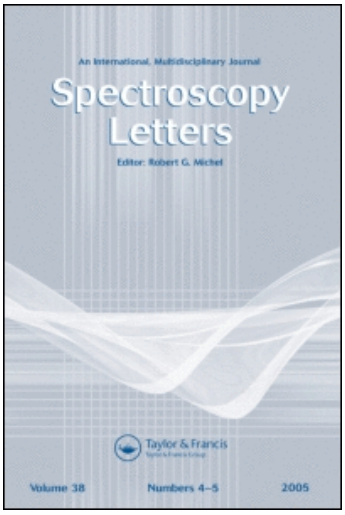

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Direct Arsenic Determination in Exposed Embryos of Zebrafish (*Danio rerio*) with Zeeman Electrothermal Atomic Absorption Spectrophotometry

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An invited paper submitted to a special issue on Green Spectroscopy and Analytical Techniques, organized by Professor Miguel de la Guardia, of the Department of Chemistry, University of Valencia, Spain, and Professor Arabinda Kumar Das, of the Department of Chemistry, University of Burdwan, West Bengal, India.

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ABSTRACT Several methods of analysis have been evaluated for the determination of the arsenic content in zebrafish embryos (*Danio rerio*). The methods have been developed based on waste minimization, while sample treatment was exploited to attend the standards of clean chemistry. The ultimate goal of developing a method to directly introduce whole single embryos into the electrothermal furnace atomic absorption spectrometer (ETAAS) was accomplished after optimization of instrument parameters and matrix modifiers. The significant matrix effects due to the complexity of the sample were overcome by the use of a palladium modifier and hydrogen peroxide as an oxidizing agent to aid in the mineralization of the sample during the pyrolysis. The results obtained from this direct method for arsenic analysis were in agreement with those from more common sample preparation methods of acid digestion or ultrasonic extraction. The speed of the ultrasonic method and use of environmentally safe reagents makes this a more favorable technique than acid digestion. The single embryo direct introduction method, however, is preferred due to its ability to measure whole single samples without any sample pretreatment, and furthermore allows for evaluation of the variability of arsenic accumulation between individual samples. The ETAAS method developed has been validated by inductively coupled plasma mass spectrometry (ICP-MS).

KEYWORDS arsenic, ETAAS, ICP-MS, zebrafish *Danio rerio*

INTRODUCTION

Arsenic is found in the environment in many toxic (mostly inorganic) and relatively nontoxic (mostly organic) forms from both natural and anthropogenic sources.[1] Although most of the arsenic found in the environment is a result of natural processes, the impact of industrial As use is still significant. Released arsenic enters the environment and its biological systems. Drinking water contamination of arsenic is a great concern especially in South East

Asia and India, but the element is considered as an environmental contaminant in many other parts of the world.[2] Exposure to arsenic is related to the appearance of non-cancer and cancer health effects. In 2004 the International Agency for Research on Cancer based on experimental and ecological studies drew overall evaluation that arsenic in drinking-water is carcinogenic to humans (group 1).[3] Studies on human population origin from different parts of the world but exposed to comparable level of arsenic in drinking water demonstrated remarkable individual varieties in susceptibility to arsenic-induced health effects.[4–7]

There is no ideal animal model organism that could be applied for pollutant accumulation studies. For the purposes of this work interest was in observing the ability of absorption of arsenic by zebrafish embryos (*Danio rerio*) as they have consistently demonstrated their usefulness as a model for vertebrate development and responses to external stimuli,[8–11] and thus are an ideal system to gauge the effects of an arsenic environment on accumulation levels of this element. Their ability to readily absorb compounds from their surrounding environment was of particular importance in their selection for this experiment. Additionally, the structure of the shell of the egg is relatively durable and allows for careful manipulation and transfer of single or small groups of embryos. This provides the unique opportunity to manipulate and perform experiments that consider only single organisms.

