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Development and characterization of an immunoaffinity column for the selective extraction of bisphenol A from serum samples

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Abstract

An immunoaffinity column (IAC) was developed by covalently coupling polyclonal antibodies against estrogenic bisphenols to CNBr-activated Sepharose 4B. The IAC showed high affinity for bisphenol A, while phenol was barely retained. Proteins in the sample matrix showed little nonspecific adsorption on the column. The best binding solvent for bisphenol A was found to be 0.01 mol l^{-1} phosphate-buffered saline (PBS) and the optimal operating temperature was 4 °C. The bound bisphenol A could be quantitatively recovered by 1 ml of methanol–water (80:20) with an average recovery of 91.8% and a relative standard deviation of 7.1% (n=6). The immunoaffinity column has been successfully used for the isolation and purification of bisphenol A from serum samples.
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1. Introduction

2,2-(4,4-Dihydroxydiphenyl)propane, commonly known as bisphenol A (BPA), is the main intermediate in the production of polycarbonate and epoxy resins [1]. Due to the wide use of these plastic materials, especially in food packing [2,3] and dental composites [4,5], human are very likely to be exposed to bisphenol A. A study by Olea et al. [4] determined 90–931 μg of bisphenol A in saliva samples collected during a 1-h period after treatment with a sealant based on bisphenol A diglycidylether methacrylate. In another study concerning bisphenol A release from lacquer coatings in food cans [3], it was found that the amount of bisphenol A extracted from the liquid of canned foods ranged from 0 to 30 μg per can. It has been reported that bisphenol A has estrogenic activity [6] and may disrupt the endocrine system of humans and wildlife [7], therefore highly sensitive and selective methods are needed to assess the potential association between human exposure to bisphenol A and possible adverse health effects.

Gas chromatography–mass spectrometry (GC–MS) or high-performance liquid chromatography (HPLC) with ultraviolet (UV), fluorescence (FLU) or mass spectrometric (MS) detection are the most commonly used techniques to determine bisphenol A in beverages [8], serum [9], plasma [10] and environmental water [11] samples. Since bisphenol A is usually present in samples at extremely low levels

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and most of the sample matrices are very complicated, especially the biological samples, a pretreatment procedure based on solid-phase extraction (SPE) or liquid–liquid extraction (LLE) is often required to remove the interfering matrix components and concentrate the targeted bisphenol A. However, these approaches generally consist of quite a few steps and consume a large amount of organic solvents.

Here we present a new sample pretreatment method based on an immunoaffinity column (IAC). Due to the high selectivity of the immunoaffinity reaction, the column shows specific affinity for bisphenol A. The binding conditions and elution protocols were all optimized. Serum samples spiked with bisphenol A were purified with the IAC and the bound bisphenol A was recovered quantitatively. The IAC proved to be a simple, practical and reliable sample pretreatment method for biological fluid samples.

2. Experimental

2.1. Reagents and instruments

CNBr-activated Sepharose 4B was purchased from Pharmacia Biotech (Sweden). 4,4-Bis(4-hydroxyphenyl)valeric acid (BHPVA) and bisphenol A (BPA) were both obtained from Aldrich (Milwaukee, WI, USA). Human serum albumin (HSA) was purchased from Sino-American Biotech (China). Bovine serum albumin (BSA) and sodium azide were obtained from Sigma (St. Louis, MO, USA). Tris-(hydroxymethyl)-amine-methane (Tris) was purchased from Beijing Kangte Physichemical Hightech (China). All the solvents used were of analytical grade or better.

Stock solutions of bisphenol A (1000 $\mu g\ ml^{-1}$) and phenol (1000 $\mu g\ ml^{-1}$) were both prepared in acetonitrile and stored at 4 °C until use.

A HZQ-F incubator shaker was supplied by Harbin Donglian Electronic Technology Development (China). An Econo-Column (0.5×20 mm) manufactured by Bio-Rad (Richmond, CA, USA) was used as the immunoaffinity column cartridge. A Cary 1E UV–Vis spectrophotometer from Varian (USA) was used to scan the absorption curve of bisphenol A and measure the absorbance of the protein solution. A Biological LP UV detector supplied by Bio-Rad was used to monitor the on-line UV signals of proteins eluted from the immunoaffinity column. An LG4-301 chromatography refrigerator from the Wanbao Group (China) was used to perform the loading of samples onto the column at 4 °C.

2.2. HPLC parameters

A HP1100 HPLC equipped with a diode-array detector (DAD) and a fluorescence detector (FLD) was used for the detection and quantification of bisphenol A and phenol. The column was a Zorbax Eclipse XDB-C_8. Acetonitrile–water (60:40) was used as the mobile phase at a flow-rate 0.5 ml min^{-1}. The detection parameters for the FLD were λ_{ex} = 230 nm, λ_{em} = 315 nm, and PMT gain 14. For the DAD detector, the signals were recorded at 227 and 280 nm.

