 kV (15, 18, 20, 23 and 25 kV) were investigated, and 18 kV was selected for better separation and shorter analysis time. Meanwhile, the cassette temperature from 15 to 35 °C with 5 °C increment was also compared, the migration time decreased with the increase of temperature, and 25 °C was finally chosen.

In conclusion, the optimum CZE conditions for the analysis of eight investigated isoflavones were: running buffer was 50 mM $BMImBF_4$ in 30 mM sodium tetraborate water solution at pH 9.5, applied voltage was 18 kV and capillary temperature was at 25 °C.

Method Validation

Linearity, LOD and LOQ

Stock solutions containing the eight reference compounds were prepared and diluted to the appropriate concentrations for the construction of calibration curves. At least six concentrations of the solution were analyzed in duplicates under the optimized CZE conditions, and then the calibration curves were constructed by plotting the peak area of the individual compound *versus* the concentration of each analyte [peak area (y) vs. concentration (x) (μg/mL)]. The limits of detection (LOD) and quantification (LOQ) under the optimum conditions were determined based on signal-to-noise (S/N) ratios of 3 and 10 respectively. Table 1 shows the linear

regression data, LOD and LOQ. The LODs and LOQs in the present study are similar to that of reported CE method [5], but much poorer than that of reported UPLC method [2].

Precision and recovery

Intra- and inter-day variations were chosen to determine the precision of the developed assay. The known concentrations of eight standard solutions were tested. For intra-day variability test, the mixed standards solutions were analyzed for six replicates within one day, while for inter-day variability test, the solutions were examined in duplicates for consecutive three days. The results of variations RSD are listed in Table 2. Intra-day RSD and inter-day RSD varied from 1.1% to 4.7%, 2.0% to 6.6%, respectively.

The recovery was used to evaluate the accuracy of the method. Known amounts of individual standards were added into a certain amount (0.5 g) of sample *P. lobata* from Guangxi. The mixture was extracted and analyzed using the method mentioned above. Three replicates were performed for the test, and the results were summarized in Table 3, which indicate that the developed method can be applied in the real sample (RP) analysis.

Determination of investigated compounds in three RP samples

Under the optimum conditions, methanol extracts of three RP samples (*P. lobata* and *P. thomsonii*) were analyzed. The electrochromatograms are shown in Fig. 3. The identification of the compounds was done by comparing their retention time with those of standards, as well as adding the individual

standard to the samples. By using the calibration curve of each standard, the content of the eight investigated compounds in the three samples was determined. The quantification results are shown in Table 4, which are conforming to reported results in the literatures [2, 3]. It is indicated that *P. lobata* contains much abundance of isoflavones than *P. thomsonii*, and puerarin is the main isoflavone in RP.

Conclusion

A simple CZE method with $BMImBF_4$ as additive was successfully developed and applied to simultaneous determination of eight isoflavones in RP. Organic solvents such as methanol and acetonitrile usually used as additive in CZE, but the total analysis time usually increased when the organic solvent was applied. $BMImBF_4$ as additive in CZE can improve the resolution with little effect on the EOF, so the total analysis time will not obvious influenced. Therefore, CZE with ILs as additive should be a promising approach for the phytochemical analysis.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21275169, 81202886 and 21175159), and project No. CQDXWL-2014-Z007 supported by the Fundamental Research Funds for the Central Universities.

References

[1] National Pharmacopoeia Committee, *Pharmacopoeia of People's Republic of China* [M].Volume I. Beijing: Chemical Industry Press, 2010, 272, 312-313.

[2] G. Du, H.Y .Zhao, Q.W. Zhang, G.H. Li, F.Q. Yang, Y. Wang, Y.C. Li and Y.T. Wang, *J. Chromatogr. A*, 2010, 1217, 705-714.

[3] G. Du, H.Y. Zhao, Y.L. Song, Q.W. Zhang and Y.T. Wang, *J. Sep. Sci.*, 2011, 34, 2576-2585.

