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Short communication

Rapid and specific high-performance liquid chromatographic method for the determination of iodide in urine

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Abstract

A rapid and specific method for the determination of iodide in urine by high-performance liquid chromatography on an anion-exchange column with electrochemical detection is described. The assay is reproducible as judged by the coefficient of variation of less than 4% at all concentrations used. The limit of detection was 0.1 μmol, and the calibration graph was linear for concentrations between 0.1 and 200 μmol. Using this method, healthy volunteers were found to excrete 69 ± 39 μmol of iodide per mole of creatinine.

1. Introduction

The determination of iodide in human biological samples has become a widely accepted tool in investigations concerning thyroid function and particularly in the monitoring of patients with excess iodine-induced hyperthyrosis, but little work has been done in this field. In a previous paper we described a procedure using a crystalline membrane specific electrode [1]. A number of high-performance liquid chromatographic methods utilizing ion-pairs chromatography with UV [2] or electrochemical detection [3,4] have been proposed. All of them are time consuming and involve a sample preparation step. Turzo et al. [5] have described a urinary iodide assay based on X-ray fluorescence spectrometry. However, although this technique may be very useful for fundamental research, it is not suitable in routine practice because specialized facilities are needed. This paper outlines a rapid, selective and sensitive HPLC method for the determination of iodide in urine that does not suffer from the above limitations.

2. Experimental

2.1. Instrumentation

The HPLC system consisted of a Dionex (Sunnyvale, CA, USA) QIC analyser equipped with a DQP-1 high-pressure pump, a 50-μl loop injector and a ECD amperometric detector (Dionex), with a silver working electrode and silver/silver chloride reference electrode.

A Dionex AG4A anion precolumn (50×4 mm I.D.) was used and the separation was achieved with a Dionex IonPac AS-4A pellicular anion-exchange column (250×4 mm I.D.). A Hitachi

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(Tokyo, Japan) D-2500 chromato-integrator was used for data acquisition.

2.2. Chemicals

Sodium iodide (analytical-reagent grade), acetonitrile (gradient grade), sodium hydrogencarbonate and anhydrous sodium carbonate (analytical-reagent grade) were purchased from Merck (Darmstadt, Germany) and 4-cyanophenol (analytical-reagent grade) from Janssen (Beerse, Belgium). Water was purified through a Milli RO and Milli Q system (Millipore, Molsheim, France).

A stock standard solution of iodide (0.1 *M*) was prepared by dissolving 1.5 g of sodium iodide in 100 ml of deionized water. This stock standard solution was stored at 4°C and remained stable for approximately 3 months. Working standard solutions were prepared just prior to use by diluting the stock standard solution with deionized water to yield final concentrations of 0.5, 5, 10, 50 and 100 μM.

2.3. Analytical procedures

The HPLC system was conditioned with a mobile phase consisting of 4.3 m*M* sodium hydrogenphosphate, 3.4 m*M* anhydrous sodium carbonate and 0.8 m*M* 4-cyanophenol at a flow-rate of 1.0 ml/min at room temperature. Prior to use, the mobile phase was filtered through a 0.45-μm MF filter (Millipore).

Amperometric detection was used with an applied potential of +200 mV (vs. Ag/AgCl) and an output range of 30 nA/V.

2.4. Sample preparation

To 1.0 ml of urine in a 15-ml screw-capped centrifuge tube were added 1 ml of acetonitrile and 3 ml of deionized water. The tubes were vortex mixed for 20 s and centrifuged at 2000 *g* for 10 min. A 50-μl volume of the supernatant was injected directly into the chromatographic system.

The same procedure was used for the preparation of 0.5, 5, 10, 50 and 100 μM calibration standards, by replacing urine with 1 ml of working standard solution.

2.5. Calculations

The concentration of iodide in urine was calculated by determining the chromatographic peak area and comparing this area with a calibration graph prepared daily utilizing two replicates at each concentration (0.5, 5, 10, 50 and 100 μM).

2.6. Creatinine determination

Urinary creatinine was determined using the kinetic Jaffé reaction [6].

