# Molecularly Imprinted Monolithic Material for the Extraction of Three Organic Acids from *Salicornia herbacea* L

**Tao Zhu, Shengnan Li, Kyung Ho Row**

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

Received 5 June 2010; accepted 11 November 2010
DOI 10.1002/app.33755
Published online 4 March 2011 in Wiley Online Library (wileyonlinelibrary.com).

**ABSTRACT:** A molecularly imprinted monolithic material was designed and prepared by *in situ* thermally initiated copolymerization for the extraction of protocatechuic acid, caffeic acid, and ferulic acid from *Salicornia herbacea* L. Field emission scanning electron microscopy and offline solid-phase extraction (SPE) were investigated for the characterization of this material. The extract samples were loaded onto and passed through the monolithic material; the target compounds were selectively retained on the material, whereas other interfering substances were quickly washed out. Chromatographic analysis was conducted on a $C_{18}$ column with ultraviolet detection at 270 nm, and an eluting solution consisting of acetonitrile, water, and acetic acid (14/86/0.5 v/v/v, pH 5.0) was used as the mobile phase at a flow rate of 0.8 mL/min. The linearity was confirmed in the concentration ranges of 0.10–200.00, 0.20–400.00, and 0.30–600.00 μg/mL for protocatechuic acid, caffeic acid, and ferulic acid, respectively, with $r^2$ greater than 0.9997. The SPE recoveries of the three organic acids ranged from 71.08 to 81.02%, and the coefficient of variation (precision) was 3.08–5.70%. This method is simple, economical, and specific and has been used successfully in the extraction of three organic acids from *S. herbacea* L. This cartridge can be used as a potential tool for the extraction of drugs from natural plants. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 1691–1696, 2011

**Key words:** high performance liquid chromatography; liquid chromatography; molecular imprinting

## INTRODUCTION

Molecular imprinting is being rapidly developed for the preparation of polymers with specific molecular recognition properties for a given compound or its analogues, and these polymers are becoming attractive as effective materials for functional separation because of their high selectivity.[1–3] A molecularly imprinted polymer (MIP) is prepared with a template molecule and functional monomers that assemble around the template and subsequently are cross-linked to one another.[4,5] Monolithic materials in high-performance liquid chromatography (HPLC) have attracted significant interest because of their ease of preparation, reproducibility, versatile surface chemistries, and fast mass transport.[6–8] They exhibit through-pores that are large enough to sustain the percolation of a stable pressure-driven flow of a mobile phase through them, and this is much more rapid than diffusion in conventional stationary phases.[9] Monolithic MIP technology, a novel method for the preparation of chromatographic materials, combines the advantages of molecular imprinting and monolithic column technology.[10] With this technique, MIPs can be synthesized directly inside stainless steel columns without the tedious procedures of grinding, sieving, and column packing.[11] Moreover, some shortcomings of MIP, such as high backpressures and low efficiencies, can be avoided.[12]

*Salicornia herbacea* L. (also known by the Korean name *Hamcho*) is an annual herb that grows in high salt marshes and salt fields in most Asian countries, such as Korea, China, and Japan.[13] *S. herbacea* L. has attracted much attention because of its nutritional value, functional properties, and many bioactive substances, such as organic acids,[14] phytosterols,[15] and polysaccharides.[16,17] It also has been used as a folk medicine to treat a variety of diseases, such as gastroenteric disorders, asthma, hepatitis, and cancer.[18] Protocatechuic acid, caffeic acid, and ferulic acid are three important antioxidant constituents of *S. herbacea* L. These three organic acids are phenolic antioxidants, and they have been shown to act as carcinogenic inhibitors and to have effects on cancer cells in *in vitro* and *in vivo* studies.[19,20] At present, in

*Correspondence to:* K. H. Row (rowkho@inha.ac.kr).
Contract grant sponsor: Basic Science Research Program (through the National Research Foundation of Korea, which is funded by the Korean Ministry of Education, Science, and Technology); contract grant number: 2010-0015731.