[4] J.K. Prasain, K. Jones, M. Kirk, M.R. Kirk, L.D. Wilson, M. S. Johnson, C. Weaver and S. Barnes, *J. Agric. Food Chem*., 2003, 51, 4213-4218.

[5] C.Y .Wang, H.Y. Huang, K.L. Kuo and Y.Z. Hsieh, *J. Chromatogr. A*, 1998, 802, 225-231.

[6] S.Y. Wei, Y. Chen and X.Y. Xu, *Chin. J. Nat. Med*., 2014, 12, 0407-0414.

[7] H.W. Ka, Q.L. George, M.L. Kong, R.N. Valentina and C. Kelvin, *J. Ethnopharmacol*., 2011, 134, 584-607.

[8] M.K. Choo, E.K. Park, H.K. Yoon and D.H. Kim, *Biol. Pharm. Bull*., 2002, 25, 1328-1332.

[9] N.R. McGregor, *Alcohol*, 2007, 41, 469-478.

[10] M. Miyazawa, K. Sakano, S. Nakamura and H. Kosaka, *J. Agric. Food Chem.*, 2001, 49, 336-341.

[11] M.C. Guerraa, E. Speroni, M. Broccoli, M. Cangini, P. Pasini, A. Minghetti, N. Crespi-Perellino, M. Mirasoli, G. Cantelli-Forti and M. Paolini, *Life Sci*., 2000, 67, 2997-3006.

[12] S. Kayano, Y. Matsumura, Y. Kitagawa, M. Kobayashi, A. Nagayama, N. Kawabata, H. Kikuzaki and Y. Kitada, *Food Chem*., 2012, 134, 282-287.

[13] L.Z. Liu, F. Feng, S.M. Shuang, Y.F. Bai, and Martin M.F. Choi, *Talanta*, 2012, 91, 83-87.

[14] Y.Y. Peng, J.J. Yuan and J.N. Ye, *Am. Lab*., 2007, 39, 13-17.

[15] G. Chen, J.X. Zhang and J.N. Ye, *J. Chromatogr. A*, 2001, 923, 255-262.

[16] F.Q. Yang, J. Zhao and S.P. Li, *Electrophoresis*, 2010, 31, 260-277.

[17] X.J. Chen, J. Zhao, Y.T. Wang, L.Q. Huang and S.P. Li, *Electrophoresis*, 2012, 33, 168-179.

[18] J. Dupont, R. F. de Souza and P. A. Suarez, *Chem. Rev.*, 2002, 102, 3667-3692.

[19] P. Wasserscheid and T. Welton, *Ionic Liquids in Synthesis*, Wiley-VCH, Weinheim, 2nd edn, 2002.

[20] S. Tang, S.J. Liu, Y. Guo, X. Liu and S.X. Jiang, *J. Chromatogr. A,* 2014, 1357, 147-157.

[21] L.P. Mercedes, M.S. Bartolomé, L. Bernhard and V. Miguel, *Electrophoresis*, 2008, 29, 94-107.

[22] M.E. Yue, J. Xu, Q.Q. Li, and W.G. Hou, *J. Food Drug Anal*., 2011, 19, 146-150.

[23] M.E. Yue and Y.P. Shi, *J. Sep. Sci.*, 2006, 29, 272-276.

[24] S.D. Qi, Y.Q. Li, Y.R. Deng, Y.Q. Cheng, X.G. Chen and Z.D. Hu, *J. Chromatogr. A*, 2006, 1109, 300-306.

[25] M. Vaher, M. Koel and M. Kaljurand, *J. Chromatogr. A*, 2002, 979, 27-32.

