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Automatic On-line Solid-phase Extraction–Electrothermal Atomic Absorption Spectrometry Exploiting Sequential Injection Analysis for Trace Vanadium, Cadmium and Lead Determination in Human Urine Samples

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A fully automated sequential injection column preconcentration method for the on-line determination of trace vanadium, cadmium and lead in urine samples was successfully developed, utilizing electrothermal atomic absorption spectrometry (ETAAS). Polyamino-polycarboxylic acid chelating resin (Nobias chelate PA-1) packed into a handmade minicolumn was used as a sorbent material. Effective on-line retention of chelate complexes of analytes was achieved at pH 6.0, while the highest elution effectiveness was observed with 1.0 mol L^{-1} HNO_3 in the reverse phase. Several analytical parameters, like the sample acidity, concentration and volume of the eluent as well as the loading/elution flow rates, have been studied, regarding the efficiency of the method, providing appropriate conditions for the analysis of real samples. For a 4.5 mL sample volume, the sampling frequency was 27 h^{-1}. The detection limits were found to be 3.0, 0.06 and 2.0 ng L^{-1} for V(V), Cd(II) and Pb(II), respectively, with the relative standard deviations ranging between 1.9 – 3.7%. The accuracy of the proposed method was evaluated by analyzing a certified reference material (Seronorm™ trace elements urine) and spiked urine samples.

Keywords Automation, sequential injection, solid phase extraction, metal determination, atomic spectrometry, chelating resin

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Introduction

Several metals and their compounds have long been recognized as important toxic agents, causing acute and chronic poisoning cases in environmental high-exposure situations. On the other hand, there are some metals, like vanadium, which are essential for the living organisms at low concentrations, but can cause a number of problems to human health when the uptake is too high. Among toxic heavy metals, cadmium and lead in urine, with low-to-moderate chronic exposure, reflect an integrated exposure over time and a total body burden.[1] According to the "Agency for Toxic Substances and Disease Registry" (ATSDR), normal cadmium and lead concentrations in human urine in the general population (≥ 6 years of age) are 0.185 and 0.677 μg L^{-1}, respectively, while the normal vanadium concentration in human urine is 0.5 μg L^{-1}.[2]

Atomic spectrometric (AS) techniques, such as flame absorption atomic spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) as well as mass spectrometry (ICP-MS), have been extensively employed for metal determination in various types of samples. However, direct trace metal determination is usually difficult due to the low analyte concentration and matrix complexity. Therefore, a preconcentration and/or separation step prior to the final measurement is usually required. The combination of on-line solid phase extraction (SPE) with ETAAS has proved to be of considerable interest, providing increased sensitivity and selectivity as well as higher recovery.[3-5] As a result, it has become one of the most commonly used and greenest alternative sample pretreatment techniques due to its simplicity, low cost, reduced sample and reagents consumption.[6] In this context, sequential injection (SI) analysis is a well-established automated-pretreatment (Auto-Pret) platform, offering great advantages in the automation and miniaturization of the analytical methods.[7-11]

Over the last few decades, research in the field of solid phase extraction has focused on the synthesis and study of new sorbent materials with improved characteristics,[12] like co-polymeric beads Oasis HLB,[13,14] carbon nanotubes,[15-17] magnetic particles,[6,18] lysine-modified mesoporous silica (Fmoc-SBA-15),[19] polytetrafluoroethylene (PTFE),[20] polyether–ether–ketone (PEEK),[21] since the selection of the appropriate one is of prime importance for the sensitivity and selectivity of an analytical method. Nobias chelate PA-1, first introduced by

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Sakamoto *et al.*[22] and Yamamoto *et al.*,[23] is a chelating adsorbent consisting of a hydrophilic methacrylate polymer backbone, which is chemically modified by amino and carboxylic groups. In the batch mode, this type of chelating adsorbent has been successfully employed for the ICP-MS determination of trace metal ions in environmental water samples (seawater, tap water)[22-35] and food samples.[36] On the other hand, the use of Nobias chelate PA-1 as an adsorbent for on-line column preconcentration is a new task in the field of on-line sample preparation with limited applications of metal determination in water samples[37] and leached solutions from ceramic ware.[9]

