Journal of Food and Drug Analysis, Vol. 16, No. 4, 2008, Pages 8-14 藥物食品分析 第十六卷 第四期

Molecular Recognition and Identification of Sulfapyridine and Sulfadimethoxine by High Performance Liquid Chromatography with Molecularly Imprinted Polymer as the Stationary Phase

CHIN-YIN HUNG[1], YUN-TZU HUANG[2] AND CHING-CHIANG HWANG[3]*

[1.] *Department of Biotechnology, National Formosa University, Yunlin, Taiwan (R.O.C.)*
[2.] *Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Hsinchu, Taiwan (R.O.C.)*
[3.] *Department of Life Science, Mingdao University, ChangHua, Taiwan (R.O.C.)*

(Received: June 26, 2007; Accepted: December 18, 2007)

ABSTRACT

Molecular imprinting technique is becoming an important tool for determining sulfonamides because of its simplicity and high separation efficiency. The creation of molecularly imprinted polymers (MIPs) for recognition of sulfapyridine and sulfadimethoxine is reported. Polymers were prepared using methacrylic acid (MAA) as the functional monomer and sulfapyridine as the template during imprinting. The recognition properties of the polymers were assessed by high performance liquid chromatography (HPLC) for their ability to retain the templates and analogous analytes. Retention time of sulfapyridine and sulfadimethoxine was approximately 8.24 and 3.89 min, respectively. In order to compare the chromatographic data from the stationary phase, both retention factor (*k'*) and separation factor (α) were given. The value of 2.39 for separation factor indicated that the MIP was able to recognize structurally subtle differences from the template molecule. Our results are discussed with regard to the amount of template, the compositions of the chromatographic mobile phase and the adsorption capacity.

Key words: molecularly imprinted polymers (MIPs), recognition, sulfapyridine, sulfadimethoxine, high performance liquid chromatography (HPLC)

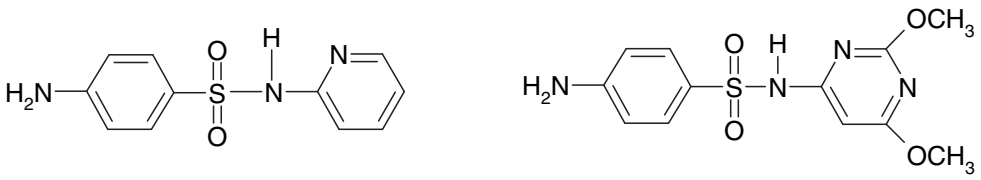
INTRODUCTION

Determination of sulfonamides has received significant attention in recent years because these pharmaceuticals are widely used for medicinal practices, including livestock diseases such as urinary tract infections, Chlamydia, rheumatic, fever and malaria. Current analytical techniques for sulfonamides determination have been developed and the most frequently employed techniques include liquid chromatography coupled with mass spectrometry (LC-MS)[(1-3)], high-performance liquid chromatography (HPLC)[(4,5)], gas-chromatography (GC)[(6)], and UV detection[(7)]. Nevertheless, these methodologies involve expensive instrumentations and tedious analytical procedures. Therefore, it is necessary to develop a cost effective, precise and rapid method for sulfonamides quantification. To approach this, we explored the feasibility of sulfonamides determination by liquid chromatography (LC) using molecularly imprinted polymers (MIPs) materials as the stationary phase. MIPs have gained great interest in the past decade and their widespread applications have become important, as shown in recent reviews[(8-11)]. Cross-linking and functional monomers in the traditional molecular imprinting are co-polymerized in the presence of the template molecule. After polymerization, the templates are removed and specific recognition sites, that are complementary to the template molecules in the resultant MIPs are created. Finally, MIPs can provide analyte-specific recognition sites. MIPs play an important role in increasing the adsorption ability towards the template and are utilized frequently in various fields, e.g. as stationary phase for HPLC[(12-14)]. Using MIP as the stationary phase in liquid chromatography is the first kind of analytical application.

