Chapter

6

〖Method of Arsenic Estimation〗

The amount of arsenic in water, soil, air, foodstuffs, and biological samples (blood, urine, nail, hair) can be estimated by a number of methods, ranging from the simplest Gutzeit's test to ICP-MS. Several points are considered before selection of any of the following methods: a) qualitative or quantitative test, b) field-based or laboratory based, c) immediately done or time consuming, d) cheap or costly, e) require less skill or experienced, and f) estimation of total arsenic or speciation.

Qualitative method: Marsh test or Gutzeit's test can be used for initial screening purpose at the field level. In addition, this method is preferred where a large number of samples need to be estimated such as in Bangladesh, India or China.

Semi-quantitative method: This method is more reliable than the qualitative

method. Like qualitative method, this method can be used for the estimation of total arsenic in the water samples of all hand pump tube wells of the country like Bangladesh, India or China, where it is a gigantic task that involves technical, institutional, and social challenges. Initial screening and regular monitoring of all the tube wells water are almost impossible using atomic absorption spectrophotometer. In this situation, kit method can be used at the field level.

Quantitative method: This method is the most preferred method. The instruments used for the estimation of total arsenic level are UV-Vis spectrophotometer, atomic absorption spectrophotometer with either hydride generator or graphite furnace, and atomic fluorescent spectrometry (AFS). Speciation of arsenic (As^{III}, As^{V}, MMA^{III}, MMA^{V}, DMA^{III}, DMA^{V}, arsenicholine, arsenibutaine, arsenosugars, etc) is estimated by high performance liquid chromatography-atomic fluorescence spectrometer (HPLC-AFS), inductively coupled plasma-mass spectroscopy (ICP-MS) and HPLC-MS/MS. When two or more instruments are joined they are called hyphenated method. These hyphenated methods are expensive.

Table 6.1 *Detection limit of arsenic in water using deferent methods.*

Method	Detection limit (ppb)
ICP-MS	1
Atomic absorption spectrophotometer with hydride generator	2
Atomic absorption spectrophotometer with graphite furnace	5
UV-VIS spectrophotometer	8
Kit method	50

(Misbahuddin and Khandker, 2011)

The limit of detection of each quantitative method is important (Table 6.1). The spectrophotometric method of total arsenic estimation is the silver diethyldithiocarbamate (SDDC) method which is more sensitive than the kit

method. The limit of detection using ICP-MS is 1 ppb whereas it is 50 ppb in kit method.

6.1 Marsh Test

The test was named from its inventor, the English chemist James Marsh (1794-1846) and was first published in 1836.

Principle: Arsenic produces arseneuratted hydrogen in presence of zinc and sulfuric acid. This arsenic is deposited on the glass.

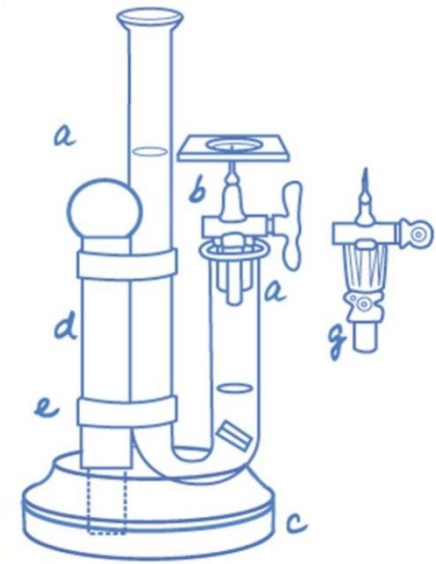

Figure 6.1 *Schematic diagram of the apparatus used in Marsh test. a) A glass tube open at both ends and about three quarters of an inch in its internal diameter. It is bent into the form of a siphon, the longer leg is about 8 inches in length and the shorter leg is about 5 inches; b) stopcock ending in a jet of fine bore; c) wooden block for the reception of the lower part of the pillar (d) with two elastic slips (e); f) horizontally piece of window-glass over the stopcock placed in such a manner as to retard slightly the combustion, the arsenic (if present) will be deposited on the glass; g) a small glass bucket.*

Procedure: A glass rod (1 inch) is to be dropped into the shorter leg followed

by a piece of zinc (1.5 x 0.5 inch) (Figure 6.1). The fluid to be examined for arsenic is mixed with 5 mL dilute sulfuric acid (acid: water 1:7) and poured into the long leg. Bubble gas appears from the zinc which is pure hydrogen, if no arsenic is present. But if the sample contains arsenic, the gas is arsenuretted hydrogen. The first portions are allowed to escape (air) by opening the stopcock and then closed. A portion of gas gives pressure of a column of fluid 7-8 inches high when the stopcock is opened. The gas is propelled with some forces through the jet. On igniting it, arsenic is deposited in the metallic state on the glass (Marsh, 1836).

