Eur Food Res Technol (2010) 230:715–722
DOI 10.1007/s00217-009-1212-z

ORIGINAL PAPER

A sensitive analytical method for the component monosaccharides of the polysaccharides from a Tibetan herb *Potentilla anserine* L. by capillary zone electrophoresis with UV detector

Lian Xia · Zhi Wei Sun · Guo Liang Li · You Rui Suo · Jin Mao You

Received: 26 September 2009 / Revised: 9 December 2009 / Accepted: 23 December 2009 / Published online: 7 January 2010

Abstract A rapid and sensitive method was optimized and validated for the separation and quantification of derivatized monosaccharides in polysaccharide from *Potentilla anserine* L. using 1-naphthyl-3-methyl-5-pyrazolone (NMP) as precolumn derivatization reagent by capillary zone electrophoresis (CZE). On the basis of the optimum conditions, nine NMP-derivatized monosaccharides achieve baseline resolution within 16 min. The developed method has been successfully applied to analyze component monosaccharides of three *Potentilla anserine* L. samples, which were obtained by gradational precipitation with 50, 70, and 90% aqueous ethanol, respectively. The polysaccharide precipitated from 50% ethanol solvent was composed of fucose, mannose, xylose, glucuronic acid, glucose, rhamnose, galacturonic acid, galactose, arabinose in molar proportion of 1:1.65:1.99:5.08:7.38:8.14:13.05:27.41:39.02; the corresponding molar proportions for polysaccharide obtained from 70% ethanol solvent were 1:1.64:1.65:4.52:13.96:9.13:26.30:10.52:18.00; fucose and galacturonic acid were not found in the polysaccharide precipitated from 90% ethanol solvent, and mannose, xylose, glucuronic acid, glucose, rhamnose, galactose and arabinose were determined with molar proportion of 1:0.87:1.77:2.78:1.69:2.58:2.49. Quantitative recoveries of the component monosaccharides in the polysaccharide were in the range of 93.3–105.1% and the relative standard deviation (RSD) values fell within 3.4–6.3%, respectively. The results demonstrated that the proposed CZE method was precise and sensitive for the analysis of the composition of polysaccharide.

Keywords *Potentilla anserine* L. · Polysaccharide · Monosaccharide · Analytical method

L. Xia · J. M. You (✉)
Key Laboratory of Life-Organic Analysis of Shandong Province, College of Chemistry Science, Qufu Normal University, 273165 Qufu, China
e-mail: jmyou6304@163.com

L. Xia
e-mail: xialian01@163.com

L. Xia · Z. W. Sun · G. L. Li · Y. R. Suo · J. M. You
Northwest Plateau Institute of Biology, Chinese Academy of Sciences, 810001 Xining, China

L. Xia · Z. W. Sun · G. L. Li
Graduate University of Chinese Academy of Sciences, 100049 Beijing, China

Introduction

Polysaccharides from natural sources are a class of macromolecules that can profoundly affect the immune system and therefore have the potential as immunomodulators with wide clinical applications [1]. Polysaccharides from various traditional medicinal herbs have been shown to be immunopotentiating both in vivo as well as in vitro [2–8]. However, polysaccharides isolated from the different natural sources showed different anti-tumor activities, depending on their monosaccharide composition, molecular mass, and chain conformation [9]. For these reasons, developing new methods is crucial to analyze the polysaccharides.

The roots of the Tibetan herb *Potentilla anserine* L. are amongst the most popular health-promoting herbs in China. For thousands of years, it has been used frequently as a crude substance, taken orally as folk medicine and

sanitarian product [10]. The *Potentilla anserine* L. have been used as astringent, anti-inflammatory, antispasmodic, haemostatic and also have been used for diarrhoea, leucorrhoea, dysmenorrhoea, arthritis, cramps, kidney stones, bleeding piles and as a mouth wash in pyorrhea, gingivitis, and sore throat [11]. Also literature reports that *Potentilla anserine* L. can be used to treat chronic liver diseases [12, 13], and several controlled trials have been done to assess the efficacy and safety of *Potentilla anserine* L. for chronic hepatitis B infection [14].

The characteristic of polysaccharide is complicated structure and high microheterogeneity, so it is difficult to be separated. Capillary electrophoresis is a kind of new separation and analytical technology developed in 1980s, because of the high chromatographic efficiencies, short analysis time and less sample consumption. Suzuki et al. [15] presented a new derivatization method in which 1-phenyl-3-methyl-5-pyrazolone (PMP) was used as the labeling reagent. The reaction of PMP with saccharides was carried out by a condensation reaction in which active carbon (C-4) in carbohydrate molecular chain reacted with the aldehyde function group of reducing sugar under a mild condition.

