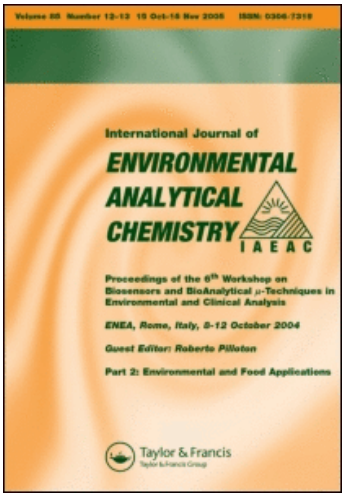

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The Hitachi Graphite Furnace–Zeeman Atomic Absorption Spectrometer as an Automated, Element-Specific Detector for High Pressure Liquid Chromatography:

The Separation of Arsenobetaine, Arsenocholine and Arsenite/Arsenate

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An interface between a high pressure liquid chromatograph and a Hitachi Zeeman graphite furnace atomic absorption spectrophotometer for the element-specific detection of trace element compounds is described. This device was used for the separation of arsenobetaine bromide, arsenocholine bromide, and inorganic arsenic (arsenate/arsenite) from each other.

KEY WORDS: Arsenobetaine, arsenocholine, inorganic arsenic, high pressure liquid chromatography, graphite furnace atomic absorption spectrometry.

INTRODUCTION

One of the prerequisites for an understanding of the harmful as well as the beneficial interactions between metallic or metalloidal trace-elements and biological systems is knowledge of the nature of the trace element compounds to which organisms may be exposed. Equally important is knowledge of the nature of the trace element compounds formed *via* these interactions and of the biochemical pathways utilized in such transfor-

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mations. The experimental techniques required for such investigations must be capable of separation, selective detection, identification and quantitation of trace element compounds with no or minimal chemical change at and below the ppm level in a generally complex matrix.

An atomic absorption spectrophotometer is an element specific detector for a gas-liquid chromatograph is well suited as a tool for the analysis of trace element compounds which can be volatilized.[1,2,3] Certain non-volatile compounds can be reduced to volatile hydrides, which can then be detected.[4] However, many trace element compounds of high molecular mass or of ionic or highly polar character are neither volatile nor easily converted to volatile derivatives.[5,6] High pressure liquid chromatography (HPLC) is the method of choice for the separation of such non-volatile compounds. HPLC is versatile, has high resolving power and does not require chemical modification of the compounds to be separated. However, the commercially available detectors for liquid chromatographs lack either selectivity, sensitivity or both for element-specific operation. Brinckman[7] and Koizumi[8] have demonstrated that a graphite furnace-atomic absorption spectrophotometer (GF-AA) can be used as an element specific detector in liquid chromatography. Brinckman[7] modified a Perkin Elmer Model AS-1 Auto Sampler System to allow on-stream or off-stream analysis of an HPLC effluent. Koizumi's procedure[8] involved the collection of samples off-stream and subsequent manual injections of the sample into the GF-AA.

EXPERIMENTAL AND DISCUSSION

We have developed a low-cost automated interface for the Hitachi Zeeman GF-AA which makes possible the use of this instrument as an element-specific detector for HPLC. The interface consists of an Altex slider injection valve with pneumatic actuators and a 40 μL sample loop, a linear actuator, and a sequence control circuit (SCC). The sample injection valve is mounted downstream from the ultraviolet and the differential refractive index detectors (Figure 1). The valve is switched by nitrogen pressure between the load and inject positions on command from the SCC. The co-analyte delivery pump and the tube through which the nitrogen flows to push the sample into the graphite cuvette are connected to the valve. The sample is introduced into the cuvette through a 1/16 inch O.D. 316 stainless steel tube which is lowered and raised by a linear actuator. All connecting nitrogen and sample lines are 1/16 inch O.D. Teflon tubing. The main components of the SCC are five LM 556 CN monostable adjustable multivibrators cascaded in series. Each one of these