The aim of this work was to develop a fully automated SI system for the on-line column preconcentration and determination of trace metal ions in biological samples using Nobias chelate PA-1 resin as the sorbent material. The proposed method was demonstrated for vanadium, cadmium and lead determination by ETAAS in human urine. As far as we are concerned, this is the first reported application of the above-mentioned resin in a minicolumn format for on-line V(V), Cd(II) and Pb(II) preconcentration and determination in biological samples. All major chemical and hydrodynamic factors affecting the analyte adsorption and elution, such as sample acidity, eluent type and its concentration, loading and elution flow rates as well as the ETAAS operating conditions were systematically studied and optimized. The accuracy of the proposed method was evaluated by analyzing a certified reference material and a spiked urine sample.

Experimental

Instrumentation

A polarized Zeeman graphite furnace atomic-absorption spectrometer, Z-2700 (Hitachi High-Technologies, Tokyo, http://www.hitachi-hitec.com/global/science/index.html), with a pyrolitically coated graphite tube platform cuvette was used throughout the measurements. Hitachi High-Technologies single element hollow cathode lamps (HCL) for vanadium, cadmium and lead operated at 10, 7.5 and 7.5 mA respectively, were used as light sources. The wavelength was set at 318.4, 228.8 and 283.3 nm for vanadium, cadmium and lead, respectively, and the monochromator spectral bandpass (slit) was set at 1.3 nm for each metal. The integrated absorbance (peak area) was used for signal evaluation throughout the study. The graphite furnace temperature/time program for each metal determination is summarized in Table 1.

An Auto-Pret SI system, programmable by a personal computer, reported previously by the authors,[9] was used for the automatic process of the proposed method. The SI system, as shown in Fig. 1, consisted of a 3-port syringe pump, equipped with a 5 mL glass syringe barrel (Hamilton PSD/4, USA, http://www.hamiltoncompany.com/home.php), an 8-port selection valve (Hamilton, USA) and a 6-port 2-position injection valve (Hamilton, USA). The pump and valves were computer controlled by a program written by Visual Basic software. A

Table 1 Graphite furnace temperature program for vanadium, cadmium and lead determination in 50 µL of eluent

Step	Temperature/°C			Ramp time/s	Hold time/s	Argon flow rate/mL min^{-1}
	V	Cd	Pb			
Drying	120	120	140	100	100	200
Pyrolysis	900	300	700	20	0	200
Atomization	2700	1500	2400	0	5	0
Cleaning	2800	2800	2500	0	10	200