The possibility to measure the arsenic content in single embryos is important as it allows for the determination of differences in uptake between each embryo within a group and between embryos in different replicas. To accomplish this objective, the Electrothermal Atomic Absorbtion Spectrometry (ETAAS) was thought of as an appropriate technique due to its direct measuring capability and applicability to the analysis of solid samples without the need of sample treatment. It is easy to use and can be considered "environmentally friendly" because of the minimal sample treatment required, the use of low amount of toxic chemicals, and the reduced waste generation. All these aspects positively contribute to the development of Green Analytical Chemistry techniques.[12–14] For developing the suitable method different alternatives have been attempted and presented. Embryos samples with established content of arsenic were not available; in consequence, the present work was performed on non-homogenous samples, meaning that they proceeded from different egg-laying. In order to evaluate random errors validation of ETAAS with Flow Injection Analysis Inductively Coupled Plasma/Mass Spectrometry (FIA-ICP/MS) was performed.

EXPERIMENTAL

Instrumentation

Experiments with ETAAS employed a Perkin Elmer 4100ZL atomic absorption spectrometer with longitudinal Zeeman background correction. THGA graphite tubes (Perkin Elmer) equipped with L' vov platforms were used. Arsenic levels were determined using the integrated absorbance of the atomic absorption signal. Liquid samples were introduced using an AS-70 auto-sampler. The auto-sampler and furnace operation were controlled using Perkin Elmer AA Winlab software, Version 4.1 SP1 (Norwalk, USA). A Perkin Elmer arsenic electrodeless discharge lamp (EDL) with wavelength 193.7 nm and instrument slit width 0.7 nm was used. A Perkin Elmer EDL System was used to stabilize the lamp current between 349–351 mA.

Experiments with ICP/MS used an HP-4500 Plus (Agilent Technologies, Analytical System, Tokyo, Japan), equipped with a Babington nebulizer, Fassel torch, and a double-pass Scott-type spray chamber cooled by a Peltier system. The ICP-MS detector used single ion monitoring at m/z 75 for data collection. Monitoring of Cl (m/z 35) species was carried out in order to evaluate possible ArCl (m/z 75) polyatomic interference. The ICP/MS experimental conditions have been summarized in Table 1. The arsenic concentration in the liquid samples was measured by coupling flow injection analysis (FIA) to the ICP/MS detector. The injected volume was 20 μL.

TABLE 1 Experimental Conditions for the Analysis of Arsenic by ICP/MS

RF Power	1350 W
Ar flow rate	Plasma gas: 15.3 L min^{-1} Nebulizer: 1 L min^{-1} Auxiliary 1.2 L min^{-1}
Sample flow rate	1 mL/min
Isotope monitored	^{75}As
Integration time	0.1 s (spectrum) per point
Points per peak	3

Ultrasonic sample extraction of water soluble arsenicals was done with an ultrasonic probe homogenizer, model Sonopuls HD 2200 (Bandelin, Germany), equipped with a converter UW 2200, BR 213 G horn as amplifier, BR 30 cup booster, and EH 3 microtube holder. A centrifuge model 5415 R Eppendorf (Hamburg, Germany) was used for the separation of aqueous extracts from insoluble pellets after ultrasonic embryos extraction.

Reagents and Standards

All solutions used were prepared with ultra-pure, deionized Milli-Q® (MilliPore Corp., USA) 18.0 MΩ · cm water made fresh daily. Required solutions of nitric acid were prepared by dilution of the commercial acid (Merck, Darmstadt, Germany), which was further purified in a Teflon sub-boiling distillation unit. Neither the water nor the nitric acid showed any detectable arsenic in the ETAAS or ICP/MS.