In our previous study, based on the photochromic characteristics of the naphthalene, a novel photochromic molecule 1-(2-naphthyl)-3-methyl-pyrazolone (NMP) has been synthesized and used to label monosaccharides [16–18]. In this study, an optimized CZE method for quantifying monosaccharides in *Potentilla anserine* L. with NMP as labeling reagent has been established. The comparison of UV responses between NMP and the commonly used PMP were also evaluated, the result indicated that NMP was superior to PMP with excellent spectral properties. The validation of the proposed method for the detection of content of polysaccharide from the hydrolyzed *Potentilla anserine* L. sample was evaluated. It was demonstrated that the established method was sensitive and reliable for the determination of the composition of carbohydrate compounds from natural source. To our best knowledge, this is the first time that the composition of polysaccharide from *Potentilla anserine* L. was comprehensively studied.

Materials and methods

Plant material

The Tibetan herb *Potentilla anserine* L. sample was purchased from Xining herb market (QiHai province, China), and was identified by professor ChangFan-Zhou at the Northwest Plateau Institute of Biology, the Chinese Academy of Sciences.

Chemicals and instruments

NMP was synthesized by author's laboratory. Sugar standards were purchased from Sigma Co (St. Louis, MO, USA). Borate was purchased from Xuzhou Chemical Regent Factory (JiangSu province, China). Water was Milli-Q ultrapure water, and the other reagents were analytical grade. The HP-3D capillary electrophoresis instrument (Agilent Company, USA) and capillary column (58.5 cm × 50 μm i.d., 50-cm effective length), (Hebei Yongnian Ruifeng Chromatography Device Co. Ltd, China) were used in this study.

Extraction of polysaccharide from *Potentilla anserine* L.

An amount of 100.0 g triturated and dry *Potentilla anserine* L. root were mixed with 100 ml ethanol/ether (50:50, v/v), and then the mixture was refluxed at 65 °C for 3 h to get rid of the lipids. After filtrated under vacuum, the sediment was redissolved in 100 ml of 85% ethanol and extracted supersonically for 30 min to remove the monosaccharides. After filtrated under vacuum, the sediment was ultrasonic extracted with deionized water for 90 min, then filtrated under vacuum, and the supernatant was collected. After concentrated under diminished pressure, the residue was redissolved by 3 times volume of 95% ethanol and kept at 4 °C overnight. Finally, the polysaccharide pellets were obtained by centrifugation at 1,788×*g* RCF for 15 min. The polysaccharide pellets were repeatedly washed with possibly less amounts of ethanol, acetone, and ether, respectively. The refined polysaccharide pellets were completely dissolved in appropriate volume of distilled water and extracted three times with $CHCl_3$-*n*-butanol (4:1, v/v). The extracts were collected and added ethanol to the ethanol concentration of 50%, then kept at 4 °C overnight, the precipitate was centrifuged and dried at 70 °C to obtain crude *Potentilla anserine* L. polysaccharides (P50) 170.67 mg. The corresponding supernatant solution was collected and added to the ethanol concentration of 70%, after standing for 24 h at 4 °C, the precipitate was centrifuged and dried at 70 °C to obtain crude *Potentilla anserine* L. polysaccharides (P70) 18.08 mg. Ethanol was continuously added to the supernatant solution to the ethanol concentration of 90%, the contents were kept at 4 °C overnight, the precipitate was centrifuged and dried at 70 °C to obtain crude *Potentilla anserine* L. polysaccharides (P90) 9.33 mg.

Hydrolysis of the polysaccharide

An amount of 5 mg each dry *Potentilla anserine* L. polysaccharide was mixed with 1 ml 3 M trifluoroacetic acid in

