Journal of Chromatography A, 1232 (2012) 37–42

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Separation of phenolic acids from natural plant extracts using molecularly imprinted anion-exchange polymer confined ionic liquids

Wentao Bi, Minglei Tian, Kyung Ho Row*

Department of Chemical Engineering, Inha University, 253 Yonghyun-Dong, Nam-Ku, Incheon 402-751, South Korea

ARTICLE INFO

Article history:
Available online 22 August 2011

Keywords:
Ionic liquid
Anion exchange
Molecularly imprinted polymer
Solid phase extraction
Phenolic acid

ABSTRACT

Polymer-confined ionic liquids were used for the separation of phenolic acids from natural plant extract by utilizing an anion-exchange mechanism. They were synthesized using molecular imprinting technique to reduce non-directional ion–ion interactions during anion-exchange and other interactions with interference substances that could decrease selectivity. A suitable sorbent for phenolic acid separation could be identified based on the adsorption behaviors of phenolic acids on different polymer-confined ionic liquids. Thus, the developed ionic liquid-based molecularly imprinted anion-exchange polymer (IMAP) achieved high recovery rates by solid-phase extraction of phenolic acids from *Salicornia herbacea* L. extract: 90.1% for protocatechuic acid, 95.5% for ferulic acid and 96.6% for caffeic acid. Moreover, the phenolic acids were separable from each other by repeated solid phase extraction cycles. The proposed method could be used to separate other phenolic acids or organic acids from complex samples.

1. Introduction

Phenolic acids are secondary metabolites and are found in many plant species. Furthermore, they have excellent antioxidant activities, higher than those of vitamins C and E against reactive oxygen [1], and because of this, they have been used to prevent or treat diseases associated with oxidative damage, such as, coronary heart disease, stroke, and cancers [2–5]. Currently, they are isolated using chromatographic methods including high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC) and solid phase extraction (SPE) [6,7]. Typically the stationary phases or sorbents used in chromatography are C_{18}, anion-exchange resin and molecularly imprinted polymer (MIP). These materials interact with target compounds via hydrophobic, anion-exchange and hydrogen bonding interactions, respectively. However, singly interacting materials cannot effectively separate phenolic acids from interference. Ionic liquid (IL)-based materials with multiple interactions have therefore been proposed as alternative stationary phases or sorbents.

IL-based materials are used for the analysis and separation of bioactive compounds [8–14] due to their excellent physical and chemical properties. They facilitate separation by mechanisms that usually involve multiple interactions, for example hydrogen bonding, hydrophobic, π–π, electrostatic, and anion-exchange interactions. Although these interactions, especially anion-exchange interaction, can significantly increase the selectivity and capacity of IL-based materials towards organic acids, non-directional ion–ion interaction during anion-exchange and other interactions of interference with ionic liquids can reduce selectivities for target structures. Accordingly, the molecular imprinting technique has been employed to increase structural selectivity.

Molecular imprinting is a promising technique for the preparation of intelligent polymer materials with the ability to recognize specific molecules [15,16]. Molecularly imprinted polymers (MIPs) produce specific recognition sites by forming complexes between template molecules and functional monomers. Furthermore, the removal of template molecules by solvent leaves binding sites in polymers that are complementary in size, shape, and functionality to those of template molecules. MIPs have specific binding capacities and selectivities for template molecules, which makes them suitable sorbents for the solid-phase extraction (SPE) of specific bioactive compounds from plant extracts [17,18]. SPE generally involves loading, washing, and eluting with loading being the most time-consuming part of the process, because it requires time to achieve adequate adsorption of the target compounds onto sorbent in the presence of interfering species. To accelerate loading and to reduce the use of organic solvents during washing, sorbents can be directly mixed with extract; target compounds then quickly adsorb, assisted by heat or ultrasonication, without excessive interference. The resulting suspensions can then be added to an empty cartridge for subsequent SPE. This work reports a new method for the separation of phenolic acids, which we refer to as ionic liquid-based molecular imprinting anion-exchange solid phase extraction

* Corresponding author. Tel.: +82 32 860 7470; fax: +82 32 872 0959.
E-mail address: rowkho@inha.ac.kr (K.H. Row).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.08.054