Sulfapyridine and sulfadimethoxine (Figure 1) are commonly used sulfonamides but adverse effects of the sulfonamides are numerous and may involve nearly all organ systems. In order to ensure the safety, the Depart-

* Author for correspondence. Fax: +886-4-8871774;
E-mail:d8844001@yahoo.com.tw

Journal of Food and Drug Analysis, Vol. 16, No. 4, 2008



(a) Sulfapyridine (SPD) (b) Sulfadimethoxine (SDM)

Figure 1. Chemical structures of the sulfapyridine and sulfadimethoxine.

ment of Health, Executive Yuan (TAIWAN) adopted a maximum sulfonamide residue level (MRL) of 0.1 ppm in foods, including meat and milk[15]. Because sulfapyridine and sulfadimethoxine are structurally related to compounds, they are ideal candidates for MIP studies.

In this paper we describe liquid chromatography with UV detection analysis of the molecular imprinting, where interactions between sulfapyridine (template) and methacrylic acid (MAA, functional monomers) occur at a low temperature of 4°C. That is to say, this method was applied to the investigation of the MIP template systems, namely, sulfapyridine, which was imprinted in methacrylic acid–ethyleneglycol dimethacrylate (MAA-EGDMA, crosslinker) network copolymers.

The analysis presented provides a means for separating sulfapyridine and sulfadimethoxine, and allows for capacity factor estimation of selective recognition sites in a polymer prepared with a given monomer/template composition. In our study, two sulfonamides were determined in less than 11 min using MIP as the stationary phase. This method could provide a valuable tool for rapid investigation of the molecular imprinting systems.

Table 1. Polymerization conditionsa of the monomeric mixtures

Polymer	Monomer	Cross-linker (mol%)	Percentage of monomer (mol%)	Percentage of template (mol%)
P1	MAA	EGDMA(80)	15	5
P2	MAA	EGDMA(85)	10	5
P3	MAA	EGDMA(90)	5	5
P4	MAA	EGDMA(93)	5	2
P5	MAA	EGDMA(95)	3	2
P6	MAA	EGDMA(95)	5	0

[a] The polymerizations were performed with MAA as the functional monomer and EGDMA as the crosslinker. Acetone was used as solvent.

Polymerization was carried at 4°C under UV radiation for 2 h.

MATERIALS AND METHODS

I. *Reagents and Chemicals*

Chemicals were of analytical grades, and all solvents are of HPLC grades. Sulfapyridine and sulfadimethoxine were purchased from Sigma (St. Louis, MO, USA). Ethylene glycol dimethacrylate (EGDMA, 98%) and methacrylic acid (MAA, 99%) were purchased from Merck (Darmstadt, Germany). EGDMA and MAA were distilled to remove the inhibitors prior to polymerization. Chloroform, acetic acid (GC grade) and 2.2'-Azo-bisisobutyronitrile (AIBN) were purchased from TCI (Tokyo, Japan). Acetronitrile, ethanol, methanol, acetone, phosphoric acid and sodium phosphate were purchased from TEDIA (Fairfield, OH, USA). Water was double de-ionized through the Milli-Q system (Millipore, Bedford, MA, USA).

II.*Preparation of Molecularly Imprinted Polymer*

The stationary phase of HPLC was directly prepared by bulk polymerization technique. The detailed recipes were given in Table 1. All the imprinted polymers were prepared in a similar way with methacrylic acid as the functional monomer and EGDMA as the cross-linker. In a conical Erlenmeyer flask, a solution was prepared by dissolving the template into 10 mL acetone. Then, MAA, EGDMA and free-radical initiator (AIBN) were added under sonication in water bath until a clear solution was obtained. The mixture was deoxygenated by a stream of nitrogen for 5 min and the flask was sealed and placed under a UV-lamp (365 nm, 100 W) at 4°C for 2 h. Following polymerization, the solvent was removed. The hard polymers were dried in a vacuum oven for 24 h at room temperature. Polymers were then ground to the required size (11~25, 25~44 μm) using a laboratory mortar grinder. The non-imprinted molecular system for control experiments was prepared identically to the process described above, except for the addition of template molecule.