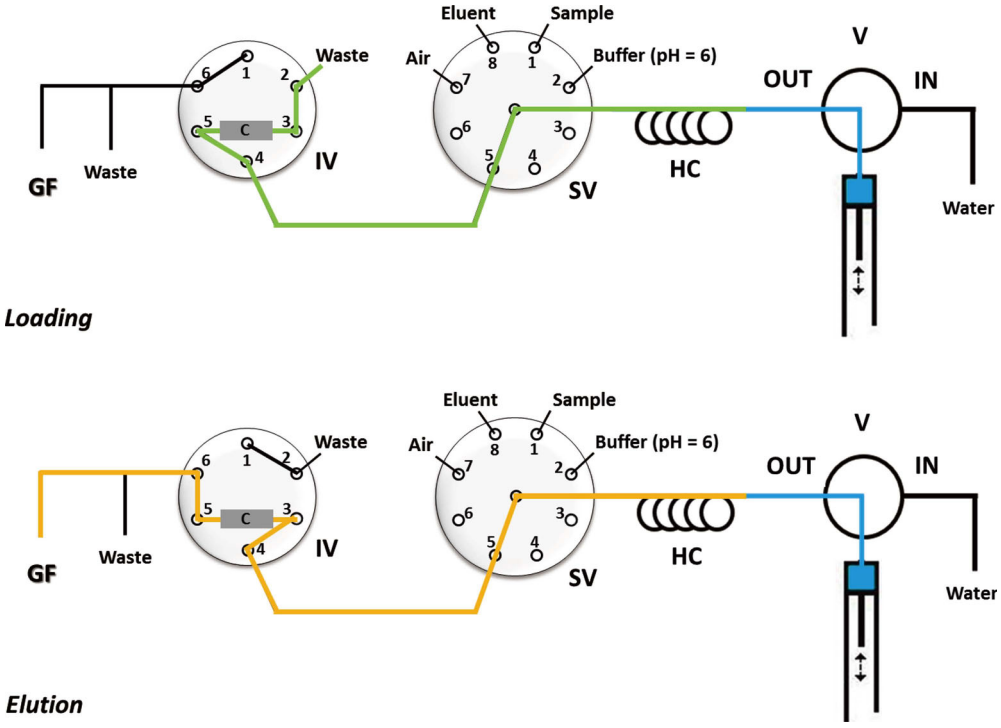

Fig. 1 Schematic diagram and the operation sequences (step 5: loading and step 11: elution) of the Auto-Pret-SI-SPE-ETAAS system for metal determination. IV, injection valve; SV, selection valve; V, valve; HC, holding coil; GF, graphite furnace; C, minicolumn packed with Nobias chelate PA-1.

Table 2 Operation sequences of the analytical method for metals determination

Step	Position			Operation	Flow rate/ μL s^{-1}	Volume/ μL	Comment
	V	SV	IV				
1	OUT	7	L	Aspirate	50	50	Aspiration of air segment
2	OUT	2	L	Aspirate	250	1000	Aspiration of buffer solution
3	OUT	5	L	Dispense	100	1000	Conditioning of the minicolumn
4	OUT	1	L	Aspirate	200	4500	Aspiration of sample solution
5	OUT	5	L	Dispense	100	4500	Loading of sample/preconcentration
6	OUT	7	E	Aspirate	250	700	Aspiration of air segment
7	OUT	5	E	Dispense	100	700	Evacuation of the minicolumn
8	OUT	8	E	Aspirate	250	1000	Aspiration of eluent segment
9	OUT	7	E	Aspirate	50	50	Aspiration of air segment
10	OUT	8	E	Aspirate	50	500	Aspiration of eluent segment
11	OUT	5	E	Dispense	50	470	Elution and transportation of the eluent up to the edge of the nozzle
				Autosampler arm into the hole of graphite tube			
12	OUT	5	E	Dispense	10	50	Injection of 50 μL of the eluent into the graphite tube
				Autosampler arm into standby position			
13	OUT	5	E	Dispense	100	1080	Evacuation of the minicolumn
14	IN	5	E	Aspirate	200	1000	Aspiration of water
15	OUT	5	E	Dispense	200	1000	Cleaning of the minicolumn and the tubing

trigger switch was placed next to the graphite furnace and, which was turned on by the movement of the autosampler arm of the graphite furnace (GF) for synchronization of the Auto-Pret-SI-SPE system with ETAAS.

PTFE tubing was used for the flow lines (0.5 mm i.d.) and the holding coil (1.5 mm i.d.). The length of tubing used for the delivery of the eluent into the graphite tube was kept as short as possible in order to minimize the dispersion and the dead volume in the proposed system.

A Horiba (Kyoto, Japan, http://www.horiba.com/) pH-meter was used for pH measurements.