The $Pd(NO_3)_2$ matrix modifier solution was made from dilution of $10.00 \pm 0.03\,g\,L^{-1}$ Pd matrix modifier solution (Merck, Darmstadt, Germany) with water to the desired final concentration. The Pb/Mg modifier solution was made from solid $Pb(NO_3)_2$ (Merck, Darmstadt, Germany) and $Mg(NO_3)_2 \cdot 6\,H_2O$ (Merck, Darmstadt, Germany) to a stock concentration of $2.5\,g\,L^{-1}$ Pb and $0.2\,g\,L^{-1}$ Mg, which was diluted with water as needed. The nickel modifier was made from solid $Ni(NO_3)_2$ (Merck, Darmstadt, Germany) to a stock solution of $2.0\,g\,L^{-1}$. Hydrogen peroxide (30%, Panreac, Barcelona, Spain) was used as an oxidizing agent for matrix effect removal. Arsenic standard solutions used were made from dilutions with water of $As_2O_5 \cdot 2\,H_2O$ (98.5%) purchased from Merck (Darmstadt, Germany) and As_2O_3 (99.5%) purchased from J. T. Baker (Deventer, Holland). Lyophilized fish candidate reference material prepared within the framework of European project SEAS (ref. SEAS G6RD-CT2001-00473) containing $42.2 \pm 0.09\,\mu g$ As g^{-1} was employed for method validation.[15]

Embryos Samples

Zebra fish embryos of age 24 hpf (hours post fertilization) were obtained and treated by ZF Biolabs, Madrid, Spain.[16] During the exposition experiment, separate groups of embryos (10–20) were exposed to solutions containing 1 mg $As^V\,L^{-1}$. Parallel blank sample experiments were also performed without arsenic addition. When the exposure period was terminated liquid growing medium was removed and then embryos were washed with Milli-Q water. The embryos were then placed in plastic containers in groups of five or individually separated. The excess of water was removed and samples were frozen at −20°C, and stored until analysis.

Procedures

Acid Digestion Protocol

The optimization of the digestion protocol was done employing the SEAS lyophilized fish candidate reference material. Approximately 10 mg of sample were placed in closed Teflon vessels and digested by adding 375 µL distilled nitric acid and 150 µL of 30% hydrogen peroxide. The samples were kept overnight at 40°C, then heated in an oven to 110°C for 2 h. Samples were allowed to cool to room temperature then diluted to a volume of 5 mL with distilled water. When digestion of embryos was implemented, the protocol was slightly modified: 5 embryos were placed in the Teflon vessels with 300 µL of distilled nitric acid and 150 µL of 30% H_2O_2. Then, samples were heated as the reference material, then allowed to cool and diluted to a final volume of 1 mL in order to maintain a concentration within the quantification limits of ETAAS. Different extracts were employed for the analysis of arsenic by ETAAS and by ICP/MS.

Ultrasonic Sample Extraction Protocol

Arsenic quantification in the aqueous extracts of the embryo samples of age 24 hpf was also measured after ultrasonic digestion. Three samples of 5 embryos were suspended into 0.5 mL of Milli-Q water and the vials were then immersed in a water bath and sonicated for 5 min at 40% of maximal amplitude. The vials were centrifuged at 4000 rpm for 20 min, at 10°C. Supernatant fractions were separated from pellets and filtered through a 0.22 µm nylon syringe filter before analysis. Arsenic content in the supernatant was then measured by ETAAS and also by ICP/MS. Different extracts were employed for the analysis of arsenic by ETAAS and by ICP/MS. When the ultrasonic sample extraction was applied to a single embryo only 100 µL of water was used to form the suspension, which was sonicated but was not centrifuged or filtered.

Direct Injection of Embryos

The arsenic content in the solid embryos was measured by direct injection in ETAAS. Solid embryos were manually injected using a glass Pasteur pipette; then 10 μL of the 2.0 g L^{-1} Pd modifier and 10 μL of 30% hydrogen peroxide were added by the autosampler. Immediately the heating furnace program showed in Table 4 was applied.

RESULTS AND DISCUSSION

ETAAS Parameters Optimization

Firstly, the ETAAS optimization to obtain the best signal for arsenic was done by measuring the fish candidate reference material extracts. The sample volume injected in the graphite tube was 20 μL. Common limitations when using ETAAS are the effects of strong matrix interferences. To avoid these difficulties modifiers such as magnesium, nickel, and palladium have been commonly employed.[17] Modifier solutions containing palladium, nickel, or magnesium/lead were tested to select the optimal and its concentration in order to obtain the most consistent and accurate response. Acid digestion of the reference material was performed according to the procedure described earlier. Optimization was carried out in the 0.01–2 g L^{-1} concentration range of the modifiers. The signal response obtained with Ni was often no better than that of the unmodified solution, therefore it was determined that this compound was an ineffective modifier. The best results were obtained when Pd at a concentration of 0.5 g Pd L^{-1} was added to the reaction mixture. Since the signal stabilizes at higher concentrations, 2.0 g L^{-1} was finally selected to ensure a sufficient modifier amount.

