The bismuth contents of various digested urine samples and prescription medicines were determined by atomic absorption spectrometry combined with hydride generation. The procedure followed was a standard addition method for urine and direct calibration for the prescription medicines. The detection limit of the method was determined to be 320 pg ml$^{-1}$ Bi with an analytical frequency of 150 h$^{-1}$. A relative standard deviation of 4.7% was found for Bi in urine at the level of 4.3 ng ml$^{-1}$ Bi. Interference caused by Ni$^{II}$, Co$^{II}$, Cu$^{II}$, Ag$^{I}$, Se$^{IV}$, Sb$^{III}$ and Hg$^{II}$ could be controlled with a masking solution of thiourea (0.2%)–KI (10%).

**Keywords:** Bismuth; hydride generation atomic absorption spectrometry; urine; prescription medicines

The analysis of urine may provide relevant information related to absorption, retention and excretion of certain metals. Bismuth-containing pharmaceuticals have been used for a long time for different medical purposes, especially for the treatment of gastrointestinal tract disturbances, such as gastritis and peptic ulcer. Incorrect use of these formulations results in considerably increased intake of the metal with significant adverse effects, such as encephalopathy and nephrotoxicity. Bismuth and its compounds are not readily absorbed by the gastrointestinal tract and most of them are excreted in feces but, once absorbed, its excretion is rapid, mainly in urine. In order to monitor the excretion of bismuth taken by oral administration of bismuth-containing pharmaceuticals, the urine may be analysed by one of several different methods which provide the required detection limits.

Hydride generation (HG) is undoubtedly one of the most sensitive and convenient analytical techniques for a group of elements, including bismuth. Since the publication by Åstrom, in 1982, reporting the combination of HG with the flow injection (FI) technique for the determination of bismuth, the potentialities of this association have been demonstrated in subsequent investigations. This versatile approach includes the use of small sample volumes, high precision, better accuracy, reduction of interference effects, in both the liquid and gaseous phases, and high analytical frequency. Therefore, HG offers the advantage of excellent sensitivity for the determination of bismuth with relatively simple instrumentation, being well suited for routine analysis. However, severe systematic errors may occur when direct analysis is considered, probably due to excessive foaming and/or the binding of bismuth to some constituents, which remove the free metal ions from the solution. Otherwise, the accuracy of the determination depends critically on the acid digestion. Improvements have been obtained with samples from patients under bismuth therapy, in which larger dilution factors could be applied, as described by Chou et al. The recovery factor is reduced at low sample dilution.

The use of modern technology, including automation or even semi-automation, is recommended for obtaining reliable analytical results. Recently, we have reported a new approach for trace determination of bismuth in metallurgical samples, including a stripping-type generator–gas–liquid separator, with excellent performance.

This paper reports a simple, fast and efficient procedure for the determination of traces of bismuth in wet-oxidised urine samples and prescription medicines, using an on-line HG system with the new device for the generation and separation of the bismuth hydride from the aqueous solution with subsequent on-line transportation to the atomic absorption cell using nitrogen.

**Experimental**

**Instrumentation**

The FI–HG manifold has been described in detail previously. All analytical measurements were carried out with a T-shaped quartz cell (17 cm long × 0.8 cm id), electrically heated and supported in the optical path of a Varian (Palo Alto, CA, USA) atomic absorption spectrometer, Model Gemini AA 12/1475, provided with a deuterium background corrector. A nitrogen flow of 120 ml min$^{-1}$ was used. This purge gas causes, simultaneously, efficient mixing and removal of the bismuthine (BiH$_3$) from the aqueous solution. The merging-zones configuration was used with small sample and reagent volumes (50 µl). Considering that the samples were decomposed with nitric acid, no background was expected. Indeed, the possible occurrence of some undesirable signals was checked and no relevant response was observed which required correction. A bismuth hollow cathode lamp was used as a light source, operating at a current of 8 mA. The wavelength was set to 223.1 nm and a slit-width of 0.2 nm was used. All analytical signals were registered on an Epson (Torrance, CA, USA) LX-800 printer and the measurements made in peak height mode. The T-quartz tube atomiser was frequently cleaned with a 10% (v/v) HF solution and then submitted to treatment with 5% di-chlorodimethylsilane (v/v) in toluene, in order to eliminate reactive sites on the glass surface and generate better conditions to improve the sensitivity and reproducibility of the signals.