Sorbent material-column preconcentration

Nobias chelate PA-1® resin (Hitachi High-Tech Fielding, Tokyo) is a reversed-phase solid-phase extraction sorbent material comprising of a hydrophilic poly(hydroxy-methacrylate) based porous resin functionalized with ethylenediaminetriacetic acid (EDTriA) and iminodiacetic acid (IDA) compounds acting as chelating groups. This type of resin selectively adsorbs transition and alkaline earth metals *via* chelation with the polyamino-polycarboxylic sites in a suitable pH range. In addition, Nobias chelate PA-1 can also retain oxyanions, such as V(V), Cr(VI) and U(VI) (but not As(III)).[25] In a pH 4 – 8, V(V) is probably retained as the vanadate anion $H_2VO_4^-$, having a similar sorption behavior with other chelating resins containing iminodiacetate groups, like Chelex 100;[38] namely there would be an applicable interaction between such oxyanions and protonated amino groups from the resin at a pH around 6 (we chose this pH for retention). It should be mentioned that Na, K, Ca and Mg ions are rarely adsorbed at a pH of less than 7,[22] which is a significant advantage for urinary analysis.

An amount of 40 mg of Nobias chelate PA-1 resin was packed firmly into a methacrylate (40 mm length, 2.6 mm i.d.) on-line preconcentration column (M&G CHEMATechs JAPAN) with glass-wool frits at both ends for sorbent immobilization. The minicolumn was attached to the 6-port injection valve (IV) between ports 3 and 5, as shown in Fig. 1. As it was revealed from the whole experimental study, the performance of the proposed minicolumn was consistent for at least 1000 preconcentration cycles without any decrease in the integrated absorbance. This is an essentially important advantage for keeping the process cost down in routine analysis.

Reagents and samples

All chemicals were of analytical reagent grade. Ultra-pure quality water, produced by an Elix 3/Milli-Q Element System (Nihon Millipore, Tokyo, Japan), was used throughout all experiments. All metal standard solutions were prepared by appropriate stepwise dilution of 1000 mg L^{-1} V(V), Cd(II) and Pb(II) stock standard solutions in 0.5 mol L^{-1} HNO_3 (Wako Pure Chemical Industries, Osaka) prior to use. The working standard solutions and samples were adjusted to pH 6.0 using an ammonium acetate buffer solution. An ammonium acetate buffer solution at a concentration level of 0.1 mol L^{-1} was prepared by dissolving an appropriate amount of solid ammonium acetate (≥ 97%, analytical grade, Sigma-Aldrich Japan, Tokyo) in water, and being adjusted to pH 6.0 with a 10% (v/v) nitric acid solution. The eluent was a 1.0 mol L^{-1} HNO_3 aqueous solution prepared from concentrated nitric acid (60% (v/v) HNO_3, analytical grade, Sigma-Aldrich Japan). Laboratory glassware was rinsed with ultra-pure water and decontaminated overnight in 10% (v/v) HNO_3.

The accuracy of the developed method was estimated by analyzing the following standard reference material (CRM): Seronorm™ Trace Elements Urine Level 1, containing trace elements in urine. Moreover, a urine sample was taken from a healthy adult and digested, using concentrated nitric acid. The digestion procedure was carried out at 130 – 140°C, using the START D microwave digestion system (Milestone General K.K., Kawasaki, Japan) under the recommendations of the manufacture.

Analytical procedure

A schematic diagram of the flow system for trace metal determination by ETAAS is presented in Fig. 1, and the operation sequences of the analytical method are summarized in Table 2. Each analytical cycle starts with the aspiration of a small volume (50 μL) of air into a holding coil (HC) in order to