Another relevant parameter for arsenic measurement using ETAAS is the thermal furnace program applied. The obtained optimal furnace program is illustrated in Table 2.

After optimization of furnace and thermal program, different methods of calibration were tested to determine the best approach. In order to compare the behavior of inorganic arsenic species during the analysis, calibration curves were prepared using arsenic acid (iAs^{V}) and arsenous acid (iAs^{III}). To study possible differences between inorganic and organic compounds, a calibration curve based on arsenobetaine was also prepared. The choice of those standards was made based on the following reasons: iAs^{V} was used as the arsenic source in the exposure experiment, and it is also the As compound obtained after the acid digestion; iAs^{III} is the first metabolite in the biotransformation process reported for many living organisms[18]; and arsenobetaine is the main arsenic compound detected in lyophilized fish used in the current work as a reference material.[15] Linear calibrations of five measurements in the concentration range of 4–80 μg L^{-1} were prepared. Obtained results did not show any impact of the standard on the absorbance, being the calibration curves similar in terms of slope and intercept ($0.0592x + 0.0088$, $R^2 = 0.9923$; $0.0611x + 0.0088$, $R^2 = 0.9961$; $0.0655x + 0.0048$, $R^2 = 0.998$ for iAs^{V}, iAs^{III}, and arsenobetaine, respectively). Therefore, iAs^{V} was used as the standard solution for all calibration measurements. The relative standard deviation value (R.S.D. %) of three replicates of 40 μg L^{-1} was below 5%.

TABLE 2 Furnace Program Optimized and Applied to the Liquid Samples

Step		Temperature [°C]	Ramp [sec]	Hold [sec]	Gas (Ar) [mL min^{-1}]
1	Drying stage 1	110	1	30	250
2	Drying stage 2	130	15	30	250
3	Pyrolysis stage	1200	10	20	250
4	Atomization	2400	0	3	0 (read)
5	Cleaning	2450	1	3	250

Analysis of Arsenic Content in the Embryos Extracts After Treatment

ETAAS measurement parameters optimized previously with candidate reference material was subsequently applied for measurement of arsenic in extracts from zebrafish embryos after acid digestion and after ultrasonic extraction. Sample preparation procedures have been detailed in previous sections. The extracts were also measured by FIA ICP-MS for validation of the developed ETAAS method, as has already been mentioned. Analysis of the pellet remaining after extraction showed that the remaining arsenic after supernatant separation was below the detection limit. Evaluation of the matrix effect was studied as before, proving that there was null influence for any of the extracts measured. It is important to highlight that the zebrafish embryos analyzed all throughout this experiment were not homogeneous

TABLE 3 **Arsenic Concentration Measured in Zebrafish Embryos Expressed as ng As/Embryo**

Sample preparation method		Determination method	
		ETAAS	FIA ICP MS
Acid digestion (batch 1)	ng As/embryo	0.6 ± 0.1	0.6 ± 0.1
	RSD (%)	13.5	11.2
Ultrasonic extraction (batch 2)	ng As/embryo	0.9 ± 0.1	0.8 ± 0.2
	RSD (%)	7.0	15.3

The extracts were obtained from embryos of the first batch of samples (a) after acid digestion; (b) after ultrasonic extraction. Both acid digestion and ultrasonic extraction were performed by six and three replicates were measured by ETAAS and other three by FIA-ICP/MS. Exposure to 1 mg iAs^V L^{-1}; N = 3 (each replicate consisted of 5 embryos).

samples because the different batches were produced in different egg-laying. Results are shown in Table 3.