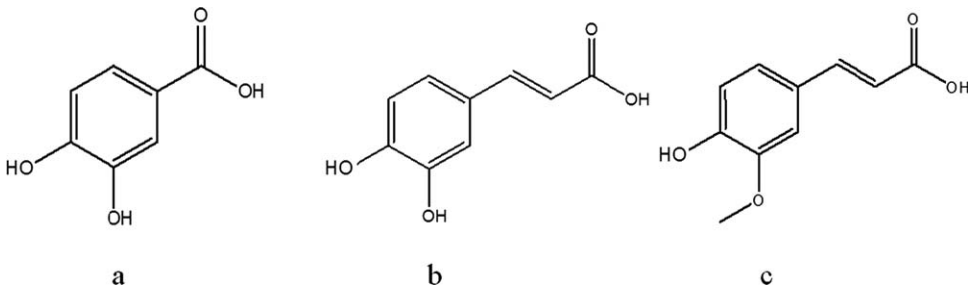


**Figure 1** Chemical structures of (a) protocatechuic acid, (b) caffeic acid, and (c) ferulic acid.

the extraction or synthesis of these three organic acids, organic solvents are mostly used for extraction.[21] However, it is also necessary to remove impurities from the organic solvent extracts. Solid-phase extraction (SPE) has been developed as an acceptable alternative to liquid–liquid extraction for the separation, purification, concentration, and solvent exchange of solutes from solution.[22,23] It is a viable alternative to conventional sample preparation methods and has been applied to natural plant extraction.[24] It is necessary to include cleanup in the SPE protocol to enhance the selectivity of the sorbent, and the choice of the sorbent is a key point in SPE because it can control analytical parameters such as the selectivity, affinity, and capacity. An MIP monolithic material can effectively improve the selectivity of organic acids with caffeic acid as the template. The chemical structures of three organic acids are shown in Figure 1.

In this work, an MIP monolithic material was prepared in a chromatographic column by an *in situ* synthesis method with caffeic acid as the template. After the obtained material was characterized and tested, an offline SPE method was established for the simultaneous analysis of three organic acids from *S. herbacea* L. with the MIP monolithic cartridge. In comparison with the other SPE method,[23,25] this monolithic MIP separation has attracted significant interest because of its ease of preparation, high selectivity, and rapid mass transfer.

## EXPERIMENTAL

### Reagents and materials

*S. herbacea* L. was obtained from Hyundai Global Silicate Co., Ltd. (Incheon, Korea). Protocatechuic acid, ferulic acid, and caffeic acid were bought from Sigma–Aldrich (St Louis, MO, United Stated) and used without further purification. Ethylene glycol dimethacrylate was purchased from Fluka (Buchs, Switzerland). Dodecanol was purchased from Acros Organics (Geel, Belgium). Cyclohexanol and 2,2′-azobisisobutyronitrile were purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan), and were refined before use. Acetonitrile, methanol, acrylamide, and acetic acid were obtained from Duksan Pure Chemical Co., Ltd. (Ansan, Korea). All other reagents used in the experiments were HPLC-grade or analytical-grade. Double-distilled water was filtered with a vacuum pump (Waters, Milford, MA, United States) and a filter (Waters, Milford, MA, United States) before use. All samples were filtered with a filter (MFS-25, 0.2-μm TF, Whatman, Piscataway, NJ, United States) before their injection into the HPLC system.

### Chromatography

The chromatography system consisted of a Waters 600s multisolvent delivery system (Waters, Milford, MA, United States), a Waters 616 liquid chromatograph (Waters, Milford, MA, United States), a Rheodyne injector (20-μL sample loop, Oak Harbor, WA, United States), and a 2487 variable-wavelength ultraviolet (UV) dual-channel detector (Waters, Milford, MA, United States). Autochro-2000 software (Younglin Co., Ltd., Anyang, Korea) was used as the data-acquisition system. The analysis was performed on an OptimaPak $C_{18}$ column (5 μm, 150 × 4.6 mm, i.d., RS Tech Corp., Daejeon, Korea) with a guard column (10 × 4.6 mm i.d.) packed with $C_{18}$ materials. HPLC separation of organic acids was conducted with acetonitrile/water/acetic acid (14/86/0.5 v/v/v, pH 5.0) as the mobile phase at a flow rate of 0.8 mL/min, and the detection was carried out at a wavelength of 270 nm.

