DEVELOPMENT AND APPLICATION OF HYDRIDE GENERATION LASER INDUCED FLUORESCENCE METHOD FOR QUANTITATIVE ANALYSIS OF BISMUTH AND GERMANIUM

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ABSTRACT

Continuous flow hydride generation coupled with laser induced fluorescence spectrometry (HG-LIF) has been investigated for the measurements of Bi (III) and Ge (IV) in aqueous samples. The developed HG-LIF method has been evaluated in terms of analytical figures of merit. The limits of detection calculated are 0.03 ng/mL and 0.1 ng/mL for Bi and Ge, respectively. The accuracy of this method has been evaluated by analysis of a NIST standard reference material. The analytical utility of masking reagents has been investigated by performing recovery studies on a multielement ICP standard reference material. The HG-LIF approach has been applied to the measurements of Bi in a commercial medicine, and different kinds of tea leaves. The analytical utility of HG-LIF technique for measurements of Ge has been evaluated by analyzing a germanium supplement. The results of these studies demonstrated that HG-LIF approach has strong feasibility for Bi and Ge measurements in environmental samples.

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LIST OF SYMBOLS AND ABBREVIATIONS

LASER	Light Amplification by Stimulated Emission of Radiation
Nd:YAG	Neodymium-doped Yttrium Aluminum Garnet
LIF	Laser Induced Fluorescence
HG	Hydride Generation
PMT	Photomultiplier tube
Nd^{3+}	Neodymium ion
Bi	Bismuth
Ge	Germanium
Sb	Antimony
Sn	Tin
Ar	Argon
H_2	Hydrogen
ppm	Parts per million
ppb	Parts per billion
ppt	Parts per trillion
LOD	Limit of detection
LOQ	Limit of quantification
RSD	Relative standard deviation
HCl	Hydrochloric acid
$NaBH_4$	Sodium borohydride
NaOH	Sodium hydroxide
L	Liter

mL	Milliliter
µg	Microgram
g	Gram
ng	Nanogram
µm	Micrometer
nm	Nanometer
µs	Microsecond
ns	Nanosecond
M	Molar
w/v	Weight per volume
v/v	Volume per volume
V	Volt
mV	Millivolt
eV	Electron volt
kW	Kilowatt
mJ	Millijoule
Hz	Hertz
min	Minute
%	Percentage
a.u	Arbitrary units

INTRODUCTION

In recent years the development of sensitive and selective analytical techniques for trace and ultra-trace analysis of metals and metalloids has gained more importance due to increased concerns over pollution of the environment and the important roles trace metals have in biological systems [1]. A number of atomic spectroscopy techniques have been developed and used formerly for the determination of different elements in environmental and biological samples. Laser Induced Fluorescence coupled with Hydride Generation sample introduction is a very sensitive method to determine trace amounts of hydride forming elements [2]. In this study, a Hydride Generation Laser Induced Fluorescence (HGLIF) method has been investigated for the trace level measurement of Bismuth (Bi), and Germanium (Ge). The toxicity and/or essentiality of these elements depend not only on their concentrations, but also on their chemical speciation [3].The goal of these studies is to evaluate the HG-LIF approach in terms of spectral characteristics and analytical figures of merit. Hydride Generation (HG) sample introduction technique can improve sensitivity and provide speciation information for certain metal and metalloid elements [4].

Laser induced fluorescence (LIF)

Atomic fluorescence is the optical emission from gas-phase atoms that have been excited to higher energy levels by photon absorption. In Resonance fluorescence, the same upper and lower energy levels are involved in the excitation-emission process so that the absorption and fluorescence wavelengths are the same, whereas in non-resonance fluorescence different combinations of energy levels are involved in the excitation-emission process so that absorption and fluorescence wavelengths are different. Laser

Induced Fluorescence (LIF) is a type of Atomic Fluorescence technique which involves the excitation of gas-phase atoms by a beam of laser radiation followed by the detection of the subsequent emission of radiation from the analyte atoms. Laser Induced Fluorescence is a very sensitive and selective analytical technique for the analysis of a number of environmentally and biologically significant elements such as Arsenic, Selenium, Antimony, Lead, and Tin [5, 6].

Laser Induced Fluorescence has several advantages over Atomic Absorption and Atomic Emission spectroscopy. First, LIF has excellent detection sensitivity because a signal is observed against a dark background [7]. Second, LIF has high selectivity, due to the use of dissimilar excitation and fluorescence wavelengths. The high selectivity of LIF provided by the narrow line laser source and the fluorescence monochromator reduces the possibility of spectral interferences. The third advantage is that LIF has a large range of linearity. The signal to noise ratio of fluorescence signals is also very high, since emitted light is read at right angles to the exciting light. Because of these differences, the sensitivity of LIF is often 10^3 times greater than absorption spectroscopy.

LIF spectroscopy is a powerful analytical technique that combines high selectivity, sensitivity and outstanding capabilities for ultra-trace analysis. By means of LIF, detection limits down to pg/mL (ppt) levels can be obtained [4, 5]. The detection limits obtained by Laser Induced Fluorescence are often comparable to ICP-MS. Advantages of LIF over ICP-MS can include a lower order of magnitude of absolute Limit of Detection (LOD), which is important in the case of limited sample volume, and high selectivity that minimizes spectral interferences [8]. It can be used in the analysis of complex samples without loss of sensitivity [9].

Despite the fact that LIF is a powerful analytical technique, it also has some limitations. One limitation is the amount of the fluorescence collected is only a small part (1 %) of the total fluorescence that can be collected. Computer modeling has been used to investigate ways to improve collection efficiency of the LIF [10]. LIF is also not appropriate for the analysis of elements that necessitate the use of resonance fluorescence, because of the large scattering background of the laser.

Bismuth

Bismuth (Bi) is a naturally occurring element that is commonly found in many cosmetics and pharmaceuticals. Bismuth has been widely used in industry and medication in recent years. The effects of Bi are influenced mainly by its concentration in materials [11]. The most important ores of Bi are bismuthinite (bismuth sulfide) and bismite (bismuth trioxide) [12].

Health effects of bismuth:

Bismuth exposure can occur through inhalation, ingestion and skin absorption. Inhalation of bismuth causes acute effects like respiratory irritation. Inhalation may also cause foul breath, metallic taste, and gingivitis [12]. Chronic exposure to Bi can result in the development of a black deposit or Bi line on gingiva [13]. In the 1970's and 1980's high levels of bismuth ingestion caused encephalopathy and headaches [14].The toxicity of Bi is mainly due to its effect on the brain by the alteration of neurons [15]. Bismuth can cause skin and eye irritation when high concentrations of Bi come in contact with the sites [16]. Bismuth and its salts can cause encephalopathy or mild kidney damage. Large doses can lead to albuminuria, bodily discomfort and sometimes serious exodermatitis

[17]. Unwanted and deadly side-effects can happen if high concentrations of Bi are introduced into the body [15].

Environmental effects of bismuth:

Bismuth metal has minimal effects on the environment and hence is usually not considered as a toxic metal. Bismuth compounds should be handled with care, because of the limited information available on the effects and fate of those compounds in the environment [12]. Highly sensitive methods are often required for the detection of Bi in the environment because of its usually very low concentrations. The concentration of Bi in colored gelatin films determines the quality of the film [18], which makes the determination of the trace amounts of Bi necessary. The techniques used previously for the determination of trace amounts of Bi included Atomic Fluorescence Spectrometry [19], Inductively Coupled Plasma – Atomic Emission Spectrometry [20], Inductively Coupled Plasma – Mass Spectrometry [21], Hydride Generation – Atomic Absorption Spectroscopy [18,22], Hydride Generation – Atomic Fluorescence Spectrometry [23, 24, 25], Hydride Generation – Inductively Coupled Plasma-Mass Spectrometry [26].

Germanium

Germanium (Ge) is an element that is found in earth's crust and has essential effects on human health [27]. Organo-Germanium compounds play an important role in the defense mechanism as antioxidants, immune modulators and immune enhancers [28]. Organo-Germanium compounds have also been observed to protect against mercury poisoning when mercury chloride was injected into the body of rats [29]. Experiments on Organo-Germanium compounds showed that they can protect against radiation damage in humans [30]. Germanium is used as a semiconductor in transistors, and integrated circuits, in

fiber optics, communication networks, wide angle lenses, and infrared night vision systems.

Health and environmental effects of germanium:

The route of exposure of Ge is by inhalation of Germanium hydride and Germanium Tetra hydride gasses. Inhalation of these gases can cause abdominal cramps, burning sensation, and redness of the eyes [12]. Due to its heavy metal nature, Germanium can cause some negative impacts on aquatic ecosystems, hence Germanium is considered to be an environmental pollutant. Highly sensitive methods are needed for the detection of Germanium, because of its very low amounts in the environment. The techniques used previously for the determination of trace amounts of Germanium included Atomic Fluorescence Spectrometry [27,31], Inductively Coupled Plasma – Mass Spectrometry [32], Hydride Generation – Atomic Absorption Spectrometry [33], Hydride Generation – Atomic Fluorescence Spectrometry [34].

Hydride generation

Sample introduction systems for atomic spectrometry methods include, Pneumatic nebulization, Ultra-sonic nebulization, and Hydride generation. Hydride generation (HG) is a common means of sample introduction for elements that form volatile hydrides like Sn, As, Sb, Pb, and Bi. The HG method is particularly useful for environmental analysis, where the concentration ranges are often close to ng/mL or ppb levels. One of several reasons for the popularity of hydride generation is its relative simplicity and the low cost of the apparatus [35]. HG has several advantages over conventional nebulization including high sensitivity, high selectivity and the ability to transport more analyte to the flame/plasma atomizer. Lower detection limits can be achieved because of the higher

sensitivity of this method. In HG the Non-hydride forming metal/metalloid elements present in the sample are not carried to the atomizer, which results in higher selectivity. In conventional nebulization, liquid analyte is usually converted to an aerosol and then the mixture is sprayed into the flame using a nebulizer. The efficiency of transport of analyte to the flame is low in the case of conventional nebulization (approximately 2 – 5 %). However, in the case of HG, as the hydride gas is carried directly to flame/plasma, the efficiency of transport of analyte is very high often approaches 100 %.

HG is a very efficient analytical technique developed to separate hydride forming metals from a range of matrices and varying acid concentrations [36]. Separating the analyte from the matrix can improve the sensitivity of the LIF detection technique and avoids physical, matrix and spectral interferences. The separation of the hydride from the matrix also allows for high efficiency of analyte introduction into the atomizer [36]. HG techniques can be useful for providing speciation information for certain metal and metalloid elements [35]. HG has been combined with several other detection techniques like Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [37,38,39], Atomic Absorption Spectrometry (AAS) [40,41], and Atomic Fluorescence Spectrometry (AFS) [42,43].

The HG reaction requires careful optimization and control to achieve high efficiency, and some reactive hydrides can be lost during transport or trapping. Another disadvantage of the method is the possibility of interferences in hydride evolution, which may take place at laboratory temperatures and in aqueous solutions [35].

Principle of hydride generation

The hydride generation process generally involves 2 steps

1. Release of hydride from the sample solution
2. Transport of the released hydride by a flow of purge gas to an atomizer [35]

A general equation showing the formation of hydride by the reaction of analyte and borohydride is as follows:

$$\text{Analyte } (l) + BH_4^- \quad \rightarrow \quad \text{Hydride } (g)$$

The HG reaction is dependent on the oxidation state of the analyte element and care must be taken to generate the specific oxidation state of the analyte. A purge gas is used to transport the released hydride to the atomizer, but it can also help in hydride release by stripping the hydride out of the sample solution. Chemical reduction by tetrahydroborate is typically used for hydride generation. An aqueous solution of $NaBH_4$ stabilized by sodium or potassium hydroxide is the most popular and most convenient reducing agent for hydride formation. A typical HG reaction involves the reduction of oxidized analyte by mixing a reagent solution of $NaBH_4$ with NaOH with an acidified sample solution. Borohydride solutions are unstable in acidic to neutral media, so the presence of NaOH is necessary to maintain BH_4^- in solution. The reactions involved in HG are as follows:

$$BH_4^- + H^+ + 3H_2O \rightarrow H_3BO_3 + 8H$$

$$M^{(m+)} + (m+n)\,H \rightarrow MHn + mH^+$$

Where M is the analyte of interest, *m* represents the oxidation state of the analyte, *n* the coordination number of the hydride and MH*n* is the hydride form of the element. The covalent hydrides investigated in these studies were Bi (BiH_3) and Ge (GeH_4) [44].

INSTRUMENTATION

Principle

Laser induced fluorescence

Atomic Fluorescence Spectroscopy (AFS) is a method that has the characteristics of both Atomic Absorption Spectroscopy (AAS) and Atomic Emission Spectroscopy (AES) methods. Laser Induced Fluorescence (LIF) is a type of AFS technique where a laser is used as the excitation source.

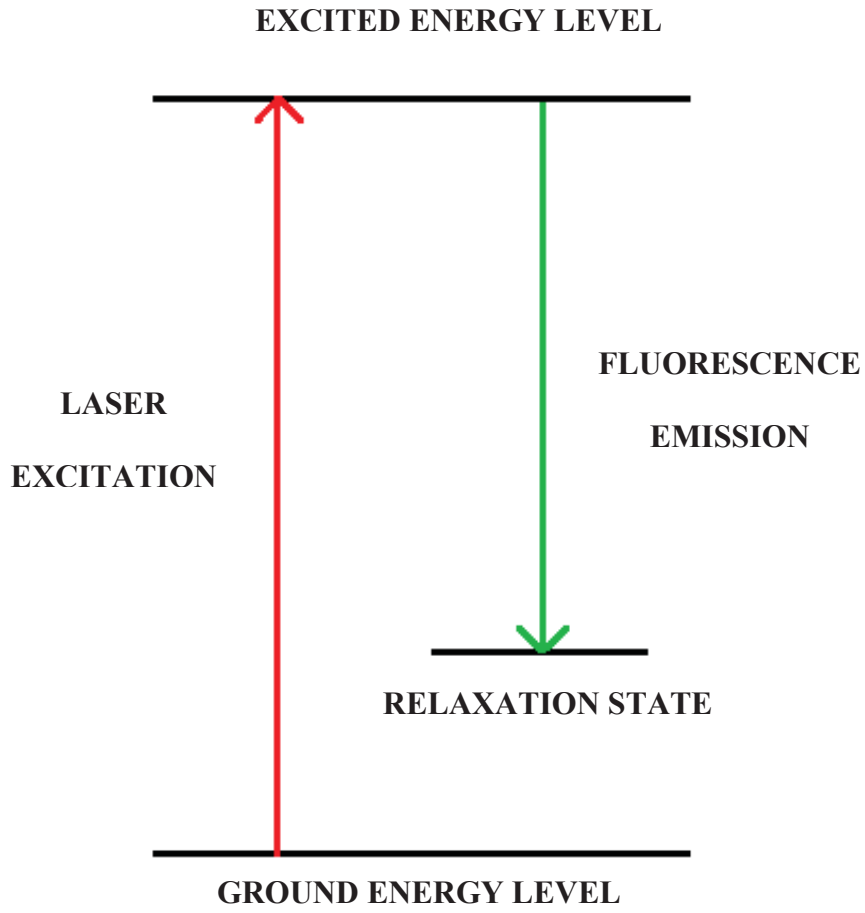


Figure 1 Schematic diagram of laser induced fluorescence

The main principle involved in LIF technique is fluorescence emission from gas phase atoms. Fluorescence emission is a two step process which involves excitation of gas phase atoms followed by subsequent relaxation. Excitation of gas phase atoms is caused by the absorption of photon energy from incident laser radiation. The wavelength is selected to be the one at which atoms have their maximum absorption. The excited state atoms then relax by re-emitting the absorbed energy in the form of fluorescence emissions. The subsequent fluorescence emission is then detected with a photo-multiplier tube. Atomic Fluorescence Spectroscopy is a useful method for quantitative assessments of elements because of its wavelength selectivity, its high signal to noise characteristics and low background noise.