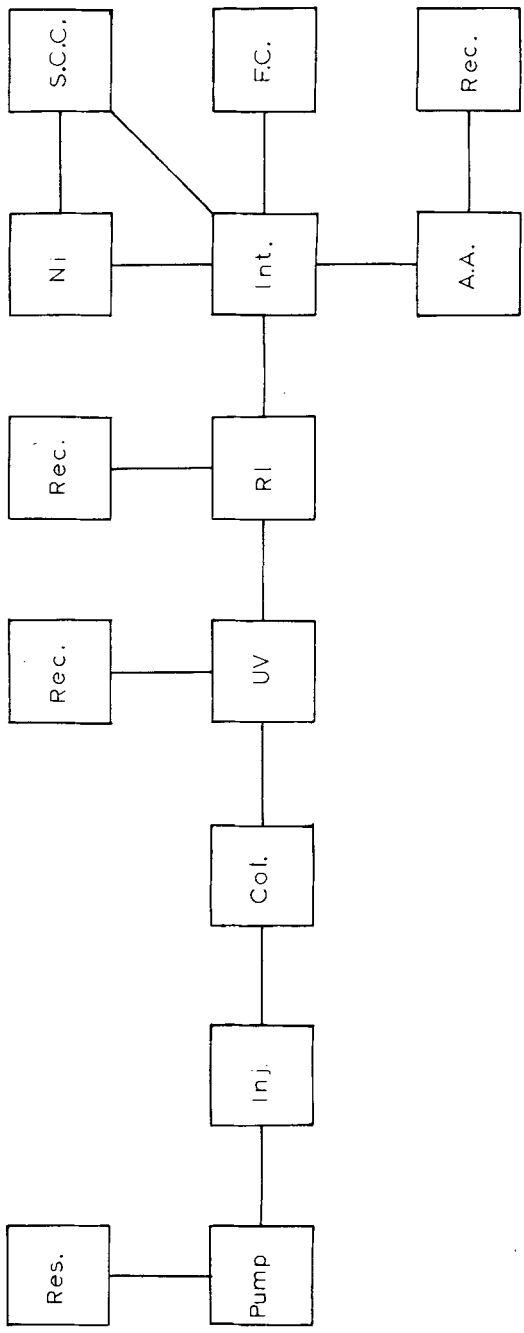

FIGURE 1 Block Diagram for the Graphite Furnace Atomic Absorption Detector for High Pressure Liquid Chromatography. (Res.) Solvent Reservoir; (Pump) L.C. Pump; (Inj.) Sample Injection Valve; (Col.) L.C. Column; (U.V.) Ultraviolet Detector; (RI) Refractive Index Detector; (Int.) Slider Injection Valve for sampling L.C. effluent; (F.C.) Fraction Collector; (A.A.) Graphite Furnace Atomic Absorption Spectrophotometer; (Ni) Ni Reservoir and Pump; (S.C.C.) Sequence Controlling Circuit; (Rec) Recorders for the respective detector.

multivibrators controls two relays which in turn provide the control for the sampling and analysis sequence.

Starting with the GF-AA ready for operation and the effluent flowing through the sample loop of the valve the interface will control the following events: (a) lowering of the sample delivery tube into the graphite cuvette; (b) switching of the sampling valve into the inject position; (c) injection of 40 μL of effluent into the cuvette; (d) switching of the sampling valve into the sampling position; (e) raising the sample delivery tube; (f) starting the GF-AA dry-cycle; (g) termination of the dry-cycle after evaporation of the solvent; (h) injection of the co-analyte if required;[9] (i) initiation of the GF-AA analysis cycle; (j) allowing sufficient time for the cuvette to cool after completion of the analysis; (k) termination of the cool-cycle; and (l) initiation of the next sampling and analysis cycle.

The parts for the interface do not cost more than $300. Although the described interface was developed for the Hitachi Zeeman GF-AA, it is adaptable to other graphite furnace-atomic absorption spectrophotometers. Details about the interface including an electronic schematics can be obtained from the authors.

The applicability of this system to the speciation of organometalloidal trace element compounds is illustrated by the separation of arsenocholine bromide, $[(CH_3)_3AsCH_2CH_2OH]^+Br^-$, arsenobetaine bromide $[(CH_3)_3AsCH_2COOH]^+Br^-$ and inorganic arsenic (arsenite and arsenate) from each other. This mixture of compounds was chosen, because arsenobetaine and arsenocholine, which probably occur in many organisms, cannot be converted to volatile arsenic compounds without drastic chemical changes, which obliterate most of the information about the nature of the original compound. Arsenobetaine was isolated from rock lobsters[10] and arsenocholine is probably the precursor from which arsenobetaine is formed.

