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Flow injection determination of bismuth in urine by successive retention of Bi(III) and tetrahydroborate(III) on an anion-exchange resin and hydride generation atomic absorption spectrometry

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Abstract

Bismuth as $BiCl_4^-$ and BH_4^- ware successively retained in a column (150 mm × 4 mm, length × i.d.) packed with Amberlite IRA-410 (strong anion-exchange resin). This was followed by passage of an injected slug of hydrochloric acid resulting in bismuthine generation (BiH_3). BiH_3 was stripped from the eluent solution by the addition of a nitrogen flow and the bulk phases were separated in a gas–liquid separator. Finally, bismutine was atomized in a quartz tube for the subsequent detection of bismuth by atomic absorption spectrometry. Different halide complexes of bismuth (namely, $BiBr_4^-$, BiI_4^- and $BiCl_4^-$) were tested for its pre-concentration, being the chloride complexes which produced the best results. Therefore, a concentration of 0.3 mol l^{-1} of HCl was added to the samples and calibration solutions. A linear response was obtained between the detection limit (3σ) of 0.225 and 80 μg l^{-1}. The R.S.D.% (n = 10) for a solution containing 50 μg l^{-1} of Bi was 0.85%. The tolerance of the system to interferences was evaluated by investigating the effect of the following ions: Cu^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, and Mg^{2+}. The most severe depression was caused by Hg^{2+}, which at 60 mg l^{-1} caused a 5% depression on the signal. For the other cations, concentrations between 1000 and 10,000 mg l^{-1} could be tolerated. The system was applied to the determination of Bi in urine of patients under therapy with bismuth subcitrate. The recovery of spikes of 5 and 50 μg l^{-1} of Bi added to the samples prior to digestion with HNO_3 and H_2O_2 was in satisfactory ranges from 95.0 to 101.0%. The concentrations of bismuth found in six selected samples using this procedure were in good agreement with those obtained by an alternative technique (ETAAS). Finally, the concentration of Bi determined in urine before and after 3 days of treatment were 1.94 ± 1.26 and 9.02 ± 5.82 μg l^{-1}, respectively.

Keywords: Bismuth; Urine; Flow injection; Ion-exchange pre-concentration; Solid-phase hydride generation; Atomic absorption spectroscopy

1. Introduction

Bismuth compounds have been used in medicine for more than 200 years in a variety of gastrointestinal disorders, because of their demulcent properties [1]. Theories concerning the mechanism by which the element promotes ulcer curing support the hypothesis that a precipitate of bismuth coats the ulcer area and isolates the underlying sensitive surface from the action of the gastric and duodenal juices [2]. Although intestinal absorption of bismuth is limited in humans, because of its low solubility and its propensity to form insoluble oxychloride salts, some absorption must occur to produce measurable concentrations in body fluids and tissues [3,4]. At the same time, the intake of bismuth-containing pharmaceuticals has been related to nephrotoxicity [5] and neurotoxicity (reversible encephalopathy) [6] and this brought about a temporary decline in the therapeutic use of bismuth compounds. However, the fact that therapy using colloidal bismuth subcitrate (CBS) [7] and other bismuth compounds [8] exert beneficial effects by cytoprotection and bactericidal activity to the intestinal bacterium *Helicobacter pylori* (amended *C. pylori*,

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which may well initiate ulcer formation by excreting acid) has re-awaking the interest in bismuth therapies.

The basic levels of bismuth in humans and the degree of bismuth absorption from pharmaceutical preparations can be monitored by the determination of bismuth content in blood and urine as well as in tissue samples. In the particular case of urine, a range between 0.8 and 1.6 $\mu g\,l^{-1}$ with a mean concentration of 1.2 $\mu g\,l^{-1}$ has been reported as normal in man [9,10]. However, bismuth concentration in human urine samples as low as 0.1 $\mu g\,l^{-1}$ has been reported [11,12], a fact that demands the implementation of sensitive and reliable analytical methods for the determination of this element at sub-$\mu g\,l^{-1}$ levels.

