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A novel homocystine–agarose adsorbent for separation and preconcentration of nickel in table salt and baking soda using factorial design optimization of the experimental conditions

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Abstract

Homocystine was for the first time, chemically linked to a highly cross-linked agarose support (Novarose) to be employed as a chelating adsorbent for preconcentration and AAS determination of nickel in table salt and baking soda. Nickel is quantitatively adsorbed on a small column packed with 0.25 ml of the adsorbent, in a pH range of 5.5–6.5 and simply eluted with 5 ml of a 1 mol l^{-1} hydrochloric acid solution.

A factorial design was used for optimization of the effects of five different variables on the recovery of nickel. The results indicated that the factors of flow rate and column length, and the interactions between pH and sample volume are significant.

In the optimized conditions, the column could tolerate salt concentrations up to 0.5 mol l^{-1} and sample volumes beyond 500 ml. Matrix ions of Mg^{2+} and Ca^{2+}, with a concentration of 200 mg l^{-1}, and potentially interfering ions of Cd^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+}, with a concentration of 10 mg l^{-1}, did not have significant effect on the analyte's signal. Preconcentration factors up to 100 and a detection limit of 0.49 μg l^{-1}, corresponding to an enrichment volume of 500 ml, were obtained for the determination of the analyte by flame AAS. Application of the method to the determination of natural and spiked nickel in table salt and baking soda solutions resulted in quantitative recoveries. Direct ETAAS determination of nickel in the same samples was not possible because of a high background observed.

Keywords: Homocystine; Agarose adsorbent; Factorial design optimization; Nickel; Food analysis

1. Introduction

Nickel is probably an essential element for humans, with several possible roles in the maintenance and production of body cells [1]. It also produces a general toxic effect on the human organism, causing nasopharynx, lung and dermatological diseases and malignant tumors [2]. The incidence of allergies to nickel has been increased rapidly in recent years. There is no way to desensitize a person with nickel allergy with shots, pills, or any other methods. Avoiding the use of nickel containing products is the key in treatment [3]. Accurate determination of nickel in food products is, hence, essential. The determination of trace metals, including nickel, in food products such as table salt and baking soda is not straightforward due to their complex matrices and low concentration levels of the metal ions. A separation/enrichment procedure prior to the final measurements will be critical in most of the cases.

Several preconcentration methods for nickel have been described in the literature [2,4–9] among them column techniques have been of most interest [5–9]. One of the problems with such techniques is optimization of the several factors that affect experimental conditions. In most cases a classical one-at-a-time method has been applied, while this method may result in wrong conclusions if there are interactions between different factors. On the other hand, the use of a technique such as factorial design can, in many cases, reduce the number of experiments and provides much more information about the effects of different variables and their interactions. The interest to the use of such optimization methods has been substantially increased in recent years [10–13].

Homocystine, a nonessential amino acid composed of two homocysteine molecules, contains sulfur, nitrogen and oxygen donor atoms. It can be well suited as a ligand for complexation of heavy metals [14]. In the best of our knowledge there is no

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report on analytical applications of homocystine or its use as a chelating agent in preconcentration techniques.

In previous works [15–17], we reported the use of a highly cross-linked agarose (Novarose) as a promising column packing for the preconcentration of metal ions. High sample flow rates were tolerated by an iminodiacetate (IDA)–Novarose column with still quantitative recoveries for a number of metal ions [18]. The selectivity of IDA–Novarose is, however, low and it cannot efficiently remove matrix ions such as Ca^{2+} and Mg^{2+} unless a washing step is used before column elution. As an alternative, we have recently proposed more selective chelators, based on the use of ligands such as dipicolyl amine and tris(2-aminoethyl)amine, containing only nitrogen donor groups [19]. The resulting adsorbents, however, could not efficiently scavenge Ni^{2+} ions and a considerable loss of this metal ion was observed.

In this work, homocystine is chemically linked to the Novarose support in order to achieve a selective chelating adsorbent for preconcentration and efficient matrix removal of nickel. The resulting adsorbent is then used for the development of an efficient, simple and selective method for the column preconcentration and flame AAS determination of nickel in saline samples. Since the recovery of the metal ion in the developed system can depend on a variety of factors, whose relative importance is unknown, a fractional factorial design is applied to the optimization of the experimental conditions [20].