III. *Apparatus*

The HPLC system used was composed of a JASCO PU-2080 pump, UV-2075 detector (both from JASCO, Tokyo, Japan), Rheodyne 7725 syringe injector with 20 μL loop (Rheodyne, Cotati, CA, USA) and Peak ABC Chromatography Workstation Ver.2.10 integrator for data analysis peak integration. This system assured that all the UV-absorbing components are detected, if present in sufficient quantity. A pH meter (Hunna, model HI 9017) with 0.01 precision was used.

IV. *HPLC Analysis*

The obtained polymers were packed into a stainless-steel column (150 mm × 4.6 mm i.d.) by a slurry packing technique with methanol (30 mL) as slurry solvent and acetone as packing solvent using an air-driven fluid pump. The particle content in each column was approximately 3.32 g. The packed columns were washed at 1 mL/min with methanol–acetic acid (9:1, v/v) until a stable baseline at 272 nm was reached.

Solution or mixture of sulfapyridine and sulfadimethoxine prepared in acetone was injected for analysis in a total volume of 20 μL and eluted isocratically at a flow-rate of 1.0 mL/min. Column void volume was measured by eluting with toluene, and absorbance was recorded at 272 nm. The mobile phase was prepared by adjusting the pH of 21mM sodium phosphate solution and mixing is with acetonitrile to the desired proportion. Each elution was repeated three-four times to assure the reproducible chromatogram.

The separation factor (α) and retention factor (k) were calculated as follows: α was determined using the relationship $\alpha = k_{SPD} / k_{SDM}$, where k_{SPD} and k_{SDM} are the retention factors of sulfapyridine and sulfadimethoxine on the MIP, respectively. The retention factors were determined as $k_{SPD} = (t_{SPD}–t_o) / t_o$ and $k_{SDM} = (t_{SDM}–t_o) / t_o$, where t_{SPD} and t_{SDM} are the retention time of sulfapyridine and sulfadimethoxine, respectively, and t_o was the elution time corresponding to the column void volume.

V. *Evaluation of the Adsorption Capacity*

HPLC analysis was employed to determine the adsorption capacity of sulfapyridine and sulfadimethoxine after the adsorption experiments. The concentrations of sulfapyridine and sulfadimethoxine standard solutions were 10 mM. The imprinted or non-imprinted polymeric receptor (0.1 g) was incubated in the standard solution and rotary for 12 h at 4°C. The mixture was centrifuged at 2000 × g for 10 min. The precipitate was transferred into a 5.0 mL volumetric flask and the amount of sulfapyridine or sulfadimethoxine in the liquid phase was analyzed by HPLC with UV detection. The adsorption percentage was then calculated.

VI. *Preparation of Standard and Sample Solutions*

Stock standard solutions of sulfapyridine and sulfadimethoxine were prepared at concentration of 1.0 mg/mL in acetonitrile. Magnetic stirring for 30 min was required to complete dissolution. The standard solution was prepared within the studied range of 0.2~0.8 mg/mL in the same solvent by appropriate dilution of the stock solution.

Calibration graphs were constructed by plotting peak area against the concentration of sulfapyridine and sulfadimethoxine under optimum conditions. Linear plots were obtained in the concentration range of 0.2~0.8 mg/mL for sulfapyridine and sulfadimethoxine.

A commercial pharmaceutical preparation was assayed. Tablets of each of sulfapyridine and sulfadimethoxine were purchased from a local pharmacy. The SPD-containing tablets have the ingredients of stearic acid, cornstarch, magnesium stearate, and cellulose while SDM-containing tablets have the ingredients of cornstarch, magnesium stearate, hydroxypropyl cellulose and microcrystalline cellulose.

The sample solutions were prepared by grinding tablets to fine powder using a mortar and pestle and the powder was transferred through a funnel into a 100 mL volumetric flask followed by washing with 50 mL acetonitrile. The contents of the flask were stirred magnetically for 1 h, after which the stir bar was removed using a magnetic rod. The solution was diluted with sodium phosphate solution and sonicated for 30 min to completely dissolve sulfapyridine and sulfadimethoxine. Undissolved inert ingredients were removed by filtering through Whatman no. 42 filter paper. The theoretical concentrations of the test solutions of SPD and SDM tablets were 0.5 mg/mL of sulfapyridine and sulfadimethoxine, respectively.