From the wide selection of analytical techniques described in the literature for the determination of bismuth, only few have adequate precision, accuracy and sensitivity (LOD $\leq$ 1 $\mu g\,l^{-1}$) to quantify it in urine samples [13–26]. Among them, atomic absorption spectrometry with hydride generation (HG-AAS) [13,14], electrothermal atomic absorption spectrometry (ETAAS) [15–21], inductively coupled plasma atomic emission spectrometry (ICP-AES) [22,23], and ICP-mass spectrometry (ICP-MS) [24–26]. Although the previously mentioned techniques were suitable for the determination of bismuth in urine samples, most of them were not able to do it directly. In order to achieve accurate, reliable and sensitive results, in most of cases pre-concentration and separations were needed when the concentration of analyte in the original material or the prepared solution were too low to be determined directly. Procedures using ICP-MS, can achieve detection limits as low as 14.6 $ng\,l^{-1}$ [24] and 9.7 $ng\,l^{-1}$ [25], making this technique probably the most indicated for direct determination of bismuth in urine (although bismuth was not found in urine from untreated individuals). However, the cost of such instrumentation may be prohibitive to many laboratories. Finally, there are significant advantages in the use of hydride generation (HG) with AAS or other spectrometric detection techniques for the determination of virtually all elements which form volatile hydrides. The generation of bismuth hydride (bismuthine, BiH_3) has become one of the most powerful and well-established techniques for the determination of sub-μg to pg of bismuth [27]. For example, the combination of HG-ICP-MS produced LODs down to the fantastic levels of 0.7 $ng\,l^{-1}$ [28]. Therefore, the combination of pre-concentration procedures and HG with a widespread technique as AAS, may result very convenient and appropriate for most laboratories to determine bismuth in urine.

In a previous work it was successfully achieved the generation of selenium hydride from selenite (SeO_3^{2-}) retained on anionic exchange resin [29,30]. The simultaneous or successive retention of selenite and borohydride followed by passage of hydrochloric acid allowed the determination of selenium at $ng\,l^{-1}$ level. The aim of this work was to extend the work done with selenium to successive retention of anionic species of bismuth (namely, $BiBr_4^-$, BiI_4^- and $BiCl_4^-$) and borohydride as a viable method for the pre-concentration of such species, with the subsequent generation of bismuthine and detection by AAS. Finally, the system was applied to the determination of Bi in urine of patients under therapy with bismuth subcitrate.

2. Experimental

2.1. Instrumentation

The detection unit used was a Perkin-Elmer model 3100 atomic absorption spectrometer. The instrument was interfaced with a DTK 286 personal computer, and programmed/controlled with the Perkin-Elmer Gem (version 3.10) and 3100 EDS/3300 (version 7.10) softwares. The atomizer was a flame-heated quartz 'T'-shaped tube. The quartz tube was cleaned weekly with acid following the procedure described by Hatfield [31]. A bismuth hollow cathode lamp (Perkin-Elmer) was used as a light source. The wavelength for Bi was 223.1 nm with a spectral band-pass of 0.2 nm.

The manifold, shown schematically in Fig. 1 was constructed from 0.8 mm i.d. PTFE tubing and Tygon 1.54 mm i.d. yellow/blue pump tubing (Cole-Parmer). The gas–liquid separator consisted of a glass device with glass beads (Perkin-Elmer, part no. B019-3772). The column consisted of a glass tube of 150 mm length and 4 mm i.d. Two PTFE reducing unions 1/4 in. × 1/8 in. (Cole-Parmer) fitted at either end of the column were used to connect it to the manifold.

A slurry of anion-exchange resin was introduced into the column with the aid of a syringe. A small amount of glass wool was placed at the ends of the column to prevent loss of the resin. The column was ready for use after washing several times alternately with borohydride and hydrochloric acid solutions. A remote controllable six positions (only four were used) Teflon-motor-switching-valve (LATEK-TMV-P) was used. Two programmable peristaltic pumps (ISMATEC-IPC and GILSON Minipuls-3) with the option of remote control were used for the propulsion of the reagents. The flows of reagents were regulated varying the pump head rotation speed and different internal diameter Tygon pump tubing (Cole-Parmer). A home-made software (Windows platform, PC compatible) was developed to control the pumps and valves and other necessary devices. An R232/RS485 card, incorporated in a Pentium I processor PC, was used to interface the periphery devices.