Arsenocholine, arsenobetaine and inorganic arsenic were separated on a micro-particulate (10 μ) C-18 reverse phase column with the sodium salts of heptanesulfonic acid (Figure 2) or dodecylbenzenesulfonic acid (Figure 3) as counter ion for the arsonium salts. The eluting solvent, mixtures of acetonitrile, water, and counter ion, is made 1.0 M with respect to acetic acid to suppress the dissociation of the carboxylic acid group in arsenobetaine and achieve greater retention on the reverse phase column. By varying the ratio acetonitrile/water in the eluent the retention volume of arsenocholine can be changed from total retention to co-elution with arsenobetaine. Arsenobetaine cannot be totally retained with heptanesulfonic acid as the counter ion. However, total retention of arsenobetaine on the top of the column was achieved with dodecylbenzenesul-

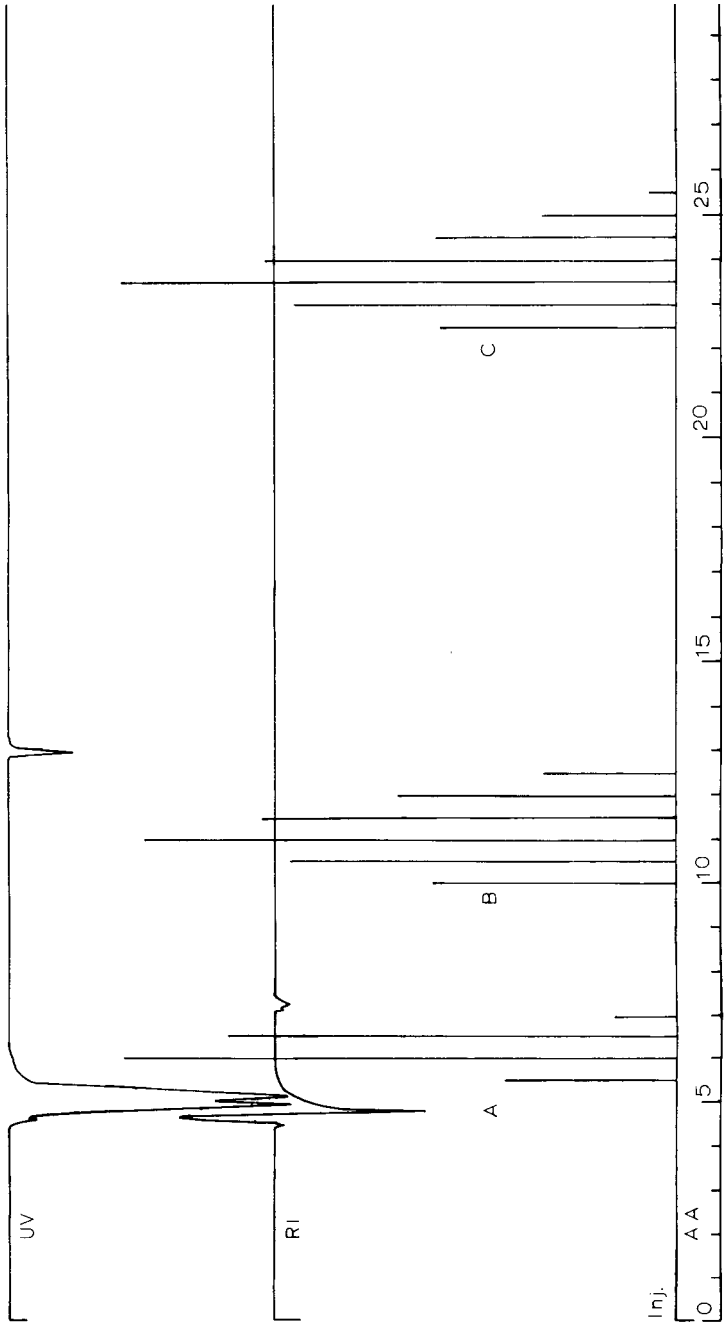

FIGURE 2 Tracing of U.V., R.I., and GF-AA Recordings using 95/5/6 H_2O/Acetonitrile/Acetic Acid and 0.005 M Heptanesulfonic Acid as Solvent. A = 1 μg arsenate/arsenite mixture; B = 1 μg arsenobetaine; C = 1 μg arsenocholine. Flow rate was 0.5 ml/min on a μ-Bondapak (C_{18}) reverse phase column. R.I. attenuation was 4X and the U.V. was 0.1 A.U.F.S.