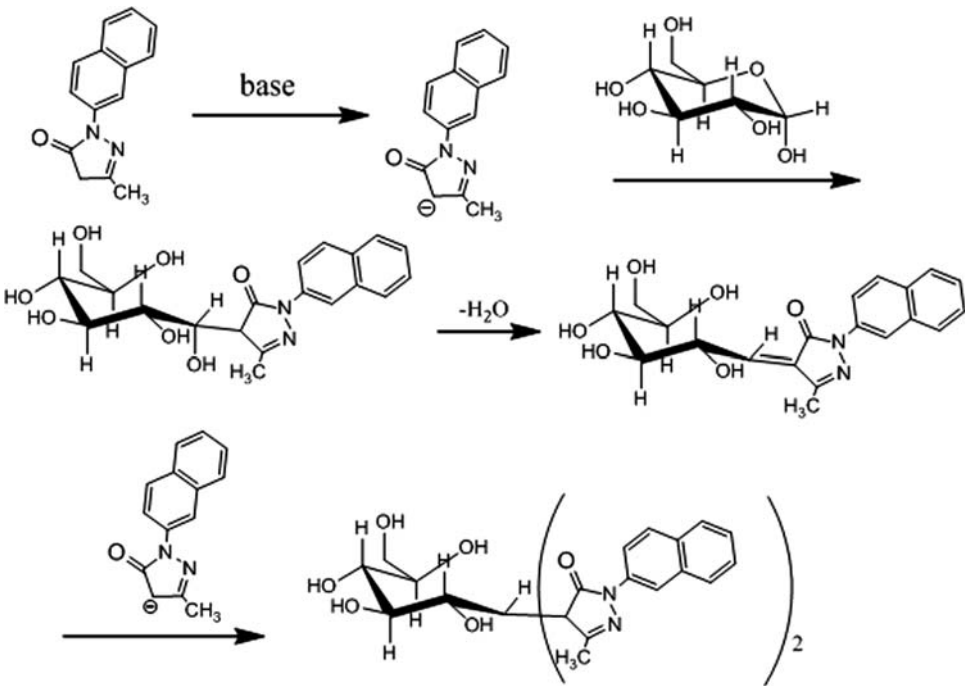

Fig. 1 Derivatization scheme of 1-(2-naphthyl)-3-methyl-5-pyrazolone (NMP) with reductive saccharides

a ampoule. The ampoule was sealed under a nitrogen atmosphere. After hydrolysis at 110 °C for 8 h, the mixture was cooled to atmosphere temperature and dried with nitrogen gas and redissolved in 1 ml deionized water, which waited for derivatization.

Preparation of the standard solution

Stock standard sugar solutions (0.01 M for each) were prepared by dissolving each standard monosaccharide in water. Working standard solutions were further obtained by appropriate dilution of the stock standard solutions with deionized water. The sample solutions were filtered through a 0.2 μm syringe filter and were degassed using an ultrasonic bath for 2 min prior to use. NMP (0.05 M) was prepared by dissolving 112 mg NMP in 10 ml acetonitrile (CAN). All the solutions prepared were stored in the dark at 4 °C until being used.

Derivatization procedure

The derivatization reaction was carried out as follows: 40 μl hydrolyzed solution of polysaccharide from *Potentilla anserine* L. was added in a ampoule, to which 150 μl 0.05 M NMP/ACN solution and 30 μl 17% ammonia solution were then added. After sealing, the solution was allowed to stand for 35 min at 70 °C in a water bath. After derivatization, the mixture was dried under nitrogen stream. The residue was redissolved in 500 μl solution of ACN/water (4:1, v/v). The derivatized sample solution was directly injected into the CZE system for analysis. The derivatization process was shown in Fig. 1 (mannose is the representative).

CZE-UV conditions

The NMP-labeled monosaccharides were analyzed by CZE equipped with a UV detector (Agilent, USA) and capillary column. The CZE conditions was as follow: borate buffer (55 mM) was adjusted to pH 9.46 with phosphoric acid (1.0 M) and treated ultrasonically for 15 min to remove gas bubbles prior to use. Other parameters were as follows: voltage of 22 kV, column temperature of 20 °C, and DAD detection of 254 nm. The samples were atmospherically introduced with an injection time of 10 s. The capillary column was flushed, consecutively by 0.1 M aqueous solution of NaOH and buffer solution for 5 min prior to injection. When buffer solution was replaced, the capillary column was flushed for 10 min.

Results and discussion

Comparison of UV responses between NMP and PMP

The comparison of UV responses between NMP and the commonly used PMP were evaluated. To make a quantitative comparison with respect to relatively UV absorbance, the standard solutions of NMP and PMP (1.0×10^{-5} mol/l) were used. The results indicated that UV absorbance for NMP exhibits obviously enhancement. The molar absorption coefficients (ε) of NMP in ACN and

MeOH are 5.58×10^4 l mol^{-1} cm^{-1} and 3.30×10^4 l mol^{-1} cm^{-1}, respectively. The ratios for the UV responses were as follows: $UV_{NMP}/UV_{PMP} = 2.55$ (ACN) and $UV_{NMP}/UV_{PMP} = 2.58$ (methanol) (data obtained using 300 Bio ultraviolet spectrophotometer were not corrected, spectrum was not shown). This was probably due to the fact that NMP molecule has the large molar absorbance, which made it more sensitive relative to that of PMP.