[26] Y.Q. Lu, D.Q. Wang, C.Y. Kong, H. Zhong and M.C. Breadmore, *Electrophoresis*, doi: 10.1002/elps.201400242 (in press).
[27] S.M. Mwongela, A. Numan, N.L. Gill, R.A. Agbaria and I.M. Warner, *Anal. Chem.*, 2003, 75, 6089-6096.
[28] K. Tian, S.D. Qi, Y.Q. Cheng, X.G. Chen and Z.D. Hu, *J. Chromatogr. A*, 2005, 1078, 181-187.
[29] Y. Wang, F. Li, F.Q. Yang, H.L Zuo and Z.N. Xia, *Talanta*, 2012, 101, 510-515.
[30] F. Li, F.Q. Yang and Z.N. Xia, *Chromatographia*, 2013, 76, 1003-1011.
[31] F. Li, R. Liu, F.Q. Yang, W. Xiao, C. Chen and Z.N. Xia, *Anal. Methods,* 2014, 6, 2566-2571.

Figure legend

Fig. 1 The structures of eight isoflavones.

Fig. 2 Effects of sodium tetraborate concentration (A), buffer pH (B) and $BMImBF_4$ concentration on the separation of 8 investigated compounds by CZE.

Capillary, 48.0 cm (40.5 cm effective length)×50 μm i.d.; Applied voltage, 20 kV; injection, 35 mbar×3 s; cassette temperature, 25 °C; detection, 260 nm; 1, ononin (◆); 2, daidzin (■); 3, genistin (▲); 4, biochanin A (◇); 5, formononetin (□); 6, puerarin (●); 7, genistein (○); 8, daizein (△).

Fig. 3 The electrochromatograms of mixture of standards (A), *P. thomsonii* from Guangxi (B) and *P. lobata* from Guangxi (C).

1, ononin; 2, daidzin; 3, genistin; 4, biochanin A; 5, formononetin; 6, puerarin; 7, genistein; 8, daizein.

Running buffer: 30 mM sodium tetraborate buffer (pH 9.5) added with 50 mM $BMImBF_4$, voltage 18 kV. Other conditions are same as in Fig. 2.

Table 1 Linear regression data, LOD and LOQ of the investigated compounds

Analytes	Linear regression data			LOD	LOQ
	Regression equation	Tested range (μg/mL)	R^2	(μg/mL)	(μg/mL)
1 ononin	y = 0.1210x + 1.1890	9.69-310.20	0.9960	3.52	9.39
2 daidzin	y = 0.2326x + 0.3291	10.94-350.00	0.9987	4.92	9.84
3 genistin	y = 0.2910x - 0.1160	10.16-325.10	0.9990	3.25	7.05
4 biochanin A	y = 0.3650x - 0.0334	10.94-350.00	0.9996	4.87	6.02
5 formononetin	y = 0.4884x + 0.2120	10.56-325.00	0.9995	3.17	5.08
6 puerarin	y = 0.4290x + 1.2210	14.85-475.30	0.9963	2.85	5.71
7 genistein	y = 0.5214x + 0.1244	8.55-275.00	0.9996	2.15	4.30
8 daidzein	y = 0.7820x + 0.0610	8.59-275.10	0.9990	1.72	3.64

Table 2 Intra- and inter-day precision (*n*=6) of the 8 investigated compounds.

Analytes	Concentration (μg/mL)	Intra-day RSD (%)	Inter-day RSD (%)
1 ononin	310.20	4.3	5.1
	77.55	4.2	2.2
	19.39	4.0	2.1
2 daidzin	350.00	2.1	3.8
	87.50	2.1	5.7
	21.87	2.6	3.0
3 genistin	325.10	2.2	3.4
	81.27	2.0	3.7
	20.32	2.6	2.5
4 biochanin A	350.00	2.0	4.7
	87.50	1.1	6.6
	21.87	2.2	3.6
5 formononetin	325.00	1.4	3.9
	81.25	1.7	2.7
	20.31	2.7	4.7
6 puerarin	475.30	1.5	3.3
	118.83	1.9	2.0
	29.71	3.6	4.0
7 genistein	275.00	1.6	2.6
	68.75	1.6	4.7
	17.18	2.6	4.7
8 daidzein	275.10	1.2	4.1
	68.78	1.6	2.1
	17.19	4.7	4.7