### Preparation of the monolithic MIP column[26]

The monolithic MIP was prepared by *in situ* polymerization in a stainless steel chromatographic column tube (45 × 9.0 mm i.d.). The template molecule (caffeic acid; 1.00 g), 0.05 g of the initiator (2,2′-azobisisobutyronitrile), 0.60 g of the functional monomers (acrylamide), and 3.0 mL of the crosslinker (ethylene glycol dimethacrylate) were dissolved in the appropriate porogenic solvents (cyclohexanol and dodecanol). The solution was ultrasonicated for 15 min and sparged with helium for 5 min to remove oxygen. The polymerization mixture was then poured into the HPLC column. Subsequently, the polymerization

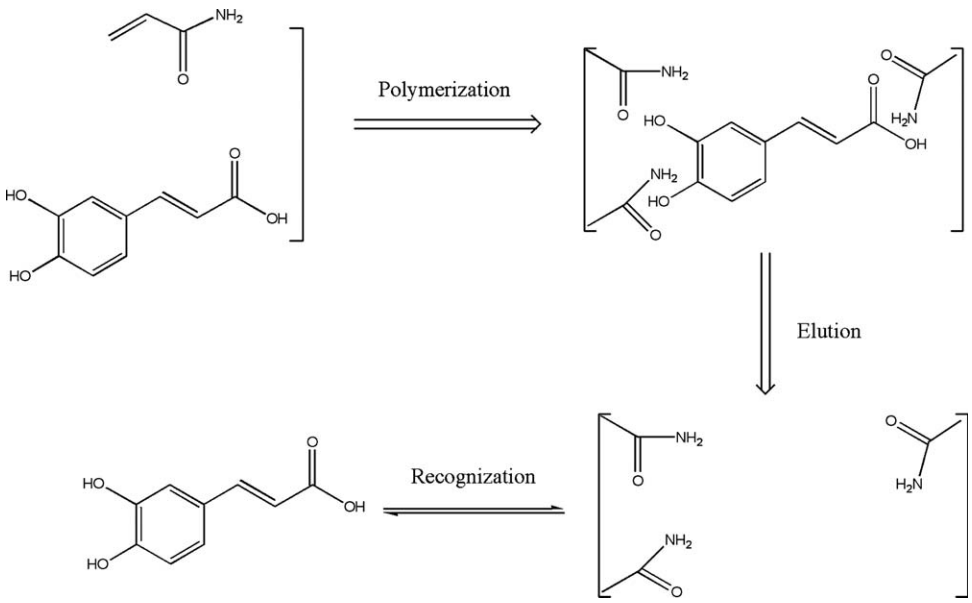


**Figure 2** Schematic principle of caffeic acid imprinted polymers.

reaction took place in the column in a water bath at 55°C for 24 h. After the polymerization, the column was connected to an HPLC pump and was washed with tetrahydrofuran and methanol/acetic acid (80/20 v/v), respectively, to remove the templates, porogenic solvents, and other compounds. A nonimprinted blank monolithic column was prepared in the absence of the template and was treated in an identical manner. The schematic principle of the imprinted polymers is shown in Figure 2.

### Characterization of the monolithic molecular imprinting materials

After the chromatographic experiments had been completed, the column was washed with methanol/acetic acid (80/20 v/v) for 30 min. The bottom column fitting was removed, and the monolith inside the column was pushed out of the tube with the pressure of the methanol mobile phase at a flow rate of 5 mL/min. The cylindrical monolith was then dried *in vacuo* at 50°C for 24 h and cut into pieces with a razor blade. The microstructures of the dried monolithic samples were observed by field emission scanning electron microscopy (FESEM; model S-4300, Hitachi, Tokyo, Japan) at 15 kV.

### Preparation of the standard solutions and sample solution

Standard stock solutions of protocatechuic acid, caffeic acid, and ferulic acid were prepared with the concentration of 1000.00 μg/mL in methanol. Calibration-standard mixture solutions with the concentration ranges of 0.10–200.00, 0.20–400.00, and 0.30–600.00 μg/mL for protocatechuic acid, caffeic acid, and ferulic acid, respectively, were prepared with appropriate aliquots of standard stock solutions. The quality-control samples were also prepared with low, medium, and high concentrations. These calibration standards and quality-control samples were freshly prepared for each analytical batch.