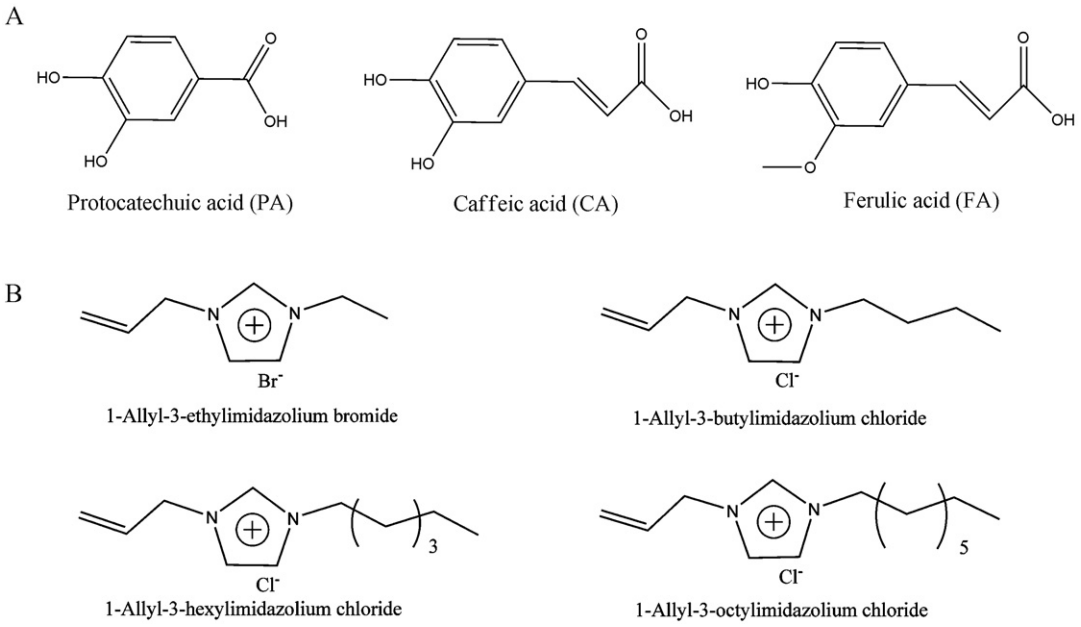

Fig. 1. Chemical structures of studied phenolic acids (A) and IL monomers (B).

(IMASPE). The devised technique was tested by separating protocatechuic acid (PA), caffeic acid (CA) and ferulic acid (FA) from *Salicornia herbacea* L. (*SHL*) extract.

PA, CA, and FA (Fig. 1A) are three important phenolic acids in *SHL*, a vegetable and medicinal herb used across East Asia [19–21]. They are considered important for preventing and treating diseases, such as, constipation, obesity, diabetes, cancer, and cardiovascular diseases [22–24]. Molecular imprinting [25] and solid phase extraction [26] have been used to separate these phenolic acids from *SHL*, but with lackluster results. Therefore, IMASPE was tested for the separation of these compounds. Initially, ionic liquid-based molecularly imprinted anion-exchange polymers (IMAPs) were produced using different functional and co-functional monomers, porogens, crosslinkers, and templates and optimized by comparing specific adsorption capacities. The resulting optimized polymer was used as a sorbent in adsorptive SPE (ASPE) to separate PA, CA, and FA from *SHL* extract. All necessary conditions were systematically optimized. The proposed method showed potential to be widely applied for the fast, convenient, and efficient isolation of various phenolic acids or other organic acids from plant extracts.

2. Experimental

2.1. Chemicals

2,2′-Azobisisobutyronitrile (AIBN) was purchased from Junsei Chemical Co. (Tokyo, Japan). 2,6-Di-tert-butyl-4-methylphenol (DBMP) (>99%), 1-allylimidazole (97%), ethyl bromide (>99%), *n*-butyl chloride (>98%), *n*-hexyl chloride (>95%) and *n*-octyl chloride were from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Protocatechuic acid (>97%), ferulic acid (>99%), caffeic acid (>98%) and ethyleneglycol dimethacrylate (EDGMA) were from Sigma–Aldrich (St. Louis, MO, USA). Methacrylic acid (MAA) was from Kanto Chemical Co., Inc. (Tokyo, Japan). Methanol, ethanol, isopropanol, *n*-hexanol and other organic solvents were from Duksan Pure Chemicals Co., Ltd. (Ansan, Korea). Distilled water was filtered using a vacuum pump and a filter (HA-0.45, both from Millipore, USA) before use. All other solvents were of HPLC or analytical grade. All samples were filtered (MFS-25, 0.2 μm TF, Whatman, USA) before being injected into the HPLC system.

