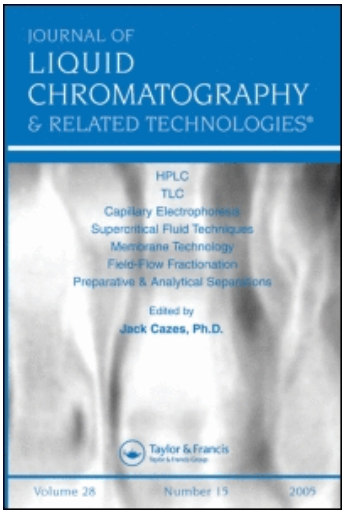

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HPLC Determination of Iodide in Scrum Using Paired Ion Chromatography with Electrochemical Detection

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HPLC DETERMINATION OF IODIDE IN SERUM USING PAIRED ION CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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Abstract

HPLC utilizing paired ion chromatography with electrochemical detection is used for the determination of iodide in serum. Serum samples are prepared by precipitation of protein, centrifugation and removal of interfering substances with a bonded phase column. The resulting sample is then analyzed. The method has successfully been applied to human serum and gives data that agrees well with values obtained by other methods. The method is accurate precise, and time conservative when compared to more classical methods.

Introduction

Serum iodide determinations are done primarily to evaluate thyroid function in man and animals. The thyroid gland utilizes inorganic iodide and protein to form organic compounds with some hormonal activity. Increased thyroid activity may be associated with elevated serum iodide levels while decreased activity results in lower levels. Additionally if a subject has been exposed to any of a number of environmental or medical sources of iodide elevated serum iodide levels will result. Elevated serum iodide levels can interfere with diagnostic tests of thyroid function. Some of these agents include radiopaque dyes used for various

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diagnostic procedures and Lugol's solution. Exposure to both of these agents will result in elevated iodide levels.(1)

Existing methods for the determination of serum iodide entail the precipitation of the protein bound iodide and a digestion of the residue by dry ashing or wet digestion.(2,3) All of these methods require extensive sample preparation, specialized equipment or extended periods of time prior to the final determination. The method described in this paper involves simple sample preparation. The final HPLC determination step is rapid and accurate. An analyst can prepare and analyze about 25-30 samples in an eight hour day.

Materials and Methods

Sample preparation: Samples of serum were placed in test tubes and diluted 1:1 with distilled water and mixed for 30 seconds. Eight ml of acetonitrile was added to the test tubes and they were mixed again. After this they were placed in a centrifuge for 10 minutes at 2000 rpms, the supernatant withdrawn and placed on previously prepared columns.

Column preparation: Sample columns were purchased from Analytichem, International and consisted of an amino bonded phase packed in 2.8 ml capacity polyethylene columns. They were prepared for use by first rinsing with 3 ml of CH_3CN followed by 3 ml of distilled water.

Sample clean-up: The previously prepared samples were placed onto the clean-up columns. After all of the supernatant was allowed to pass through the columns, the iodide containing fraction was eluted with 1.0 ml of 0.2N KH_2PO_4.

HPLC: All analyses were performed on a high performance liquid chromatograph system. The solvent delivery system was a M590 pump (Waters Associates). Samples were injected using a Model 7125 Loop Injector (Rheodyne) or a Model 710B WISP (Waters Associates). The HPLC detector used was a Model LC-4B Amperometric Detector equipped with a Ag electrode (Bioanalytical Systems) operated at + 0.01 volts. The column temperature was maintained at 25°C. The HPLC column used was a 3.9 mm I.D. x 30 cm long column packed with 10μ Spherisorb ODS (HPLC Technology).

The mobile phase was previously used for the determination of iodide in milk (4) and consisted of 12.78 g of Na_2HPO_4, 3.2 g of hexadecyltrimethyl ammonium chloride, 1.4 g of Na_2EDTA added to 2.8 ℓ of HPLC grade water. This mixture was combined with 1 ℓof HPLC grade acetonitrile, mixed and thoroughly degassed.