Optimization of derivatization procedure

The derivatization yields for the reaction between NMP and carbohydrates were notably different in various solvent systems. Thus, five different solvent solutions, including CAN, methanol (MeOH), tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), and dimethylsulfoxide (DMSO) were investigated in this study. The results indicated that the derivatization yields were basically identical in ACN, DMF and DMSO, which were much higher than those in THF and MeOH. Considering the subsequent processing, ACN was chosen as the solvent for derivatization reaction. Lin et al. [19] reported that NaOH was replaced by ammonia as a catalyst to improve the derivatization conditions of PMP with carbohydrates. The same result was obtained when 17% ammonia was used as a catalyst in this study. Moreover, the subsequent processing was also simplified because the reaction solution could be dried directly with a stream of nitrogen. It was found that the yields increased with the rise of temperature when the temperature was below 70 °C; above 70 °C, the yields of derivatization for glucuronic acid, galacturonic acid, arabinose, xylose and mannose decreased significantly. Therefore, 70 °C was adopted as the optimum derivatization temperature. To ensure the derivatization reaction proceeds completely, NMP with the molar amount of 5–6 times to monosaccharides was used, and the chosen reaction time was 35 min.

Optimization of separation conditions

To achieve optimal separation, the choice of buffer solution was tested (carbonate buffers, phosphate buffers and borate buffers). The results indicated that borate buffer gave the optimal resolution for the tested monosaccharides derivatives in the pH range of 8–10.

The separation of NMP-labeled carbohydrates was very sensitive to borate buffer concentrations. In this study, borate concentration in the range of 40–65 mM was evaluated. The results indicated that the migration time of NMP-labeled carbohydrates generally increased with a gradual increasing of buffer concentrations. Taking the shorter run-time and good resolution into consideration, 55 mM borate buffer solution was selected and the maximum resolution was obtained.

The effect of pH on the separation was also investigated in the pH range from 8.0 to 10.0 using borate buffer solutions as background electrolytes. In the pH range of 8.0–9.4, the resolution increased with increasing buffer pH. At further higher pH values in the pH range of 9.5–10.0, a remarkable decreased in resolution with broadening peak for each NMP-labeled carbohydrate was observed. The highest separation efficiency coupled with a good baseline resolution can be obtained when borate buffer solution pH was adjusted to 9.46.

Effect of capillary temperature and working voltage on the resolution of the NMP-labeled carbohydrates was investigated. The results indicated that the resolution was not remarkably improved with increasing temperature from 10 to 30 °C. With high temperature applied in capillary column, the overheat phenomena will be produced; therefore the applied voltage should be reduced to decrease the Joule heat. In this experiment, capillary working temperature was set at 20 °C. Migration time of NMP-labeled carbohydrate decreased remarkably with increasing working voltage. To decrease the Joule heat, 22 kV working voltage was selected.

The addition of organic modifier was important to the improvement of viscosity of medium, dielectric constant and structure of electric double layer of capillary wall [20]. In this study, various additives, such as isopropanol, ACN, and MeOH were examined. The results indicated that no significant influences on resolution were observed when the organic modifier including isopropanol, ACN, and MeOH were used. So the organic modifier was not used in subsequent experiments.

Separation of monosaccharides

Based on the optimal conditions for the separation of NMP-labeled monosaccharide, nine NMP-derivatized monosaccharides achieved baseline resolution within 16 min, and the chromatogram was shown in Fig. 2. The NMP derivatized monosaccharides were eluted in the order of xylose, arabinose, glucose, rhamnose, mannose, fucose, galactose, glucuronic acid, and galacturonic acid. Moreover, the number of theoretical plates for the saccharides were ranging from 1.75×10^5 to 3.53×10^5. Satisfactory selectivity was achieved for neighboring peaks in the range of 1.01–1.06, and the resolution of the nine labeled monosaccharides for the proposed method was no less than 1.27.