2.2. HPLC analysis and characteristic analysis

The HPLC system comprised a M930 solvent delivery pump (Young Lin Co. Korea), a UV detector (M 720 Absorbance Detector, Young-In Scientific Co., Korea) and an integrated data system (Autochrowin. Ver. 1.42, Young Lin Co., Korea). Injection valves with 10.0 μL sample loops were used. HPLC analysis was performed on a commercial C_{18} column (4.6 × 150 mm, 5.0 μm) from RStech Co. (Daejeon, Korea). The mobile phase was acetonitrile/water/trifluoroacetic acid = 20/80/0.1 (v/v/v) at a flow rate of 0.5 mL min^{-1}. The UV detector was set at was at 270 nm, and the injection volume was 5.0 μL.

FT-IR spectra were obtained using KBr pellets on a Vertex 80V (Bruker, Billerica, MA, USA) between 400 and 4000 cm^{-1} at a scan rate of 20 scans min^{-1}. Field emission-scanning electron microscopy (FE-SEM) was conducted on an S-4200 microscope (Hitachi, Ontario, Canada). BET surface areas (N_2 atmosphere at −195.85 °C) were measured using an ASAP2020 surface area and porosimetry analyzer (Micromeritics, Norcross, GA, USA).

2.3. Preparation of IMAP

IL monomers were prepared as previously described [27]. Briefly, to a dried 100.0 mL flask were added 1-allylimidazole (10.8 g, 0.10 mol), alkyl halide (0.11 mol) and DBMP inhibitor (0.10 g). The reaction mixture was stirred at 45 °C under nitrogen for 24 h, and the resulting viscous liquid was washed with excess ethyl ether, and dried under vacuum at room temperature to give the purified product as a transparent viscous liquid. The chemical structures of these IL monomers are shown in Fig. 1B.

Phenolic acid-imprinted polymers were prepared by thermal-initiated polymerization in 4.0 mL glass vials [28]. The polymerization mixture comprised 0.3 mmol phenolic acid, 1.0 mmol IL monomers, 3.0 mmol EDGMA (cross linker), and 1% (w/w) AIBN, dissolved in an appropriate porogen (alcohol:water = 9:1, v/v). The solution was sonicated for 10.0 min,

Table 1
Studied polymers in this experiment.

	No.	IL monomer	Porogen type	Porogen amount (mL/mmol)	Monomer/ crosslinker ratio (mmol/mmol)	Template	Template/ monomer ratio (mmol/mmol)	IL/MAA ratio (mmol/mmol)
ILAEP	P1	1-Allyl-3-ethylimidazolium bromide	Ethanol/H_2O	0.25	1/3	–	–	–
	P2	1-Allyl-3-butylimidazolium chloride						
	P3	1-Allyl-3-hexylimidazolium chloride						
	P4	1-Allyl-3-octylimidazolium chloride						
	P5		Isopropanol/H_2O					
	P6		*n*-Butanol/H_2O					
	P7		*n*-Hexanol/H_2O					
	P8	1-Allyl-3-ethylimidazolium bromide	*n*-Butanol/H_2O	0.125	1/3			
	P9			0.2				
	P10			0.3				
	P11			0.35				
	P12			0.2	1/1			
	P13				1/2			
	P14				1/4			
	P15				1/5			
ILMIAEP	P16	1-Allyl-3-ethylimidazolium bromide	*n*-Butanol/H_2O	0.2	1/3	PA	1/3	–
	P17					CA		
	P18					FA		3/0
	P19						1/1	–
	P20						1/2	
	P21						1/4	
	P22						1/5	
	P23						–	2/1
	P24							1/2
	P25							0/3

sparged with helium for 5.0 min to remove oxygen, and then polymerized at 60.0 °C in a water bath for 24.0 h. After polymerization, the polymers were ground and repeatedly suspended in acetone to remove small particles. The polymer particles were dried under vacuum, placed into a glass column, and washed with 6.0 mL ethanol and 6.0 mL ethanol–HCl (90:10, v/v) to remove the templates. After balancing with methanol and drying in a drying oven (50 °C) for 24 h, the particles were stored at ambient temperature. Non imprinted blank polymers (NIP, without a template) were similarly prepared. The synthesized polymers are listed in Table 1.