The precision of the method was evaluated by analyzing SPD-containing and SDM-containing test solution six times on the same condition, and then calculating the relative standard deviation (RSD) values of the recovery results. The recoveries compared to the label value were calculated.

RESULTS AND DISCUSSION

I. *Selectivity of the Molecularly Imprinted Polymers (MIPs)*

The polymers were prepared as discussed in the experimental part. Processing at low temperature (4°C) was performed to confirm the hydrogen bonding between template and functional monomers. The molecular recognition ability of the sulfapyridine-imprinted polymer was evaluated by liquid chromatography. In order to obtain highly selective MIP, formation of stable complexes (Figure 2) between templates and functional monomers in the reaction mixture is crucial. Therefore the following properties were investigated and optimized: influence of cross-linker, functional monomer and template content on the synthesized polymers. Furthermore, considering the retention and selectivity factors reported in Table 2, increasing EGDMA concentration in the monomer mixture to the 95 mol% (P5) led to a significant increase in the selectivity factors. However, decreasing the EGDMA concentration (P1~P4) results in lower selectivity factor, which might result from the reduction of cross-linking level required for the preservation of the recognition structure.

As shown in Figure 3 and Table 3, the retention time of imprinted molecule, SPD, was longer than that of SDM,

Journal of Food and Drug Analysis, Vol. 16, No. 4, 2008

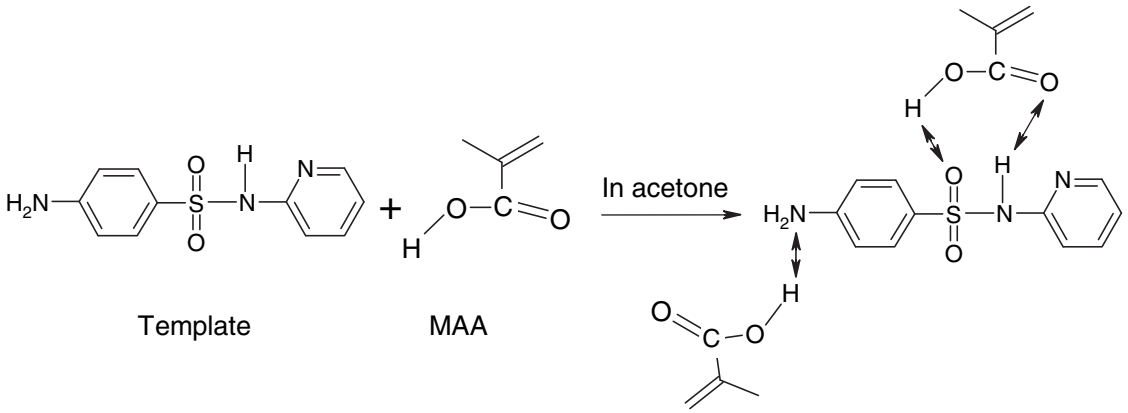


Figure 2. Schematic representation of pre-complexation imprinting.

Table 2. The chromatographic data, retention time and separation factor (α) of SDM and SPD used P1~P6 as the stationary phase

	Retention time		Retention factor		Separation
Polymer	SDM	SPD	SDM	SPD	factor (α)
P1	No resolution		----------		---------
P2	No resolution		----------		---------
P3	4.30	8.58	4.66	10.29	2.20
P4	4.29	8.67	4.65	10.41	2.23
P5	3.89	8.24	4.11	9.84	2.39
P6[a]	1.07	1.09	0.41	0.43	1.04