Advantage: Marsh test does not require any instrument to estimate the presence of arsenic. It can be done by a less skill person within a short time. This method is suitable for field level detection of arsenic.

Disadvantage: Marsh test is a qualitative method. Now-a-days this method is rarely used. There may be false positive result due to presence of antimony.

6.2 Gutzeit's Test

Gutzeit's test is named from a German chemist, Max Adolf Gutzeit (1847-1915).

Principle: Arsine is formed from arsenic compounds by the addition of zinc granules to concentrated sulfuric acid (Nadeau, 1952). The arsine is detected on a strip of filter paper as gray spot (moistened with silver nitrate) or yellow to reddish-brown spot (moistened with mercuric chloride).

Procedure: A wide-necked bottle (200 mL) or conical flask is closed by a rubber bung perforated with one hole, in which it is held vertically a narrow glass tube (3.5 x 1 inch) (Figure 6.2). The tube is loosely packed with a wad of

glass wool impregnated with lead acetate to remove hydrogen sulfide from the evolved gases. It is closed at the upper end by a rubber stopper perforated by a hole (5 mm diameter). The top surface of this stopper is flat on which a mercuric chloride paper is laid during estimation. The paper is kept in place by a loose cape made of a disc of glass (5 cm).

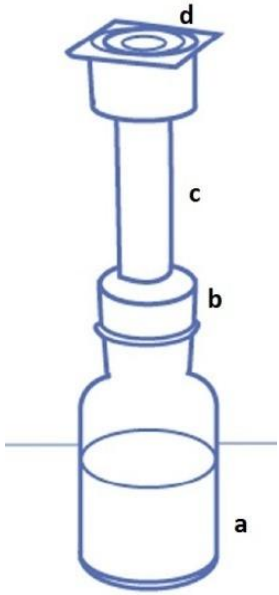

Figure 6.2 *Schematic diagram of the apparatus used in Gutzeit's test. a) A glass bottle containing arsenic, zinc and sulfuric acid; b) rubber with bore; c) narrow glass tube containing glass wool impregnated with lead acetate; d) a filter paper at the top of the glass tube containing either silver nitrate or mercuric chloride.*

Advantages: Gutzeit's test does not require any instrument to detect the presence of arsenic in the sample. Simply, a bottle or conical flask, filter paper, glass wool, silver nitrate, and mercuric chloride are required. It can be done by a less skill person within a short time. This method is suitable for field level.

Disadvantages: Gutzeit's test is a qualitative method. Silver nitrate is light sensitive. The sensitivity of this method is about 1 μg. It is only applied for the

estimation of arsenic in water or urine.

6.3 Field Test Kits

Several commercial field test kits (E. Merck kit, HACH kit, Arsenator 36, Wagtach Digital Arsenator, CMC kit, NIPSOM kit, ITN-BUET kit and GPL kit, etc) are available for the determination of total arsenic in tube wells water (Figure 6.3; Table 6.2). The field test kit must be simple, cheap, accurate, precise, safe, rapid and reliable. The existing kits do not fulfil all the criteria.

Figure 6.3 *Different kit methods for rapid estimation of arsenic.*

Principle: As^{V} is converted to the As^{III} form in water by the addition of potassium iodide and stannous chloride. Addition of zinc and hydrochloric acid to the water sample liberates nascent hydrogen that reacts with the As^{III} to

release arsine gas. The arsenic reacts with mercuric bromide paper to form complex salts of arsenic and mercury, producing yellow to brown stain depending on the arsenic concentration in the sample. This coloration is due to the formation of the compounds $H(HgBr)_2As$ (yellow), $(HgBr)_3As$ (brown) and Hg_3As_2 (black). At low concentration of arsenic a yellow stainning is produced while high level gives a black stainning (Pande et al., 2001).

In case of Merck kit test strip: If the strip turns yellow, then it means arsenic concentration is 100 pp or more; turns pale yellow means arsenic concentration is in between 1-100 ppb; if remains white it means there is no arsenic. That is, the range of arsenic is very wide.

