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Determination of iodide in serum and urine by ion-pair reversed-phase high-performance liquid chromatography with coulometric detection

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Abstract

An HPLC method is described for the determination of iodide in serum and urine using ion-pair chromatography with coulometric detection. After adding hexadecyltrimethylammonium chloride, the ions pairs formed with the iodide in the sample are extracted using an organic solvent. The solvent is then evaporated and the dry residue obtained is mixed with an appropriate volume of mobile phase so as to concentrate the sample prior to injection into the chromatograph. For a sample of 0.5 ml of serum, the method features a limit of detection (signal-to-noise ratio of 3) of 0.2 $\mu g\,l^{-1}$, sufficient to be applied in paediatric assays for the diagnosis of both iodide deficiency and excess.

1. Introduction

In mammals, inorganic iodine is essential for the biosynthesis of thyroid hormones [1]. Determination of iodide in biological media is therefore a requisite for any metabolic, nutritional or epidemiological study in this area [1–3]. In addition, the determination of iodide in serum and/or urine is useful for the clinical diagnosis of transient thyroid dysfunction [4–8]. With infants, where iodide-induced cases of hypothyroidism are frequently encountered [9–12], urine collection is not always easy and therefore blood assay is preferable. However, as blood sampling in paediatrics is limited to a few microlitres, the assay method must be sufficiently sensitive.

Several methods have been described for the determination of iodide in biological fluids, among which the best known is the spectrophotometric assay based on the Sandell and Kolthoff reaction [13]. Although this method exhibits the required sensitivity, it includes extensive sample preparation and suffers from several potential sources of interference. Methods with neutron activation analysis (NAA) [14] also possess the required sensitivity but are not

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suitable for routine practice. Hurst et al. [15] proposed a high-performance liquid chromatographic (HPLC) method using ion-pair chromatography with electrochemical detection. Although apparently simpler and more practical than the preceding methods, its limit of detection of 4 $\mu g l^{-1}$ falls short of the sensitivity required for paediatric applications. Buchberger [16] reported a method for the determination of iodide by ion chromatography with post-column reaction detection. However, this method still requires 2 ml of serum sample.

In this paper, we describe a method for determining iodide in serum by HPLC coupled with coulometric detection that is sufficiently sensitive for paediatric applications. It requires only 0.05–0.10 ml of serum sample for the diagnosis of excess iodide and a maximum of 0.50 ml of sample for diagnosis of hypoiodaemia. This method has also been applied to the determination of iodide in urine.

2. Experimental

2.1. Blood and urine samples

Blood and urine samples (venous blood collected in a dry tube and 24-h urine) were residues of tests made during pre-operative examinations of children hospitalized in the otolaryngology ward of the Trousseau Hospital for tonsillectomy or adenoidectomy.

2.2. Reagents

The following reagents were used without prior purification: Na_2HPO_4, potassium iodide (KI) and acetonitrile from Merck (Darmstadt, Germany), hexadecyltrimethylammonium chloride (HDTA) from Kodak (Paris, France) and orthophosphoric acid from Prolabo (Paris, France).

2.3. Apparatus and columns

The chromatograph featured an Isochrom LC pump (Spectra-Physics, Courtaboeuf, France) connected to a Rheodyne Model 7125 injector equipped with a 100-μl loop injector. The ODS Hypersil 5-μm column (250 × 4.6 mm I.D.) from Spectra-Physics is protected by a PSF 25 C_{18} 5-μm precolumn (SFCC Shandon, Eragny, France). Coulometric detection was carried out with a Model 5011 cell driven by a Coulochem 5100A module (ESA, Cergy-St. Christophe, France) connected to a C-R1B Chromatopak integrator (Shimadzu). During our tests we also used a Model 400 electrochemical detector (EGG Princeton Applied Research, Ivry, France).

The mobile phase consisted of water–acetonitrile (65:35, v/v) containing 0.023 *M* Na_2HPO_4 and 1.3 m*M* HDTA. The pH of the mobile phase was adjusted to 6.8 ± 0.1 with phosphoric acid. Sep-Pak C_{18} cartridges (Millipore Waters, St. Quentin-en-Yvelines, France) were used to clean up the samples prior to injection into the chromatograph.