The LIF instrumentation in this project consists of

1. Nd:YAG laser (Pulsed laser)
2. Tunable dye laser
3. Frequency doubling crystal
4. Raman shift cell
5. Optics for focusing and collecting light
6. H_2 Flame
7. Hydride generation system
8. Monochromator
9. Photo-multiplier tube
10. Box-car integrator
11. Oscilloscope and computer to view and record the spectrum
12. Argon gas system

Nd:YAG laser

A Nd:YAG pulsed laser is used as an excitation source in LIF (DCR 2A, Spectra Physics & Continuum, Surelite SLII-10).

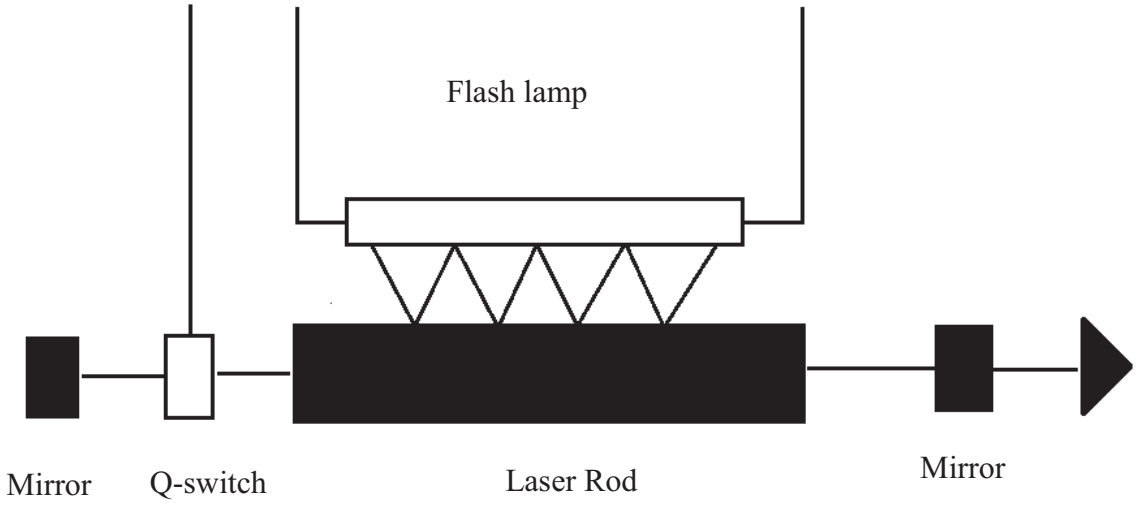


Figure 2 Schematic diagram of Nd:YAG laser

An Nd:YAG laser consists of four major components including a flash lamp, laser rod, Q-switch and mirrors. The main function of flash lamp is to optically pump the Nd:YAG laser. The optical resonator is a closed cavity with a neodymium-doped yttrium aluminum garnet crystal ($Nd:Y_3Al_5O_{12}$) as a lasing medium. Nd^{3+} ions in the gain medium absorb pumping light from flash lamp and they become excited. These excited ions emit photons with the same energy as the laser atomic transition wavelengths. In this process, when a photon passes through the lasing medium and when the frequency of the photon is equal to that of lasing medium, the photon becomes amplified due to the

simulation of the decay of the other ions from the upper energy state to the lower energy state. The mirrors are arranged in a way to make the amplified light reflect back into the resonant cavity to increase the amplification.

Nd:YAG lasers are one of the most common types of lasers and are used in LIF measurements because of their consistency, efficiency, compactness, and high laser energy production. The fundamental wavelength for a Nd:YAG laser is 1064 nm in the infra-red region, which can be converted to second, third, and fourth harmonic wavelengths.

Table 1 Wavelengths of Nd:YAG laser

Type		Wavelength (nm)	Pulse width (ns)
Fundamental		1064	7
Harmonics	Second	532	4 – 6
	Third	355	4 – 6
	Fourth	266	4 – 6

The fundamental wavelength (1064 nm) is not directly useful for many applications. Different birefringent materials like KDP (Potassium Dihydrogen Phosphate) and KD*P (Potassium Dideuterium Phosphate) can be used to convert the fundamental wavelength to other harmonic wavelengths like 532 nm (second harmonic) and 355 nm (third harmonic) which have two and three times the photon energy of the fundamental wavelength and are useful for many applications, including dye laser pumping.

Table 2 Energy of fundamental and harmonic wavelengths of Nd:YAG laser

Wavelength (nm)	Energy (mJ)
1064	650
532	280
355	170

Table 3 Specifications of laboratory Nd:YAG laser (Surelite Laser)

Pulse energy	40 mJ at 355 nm
Pulse duration	4-6 ns
Pulse rate	10 Hz/sec
Energy stability	+/- 2 %
Beam diameter	<10 mm
Beam divergence	0.5 mrad
Flash lamp lifetime	10* 10^6 shots

Tunable dye lasers

A Tunable dye laser uses an organic dye as the gain or lasing medium and is used to change a laser emission wavelength in a given spectral range. The wavelength of operation of a dye laser can be altered in a controlled manner and hence it can be used over a wide range of wavelengths [45]. The wide bandwidth of Tunable dye lasers makes them particularly suitable for a wide range of wavelengths. Dye molecules absorb pump laser light at one wavelength and re-emit at a different wavelength. Organic dyes have

broad fluorescence bands. These bands are excited by a pump laser (Nd:YAG laser) and a laser output wavelength is selected using gratings and prisms. The combination of tunable dye lasers and Laser Induced Fluorescence has been shown to provide high sensitivity for many elements [46].

Frequency doubling crystal

Frequency doubling or second harmonic generation is an optical process, in which two photons passing through a non-linear crystal are efficiently combined to form new photons with double the frequency of the initial photons. This process is used for the conversion of fundamental wavelength (1064 nm) to the second harmonic wavelength (532 nm).

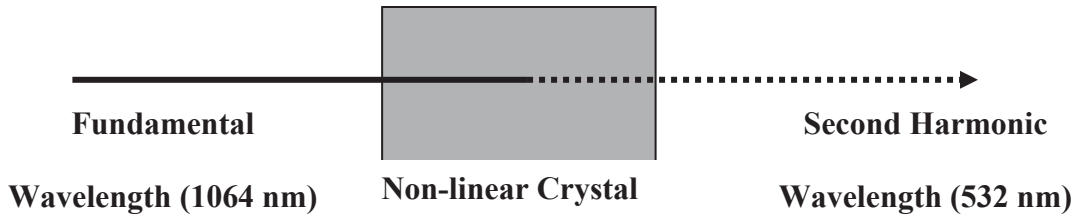


Figure 3 Configuration of frequency doubling crystal

While interacting with the non-linear crystal, the high intensity fundamental wave generates a nonlinear polarization in the crystal, which provides a new wave at double the fundamental frequency. The generated new photons have half the wavelength and double the energy of the original photons. Frequently used nonlinear crystal materials include lithium niobate ($LiNbO_3$), lithium triborate (LiB_3O_5), lithium tantalate ($LiTaO_3$), and potassium titanyl phosphate ($KTiOPO_4$).

Raman shift cell

A Raman cell is designed to convert the output of a dye laser into the ultra-violet range. Wavelength alteration is achieved by Stimulated Raman Scattering (SRS). The Raman cell is filled with either nitrogen or hydrogen gas at high pressure. The Raman cell produces multiple Stokes and Anti-stokes beams concurrently. The fundamental beam is focused into the entrance window of the gas cell and a lens is used after the cell's exit window to collimate the emerging beams. Shifted beams need to be separated from the fundamental beams as they are collinear. Separation of beams is usually achieved by use of Pellin-Broca prisms.

Optical systems

Lenses and mirrors of specific focal length are used for focusing and collecting the laser and fluorescence emissions. Different focal length lenses are chosen depending upon the wavelength of the element.

Hydrogen (H_2) flame

The hydrogen flame uses hydrogen gas and air present in the atmosphere as the fuel-oxidant mixture. The hydrogen flame is an invisible flame and has low background noise. A H_2-Air flame sustained on a quartz tube ($1/4^{th}$ inch o.d.) was used as the atomizer for LIF spectrometry. The laser beam passed through the flame at a height of 2-3 mm above the outlet of the quartz tube. The height of the flame was adjusted with a micrometer stage to optimize the fluorescence signal.

Hydride generation system

The hydride generation system used in these studies was a continuous flow hydride system. The continuous flow hydride generation system consists of the following components: a four-channel peristaltic pump (Model RP-1, Rainin Instrument Co. Inc., Emeryville, CA, USA) with Tygon or PVC tubing, a U-shaped gas – liquid separator made of pyrex glass, a Nafion tube dryer (MD Series, Permapure Inc., Toms River, NJ, USA) and reaction coils of various lengths (1-2 m, 1mm i.d.) of Teflon tubing.

Two channels of the four channel peristaltic pump were used to pump the sodium tetrahydroborate and acidified samples. The flow rates of sodium tetrahydroborate and acidified sample were controlled by the pump speed and by use of tubing with different diameters. These solutions were combined in a mixing chamber, and sent through a reactions coil to the gas-liquid separator (GLS). The GLS separates the gas from the liquid waste and purges the volatile hydrides towards the atomizer. An argon gas stream was used to carry the volatile hydrides to the flame. The flow rates of all the gases and solutions used are shown in Table 4.

Table 4 Operating conditions for the hydride generation system

Parameter	HG-LIF Bi	HG-LIF Ge
Acidified sample flow rate	4.4 mL/min	5.7 mL/min
$NaBH_4$ flow rate	1.8 mL/min	2.3 mL/min
Argon flow rate (flame)	600 mL/min	600 mL/min
Hydrogen flow rate (flame)	15 a.u.	15 a.u.

Monochromator

A monochromator is an optical device that helps in selecting a desired wavelength from a range of wavelengths. In these studies, a monochromator (1000 μm vertical slits, Spectra Pro-275, f/3.5, Action Research Corp.) was used to isolate fluorescence emissions. Fluorescence emissions were collected at 90^{o} to the laser beam direction and were transmitted to the entrance slit of monochromator. From the entrance slit, the fluorescence emissions were directed towards a diffraction grating. The grating disperses the light by diffracting different wavelengths at different angles. By adjusting the angle of the grating, the desired wavelength was isolated. The isolated fluorescence emission is directed to a focusing mirror, exit slit and to the photo-multiplier tube (PMT) detector. The slit widths of the entrance and exit slits of the monochromator were adjusted to provide the best signal-to-noise ratio.

Table 5 Specifications of laboratory Czerny Turner monochromator

Focal length	275 mm
Slit width	Up to 3000 μm
Grating	1200 grooves/mm
Resolution	0.05 nm
Focal plane	27 mm wide * 14 mm high

Photomultiplier tube

A photo-multiplier tube is a light detector that consists of a photocathode, an electron multiplier and a series of dynodes. In this study, the isolated fluorescence emission from the monochromator was detected by a photo-multiplier tube (PMT, R955, Hamamatsu Corp.). A photo-multiplier tube amplifies a single incident photon to approximately 10^6 electrons. A typical PMT consists of the following components: a vacuum tube, several dynodes and an anode. The vacuum tube contains a photosensitive metal called a photo-cathode. When a photon with enough energy strikes the photo-cathode, it emits a photo-electron due to the photoelectric effect. These photoelectrons are accelerated towards a dynode which generates 2-5 secondary electrons for each incident electron. When these secondary electrons hit another dynode, additional secondary electrons are generated. As there are a series of dynodes, many secondary electrons are produced and when all these secondary electrons reach the anode it generates an electrical pulse that can be detected by the oscilloscope.

Boxcar integrator/ Gated integrator

A boxcar integrator amplifies and integrates the input signal during a predefined gatewidth, starting at a predefined trigger, ignoring the noise and interference that could be present at other times. Each of these analog input signals can then be averaged by using an analog averager. The boxcar integrator consists of three components: a gate generator, a gated integrator, and an analog averager. The gated generator provides a delay from a few ns to 100 ns. The delay gate can be adjusted from 2 ns to 15 μs. The gated generator is usually triggered externally. The gated integrator integrates and amplifies the input signal during the delay gate. The output signal from the gated

integrator is then normalized to give a voltage. The output voltage corresponds to the average of the input signal during the time the gate is open (Sampling gate). The exponential averaging technique is useful for measuring small signals in the presence of noisy backgrounds.

Oscilloscope

An oscilloscope is basically a graph displaying device, which converts the waveform of detector signal into an electrical signal and draws a graph of the electrical signal. In most application the graph shows how fluorescence signals (Y – axis) change over time (X – axis). The oscilloscope has a time base control that adjusts the scale of X – axis in seconds and a vertical control that adjusts the Y – axis in volts. By using an oscilloscope the time and voltages values of a signal can be determined.