*S. herbacea* L. powder (3 g) was weighed and mixed with 30.0 mL of methanol. Then, the mixture was shaken with ultrasound for 2 h. After centrifugation, the extract was collected as a stock sample solution.

### SPE by the MIP monolithic cartridge

The cylindrical MIP monolithic column (45 mm × 9.0 mm i.d.) was dried *in vacuo* at 50°C for 24 h and cut into small pieces (15 mm × 9.0 mm i.d.) with a razor blade. Then, these small pieces were put into a plastic syringe tube for SPE. Each cartridge was equilibrated with 3 mL of methanol and then conditioned with 3 mL of water. After the conditioning step, 0.5 mL of the stock extraction sample solution was loaded onto and passed through the cartridge. After they were washed once with 3 mL of water, the analytes were then eluted with 3 mL of methanol. The eluent was evaporated to dryness, redissolved in 0.5 mL of acetonitrile/water/acetic acid (14/86/0.5 v/v/v, pH 5.0), and injected into the HPLC apparatus for analyte quantification.

## RESULTS AND DISCUSSION

### Morphological characteristics of the imprinted monolith

Morphological analysis of the polymers was investigated with FESEM in this study. FESEM has been

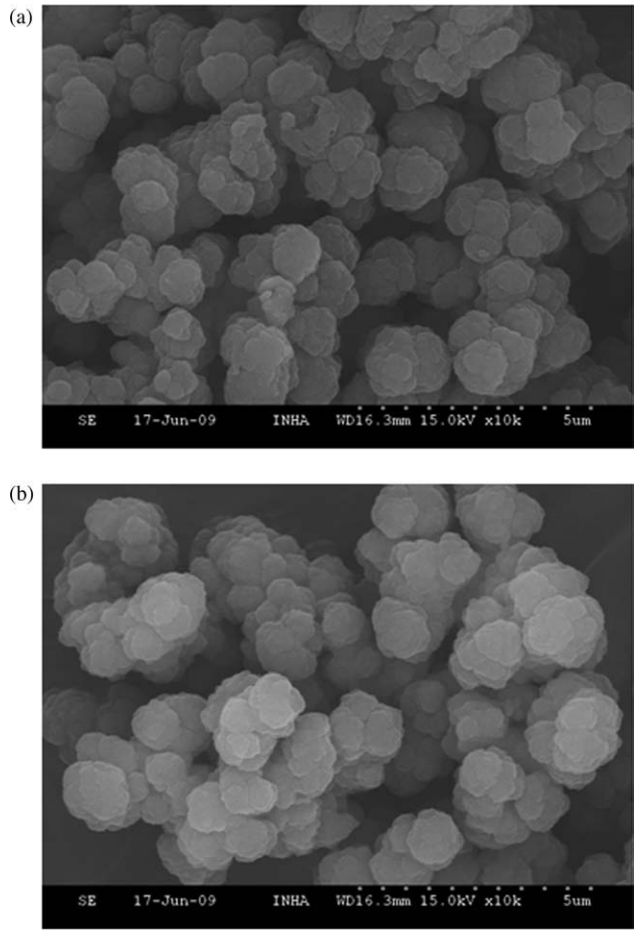


**Figure 3** FESEM images of (a) an MIP and (b) a nonimprinted blank polymer.

successfully used to observe the morphologies of block polymers, which are important parameters that are used to evaluate polymerization stability and reproducibility. According to Figure 3(a,b), there were no significant differences between the caffeic acid MIP and nonimprinted blank polymers; also, many macropores and flow-through channels were inlaid in the network skeletons of these polymers. These macropores and channels allowed the mobile phase to flow through the monolith with low flow resistance and thus enabled fast mass transfer of the solutes. Moreover, the low backpressure allowed their operation at higher flow rates. The polymerization temperature, the solvent type, and the composition were the three greatest factors affecting the pore properties of the monolithic column. In contrast, the backpressure of the packed column was higher over the whole range of flow rates because of the irregular shapes and nonuniform sizes of the packed particles.