2. Experimental

2.1. Apparatus

A flame atomic absorption spectrometer (Shimadzu AA-670, Japan) employing an air-acetylene flame was used for determination of nickel and other elements under manufacture's recommended conditions (conditions for Ni: lamp current 4 mA, slit 0.15 nm, wavelength 232 nm). No background correction was used. An ETAAS (Shimadzu AA6650, Japan) with a deuterium lamp background correction was used for determinations of nickel in food samples. Hollow-cathode lamps were employed as radiation sources. For pH determinations, a Jenway model 3020 with a combined glass electrode was used after calibration against standard Merck buffers. Polpropylene tubes with 5.5 mm i.d. were used with two end frits for holding the adsorbent. A peristaltic pump (Eyla, Japan) was used for pumping solutions through columns.

2.2. Chemicals and reagents

Homocystine and other chemicals and reagents were of analytical reagent grade (Merck, Germany) and used as received. Doubly distilled water, prepared by a totally glass Fisons (UK) double distiller and was used for all the dilutions. Cross-linked and epoxy activated agarose (Novarose) was a gift from Inovata AB (Stockholm, Sweden) with 40–60 μm particle size. Before use, Novarose was thoroughly washed with a 20 ml portion of water, hydrochloric acid (1 mol l^{-1}) and acetate buffer (0.1 mol l^{-1}, pH 5.5), respectively. The table salt and baking soda samples were productions of Tehran Chemical Co. (Tehran, Iran) and Sabzi-e-Iran (Shahriar, Iran), respectively.

2.3. Methods

Stock metal ion solutions (1000 mg l^{-1}) were prepared by dissolving required amounts of their chloride or nitrate salts in 10 ml of concentrated hydrochloric acid and diluted to 250 ml by water. Working solutions were prepared by dilution of the stocks. The test solutions were usually buffered with a 0.01 mol l^{-1} acetic acid/ammonium acetate buffer and pH adjusted with dropwise addition of 0.1 mol l^{-1} solutions of HCl or NaOH before enrichment on the column.

For packing of columns, a suspension of the adsorbent was pipetted into an empty column with a frit at the bottom. A gentle vacuum was applied for faster settling of the particles before mounting the top frit. For column pretreatment, 10 ml 1 mol l^{-1} hydrochloric acid, 15 ml double distilled water, and 10 ml 0.1 mol l^{-1} acetate buffer (pH 5.5) solutions were passed through it.

Measurements of capacity for the chelating adsorbent were performed in column mode. A 100 ml portion of a 100 mg l^{-1} metal ion solution at pH 5.5 (buffered in 0.01 mol l^{-1} ammonium acetate/acetic acid buffer) with a flow rate of 4 ml min^{-1} was used for saturation of the column. The column effluent was tested occasionally in order to ensure saturation of the column with analyte. After washing with 5 ml acetate buffer (0.01 mol l^{-1}) to displace non-bound metal ion from the void, the column was eluted with 10 ml of 1 mol l^{-1} hydrochloric acid and determined by flame AAS.

Preconcentration and recovery experiments were performed by pumping a known volume of a buffered test solution through a pretreated 0.25 ml column. The column was then washed with a 5 ml 0.01 mol l^{-1} acetate buffer and eluted by 5 ml 1 mol l^{-1} hydrochloric acid (unless otherwise stated). The eluate was collected in small capped vessels and measured by flame AAS against matched standards.

The detection limit of the method was calculated from the amount of Ni^{2+} causing an absorbance of more than three times the standard deviation of a blank that was treated in the same way.

For preparation of the table salt and baking soda solutions, a known amount (about 17 g) of the powdered samples were dissolved in 8 ml concentrated HCl and diluted to 1 l with doubly distilled water. The baking soda sample was swirled for some time in order to remove the produced gasses before dilution to the volume. The required volumes of the samples were then pH adjusted by dropwise addition of 1 mol l^{-1} sodium hydroxide solution and analyzed with essentially the same method as the test samples.