Figure 4 Schematic diagram of HG-LIF Bi

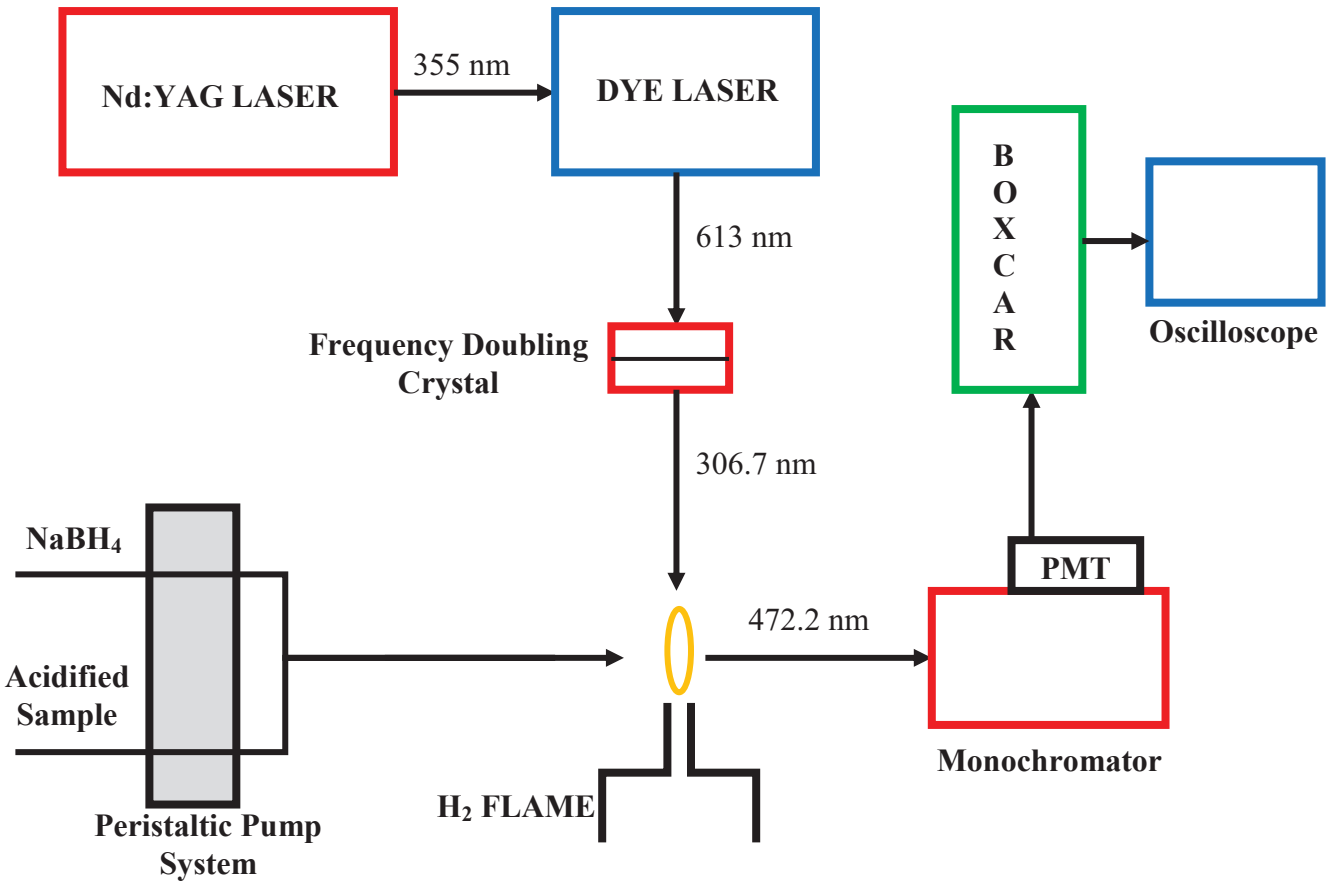
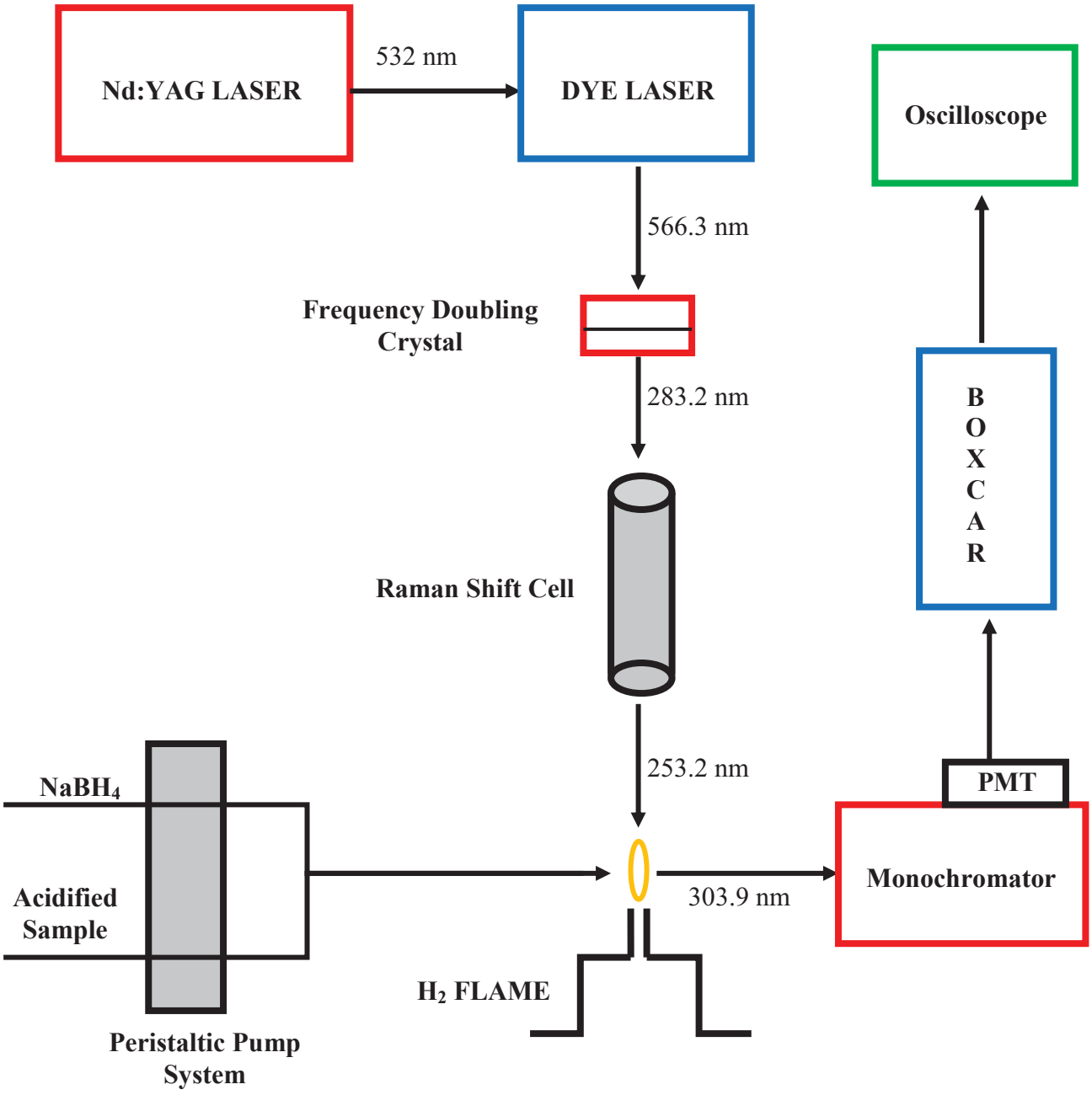


Figure 5 Schematic diagram of HG-LIF Ge



EXPERIMENTAL SECTION

Reagents and standards

All the reagents used in these studies were of analytical grade or better. The stock solution of Ge was an atomic absorption standard solution (Acros Organics – 1 mg/mL Ge in 2 % KOH). The standard stock solutions of Bi (Fluka Analytical – 1 mg/mL ± 0.004 mg/mL), and Sn (Spex Certiprep – 1 mg/mL in 20 % HCl) were commercially available in a concentration of 1000 μg/mL. Calibration standards were freshly prepared by appropriate dilutions of the stock solution. The reagents that were used are as follows: hydrochloric acid (Fisher – trace metal grade), sodium borohydride (Sigma Aldrich – AF granules, 10 – 40 mesh, 98 %), sodium hydroxide (Fisher Scientific – ACS grade), nitric acid (EMD – Omni trace 69.0 – 70 %), O-phosphoric acid (Fisher Scientific – ACS grade, Pharmaco-AAPER – ACS reagent grade), Hydrogen peroxide (Fisher Scientific – Certified ACS), Ascorbic acid (Fisher Scientific), Thiourea (Sigma Aldrich – ACS grade), Urea (Fisher Scientific – Reagent grade), Multi-element Standard solution 4 for ICP (Fluka Analytical – 51844), and Multi-element Standard solution V for ICP (Fluka Analytical – 54704). Sodium borohydride was used as the reducing solution and was freshly prepared by dissolving $NaBH_4$ in de-ionized water with NaOH added as a stabilizer. The diluted hydrochloric and phosphoric acid solutions were prepared by appropriate dilution of concentrated hydrochloric and phosphoric acids, respectively, in de-ionized water. The glassware was thoroughly cleaned with de-ionized water prior to use.

Certified reference material and samples

The certified reference material NIST 1643e (Trace elements in water) was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Bi containing medication and Tea samples were purchased from a local market in Youngstown city and prepared as indicated below.

Sample preparation HG-LIF Bi

A 1 g mass of tea sample/Bi containing medication was weighed into a 50 mL beaker and 10 mL of concentrated HNO_3 was added. The beaker was covered with a watch glass and the mixture was then heated on a hot plate until the volume of the solution was reduced to 2-3 mL. 1 g of urea and 10 mL of water was added to the beaker and heated on a hot plate to remove nitrogen oxides. When 3-5 mL of digest solution remained in the beaker after cooling, 10 mL of concentrated HCl and 2 mL of H_2O_2 were added to assist further digestion and the mixture was then heated on a hot plate until about 1-2 mL of sample was left. The sample was initially diluted to 30 mL, filtered and then diluted to a final volume (as mentioned below) before analysis by HG-LIF.

Bi containing medication samples were diluted by a factor of 10^6 before being analyzed by HG-LIF approach. Tea samples were diluted by a factor of 10^2 before being analyzed by HG-LIF technique.

Sample preparation HG-LIF Ge

A germanium supplement capsule was digested using 5 mL of concentrated HNO_3 and the solution was heated until 2-3 mL of solution remained in the beaker. The sample was then diluted by a factor of 4×10^6 before being analyzed by HG-LIF method.

Experimental setup for HG-LIF Bi

A Q-switched Nd:YAG laser (DCR 2A, Spectra Physics) operating at 355 nm and a repetition rate of 10 Hz was used as the pump laser source. For Bi measurements, the 355 nm output was used to pump a tunable dye laser (Northern Lights, Dakota Technologies Inc.) operating on Rhodamine 610 near 613 nm. The dye laser output was frequency-doubled in a Barium Borate (BBO) crystal to produce tunable radiation near 306.7 nm at pulse energies of approximately 1 μJ/pulse. The laser had a pulse width of approximately 5 ns and was reported to have a linewidth of < 1 cm^{-1}, which corresponds to a spectral resolution of < 0.013 nm at the frequency-doubled wavelength. The dye laser is compact (30 cm x 6.25 cm x 11.25 cm) and serves as a convenient and effective source for laser induced fluorescence spectrometry measurements of Bi. The laser light passes through several optics and into the flame atomizer to cause excitation of analyte atoms. The laser excited atoms emit fluorescence light that is directed towards the monochromator by a 25 cm focal length lens. The monochromator (Czerny-Turner spectrograph) isolates the desired wavelength and directs it to the photo-multiplier tube (PMT). The photo-multiplier tube converts the light signal into an electrical signal and is recorded by an oscilloscope. The excitation wavelength used for Bismuth was 306.7 nm and the fluorescence wavelength was 472.2 nm.

Table 6 Experimental conditions used for HG-LIF Bi

YAG LASER	Q-switched Nd:YAG laser
Wavelength	355 nm
Pulse width	5 ns
Repetition rate	10 Hz
Energy used	40 mJ to 200 mJ
DYE Laser	
Dye solution	Rhodamine 610
LENS	Quartz made, 25 cm focal length
Hydride Generation	
HCl Concentration	10 % (v/v)
$NaBH_4$ + NaOH Concentration	2 % (w/v) + 0.4 % (w/v)
DETECTION SYSTEM	
Grating	Czerny-Turner, 1800 grooves mm^{-1}
Slit width	2000 μm
Slit height	4 mm
PMT Voltage	-1100 V
Boxcar – Integrator	
AVERAGING	30 a.u.

Experimental set up for HG-LIF Ge

A Q-switched Nd:YAG laser (Continuum, SureLite SLII-10) operating at a repetition rate of 10 Hz, in the 2nd harmonic (wavelength equal to 532 nm) was used to pump a dye laser (Lambda Physik). The dye laser produced radiation near 283.2 nm by frequency doubling of 566-567 nm radiation produced using Rhodamine 590 laser dye in a BBO crystal. The anti-stokes radiation near 253.3 nm was produced by sending the frequency doubled radiation at 283.2 nm through a Raman cell filled with hydrogen gas. The laser had a pulse width of approximately 5 ns and is reported to have a line width of < 1 cm^{-1}, which corresponds to a spectral resolution of < 0.013 nm at the frequency-doubled wavelength. The laser radiation at 253.3 nm was used to excite analyte atoms in the flame atomizer. The laser excited atoms emit fluorescence light at 303.9 nm which is directed towards the monochromator by a 25 cm focal length lens. The monochromator (Czerny-Turner spectrograph) isolates the desired wavelength of 303.9 nm and directs it to the PMT. The photo-multiplier tube converts the light signal into an electrical signal and is recorded by an oscilloscope.

The calibration curve measurements of Ge and the Ge supplement capsule analysis were performed using laser pulse energies of approximately 18 μJ/pulse and 51 μJ/pulse, respectively.

Table 7 Experimental Conditions used for HG-LIF Ge

LASER	Q-switched Nd:YAG laser
Wavelength	532 nm
Pulse width	5 ns
Repetition rate	10 Hz
Energy used	40 mJ to 200 mJ
DYE Laser	
Dye solution	Rhodamine 590
Frequency Doubling Crystal	BBO crystal
Raman Cell	Filled with Hydrogen gas
LENS	Quartz made, 25 cm focal length
Hydride Generation	
H_3PO_4 Concentration	2 M
$NaBH_4$ + NaOH Concentration	0.5 % (w/v) + 0.1 % (w/v)
DETECTION SYSTEM	
Grating	Czerny-Turner, 1800 grooves mm^{-1}
Slit width	2000 µm
Slit height	4 mm
PMT Voltage	-1200 V
Boxcar – Integrator	
AVERAGING	300 a.u.

RESULTS AND DISCUSSON

Bismuth

Fluorescence intensity scans

Fluorescence intensity scans were taken from 452 nm to 492 nm to determine the optimum wavelength. Three different scans, one for each of blank, 0.5 ppb Bi, and 1 ppb Bi were taken. The scans not only show the wavelength of maximum intensity, 473.0 nm, but also show that the blank has no significant Bi contamination. The fluorescent signal intensities for solutions containing 0.5 ppb and 1 ppb Bi show that the fluorescent signal intensity is proportional to the concentration of Bi.

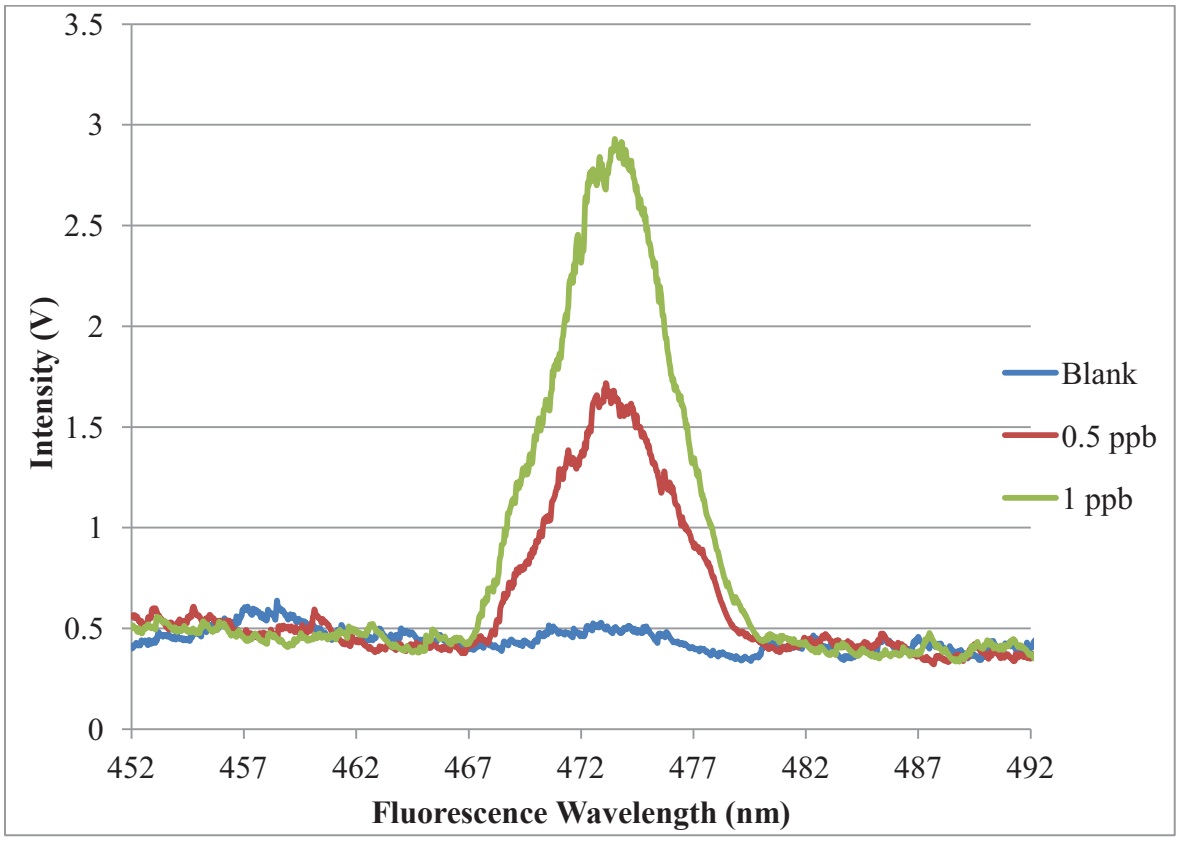


Figure 6 Fluorescence intensity scans

Influence of hydrochloric acid concentration on fluorescence intensity of Bi

To determine the optimal acidity conditions for the generation of Bi hydride, the influence of different concentrations of HCl on fluorescence intensity of Bi was investigated. The fluorescence intensity for 1 ng/mL Bi was measured at 5 % (v/v), 10 % (v/v), 15 % (v/v), 20 % (v/v), and 25 % (v/v) HCl concentrations. The fluorescence signal was relatively constant over this range of HCl concentrations. Figure 7 shows that 10 % (v/v) HCl gave the best signal even though the signal was almost equal for all the concentrations. A concentration of 10 % (v/v) hydrochloric acid was used during subsequent Bi fluorescence measurements.