2.4. Calibration

The calibration scale for the determination of iodide in serum was prepared from 2 mg/l^{-1} of an aqueous solution of KI (KI_s) and a pool of normal sera (taken from volunteers in our laboratory). The calibration graph was made up of four points: (i) point 0 = serum–water (9.50:0.50, v/v); (ii) point 1 = serum–KI_s (9.50:0.50, v/v); (iii) point 2 = serum–KI_s–water (9.50:0.25:0.25, v/v/v); (iv) point 3 = serum–KI_s–water (9.50:0.10:0.40, v/v/v).

For the assay of iodide in urine, the same range of concentrations was used with doubly distilled water instead of serum.

2.5. Sample preparation

According to the case, samples of serum, urine or range point (0.050–0.500 ml of serum; 0.020 ml of urine; 0.100 ml of calibration sample) were placed in test-tubes and 1 ml of acetonitrile was added before mixing for 2 min. After 10 min of centrifugation at 5000 g, the supernatant was collected into a clean tube and 0.500 ml of an aqueous solution of HDTA was added to 0.500 g

l^{-1} and mixed for 2 min. The ion pairs thus formed were extracted using 2 ml of dichloromethane after 20 min of mixing. After centrifugation at 5000 *g* for 10 min, the organic phase was withdrawn and evaporated to dryness under a stream of nitrogen. The residue was mixed with 2×1.0 ml of dichloromethane and purified by passage twice in 1-ml portions through a Sep-Pak C_{18} cartridge prepared in advance by soaking it in a solution of dichloromethane for 24 h. Lastly, the 2 ml of organic eluate were mixed before being evaporated to dryness under a stream of nitrogen. The dry residue was then mixed with 0.120 ml of mobile phase and 0.100 ml were injected into the chromatograph.

3. Results

Fig. 1A represents the linear voltammogram for an aqueous solution of iodide obtained after direct injection (without column) into the coulometric cell. This voltammogram features two oxidation waves. Fig. 1B shows the cyclic voltammogram for the oxidation of the iodide obtained after direct injection into the amperometric detection cell of the Model 400 EGG electrochemical detector. These results indicate that (i) the oxidation mechanism is identical for the two types of working electrodes used, making allowances for the difference in potential (different reference electrodes and scanning conditions); and (ii) the two oxidation waves for the iodides are reversible.

Figs. 2 and 3 show the chromatographic profiles of serum and urine obtained by the proposed method. The identity and purity tests of the iodide peak are based on its capacity factor and hydrodynamic voltammogram.

During coulometric detection, the best signal-to-noise ratio was obtained at an applied potential of 0.65 V vs. a palladium reference electrode. Under these conditions, the detection limit of the method, for a signal-to-noise ratio of 3 and a serum sample of 0.500 ml, is 0.2 $\mu g\ l^{-1}$ of iodide.

The method is linear from 1.50 to 152 $\mu g\ l^{-1}$

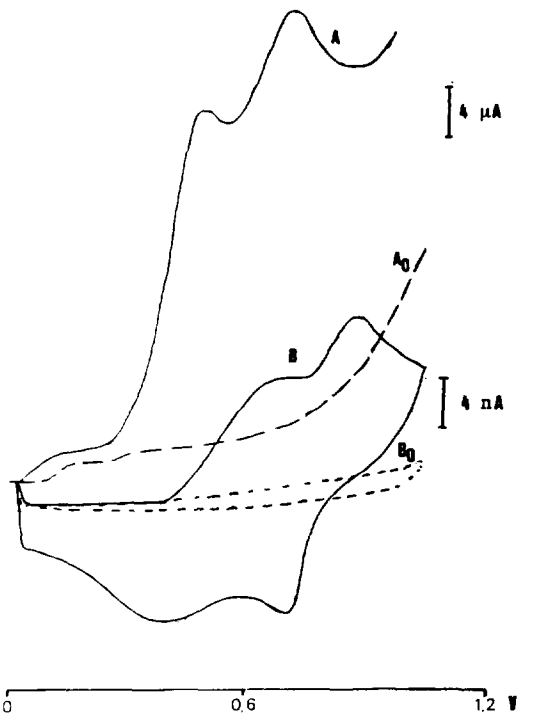

Fig. 1. Oxidation of KI aqueous solution (100 $mg\ l^{-1}$) with a porous graphite (PG) and a vitreous carbon (VC) electrode. (A) Linear voltammogram (scan rate 10 $mV\ s^{-1}$) obtained with the Coulochem 5011 cell (PG electrode); (A_0) linear voltammogram obtained with the mobile phase alone under the same conditions as for (A); (B) cyclic voltammogram (scan rate 50 $mV\ s^{-1}$) of the same solution obtained with a Model 400A EGG detector (VC electrode); (B_0) cyclic voltammogram obtained with the mobile phase alone under the same conditions as for (B).