Looking at the results it can be stated that the efficiency of the examined digestion procedures is comparable. The variances ratio F-test applied to evaluate significant differences between the two applied methods let us accept the null hypothesis that there is no significant difference between the methods and any difference in the sample variations/standard deviations are due to random errors. Reproducibility calculated on triplicate was generally less than 15% RSD (%), but higher than for the fish reference material. It can be concluded that ultrasonic digestion is equally effective as acid digestion. Considering that this sample pretreatment method is faster than the overnight digestion and does not require the use of harsh, environmentally damaging reagents, makes it a more favorable sample preparation technique from practical and environmental considerations. The differences in the total arsenic concentration in solution obtained after applying the two methods of sample digestion have been then attributed to differences in the element content in embryos originating from different batches of samples (batch 1 for acid digestion and batch 2 for ultrasonic extraction) belonging to different egg-laying by different zebrafish females.

Optimization of ETAAS for the Individual Embryos Injection

Elaboration of a simple, direct method of arsenic assessment would provide a powerful tool for further studies, like unique information about individual arsenic accumulation can be traced. Simple and quick method in combination with very short embryonic development of zebrafish can give the possibility of bringing information about arsenic metabolism in a very short time. The development of a method based on the direct introduction of single embryos in an electrothermal furnace was accomplished.

The optimization of ETAAS conditions for the introduction of solid samples was carried out by directly injecting control embryos, that is, embryos not exposed to arsenic. Initial measurements were done by injecting one control embryo, 5 µL of 50 µg L^{-1} iAs^V, 5 µL water and 10 µL 2.0 gL^{-1} Pd modifier. As was expected, a strong matrix effect was observed by means of absorbance peak broadening and consequent lowering of the signal. There also seemed to be a visible residue remaining in the graphite tube after 3–4 analyses. At this point, the water diluent was replaced with the same volume of 30% hydrogen peroxide expecting that this oxygenating agent would cause a more efficient pyrolysis and decrease the amount of residue remaining after measurement. Satisfying results were obtained and other oxidizing agents were not considered. The final optimized solution mixture consisted on the single embryo, 10 µL of the 2.0 g L^{-1} Pd modifier, 10 µL of 30% hydrogen peroxide and 5 µL of 50 µg L^{-1} iAs^V solution; when the exposed embryos were analyzed, iAs^V solution was replaced by water. Peak profiles of Fig. 1 showed that these considerable matrix effects have been almost completely overcome. The

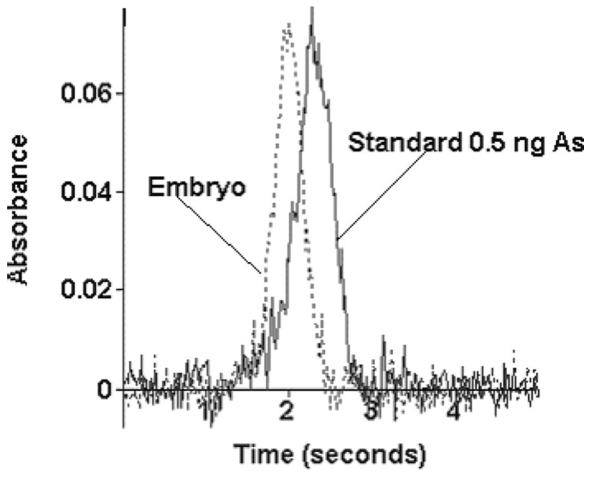

FIGURE 1 **Arsenic signal peak profiles for direct injection of Zebrafish embryos and measured by ETAAS. (a) Standard 0,5 ng As^V; (b) Embryo + 0,5 ng As^V.**

TABLE 4 Furnace Program Optimized and Applied to *Zebrafish* Single Embryo Samples

Step		Temperature [°C]	Ramp [s]	Hold [s]	Gas (Ar) [mL min^{-1}]
1	Manual injection of embryo	20	1	15	—
2	Drying stage 1	110	1	30	250
3	Drying stage 2	130	15	30	250
4	Pyrolysis stage 1	600	25	45	250
5	Pyrolysis stage 2	1200	25	45	250
6	Atomization	2200	0	3	0 (read)
7	Cleaning	2450	1	5	250

removal of matrix interference was tested by performing external and standard addition calibration curves by spiking the embryo with the modifier + the the oxidizing reagent + increasing iAs^{V} concentrations in the range of 5–100 µg L^{-1}. Comparable curves were obtained by applying the two calibration approaches. This result was highly beneficial as the ultimate goal of this work was the direct measurement of arsenic content in single zebrafish embryos exposed to arsenic for the study of the individual accumulation where standard addition method could not be applied.