Advantages: Field test kit is simple, low-cost method for initial screening of arsenic in hand pump tube well in shortest possible time.

Table 6.2 *Field kits used in Bangladesh.*

Name	Country of origin
E. Merck field kit	Germany
HACH kit	USA
NIPSOM kit	Bangladesh
ITN-BUET kit	Bangladesh
GPL kit	Bangladesh
AIIH&PH kit	India
CMC kit	China
AAN-Hironaka kit	Japan
Aqua kit	India
Arsenator 36	Austria
Wagtech Digital Arsenator	UK
PeCo 75	Austria

Disadvantages: Field kit provides a semi-quantitative result and the re-liability of this type of field kit is questionable because of poor accuracy (Rahman et al., 2002). Ten to seventeen per cent of the samples show false negative.

Reproducibility of result by the kits at the lower level of arsenic is found unsatisfactory. Field test kit produces arsine gas which is toxic. Mercuric bromide paper used is also toxic. Silver nitrate is light sensitive. This method provides qualitative and semi-quantitative method of total arsenic estimation. Kit method is not able to detect arsenic level of 10 ppb. There is inacuracy of data when the concentration of arsenic is 100 ppb or more.

6.4 Spectrophotometric Method

This is the most widely acceptable method for the estimation of total or speciation of arsenic in water, hair, nail and urine. This method was reported in 1959 (Powers et al., 1959).

Principle: This method consists of digestion of sample and generation of arsenic. In the process of digestion, the sample containing arsenic is to be digested with four acids: sulfuric acid, nitric acid, hydrochloric acid and perchloric acid. Digestion is considered to be completed when there apprears white fume. After digestion, the inorganic arsenic in a sample is reduced by acid zinc reaction to arsine (AsH_3) which is scrubbed through lead acetate impregnated glass wool and is absorbed in SDDC dissolved in pyridine. This step is done with the help of arsenic generator (Figure 6.4). The color developed due to arsine-SDDC and the reading is taken at 535 nm (Figure 6.5) by spectrophotometer. 1-Ephedrine in chloroform has been found to be a suitable solvent for SDDC if the analyst finds the odor of pyridine objectionable. In case of speciation, the sample must be passed through a column in order to separate MMA, DMA, and inorganic arsenic. Then these are estimated by spectro-photometer using the procedure same as total arsenic.

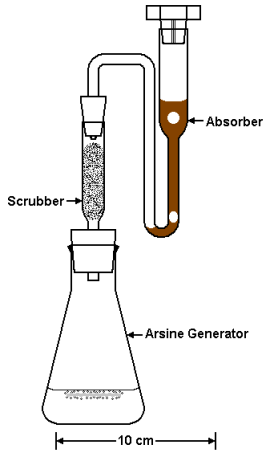

Figure 6.4 *Schematic diagram of arsine generator.*

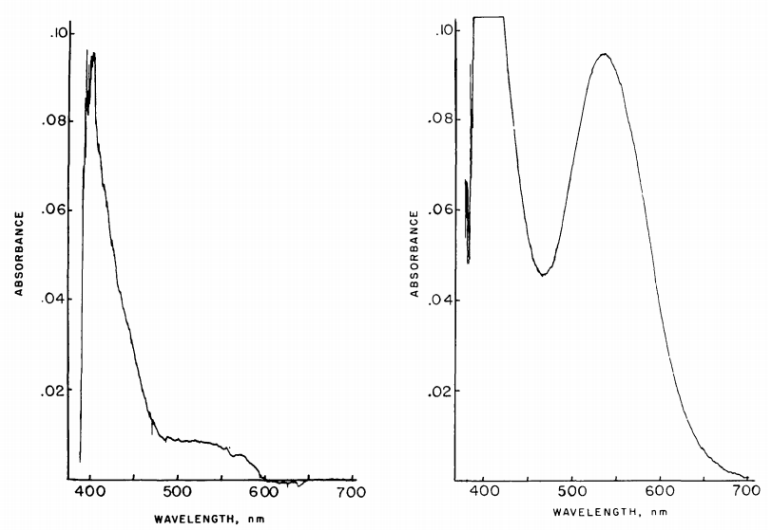

Figure 6.5 *Absorbance spectrum of reagent blank (left) and arsenic (2 µg; right) recorded against a SDDC method using spectrophotometer.*

Advantage: The limit of detection of total arsenic in water using SDDC is 10 ppb.