[a] P6 was used as blank polymer

indicating that imprinted molecules exert stronger interaction with the MIPs. A separation factor of 2.39~2.74 was obtained. Thus, it is quite obvious that the retention time and retention factor of the imprinted molecules were higher than SDM in the MIPs, indicating that the strong interaction between MIPs and imprinted molecules resulted in the separation of SPD and SDM. Moreover, molecular structure analyses indicated that both SPD and SDM contain benzenesulfonylamino group which is matching with the functional groups in MIPs. However, in addition to benzenesulfonylamino group, SDM also comprises two methoxy side chain groups with similar hydrophobic intensity. For this reason, methoxy side chain groups can't interact with the cavities of MIPs and the space structure

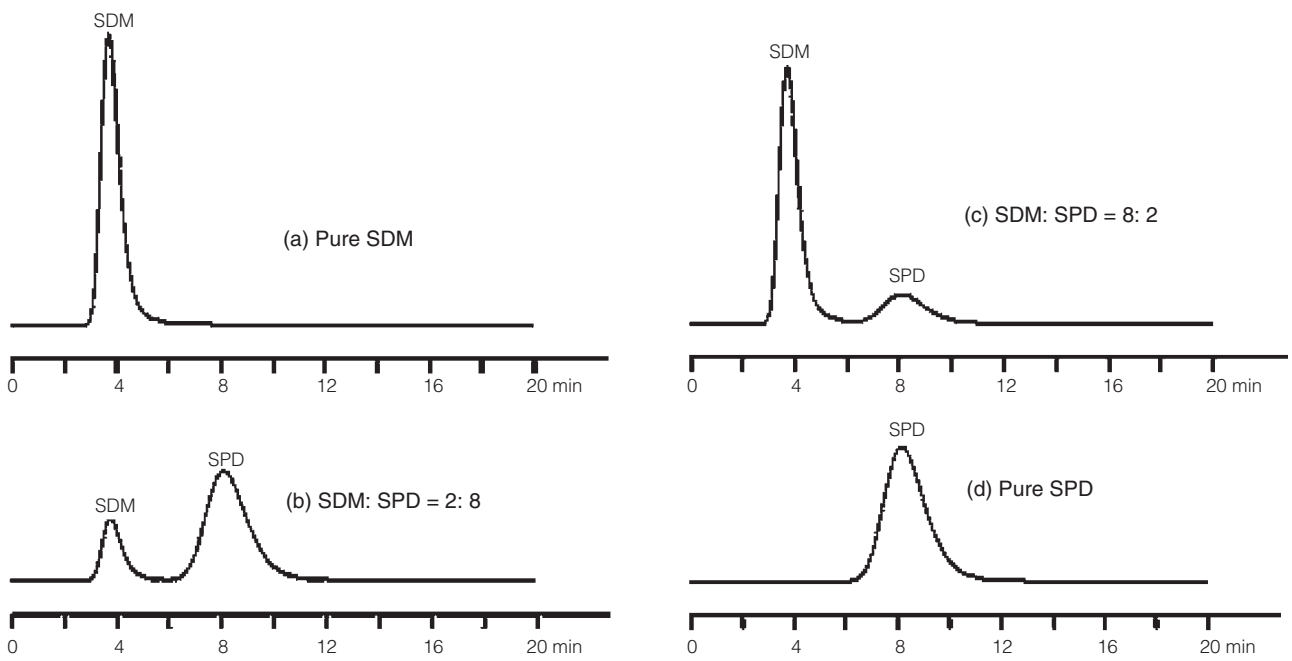


Figure 3. Chromatograms for SDM and SPD on the imprinted polymers.
Conditions: mobile phase = buffer solution/acetonitrile (3/2, v/v), injection volume = 20μL, column dimensions = 150 mm×4.6 mm i.d. Analyte detection was performed at λ = 272 nm. The void volume marker was toluene.

of SDM will not match with the MIPs of SPD. Therefore, the MIPs of SPD can separate SPD from SDM effectively. Prepared MIPs of SPD polymerized by low temperature UV-inducement had the characteristics of selective specificity to the imprinted molecules, not only due to the complementary functional groups, but also due to the matched space structures; hence they can be successfully employed for molecular recognition studies. Furthermore, comparing the retention behaviors of MIP and blank polymers, we observed that MIP always had more definite evidence of imprinting than the blank polymers. In addition, retention factors of the sulfapyridine and sulfadimethoxine on the blank polymer are negligible ($k < 0.1$), leading to no imprinting effect.