2.2. Reagents

All chemicals were of analytical reagent grade, unless otherwise stated. Double distilled 18 MΩ cm specific resistivity obtained in a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the experiment. All the laboratory materials were carefully cleaned before use by first washing with a neutral detergent solution and then rinsing

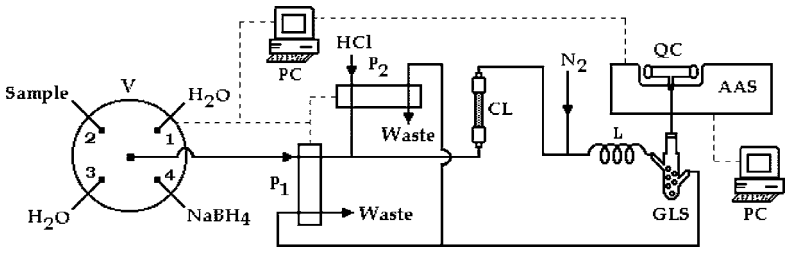

Fig. 1. Schematic diagram of the manifold and the instrumentation set up for bismuth pre-concentration and hydride generation from a solid phase. V, a six positions (only four ere used) Teflon-motor-switching-valve; P_1 and P_2, peristaltic pumps; CL, column packing with strong anionic-exchange resin; L, stripping coil; QC, quartz cell; GLS, gas–liquid separator; PC, computer; AAS, 3100 atomic absorption spectrometer.

with both distilled and distilled/de-ionized water. Bismuth nitrate standard solution for AAS (1 ml = 1.00 mg of bismuth) prepared in nitric acid 1 mol l^{-1} was from BDH laboratory reagents (Poole, UK). Working standard solutions were prepared daily by diluting appropriate volumes of this stock in 0.3 mol l^{-1} of HCl. Sodium borohydride solution 0.08% (w/v) in 0.1% (w/v) sodium hydroxide solution was prepared just prior to use by dissolving an appropriate amount of $NaBH_4$ (98.9% purity, Sigma) in 500 ml of sodium hydroxide 0.1% (w/v) (original solid from Baker Analyzed Reagent). The hydrochloric acid solutions were prepared by diluting appropriate volumes of concentrated hydrochloric acid (37.8%, Fisher Scientific) in de-ionized water. The resin used was Amberlite IRA-410 (Aldrich), which is strongly basic anion-exchange resin with a styrene–divinylbenzene skeleton.

A standard reference material (SRM; Seronorm™ trace elements urine, lot 403125) with 25 μg l^{-1} added amount of bismuth (Nycomed AS, Oslo, Norway) was used in accuracy studies. Finally, hydrogen peroxide (30%, w/w, Sigma) and nitric acid (70%, Sigma) were used for samples digestion.

2.3. Urine samples collection and preparation

Urine samples were obtained from 14 healthy male laboratory employees or students (26 ± 10 years old) with normal renal function. The volunteers accepted to take 250 mg of bismuth subcitrate (BS) once a day for 3 days before bed. Urine samples were collected during 24 h following the treatment and stored in plastic containers without adding preservatives. To their knowledge, they had never taken bismuth medication. The samples of urine were digested as follows. In a 100 ml beaker, 25 ml of urine sample accurately measured, was treated with a mixture of 5 ml H_2O_2 and 2.5 ml of concentrated HNO_3, and was placed on a hot plate. The sample was moderately heated up to the disappearance of the amber color. Then, the sample was evaporated almost to dryness. Thereafter, fresh portions of 2.5 ml of concentrated HNO_3 were added to the dark residue and heated to dryness. This procedure was repeated until a white ash was obtained. Finally, the residue was dissolved in 2.5 ml of 3.0 mol l^{-1} of HCl and diluted exactly to 25 ml with de-ionized water. Blanks were prepared with the same reagents, without the samples, undergoing an identical process of mineralization.

2.4. Procedure

All the experiments were carried out using the manifold shown in Fig. 1. The steps followed and the optimal conditions for bismuth determination are summarized in Table 1. The valve position and the pumps speed were controlled using the software designed, which also allows to interface the spectrometer to instruct it to start the read step.