2.4. Adsorption

5.0 mg of sorbents were stirred with 1.0 mL of each phenolic acid standard aqueous solution at 0.1 mg mL^{-1} in vials at room temperature until the concentration of free phenolic acids stopped decreasing and equilibrium adsorptions were obtained. Amounts of phenolic acids adsorbed on polymers were calculated by subtraction. Repeatability was assessed over five phenolic acid adsorptions. Two-sided *t*-tests were used to evaluate the data obtained from independent samples.

2.5. Real samples extraction

Dry *SHL* powder (5.0 g) was extracted using 100.0 mL distilled water in a flask with stirring at about 80 °C for 5.0 h. After centrifugation, the supernatant was filtered through a 0.2 μm hydrophilic membrane. Clear *SHL* extract (1.0 mL) was then mixed with IMAP (100.0 mg) under ultrasonication at room temperature for 15.0 min. The resulting suspension was then transferred to an empty polypropylene SPE cartridge, washed and eluted, to separate the phenolic acids from interferences. All significant variables were investigated.

3. Results and discussion

3.1. Morphological characterization

The FT-IR spectra of IAP (P9) and IMAP (P18) showed peaks at 1634 cm^{-1}, characteristic of imidazolium groups [29], and at 1730 cm^{-1}, attributable to the C=O group of EDGMA. No strong band characteristic of $C{=}CH_2$ was observed between 3075 and 3090 cm^{-1}. These data indicate the successful preparation of the intended polymers.

The structures of IAP (P9) and IMAP (P18) were studied by SEM and BET. Fig. 2 shows monolithic structures (Fig. 2A) that likely arose due to their similar synthesis of monolithic columns. When polymers were magnified to *ca.* 600 nm (Fig. 2B), P9 and P18 showed sub-porous structures, and an investigation by BET found surface areas of 50.7 m^2 g^{-1} and 72.4 m^2 g^{-1} and average pore sizes of 50.2 Å and 45.6 Å, respectively, which were attributed cavity formation by molecular imprinting, which increased the surface area of IMAP as average pore size decreased.

3.2. Optimization of IMAP

Sorbent selection is crucial for efficient SPE. Therefore, all factors affecting the properties of IMAP were investigated. Because loading involved the adsorption of phenolic acids on sorbents, adsorbed amounts indicate the performance of each material.

The functional groups of polymers affect their selectivity towards and adsorption capacity of target compounds. ILs, as functional monomers, were evaluated for their abilities to adsorb PA, CA, and FA. An IL structure, including cations and anions, significantly influenced their physicochemical properties and thus adsorption capacity. However, IL anion-exchange polymers (IAP) were to be regenerated by anion metathesis. Hence, P1, P2, P3, and P4, each with Cl^- anions, were evaluated with respect to