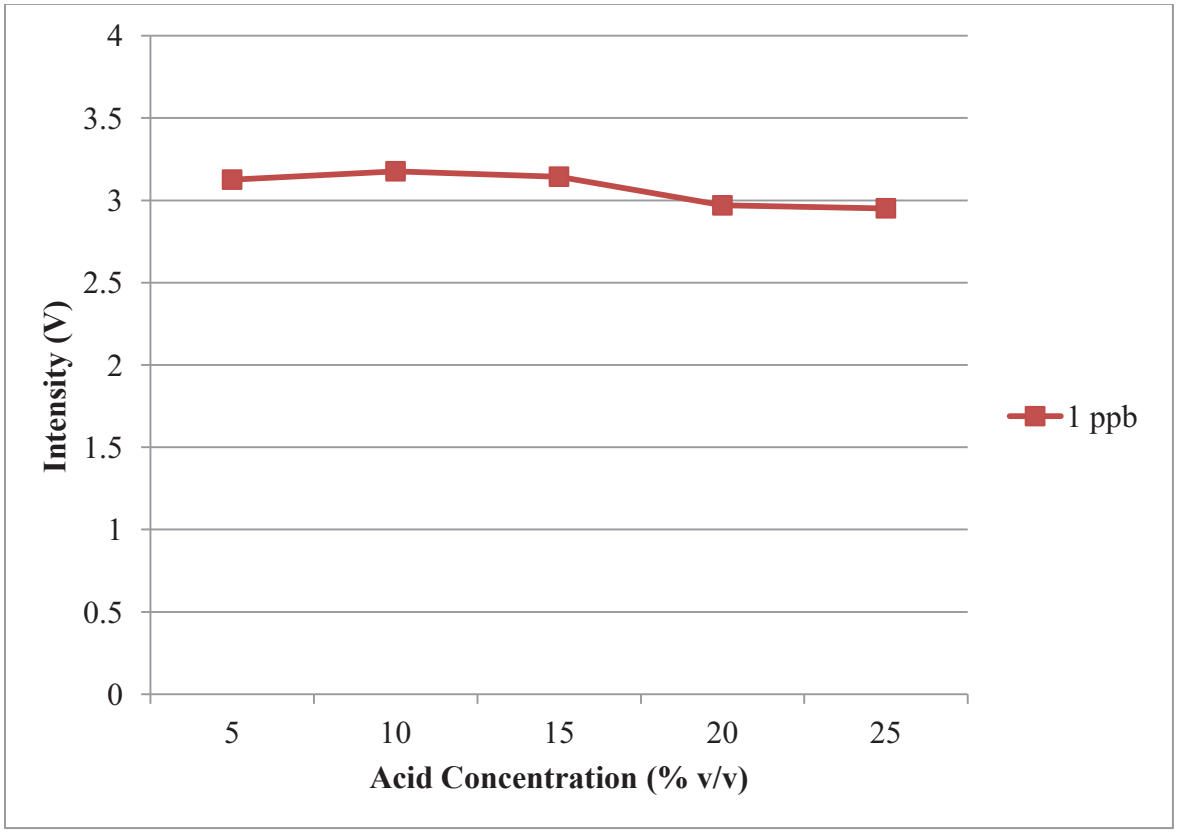


Figure 7 Influence of hydrochloric acid concentration on fluorescence intensity of Bi

Influence of sodium tetrahydroborate concentration on fluorescence intensity of Bi

To optimize the amount of reducing agent, the effect of sodium tetrahydroborate concentration on fluorescence signal intensity of Bi was investigated. To improve stability, Sodium tetrahydroborate solutions were prepared in 0.4 % (w/v) sodium hydroxide. In this study, it was found that different concentrations of $NaBH_4$, 0.5, 1.0, 1.25, 1.5, 1.75 and 2.0 % (w/v), affected the fluorescence signal of 1 ng/mL Bi. The results shown in Figure 8 indicate that the fluorescence signal increased upon increasing $NaBH_4$ concentration. From these results, it was determined that 2.0 % $NaBH_4$ in 0.4 % (w/v) NaOH; and 10 % (v/v) HCl provided the best results for HG-LIF Bi measurements.

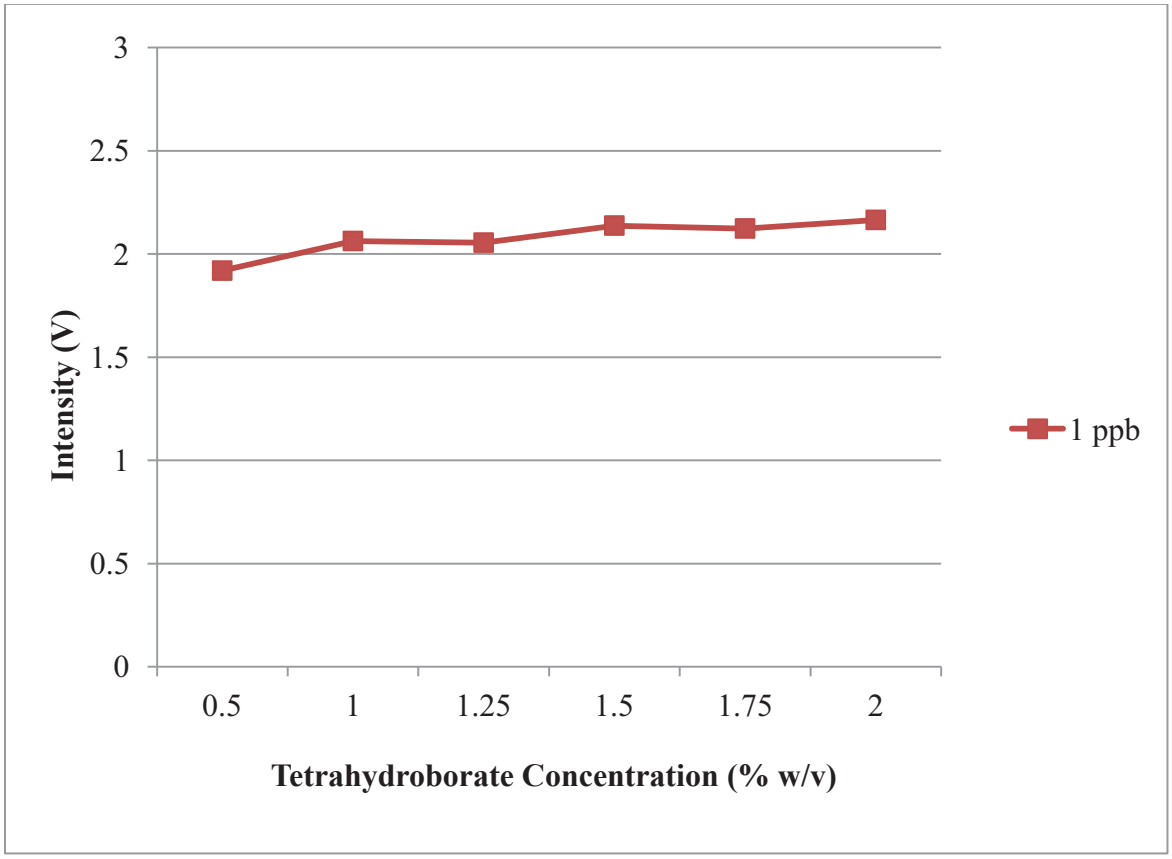


Figure 8 Influence of sodium borohydride concentration on fluorescence intensity of Bi

Influence of peristaltic pump flow rates on fluorescence intensity of Bi

The effect of the peristaltic pump flow rate on the magnitude of 1 ng/mL Bi fluorescence signal was studied. As seen from Figure 9, the signal of Bi increased gradually when the peristaltic pump speed was increased between 10 and 30 (rpm). A pump speed of 15 rpm was selected as the optimum rate for the rest of the study.

In this study, argon was used as carrier gas to carry the volatile hydride to the flame atomizer and a hydrogen flame was used for the atomization of the hydrides.

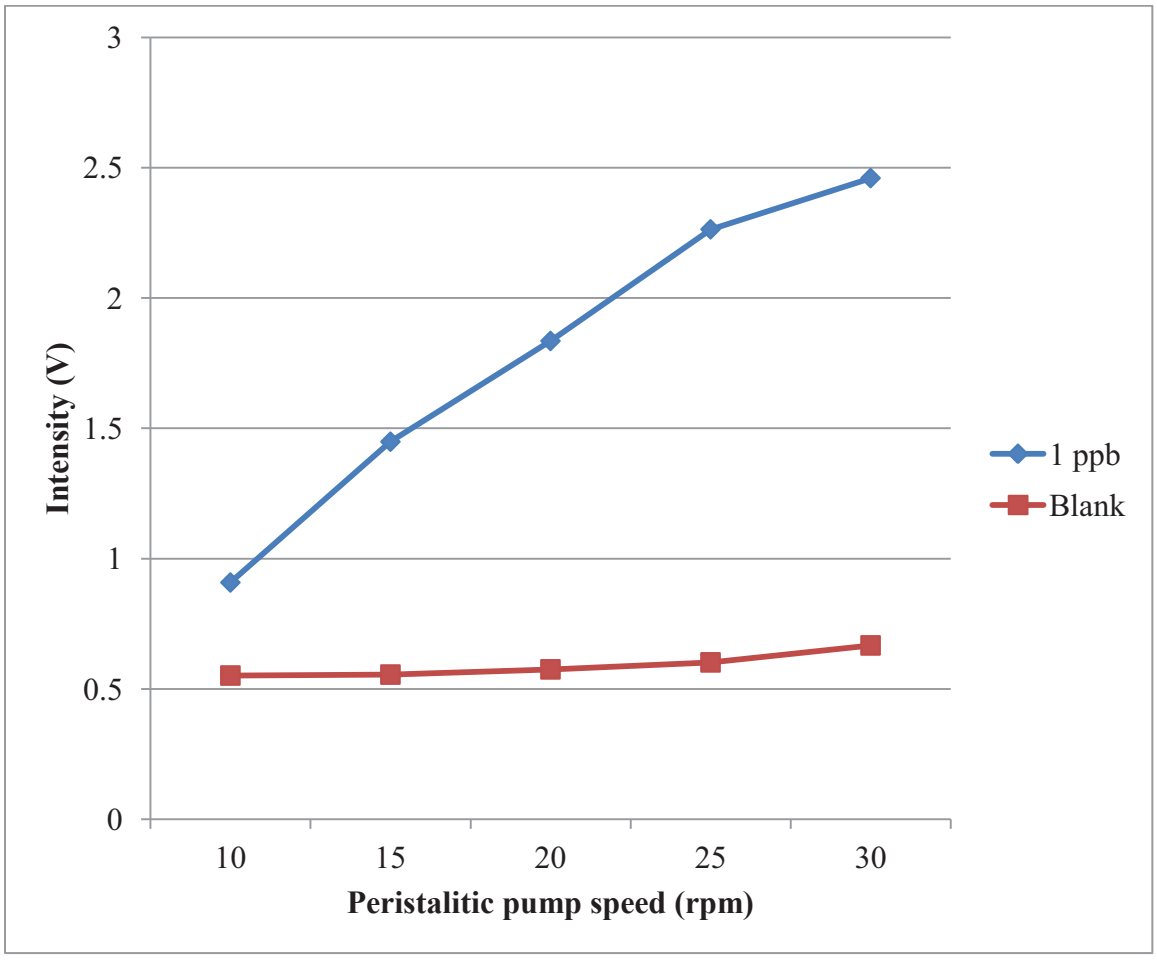


Figure 9 Influence of peristaltic pump flow rates on fluorescence intensity of Bi

Table 8 shows the corresponding volumetric flow rates for the solutions.

Table 8 Peristaltic pump flow rates of acid/sample and $NaBH_4$

Pump Flow rate (rpm)	Acid/Sample (mL/min)	$NaBH_4$ (mL/min)
10	3.1	1.2
15	4.4	1.8
20	5.7	2.3
25	7.1	2.8
30	8.4	3.3

Influence of monochromator slit width on fluorescence intensity of Bi

Figure 10 shows the influence of monochromator slit width on the magnitude of the fluorescence signal of the blank and 1 ng/mL Bi. The results indicated that the blank signal increased slightly with increased slit width. As seen from Figure 10, the signal of 1 ng/mL Bi increased linearly when the slit width was increased from 1000 to 2000 µm. An increase in the magnitude of signal after 2000 µm was not linear and 2000 µm slit width was selected for all other fluorescence measurements.