2.4. Preparation of homocystine–Novarose adsorbent

About 0.6 g of the ligand was dissolved in 40 ml 0.5 mol l^{-1} Na_2CO_3 and the solution's pH was adjusted by 0.1 mol l^{-1} sodium hydroxide on 12.2. The solution was then added to a magnetically stirred suspension of 2 ml of epoxy activated

Table 1
The factors included in the fractional factorial design

Symbol	Parameter	Low (−)	High (+)
A	pH	5.5	6.5
B	Ionic strength (mol l^{-1})	0.01	0.5
C	Column length (mm)	5	15
D	Flow rate (ml min^{-1})	5.5	10
E	Sample volume (ml)	25	150

Novarose particles in 10 ml water and agitated for 24 h. The mixture was then filtered and the adsorbent was washed by 20 ml portions of 0.5 mol l^{-1} HCl, water, ethanol and water again, respectively. Deactivation of the remaining active sites was made by suspension of the adsorbent in 25 ml of a 1 mol l^{-1} sodium hydroxide solution and agitation for an extra 24 h. Finally the adsorbent was washed by water and ethanol and stored at 4 °C in 20% ethanol.

2.5. The fractional factorial design test

For performing the factorial design test, five parameters were chosen as variables and tested at two levels. The parameters were pH, ionic strength, column length, flow rate and sample volume. The high and low levels defined for them are listed in Table 1.

For five variables with two levels for each, there are 2^5 or 32 possible combinations in a full factorial design. An incomplete design allows one to select an effective sample from the total experiments by a procedure based on the advantages of randomized testing and balancing of two-factor interactions [10]. In this case we selected $2^{5-1} = 16$ experiments using the computer program STATGRAPHICS [21]. We also added three center points in order to have an estimate from the experimental error. An ANOVA test was used in order to identify the effect of individual factors and their second order interactions. Higher order interactions were assumed to be negligible in the half fraction design applied. The recovery was defined as the dependent variable and the five selected factors as independent variables in this test.

3. Results and discussion

3.1. Synthesis and capacity of the adsorbent

Homocystine contains two $-NH_2$ groups that are sufficiently strong nucleophils to react, in an alkaline pH, with the epoxy groups of the activated Novarose. The capacity for copper at pH 5.5 is usually considered as a measure of the progress of the reaction [22]. The binding capacity of the synthesized adsorbent was measured for five metal ions at pH 5.5. As it is shown in Table 2 the capacity for copper is 48.61 (±1.41) μmol/ml packed adsorbent and for the other metal ions is in the order of $Cu^{2+} > Ni^{2+} > Pb^{2+} > Hg^{2+} > Cd^{2+}$. The results for copper indicate a reasonably good progress of the reaction and the diversity of the capacities for different metal ions indicates a good selectivity of the adsorbent. The capacity for Ni^{2+} is 18.69 (±0.13) μmol/ml that seems to be sufficient for the accumulation of trace concentrations of this metal ion in natural samples.

Table 2
Capacity of the homocystine–Novarose adsorbent for different metal ions[a]

Metal ion	Capacity (μmol ml^{-1})	S.D.[b]
Cu^{2+}	48.6	1.4
Ni^{2+}	18.7	0.1
Pb^{2+}	11.2	0.2
Cd^{2+}	3.8	0.1
Hg^{2+}	4.6	0.2

[a] Experimental conditions: sample volume 100 ml, pH 5.5, metal ion concentration 100 mg l^{-1}, acetate buffer concentration 0.01 mol l^{-1}, flow rate 4.0 ml min^{-1}, eluent, 10 ml HCl (1 mol l^{-1}), 0.5 ml column.
[b] Standard deviation for triplicate measurements.

The rest of the work was focused on nickel and optimization of the experimental conditions for its preconcentration. However, the adsorbent can probably be used for the preconcentration of copper and some other heavy metals as well.

3.2. Effect of pH on the recovery

Effect of pH on the accumulation and recovery of Ni^{2+} from the column was studied in a pH range of 2.0–9.0. As shown in Fig. 1, the recovery increases rapidly with increasing pH, as expected [18], and gets to its maximum over a pH range of 5.5–6.5. A gradual decrease of recovery is observed in pH levels higher than 6.5, probably due to partial hydrolysis of the analyte. For subsequent experiments, pH 6.0 was usually used as the working pH.