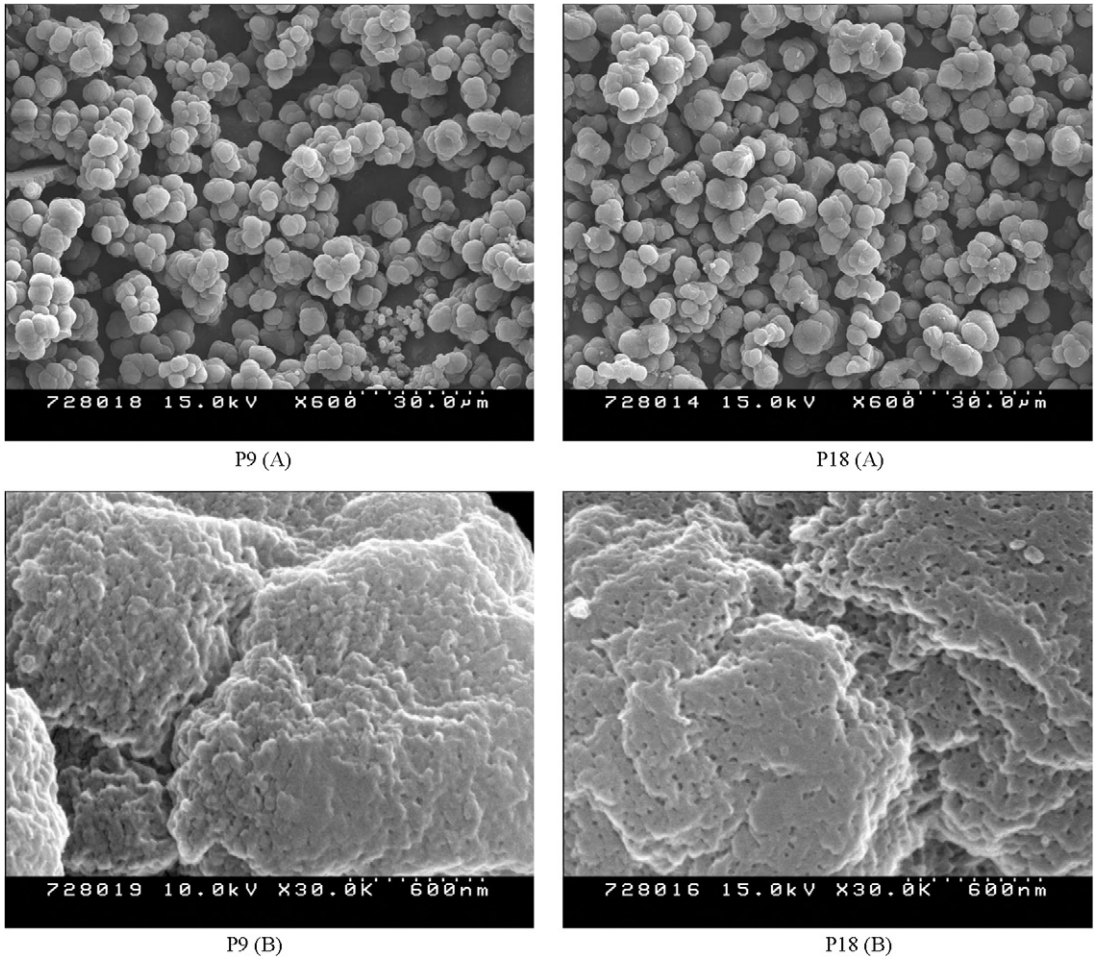

Fig. 2. SEM images of IAP (P9) and IMAP (P18).

their cations. Increasing alkyl chain length influenced adsorption, with P1 being the most efficient sorbent. Although hydrophobic interactions increased from ethyl to octyl at the 1-position of the imidazolium ring, long alkyl chains blocked the pores of polymer due to greater increased hydrogen bonding acidity. Therefore, P1 was selected for further optimization.

Pore size affects the arrangement of the functional groups, which are located within the pores [30]. Several alcohols (ethanol, isopropanol, *n*-butanol and *n*-hexanol) which can dissolve PA, CA, and FA were chosen as porogens given the properties of the target compounds. Adsorption of phenolic acids improved greatly as alkyl chain length increased from ethanol to *n*-butanol. No obvious increase in adsorption was observed when *n*-hexanol was used as a porogen. The volume of porogen per amount of monomer and crosslinker was studied in the range of 0.125–0.35 mL/mmol. Adsorbed amounts increased with increasing the amount of porogen between 0.125 and 0.20 mL/mmol, and decreased with further increases. This could be explained by different pore sizes in polymers arising as the type and amount of porogen varied. Generally, increasing the volume and alkyl chain length of the porogen increased pore size and reduced surface area. Using less and porogen with shorter alkyl chains produced smaller pores and increased surface area. Small pores prevent target compounds from penetrating the polymer, while large pores mean a low surface area and reduced adsorption capacity. Therefore, P9 was chosen for subsequent experiments.

Increasing the amount of cross linker decreased polymer pore sizes. Cross linker also affected the distribution of active sites in IAP. If the functional groups are too close to each other, target compounds cannot effectively interact with the functional groups due to steric hindrance. In the series of IAPs prepared at different monomer: cross linker ratios (1:1, 1:2, 1:3, 1:4, and 1:5 mmol/mmol), adsorbed amounts were highest at a monomer:cross linker ratio of 1:3 (mmol/mmol). Overall, P9 was considered optimal for the maximized adsorptions of PA, CA, and FA.

The shapes of MIP cavities, which are determined by template molecules, mainly determine molecular recognition. Therefore, PA, CA, and FA were assessed as templates. P18, employing FA, showed the highest adsorption of phenolic acids, which was attributed to FA having a larger molecular structure than the other phenolic acids. Monomer:template ratio can also affect recognition and selectivity. An excess of functional monomer relative to template will result in steric mismatch in the imprinted polymer and excessive binding sites. On the other hand, insufficient monomer will reduce self-assembly and result in low selectivity. The template:monomer ratio was therefore tested between 1:1 and 1:5 (mmol/mmol). Unlike previous tests, the polymer with lowest adsorption capacity was considered most suitable. Multiple interactions between the template and the functional groups of the ILs result in one molecular template being bound to several functional groups, reducing adsorption capacity. The lowest amounts of adsorbed phenolic acid were obtained at a template:monomer ratio of 1:3 (mmol/mmol) (P18). Therefore P18 was considered to have the highest selectivity.