2.3. Production and purification of polyclonal antibodies

The polyclonal antibody against bisphenol A was produced according to the procedure described in a previous study [12]. In brief, 4,4-bis(4-hydroxyphenyl)valeric acid was coupled to BSA to prepare the complete antigen, which was used to immunize rabbits. The obtained antiserum was purified using a saturated ammonium sulfate method [13]. To 2 ml of antiserum diluted with the same volume of saline, 4 ml of saturated ammonium sulfate solution (pH adjusted to 7.4) was added dropwise under gently magnetic stirring at 4 °C. The obtained turbid solution was transferred to a centrifuge tube and allowed to stand for more than 1 h or overnight. Then the solution was centrifuged at 13 000 rpm for 10 min at 4 °C. The supernatant was discarded and the precipitates were dissolved in 2 ml of saline. To this solution, 1 ml of saturated ammonium sulfate solution was added under magnetic stirring and allowed to stand for 1 h. Then the solution was centrifuged at 13 000 rpm for 10 min at 4 °C and the supernatant was discarded. The precipitates were dissolved in 1 ml of saline and dialyzed against 0.01 mol l^{-1} phosphate-buffered saline (PBS) until no sulfate ion could be detected in the dialysis solution

with 0.5 mol l^{-1} $BaCl_2$ acidified with HCl. The immunoglobulin (IgG) concentration in the obtained solution was measured with a 1 cm cell using a Cary 1E UV–Vis spectrophotometer and calculated based on the UV absorption difference between 280 and 260 nm. The formula used for the calculation was [14]

$$C_{\text{protein}}\ (\text{mg ml}^{-1}) = 1.45A_{280\ \text{nm}} - 0.74A_{260\ \text{nm}}$$

2.4. Generation of the immunoaffinity column

The immunosorbent was produced according to the manufacturer's instructions and related literature [13–15]. Briefly, the purified antibody solution containing 14 mg of the antibody protein obtained as described in Section 2.3 was first dialyzed against the coupling buffer (0.1 mol l^{-1} $NaHCO_3$, pH 8.3, containing 0.5 mol l^{-1} NaCl) overnight and the solution volume was brought up to 5 ml. 0.5 g freeze-dried powder of CNBr-activated Sepharose 4B was weighed into a clean beaker and suspended in 40 ml of 1 mmol l^{-1} HCl to remove the protecting groups. The suspension was transferred to a sintered glass filter (pore size 4–7 μm) and the gel was washed with about 200 ml of 1 mmol l^{-1} HCl in three to four fractions. Then the outlet of the filter was sealed and the antibody solution was added to the gel. The obtained gel solution was pipetted into a conical flask and the reaction proceeded in the incubator shaker at 20 °C for 3 h.

The resulting immunosorbent was transferred to an Econo-Column cartridge (0.5×20 mm) and the effluent was measured with an on-line UV detector and collected in a measuring cylinder. The column was washed with the coupling buffer until the UV absorbance of the effluent at 280 nm was zero. The absorbance of the well-mixed collected solution was measured with a Cary 1E UV spectrophotometer to determine the protein content. The excess active groups on the immunosorbent were capped with the blocking buffer (0.1 mol l^{-1} pH 8.0 Tris–HCl buffer). To ensure complete blocking, the outlet of the column was sealed and the gel was immersed in two column volumes of the blocking buffer and allowed to stand for 2 h. Then the column was washed alternatively with no less than five column volumes of 0.1 mol l^{-1} pH 4.0 acetate buffer containing 0.5 mol l^{-1} NaCl and 0.1 mol l^{-1} pH 8.0 Tris–HCl buffer containing 0.5 mol l^{-1} NaCl for three cycles. Finally, the column was washed with 0.01 mol l^{-1} PBS and stored in 0.01 mol l^{-1} PBS containing 0.02% NaN_3 at 4 °C until use. The coupling efficiency was estimated by comparing the protein content of the above collected solution after the coupling reaction and the original antibody solution.

2.5. Optimization of the binding conditions of bisphenol A on the immunoaffinity column

To determine the optimal binding conditions, solutions of mixtures of bisphenol A and phenol were prepared in different solvents, including 10% acetone, pure water, 0.01 mol l^{-1} PBS and the coupling buffer (0.1 mol l^{-1} $NaHCO_3$, pH 8.3, containing 0.5 mol l^{-1} NaCl). The influence of nonionic surfactants such as Tween-20 and Triton X-100 on the binding efficiencies was also investigated.