**Reagents**

All reagents used in this work were of analytical-reagent grade. Distilled, deionized water was used throughout. A reference bismuth stock solution (1000 µg ml$^{-1}$) was prepared from metallic bismuth (J. T. Baker, Phillipsburg, NJ, USA, 99.99%) treated with concentrated HCl and HNO$_3$ until dissolution and then diluted to 1000 ml with 1.0 mol l$^{-1}$ HCl. Appropriate dilution was made from the stock solution with 1.0 mol l$^{-1}$ HCl.

Sodium tetrahydroborate was prepared by the dissolution of NaBH$_4$ powder (Merck, Elmsford, NY, USA) in 0.05 mol l$^{-1}$ KOH and stored in a high density polyethylene flask (Nalgene) under refrigeration. This reducing solution is stable for at least 2 weeks, proved by observing the peak height and peak profile.
of the absorption signal. Interferent ions and masking agent solutions were prepared by dissolving the appropriate salts in acid or distilled, deionized water.

Samples

Bismuth-containing pharmaceuticals

Bismuth subsalicylate (powder) Van Roosmalen. A portion (0.10 g) of this medicine was dissolved in 10 ml of 5 mol l\(^{-1}\) HCl and evaporated to dryness. The residue was dissolved and diluted to 100 ml with 1.0 mol l\(^{-1}\) HCl.

Bismuth subnitrate (tablet), Roter. One tablet (1.03 g) was dissolved in 10 ml of 5 mol l\(^{-1}\) HCl and 2 ml of concentrated HNO\(_3\) and evaporated on a hot plate to dryness. The residue was dissolved in 1.0 mol l\(^{-1}\) HCl and diluted to 1000 ml with the same acid.

Urine

To 10.0 ml of urine sample were added 10 ml of concentrated HNO\(_3\) in a beaker that was then covered and this mixture was carefully heated on a hot plate at a temperature of 110–120 °C for about 1 h in order to permit contact of the acid with the sample and reduce the volume, resulting in a clear yellow solution. At this point, the cover was removed and the oxidation proceeded with evaporation of the solution to dryness. To the residue was added 3 ml of concentrated HCl and the resulting solution was again heated to dryness at the same temperature. Finally, the digests were all treated by heating with 1.0 mol l\(^{-1}\) HCl, transferred to a 10.0 ml flask and the volume completed with the same acid.

Results and discussion

Bismuth-containing pharmaceuticals

Even considering that the main interest of this investigation was the determination of bismuth in urine, studies have been made in order to determine the amount of Bi in some pharmaceutical preparations using this system as an alternative procedure to flame atomic absorption technology. Two bismuth-containing compounds currently used to control gastrointestinal diseases were evaluated: bismuth subsalicylate and bismuth subnitrate. Both are administered orally to patients in order to act as a protector to the injured gastrointestinal tract, causing lowering of acidity, protection of the mucosa from gastric acids and from the erosive properties of aspirin and alcohol and, in addition, providing antimicrobial activity.\(^{12}\)

Samples were prepared as described and after the required dilution, appropriate aliquots were injected into the HG system. The results obtained were compared with those obtained by FAAS. The analytical calibration curve for bismuth analysis in these two medicines, using the proposed procedure showed linear response from 0 to 100 ng ml\(^{-1}\) Bi, with a detection limit of 320 pg ml\(^{-1}\) Bi, considering a signal to noise ratio of 3 and an analytical frequency of 150 h\(^{-1}\). Table 1 shows the results obtained for the bismuth content in these medicines by HG and also by FAAS. Good reproducibility of the bismuth determination is observed, considering the relative standard deviation of each sample.

### Table 1 Determination of bismuth in medicines (\(n = 5\))

<table>
<thead>
<tr>
<th>Medicines</th>
<th>Declared value</th>
<th>HGAAS (%)</th>
<th>FAAS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bismuth subsalicylate (5 g)</td>
<td>101</td>
<td>99 (1.0)</td>
<td>101 (2.0)</td>
</tr>
<tr>
<td>Bismuth subnitrate (1 tablet)</td>
<td>206</td>
<td>214 (2.3)</td>
<td>214 (2.8)</td>
</tr>
</tbody>
</table>

Urine samples

Initially the bismuth content in the urine of a patient who had not taken any kind of bismuth-containing medicines during the past 12 months before sampling was determined. Even considering the analytical interest, these tests were developed in such way to show the analytical potential as a tool for the clinical laboratory. As reported by Chou et al.\(^{1,4}\) a preliminary reference range for urinary bismuth was found to be less than 17 ng ml\(^{-1}\) Bi, but for patients submitted to bismuth therapy for peptic ulcers, for instance, the level of bismuth varied from 5 to 1460 ng ml\(^{-1}\) Bi. A reference interval of 0.3–4.6 ng ml\(^{-1}\) Bi for urine was reported by Froomes et al.\(^{1}\)