Disadvantages: Several metal ions-chromium, copper, mercury, molybdenum and antimony may interfere in the determination of arsenic in water. It is more expensive than Gutzeit's method but cheaper than HG-AAS or HPLC-HG-AFS.

6.5 Atomic Absorption Spectrometer

Arsenic can be estimated by atomic absorption spectrometer (flame, with hydride generator or graphite furnase). Usually atomic absorption spectrometer (flame) is not recommended for arsenic estimation. Atomic absorption spectrometer (graphite furnase) is better than atomic absorption spectrometer (flame). Atomic absorption spectrometer (with hydride generator) is the most sensitive method among these.

Principle: This procedure is used for the quantitative determination of arsenic employing the absorption of optical radiation (light) by free atoms in the gaseous state (Figure 6.6). A sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law.

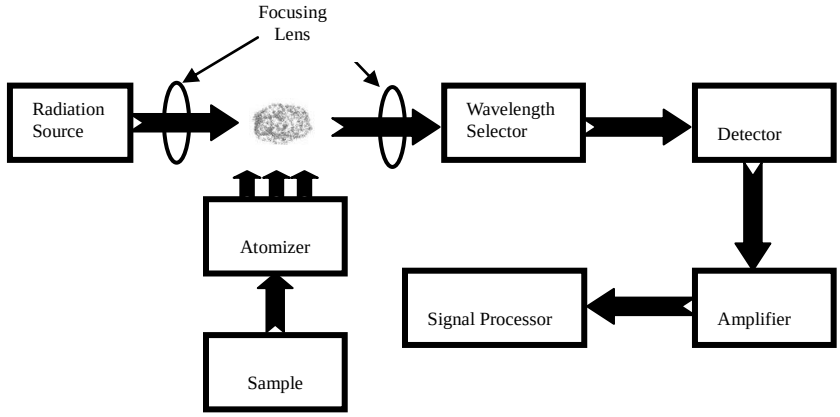

Figure 6.6 *Schematic diagram of atomic absorption spectrometer.*

Advantages: It is more sensitive than SDDC method.

Disadvantages: It is less sensitive than atomic absorption spectrometer hydride generator. Flame atomizer is not recommended for estimation of arsenic. However, graphite furnace spectrophotometer may be used.

6.6 Hydride Generation Atomic Absorption Spectrometer (HG-AAS)

Principle: HG-AASinduces the reaction of sodium borohydride with reduced arsenic species (As^{3+}) to produce volatile hydrides which are purged from solution and detected by spectrometry.

Advantages: The limit of detection is 0.02 ppb. It is more sensitive than atomic absorption spectrometer (flame).

Disadvantages: Speciation of arsenic cannot be estimated by this method. In addition, the complexity of the sample matrix can alter the efficiency of the reduction procedure or the hydride generation reaction. The interferences from transition metals, dissolved organic carbon, and salinity are very well documented and allow for significant biases associated with complex matrices, especially at trace levels.

6.7 Voltammetric Stripping Technique

Voltammetric stripping technique is useful for on-site analysis, providing accurate measurements of low concentrations with rapid analysis times and low cost/weight instruments (Feeney & Kounaves, 2002). It provides an effective and reliable method to analyze arsenic at the ppb levels found in drinking water. The method involves cathodic or anodic stripping voltammetry using a pulsed

wave form.

6.8 HPLC-HG-AFS

HPLC-HG-AFS method uses ion-exchange liquid chromatography coupled on-line to atomic fluorescence spectrometry through continuous hydride generation. HPLC is used for chromatographic separation of speciation of arsenic compounds (As^{III}, As^{V}, MMA, DMA) (Figure 6.7).