Furthermore, addition of the modifiers increases the life time of the graphite tubes, allowing them to be used for at least 300 analyses.

The heating furnace program was finally modified by adding an initial hold step at room temperature in order to circumvent the auto-sampler and allow for manual embryo injection. Also, the times of the different steps have been increased as well as a previous pyrolysis step at 600°C in order to totally mineralize the high carbon content of the solid sample. The details of the final conditions of the furnace program for embryos are shown in Table 4. After measuring 3 samples, a blank was run to ensure that no residual arsenic remained in the graphite tube. The detection limit of the method was established as 2 ng As g^{-1}.

Analysis of Arsenic Content in the Individual Embryos Exposed to As^{v}

Another embryo batch (batch 3) was exposed to 1 mgL^{-1} As^{V} and then analyzed by individual injection in ETAAS applying the developed method explained in the previous section. The weight of the individual embryos was controlled and varied in the range of 0.73–1.06 mg, with the mean value of 0.9 mg and R.S.D. 7.6%. The mean value of As for 10 embryos was 0.50 ± 0.15 ng/embryo (RSD = 30%). A large variability on the arsenic content between individual measurements is deduced from this result. These divergences raised the doubt that a possible cause of the variability could be due to the difficulty in exactly reproducing the embryo manual injection, that is, a problem related with the solid sample introduction method developed. Another possible explanation of the observed differences in arsenic content could be the individual natural variability in the absorption of this element. In order to clarify these doubts, a new batch of samples (batch 4) was prepared and exposed to As^{V}. Then, 10 embryos were individually treated following the procedure explained in an earlier section. (ultrasonic extraction) and analyzed by FIA-ICP/MS. This second set of measurements gave an arsenic content of 0.30 ± 0.10 ng/embryo (RSD = 25%), a similar variability in single embryos than the analysis performed by ETAAS. Then, analysis of arsenic content in the single embryos revealed high variation in the element accumulation. However, variances ratio F-test showed there was not significant difference between the two compared methods. With these evidences it is possible to postulate that the high variability in arsenic accumulation is likely caused by biological factors that differ between embryos, which may have an impact on arsenic uptake and its accumulation. Then, the development of this method of analysis allows for the monitoring of differences in arsenic absorbance between embryos and ensures that the content of the entire organism is measured. This feature is very interesting for this unique sample and for future toxicological and model studies.

CONCLUSION

Solid sampling ETAAS methods have become incredibly useful in decreasing sample preparation time, sample handling, and losses of analyte, as well as allowing for direct determination of elemental content from a native sample. In the presented method for direct determination of arsenic content in single zebrafish embryos, optimization of the furnace program and matrix modifiers became the

main concern to obtain analytically useful results and circumventing the prevalent matrix effects associated with complex biological samples. The method used in this work was successful in direct arsenic determination; however, further steps should be taken to minimize difficulties in the sample introduction stage due to the delicate nature of the embryos. Moreover, the developed method can be easy applied to other elements. Whether determination of arsenic is desired, disregarding the differences between embryos, an ultrasonic digestion method combined with FIA-ICP/MS detection is recommended due to its speed, simplicity, and reproducible results. The high variability of the arsenic content found between embryos is attributed to the different capability of each biological sample to accumulate the analyte.

It must also be emphasized that the described methods are environmentally friendly. Replacing traditional sample acidic digestion method by ultrasonic extraction contributes to decreased consumption of acids in the laboratory, and generation of hazardous residues can be reduced. Moreover, these methods are less time consuming and laborious, especially in the case of solid samples introduction in the ETAAS where no sample treatment is needed and no residues are generated. These advantages can became fundamental for routine analysis of a large number of samples.