Validation

The CZE method was validated in terms of linearity, reproducibility, detection limits, and precision. The linear calibration regression equations were established with the

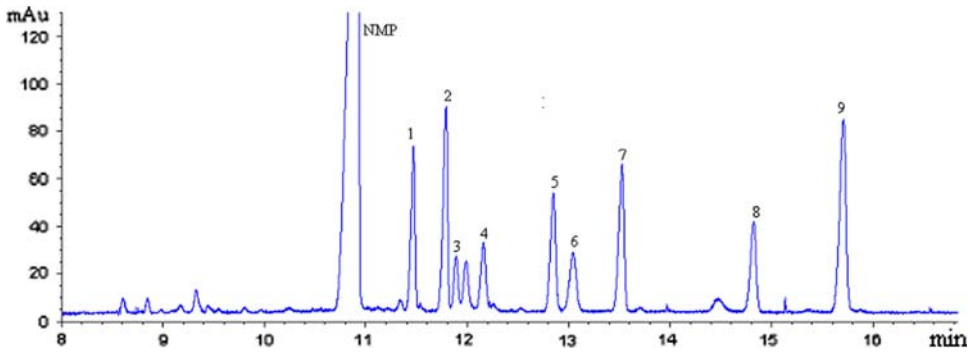

Fig. 2 Chromatograms of nine derivatized carbohydrates by CZE *1* xylose; *2* arabinose; *3* glucose; *4* rhamnose; *5* mannose; *6* fucose; *7* galactose; *8* glucuronic acid; *9* falacturonic acid

peak area versus the concentration of monosaccharide derivatives ranging from 250 to 5 μmol/l. The linear regression equations, migration time, correlation coefficients, and detection limits for all the monosaccharide derivatives were listed in Table 1. As a consequence, the good linearity (correlation coefficient $r > 0.9991$) between Y (peak area) and X (concentration of the standards) was achieved in the tested range. Furthermore, detection limits of each tested analyte was obtained by the injecting of gradational dilutions of a standard mixture derivatized as mentioned above in the derivatization procedure, followed by the comparison of peak height with baseline noise level and a signal-to-noise ratio (S/N) of three assigned the detection limit. The results showed that the detection limits of the monosaccharides was in the range from 0.85 to 2.19 μmol/l (Table 1), indicating that the sensitivity of the method was satisfactory.

Moreover, method precision was also determined by measuring the repeatability (intra-day variability) and intermediate precision (inter-day variability) of the migration times and peak areas for each tested monosaccharide derivatives. The precision of the method was calculated as the relative standard deviation for six successive injections of each tested monosaccharide derivatives (100 μmol/l) under the same optimum conditions and the results were summarized in Table 2. The results showed that the intra-day reproducibility were less than 0.48% for the migration time and 4.8% for the peak areas, and the inter-day were less than 0.72% for the migration time and 6.3% for the peak areas, indicating that the method precision was satisfactory.

The recovery efficiency of the method was determined by the addition of 10 μl standard monosaccharides solution (1.0×10^{-2} mol/l) into the hydrolysate of *Potentilla anserine* L. polysaccharide under the optimum derivatization and capillary electrolysis conditions. The results show that the recoveries of all the nine monosaccharides ranged between 93.3 and 105.1% and the RSD values fell within 3.4–6.3%, respectively. Such results further demonstrated that this method was precise and practical for the analysis of the polysaccharide from *Potentilla anserine* L.

Analysis of the polysaccharide from *Potentilla anserine* L.

This experiment was designed to develop a rapid, sensitive, repeatable, and accurate analysis method for the quantification of the component carbohydrates in the polysaccharide from *Potentilla anserine* L. In order to evaluate the applicability of the proposed method, the isolated polysaccharide was hydrolyzed with trifluoroacetic acid (TFA), dried, and NMP-labeled as described in the experimental section and

Table 1 Migration time, linear regression equations, correlation coefficients(r), and detection limits of the proposed CZE method ($n = 6$)

Component	Migration time/min	Regression equation	Correlation coefficient	Detection limits/μmol/l ($S/N = 3$)
Xylose	11.456	$Y = 1.906X - 0.257$	0.9996	0.89
Arabinose	11.785	$Y = 2.046X + 0.425$	0.9997	0.85
Glucose	11.885	$Y = 1.419X + 0.788$	0.9991	1.26
Rhamnose	11.985	$Y = 1.540X - 1.634$	0.9980	1.49
Mannose	12.845	$Y = 1.732X - 1.765$	0.9989	1.34
Fucose	13.037	$Y = 1.872X - 0.305$	0.9995	1.60
Galactose	13.520	$Y = 2.231X - 0.301$	0.9998	1.02
Glucuronic acid	14.818	$Y = 1.273X - 1.890$	0.9998	2.19
Galacturonic acid	15.704	$Y = 2.157X - 3.327$	0.9996	1.44