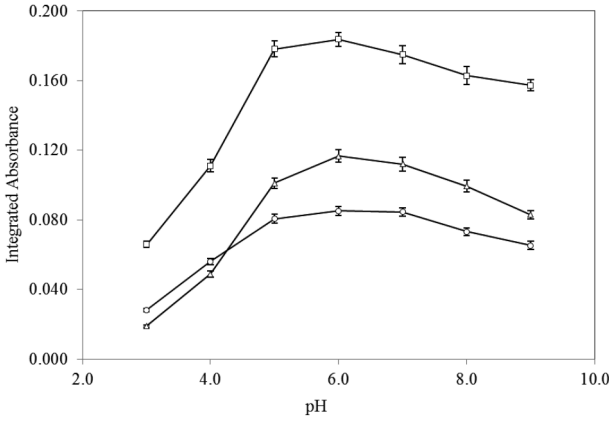

Fig. 2 Effects of the pH on the integrated absorbance values of 2.0 μg L^{-1} V(V) (△), 50.0 ng L^{-1} Cd(II) (□) and of 0.3 μg L^{-1} Pb(II) (○). Volume of a loaded standard solution: 3.0 mL; all other experimental parameters as in Table 2.

eliminate any dispersion of aqueous solutions with the solution of the carrier (water). Initially, conditioning of the minicolumn takes place by delivering 1000 μL of an ammonium acetate buffer solution (pH 6) in order to activate the sorbent material. In steps 4 and 5, a volume of 4500 μL of the standard or sample is aspirated into the HC and, consecutively, dispensed through the minicolumn (C) at a flow rate of 100 μL s^{-1} for analyte preconcentration. Thereafter, valve IV is switched to the "elution" position, and the minicolumn is evacuated by a segment of 700 μL of air. In the following steps (8, 9 and 10), 1000 μL of the eluent (1.0 mol L^{-1} HNO_3), 50 μL of air and 500 μL of 1.0 mol L^{-1} HNO_3 are aspirated sequentially into the HC for elution purposes. The elution is performed in the reverse direction than that of the loading procedure in order to avoid any analyte dispersion into the segment of the eluent. Next, 470 μL are dispensed through the minicolumn in order to elute the retained analyte and transport the front portion of the HNO_3 segment up to the edge of the nozzle of the autosampler. After that, the arm of the autosampler of ETAAS moves towards the dosing hole of the graphite tube, and presses the trigger switch, which is placed at an appropriate position next to the furnace. Once the trigger switch is pressed (step 12), the front 50 μL portion of the eluent segment, containing the highest analyte amount, is dispensed into the graphite tube for atomization and measurement. The atomization program of ETAAS runs in parallel, and is synchronized with the program of Auto-Pret-SI. During the following steps (13, 14 and 15), a thorough cleaning of the minicolumn, syringe and tubing is accomplished so as to eliminate any possible sample carryover. Five replicate measurements are made in all instances.

Results and Discussion

Optimization study

The chemical and hydrodynamic parameters associated with the loading and elution procedures of the proposed method were investigated, so that optimum analytical conditions could be reached. Standard aqueous solutions of V(V), Cd(II) and Pb(II) at 2.0, 0.050 and 0.3 μg L^{-1} concentration levels, respectively, at a fixed loading sample (each standard solution) volume of 3.0 mL were used for the optimization study.

Effect of the pH. The pH value of the sample solution plays a key role to the quantitative adsorption of target metal ions on the surface of the chelating resin, controlling the preconcentration process. The effect of the pH on the integrated absorbance values for V(V), Cd(II) and Pb(II) was studied in the range of 3.0 to 9.0 by adjusting it by adding either a 10% (v/v) HNO_3 solution or 0.1 mol L^{-1} ammonium acetate as a buffer solution. The obtained results are presented in Fig. 2. The absorbance increased with an increase of the pH up to 5.0, while higher sensitivity was achieved at between 5.0 and 7.0, where the absorbance was leveled off for all metals. At higher pH values the signals decreased due to possible hydrolysis, which leads to the precipitation of metal hydroxides, as has been reported previously.[9,22] The low signals at lower pH values are attributed to the protonation of EDTriA and IDA functional groups, resulting in inefficient complex formation. Consequently, a pH value of 6.0 ± 0.2 was selected for all subsequent experiments.