The pH of the resulting mobile phase was then adjusted to 6.8 (± 0.1) with 85% H_3PO_4. The mobile phase was flowing at 2.0 ml/min. The iodide standard was ultrapure potassium iodide that was prepared as a stock solution containing 1.0 ng of iodide per µl.(Alfa).

Analysis

Fifty microliter quantities of a diluted iodide standard (.04 ng/µl) were injected in duplicate at an instrument setting of 2 nafs (nanoamps full scale). Varying portions of sample ranging from 5 to 100 µl were injected depending on the concentration of iodide in the sample. The peak height of samples were compared to peak height of standards to arrive at the final iodide concentration.

Results

The methodology described in this communication was evaluated through the use of accuracy and precision studies. The dilute iodide standard (.04 ng/µl) was injected ten times and a pooled serum extract was also injected ten times to arrive at precision data. These data are summarized in Table 1.

Measured amounts of iodide standard were added to a pooled serum sample. This standard was added prior to any sample cleanup and therefore, was subjected to the same conditions as were the samples. These data are summarized in Table 2.

Table 1

Iodide Precision Study

Matrix	Conc.	%Cv
Iodide Standard	2 ng	0.7
Serum Extract	4.3 µg/dl	1.4

Table 2

n = 3

Recovery of Added Iodide to Pooled Serum

Amt. Iodide Added (µg/dl)	Amt. Iodide Recovered (µg/dl)	% Recovery
0	4.30	-
0.5	4.77	94.0
1.0	5.21	91.0
1.5	572	94.4

The results indicate an average recovery of 93.1% for triplicate recovery studies. Based on twice the S/N ratio with all other conditions remaining the same this method has a lower limit detection of 0.4 µg/dl.

Varying concentrations of iodide were injected in duplicate over a 200 fold range from 500 pg to 100 ng in increments ranging from 1-10 ng. Data points correlated well linearly with a regression coefficient of 0.988.

To arrive at an optimum applied Eo for use in this study, the same concentration of iodide was injected at Eo (applied) ranging from 0 volts to + 0.150 volts. The results indicate that as applied voltage increases peak height decreases. The applied Eo of 0 volts gave a maximum peak height but the noise was excessive therefore, +0.010 volts was used as a compromise between noise and detector response. Over the range from 0 to +0.150 volts a decrease in peak height of about 30% was seen.

A particular advantage of the method results from the combination of the Ag electrode and the applied voltage which allows the detector to be selective for iodide and ignore possible anions. Figure 1 shows several anions using UV detection at 210 nm while Figure 2 shows the same anions using the Ag electrode. Figure 3 shows the chromatogram for a human serum extract.

Finally, Table 3 outlines some data obtained from the serum of six subjects.

Pooled serum samples were run everyday for a two week period to also evaluate the method's precision. These data indicated an average concentration of 4.51 µg/dl with a variance of 0.17 µg/dl and a coefficient of variation of 3.6%

These serum iodide values show good agreement with the literature values from several sources which indicate the values for serum iodide range from 3 to 8 µg/dl.(5)

Discussion

All of the current clinical procedures involve the determination of Protein Bound Iodine (PBI). It is therefore necessary to convert the iodine to its inorganic form by either wet or dry ashing with the final determination based on the catalytic activity of iodide (Kolthoff-Sandell reaction). This is a redox reaction between cerium (IV) and arsenic (III) which is catalyzed by iodide. If all parameters are kept constant, the concentration of iodide is proportional to the change in absorbance at 420 nm. This assay is not without problems due to its