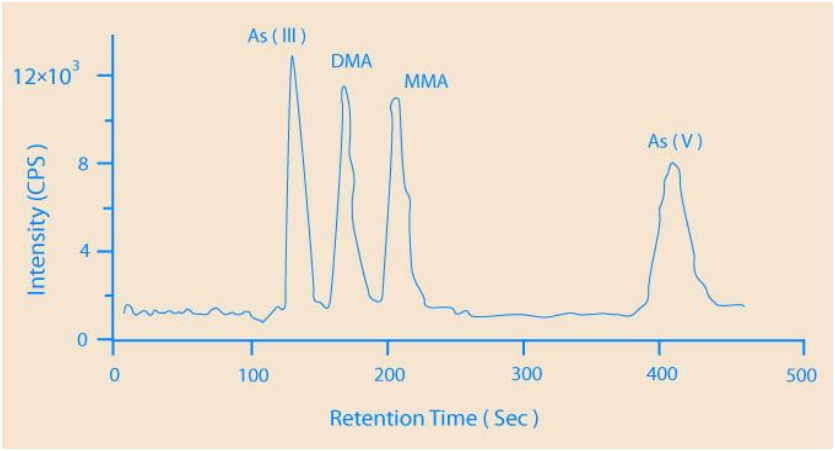

Figure 6.7 *Speciation of arsenic using HPLC-HG-AFS (Le, 2001).*

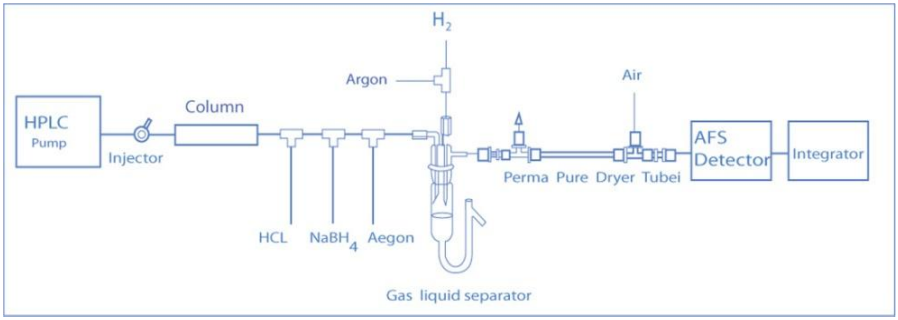

Figure 6.8 *Diagrammatic scheme of HPLC-HG-AFS (PS Analytical application note 024).*

Procedure: Sample containing arsenic is injected into the HPLC system containing strong anion exchange columns and a gradient is performed by changing from 10 mM to 60 mM phosphate buffer solution (Figure 6.8). Hydrochloric acid and $NaHB_4$ are added by peristaltic pump for reaction with arsenic for hydride generation. The flow rate of the mobile phase is 1 mL/min. The volatile arsine is produced which is carried by argon gas to the gas liquid separator and finally to the atomic fluorescence detector.

Advantages: The limit of detection is as low as 0.1 ng for arsenic and its metabolites. All compounds are detected within 10 min after injection of the sample into the HPLC pump.

6.9 ICP-MS

The inductively coupled plasma mass spectrometry (ICP-MS) is a technique that combines a high-temperature (95,000-10,000 K at atmospheric pressure) inductively coupled plasma source with a mass spectrometer. It allows the analyst to identify and quantify the multiple elements including arsenic at high speed. The technique was commercially introduced in 1983. An aerosol of the sample is introduced into the plasma source where vaporization, atomization and ionization of the analyte occur nearly simultaneously. Elemental ions are passed into a mass spectrometer.

Procedure: The instrument should be started according to the standard operating procedure. After ~30 min warm-up, tune ICP-MS normally. The performance should be check with default specifications. The peristaltic pump is used to introduce arsenic at a concentration of 10 ng/g in mobile phase directly into the nebulizer (Figure 6.9). One has to ensure that the signal for m/z 75 response is within normal range. The nebulizer tube is connected to a 3-way

tee. Internal standard should be delivered via peristaltic pump with a flow rate of approximately 0.04 mL/min into one port of the tee. The flow from the HPLC column should be connected to the third port of the tee. All connections should use PEEK fittings. ICP-MS is connected with HPLC. The flow (1 mL/min) of HPLC is started. Ensure proper flow and adequate drainage of ICP spray chamber (>1 mL/min). At the sametime check for leaks. Allow time for column and plasma to equilibrate. Set ICP-MS acquisition method for time-resolved collection of m/z 72 and 75 with integration (dwell) times of 0.2 and 0.8 s, respectively, and 1 replicate (read) per point. Analyze a blank (mobile phase only) solution to verify that mobile phase and chromatography vials are arsenic-free. Monitor instrument conditions to ensure operation is stable and within normal functioning range.