In this study, the HG procedure was applied to the wet-oxidised urine samples after spiking with increasing amounts of bismuth in different aliquots of urine from this patient. Standard addition was used and evaluated, because of the expected low concentration for bismuth. The net numerical results obtained revealed a value of 1.6 ng ml\(^{-1}\) Bi with a standard deviation of 0.2 (\(n = 5\)). This value was considered as a reference to identify any variation in the amount of circulating bismuth in the urine. To the same patient a single dose of bismuth subsalicylate was orally administered, equivalent to 58 mg Bi, and continuous random frequency sampling of the urine was followed by determining the concentration of excreted bismuth. By monitoring this excretion for about 8 d a profile of the bismuth eliminated through 204 h was obtained. Fig. 1 illustrates the results obtained in this experiment with the method of standard addition. This experiment shows that 48 h after taking the medicines most of the bismuth has been eliminated, but even after 8 d the circulating amount of bismuth is still above the reference value. To illustrate the analytical performance of this procedure the results obtained in one sample collected at 16 h after ingestion of bismuth are considered. Fig. 2 includes the analytical curve for the standard addition applied to the sample.

![Fig. 1 Profile of bismuth excreted by urine during 8 d. Concentration determined by the technique of standard addition.](image-url)

![Fig. 2 Method of standard addition applied to the urine sample spiked with bismuth.](image-url)
collected 16 h after taking the medicine and Fig. 3 shows the respective analytical signal in peak height for bismuth. Results show a concentration of 5.8 ng ml$^{-1}$ Bi with an RSD of 1.7%.

The accuracy of these measurements was assisted by analytical determinations of bismuth concentrations in each sample of urine with a procedure described by Gladney,\textsuperscript{13} using ETAAS and Ni(NO$_3$)$_2$ as chemical modifier. These results showed excellent agreement with those obtained by HG. Table 2 presents the comparative values for a sample of urine collected 24 h after ingestion of bismuth subsalicylate as being 4.3 ng ml$^{-1}$ Bi with an RSD of 4.7%.

Interferences caused by organic components of urine were not expected to occur because of the oxidation with HNO$_3$. Thus only the presence of metals\textsuperscript{14} was considered. Under the experimental conditions, Al$^{3+}$, Ca$^{2+}$, Fe$^{3+}$, Mg$^{2+}$, Mn$^{2+}$ and Zn$^{2+}$ did not cause any interference even when present in 5000- to 10000-fold excess. Small concentrations of Ni$^{2+}$, Co$^{2+}$, Cu$^{2+}$ and Se$^{4+}$ caused severe depression of the bismuth signal. To overcome these interfering effects various reagents and mixtures were investigated.\textsuperscript{11} Using a masking solution of thiourea (0.2%)–KI (10%), the recovery of the analytical signal in the presence of such ions was obtained with success.

**Conclusion**

Bismuth may be conveniently determined by FI–HGAAS in urine samples and prescription medicines after a wet-oxidation procedure. Excellent sensitivity and reproducibility was obtained because of the low residence volume (≈200 μl) inside the chamber of the generator–gas–liquid separator. As has been reported for other hydride-forming elements, we have also observed interferences caused by various metal ions in solution,\textsuperscript{11} but any possible interference is efficiently controlled with a solution of thiourea (0.2%)–KI (10%). Results obtained using the standard addition method indicated that the proposed procedure overcame problems related to the low concentration of bismuth in urine and, in addition, presented excellent precision and accuracy, as may be verified by comparative methodologies. In spite of the fact that we used an addition procedure, we did not observe any matrix interference and the analytical curve for the standard addition itself showed no slope differences when compared with the aqueous calibration curve.

Under these conditions, aqueous standards may be used for the quantification of bismuth, avoiding the time consuming standard addition steps. No tedious separation step is necessary and, when required, wet-oxidised digests may be dissolved and diluted to a smaller aqueous volume and a sample injection volume of 100 μl used instead of the 50 μl, representing an enrichment factor, with an increased mass of bismuth being transported to the reaction chamber, improving the analytical figure of merit, in addition to the excellent limit of detection of 320 pg ml$^{-1}$ Bi. Sample throughputs of 150 h$^{-1}$ with a recovery factor of 95.0% and 97.5% at the levels of 40 and 80 ng ml$^{-1}$ Bi, respectively, are described in this work.

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**References**