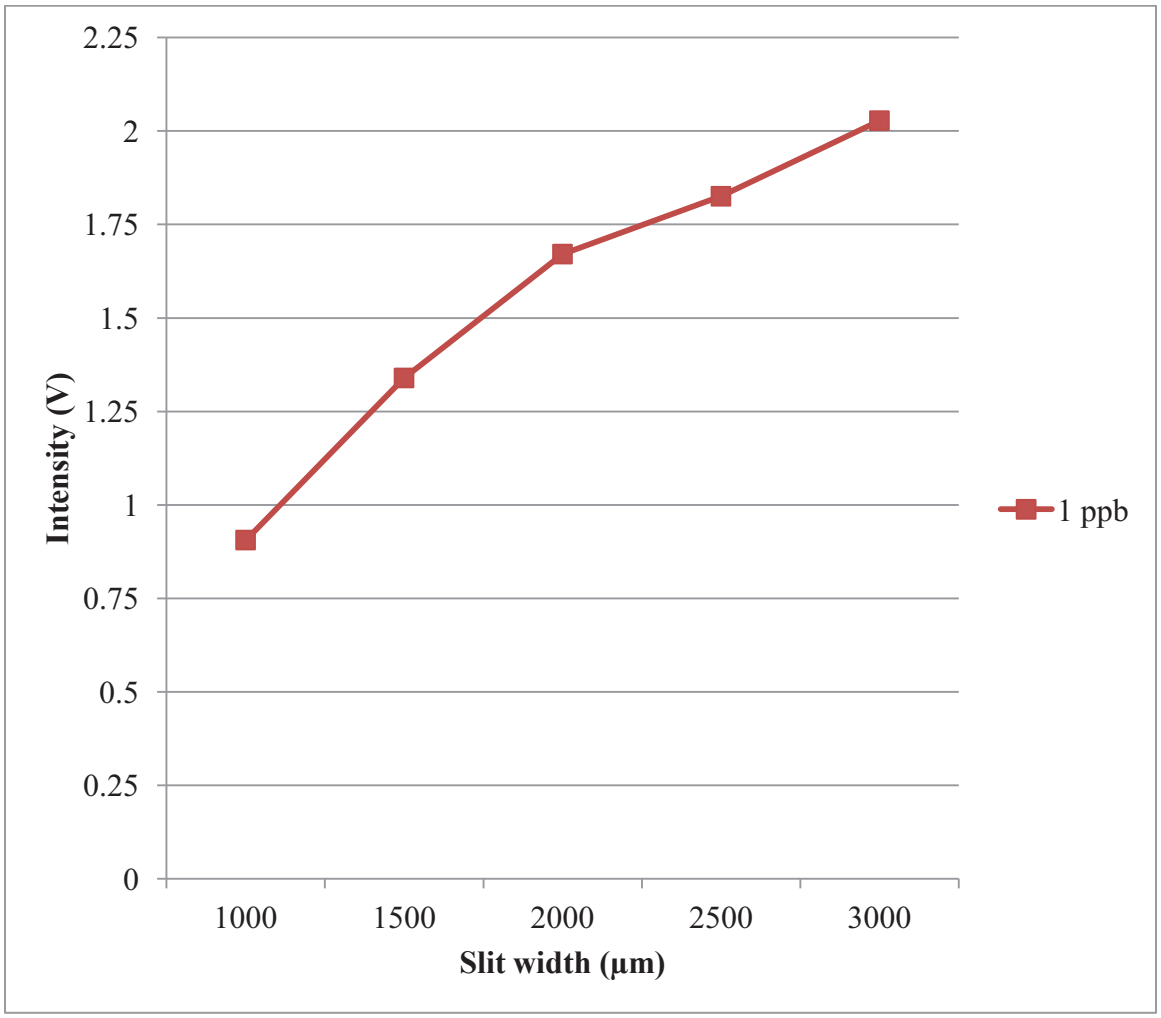


Figure 10 Influence of monochromator slit width on fluorescence intensity of Bi

Influence of masking reagent concentration on fluorescence intensity of Bi

The use of masking agents helps to nullify the effect of interference elements on hydride generation during real sample analysis [20]. A masking reagent of thiourea-ascorbic acid was chosen and the optimal concentration of masking reagent was investigated. Figure 11 shows that the addition of masking reagent has no influence on the fluorescence signal of Bi and 0.3 % (w/v) thiourea-ascorbic acid was prepared for real sample analysis. In this study, while analyzing real samples, all the solutions including calibration standards and real samples were made in 0.3 % (w/v) thiourea-ascorbic acid.

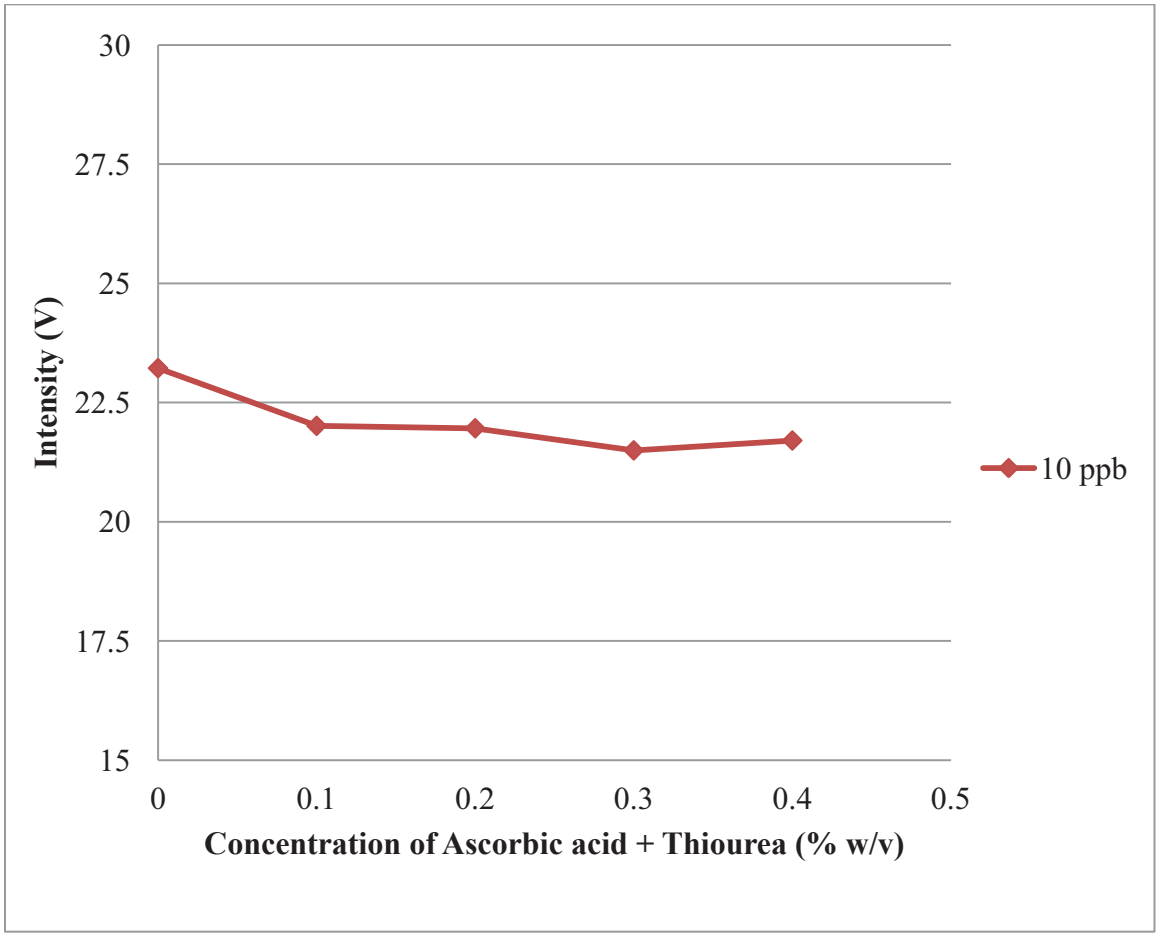


Figure 11 Influence of masking reagent concentration on fluorescence intensity of Bi

Table 9 Optimal conditions for HG-LIF Bi

Parameters	Bi
Acid concentration	10 % (v/v)
Tetra hydro borate concentration	2.0 % (w/v) in 0.4 % (w/v) NaOH
Peristaltic pump flow rate	15 a.u.
Monochromator slit width	2000 μm
Masking reagent concentration (Thiourea-Ascorbic Acid)	0.3 % (w/v) + 0.3 % (w/v)

Calibration and analytical figures of merit

In order to determine the sensitivity and limit of detection of the HG-LIF method, calibration curve measurements were performed and typical calibration curves for Bi are shown in Figures 12 & 13. The fluorescence intensity of Bi was found to be linearly proportional to the concentration of Bi. All the calibration curves showed good linearity. After optimizing the HG-LIF response as a function of HG reagents, the Limit of Detection (LOD) was calculated by using the standard deviation of the fluorescence signal of 16 blanks and the slope of the corresponding calibration curves.

$$\mathbf{LOD = 3\sigma/m}$$

Where, σ is the average standard deviation (noise) and m denotes slope of calibration line (sensitivity).

The limit of detection for Bi fluorescence at 472.2 nm was determined to be 0.03 ng/mL.

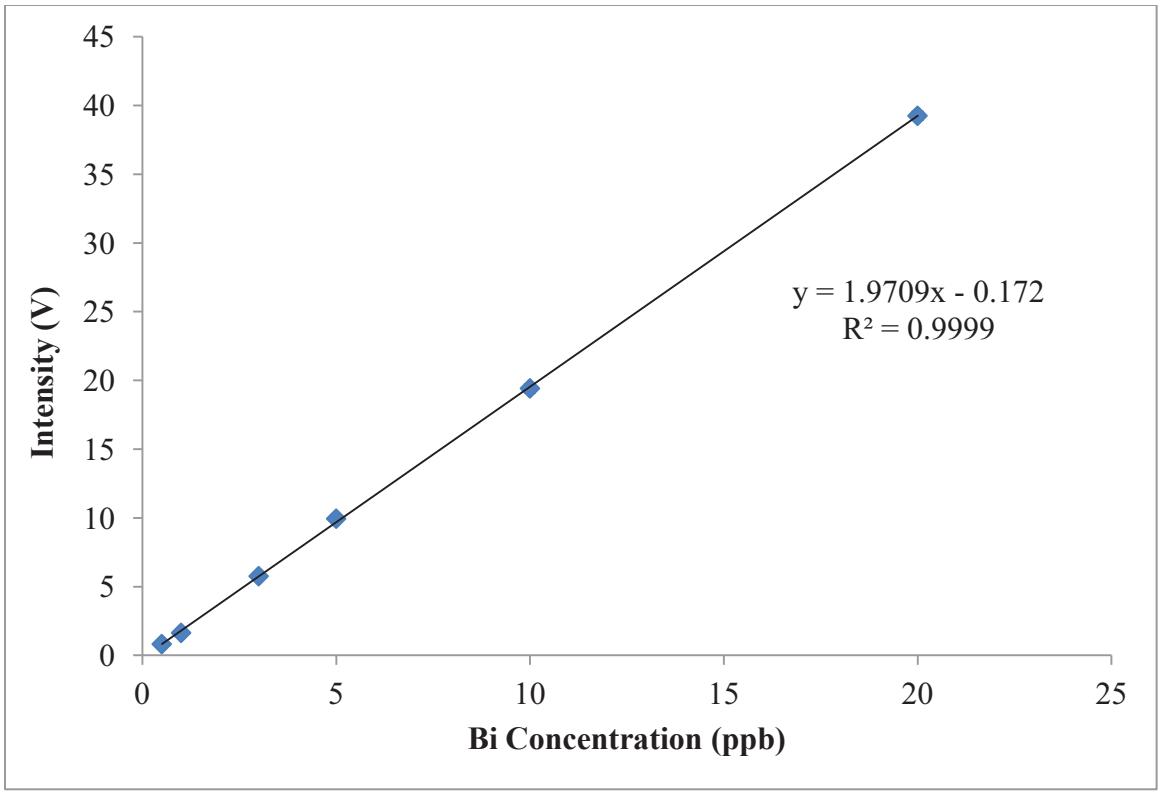


Figure 12 Calibration curve HGL-LIF Bi

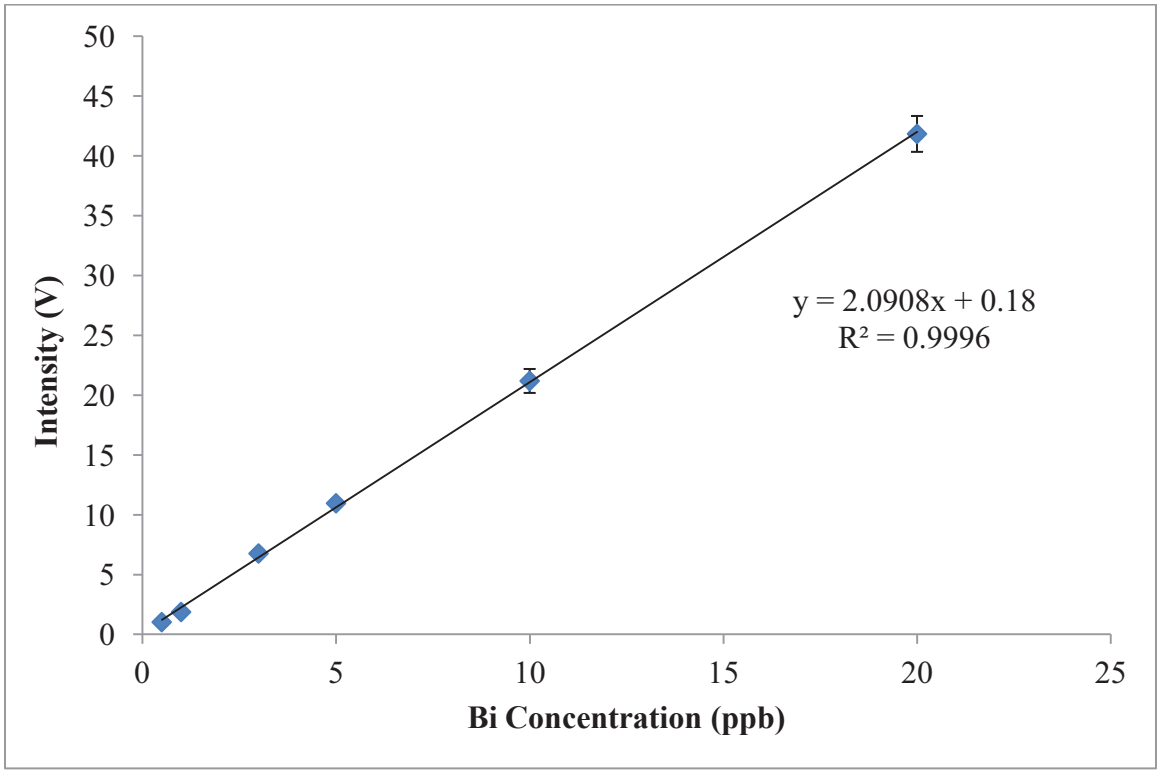


Figure 13 Calibration curve HG-LIF Bi

At high concentration of Bi sometimes the fluorescence emission exceeded the capacity of the photo-multiplier tube. Hence, Neutral Density (ND) filters were used to limit the amount of light reaching the detector. When using these filters, the actual signal intensity was determined by multiplying the observed intensity with the filter factor of the corresponding filter. In this study, ND 0.3, ND 0.5, ND 0.6, ND 2 filters were used and the corresponding filter factors are summarized in Table 10.

Table 10 Filter factors of the ND filters at different wavelengths

Wavelength (nm)	ND 0.3 filter	ND 0.5 filter	ND 0.6 filter	ND 2 filter
472.2	1.646	3.058	3.638	66.973
303.9	1.7181	-	4.198	115.2

Table 11 shows the analytical figures of merit for Bi measurements using HG-LIF technique.

Table 11 Analytical figures of merit for HG-LIF Bi

Analytical Figures	Bi
Linear Range (ng/mL)	0.05 to 20
Limit of Detection (ng/mL)	0.03
Limit of Quantification (ng/mL)	0.1
Relative Standard Deviation (%) at 3 ppb Bi	0.1

Analysis of a multielement standard and a standard reference material

Laser Induced Fluorescence has high spectral selectivity resulting from both the laser excitation source and the monochromator. The high selectivity of the HG-LIF method is expected to result in high accuracy for Bi, even in complex sample matrices. To check the accuracy of the method, a standard reference material (NIST 1643e Trace elements in water) that contains low concentrations of several elements, was analyzed. The certified value of Bi in the standard reference material was 14.09 ± 0.15 ng/mL. The result of HG-LIF determination was 12.8 ± 1.7 ng/mL. The good agreement of these values demonstrates the accuracy of the HG-LIF approach.

A Multielement Standard Solution V for ICP was analyzed by the HG-LIF method, and recovery experiments were carried out after a spike of 10 ng/mL Bi. Recovery studies were carried out with and without the addition of 0.3 % thiourea-ascorbic acid masking agents. The recoveries with and without masking agents were 97.5 % ± 0.3 %, and 55.5 % ± 0.9 % respectively. These results demonstrate the need for masking reagent while analyzing real samples.