Firstly, the immunoaffinity column was conditioned with no less than two column volumes of one of the above solvents, then 1 ml of a solution of a mixture of bisphenol A (4.0 μg ml^{-1}) and phenol (9.0 μg ml^{-1}) in the same solvent was loaded onto the column. At the moment the mixture solution entered the gel, the effluent from the column was collected in 1.5 or 0.5 ml Eppendorf tubes with 1 or 0.5 ml per tube. Then the column was washed with 1 ml×10 of the loading solvent and the eluate was also collected in the same manner. All the solutions collected in the Eppendorf tubes were vortex mixed before they were injected into the HPLC to determine the concentrations of phenol and bisphenol A in each fraction. After the experiment for each solvent, methanol–water (80:20) was used to remove the bisphenol A remaining on the column. The binding experiments of the IAC at 4 °C were carried out in a 4 °C chromatography refrigerator.

2.6. Breakthrough volume and recovery test

The immunoaffinity column was conditioned with 0.01 mol l^{-1} PBS at 4 °C. Then 10 ml of the mixture solution of bisphenol A and phenol in 0.01 mol l^{-1} PBS was applied to the column and the effluent was

collected and measured by HPLC until bisphenol A appeared in the effluent.

In the 4 °C chromatography refrigerator, 4.0 ml of 100 ng ml^{-1} bisphenol solution was applied to the well-equilibrated immunoaffinity column. The effluent was discarded. Then 1 ml of pure water was used to wash the column and the eluate was discarded. Thereafter, the immunoaffinity column was taken out of the refrigerator and the bound bisphenol A was eluted with methanol–water (80:20) at room temperature. The fraction that contained bisphenol A was collected and evaporated to dryness on a 50 °C water bath under a gentle stream of nitrogen. The residue was redissolved in 400 μl of acetonitrile–water (60:40) (the HPLC mobile phase) and injected into the HPLC for quantification. A control solution of 1.0 μg ml^{-1} bisphenol A in acetonitrile–water (60:40) was prepared and analyzed by HPLC to determine the recovery.

2.7. Interference of matrix proteins

Human serum albumin was used to investigate the influence of proteins in the sample matrix on the properties of the immunoaffinity column. Four milliliters of a synthesized solution of 1.0 μg ml^{-1} bisphenol A and 10.0 mg ml^{-1} human serum albumin in 0.01 mol l^{-1} PBS was applied to the column at 4 °C and the effluent was collected in a 1.5 ml Eppendorf tube at 1 ml per tube. Then 1 ml of pure water was used to wash the column and the eluate was also collected. Thereafter, the column was taken out of the chromatography refrigerator and methanol–water (80:20) was used to elute the bound bisphenol A at room temperature; the eluate was also collected. The UV absorbance of the collected fractions was measured to determine the protein content of each fraction.

2.8. Application to serum samples

Blood collected from BALB/c mice was first allowed to stand overnight at 4 °C before being centrifuged at 3000 rpm for 15 min. Then the supernatant was transferred to another centrifuge tube and centrifuged at 12 000 rpm for 10 min. The obtained supernatant was diluted 1:25 with 0.01 mol l^{-1} PBS and spiked with bisphenol A at a concentration of 100 ng ml^{-1}. For the blank sample, the serum was simply 25-fold diluted with 0.01 mol l^{-1} PBS. The synthesized sample was allowed to equilibrate at 4 °C for more than 1 h before being applied to the immunoaffinity column.

In the 4 °C chromatography refrigerator, 4.0 ml of spiked or unspiked serum sample was applied to the immunoaffinity column that had been preconditioned with 0.01 mol l^{-1} PBS at 4 °C. The following steps were similar to those described in Section 2.6. The recovery of bisphenol A in serum samples was determined by subtracting the result obtained for the unspiked serum sample from that obtained for the spiked serum sample and then comparing the amount of bisphenol A obtained with the result for the control solution.

3. Results and discussion

3.1. Generation of the IAC

The specificity of the polyclonal antibody has been demonstrated in a previously reported competitive inhibition ELISA [12]. Compared with the hapten 4,4-bis(4-hydroxyphenyl)valeric acid, the cross reactivities of the antibody with bisphenol A and phenolphthalein were 53 and 23%, respectively. The cross reactions of phenol, hydroquinol and *p*-hydroxybenzoic acid were all less than 1%.

The coupling efficiency of the polyclonal antibody to the CNBr-Sepharose 4B, defined as the percentage of immobilized antibody accounted for of the original amount [13], was observed to be 86±3% (mean of two coupling experiments). The coupling reactions were performed twice. One was generated from 0.5 g of freeze-dried powder of CNBr-activated Sepharose 4B with a final gel volume of about 2.0 ml, and the column is referred to as IAC_1. The other was generated from 0.8 g of freeze-dried powder, with a final gel volume of about 3.5 ml. The obtained column is referred to as IAC_2.