3. Results and discussion

3.1. Preparation of the complex halides

In order to achieve its retention in the anionic exchange resin, it is necessary that bismuth be present in solution in anionic form. The chemistry of bismuth in aqueous solution shows that it exists mainly as Bi^{3+}, because the Bi^{5+}–Bi^{3+} couple is strongly oxidizing (E° = +2.1), able to oxidize water to oxygen [33]. The halide complex of Bi^{3+} (general formula, BiX_n^{3-n}) have been extensively studied in aqueous solution and information about individual species has been obtained from ultraviolet and Raman spectroscopy [33,34]. Among the anionic complexes that bismuth can form [33] the halides complexes result to be very easy to prepare and convenient for the objectives of this work. Therefore, the primary studies were devoted to prepare the iodides, bromides and chlorides complexes of bismuth. Bismuth trifluoride is rather insoluble in water, and complex fluorides of bismuth are not well known [33].

The first anionic species studied were the iodides complex. The addition of an excess of a soluble iodide to an acid solution of bismuth produces a yellow-to-orange color due to iodides complexes of bismuth, chiefly BiI_4^-, BiI_6^{3-}, and BiI_7^{4-} [34]. In the presence of 0.01 mol l^{-1} I^-, Bi is present almost entirely as BiI_4^-, and in 0.1 mol l^{-1} I^-, approximately 30% as BiI_4^- and around 70% as BiI_6^{3-} according to one set of formation constants [33]. The different iodides were prepared by varying the concentration of KI between 0.01 and

Table 1
Steps followed for bismuth determination (also see Fig. 1 and text)

Valve position	Time (s)	Pump 1 (rev min^{-1})/flow rate (ml min^{-1})	Pump 2 (rev min^{-1})/flow rate (ml min^{-1})	Comments
1	10	60/8	0/0	Purified water is pumped through the column to wash the system and to set up the baseline. Drains from gas–liquid separator are pumped to waste
2	30	45/5	0/0	The sample is pumped through the column for the analyte retention. Drains from gas–liquid separator are pumped to waste
3	20	60/8	0/0	Purified water is pumped through the column, in order to flush interfering species present in the sample. Drains from gas–liquid separator were pumped to waste
4	30	45/5	0/0	0.08% (w/v) $NaBH_4$ is pumped through the column for BH_4^- retention. Drains from gas–liquid separator are pumped to waste
1	20	60/8	0/0	Purified water is pumped through the column to remove excess of BH_4^- in order to ensure that the reaction will take place in the column when the acid is introduced. Drains from gas–liquid separator were pumped to waste
1	30	0/0	50/6	5 mol l^{-1} HCl is pumped through the column and the bismuthine is generated. The BiH_3 is stripped from the eluent solution with the aid of a nitrogen flow at 110 ml min^{-1} and the bulk phases is separated in the glass gas–liquid separator. Spectrometer is directed to read and BiH_3 is atomized in a quartz tube heated at 900 °C and bismuth atomic absorption is measure at 223.1 nm. Drains from gas–liquid separator are pumped to waste
1	20	60/8	0/0	Purified water is pumped through the column to remove the acid remaining in the column. Drains from gas–liquid separator are pumped to waste

0.16 mol l^{-1}. A concentration of 0.1 mol l^{-1} of HNO_3 was found to be sufficient as acid medium, necessary to achieve the efficient formation of the complexes. Once the complexes were ready, the analyte was determined in each solution following the procedure described earlier. The results of this experiment are shown in trace A in Fig. 2; it can be seen that the signal increases as the KI concentration increases up to 0.12 mol l^{-1}, then it slightly decreases. These results are in agreement with the fact that iodides with higher negative charge, which are formed at elevated iodide concentrations, are more effectively retained in the resin. The small reduction in the signal observed at the highest KI concentration probably may be explained by the interference in the retention of the analyte complexes caused by the iodide ions. Finally, an elevated memory effect was observed when a successive determination was performed without introduction of analyte solution. The results of this study are shown by trace B in Fig. 2. Therefore, a cleaning step was necessary in other to avoid the memory effect. The column was cleaned between determinations by passing through it a solution of 2% (w/v) sodium borohydride in 0.1% (w/v) NaOH, followed by 8 mol l^{-1} HCl and finally purified water for 30 s each at a flow rate of 8 ml min^{-1}.