Y peak Area, X concentration of monosaccharides, μmol/l

Table 2 Precision of the migration time and peak area of analytes in the present method

Component	Intra-day precision (RSD $n = 6$)		Inter-day precision (RSD $n = 6$)	
	Migration time (%)	Peak area (%)	Migration time (%)	Peak area (%)
Xylose	0.48	4.8	0.72	5.7
Arabinose	0.48	4.6	0.69	6.3
Glucose	0.46	3.4	0.61	3.6
Rhamnose	0.44	3.5	0.63	4.4
Mannose	0.44	3.8	0.43	5.1
Fucose	0.46	4.3	0.70	6.3
Galactose	0.47	3.2	0.54	4.8
Glucuronic acid	0.48	4.2	0.67	5.4
Galacturonic acid	0.45	3.3	0.49	5.2

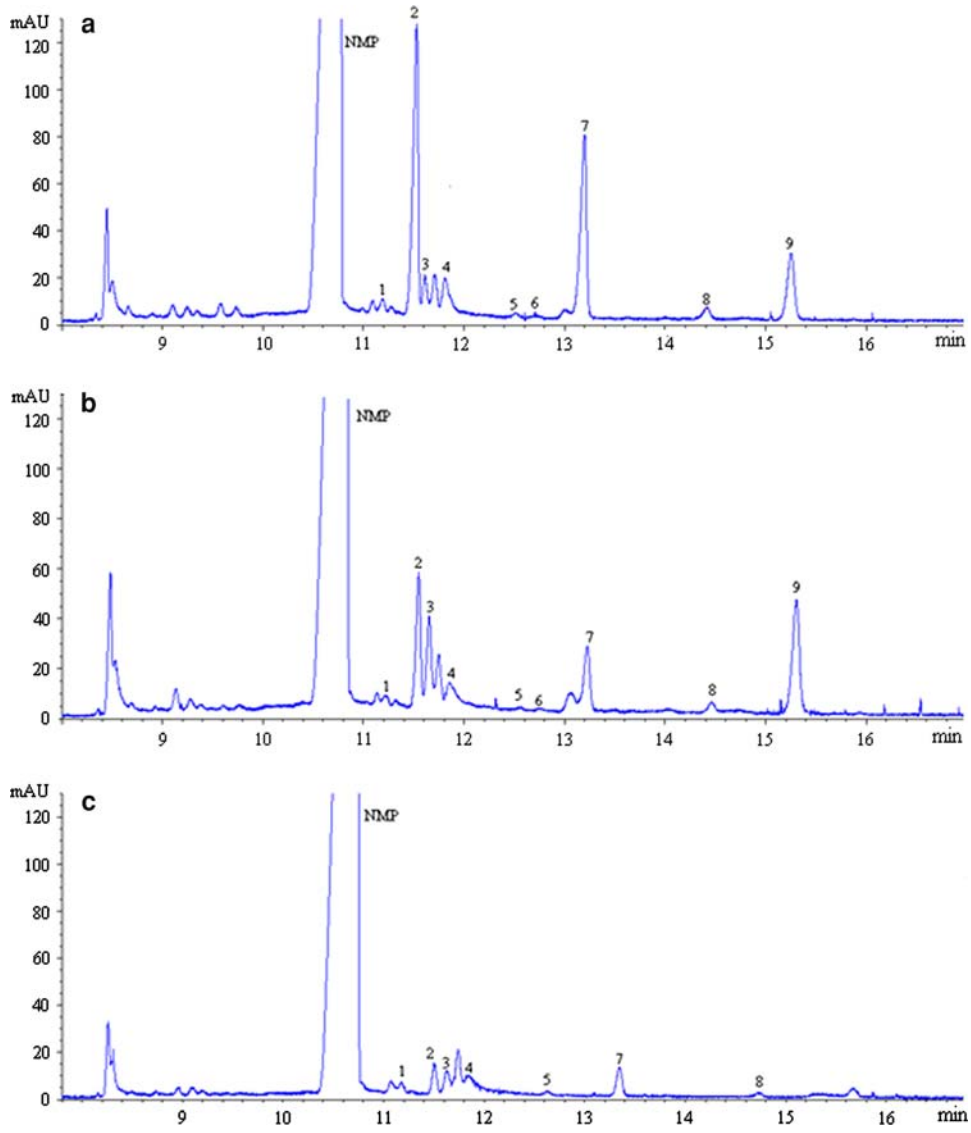

Fig. 3 Chromatograms of saccharides from *Potentilla anserine* L. sample by CZE **a** P50, **b** P70, **c** P90)