of serum iodide ($y = 0.527x + 0.008$, $r = 0.994$, $n = 6$, where y is the peak area (Vs) and x the iodide concentration in $\mu g\ l^{-1}$). The within-run precision ($n = 5$) for a pooled serum sample was 4.0% at the 15 $\mu g\ l^{-1}$ of iodide level. The between-run precision ($n = 5$) for the same sample was 4.3%. The within-run precision ($n = 5$) for a pooled serum sample spiked with an aqueous solution of KI at the 100 $\mu g\ l^{-1}$ of iodide level (final concentration) was 4.3%. The between-run precision ($n = 5$) for the last sample was 5.8%.

The recovery was determined by comparing

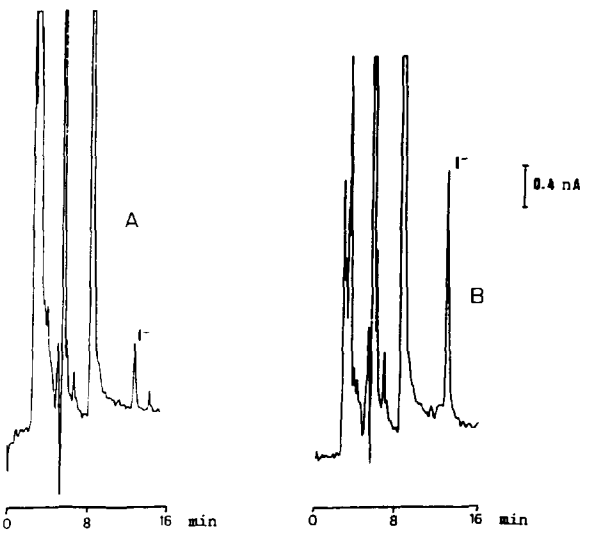

Fig. 2. Chromatographic profiles: (A) serum from a euthyroid child (0.100-ml sample); (B) serum (A) spiked (9 volumes of serum for 1 volume of KI aqueous solution at 500 $\mu g\, l^{-1}$).

the results obtained for two duplicate samples with equivalent iodide concentrations. The first two samples were aqueous solutions of KI containing 10 and 200 $\mu g\ l^{-1}$ of iodide. The other two were obtained by spiking two aliquots from the same serum pool. After treating the four samples under the same conditions, we calculated the ratios (serum sample/aqueous sample) of the iodide peak areas recorded and found that recovery was 97% and 99%.

The result obtained with this method for six children aged from 0.9 to 5.2 years (mean = 1.6 years) was $7 \pm 5\ \mu g\ l^{-1}$ of serum iodide. Excretion of iodide in 24-h urine from thirteen children aged from 1.1 to 12.3 years (mean = 4.6 years) was 80–350 μg per 24 h.

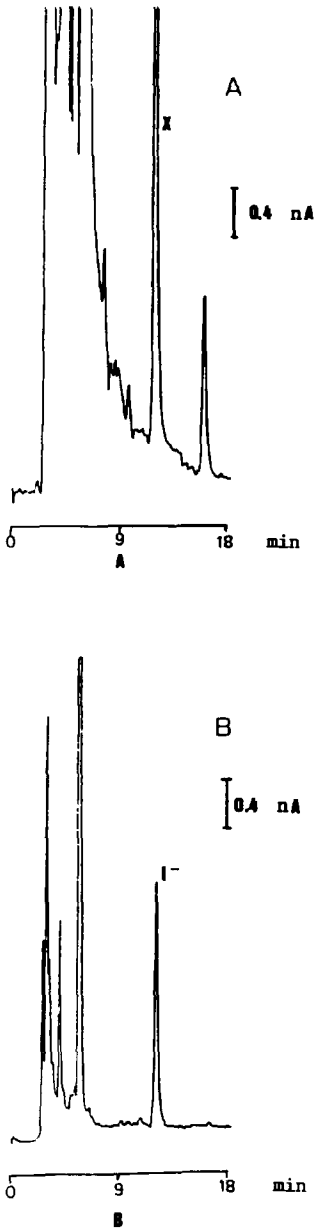

Fig. 3. Urine chromatographic profiles: (A) urine sample treated with Odink's et al. method [24]; (B) the same sample treated by the proposed method.