Analysis of real samples

Once the optimal conditions for the generation and collection of Bismuth hydride gas had been established, the proposed HG-LIF method was applied to determine trace levels of Bi in real samples. To demonstrate the accuracy of the HG-LIF method, a Bi containing medication was analyzed. The certified value of Bi in the Bi containing medication was 10.08 mg/mL; therefore the Bi concentration in the digested solution was 10.08 μg/mL. The result of HG-LIF determination was 10.44 ± 0.15 μg/mL. The results indicated that the developed HG-LIF method is both accurate and precise.

Determination of Bi in different kinds of tea leaves

In another application of the HG-LIF technique to complex samples, different kinds of tea leaves were analyzed for Bi content. The results of the analyses are summarized in Table 12. As can be seen, the contents of Bi in decaffeinated tea samples were low, especially for decaffeinated breakfast tea. The means of Bi contents were 0.34 μg/g and 0.04 μg/g in decaffeinated green tea and decaffeinated black tea, respectively. In earl-grey tea and breakfast tea, the means of Bi contents were 0.88 and 1.23 μg/g respectively. The results show that the amount of Bi in caffeinated tea samples was greater than that in decaffeinated tea samples, and suggest the decaffeination process may lower the content of this element.

Table 12 Bismuth contents of different kinds of tea leaves

Sample	Number of samples	Mean (μg/g)	Standard Deviation
Green tea (decaf)	4	0.34	0.08
Black tea	5	1.23	0.03
Black tea (decaf)	4	Detected	-
Earl Grey tea	4	0.88	0.02

Germanium

Effect of acidity on fluorescence signals of Ge

In the study of Ge by Hydride Generation LIF method, the fluorescence signals were affected by the type of acid medium. Previously, different optimal acidity conditions have been reported for the generation of Germanium hydride [32]. In this study, the effects of O-phosphoric acid and hydrochloric acid on the HG-LIF measurements of Ge were investigated.

Influence of O-phosphoric acid concentration on fluorescence intensity of Ge

To determine the optimal acidity conditions for the generation of Ge hydride, the influence of different concentrations of O-phosphoric acid on fluorescence intensity of Ge was studied. The fluorescence intensity for 100 ng/mL Ge was measured at 0.5 M, 1 M, 2 M, and 3 M phosphoric acid concentrations and the results obtained are shown in Figure 14. The fluorescence signal was observed to increase slightly and reached a maximum at 2 M phosphoric acid, and then decrease slightly after 2 M phosphoric acid. The results indicated that 2 M phosphoric acid gave the best signal and hence 2 M phosphoric acid was used for all subsequent Ge measurements.

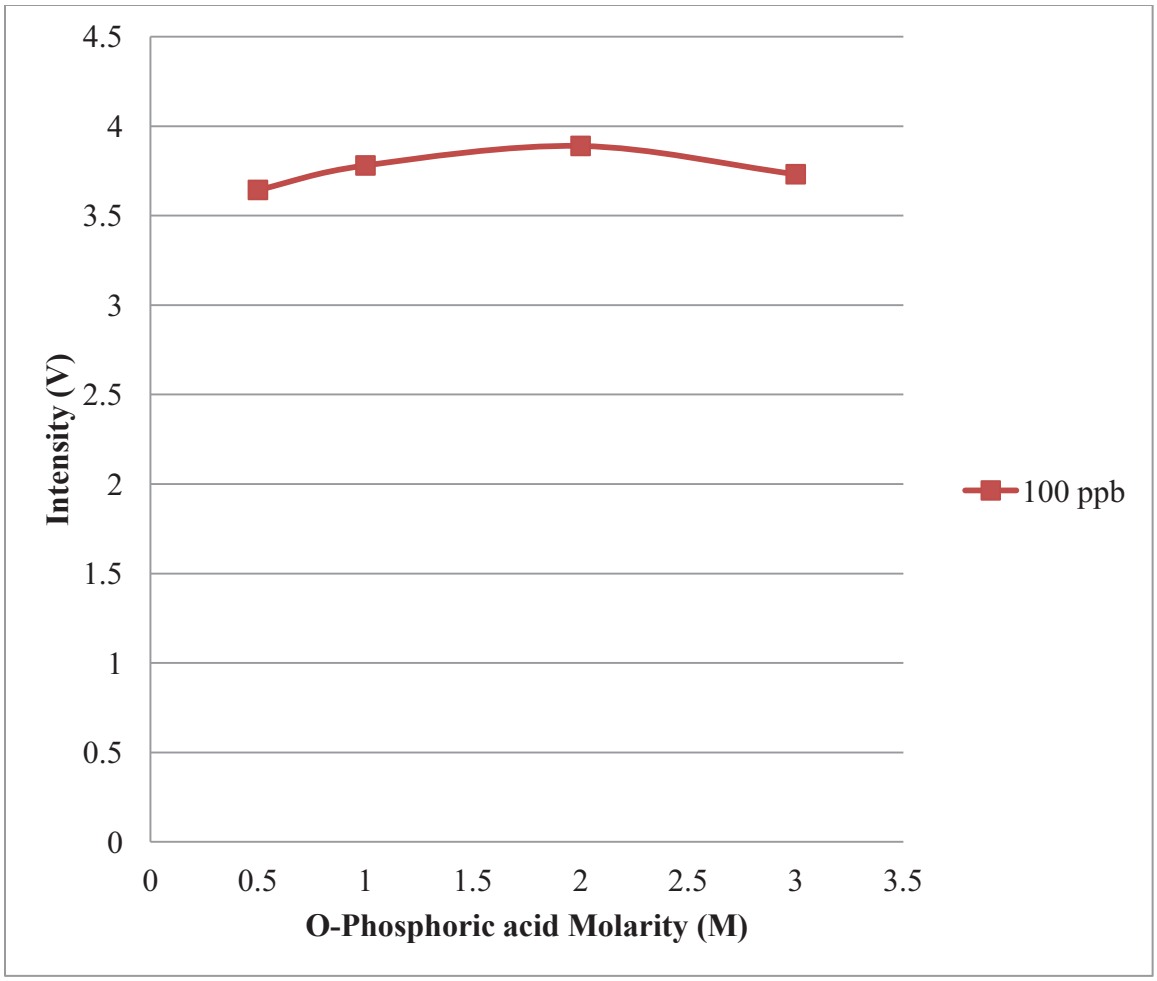


Figure 14 Influence of O-phosphoric acid concentration on fluorescence intensity of Ge

Influence of hydrochloric acid concentration on fluorescence intensity of Ge

To optimize the acidity conditions for the generation of Ge hydride, experiments were carried out using different concentrations of hydrochloric acid ranging from 0.5 % (v/v), 1 % (v/v), 2 % (v/v), 3 % (v/v), and 4 % (v/v). The results obtained for different hydrochloric acid concentrations are shown in Figure 15. The maximum intensity was found at 2 % (v/v) hydrochloric acid and the intensity dropped rapidly after 2 % (v/v) hydrochloric acid.

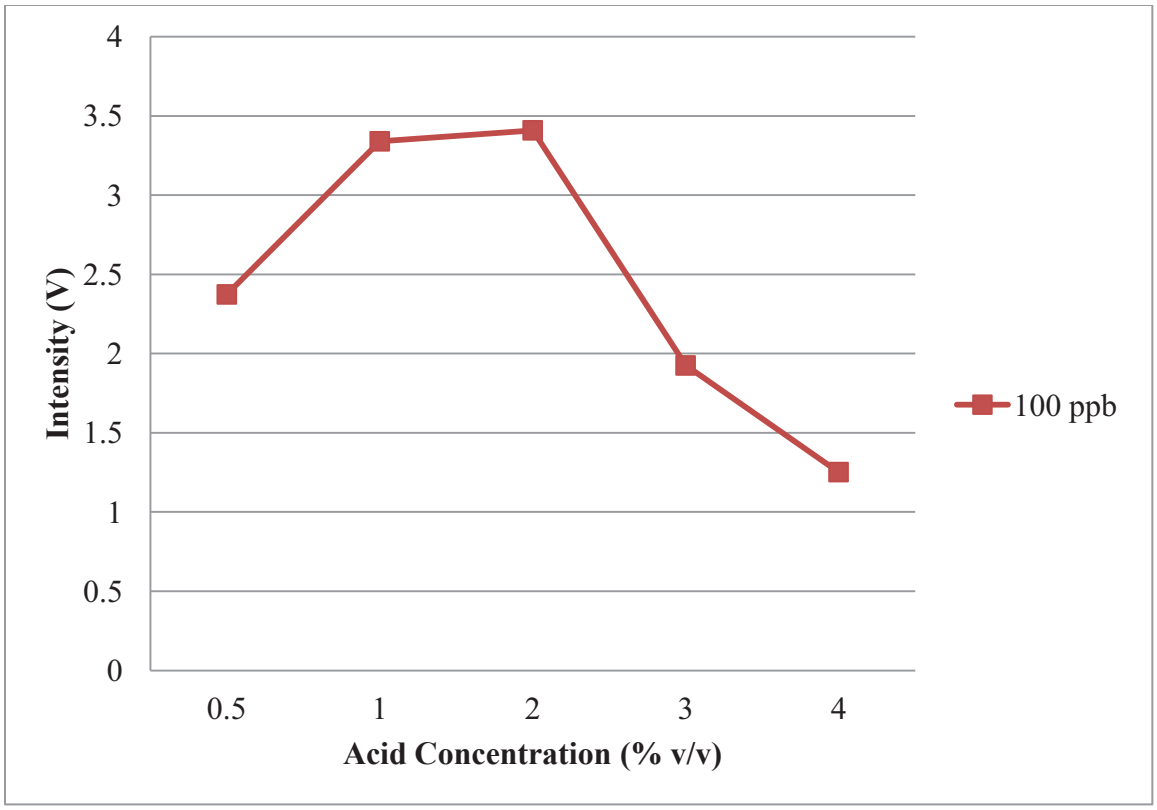


Figure 15 Influence of hydrochloric acid concentration on fluorescence intensity of Ge

Influence of sodium tetrahydroborate concentration on fluorescence intensity of Ge

To optimize the amount of reducing agent, the effect of sodium tetrahydroborate on fluorescence signals of Ge was studied by using 2 M Phosphoric acid. The results obtained for different concentrations of sodium tetrahydroborate are shown in Figure 16. The results show that the fluorescence signal of Germanium reached a maximum when 0.5 % (w/v) sodium tetrahydroborate was used. When the concentration of borohydride was increased after 0.5 % (w/v) the fluorescence signal intensity was found to decrease gradually. From the results, it was determined that 0.5 % (w/v) $NaBH_4$ in 0.1 % NaOH as stabilizer and 2 M H_3PO_4 provided the best results for HG-LIF Ge.

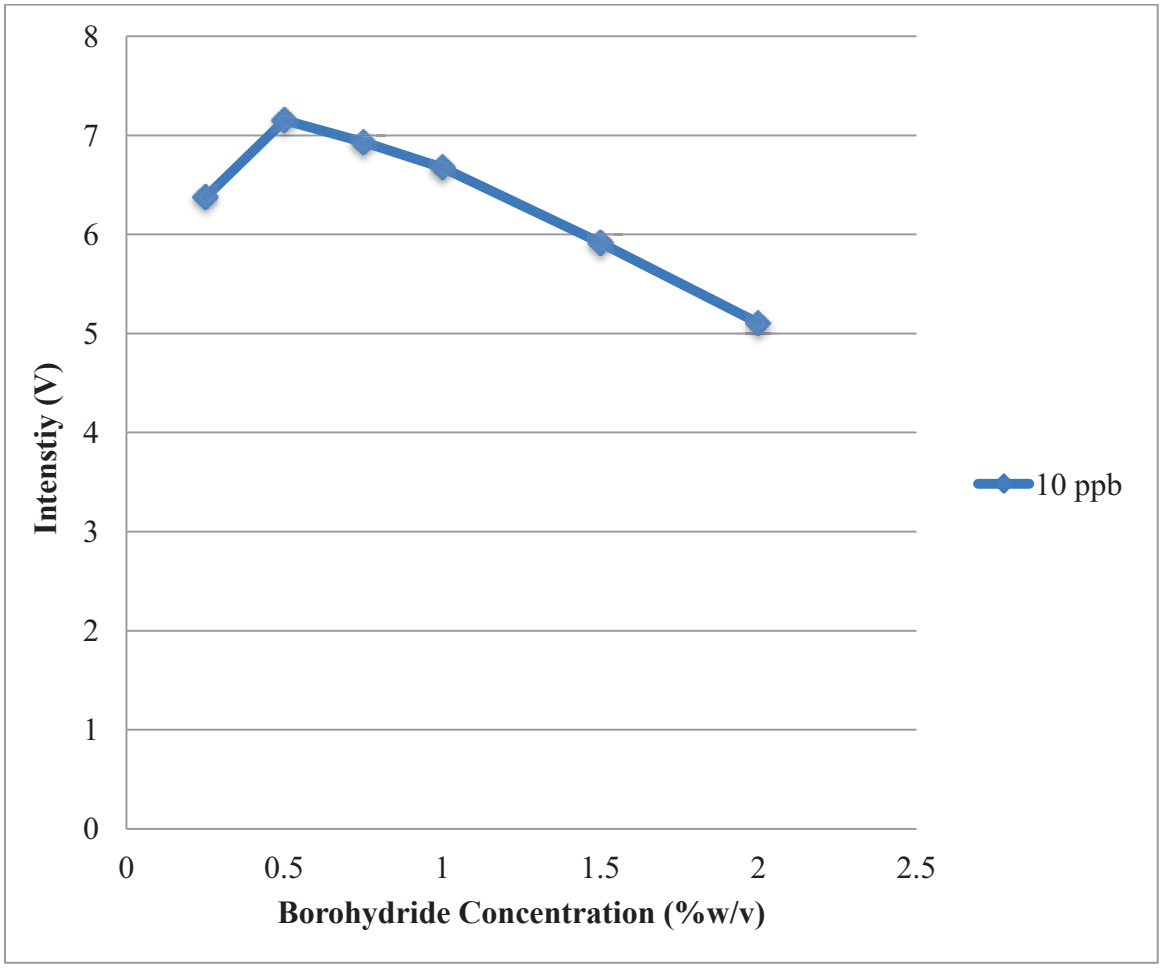


Figure 16 Influence of tetrahydroborate concentration on fluorescence intensity of Ge

Influence of peristaltic pump flow rate on fluorescence intensity of Ge

The effect of the peristaltic pump flow rate on the magnitude of 100 ng/mL Ge fluorescence signal was studied. As seen from Figure 17, the signal of Ge increased gradually when the peristaltic pump speed was increased between 15 and 25 (rpm). A speed of 20 (rpm) was selected as the optimum flow rate for the rest of the study.

In this study, argon was used as carrier gas to carry the volatile hydride to the flame atomizer and hydrogen flame was used for the atomization of the hydrides.