finally, the released monosaccharide derivatives were analyzed by the described CZE method under the optimized conditions. Figure 3 shows a typical chromatogram of the polysaccharide sample and the detected contents were listed in Table 3. As it can be seen, the NMP derivatives of the component monosaccharides released from the

Table 3 Determination of the component monosaccharides in the *Potentilla anserine* L. polysaccharide ($n = 3$)

Component	P50 (μmol/mg)		P70 (μmol/mg)		P90 (μmol/mg)	
	Content	RSD%	Content	RSD%	Content	RSD%
Xylose	0.079	5.3	0.052	5.5	0.050	5.9
Arabinose	1.549	5.0	0.570	6.3	0.142	6.1
Glucose	0.293	4.9	0.441	4.1	0.159	3.7
Rhamnose	0.323	4.9	0.289	5.8	0.097	6.2
Mannose	0.066	5.2	0.052	4.7	0.057	4.9
Fucose	0.040	5.6	0.032	6.0	–	–
Galactose	1.088	3.7	0.333	4.5	0.147	5.4
Glucuronic acid	0.202	4.2	0.143	5.4	0.101	6.3
Galacturonic acid	0.518	3.4	0.832	5.3	–	–

–: Not detected

polysaccharide sample could be still baseline separated and the component monosaccharides could be identified by comparing with the chromatogram of the mixture of standard monosaccharides (Fig. 2). The results showed that the polysaccharide precipitated from 50% ethanol solvent was a typical heteropolysaccharide and was composed of xylose, arabinose, glucose, rhamnose, mannose, fucose, galactose, glucuronic acid, and galacturonic acid and in the molar contents of 0.079, 1.549, 0.293, 0.323, 0.066, 0.040, 1.088, 0.202 and 0.518 μmol/mg, respectively, and their corresponding mole percentages were 1.9, 37.2, 7.0,7.6, 1.6, 1.0, 26.6, 4.8, and 12.3% (mol%); the corresponding molar contents of polysaccharide obtained from 70% ethanol solvent were 0.052, 0.570, 0.441, 0.289, 0.052, 0.032, 0.333, 0.143, 0.832 μmol/mg, and their corresponding molar percentages were 1.9, 20.8, 16.1, 10.5, 1.9, 1.2, 12.1, 5.2, 30.3%; fucose and galacturonic acid were not found in the polysaccharide precipitated from 90% ethanol solvent, and mannose, xylose, glucuronic acid, glucose, rhamnose, galactose, and arabinose were determined with molar contents of 0.050, 0.142, 0.159, 0.097, 0.057, 0.147, 0.101 μmol/mg, and their corresponding mole percentages were 6.6, 18.9, 21.1, 12.9, 7.6, 19.5, 13.4, respectively (Table 3). It was clear that the predominantly composition monosaccharides in the polysaccharide from *Potentilla anserine* L. were arabinose, glucose, rhamnose, galactose, and galacturonic acid more than 90% (mol%) of total carbohydrates, and more than 17% of total carbohydrates were uronic acids.

Conclusions

A fast and sensitive method with the derivatization reagent of NMP has been optimized and validated for the separation and quantification of monosaccharides from *Potentilla anserine* L. using CZE. The proposed CZE method provided a rapid, repeatable, accurate, and economic alternative for the separation of the natural monosaccharides. The proposed method was particularly suitable for determining the component monosaccharides of the polysaccharide from *Potentilla anserine* L. and could also be applied to routine analysis of monosaccharides in real-life samples, such as other plant polysaccharides, fruit juices, wines, etc. Such information would facilitate the use of the *Potentilla anserine* L. in food, pharmaceutical and other technical applications, which would contribute to the sustainable use of *Potentilla anserine* L. agricultural resource.

Acknowledgments This work was supported by the National Science Foundation under Grants # 20075016 and 30370218.