3.3. Effect of volume and concentration of eluent

For the achievement of a maximum preconcentration factor, it is often desirable to use a minimum volume of an eluent for the column elution. A lower eluent concentration also reduces the consumption of chemicals and the risk of possible contaminations. The minimum amount of HCl required for a complete elution of nickel was tested by using various HCl volumes and concentrations. Using acid volumes between 2 and 10 ml indicated that a minimum volume of 5 ml suffice. Using concentrations between 0.01 and 2 mol l^{-1} of the eluent also indicated that a HCl concentration of 0.5 mol l^{-1} is sufficient, for the achievement of a quantitative recovery.

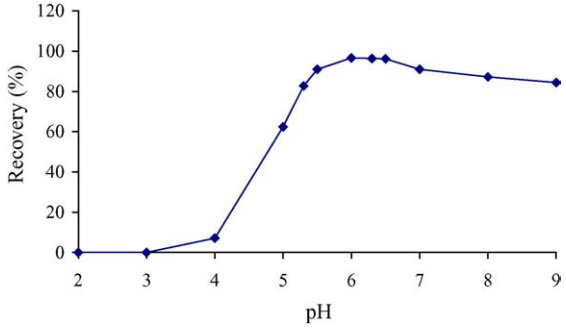

Fig. 1. Effect of pH on the recovery of Ni^{2+} from a 0.25 ml Homocystine–Novarose column. Experimental conditions: sample volume 30 ml, Ni^{2+} concentration 0.5 mg l^{-1}, acetate buffer concentration 0.01 mol l^{-1}, flow rate 5.0 ml min^{-1}, eluent 10 ml HCl (1 mol l^{-1}).

Table 3
Results of the ANOVA test for recovery

Source	Sum of squares	d.f.[a]	Mean square	*F*-ratio	*p*[b]
A: pH	175.79	1	175.79	3.25	0.1144
B: ionic strength	79.45	1	79.45	1.47	0.2648
C: column length	432.67	1	432.67	8.00	0.0255
D: flow rate	1596.18	1	1596.18	29.52	0.0010
E: volume	74.34	1	74.34	1.37	0.2794
AB	69.89	1	69.89	1.29	0.2930
AC	237.48	1	237.48	4.39	0.0744
AD	53.15	1	53.15	0.98	0.3545
AE	439.15	1	439.15	8.12	0.0247
BC	226.00	1	226.00	4.18	0.0802
BD	115.29	1	115.29	2.13	0.1876
BE	169.27	1	169.27	3.13	0.1202
CD	202.75	1	202.75	3.75	0.0940
CE	121.13	1	121.13	2.24	0.1781
DE	48.36	1	48.36	0.89	0.3758
Total error	378.53	7	54.07		

[a] d.f., degrees of freedom.
[b] *p*, probability for *F*-test.

3.4. The factorial design and optimization

The relative importance of different variables and the possibility of interactions between them were studied using a half fraction factorial design test. The low and high levels for the factors were selected according to our previous experiences and some preliminary experiments. Although pH was already studied (see Fig. 1), it was included in the test in order to study its possible interactions with other factors. The low and high pH levels were hence, defined in the range that quantitative recoveries were already obtained, i.e. pH 5.5–6.5. The other factors studied were ionic strength, column length, flow rate, and sample volume (see Table 1).

By performing the 19 runs (including three center points) in randomized order, recoveries in the range of 40.39–105.27% were obtained. Table 3 depicts the data of the ANOVA test performed by the STATGRAPHICS program. Fig. 2 represents the standardized Pareto chart which is a horizontal bar-chart. The length of each bar on the chart is proportional to the absolute

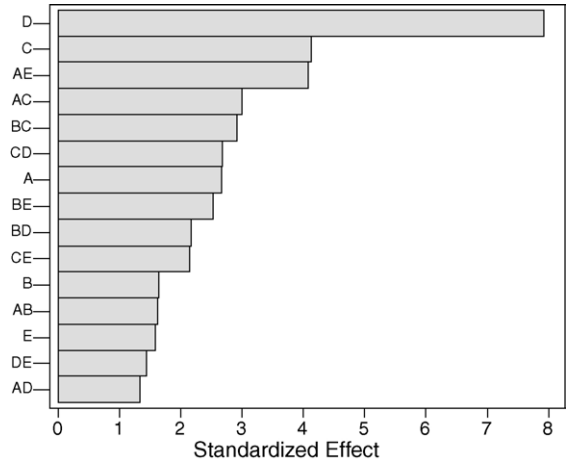

Fig. 2. Standardized Pareto chart for recovery.