3.2. Optimization of the binding conditions of bisphenol A on the IAC

In a preliminary study, solutions of mixtures of phenol and bisphenol A in various solvents were

used to assess the performance of the IAC. Before the sample was placed on the IAC, it was necessary to wash and precondition the column with a suitable solvent [15]. It is helpful to wash out possible impurities on the immunosorbent and solvate the surface of the gel beads, making the active binding sites more accessible to bisphenol A in the samples. Using the same solvent to precondition the column and load the sample can minimize the possible interface effect between two different solvents and ensure optimal retention. Such a solvent may also be called a binding solvent. The influence of the properties of the binding solvent, such as polarity, pH, ionic strength and temperature, on the interactions between the affinity ligands and target compounds and the stability of the complex formed were all carefully investigated.

First, different binding solvents, including 10% acetone, pure water, 0.01 mol l^{-1} PBS and the coupling buffer, were tested and compared at room temperature. The elution profiles of phenol and bisphenol A in these solvents are shown in Fig. 1. Fig. 1a and e show the results obtained with IAC_2, the other experiments were conducted on IAC_1. As can be seen in Fig. 1a, in 10% acetone, neither phenol nor bisphenol A was retained on the column. In pure water (Fig. 1b), phenol was still not retained, and bisphenol A did not appear until the phenol was completely eluted. This indicates that, in pure water, bisphenol A is more specifically retained on the column, but an organic solvent has disadvantages for the retention of bisphenol A. Fig. 1c and d show the results for solvents of higher ionic strength and different pH. It can be seen that in 0.01 mol l^{-1} PBS, bisphenol A was bound to the column more strongly than in pure water. Not only did the retention volume of bisphenol A increase, but also the bound bisphenol A was eluted more slowly. However, as shown in Fig. 1d, using the coupling buffer, in which the antibody was linked to Sepharose-4B as the binding solvent, the retention of bisphenol A was similar to the results obtained with pure water. The above results indicate that both ionic strength and pH have a great influence on the binding efficiency of the IAC. Under neutral conditions (pH 7.4) and high ionic strength (0.01 mol l^{-1} PBS), the binding of bisphenol A on the IAC was more stable. Another advantage of the use of a binding solvent of high ionic strength is that it may inhibit the nonspecific adsorption caused by electrostatic attraction [16].

The influence of nonionic surfactants on the retention properties of the immunoaffinity column was also examined. Two types of nonionic surfactant, i.e. Tween 20 and Trition X-100, were tested. It turned out that, in 0.05% Tween 20, phenol and bisphenol A were completely separated (Fig. 1e), but in 0.015 or 0.009% Triton X-100, the two compounds co-eluted (Fig. 1f). The critical micelle concentration (cmc) of Triton X-100 is 0.009% [17]. It was found that, at the cmc of Triton X-100, the separation of the two compounds was slightly better than at 0.015%, but, at both concentrations, bisphenol A was not completely retained. A concentration of 0.025% Tween 20 was also tested. It turned out that, at a lower concentration of Tween 20, the retention efficiency of bisphenol A on the column decreased.

From the above experimental results, it can be seen that 0.01 mol l^{-1} PBS and 0.05% Tween 20 are the preferred binding solvents for the generated immunoaffinity column. Phenol was barely retained on the column in all the solvents tested.

According to the above results at room temperature, 0.01 mol l^{-1} PBS was used to test the influence of temperature on the binding reaction. The immunoaffinity column was installed in the 4 °C chromatography refrigerator. The elution profile was measured in the same way as at room temperature. It was found that the retention volume of phenol was almost the same as at room temperature, but the retention volume of bisphenol A was significantly improved. The peak of bisphenol A was not observed even after 7 ml of effluent volume. This allows thorough washing of the matrix components with the loading solvent or other suitable solvent before the bound bisphenol A is finally eluted.