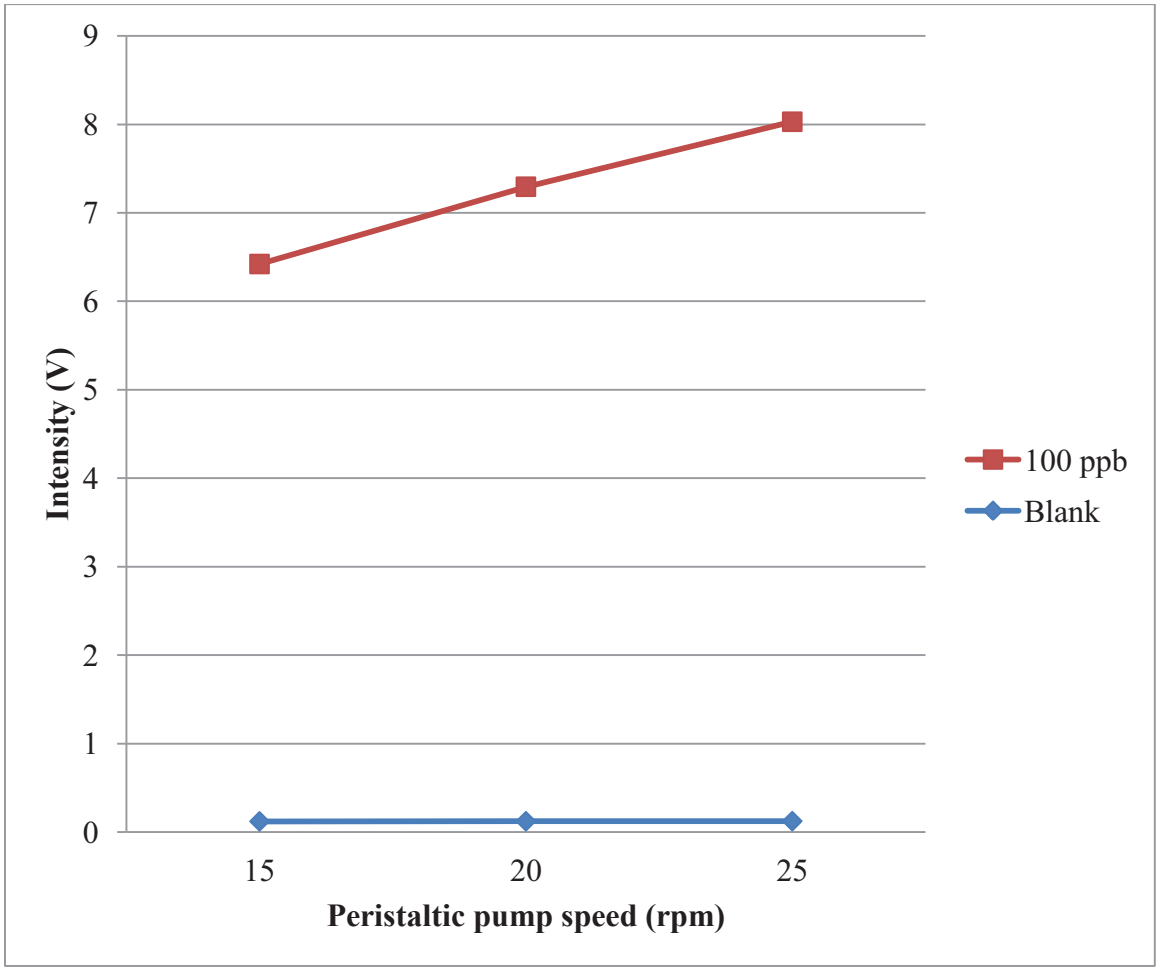


Figure 17 Influence of peristaltic pump flow rate on fluorescence intensity of Ge

Influence of masking reagent concentration on fluorescence intensity of Ge

The use of masking agents helps to reduce the effect of interferences on hydride generation during real sample analysis [20]. A masking reagent of thiourea-ascorbic acid was chosen and the optimal concentration of masking reagent was investigated. Figure 18 shows that the addition of masking reagent has no influence on the fluorescence signal of Ge and 0.3 % (w/v) thiourea-ascorbic acid was used for real sample analysis. In this study, while analyzing real samples, all the solutions including calibration standards and real samples were made in 0.3 % (w/v) thiourea-ascorbic acid.

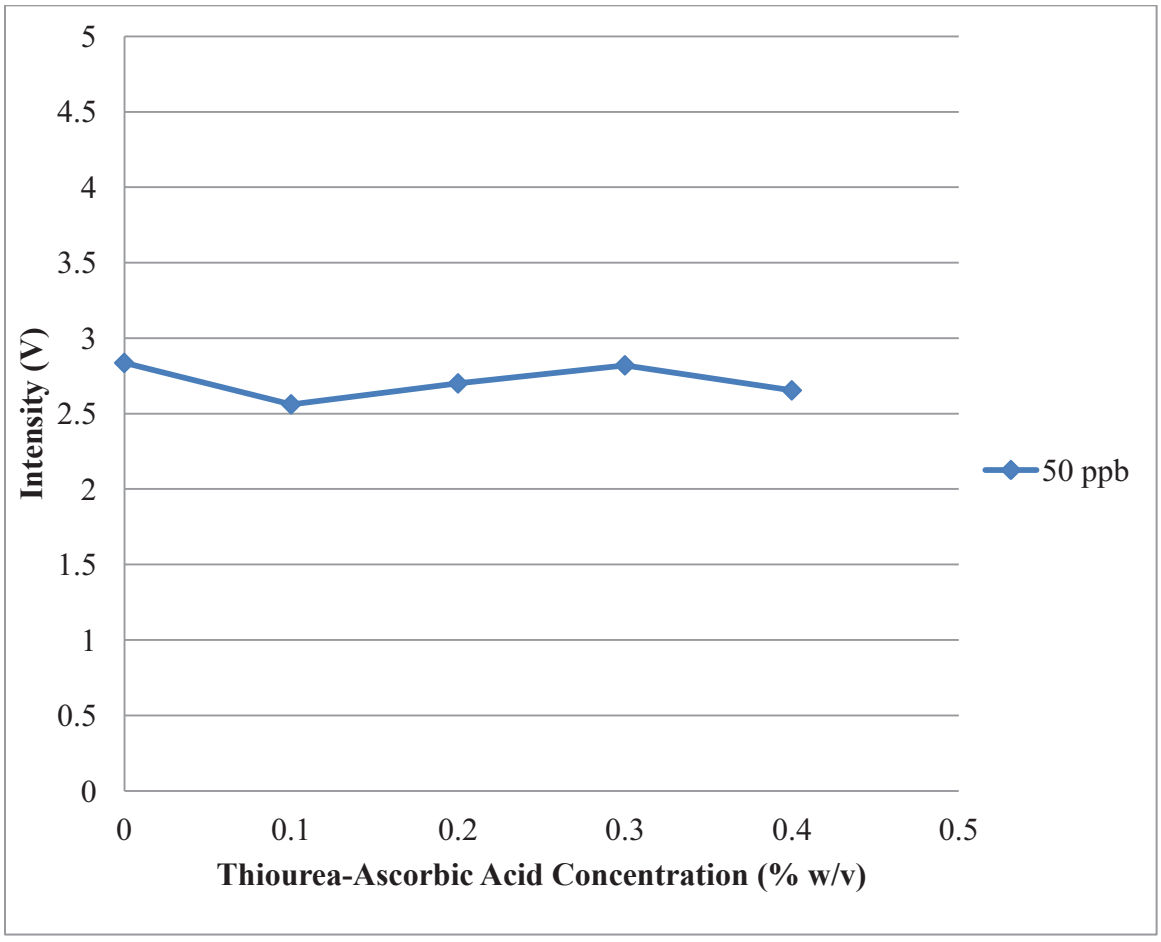


Figure 18 Influence of masking reagent concentration on fluorescence intensity of Ge

Table 13 Optimal conditions for HG-LIF Ge

Parameters	Ge
Acid Concentration	2 M Phosphoric acid
Sodium tetrahydroborate Concentration	0.5 % (w/v) in 0.1 % (w/v) NaOH
Peristaltic pump flow rate	20 a.u.
Masking Reagent Concentration (Ascorbic acid + Thiourea)	0.3 % (w/v) + 0.3 % (w/v)

Calibration and analytical figures of merit

In order to determine the sensitivity and limit of detection of the HG-LIF method, calibration curves were performed and typical calibration curves for Ge are shown in Figures 19, 20. Fluorescence intensity of Ge was found to be linearly proportional to the concentration of Ge. All the calibration curves showed good linearity. After optimizing the HG-LIF response as a function of HG reagents, the Limit of Detection (LOD) was calculated by using the standard deviation of the fluorescence signal of 16 blanks and the slope of the corresponding calibration curves.

$$\mathbf{LOD = 3\sigma/m}$$

Where, σ is the average standard deviation (noise) and m denotes slope of calibration line (sensitivity).

Under optimal conditions, the limit of detection obtained for Ge at 303.9 nm was 0.1 ng/mL.

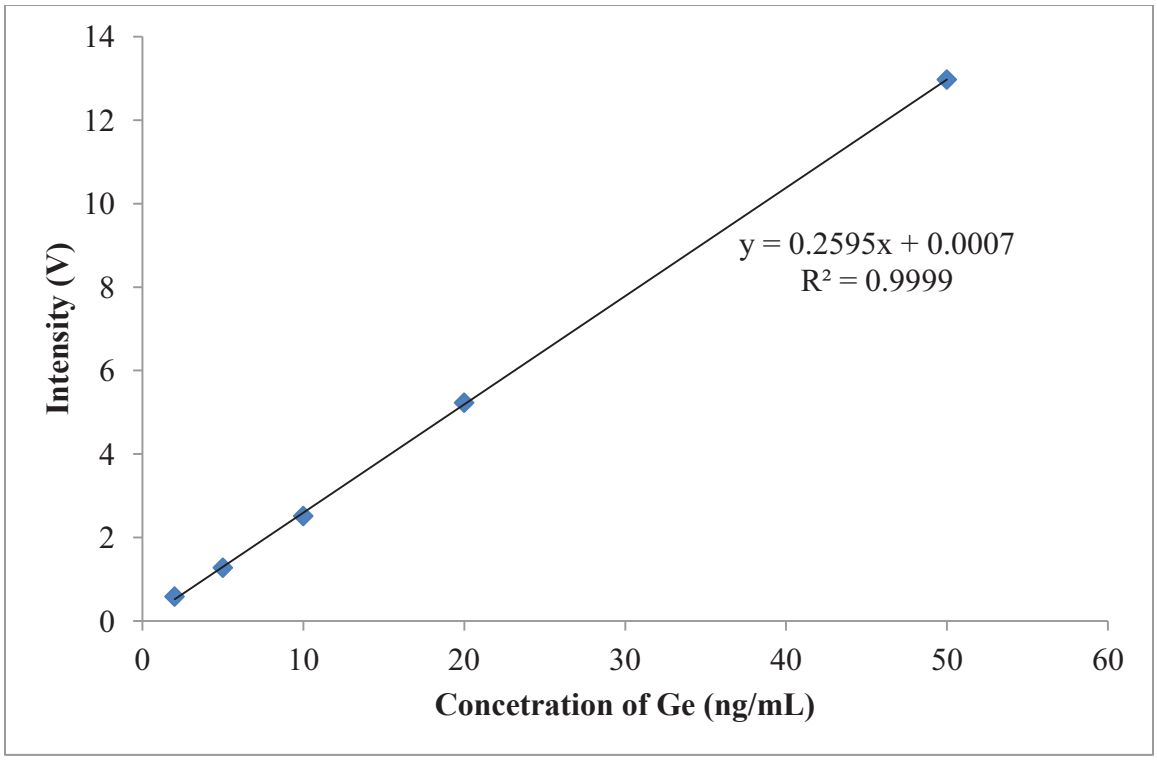


Figure 19 Calibration curve plot 1 HG-LIF Ge

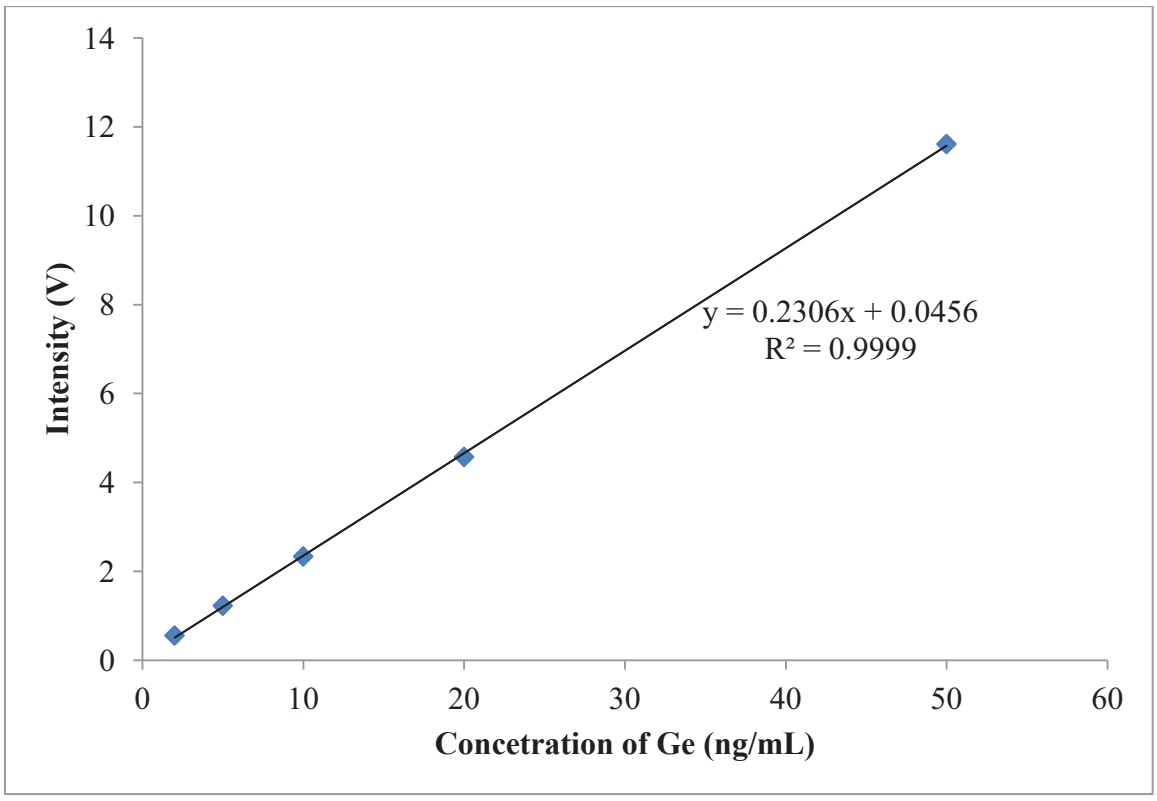


Figure 20 Calibration curve plot 2 HG-LIF Ge

Table 14 Analytical figures of merit for HG-LIF Ge

Analytical Figures	Ge
Linear Range (ng/mL)	1 to 50
Limit of Detection (ng/mL)	0.1
Limit of Quantification (ng/mL)	0.3
Relative Standard Deviation (%) at 10 ppb Ge	0.1

Analysis a certified reference material and multielement standards

The analytical utility of the HG-LIF technique for Ge measurements has been investigated through measurements of Ge in different sample matrices. It has been reported that HG based measurements of Ge can be affected by other metal ions in the sample matrix, but also that these matrix effects can be reduced or eliminated using reagents such as thiourea and ascorbic acid. Measurements of Ge were performed in samples with and without potentially interfering ions. Measurements of Ge were performed on a standard reference material (NIST SRM 1643e trace elements in water) that contained potential interfering elements. To 1 mL, 2 mL, and 3 mL of NIST standard, 20 ng/mL of Ge standard was added and recovery studies were performed. Good recovery was obtained even in the presence of potentially interfering elements when 0.3 % masking agent was used. The results were shown in Figure 21.