Table 4
Effects of sample flow rate and column length on the recovery of Ni^{2+}

Experiment no.	Flow rate (ml min^{-1})	Column length (mm)	Recovery[a] (%)
1	5.5	10	99.8 (±0.9)
2	8.1	10	100.4 (±1.2)
3	10.0	10	99.8 (±0.9)
4	12.5	10	101.3 (±1.3)
5	8.0	15	99.5 (±0.9)
6	8.0	10	101.1 (±0.7)
7	8.0	5	94.4 (±0.7)
8	8.0	3	82.86 (±3.34)

Experimental conditions are as in Fig. 1.
[a] The numbers between parentheses are standard deviations for three replicates.

value of its associated estimated effect or the standardized effect. The most important effects correspond to factor D (flow rate), factor C (column length) and the AE interaction (interaction between pH and sample volume). These effects show *p*-values less than 0.05, indicating that they are statistically significant at a 95.0% confidence level. Hence, the effects of these factors were studied more carefully.

3.5. Effect of sample flow rate and column length

Effect of sample flow rate, the most significant factor, on the recovery was studied by variation of the flow rate, keeping other conditions constant. As it is shown in the upper part of Table 4, quantitative recoveries were obtained up to the maximum possible flow rate used (12.5 ml min^{-1}) for a 10 mm column. By using higher flow rates a high back-pressure was encountered. It may be concluded that the significance of this factor is shown up only in a short column.

Study of the effect of column length in a flow rate of 8 ml min^{-1}, indicated some loses of the analyte in 5 mm and shorter columns. The results have been shown in the lower part of Table 4.

No significant interaction between the effects of flow rate and column length (CD interaction) is observed in the ANOVA table, i.e. they are additive factors. The two factors are also principally interrelated and both affect the so-called "contact time" of sample with the column. The contact time here refers to the time that an infinitesimal sample volume spends in a packed column. Fig. 3 shows the effect of this new factor (the contact time) on the column recovery. One can conclude that for achievement of quantitative recoveries the spending time of the analyte in the homocystine–Novarose column, should not be less than about 1.4 s.

3.6. Effect of sample volume at different pH

The results of the ANOVA table (Table 3) indicate that the interaction between sample flow rate and pH is significant. In order to find out the combination effects of these two factors the effect of sample volume on the recovery of Ni^{2+} was studied at two different pH levels of 5.5 and 6.5. As Fig. 4 depicts, no significant changes in the recovery are observed after enrichment

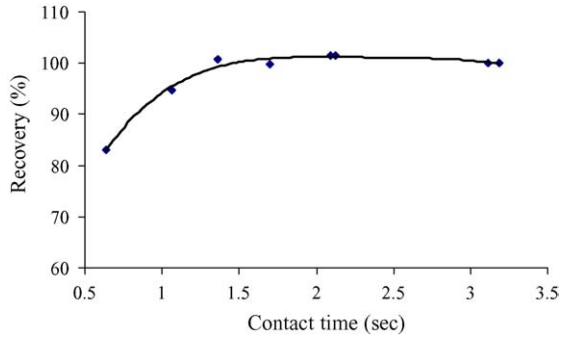

Fig. 3. Effect of contact time on the recovery of Ni^{2+}. The data have been extracted from Table 4.

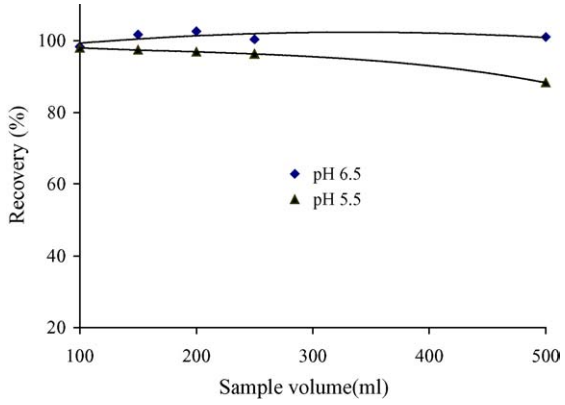

Fig. 4. Effect of sample volume at different pH. Sample flow rate 8 ml min^{-1}, eluent 5 ml of 1 mol l^{-1} HCl and other experimental conditions are as in Fig. 1.

of up to 500 ml sample at pH 6.5. On the other hand, at the lower pH level, less sample volumes can be applied and, as a result, lower preconcentration factors are achieved. This may be explained by the more stability of the Ni–homocystine complexes at the higher pH level. Therefore, pH 6.5 was used in subsequent experiments.