Table 3 Recoveries for the assay of 8 compounds in *P. lobata* (Guangxi)

Analytes	Original (μg)	Spiked (μg)	Found [a] (μg)	Recovery [b] (%)	RSD (%)
1 ononin	0.56	3.52	4.03	98.5	5.6
2 daidzin	2.10	14.57	15.66	93.1	4.3
3 genistin	12.51	3.02	15.28	91.5	4.1
4 biochanin A	3.48	3.81	7.43	103.6	5.0
5 formononetin	-[c]	3.47	3.73	107.5	5.9
6 puerarin	110.66	5.78	116.42	99.7	4.3
7 genistein	1.17	1.76	2.76	90.8	4.0
8 daidzein	2.11	3.05	5.25	103.1	4.2

[a] The data was present as average of three determinations;

[b] Recovery (%) = 100×(amount found-original amount)/amount spiked;

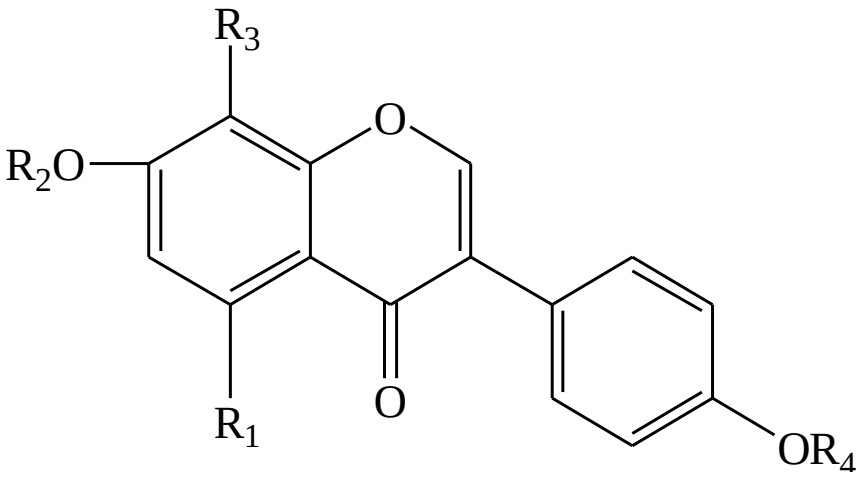
[C] Under the limit of quantitation.

Table 4 Contents (mg/g) of investigated compounds in RPs.

Analytes	*P. thomsonii* (Guangxi)	*P. lobata* (Guangxi)	*P. lobata* (Hubei)
1 ononin	- [a]	+ [b]	+
2 daidzin	0.20	3.05	4.19
3 genistin	-	0.19	0.39
4 biochanin A	-	1.51	1.83
5 formononetin	-	+	+
6 puerarin	1.08	39.48 [c]	49.67 [c]
7 genistein	+	0.16	+
8 daidzein	0.19	0.56	0.42

[a] Under the limit of detection; [b] Under the limit of quantification;

[c] The extracts were diluted for 8 times for the quantification of puerarin in *P. lobata*.



Compounds	R_1	R_2	R_3	R_4	Formula	Molecular Weight	pKa	logP
1 ononin	-H	-glc	-H	$-CH_3$	$C_{22}H_{22}O_9$	430.40	12.71±0.70	0.597±0.905
2 daidzin	-H	-glc	-H	-H	$C_{21}H_{20}O_9$	416.38	9.65±0.30	0.369±0.908
3 genistin	-OH	-glc	-H	-H	$C_{21}H_{20}O_{10}$	432.38	6.12±0.20	0.942±0.212
4 biochanin A	-OH	-H	-H	$-CH_3$	$C_{16}H_{12}O_5$	284.26	6.50±0.20	3.341±1.134
5 formononetin	-H	-H	-H	$-CH_3$	$C_{16}H_{12}O_4$	268.26	6.99±0.20	2.860±1.131
6 querarin	-H	-H	-glc	-H	$C_{21}H_{20}O_9$	416.38	6.46±0.20	0.408±1.243
7 genistein	-OH	-H	-H	-H	$C_{15}H_{10}O_5$	270.24	6.51±0.20	3.114±1.137
8 daidzein	-H	-H	-H	-H	$C_{15}H_{10}O_4$	254.24	7.01±0.20	2.632±1.134