MAA is widely used to manufacture MIPs used for the separation of phenolic acids [16]. The effect of copolymerization with MAA on

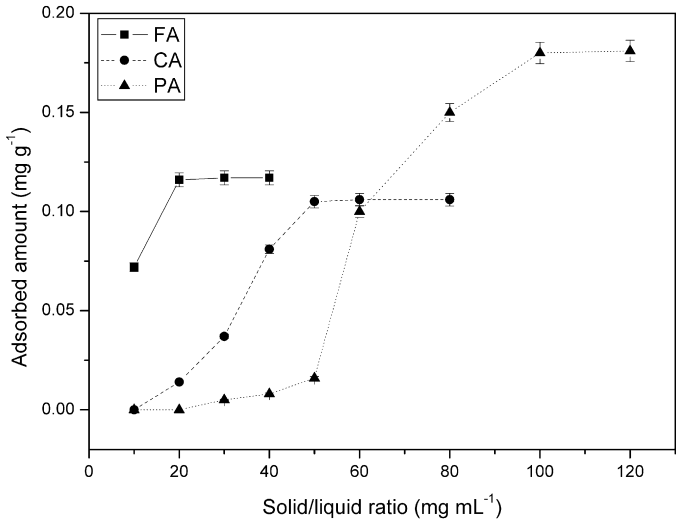

Fig. 3. Effect of solid/liquid ratio on adsorbed amounts of phenolic acids on IMAPs.

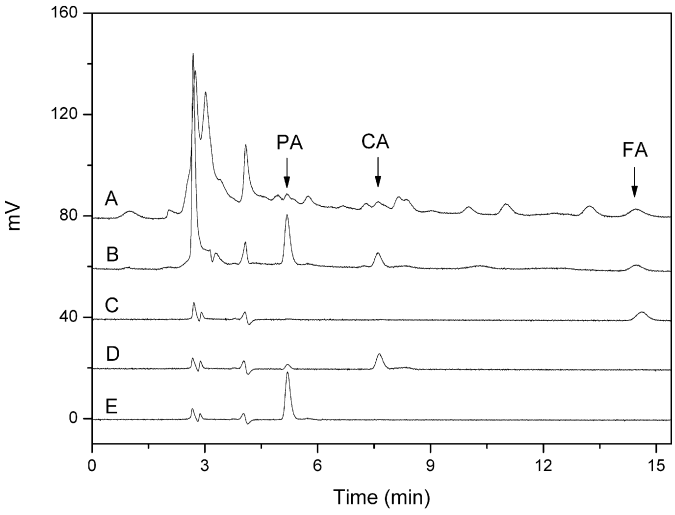

Fig. 4. Chromatograms of *SHL* extract (A), ASPE (B) and multiple ASPE (C–E).

adsorption capacity was tested at IL:MAA ratios of 3:0, 2:1, 1:2, and 0:3 mmol/mmol during the synthesis of IMAP. With increasing MAA content, the adsorptions of PA, CA, and FA decreased. Therefore MAA was less efficient than IL and P18 was the most suitable sorbent for the IMASPE of PA, CA, and FA from *SHL*.

3.3. SPE of phenolic acids by IMAP

ASPE involved adsorptive loading, washing and elution. Each step was separately optimized to isolate PA, CA, and FA from *SHL* extract.

3.3.1. Adsorptive loading

Adsorptive loading was achieved by mixing the sorbent with phenolic acid solutions. Heating and ultrasonication were examined to accelerate the adsorption. The temperature of adsorptive loading was tested at 25.0–55.0 °C. Adsorbed amounts of PA, CA, and FA decreased with increasing temperature, possibly because of chemical interactions and physical adsorption between the analyte and the SilLs. Desorption of the compounds was expedited by decreasing ion–ion and hydrogen bonding interactions with increasing temperature. Therefore, lower temperatures were favorable. Ultrasonication was then tested at 40.0–80.0 W, and 60.0 W showed the best trade-off between insufficient dispersion at low power and the heat generated at high power. Therefore, 60.0 W was optimal for rapid adsorption equilibration.