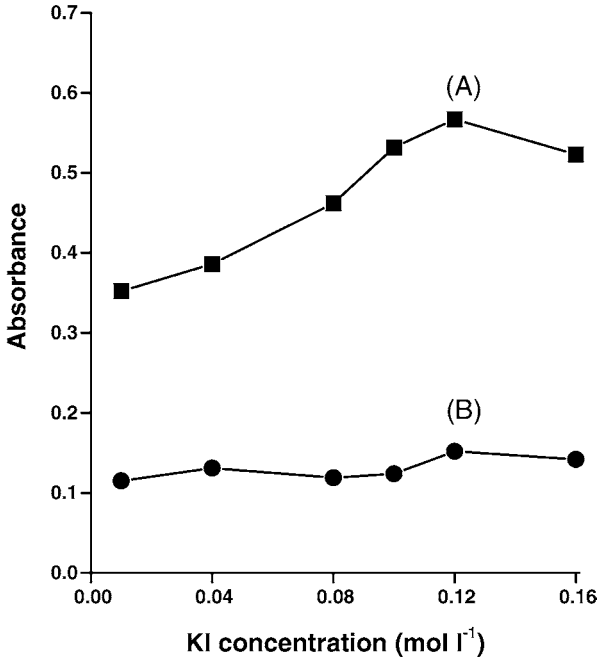

Fig. 2. Effect of the KI concentration used to prepare the iodides complexes of bismuth in solutions containing 50 μg l^{-1} of Bi and 0.1 mol l^{-1} of HNO_3.

The following anionic species studied were the bromides complexes. The different bromides were prepared by varying the concentration of KBr between 0.1 and 0.6 mol l^{-1}. In this case likewise in the case of the iodides a concentration of 0.1 mol l^{-1} of HNO_3 in the sample solution was found to be sufficient to achieve the efficient formation of the bromide complexes. The results obtained after determination of bismuth in each solution are shown in Fig. 3A. In this case, the analytical signal increased as the KBr concentration increased to 0.3 mol l^{-1}, thereafter, the signal decreased. Once again, the increase in the signal may be explained by the formation of bromides further negatively charged (taking into account the stability constants [33]) and the diminution of the signal by the interference caused by the excess of Br^- presents. The memory effect (Fig. 3B) in this case was higher that in the case of iodides, however, the same cleaning step as that performed previously was enough to clean the column.

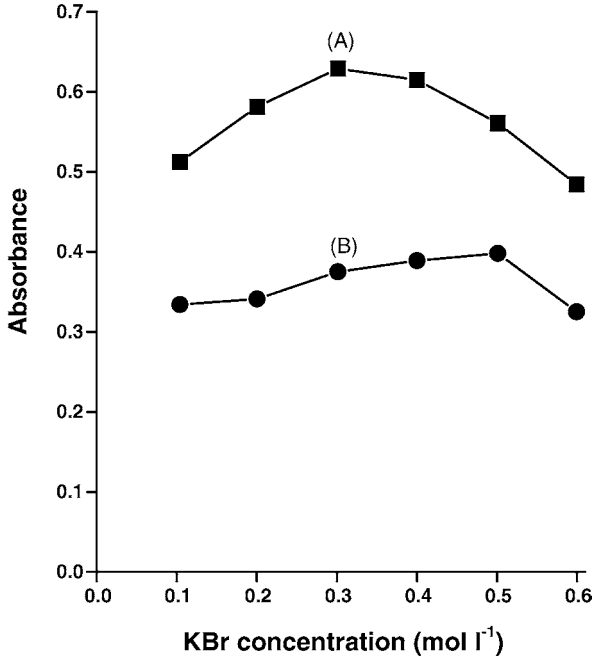

Fig. 3. Effect of the KBr concentration used to prepare the bromides complexes of bismuth in solutions containing 50 μg l^{-1} of Bi and 0.1 mol l^{-1} of HNO_3.

The third and last types of complexes of bismuth studied were the chlorides. The different chlorides were prepared by varying the concentration of HCl between 0.1 and 0.6 mol l^{-1}. The results of the determination of bismuth of this final study are represented in trace A in Fig. 4. In this case, the signal was higher than that for the other halides and without major variations within the range of HCl concentration studied. However, a slight maximum in the signal was observed at 0.3 mol l^{-1} of HCl. Additionally, in this case the memory effect was negligible (Fig. 4B), making the cleaning step unnecessary. Therefore, in the further studies a concentration of 0.3 mol l^{-1} of HCl was added to samples and standards to achieve the formation of the chloride complexes of bismuth, mainly $BiCl_4^-$ according to the stability constants [33].