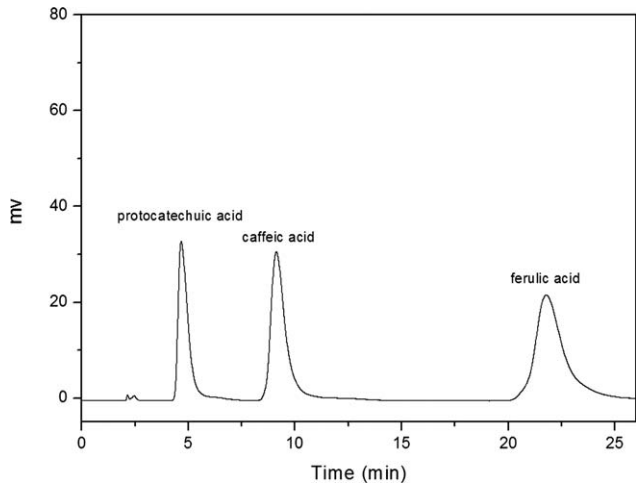


**Figure 4** Chromatogram of standard samples [mobile phase = 14/86/0.5 v/v/v acetonitrile/water/acetic acid (pH 5.0), flow rate = 0.8 mL/min, column = $C_{18}$ (5 μm, 150 mm × 4.6 mm i.d.; RS Tech), UV wavelength = 270 nm, injection volume = 10 μL].

## Optimization of the chromatographic conditions

The chromatographic conditions were optimized to improve the separation and chromatographic efficiency. The acetonitrile–water system was tested as the elution solution to simplify the operation. In this experiment, the acetonitrile content and pH value in the mobile phase greatly affected the resolution and retention time of the three organic acids on the $C_{18}$ column. The pH value was controlled from 3.0 to 7.0 through changes in the amount of acetic acid. After some experimentation, the optimum conditions for the mobile phase were obtained: acetonitrile/water/acetic acid (14/86/0.5 v/v/v, pH 5.0) at a flow rate of 0.8 mL/min on the $C_{18}$ column with detection at 270 nm. Figure 4 shows a representative chromatogram of standard samples of the three organic acids. The analytes were separated well under the optimum chromatographic conditions with retention time of 4.85, 9.93, and 22.51 min for protocatechuic acid, caffeic acid, and ferulic acid, respectively.

**TABLE I**
**Calibration Curves (n = 7), LODs, and LOQs for Three Organic Acids**

| | Regression equation | $r^2$ | Linear range (μg/mL) | LOD (μg/mL) | LOQ (μg/mL) |
|---|---|---|---|---|---|
| Protocatechuic acid | $Y = 20.87X - 24.10$ | 0.9998 | 0.10–200.00 | 0.04 | 0.10 |
| Caffeic acid | $Y = 15.01X - 43.51$ | 0.9998 | 0.20–400.00 | 0.08 | 0.20 |
| Ferulic acid | $Y = 12.92X - 51.41$ | 0.9997 | 0.30–600.00 | 0.12 | 0.30 |

**TABLE II**
**Intraday and Interday Precision, Accuracy, and Recovery of Three Organic Acids from *S. herbacea* L.**

| | | Intraday | | | Interday | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration (μg/mL) | Measured concentration (μg/mL) | Accuracy (%) | Precision RSD (%) | Measured concentration (μg/mL) | Accuracy (%) | Precision RSD (%) | SPE recovery (%) |
| Protocatechuic acid | 1.00 | 1.04 | 104.00 | 5.02 | 1.03 | 103.00 | 5.36 | 73.61 |
| | 25.00 | 25.09 | 100.34 | 5.11 | 25.37 | 101.47 | 4.89 | 79.58 |
| | 100.00 | 98.64 | 98.64 | 4.24 | 97.55 | 97.55 | 4.66 | 81.02 |
| Caffeic acid | 2.00 | 2.06 | 103.00 | 4.65 | 2.07 | 103.50 | 5.70 | 75.12 |
| | 50.00 | 52.35 | 104.69 | 3.08 | 51.02 | 102.04 | 4.91 | 80.35 |
| | 200.00 | 198.72 | 99.36 | 4.32 | 199.52 | 99.76 | 5.08 | 77.39 |
| Ferulic acid | 3.00 | 3.11 | 103.67 | 5.63 | 3.14 | 104.67 | 5.45 | 71.08 |
| | 75.00 | 76.09 | 101.45 | 4.07 | 77.17 | 102.89 | 4.21 | 78.34 |
| | 300.00 | 293.55 | 97.85 | 4.75 | 295.08 | 98.36 | 4.37 | 76.88 |