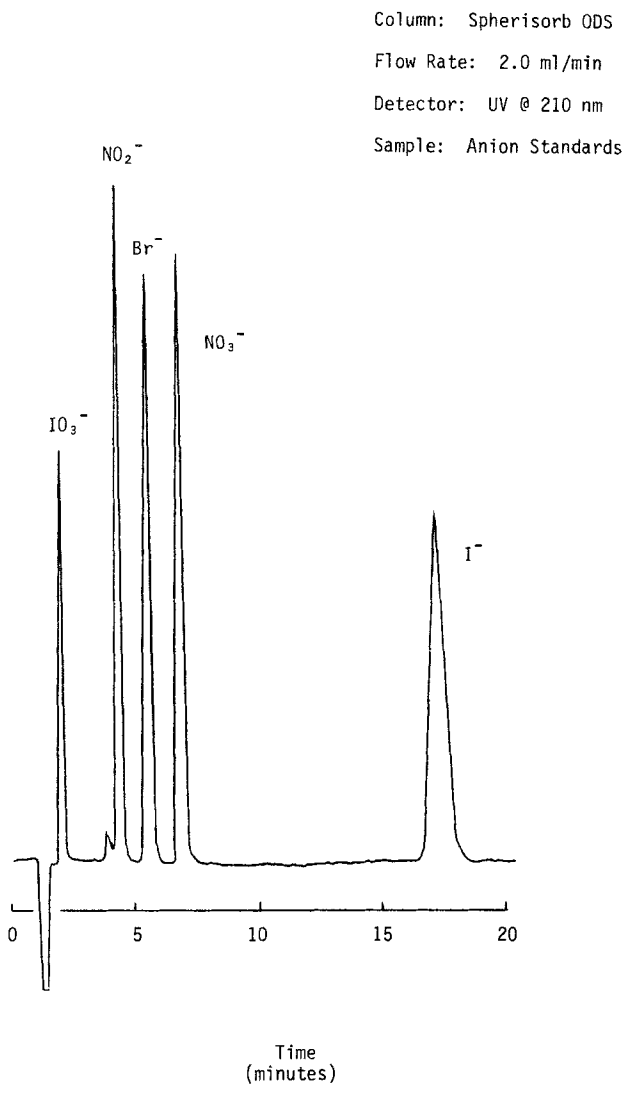

Figure 1
Chromatogram of Anion Standards with UV Detection

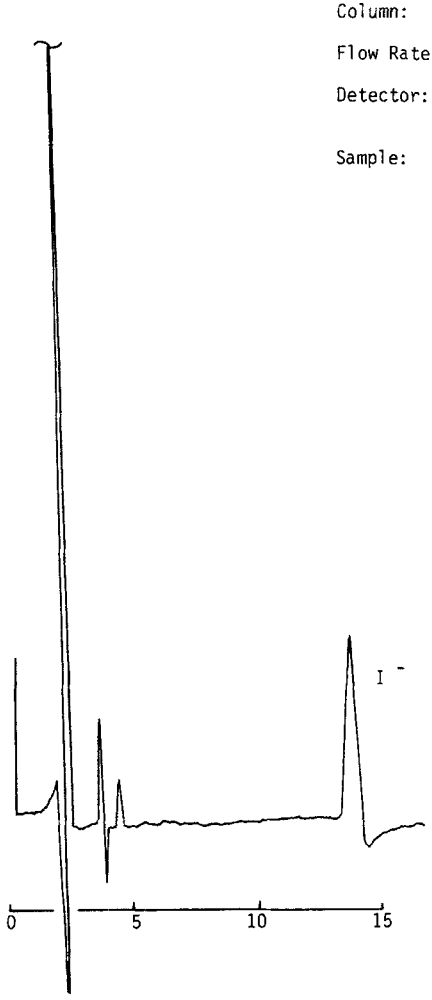

Figure 2

Chromatogram of Standards with Electrochemical Detection

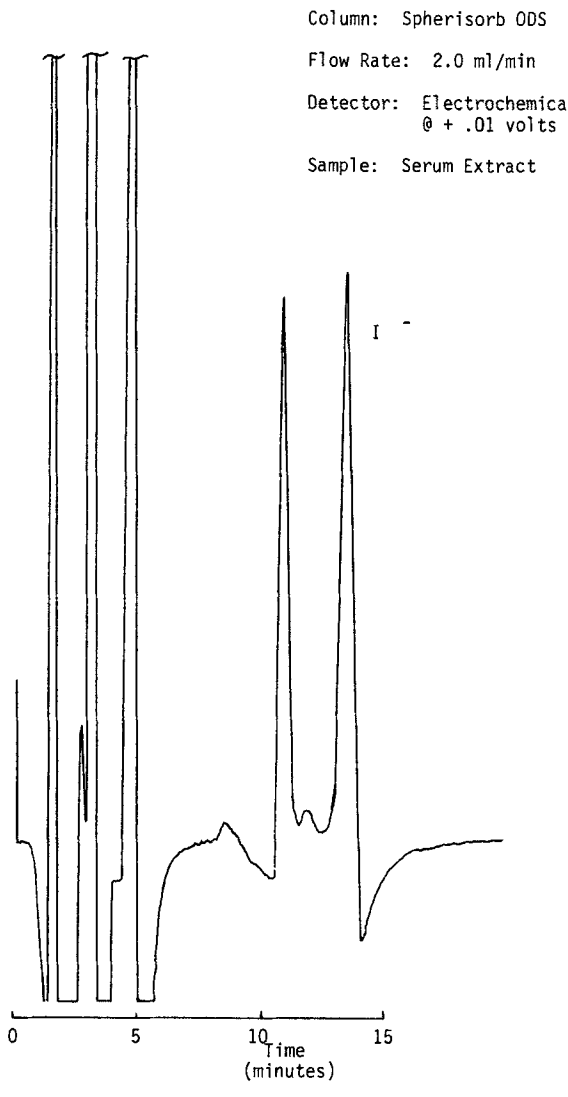

Figure 3

Chromatogram of Serum Extract

Table 3

Serum Iodide Concentration of Six Subjects

Subject	Iodide Conc. (µg/dl
1	10.41
2	8.30
3	9.42
4	11.72
5	7.10
6	6.54

catalytic nature. All parameters must be kept constant or erroneous data can result. Additionally, the entire detection system cannot come in contact with metals since any contamination will reduce the cerium (IV) and cause a decolorization of the solution.(6)

In some cases, it is not desirable to analyze serum for total iodide but to analyze for butanol-extractable iodine (BEI) which eliminates interferences. This procedure involves the extraction of an acidified serum with butanol and a back extraction of the butanol with Blau reagent to eliminate interfering substances.(3,4) The butanol fraction is removed by evaporation and the extract is then digested prior to its final determination. Preliminary studies in our laboratory indicate that this assay can be accomplished by extraction of the serum with butanol, removal of the butanol followed by the addition of buffer prior to final HPLC determination.

The concept of ion chromatography using a variety of detectors, mobile phases and supports has been well documented.(7,8) The present study demonstrates that the use of a silver electrode provides a selective and sensitive surface for the detection of iodide and for the determination of serum iodide. The results indicate that there is little contamination of the electrode surface after long periods of use. In our laboratory the electrode was used for over 600 injections of standard and sample before cleaning. Even at that point, the cleaning consisted of repolishing the electrode with alumina and washing with water and methanol. The electrode was then reassembled and used. A precolumn was used prior to the

analytical column and was packed with large particle C_{18}.(9) This seems to add to column and electrode longevity. The column was used for the same number of injections and showed no deterioration using either peak height or column plates. The disodium EDTA was added to the mobile phase to passivate the system and is recommended by the manufacture of the detector.(10) Reduction in the baseline noise before and after the addition of this compound supports this observation. Additional noise reduction was accomplished by temperature control.

The procedure described provides an attractive alternative for the determination of iodide in serum and was successfully applied to determination of serum iodide in other species. These data will be published elsewhere.

The method described eliminates many of the problems associated with this assay. It seems as if the addition of water and acetonitrile serves to liberate the iodide and reinforce the 1948 study of Taurog and Charkoff who reported that iodide in plasma is not stably bound to protein.(11)

The data presented indicate that the method described provides a useful alternative to the current methods for the determination of serum iodide. It is accurate and precise with excellent recovery of iodide. Iodide determinations are routinely not done by most laboratories due to the complexity of the assay. This assay would permit serum iodide determinations to become feasible in laboratories with HPLC equipment.

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