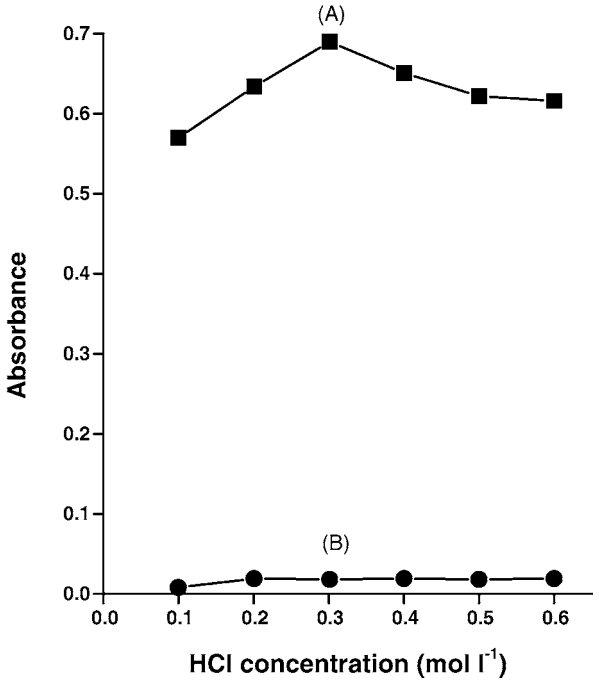

Fig. 4. Effect of the HCl concentration used to prepare the chlorides complexes of bismuth in solutions containing 50 μg l^{-1} of Bi.

3.2. Parameter optimization

The multi-cycle alternating variable search method [32] was used for the optimization of the following parameters: the borohydride concentration, the flow rate of the borohydride solution was passed through the column, the HCl concentration, and the stripping/carrier gas flow rate. The figure of merit for the optimization process was maximum net absorbance (i.e. blank subtracted). Other parameters that were studied included the dimensions of the column, the nature of the anion-exchange resin, and the flow rate of the sample solution.

The optimum conditions are given in Table 1. To achieve the best sensitivity, four cycles of the optimization process were necessary. The results of the last cycle for each parameter are presented below. At a flow rate of 5 ml min^{-1}, different amounts of BH_4^- were passed through the column by varying the concentrations of sodium borohydride within the range 0.02–0.14% (w/v). The maximum signal was obtained at 0.08% (w/v); thereafter, the signal keeps approximately constant as the $NaBH_4$ concentration increased. The signal also depended on the flow rate that the borohydride solution was passed through the column. Therefore, the same amount of $NaBH_4$ was introduced in the column by varying the flow rate and the length of time that the reagent was passed through the column within the ranges of 1.25–15 ml min^{-1} and 10–120 s, respectively. The best results (sensitivity and reproducibility) were obtained for flow rates lower than 5 ml min^{-1}. Flow rates higher than 5 ml min^{-1} produced progressive loss of sensitivity and reproducibility. This dependence is possibly due to a better distribution of the BH_4^- within the column at lower flow rates. In order to reduce the time of the analysis, a flow rate of 5 ml min^{-1} was selected as optimum.

The effect of the hydrochloric acid concentration used for the generation of bismutine from the column was study by varying the concentrations of HCl within the range 1.0–7.0 mol l^{-1}. The results shown that the signal increased constantly as the HCl concentration increased to 5 mol l^{-1}, then decreased. These results indicate that HCl concentrations lower than 5 mol l^{-1} are insufficient to complete the generation of the analyte retained in the column. On the other hand, the decrease of the signal at concentrations higher than 5 mol l^{-1} may be due to dilution of the analyte by the ex-

cessive generation of hydrogen, which is byproduct of the reaction.

When no carrier gas was used, no signal was observed, indicating that the hydrogen byproduct by itself was insufficient for stripping and transport of the generated bismutine to the quartz cell. When the nitrogen flow was increased from 0 to 90 ml min^{-1}, an increase in the signal was observed. The signal reached a plateau within the range 90–150 ml min^{-1}, thereafter the signal decreased. A flow rate of 110 ml min^{-1} was chosen as optimal.