The affinity interaction between antigen and antibody is generally thought to be a comprehensive combination of various noncovalent bondings, including electrostatic attraction, van der Waals' attractive force, hydrogen bonding, hydrophobicity, etc. [16]. Among the above four types of forces, hydrogen bonding is the most sensitive to the environmental temperature. This is because the formation of hydrogen bonds is an exothermic process. Therefore, operating at low temperature (a cool antibody) is

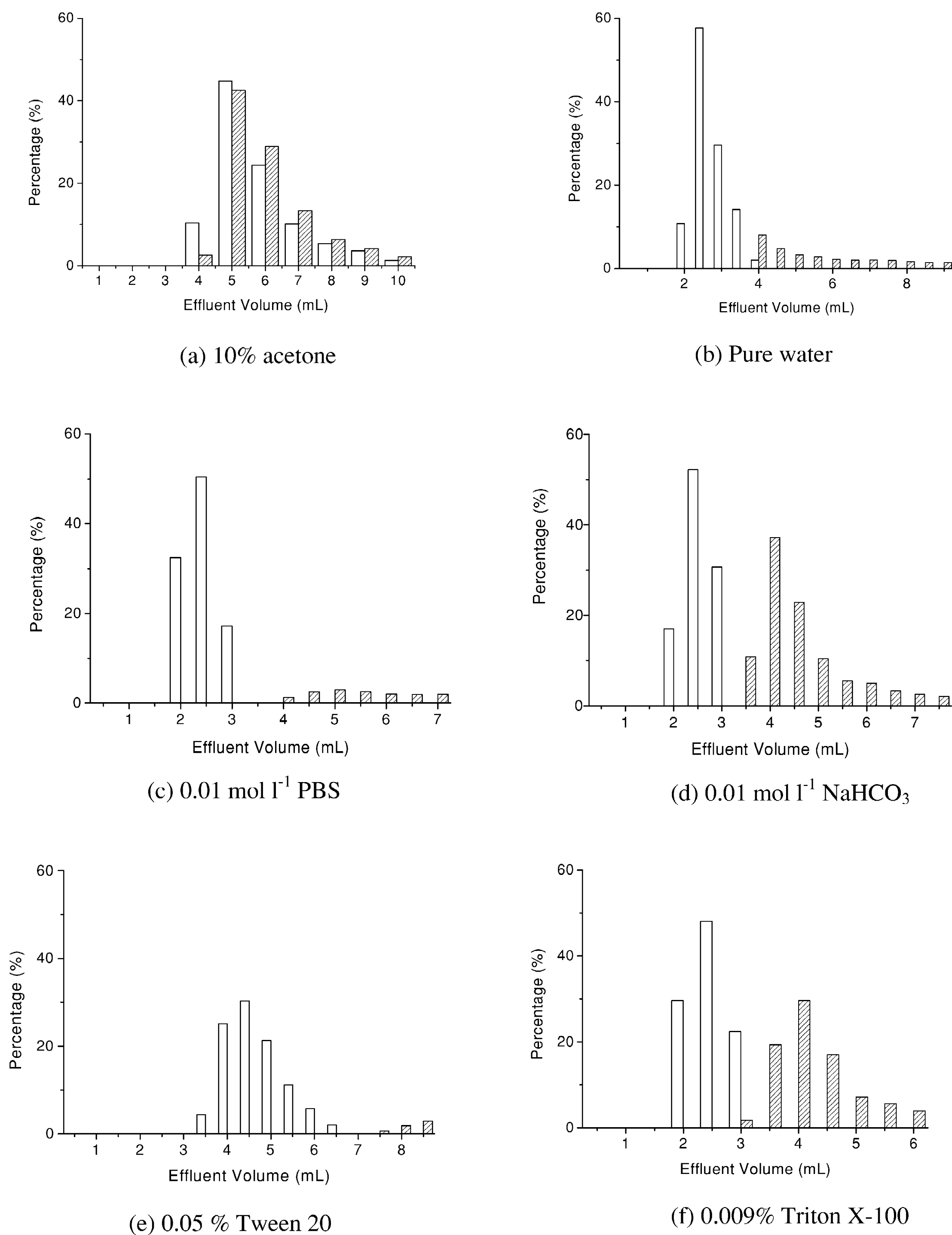

Fig. 1. Elution profiles of bisphenol A and phenol in different loading solvents. (a, e) Results obtained with IAC_2 (gel volume ca. 3.5 ml); the other experiments were all conducted on IAC_1 (gel volume ca. 2.0 ml). All experimental results were obtained at room temperature. (□) Phenol (9.0 μg ml^{-1}); (■) bisphenol A (4.0 μg ml^{-1}).

quite favorable for the stability of the antibody–antigen complex. The above experimental results also indicate that hydrogen bonding was one of the most important forces in the recognition and retention process of bisphenol A on the IAC. Unless otherwise stated, the following results were all obtained with IAC_1 at 4 °C.

3.3. Breakthrough volume and recovery test

The breakthrough volumes of bisphenol A at concentrations of 4.0 and 1.0 μg ml^{-1} were measured and the results are shown in Fig. 2. It can be seen from Fig. 2 that, at a concentration of 1.0 μg ml^{-1}, the breakthrough volume was about 0.5–1.0 ml larger than that at 4.0 μg ml^{-1}. Since the content of bisphenol A in real samples is generally very low (ng ml^{-1} or lower), according to the above results, the sample loading volume was chosen to be 4.0 ml.