Effect of the eluent. The quantitative desorption of the retained analytes from the surface of a sorbent material strongly depends on the eluting agent and its concentration. An ideal eluent should offer fast and sufficiently strong elution ability in a volume as small as possible so as to achieve a high preconcentration ratio. As revealed from preliminary experiments, comparing nitric and hydrochloric acids, a solution of 500 μL of 1.0 mol L^{-1} HNO_3 could effectively elute the retained metals. Thus, nitric acid was adopted as the eluent. The effect of the nitric acid concentration on the sensitivity of the method was studied in the range 1.0 – 2.5 mol L^{-1}. The obtained results showed that the absorbance was not influenced by the nitric acid concentration in the studied range for all metals. Therefore, 1.0 mol L^{-1} HNO_3 solution was selected as the eluent for subsequent experiments, while considering the fact that higher nitric acid concentrations might reduce the lifetime of the graphite tube of ETAAS.

Effect of the loading flow rate. In on-line sorbent extraction systems, the loading flow rate affects the preconcentration efficiency as well as the time of analysis through complex formation, and its retention on the sorbent surface. Both the exchange kinetics and the back-pressure of the column depend on the speed that the sample solution passes through the minicolumn. The effect of the loading flow rate on the absorption of the analytes was studied in the range between 10.0 and 100.0 μL s^{-1}. The experimental results showed that the sample loading flow rate had no significant effects on the integrated absorbance values for all metals in the studied area, indicating that each sorption was very fast within the studied range. Thus, a loading flow rate of 100 μL s^{-1} was selected for the preconcentration step, considering not only the time of analysis and the sampling frequency, but the sensitivity as well.

Effect of the elution flow rate. The effect of the elution flow rate on the sensitivity of the method was examined within the range of 10.0 – 100 μL s^{-1}. As is shown in Fig. 3, a higher absorbance for all metal ions was achieved at flow rates of between 10.0 and 50.0 μL s^{-1}. The reduced analytical signals for values higher than 50.0 μL s^{-1} are attributed to the short contact time of the eluent with the sorbent material, possibly due to the slow elution kinetics and the higher dispersion of the analytes into the segment of eluent. Therefore, a flow rate of 50.0 μL s^{-1} was adopted for further experiments as a compromise between the sensitivity and the time of analysis.

Effect of the injection volume of eluent into the graphite furnace. Since the ETAAS signal depends on the analyte mass, there is an effective degree of control on the recorded absorbance by controlling the injected sample volume. Generally, a larger injected volume of sample contains a greater absolute amount of the analyte available for atomization, which results in a higher

sensitivity of the method. For very low concentrations, the maximum volume of the analyte can be used, while for higher concentrations the sample volume can be reduced. On the other hand, the maximum volumes of the sample that can be used depend on the graphite tube configuration. Without a graphite platform, sample volumes at up to 100 µL can be used, while with the platform in place, a volume of up to 50 µL is recommended.

In the proposed method the first portion of the zone of the eluent is injected into the graphite tube, as presented in the analytical procedure section. Taking into account that the concentration of the analytes along the zone of the eluent is varied, and a higher concentration is in the front side, the effect of the injection volume of the eluent on the absorbance values for the three analytes was studied in the range of 10 - 50 µL. A volume of 50 µL gave a higher sensitivity, and was used throughout the experiments.

Effect of the sample volume. An efficient and sensitive volume-based on-line preconcentration system largely depends on the sample volume, which is directly related to the amount of analyte retained on the surface of the sorbent material during the preconcentration procedure. The influence of the sample volume on the sensitivity of the method was studied in the range between 0.5 and 4.5 mL under the optimized conditions. The experimental results confirmed a practically proportional increase of the recorded signal with the increase of the sample volume up to at least 4.5 mL. Therefore, a sample volume of 4.5 mL was selected for the proposed method as a compromise between the sensitivity and low consumption of the sample as well as the analysis time. On the other hand, for higher sensitivity and lower detection limits, a larger sample volume could be adopted by increasing the total time of the analysis.