Concerning arsenic accumulation, the concentrations measured up to now are rather low, but it cannot be concluded at this point that there is not accumulation by zebrafish embryos. It is still needed to make an exhaustive evaluation of other experimental parameters, such as lower exposure times due to the rapid metabolism of the zebrafish embryos, exposure to other arsenic species, and so on. in order to complete the data for bioaccumulation and model purposes.

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REFERENCES

1. Hale, M.; Carranza, E. J. M.; Duker, A. A. Arsenic geochemistry and health. *Environment International* **2005**, *31*, 631–641.
2. Sedley, P. L.; Kinninburgh, D. G. A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry* **2002**, *17*, 517–568.
3. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans: Some drinking water disinfectants, including arsenic. **2004**, *84*, 1–477.
4. Basu, A.; Mahata, J.; Gupta, S.; Giri, A. K. Genetic toxicology of aparadoxical human carcinogen, arsenic: A review. *Mutat. Res.* **2001**, *488*, 171–194.
5. Vahter, M. Methylation of inorganic arsenic in different mammalian species and population groups. *Sci. Prog.* **1999**, *82*, 69–88.
6. Abernathy, C. O.; Liu, Y. P.; Longfellow, D.; Aposhian, H. V.; Beck, B.; Fowler, B.; Goyer, R.; Menzer, R.; Rossman, T.; Thompson, C.; Waalkes, M. Arsenic: Health effects, mechanisms of actions, and research issues. *Environ. Health Perspect.* **1999**, *107*, 593–597.
7. Hopenhayn-Rich, C.; Browning, S. R.; Hertz-Picciotto, I.; Ferreccio, C.; Peralta, C.; Gibb, H. Chronic arsenic exposure and risk of infant mortality in two areas of Chile. *Environ. Health Perspect.* **2000**, *108*, 667–673.
8. Herrmann, K. Teratogenic effects of retinoic acid and related substances on the early development of the zebrafish (Brachydanio rerio) as assessed by a novel scoring system. *Toxic. In Vitro* **1995**, *9*, 267–283.
9. Lele, Z.; Krone, P. H. The zebrafish as a model system in developmental, toxicological and transgenic research. *Biotechnology Advances* **1996**, *14*, 57–72.
10. Goldsmith, P. Zebrafish as a pharmacological tool: The how, why and when. *Current Opinion in Pharmacology* **2004**, *4*, 504–512.
11. Westerfield, M. *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio)* 4th ed.; University of Oregon Press: Eugene, 2000.
12. Fabbri, D.; Baravelli, P.; Chiavari, G.; Prati, S. Profiling fatty acids in vegetable oils by reactive pyrolysis-gas chromatography with dimethyl carbonate and titanium silicate. *J. Chrom. A* **2005**, *1100*, 218–222.
13. Cava-Montesinos, P.; Ródenas-Torralba, E.; Morales-Rubiom, A.; Cervera, M. L.; de la Guardia, M. Cold vapour atomic fluorescence determination of mercury in milk by slurry sampling using multicommutation. *Anal. Chim. Acta* **2004**, *506*, 145–153.
14. Melchert, W. R.; Infante, C. M. C.; Rocha, F. R. P. Development and critical comparison of greener flow procedures for nitrite determination in natural waters. *Microchemical J.* **2007**, *85*, 209–213.
15. Sanz, E.; Muñoz-Olivas, R.; Camara, C. Evaluation of a focused sonication probe for arsenic speciation in environmental and biological samples. *J Chrom. A* **2005**, *1097*, 1–8.
16. http://www.zfbiolabs.com/compania/zfbiolabs.html
17. Niedzielski, P.; Siepak, M.; Siepak, J. Comparison of modifiers for determination of arsenic, antimony and selenium by atomic absorption spectroscopy with atomization in graphite tube or hydride generation and in-situ preconcentration in graphite tube. *Microchim J.* **2002**, *72*, 137–145.
18. Thomas, D. J.; Waters, S. B.; Styblo, M. Elucidating the pathway for arsenic methylation. *Toxicol. Appl. Pharmacol.* **2004**, *198*, 319–326.