After loading of the bisphenol A sample (100 ng ml^{-1} in 0.01 mol l^{-1} PBS), the column was washed with 1.0 ml of pure water to remove any nonspecifically retained components and reduce the salt concentration in the final collected fraction of bisphenol A. The use of a methanol–water or ethanol–water mixture as the eluting solution for IAC has been reported previously [18–20]. In our experiment, it was found that methanol–water (80:20) was a suitable solvent to elute the bound bisphenol A from the column. The average recovery of bisphenol A was found to be 91.8±7.1% ($n=6$).

A control solution was used to measure the loss of bisphenol A during evaporation on a 50 °C water bath under a flow of nitrogen. The recovery was observed to be >97% ($n=3$) compared with the original solution. This demonstrates that the loss during this process is negligible.

To investigate possible contamination from the plastic Eppendorf tubes, two tubes were filled with 1.0 ml of methanol and allowed to stand overnight. Then the methanol was evaporated and the residue was reconstituted in 200 μl of acetonitrile–water (60:40) and analyzed by HPLC. No bisphenol A was found in the extract.

3.4. Effect of the sample matrix on the IAC

To determine if the matrix components of the sample affected the performance of the IAC, HSA samples in 0.01 mol l^{-1} PBS spiked with bisphenol A were applied to the IAC. The elution profiles of the proteins are shown in Fig. 3. The X-axis represents the effluent volume, and the Y-axis the percentage of protein in each fraction of the eluate.

It can be seen from Fig. 3 that when the sample containing HSA passes through the IAC, most of the protein is not retained and passes out of the column directly with the solvent. Washing with 1.0 ml pure water further removes the remaining protein from the

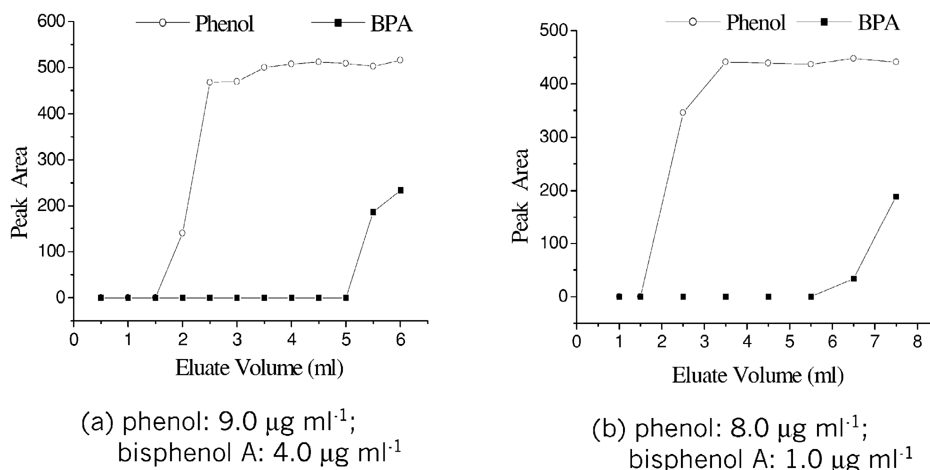

Fig. 2. Elution profiles for the continuous loading of a mixture of bisphenol A and phenol in 0.01 mol l^{-1} PBS at 4 °C.

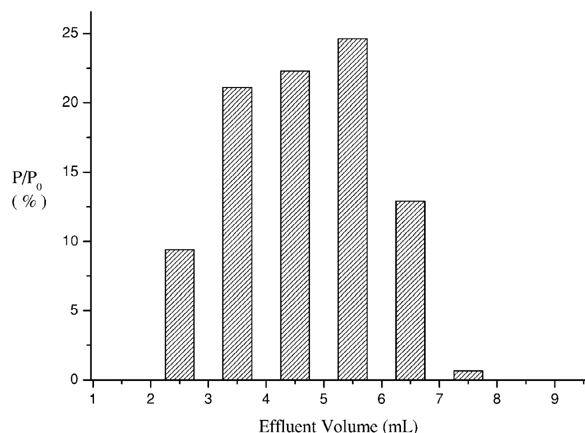

Fig. 3. Protein content of each fraction of the effluent when bisphenol A in 0.01 mol l^{-1} PBS containing 10 mg ml^{-1} HSA was loaded onto the IAC at 4 °C. P/P_0 (%): percentage of the applied protein.

column. When bisphenol A was eluted with methanol–water (80:20), the concentration of protein in the eluate (fraction 7.5–8.5 ml) declined to a very low level. To further check if any protein residue was present on the IAC, 3.0 mol l^{-1} pH 6.1 KSCN was used to elute the IAC and the absorbance of the collected solution was measured at 280 nm. No significant protein residue was observed. This indicates that the IAC showed little affinity for matrix proteins. This was most probably due to the hydrophilic properties of the surface of the Sepharose gel beads [21].