Based on previous optimization, IMAP was mixed with *SHL* extract (containing 0.020 mg mL^{-1} PA, 0.011 mg mL^{-1} CA, and 0.012 mg mL^{-1} FA) for adsorptive loading. Phenolic acids were then allowed to adsorb to the polymer with assistance by ultrasonication. The duration of ultrasonication can also affect adsorption, with longer durations that would aid adsorption being traded off against the increasing generation of heat that would decrease adsorption. Therefore, ultrasonication durations between 5.0 and 30.0 min were tested. Adsorption was best for 15.0 min of ultrasonication, and therefore 15.0 min was used in subsequent tests.

The relative amount of solid (IMAP) and liquid (extract) used during extraction also affects adsorption (Fig. 3), as adsorption increases with increasing amounts of IMAP. As the amount of sorbent is increased, target compounds are more likely to come into contact with IMAP, which would increase adsorption. Given economic considerations, 0.181 mg g^{-1} PA, 0.106 mg g^{-1} CA, and 0.117 mg g^{-1} FA were considered optimal at a solid:liquid ratio of 100:1 (mg mL^{-1}).

3.3.2. Washing

The suspension obtained during adsorptive loading was added to cartridges for SPE. 1.0 mL of water was first used to remove any remaining *SHL* extract from the SPE cartridges, and then different volumes (1.0–4.0 mL) of methanol, ethanol, isopropanol, acetonitrile, and acetone were used to wash interferences. Methanol was the most effective washing solvent, and most interferences were removed with 1.0 mL.

3.3.3. Elution

Aqueous HCl (0.5 mol/L) was used to elute phenolic acids from IMAP and to regenerate the sorbent. The elution solution was tested at different volumes (1.0–4.0 mL). Using more than 1.0 mL did not increase the elutions of PA, CA, or FA. Therefore, 1.0 mL of aqueous HCl was considered optimal (Fig. 4A and B). Overall, 90.1% PA, 95.5% CA, and 96.6% FA recovery yields were obtained from *SHL* extract.

3.3.4. Multiple ASPE

IMASPE can not only separate the target compounds from interference, but can also separate phenolic acids from each other. Fig. 3 shows that the phenolic acids were adsorbed onto IMAP in the order: FA > CA > PA because of competitive adsorption and structure recognition by the sorbent. For separate elution of the phenolic acids, 20 mg IMAP first was used to adsorb FA. It was then removed from the extract into an SPE cartridge. 30 mg of polymer was then added to the remaining extract to adsorb CA, and processed as for FA. Finally, PA was adsorbed onto additional IMAP and then placed in an SPE cartridge. These three SPE cartridges were washed and eluted using previously optimized conditions (Fig. 4C–E). These optimal conditions allowed 82.1% PA, 83.6% CA, and 96.0% FA (based on single ASPE) to be recovered from *SHL* extract.

3.4. Recycling of IMAP

IMAP was recycled for further separation of PA, CA, and FA from *SHL* extract by single ASPE. Recovery yields of PA, CA, and FA by IMAP over four cycles from SHL extract were 90.1–84.7%, 95.5–87.2% and 96.6–88.3%, respectively. Recovery yields decreased little, demonstrating the stability of the sorbent.

3.5. Analytical performance

To evaluate the proposed IMASPE method, a series of experiments were designed to assess its linearity, precision, detection limits and other characteristics under optimized conditions.

Table 2
Linear range, LODs and repeatability of the method.

Analyte	Linear range ($mg\,mL^{-1}$)	r	RSD (%)	LODs ($ng\,mL^{-1}$)
PA	0.1×10^{-3}–0.1	0.9992	3.5	20.0
CA	0.1×10^{-3}–0.1	0.9994	3.6	37.0
FA	0.1×10^{-3}–0.1	0.9993	4.1	45.0

All analytes had good linearity, with correlation coefficients, r, between 0.9992 and 0.9994 (Table 2). Precision was determined over five separations of PA, CA, and FA from extract, RSD was 3.5–4.1%. Based on a signal-to-noise ratio of 3, the limit of determinations (LOD) for the three phenolic acids was 20.0–45.0 $ng\,mL^{-1}$. These results demonstrate the stability of the proposed method and its potential to be widely applied to the determination of other medicinal products.

4. Conclusion

IMAP was used as selective sorbent during the ASPE of the phenolic acids (PA, CA, and FA) from *SHL* extract. The technique employed multiple chemical interactions and structure recognition to achieve excellent selectivity. ASPE was developed to apply IMAP sorbent to the separation of phenolic acids from interferences and from each other. Multiple ASPEs separated the phenolic acids from each other but with lower recovery yields than single ASPE. Future work will be undertaken to improve the recovery yield of the multiple ASPE method. Nevertheless, IMASPE was found to be an efficient and potentially applicable to the separation of other phenolic acids and organic acids from complex biologic samples.