3. Results and discussion

3.1. High-performance liquid chromatography

Typical chromatograms of a 10 μM iodide standard and urine from a patient with excess iodine-induced hyperthyrosis are shown in Fig. 1. The retention time for iodide was 6.1 min. The peak shapes were symmetrical, with no evidence of tailing. The detector response was

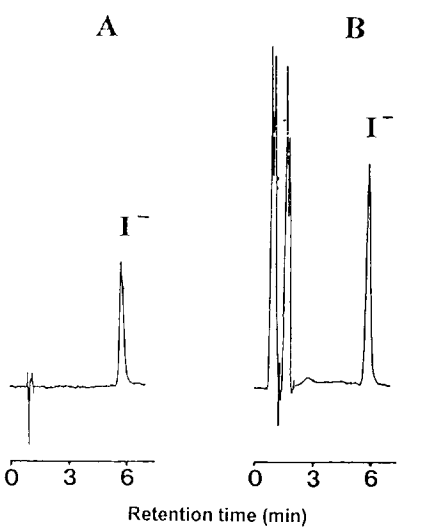

Fig. 1. Typical chromatograms of (A) a 10 μM iodide standard and (B) a twenty-fold diluted urine sample from a patient with excess iodine-induced hyperthyrosis.

Table 1
Retention times of compounds tested for interference

Compound	Retention time (min)
Iodide	6.13
Bromide	1.62
Chloride	1.04
Fluoride	4.03
Ascorbic acid	1.28
Oxalate	N.D.[a]
Monometallic phosphate	2.82
Dimetallic phosphate	2.21
Acetonitrile	N.D.
Sulfide	2.28
Sulfate	N.D.
Nitrite	N.D.
Creatinine	N.D.
Urea	N.D.

[a] N.D. = not detectable.

linear over the range 0.1–200 μM. At a required signal-to-noise ratio of greater than 5 : 1, the minimum detectable concentration of iodide in urine was 0.1 μM. During the investigation of applied potentials, it was found that an applied potential of +250 mV could afford the best sensitivity. Unfortunately, when using this potential with urine, an endogenous compound (not determined) interfered with iodide. The best results, taking into account sensitivity and specificity, were obtained with an applied potential of +200 mV.

3.2. Specificity

Thiocyanate ion has been identified as a substance interfering in numerous methods used for the measurement of urinary iodide [1,7,8]. When using an iodide-selective electrode, significant and increasing errors may be observed with chloride [9]. A recent study proposed the elimination of chlorides before the assay [10], but did not bring any solution to the problem of other interfering ions such as sulfides or thiocyanates. Ascorbic acid has also been described as a serious interferent in urinary iodide determinations, based on the ceric–arsenious acid reaction [11]. This lack of specificity, which seems to be the main problem in iodide determination, led us to test several compounds for potential interference. Table 1, which summarizes the retention times of these substances, indicates that no interference was observed.

3.3. Stability

The stability of iodides was determined by measuring the concentration changes in iodide solutions and in urine with time. The stock standard solution of iodide ($0.1M$) was stable for at least 3 months when stored in the dark at 4°C. For working standard solutions and urine samples, no significant stability problems were observed after storage for 2 weeks at +4°C, as the iodide concentration changed by less than 5%.

3.4. Recovery and precision

When recovery tests were carried out by adding iodide (10, 50 and 100 μM) to a pooled urine sample, the recoveries were 99.4, 98.9 and 99.8%, respectively.

Within-day and between-day precisions of the

Table 2
Precision of the assay ($n = 5$)

Sample[a]	Within-day assay		Between-day assay	
	Mean (μmol)	C.V. (%)	Mean (μmol)	C.V. (%)
a	0.47	2.1	0.45	3.2
b	61.35	2.3	60.51	3.4
c	106.29	2.8	105.47	3.0

[a] Sample a was a urine sample from a healthy subject. Samples b and c were obtained from two different patients with excess iodine-induced hyperthyrosis.

method were determined by repeated analyses of three urine samples five times a day for five consecutive days. These data are summarized in Table 2.

3.5. Application of the method to human urine

Because of the physiologically variable concentrations of urine, the results are to be expressed either per 24h or per mole of creatinine. Taking into account that most of the patients whose urine will be analysed by this assay are ambulatory, the iodide-to-creatinine ratio is certainly the most suitable mode of expression of the results. This is supported by the fact that when expressed per mole of creatinine, iodide excretion is independent of sex [4].

For quality control of the method, we analysed 1-ml aliquots of three urine samples with low, medium and high iodide concentrations. These quality control samples were stored frozen, then thawed and assayed in duplicate with each set of ten samples analysed.

The method described was used to assay iodide concentrations in urine samples from 30 human volunteers aged from 30 to 45 years, all apparently healthy subjects. The values found were 69 ± 39 μmol of iodide per mole of creatinine. These results are in good agreement with literature data [4]. Throughout our clinical studies, a 10–100-fold increase in urinary iodide values was observed in patients with excess iodine-induced hyperthyrosis.

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