Two different strongly basic resins, Amberlite IRA-410 and Amberlyst A26, were separately packed in six columns of three different lengths (50, 150, and 200 mm) and two different internal diameters (2 and 4 mm), respectively. Both resins have styrene–divinylbenzene skeletal structures, however, Amberlite IRA-410 is a gel-type resin and Amberlyst A26 is a porous or macroreticular resin. The resulting columns were tested for bismuth chlorides retention and subsequent bismutine generation. The best results were observed with larger column sizes (lengths and i.d.) with both resins, i.e. when the amount of resin was increased. However, lengths above 150 mm did not produce any improvement in the signal. The Amberlite IRA-410 resin produced better results than the Amberlyst A26 resin. Therefore, 150 mm × 4 mm column packed with Amberlite IRA-410 gel-type resin was chosen for further experiments.

Finally, the effect of sample solution flow rate was study. The same amount of analyte was introduced in the column by varying the flow rate and the length of time that a solution of 50 μg l^{-1} of Bi was passed through the column. The flow rate and the time were varied within the ranges of 1.25–15 ml min^{-1} and 10–120 s, respectively. The signal slightly falls down for flow rates higher than 7.5 ml min^{-1}. Additionally, a very poor reproducibility was observed at flow rates higher than 10 ml min^{-1}. A flow rate of 5 ml min^{-1} was selected as optimum for further experiments.

3.3. Effect of interferences

It is well known that the hydride generation technique for bismuth determination is susceptible to severe interference caused by the presence of transition metals, mainly those of the groups VIII, IB and IIB [27]. The tolerance of the system to interferences was evaluated by investigating the effect of the ions that are known to be the primary interfering species in the bismutine generation reaction. The following cations were studied: Cu^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, and Mg^{2+}. The tolerance limits to the interferences, expressed as the maximum concentration of the interfering element added to a Bi solution which differed less than 5% to the signal of a solution of Bi alone were determined. The results of this study are shown in Table 2. The most severe depression was caused by Hg^{2+} for which concentration of 60 mg l^{-1} caused a 5% depression on the signal. For the other cations, concentrations of between 1000 and 10,000 mg l^{-1} could be tolerated.

Table 2
Interferences from diverse elements

Cation	Added as	Tolerance limit[a]
Cu^{2+}	$Cu(NO_3)_2$	4000
Co^{2+}	$CoCl_2{\cdot}6H_2O$	10000
Fe^{3+}	$Fe(NO_3)_3{\cdot}9H_2O$	10000
Ni^{2+}	$Ni(NO_3)_2{\cdot}6H_2O$	1000
Cd^{2+}	$Cd(NO_3)_2{\cdot}4H_2O$	1000
Pb^{2+}	$Pb(NO_3)_2$	8000
Hg^{2+}	$Hg(NO_3)_2$	60
Zn^{2+}	$ZnSO_4{\cdot}7H_2O$	2000
Mg^{2+}	$Mg(NO_3)_2{\cdot}6H_2O$	10000

[a] Maximum concentration of the interfering element (mg l^{-1}) added to a Bi solution which differed less than 5% to the signal of a solution of Bi (50 μg l^{-1}) alone.

3.4. Analytical performance

The calibration equation and the other performance figures of merit are summarized in Table 3. The system responded linearly from the detection limit up to 80 μg l^{-1}. The precision of the procedure, calculated as the R.S.D.% of 10 determinations of 10 and 50 μg l^{-1} of Bi solutions, was 1.55 and 0.85%, respectively. The limit of detection (LOD) defined as the concentration giving a signal equal to three times the standard deviation of the blank signal, was 0.225 μg l^{-1}. Lower LODs can be achieved using larger sample volume, i.e. LODs of 45 ng l^{-1} for 12.5 ml and 23 ng l^{-1} for 25 ml.