3.7. Analytical performance

The preconcentration procedure for trace metals can be strongly affected by other constituents that are naturally present in a sample. For this reason, the reliability of the proposed method was examined by recovery measurements in the presence of possible interfering ions. The interfering metal ions were added to some model samples as their nitrate, chloride, sulfate and acetate salts. Table 5 shows the effect of diverse ions on the column recovery of Ni^{2+}. As the results illustrate, in no case a significant effect is observed. The stated ion concentrations in the table are the maximum concentrations used. The method, hence, may tolerate higher concentrations of the matrix ions as well.

Table 5
Effect of some diverse ions on the recovery of Ni^{2+}

Matrix ion	Concentration (mg l^{-1})	Matrix ion/Ni^{2+} mass ratio	Ni^{2+} recovery (%)	S.D.[a]
Na^{+}	11500	23000	99.1	0.5
Mg^{2+}	200	400	100.1	0.5
Ca^{2+}	200	400	100.6	0.8
Mn^{2+}	10	20	100.9	1.4
Cd^{2+}	10	20	99.8	0.6
Zn^{2+}	10	20	100.5	0.6
Cu^{2+}	10	20	98.9	0.9

Sample pH 6.5 and other experimental conditions are as in Fig. 1.
[a] Standard deviation of recovery for three replicates.

For a sample volume of 500 ml and a preconcentration factor of 100, a detection limit of 0.49 μg l^{-1} was obtained for the Ni^{2+} measurements with a flame AAS instrument. By the use of more sensitive techniques such as ET-AAS much lower detection limits can, certainly, be obtained. An estimate of the precision of the preconcentration technique (expressed as RSD) was calculated by eight replicated analysis at Ni^{2+} levels of 0.5 and 0.05 mg l^{-1} to be 1.5 and 4.4%, respectively.

3.8. Application for real samples

The proposed method was applied to the determination of nickel in table salt and baking soda samples. Direct nickel determination in the samples dissolved in double distilled water was not possible by ETAAS due to a very high background absorbance observed. The validation of the method was done by spiking the samples solutions by a known amount of nickel and recovery measurement after sample preconcentration on the column. The results (shown in Table 6) indicate good agreements between the added and recovered nickel in the samples. This indicates that despite of the high ionic strengths of the sample solutions (approximately, 0.3 mol l^{-1} for the table salt and 0.2 mol l^{-1} for the baking soda) quantitative recovery of nickel has been achieved by the system.

Table 6
Application of the homocystine–Novarose adsorbent for the determination of nickel in food samples

Sample	Sample volume (ml)	Nickel added (μg l^{-1})	Nickel found[a] (μg l^{-1})[b]	Nickel found[c] (μg g^{-1})[b]
Table salt (17.55 g l^{-1})	500	–	2.26 (±0.19)	0.128 (±0.013)
	100	10	11.76 (±2.9)	
Baking soda (16.8 g l^{-1})	100	–	2.68 (±1.53)	0.159 (±0.093)
	100	10	11.64 (±0.88)	

Sample pH 6.0 and other experimental conditions are as in Fig. 1.
[a] Measured by ETAAS after preconcentration on the column.
[b] The numbers between parentheses are confidence limits for a p-value of 0.05 and $n = 3$.
[c] Calculated concentrations in the dry samples.

It is concluded that the chemical bond between the ligand and the epoxy groups of the agarose support results in a stable adsorbent that is efficient for the quantitative recovery of nickel from high salt samples. In comparison with other agarose-based adsorbents reported elsewhere [15–19], homocystine–agarose possesses a higher selectivity towards Ni^{2+}. In saline solutions, a more selective adsorbent will be superior as it removes matrix ions more efficiently. The proposed method is sensitive, precise, selective and free from common inorganic interferences. A single packed column with the adsorbent did not show any changes in its properties after about 300 times of enrichment–elution cycles during more than 4 months of usage.

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