## Method validation

### Calibration curves, limits of detection (LODs), and limits of quantification (LOQs)

We demonstrated the linearity of the method over concentration ranges of 0.10–200.00, 0.20–400.00, and 0.30–600.00 μg/mL for protocatechuic acid, caffeic acid, and ferulic acid, respectively, by assaying seven data points in triplicate on three separate occasions. We obtained the standard calibration curve by plotting the peak area versus the concentration. The sensitivity of the method was expressed with LOD and LOQ at signal-to-noise ratios of 3:1 and 10:1, respectively. The data are shown in Table I.

### Precision and accuracy

The accuracy and precision of the method were expressed with five replicate analyses of the quality-control samples with three different concentrations of the three organic acids on the same day and consecutive days. The intraday and interday relative standard deviations (RSDs) of the proposed method were less than 5.63% and 5.70%, respectively. The results are shown in Table II. The results also indicated that the assay method was reproducible for replicate analyses of the three organic acids within the same batch and in different batches.

### SPE recovery

The SPE recovery of the three organic acids was examined at low, medium, and high concentrations in five replicates. It was determined by a comparison of the peak areas of the three organic acids of the methanol extracts and MIP monolithic SPE extracts. Recovery data are summarized in Table II. The data indicated that the extraction recovery of the organic acids was independent of the concentration.

## Application to extraction from *S. herbacea L.*

The behavior of the proposed MIP monolith and nonimprinted blank monolith was investigated through the extraction of the three organic acids from *S. herbacea* L. Because there was no template in the nonimprinted blank monolith, this material could not be selective and effectively extract these three organic acids. The application of the development method was executed through the extraction of 30 g of *S. herbacea* L. by the MIP monolithic cartridge, and the chromatograms of the sample extracts were obtained without SPE [Fig. 5(a)] and with the extraction of the MIP monolithic cartridges [Fig. 5(b)]. Comparing these two chromatograms, we observed no interfering peaks around the retention times of the three organic acids [Fig. 5(b)]. This indicated that the SPE process with the MIP monolithic cartridge played an important role in this experiment, and the cartridge had

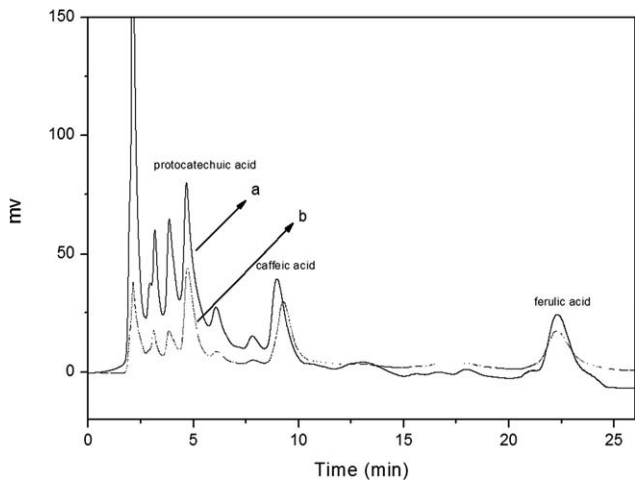


**Figure 5** Chromatograms of methanol extracts (a) without and (b) with SPE [mobile phase = 14/86/0.5 v/v/v acetonitrile/water/acetic acid (pH 5.0), flow rate = 0.8 mL/min, column = $C_{18}$ (5 μm, 150 mm × 4.6 mm i.d.; RS Tech), UV wavelength = 270 nm, injection volume = 10 μL].

good selectivity for the three organic acids. These results were conducive to the quantitative analysis of this drug. With this established method, the extract yields were 0.646, 0.511, and 0.378 mg/g for protocatechuic acid, caffeic acid, and ferulic acid, respectively.