4. Discussion

The separation and determination of iodide present in a mixture by ion or ion-pair chromatography is not particularly difficult. In fact, several such methods have already been published [13,15,16,17–23]. However, in biological media and especially in blood, this type of determination is not at all easy, as iodide ions are present only at trace levels (a few $\mu g\ l^{-1}$) [1]. As is the case with all such elements, the problems inherent to the determination arise at the stage of sample preparation (extraction,

purification, concentration, etc.) on the one hand, and at the stage of detection on the other. The HPLC method for the determination of iodide in serum with amperometric detection proposed by Hurst et al. [15] is undoubtedly the simplest of the different methods described in the literature, but it is not sensitive enough to be used in paediatrics. In fact, their method includes clean-up of the sample on an ion-exchange resin prior to injection into the chromatograph. This clean-up step leads to dilution of the starting sample, which is detrimental to the method sensitivity. As the average serum iodide concentrations in euthyroid adults are ca. 10 $\mu g\ l^{-1}$ [1], we can deduce that this method requires a minimum of 2–3 ml of serum to obtain a reasonably precise result.

The method we propose makes use the same principle of chromatographic separation as that of Hurst et al. The main difference concerns preparation of the sample before injection into the chromatograph. By extracting the iodide in the form of ion pairs, we concentrate the sample and thereby improve the limit of detection by a factor of at least 20 compared with Hurst's et al. method. With this approach, we need a sample of only 0.10 ml (or even 0.05 ml) of serum for the diagnosis of excess iodide and a maximum of 0.50 ml for the investigation of hypoiodaemia. The method is therefore readily applicable in paediatrics.

The results obtained with the proposed method concur with those published by other workers using different assay principles [1,13,16]. Hurst et al. [15] reported an average iodide concentration in six subjects of $45.1 \pm 1.7\ \mu g\ l^{-1}$. They indicated that these results agree with those published in the literature. This is surprising because, according to all the other workers, including those [1] quoted by Hurst et al., the average iodide concentration in serum observed in euthyroid adults did not exceed 10–15 $\mu g\ l^{-1}$ [1,13,16].

Lastly, the proposed method was applied to the assay of iodide in urine and the results obtained were found to agree with those published by other workers [1,14].

Odink et al. [17] used Hurst's et al. method to assay iodide in urine after clean-up the sample on 40-mm-long, 3-ml disposable ODS extraction columns. Our chromatographic conditions being similar to theirs, we injected several urine samples according to the protocol described by those workers. The chromatograms recorded show that for some samples, unidentified substances may co-elute with iodide (peak X, Fig. 3A). In fact, the peak recorded, corresponding to the capacity factor of iodide, has a different hydrodynamic voltammogram (curve X, Fig. 4). After treating the sample by the proposed method, the intensity of the peak is lower (peak I^-, Fig. 3B) and its hydrodynamic voltammogram can be superimposed on that of the iodide (curve I^-, Fig. 4).

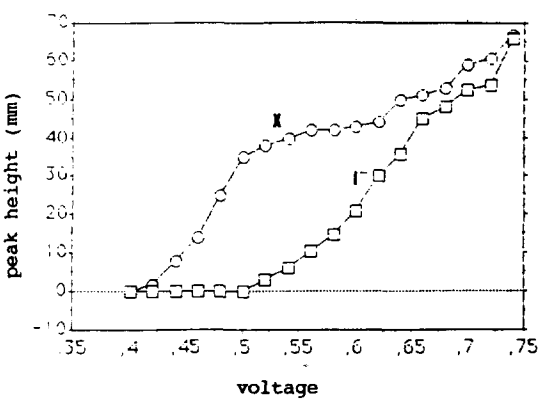

Fig. 4. Hydrodynamic voltammograms of peak X in Fig. 3A and peak I^- in Fig. 3B.

The proposed method is currently being applied to the study of iodide concentrations in serum and urine depending on age and sex.

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