1643e NIST SRM recovery study

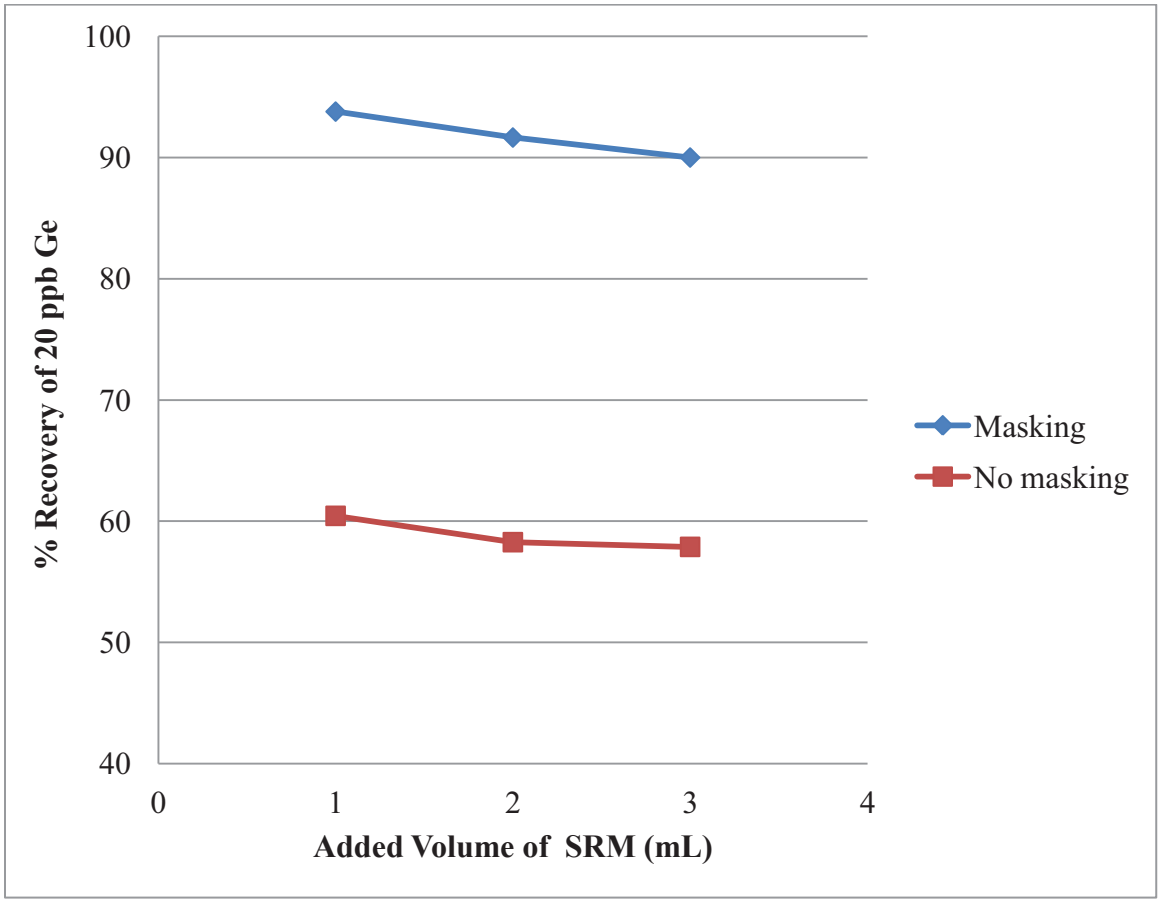


Figure 21 NIST 1643e SRM recovery study

In another study, measurements of Ge spike additions to a multielement standard solution (ICP Multielement Standard IV, Sigma Aldrich) were performed. When the masking agents were utilized, the HG-LIF measurements demonstrated a 94.3 +/- 0.2 % recovery. By comparison, measurements of Ge in the same sample matrix without masking agents showed lower recoveries (54.7 +/- 0.8 %) indicating loss of accuracy and matrix effects by the other elements. These results demonstrate the effectiveness of thiourea and ascorbic acid masking agents in controlling matrix effects caused by interfering ions.

ICP SRM 51844 recovery study

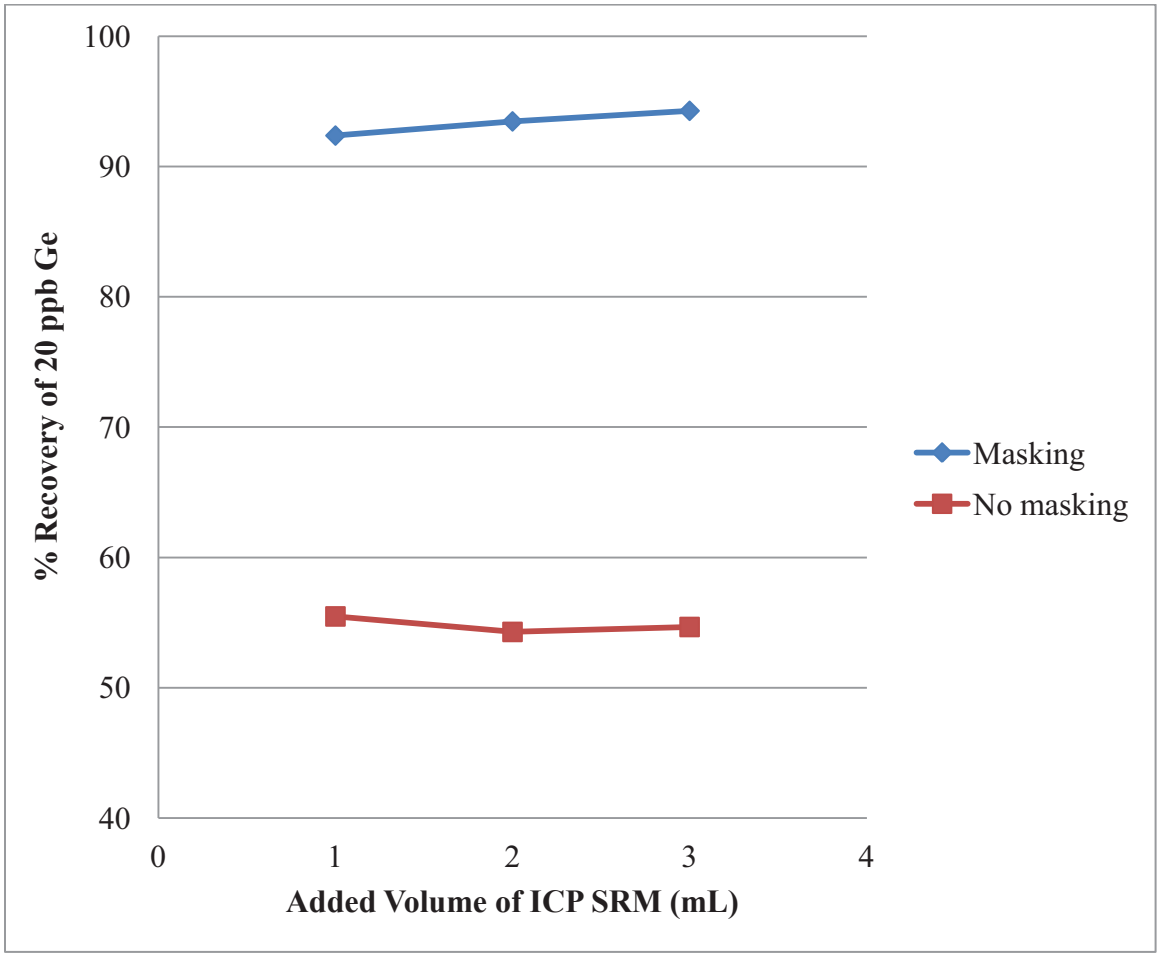


Figure 22 ICP SRM IV recovery study

Germanium supplement capsule analysis

To further evaluate the analytical utility of HG-LIF method for Ge, measurements were performed on supplement capsules from 2 different vendors. The certified value of Ge in the supplement capsules from both the vendors is 64.2 mg/capsule. The values of Ge determined by using HG-LIF technique were summarized in table 15. For comparison the supplement capsules were also analyzed using an ICP spectrometer and the values are shown in the table 15. A good agreement between the certified values and the values of

Ge determined by using HG-LIF and ICP techniques was observed. These results demonstrated the analytical utility of HG-LIF approach for trace level measurements of Ge in environmental samples.

Table 15 Germanium contents of supplement capsules from different vendors

Certified value of Ge supplement capsule (mg/capsule)	HG-LIF determination (mg/capsule)	ICP determination (mg/capsule)
Vendor 1 (64.2 mg/capsule)	63.1 +/- 1.8	57.6 +/- 2.7
Vendor 2 (64.2 mg/capsule)	62.7 +/- 1.9	56.9 +/- 2.5

CONCLUSIONS AND FUTURE DIRECTIONS

These studies have demonstrated the analytical capabilities of HG-LIF techniques for measuring trace Bi and Ge. The current techniques allow measurements in the ppt to ppb range and have limits of detection of 0.03 ppb and 0.1 ppb for measurements of Bi and Ge, respectively. Measurements in different sample matrices have demonstrated the accuracy of the approaches as well as the effectiveness of the masking agents for controlling the effects of interfering elements. Overall the results of the studies show that LIF detection combined with hydride generation sample introduction provides good analytical performance and has good feasibility for measuring Bi and Ge in environmental samples at environmentally relevant concentrations. Future efforts may focus on applying the HG-LIF method to measurements of Ge in different kinds of samples such as fly ash, metal ores and tea samples.

REFERENCES

1. Vandecasteele, C.; Block, B. C. *Modern Methods for Trace Element Determination,* Wiley, New York, **1993.**

2. Ezer, M. *Intern. J. Environ. Anal. Chem.* **2010,** 90, 697.

3. Doull, D.; Klaassen, D. C.; Amdur, O. M. *Toxicology,* Macmillan, New York, 2nd edn, **1980.**

4. Ezer, M.; Simeonsson, J. B. *Fresen. Environ. Bull.* **2009,** 18, 1572.

5. Pacquette, H. L.; Elwood, S. A.; Ezer, M.; Simeonsson, J. B. *Applied Spectroscopy.* **2000,** 54, 89.

6. Pacquette, H. L.; Elwood, S. A.; Ezer, M.; Simeonsson, J. B. *J. Anal. At. Spectrom.* **2001,** 16, 152.

7. Zare, R. N. *Annual Review of Analytical Chemistry,* Palatto, California, **2012,** 5, 14.

8. Bolshov, M. A. *Fresenius J. Anal. Chem.* **1996,** 355, 549.

9. Enger, J.; Marunkov, A.; Chekalin, N.; Axner, O. *J. Aanal. At. Spectrom.* **1995,** 10, 539.

10. Fransworth, P.B.; Smith, B.W.; Omentto, N. *Spectrochim. Acta.* **1990,** 45 B, 1151.

11. Dasilva, J. B. B.; Giacomelli, M. B. O.; Curtius, A. J. *Analyst.* **1999,** 124, 1249.

12. Bismuth's health and environmental effects. *Www.lenntech.com.* Retrieved on 26 July 2012.

13. 'Bismuth line' in the free dictionary's medical dictionary. *Farlex. Inc.* Retrieved on 26 July 2012.

14. Bruinink, A.; Reiser, P.; Mueller, M.; Gaebwiler, B. H.; Zhinder, G. *Tox. In vitro.* **1992,** 6, 4, 285.

15. Abramson, J. et al. *N. Engl. J. Med.* **1993,** 329, 1742.

16. Levantine, A.; Almeyda, J. *Brit. J. Dermatol.* **1973,** 89, 105.

17. Fowler, B. A. *Handbook on the toxicology of metals,* Elsevier, Amsterdam, **1986.**

18. Chen, S. Y.; Zhang, M. *Spectrosc. Spectral. Anal.* **1995**, 15, 85.

19. Vanhoe, H.; Dams, R.; Versieck, J. *J. Anal. Atomic Spectrom.* **1994,** 9, 23.

20. Chen, S.; Zhang, Z.; Yu, H. *Anal. Bioanal. Chem.* **2002,** 374, 126.

21. Feng, Y. L.; Chen, H. Y.; Chen, H. W.; Tian, L. C. *Fresenius J. Anal. Chem.* **1998,** 361, 155.

22. Zhang, L. S.; Combs, S. M. *J. Anal. At. Spectrom.* **1996,** 11, 1043.

23. Zhang, L.; Fu, N.; Fang, Z.; Feng, Y.; Ke, L. *Food Chem.* **2011,** 124, 1185.

24. Reyes, M. M. N.; Cervera, M. L.; Guardia, D. L. M. *Anal. Bioanal. Chem.* **2009,** 394, 1557.

25. Wang, F.; Zhang, G. *Appl. Spectrosc.* **2011,** 65, 315.

26. Zhe-Min, N.; Bi, H. *J. Anal. At. Spectrom.* **1995,** 10, 747.

27. Jiang, S. L.; Shi, C. H.; Wu, J. G. *J. Food Qual.* **2007,** 30, 482.

28. Dagnall, R. M.; Kirkbright, G. F.; West, T. S.; Wood, R. *Analyst.* **1970,** 95, 425.

29. Asai, K. *Miracle Cure – Organic Germanium,* Japan Publications Inc., Tokyo, Japan, **1980.**

30. Goodman, S. *Germanium: The Health and Life Enhancer,* Thorsons Publishers Ltd., Welling Borough, Northampton Shire, England, **1988.**

31. Boyukbhayram, A. E.; Volkan, M. *Spectrochim. Acta. Part B.* **2000,** 55, 1071.

32. Guo, X. M.; Guo, X. M. *Anal. Chim. Acta.* **1998,** 373, 303.

33. Andreae, O. M.; Froelich, N. P. *Anal. Chem.* **1981,** 53, 287.

34. Nakahara, T.; Wasa, T. *Microchem. J.* **1994,** 49, 202.

35. Dedina, J.; Tsalev, D. M. *Hydride Generation Atomic Absorption Spectrometry,* John Wiley & Sons, New York, **1995.**

36. Aaron, H. *Determination of As, Se, and Hg in water by Hydride Generation/ Cold water Atomic Absorption Spectroscopy,* Perkin Elmer Inc., Ontario, Canada.

37. Santosa, S. J.; Mokudai, H.; Tanaka, S. *J. Anal. At. Spectrom.* **1997,** 12, 409.

38. Rayman, P. M.; Abou-Shakra, R. F.; Ward, N. I. *J. Anal. At. Spectrom.* **1996,** 11, 61.

39. Klaue, B.; Blum, D. J. *Anal. Chem.* **1999,** 71, 1408.

40. Veber, M.; Cujes, K.; Gomiscek, S. *J. Anal. At. Spectrom.* **1994,** 9, 285.

41. Tyson, J. F.; Sundin, N. G.; Hanna, C. P.; McIntosh, S. A. *Spectrochim. Acta. Part B.* **1997,** 52, 1773.

42. Moreda-Pineira, J.; Cervera, L. M.; Guardia, D. L. M. *J. Anal. At. Spectrom.* **1997,** 12, 1377.

43. D'Ulivo, A.; Lampugnani, L.; Pellegrini, G.; Zamboni, R. *J. Anal. At. Spectrom.* **1995,** 10, 969.

44. Nakahara, T. *Prog. Analyst. Atom. Spectrosc.* **1983,** 6, 163.

45. Koechner, W.; Bass, M. *Solid State Lasers.* Springer, New York, **2003.**

46. Fassel, J. D.; Crouch, S. R. *Spectrochemical Analyst,* Englewood Cliffs, NJ, **1988.**