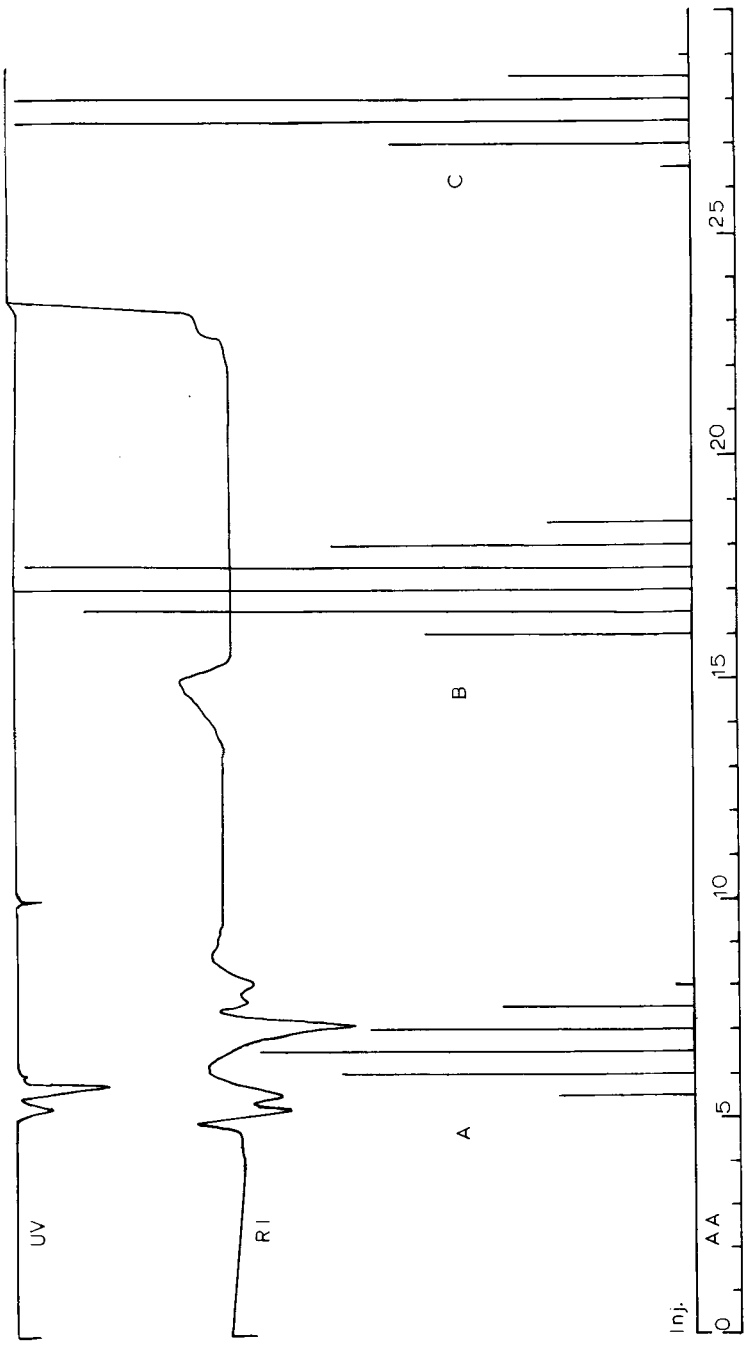

FIGURE 3 Tracing of U.V., R.I., and GF-AA recordings using 2/3/0.6 H_2O/Acetonitrile/Acetic Acid and 0.005 M sodium dodecylbenzenesulfonate. The solvent was changed to Acetonitrile at minute 17. A = 1 μg mixture of Arsenite/Arsenate; B = 1 μg Arsenobetaine; C = 1 μg Arsenocholine. Flow rate was 0.5 ml/min. R.I. attenuation was 4X on a Water's model 202 differential refractometer and the U.V. sensitivity was 0.1 A.U.F.S. on a Model 25 Beckman spectrophotometer.

fonate. The procedure is useful for the pre-concentration of arsenobetaine from very dilute solutions. Arsenite and arsenate were not retained at all with either of the sulfonates on the C-18 reverse phase column and eluted with the solvent front.

Even though arsenocholine and arsenobetaine were recrystallized several times, impurity peaks arising from the solvent and/or the samples are recorded by the refractive index and U.V. detectors. As shown in Figure 2, the GF-AA responds to the arsenic compounds even though there is insufficient response by R.I. or U.V. detectors. The GF-AA detector shows signals corresponding only to the arsenic containing compounds. The retention times for and the quantities of these compounds present in the mixture can be obtained from the signals. No peaks other than those indicating the presence of arsenic compounds will appear in the chromatogram facilitating the quantitation and identification of arsenic compounds even in complex matrices which would produce a large number of R.I. and U.V. signals. Figure 3 demonstrates this in the selective detection of arsenate/arsenite in the complex signal output from the impurities detected by the R.I. and U.V. detectors.

The interface together with HPLC-GFAA system with its ability to correct for high background will be employed to identify the organic arsenic compounds occurring in various organisms.

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