The percentage recoveries of spikes added to the urine samples prior to digestion are shown in Table 4. The values range from 95 to 101, indicating that bismuth can be quantitatively recovered from urine in all the steps of the procedure. To further confirm the accuracy and check the reliability of the analytical procedure, bismuth was determined in a standard reference material (Seronorm™ trace elements urine). The concentration found (24.93 ± 0.09 μg l^{-1}) was in good agreement with the indicated value of 25 μg l^{-1}. Finally, the accuracy was verified comparing the results obtained in the determination of bismuth in six different urine samples us-

Table 3
Analytical performance of the system

Regression equation: $A = b + mC$[a]	r[b]	LOD (3σ, μg l^{-1})[c]	R.S.D. (%)[d]	
			10	50
$A = 0.009 + 1.428 \times 10^{-2}C$	0.9978	0.225	1.55	0.85

[a] A is absorbance; b is intercept; m is slope; C is concentration of Bi in μg l^{-1}.

[b] Regression coefficient.

[c] LOD (3σ) is the detection limit, calculated for 3S.D./m, where S.D. is the within-run standard deviation of a blank determination (n = 10) for 2.5 ml of sample.

[d] R.S.D. (%) is the relative standard deviation for 10, and 50 μg l^{-1} of Bi (n = 10).

Table 4
Recovery of bismuth spiked in urine samples (25 ml)

Sample	Bismuth added[a] ($\mu g\,l^{-1}$)	Bismuth found[b] ($\mu g\,l^{-1}$)	Recovery (%)
1	0	1.47 ± 0.07	–
	5	6.86 ± 0.13	97 ± 2
2	0	2.73 ± 0.11	–
	20	21.65 ± 0.66	95 ± 3
3	0	1.23 ± 0.05	–
	50	51.85 ± 2.41	101 ± 5

[a] Added prior to digestion.
[b] Average of triplicate digestions, ± terms are standard deviations.

Table 5
Determination of bismuth in urine samples of six individuals after 3 days of treatment with BS by ETAAS [19] and by the procedure developed here ($n = 3$)

Sample	Bismuth concentration ($\mu g\,l^{-1}$)	
	ETAAS	This work
1	14.25 ± 0.65	13.95 ± 0.35
2	11.34 ± 0.56	11.73 ± 0.32
3	15.28 ± 0.73	15.90 ± 0.47
4	8.45 ± 0.36	9.01 ± 0.25
5	12.35 ± 0.61	11.97 ± 0.37
6	7.04 ± 0.31	5.66 ± 0.18

ing the procedure developed in this work with those obtained by an alternative technique (ETAAS [19]). The results of this study are shown in Table 5. The Student's *t*-test at 95% confidence level showed that none significant difference was observed between the mean values obtained.

3.5. Determination of bismuth in urine samples

The method developed was applied to the determination of bismuth in urine samples, obtained from 14 healthy male laboratory employees or students. The volunteers had taken 250 mg of bismuth subcitrate (BS) once a day for 3 days. The concentration of bismuth found in urine $\mu g\,l^{-1}$ after 0, 1, 2, and 3 days of treatment were: 1.94 ± 1.26 (within the range 0.52–3.89), 4.54 ± 2.68 (within the range 0.12–8.25), 8.14 ± 4.88 (within the range 0.79–15.23), and 9.02 ± 5.82 (within the range 0.20–19.61), respectively. In this study, the mean levels of bismuth after the intake of a therapeutic dose of BS increased markedly in most subjects. Even though, a wide spread in the individual values was observed, a fairly constant mean value of Bi in urine was found after the second day of treatment. Additionally, these results show that the bismuth uptake and its elimination greatly depend on the individual concerned. Finally, the concentrations of Bi determined in the individuals studied in this work before treatment with BS are in good agreement with those found for other authors in normal human urine [10–12].

4. Conclusions

The analytical performance of HG-AAS for the determination of bismuth can be improved by the use of an anion-exchange resin to co-immobilize bismuth and borohydride followed by passage of acid to generate bismuthine for its atomization in quartz tube. The selective retention of the analyte also gives rise to improved tolerance to cationic interferences, good reproducibility and accuracy. If better sensitivity were required, it can be attained with larger sample volumes, but this would increase the time of analysis. The proposed methodology is perfectly applicable for the determination of bismuth in human urine samples of normal individuals, as well as monitoring of patients after treatment with bismuth. This fully automatic system could be utilized either with simple or advanced spectrometric detection techniques.

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