References

1. Tzianabos AO (2000) Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. Clin Microbiol Rev 13:523–533
2. Yu F, Sheng JC, Xu J, An XX, Hu QH (2007) Antioxidant activities of crude tea polyphenols, polysaccharides and proteins of selenium-enriched tea and regular green tea. Eur Food Res Technol 225:843–848
3. Gao Y, Zhou S, Jiang WQ, Huang M, Dai X (2003) Effects of ganopoly (A *Ganoderma lucidum* polysaccharide extract) on the immune functions in advanced-stage cancer patients. Immunol Invest 3:201–215
4. Chen HX, Zhang M, Qu ZS, Xie BJ (2008) Antioxidant activities of different fractions of polysaccharide conjugates from green tea (*Camellia sinensis*). Food Chem 106:559–563
5. Han SB, Yoon YD, Ahn HJ, Lee HS, Lee CW, Yoon WK, Park SK, Kim HM (2003) Toll-like receptor-mediated activation of B cells and macrophages by polysaccharide isolated from cell culture of *Acanthopanax senticosus*. Int Immunopharmacol 3:1301–1312
6. Ando I, Tsukumo Y, Wakabayashi T, Akashi S, Miyake K, Kataoka T, Nagai K (2002) Safflower polysaccharides activate the transcription factor NF-κB via Toll-like receptor 4 and induce cytokine production by macrophages. Int Immunopharmacol 2:1155–1162
7. Shimizu N, Tomoda M, Kanari M, Gonda R (1991) An acidic polysaccharide having activity on the recticuloendothelial system from the root of *Astragalus mongholicus*. Chem Pharm Bull 39:2969–2972
8. Cui R, He JC, Wang B, Zhang F, Chen GY, Yin S, Shen H (2003) Suppressive effect of *Astragalus membranaceus Bunge* on chemical hepatocarcinogenesis in rats. Cancer Chemother Pharmacol 51:75–80
9. Jin Y, Zhang LN, Zhang M, Chen L, Cheung PCK, Qi VEC, Lin Y (2003) Antitumor activities of heteropolysaccharides of *Poria cocos* mycelia from different strains and culture media. Carbohydr Res 338:1517–1521
10. The Academy of JiangXi New Medicine (1997) Traditional Chinese drugs dictionary. Shanghai, China
11. Wu ZY (1990) Xinhua herbal scheme. Shanghai, China
12. Li XY, Yuan HL, Liu YB, Cai GM, Zhang L, Che H (2004) Analysis of benzene series residues in *Potentilla ansterina* L.

extraction by headspace-GC. Lishizhen Med Maieria Med Res 15:484–485
13. Zhao YL, Cai GM, Hong X, Shan LM, Xiao XH (2008) Anti-hepatitis B virus activities of triterpenoid saponin compound from *Potentilla anserine* L. Phytomedicine 15:253–258
14. Zhang XQ, Zhao YL, Shan LM, Wei ZM, Cai GM (2004) Study on protective mechanism of JMS on chemical liver injury. Pharm J Chin People's Lib Army 20(4):259–261
15. Suzuki S, Tanaka R, Takada K, Inoue N, Yashima Y, Honda A, Honda S (2001) Analysis of sialo-*N*-glycans in glycoproteins as 1-phenyl-3-methyl-5-pyrazolone derivatives by capillary electrophoresis. J chromatogr A 910:319–329
16. Sun ZW, Liu LJ, Hu BJ, Sheng X, Wang XY, Suo YR, You JM (2008) Preparation of 1-(2-naphthyl)-3-methyl-5-pyrazolone as pre-column derivatization reagent for separation and determination of saccharides using HPLC–MS. Chin J Chromatogr 26:200–205
17. You JM, Sheng X, Ding CX, Sun ZW, Suo YR, Wang HL, Li YL (2008) Detection of carbohydrates using new labeling reagent 1-(2-naphthyl)-3-methyl-5-pyrazolone by capillary zone electrophoresis with absorbance (UV). Anal Chim Acta 609:66–75
18. Sheng X, Ding CX, Liu LJ, Suo YR, Sun ZW, You JM (2008) Separation of derivatized carbohydrates by capillary zone electrophoresis. Chin J Anal Chem 36(3):280–284
19. Lin X, Wang ZF, Huang LJ, Bai Q, Jia F (2006) An improved PMP derivatization method for analyzing monosaccharide composition. Chem J Chin Univ 27:1456–1458
20. Ming YF, Sun YX, You JM (2003) Separation of amino acids by capillary zone electrophoresis using 9-(2-Carbazole) ethyl chloroformate as derivatization agent. Chem Res 14:9–12