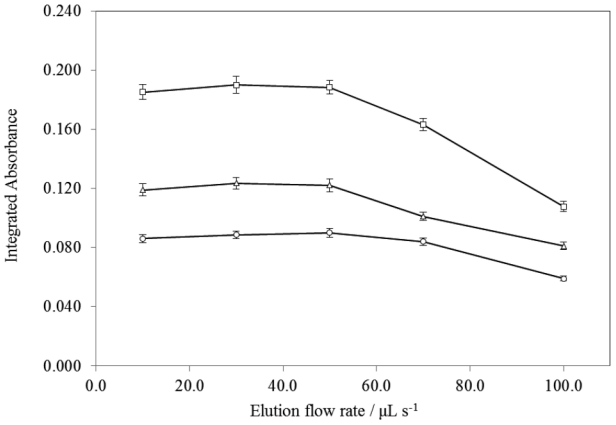

Fig. 3 Effects of the elution flow rate on the absorbance values of 2.0 µg L^{-1} V(V) (△), 50.0 ng L^{-1} Cd(II) (□) and of 0.3 µg L^{-1} Pb(II) (○). Volume of a loaded standard solution: 3.0 mL; all other experimental parameters as in Table 2.

Interferences

The interferences of commonly ions existing in biological fluids, like urine, as well as some heavy metals were examined regarding their competition for the active sites on the sorbent, for the determination of 1.0 µg L^{-1} V(V), 15.0 ng L^{-1} Cd(II) and 1.0 µg L^{-1} Pb(II) using the optimized procedure. A variation in the recovery greater than ± 5% was considered to be interference. The metal ions of Al(III), Cr(VI), Fe(III), Mn(II) and Zn(II) can be tolerated at least up to 2.0 mg L^{-1} for vanadium, cadmium and lead determination, as was revealed from the experimental results. Moreover, for each analyte, the presence of the other two metals can be tolerated up to 1.0 mg L^{-1}. In addition, common cations, such as Na(I), K(I), Ca(II) and Mg(II) do not interfere up to a concentration level of 500 mg L^{-1}.

Analytical characteristics

The analytical performance characteristics of the developed Auto-Pret-SI-SPE-ETAAS method for V(V), Cd(II) and Pb(II) determination under the optimal conditions are summarized in Table 3. For a sample consumption of 4.5 mL, the sampling frequency was 27 h^{-1} and the enhancement factors (calculated by the ratio of the slopes of the calibration curves obtained with and without preconcentration using ETAAS) were 21, 37 and 12 for vanadium, cadmium and lead, respectively. The detection limits (c_L), based on the 3s criterion (according to IUPAC[39]), were found to be 3.0, 0.06 and 2.0 ng L^{-1} for vanadium, cadmium and lead determination, respectively, while the precision of the method, evaluated as the relative standard deviation (RSD), was between 1.9 and 3.7%. The analytical performance of the Auto-Pret-SI-SPE-ETAAS method for cadmium determination with ICP-MS in terms of the detectability and sample consumption is better[9,40] than and comparable[30,37] to others reported in the literature. In addition, the proposed method presents a 3-orders of magnitude better detection limit for cadmium determination than the previous reported one using a similar SI-SPE-ETAAS system.[9]

The accuracy of the proposed method was estimated by analyzing the standard reference material, Seronorm™ Trace

Table 3 Analytical performance characteristics of the analytical method for vanadium, cadmium and lead determination under the optimized conditions