3.5. Application to real serum samples

Chromatograms of spiked and nonspiked serum samples purified with the IAC and the corresponding control solution are shown in Fig. 4. It can be seen

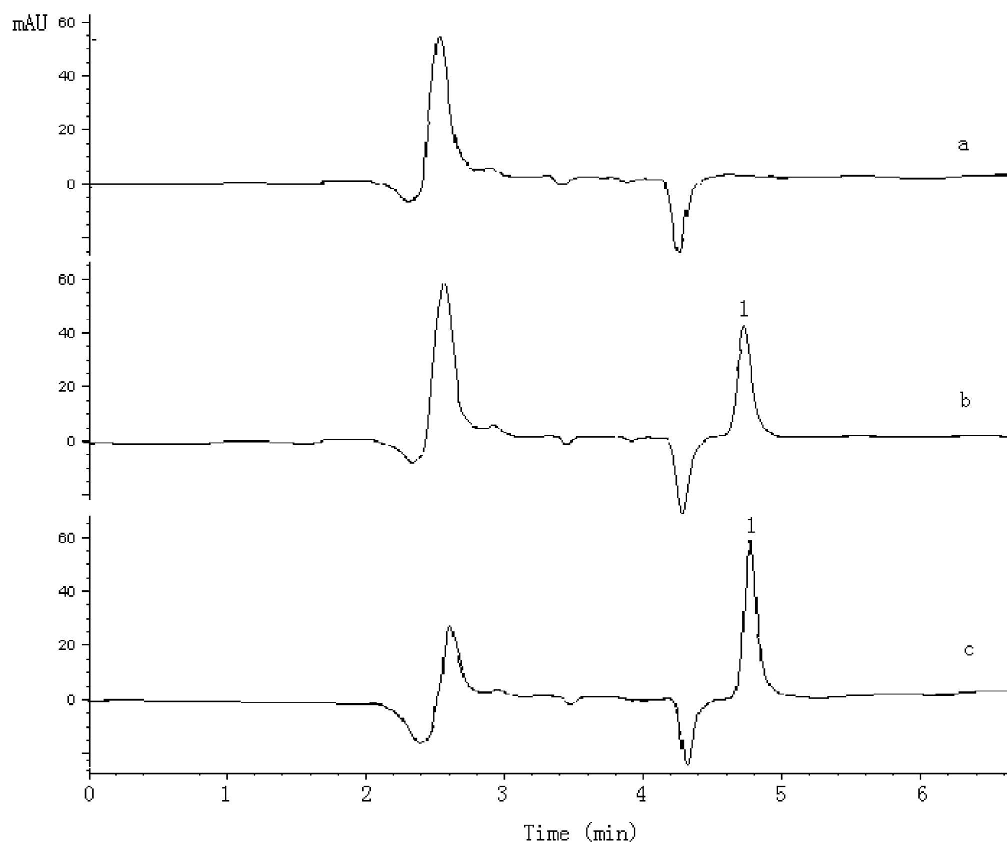

Fig. 4. Chromatograms of the bisphenol A (peak 1) collected from a nonspiked serum sample (a), a spiked serum sample (b) and the corresponding standard sample (c) after purification with the immunoaffinity column and redissolving in acetonitrile–water (60:40).

from Fig. 4 that, after purification by the immunoaffinity column, most of the matrix components in the serum samples have been removed. There are no significant interfering peaks in the chromatograms.

In our preliminary experiments with the serum sample, the serum was separated from the blood cells by centrifuging at 3000 rpm for 15 min and then diluted 1:25 with 0.01 mol l^{-1} PBS. It was observed that the flow-rate of the sample in the immunoaffinity column was slower than that of the standard solution. Moreover, the recovery of bisphenol A was only 65–75%. When the second centrifugation step was added, the flow-rate was enhanced to the same as the standard sample and the recovery was improved to above 90%. The most probable reason for the above results was that matrix impurities such as cellular fragments had blocked the voids between the gel beads and thus hindered the binding of bisphenol A to the immunosorbents.

3.6. Stability of the immunoaffinity column

The generated IAC was a gravity-flow column with a flow-rate of about 0.2–0.3 ml min^{-1}. It could be used multiple times (>20 times) and the performance was stable for at least 3 months. When in frequent use, it was simply stored in 0.01 mol l^{-1} PBS at 4 °C. For relatively long-term storage (>1 week), it was stored in 0.01 mol l^{-1} PBS containing 0.02% NaN_3 at 4 °C until use.