II. *Effect of the Mobile Phase on Liquid Chromatography*

The binding of the template sulfapyridine and the closely related to sulfadimethoxine was tested using several mobile phases in liquid chromatography in order to select suitable solvents for elution. It is well known that the chromatographic behavior of a system can be changed with the mobile phase composition, and this is often manipulated to improve the separation performance in chromatography. In addition, to predict and optimize the retention properties of the components to be separated as a function of the mobile phase composition, five different mobile phases were tested. The results obtained from different mobile phase compositions are summarized in Table 4. These findings clearly indicated that the separation selectivity for the two compounds investigated is dependent on the mobile phase. The type of mobile phase was found to have significant effect on the retention of solutes. Moreover, Table 4 apparently showed that the optimization proportion of buffer solution to the acetonitrile was 3:2 (v/v). The molecular recognition mechanism of the imprinted polymer is based on the hydrogen bonding between the carboxyl group on binding sites present in the polymeric matrix and the template molecule; strong ionic interactions can also be formed with basic functional groups (secondary amine). It follows that the polarity of the mobile phase directly influences the parti-

Table 3. Chromatographic data, retention time and separation (α) of SDM and SPD used P5 as the stationary phase. Composition of solution with a total of 1g/L

Solution No.	Concentration in sample		Retention time,min (retention factor)[a]		α
	SDM	SPD (g/L)	SDM	SPD	
1	0.5	0.0	3.90	------	
2	0.2	0.8	3.89 (4.12)	8.46(10.14)	2.46
3	0.3	0.7	3.90(4.13)	8.63 (10.36)	2.50
4	0.4	0.6	3.56(3.69)	8.47(10.14)	2.74
5	0.5	0.5	3.89(4.11)	8.24(9.840)	2.39
6	0.6	0.4	4.01(4.27)	8.57(10.28)	2.40
7	0.7	0.3	3.94(4.19)	8.51(10.20)	2.43
8	0.8	0.2	3.90(4.13)	8.49(10.17)	2.46
9	0.0	0.5	-------	8.35	

[a]The void volume of the column was about 31.46% by the injection of toluene and the retention time of toluene was 0.76 min.

Table 4. Effect of mobile phase on liquid chromatography

Mobile phase 23mM buffer: Acetonitrile (v/v)	Retention time		Separation (R_{SPD}-R_{SDM})	Separation Factor (α)
	SDM[a]	SPD[b]		
1:2 (pH 3.99 ± 0.01)	No resolution		----------	----------
2:2 (pH 3.96 ± 0.01)	No resolution		----------	----------
3:2 (pH 3.72 ± 0.01)	3.89	8.24	4.35	2.39
4:2 (pH 3.75 ± 0.01)	4.59	9.24	4.65	2.21
5:2 (pH 3.73 ± 0.01)	4.51	9.02	4.51	2.20

[a]pKa= 6.0 [b]pKa= 8.4

tion of the template in the stationary phase. In this study, the mobile phase was a mixture of buffer solution and acetonitrile. Polar substances were used to weaken the interaction between target molecules and the stationary phase so that target molecules can be released from the imprinting cavity of the stationary phase. It is believed that acetonitrile molecules were likely to bind onto the stationary phase, so that the mobile phase in which increasing acetonitrile present in the mobile phase causes decrease in the retention time.