	Vanadium[a]	Cadmium[b]	Lead[a]
Sample volume/mL	4.5	4.5	4.5
Sampling frequency, f/h^{-1}	27	27	27
Enhancement factor	21	37	12
Linear range	0.011 - 3.0	0.20 - 40.0	0.007 - 2.0
Detection limit, c_L/ng L^{-1}	3.0	0.06	2.0
Precision, RSD, % (n = 10)	2.2% (at 1.0 µg L^{-1})	1.9% (at 10.0 ng L^{-1})	3.7% (at 0.8 µg L^{-1})
Regression equation ([Pb], [V] in µg L^{-1}, [Cd] in ng L^{-1}, n = 9)	A = (0.0925 ± 0.0015) [V] + (0.0014 ± 0.0019)	A = (0.0057 ± 0.0000) [Cd] + (0.0015 ± 0.0019)	A = (0.1272 ± 0.0049) [Pb] + (0.0016 ± 0.0051)
Correlation coefficient (r)	0.9996	0.9997	0.9989

a. Concentration in µg L^{-1}, b. Concentration in ng L^{-1}.

Table 4 Determination of vanadium, cadmium and lead in CRM, Seronorm™ Trace Elements Urine L^{-1}

Analytes	Certified value/μg L^{-1}	Found[a]/ μg L^{-1}	Relative error, %	t_{exp}
V	0.66 ± 0.08	0.63 ± 0.04	4.5	1.299
Cd	0.20 ± 0.04	0.194 ± 0.01	3.0	1.039
Pb	0.66 ± 0.13	0.62 ± 0.06	6.1	1.155

a. Mean value ± standard deviation based on three replicates.
t_{crit} = 4.303 at 95% probability level.

Elements Urine Level-1, containing trace elements in urine. The student *t*-test was employed to examine any possible statistically significant differences between the obtained and certified values of the determined metals.[41] The t_{exp} values were calculated by the following equation:

$$t_{exp} = (\bar{x} - \mu)\frac{\sqrt{n}}{s}, \quad (1)$$

where $\bar{x}$ is the sample mean value, μ is the certified value, s is the sample standard deviation of the sample values and n is the replicate determinations (n = 3). In this case, there are two degrees of freedom (n – 1), and therefore the t value obtained from the table of student t-destribution, t_{crit}, is 4.303 at the 95% probability level. The analytical values and t_{exp} values for V, Cd and Pb determinations in the above CRM are given in Table 4. Since all texp values are lower than, t_{crit} = 4.303, no statistically significant differences were found at the 95% probability level, indicating the applicability of the developed method in urine type samples.

Analytical applications

The method was applied for the determination of V, Cd and Pb in urine samples collected by an adult healthy person. The analysis was completed under the optimum conditions of the proposed method and the recovery estimated by the standard addition. The results are presented in Table 5. The recoveries of the three metals varied within the range of 94.0 - 98.0%, showing that the proposed method can be successfully applied to the simultaneous determinations of V, Cd and Pb in urine samples with good performance.

Table 5 Analytical results of cadmium, lead and vanadium determination in urine sample by the Auto-Pret-SI-SPE-GFAAS method

Analyte	Added[a]	Found[a,b]	Recovery, %
V	—	0.45 ± 0.02	—
	0.50	0.94 ± 0.04	98.0
	1.00	1.42 ± 0.05	97.0
Cd	—	21.42 ± 0.65	—
	10.00	30.85 ± 1.10	94.3
	15.00	35.92 ± 1.20	96.7
Pb	—	1.05 ± 0.05	—
	0.50	1.52 ± 0.05	94.0
	1.0	2.02 ± 0.07	97.0

a. Concentration in μg L^{-1} for V and Pb determination and in ng L^{-1} for Cd determination.
b. Mean value ± standard deviation based on three replicates.

Conclusions

A new on-line Auto-Pret-SI-SPE method has been developed for metal determination by ETAAS. The use of Nobias chelate PA-1resin as an adsorbent in a handmade minicolumn format has been demonstrated for the first time for the on-line column preconcentration of vanadium, cadmium and lead in urine samples. The chemical stability, large surface area and fast kinetics of the proposed sorbent material as well as its long lifetime (> 1000 analytical cycles) make it very attractive for on-line SPE systems. The proposed method is considered to be a simple, rapid, accurate and low-cost approach for the routine monitoring of trace-level concentrations of metal species in biological samples.

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