It was mentioned in Section 3.2 that 0.05% Tween 20 was another "good" binding solvent for the generated immunoaffinity column. However, in a further study, it was found that using 0.05% Tween 20 as the loading solvent, bubbles often formed in the gel, which may affect the performance and lifetime of the IAC. Therefore, 0.01 mol l^{-1} PBS was used to carry out the later experiments.

4. Conclusions

Conventional pretreatment methods for serum samples usually involve solid-phase extraction or liquid–liquid extraction and then multiple purification steps with silica or aluminum oxide. These are not only time-consuming, but also consume large amounts of organic solvents. New sample pretreatment techniques based on immunoaffinity chromatography or receptor-affinity chromatography have received more attention in recent years. From the above experimental results, it can be seen that the generated immunoaffinity column is more selective than the commonly used solid-phase extraction based on C_{18}. In addition, the IAC method involves fewer operating steps and consumes much smaller volumes of organic solvents. It is a very simple and practical method for the purification of bisphenol A in serum samples.

Further application of the immunoaffinity column for the isolation and purification of bisphenol A from saliva, milk, urine and other aqueous samples is at present under study by our group. Post-immunoaffinity column derivatization and GC–MS detection is also under examination to further confirm the purification effect and to enhance the sensitivity.

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References

[1] C.A. Staples, P.B. Dorn, G.M. Klecka, S.T. O'Block, L.R. Harris, Chemosphere 36 (1998) 2149.
[2] N. Ben-Jonathan, R. Steinmetz, Trends Endocrinol. Metab. 9 (1998) 124.
[3] J.A. Brotons, M.F. Olea-Serrano, M. Villalobos, V. Pedraza, N. Olea, Environ. Health Perspect. 103 (1995) 608.
[4] N. Olea, R. Pulgar, P. Perez, F. Olea-Serrano, A. Rivas, A. Novillo-Fertrell, V. Pedraza, A.M. Soto, C. Sonnenschein, Environ. Health Perspect. 104 (1996) 298.
[5] H.J. Shintani, Liq. Chromatogr. 18 (1995) 613.
[6] A.V. Krishnan, P. Stathis, S.F. Permuth, L. Tokes, D. Feldman, Endocrinology 132 (1993) 2279.
[7] C. Sonnenschein, A.M. Soto, J. Steroid Biochem. Mol. Biol. 65 (1998) 143.
[8] P. Varelis, D. Balafas, J. Chromatogr. A 883 (2000) 163.
[9] J. Sajiki, K. Takahashi, J. Yonekubo, J. Chromatogr. B 736 (1999) 255.
[10] K. Inoue, A. Yamaguchi, M. Wada, Y. Yoshimura, T. Makino, H. Nakazawa, J. Chromatogr. B 765 (2001) 121.
[11] M. Seifert, G. Brenner-Weiss, S. Haindl, M. Nusser, U. Obst, B. Hock, Fresenius J. Anal. Chem. 363 (1999) 767.

[12] M. Zhao, Y. Li, Z. Guo, X. Zhang, W. Chang, Talanta 57 (2002) 1205.
[13] D. Ba (Ed.), Contemporary Immunological Technology and Application, Joint Press of Beijing Medical Sciences University and China Union Medical College, Beijing, 1998, p. 312.
[14] Y. Xu (Ed.), Immunoassay Techniques, 2nd ed., Science Press, Beijing, 1997, p. 369.
[15] J.K. Huwe, W.L. Shelver, L. Stanker, D.G. Patterson Jr., W.E. Turner, J. Chromatogr. B 757 (2001) 285.
[16] T. Yang, X. Yin (Eds.), Practical Immunology, Changchun Press, Changchun, 1994, p. 359.
[17] Handbook of Analytical Chemistry, Vol. 2: Chemical Analysis, 2nd ed., Chemical Engineering Press, Beijing, 1997, p. 246.
[18] J.F. Lawrence, C. Ménard, M.-C. Hennion, V. Pichon, F. LeGoffic, N. Durand, J. Chromatogr. A 752 (1996) 147.
[19] I. Ferrer, V. Pichion, M.-C. Hennion, D. Barceló, J. Chromatogr. A 777 (1997) 91.
[20] P.B. Carrasco, R. Escola, M.-P. Marco, J.M. Bayona, J. Chromatogr. A 909 (2001) 61.
[21] M.M. Rhemrev-Boom, M. Yates, M. Rudolph, M. Raedts, J. Pharm. Biomed. Anal. 24 (2001) 825.