Acknowledgements

This research was supported by the Basic Science Research Program (2011-0010673) and the International Research & Development Program (2010-D00016) of the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.08.054.

References

[1] R. Tsao, Z. Deng, J. Chromtogr. B 812 (2004) 85.
[2] L.S. Chua, N.A. Latiff, S.Y. Lee, C.T. Lee, M.R. Sarmidi, R.A. Aziz, Food Chem. 127 (2011) 1186.
[3] A.H. Laghari, S. Memon, A. Nelofar, K.M. Khan, A. Yasmin, Food Chem. 126 (2011) 1850.
[4] I.S.L. Lee, M.C. Boyce, M.C. Breadmore, Food Chem. 127 (2011) 797.
[5] P.V. Hung, D.W. Hatcher, W. Barker, Food Chem. 126 (2011) 1896.
[6] K. Pyrzynska, M. Biesaga, Trends Anal. Chem. 28 (2009) 893.
[7] S. Bostyn, B. Cagnon, H. Fauduet, Talanta 80 (2009) 1.
[8] H. Qiu, M. Takafuji, X. Liu, S. Jiang, H. Ihara, J. Chromatogr. A 1217 (2010) 5190.
[9] W. Bi, J. Zhou, K.H. Row, Anal. Chim. Acta 677 (2010) 162.
[10] W. Bi, M. Tian, K.H. Row, J. Sep. Sci. 33 (2010) 1739.
[11] W. Bi, M. Tian, K.H. Row, Phytochem. Anal. 21 (2010) 496.
[12] W. Bi, K.H. Row, Chromatographia 71 (2010) 25.
[13] M. Tian, W. Bi, K.H. Row, Anal. Bioanal. Chem. 399 (2011) 2495.
[14] W. Bi, J. Zhou, K.H. Row, Talanta 83 (2011) 974.
[15] C. Michailof, P. Manesiotis, C. Panayiotou, J. Chromatogr. A 1182 (2008) 25.
[16] H. Li, Y. Liu, Z. Zhang, H. Liao, L. Nie, S. Yao, J. Chromatogr. A 1098 (2005) 66.
[17] R. Mohamed, P. Mottier, L. Treguier, J. Richoz-Payot, E. Yilmaz, J.C. Tabet, P.A. Guy, J. Agric. Food Chem. 56 (2008) 3500.
[18] L. Peng, Y. Wang, H. Zeng, Y. Yuan, Analyst 136 (2011) 756.
[19] Y.C. Jo, K.S. Lee, S.M. Chon, D.S.J. Byun, Korean Soc. Med. Crop Sci. 10 (2002) 100.
[20] Y. Ren, X.Y. Pan, J. Zhejiang Agric. Sci. 6 (1997) 294.
[21] K. Shimizu, N. Ishikawa, J. Tang, S. Muranaka, W. Cao, Jpn. J. Trop. Agric. 47 (2003) 132.
[22] M. Kapiszewska, E. Sołtys, F. Visioli, A. Cierniak, G. Zajac, J. Physiol. Pharmacol. Suppl. 56 (2005) 183.
[23] J.C. Stoclet, T. Chataigneau, M. Ndiaye, M.H. Oak, J. El Bedoui, M. Chataigneau, V.B. Schini-Kerth, Eur. J. Pharmacol. 500 (2004) 299.
[24] F. Hakimuddin, G. Paliyath, K. Meckling, Breast Cancer Res. Treat. 85 (2004) 65.
[25] T. Zhu, S. Li, K.H. Row, J. Appl. Polym. Sci. 121 (2011) 1691.
[26] D. Han, M. Tian, K.H. Row, Nat. Prod. Commun. 5 (2011).
[27] J. Tang, W. Sun, H. Tang, M. Radosz, Y. Shen, Macromolecules 38 (2005) 2037.
[28] H. Yan, F. Qiao, K.H. Row, Anal. Chem. 79 (2007) 8242.
[29] X. Liang, Q. Chen, X. Liu, S. Jiang, J. Chromatogr. A 1182 (2008) 197.
[30] K. Farrington, E. Magner, F. Regan, Anal. Chim. Acta 566 (2006) 60.