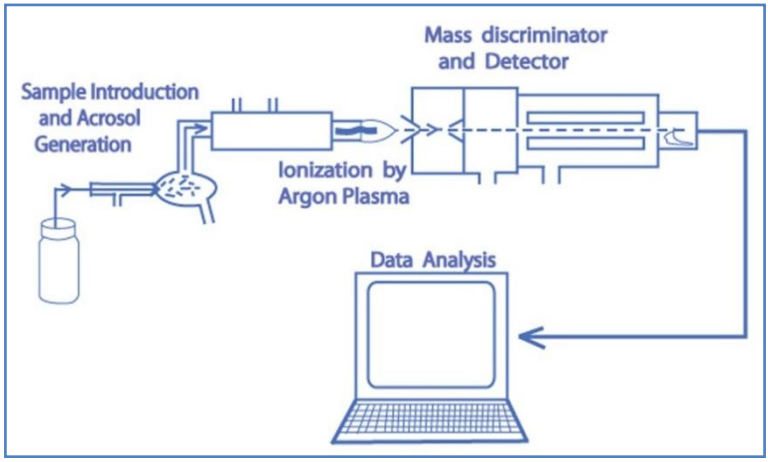

Figure 6.9 *Schematic diagram of ICP-MS.*

Advantages: ICP-MS offers advantages over general speciation analysis including: multi-element and multi-isotope detection (Figure 6.10). The estimation can be done quickly. Detection limit for arsenic is better than that obtained by graphite furnace atomic absorption spectroscopy.

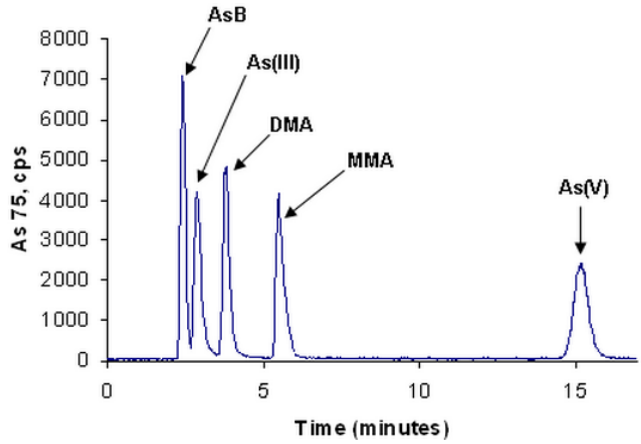

Figure 6.10 *Typical HPLC-ICP-MS chromatogram of an arsenic multianalyte working standard solution (5 ng/g).*

Limitations: This method is costly. Arsenic estimation may have a spectral interference under certain conditions. Argon from plasma gas and chlorine from the sample matrix may combine to form $^{40}Ar^{35}Cl$ which has the same nominal mass-to-charge ratio as arsenic. The monitoring signal at m/z 75 comes from two sources (the arsenic and the argon chloride interference) (B'Hymer & Caruso, 2004). It generally requires a clean room environment for ultra-low detection limit.

References

[1] B'Hymer, C., & Caruso, J. A. (2004). Arsenic and its speciation analysis using high-performance liquid chromatography and inductively coupled mass spectrometry. *Journal of Chromatography*, 1045, 1-13.

[2] Feeney, R., & Kounaves, S. P. (2002). Voltammetric measurement of arsenic in natural waters. *Talanta*, 58(1), 23-31.

[3] Le, C. (2001). Speciation of arsenic in water and biological matrices. American Water Works Association.

[4] Marsh, J. (1836). Account of a method of separating small quantities of arsenic from substances with which it may be mixed. *Edinburgh New Philosophical Journal*, 21, 229-236.

[5] Misbahuddin, M., & Khandker, S.. Drinking water contaminants in Bangladesh: Focuses on arsenic, fluoride, pesticides, manganese and cyanobacteria. LAP LAMBERT Academic Publishing, Germany, 2011.

[6] Nadeau, G. (1952). Gutzeit's arsenic test. *Canadian Medical Association Journal*, 66(5), 489.

[7] Pande, S. P., Deshpande, L. S., & Kaul, S. N. (2001). Laboratory and field assessment of arsenic testing field kits in Bangladesh and West Bengal, India. *Environmental Monitoring Assessment*, 68, 1-18.

[8] Powers, G. W., Martin, R. L., Piehl, F. J., & Griffin, J. M. (1959). Arsenic in naphthas. *Analytical Chemistry*, 31(9), 1589-1593.

[9] Rahman, M. M., Mukherjee, D., Sengupta, M. K., Chowdhury, U. K., Lodh, D. C., Roy, S., & Chakraborti, D. (2002). Effectiveness and reliability of arsenic field testing kits: Are the million dollar screening projects effective or not? *Environmental Science Technology*, 36, 5385-5394.

✶ Myth

Presence of arsenic in drinking water can be detected by immersing guava leaf.