## CONCLUSIONS

The MIP monolithic material, prepared by an *in situ* method in a chromatographic column with caffeic acid as the template, was successfully applied to the extraction of three organic acids from *S. herbacea* L. extract. The SPE analysis method and FESEM were used to evaluate the characteristics of the new material. The results showed that this SPE method exhibited high specificity and sensitivity as well as the required precision, accuracy, and recovery; thus, its acceptability for drug assays was demonstrated. It was shown to be a better cleanup method: it effectively eliminated the interfering peaks and resulted in high recovery of the three organic acids. Therefore, this MIP monolithic cartridge is a potential tool for the extraction of drugs from natural plants in the near future.

## References


1. Mahony, J. O.; Nolan, K.; Smyth, M. R.; Mizaikoff, B. Anal Chim Acta 2005, 534, 31.
2. Kindschy, L. M.; Alocilja, E. C. Biosens Bioelectron 2005, 20, 2163.
3. Yan, H.; Row, K. H.; Yang, G. Talanta 2008, 75, 227.
4. Liu, X.; Ouyang, C.; Zhao, R.; Shangguan, D.; Chen, Y.; Liu, G. Anal Chim Acta 2006, 571, 235.
5. Huang, X.; Qin, F.; Chen, X.; Liu, Y.; Zou, H. J Chromatogr B 2004, 804, 13.
6. Guiochon, G. J Chromatogr A 2007, 1168, 101.
7. Buchmeiser, M. R. Polymer 2007, 48, 2187.
8. Zou, H.; Huang, X.; Ye, M.; Luo, Q. J Chromatogr A 2002, 954, 5.
9. Yang, G.; Liu, H.; Bai, L.; Jiang, M.; Zhu, T. Microporous Mesoporous Mater 2008, 112, 351.
10. Yan, H.; Row, K. H. J Ind Eng Chem 2007, 13, 552.
11. Huang, X.; Zou, H.; Chen, X.; Luo, Q.; Kong, L. J Chromatogr A 2003, 984, 273.
12. Li, H.; Liu, Y.; Zhang, Z.; Liao, H.; Nie, L.; Yao, S. J Chromatogr A 2005, 1098, 66.
13. Jo, Y. C.; Lee, K. S.; Chon, S. M.; Byun, D. S. J Med Crop Sci 2002, 10, 100.
14. Oh, J. H.; Kim, E. O.; Lee, S. K.; Woo, M. H.; Choi, S. W. Food Sci Biotechnol 2007, 16, 90.
15. Lee, Y. S.; Lee, H. S.; Shin, K. H.; Kim, B. K.; Lee, S. Arch Pharm Res 2004, 27, 1034.
16. Im, S. A.; Lee, Y. R.; Lee, Y. H.; Oh, S. T.; Gerelchuluun, T.; Kim, B. H.; Kim, Y.; Yun, Y. P.; Song, S.; Lee, C. K. J Ethnopharmacol 2007, 111, 365.
17. Kong, C. S.; Kim, Y. A.; Kim, M. M.; Park, J. S.; Kim, J. A.; Kim, S. K.; Lee, B. J.; Nam, T. J.; Seo, Y. Toxicol In Vitro 2008, 22, 1742.
18. Jang, H. S.; Kim, K. R.; Choi, S. W.; Woo, M. H.; Choi, J. H. Ann Nutr Metab 2007, 51, 119.
19. Lin, H. H.; Chen, J. H.; Huang, C. C.; Wang, C. J. Int J Cancer 2007, 120, 2306.
20. Saija, A.; Tomaino, A.; Trombetta, D.; Pasquale, A. D.; Uccella, N.; Barbuzzi, T.; Paolino, D.; Bonina, F. Int J Pharm 2000, 199, 39.
21. Ying, Y.; Zhang, W.; Cao, S. Chin J Anal Chem 2007, 35, 1726.
22. Yan, H.; Tian, M.; Row, K. H. Sep Sci Technol 2009, 44, 359.
23. Zhu, T.; Row, K. H. Chromatographia 2009, 69, 1477.
24. Pukalskas, A.; Beek, T. A.; Waard, P. J Chromatogr A 2005, 1074, 81.
25. Zhu, T.; Row, K. H. Chin J Chem 2010, 28, 1463.
26. Yan, H.; Row, K. H. Sep Sci Technol 2006, 41, 1841.