III. *Adsorption of Sulfapyridine and Sulfadimethoxine*

The imprinted sulfapyridine polymer was prepared using EGDMA as the cross-linking agent which efficiently adsorbed sulfapyridine when contacted with sulfapyridine in the buffer/acetonitrile mixture (3/2, v/v). The adsorption was so complete that 82% of the initially charged sulfapyridine was adsorbed to the imprinted sulfapyridine polymer (Figure 4). By this procedure, 82% of the sulfapyridine was removed from the liquid phase in equilibrium. In contrast, non-imprinted polymer (blank polymer) was much less active. Sulfadimethoxine, whose chemical structure is close to sulfapyridine (Figure 1), was also adsorbed by the molecularly imprinted polymer (Figure 4). Sulfadimethoxine has primary and secondary amines groups which can form a strong hydrogen-bonding complex with MAA or a remaining carbonyl group. This binding may coordinate two or more functional groups of the polymer in a favorable position. However, sulfadimethoxine seemed to have difficulty in diffusing into the molecularly imprinted sites; it would encounter interference due to steric hindrance. Therefore binding strength between the MIP site and sulfadimethoxine is weaker than binding with the well-positioned MIP and sulfapyridine functional groups. This causes a relatively low adsorption of sulfadimethoxine from the MIP. Thus, the selectivity for sulfapyridine adsorption against sulfadimethoxine adsorption was enormously improved by the molecular imprinting technique.

IV. *Quantitative Analysis of Commercial SPD and SDM Tablets*

Chromatogram peaks were identified in appropriate solutions of the constituents under investigation. With buffer-acetonitrile (3:2, v/v) as mobile phase, the retention time obtained was approximately 8.35 min for sulfapyridine and 3.90 min for sulfadimethoxine. The complete analysis time for a sample was less than 11 min. The peaks of analyzed components were extremely well resolved and did not reveal any overlap. Short assay time is quite important during the analysis of a series of samples.

Preparation of the calibration graphs using polynomial regression has led to superior analytical results during validation. A calibration graph was established for sulfapyridine and sulfadimethoxine using seven concentrations (0.2~0.8 mg/mL). The r value (correlation coefficient) of this graph was 0.9989 for sulfapyridine and 0.9991 for sulfadimethoxine. Linearity is maintained over a wide range of concentrations. The recoveries, accuracy and relative standard deviation, are listed in

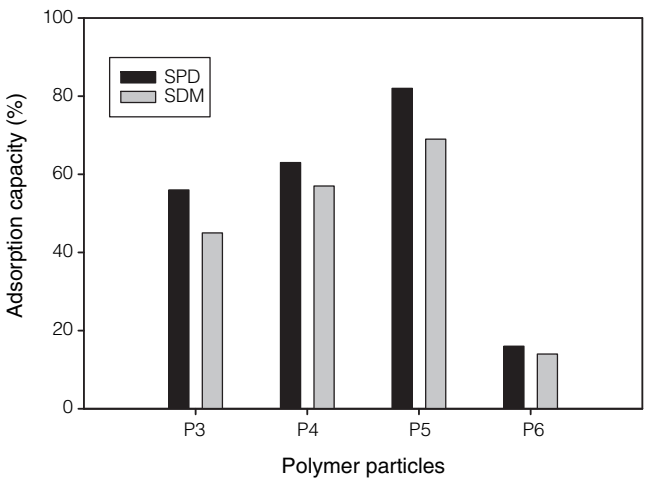


Figure 4. Different adsorption results of SPD and SDM from the MIPs (P3~P6) under 4°C polymerized. P6 used as blank polymer.

Table 5. Results of amount found, relative standard deviation(RSD) and recoveries

Sample	Amount found (mg/tablet)	RSD(%)	Recovery(%)
SPD-Tablet (50 mg/tablet)			
SPD-Tablet 1	49.83	2.23	99.66
SPD-Tablet 2	50.15	4.15	100.30
SPD-Tablet 3	49.92	3.02	99.84
SDM-Tablet (50 mg/tablet)			
SDM-Tablet 1	50.21	1.87	100.42
SDM-Tablet 2	50.35	3.56	100.70
SDM-Tablet 3	49.85	4.23	99.70

Table 5. The recovery was 99.66~100.30% for sulfapyridine and 99.70~100.70% for sulfadimethoxine. Values for both tablets were within 95~105% of the label value. These results with the declared constituent concentrations and statistical analysis show that they are characterized by high repeatability.