Fig. 1

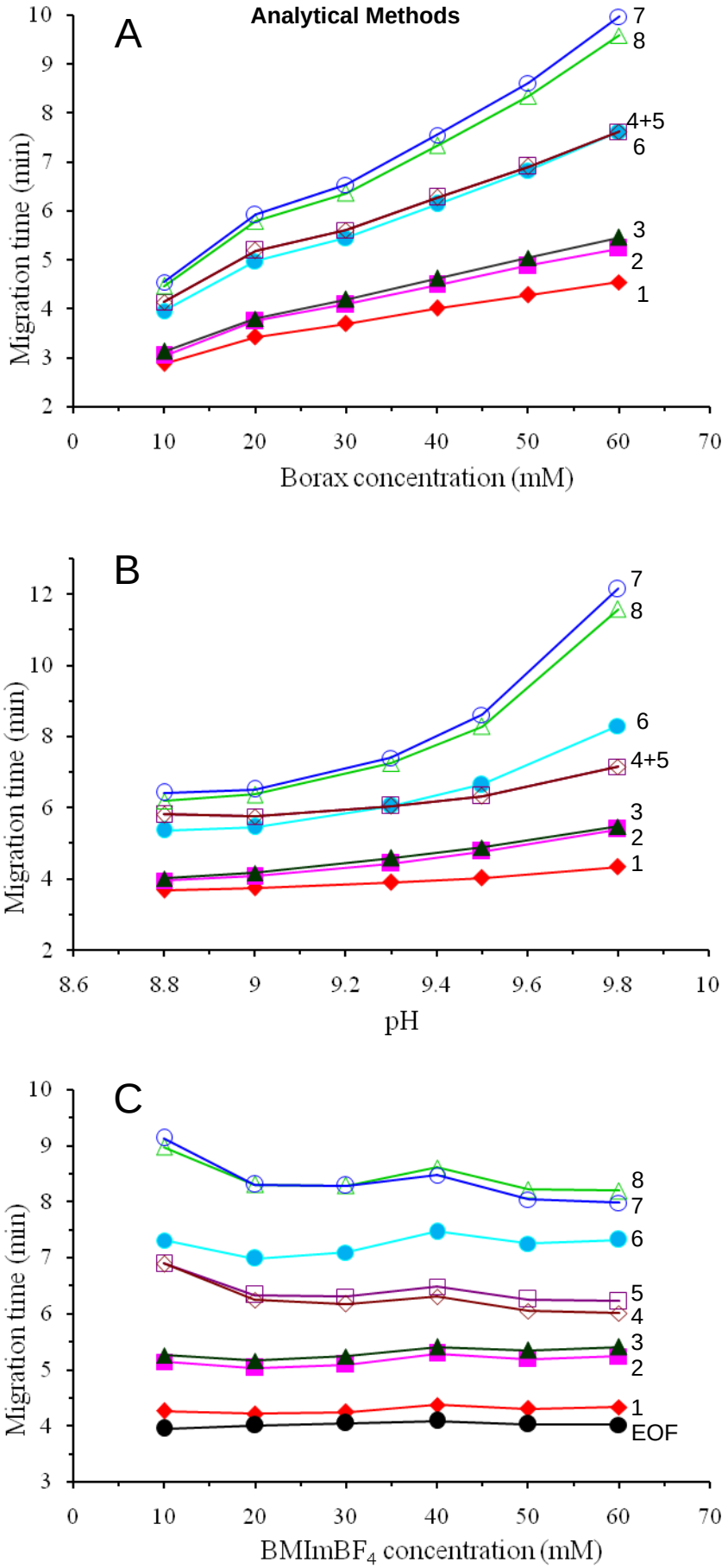


Fig. 2

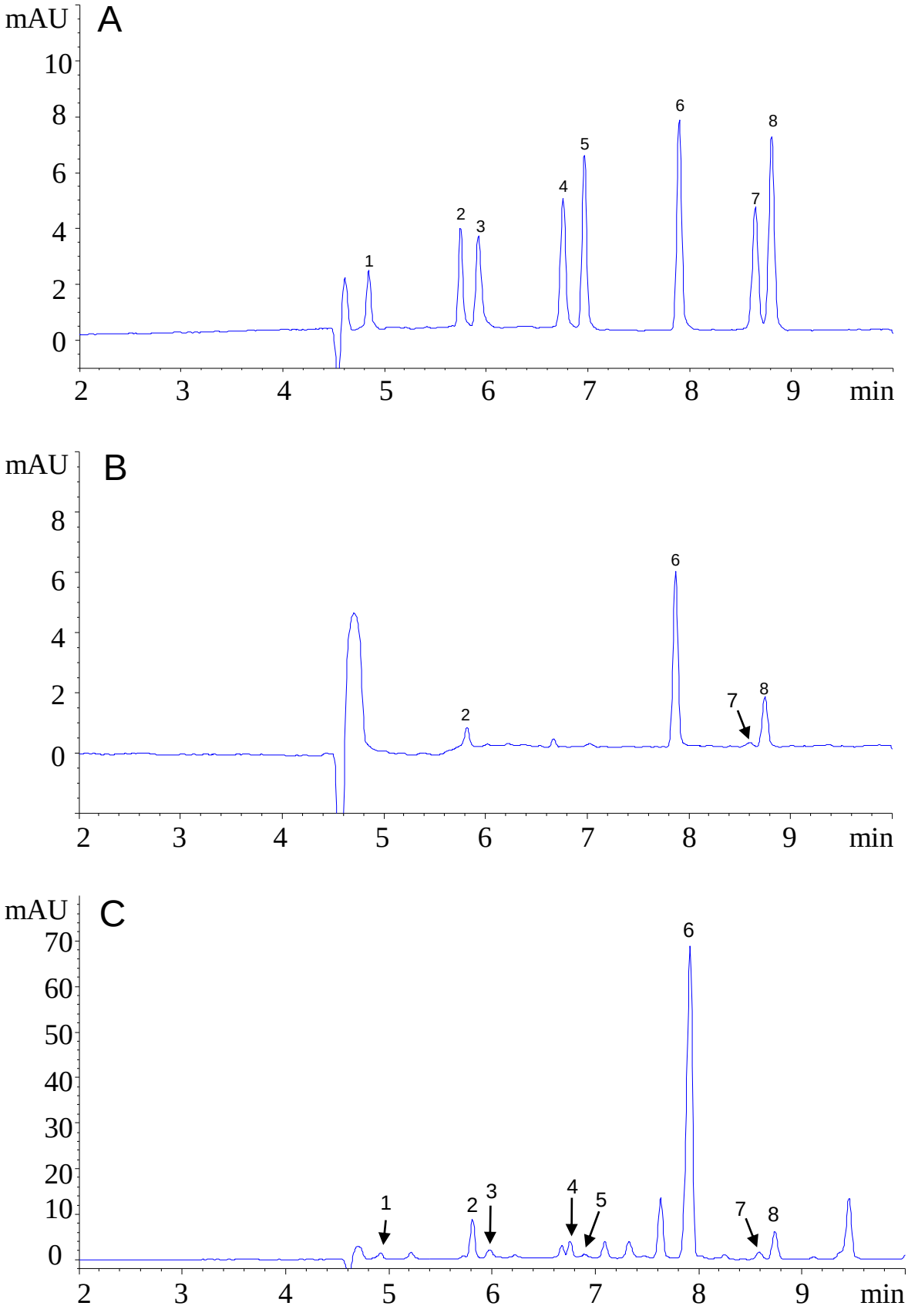
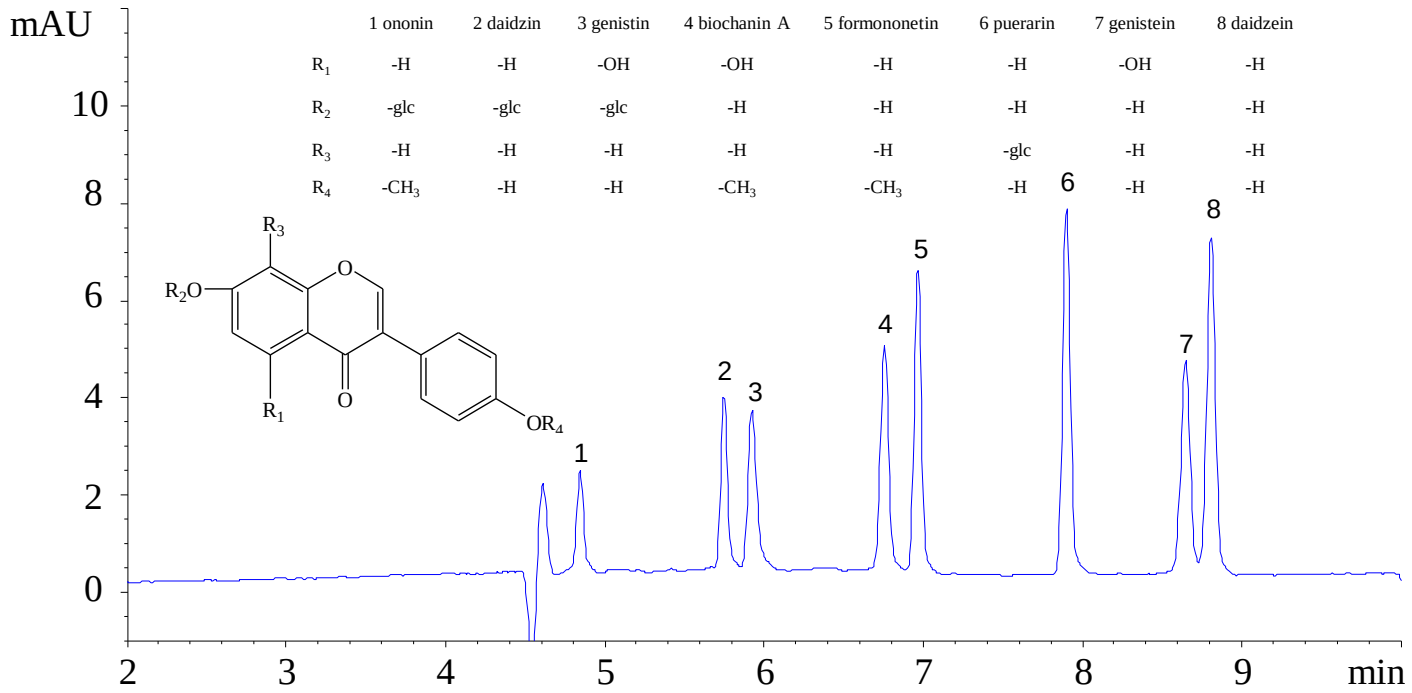


Fig. 3



Highlights:

1) A simple CZE method with ionic liquid as additive was developed for the simultaneous determination of eight isoflavones.

2) The developed method was applied to determine eight isoflavones in Radix Puerariae.

3) Ionic liquid shows potential applications as additive in CZE analysis of natural products.