In our opinion, this is a rapid and simple method of choice for sulfapyridine and sulfadimethoxine determination applying molecularly imprinted polymer. On the other hand, it does not require derivatization and complex instrumentation.

CONCLUSIONS

The non-covalent molecular imprinting of sulfapyridine using methacrylic acid functional monomer has been reported. Besides, suitable imprinted-stationary phases for the separation and the selective recognition of sulfapyridine and sulfadimethoxine have been provided.

HPLC method with UV detection has been developed for the determination of sulfapyridine and sulfadimethoxine in tablets. Our results obtained successfully in the validation process and drug analysis are encouraged indicating that the system is suitable for routine tests in the future. Under the conditions established, there is no interference. In addition, good separation is achieved for the constituents of interest. The method which is characterized by good precision and accuracy enabled successful separation and quantitative determination of sulfapyridine and sulfadimethoxine in tablets.

ACKNOWLEDGEMENTS

The authors wish to thank MingDao University and National Formosa University for financial support of the study.

REFERENCES

1. Fuh, M. R. and Chan, S. A. 2001. Quantitative determination of sulfonamide in meat by liquid chromatography- electrospray – mass spectrometry. Talanta. 55: 1127-1139.
2. Haller, M. Y., MÜller, S. R., McArdell, C. S., Alder, A. C. and Suter, M. J. F. 2002. Quantification of veterinary antibiotics(sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. J. Chromatogr. A 952: 111-120.
3. Heinig, K. and Henion, J. 1999. Fast liquid chromatographic–mass spectrometric determination of pharmaceutical compounds. J. Chromatogr. B 732: 445-458.
4. Jen, J. F., Lee, H. L. and Lee, B. N. 1998. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography. J. Chromatogr. A 793: 378-382.
5. Patthy, M. 1983. Trace analysis of sulfaquinoxaline in animal tissues by high-performance liquid chromatography. J. Chromatogr. B 275: 115-125.
6. Reeves, V. B. 1999. Confirmation of multiple sulfonamide residues in bovine milk by gas chromatography–positive chemical ionization mass spectrometry. J. Chromatogr. B 723: 127-137.
7. Zayas-Blanoco, F., Garcia-Falcon, M. S. and Simal-Gandara, J. 2004. Determination of sulfamethazine in milk by solid phase extraction and liquid chromatographic separation with ultraviolet detection. Food Control. 15: 375-378.
8. Theodoridis, G., Zacharis, C. K., Tzanavaras, P. D., Themelis, D. G. and Economou, A. 2004. Automated sample preparation based on the sequential injection principle Solid-phase extraction on a molecularly imprinted polymer coupled on-line to high-performance liquid chromatography. J. Chromatogr. A 1030: 69-76.
9. Kobayashi, T., Murawaki, Y., Reddy, P. S., Abe, M. and Fujii, N. 2001.Molecular imprinting of caffeine and its recognition assay by quartz-crystal microbalance, Anal. Chem. Acta 435: 141-149.
10. Otsu, T., Yoshida, M. and Kuriyama, A. 1982. Living radial polymerizations in homogeneous solution by using organic sulfides as photoiniferters. Polym. Bull. 7: 45-50.
11. Otsu, T. 2000. Iniferter concept and living radical polymerization. J. Polym. Sci. Part A: Polym. Chem. 38: 2121-2136.
12. Kugimiya, A. and Takeuchi, T. 2001. Surface plasmon resonance sensor using molecularly imprinted polymer for detection of sialic acid, Biosens. Bioelectron. 16: 1059-1062.
13. Hwang, C. C. and Lee, W. C. 2001. Chromatographic resolution of the enantiomers of phenylpropanolamine by using molecularly imprinted polymer as the stationary phase. J. Chromatogr. B 765: 45-53.
14. Hwang, C. C. and Lee, W. C. 2002. Chromatographic characteristics of cholesterol-imprinted polymers prepared by covalent and non-covalent imprinting methods. J. Chromatogr. A 962: 69-78.
15. No.22. Veterinary Drug Residue Limits in Foods. 2006. Public Announcement by Department of Health, Executive Yuan, Taiwan.