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Urine iodide determination by ion-pair reversed-phase high performance liquid chromatography and pulsed amperometric detection

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ABSTRACT

A sensitive and specific ion-pair reversed-phase high performance liquid chromatography (HPLC) method for urinary iodine analysis is described. This method is based on pulsed amperometric detection (PAD) using a silver working electrode (HPLC–PAD), which improves peak shape, electrode stability as well as linearity and reproducibility. A two-step extraction process consisting of solid phase extraction (SPE) and liquid–liquid extraction with dichloromethane was added in order to improve sample purification which is essential with the use of PAD. Treated samples were eluted on a C18 column, using a phosphate buffer containing ion-pairing reagent tetrabutylammonium and 5% MeOH. The calibration standard curves were linear up to 500 μg/L and within-run and between-run coefficients of variation (CVs) were $<6\%$ with the quantification limit fixed at 6 μg/L. Accuracy, expressed as recovery, ranged from 94% to 104%. Comparison with the Technicon AutoAnalyzer acid digestion (AA) method resulted in a high correlation ($r=0.9916$). Due to a low quantification limit and high sample throughput, the proposed technique appears suitable for both epidemiological and clinical follow-up studies.

1. Introduction

Iodine is an essential micronutrient utilized by the thyroid gland for the biosynthesis of thyroxin (T4) and triiodothyronine (T3). These hormones play a significant role in mental development, growth and basic metabolism. Iodine deficiency may lead to severe brain development delay. Contrarily, excessive iodine intake may cause goiter, hypothyroidism or hyperthyroidism [1]. Given that iodine deficiency is still endemic in many parts of the world [2], a reliable method is needed to evaluate dietary iodide intake and assess iodine status on a population-based level. As urinary iodine concentration reflects current dietary iodide intake, this parameter has been used as a marker of iodine status in population studies [3].

Several methods for measuring iodine are currently available, such as spectrophotometric methods based on the catalytic effect of iodide on the oxidation of As (III) by Ce (IV) (Sandell–Kolthoff reaction) [4–6], inductively coupled plasma mass spectrometry (ICP-MS) [7,8], neutron activation analysis (NAA) [9–11], and introduced more recently, electrospray ionization tandem mass spectrometry (ESI–MS–MS) [12]. However, most applied analytical methodologies are time-consuming and expensive, with the exception of capillary electrophoresis (CE) with direct UV detection [13].

Ion chromatography coupled with electrochemical detection, especially ion-pair reversed-phase high performance liquid chromatography (HPLC) provides an alternative to the above mentioned approaches, offering several advantages over standard ion chromatography [14–20]. First, there is no need to purchase expensive, special purpose ion-exchange columns and in addition, investment in extra-chromatographic instrumentation is seldom necessary, as separations can typically be performed with conventional HPLC systems. The electrochemical detection is commonly conducted using a silver electrode due to its high selectivity and very low detection potential [21,22]. However, when iodide comes in contact with the anodically poised Ag electrode, a current will flow with the concomitant precipitation of silver iodide on the electrode surface, leading over time to chromatographic postpeak distorsion, poor reproducibility, reduced linearity and signal drifts [22]. Recently, pulsed amperometric detection (PAD) using a silver electrode has been proposed in order to electrochemically clean the electrode surface, thereby improving the reproducibility of electrode responses [23–27]. The application of PAD to biological samples such as urine [26] and serum [27] was reported in the literature. However, applying the coupled ion-pair HPLC–PAD to biological samples has not yet been described, PAD being often coupled with anion exchange chromatography instead of ion-pair HPLC.

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We propose a simple, selective, and sensitive ion-pair HPLC method coupled with PAD using a conventional silver working electrode for routine urinary iodide measurements. This method is devoid of the aforementioned limitations. Our results were compared to those obtained by means of the automated Sandell–Kolthoff spectrophotometric method (Technicon AutoAnalyzer II) (AA) [4], which has been used for over 25 years in our laboratory for iodine status surveys in Belgium [2,28]. This method has been periodically subjected to routine external quality control.

2. Materials and methods

2.1. Chemicals and reagents

HPLC–PAD: Analytical-grade disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) was obtained from VWR (Leuven, Belgium), while $EDTANa_2H_2$ (Titriplex III), di-*n*-butylamine, dichloromethane and ion-pairing reagent tetrabutylammonium phosphate (TBAP) were purchased from Sigma-Aldrich (Steinheim, Germany), with methanol and analytical-grade potassium iodide being obtained from Merck (Darmstadt, Germany). To investigate potential interferences with other ions, analytical-grade NaCl, NaBr, NaF, $Na_2S_2O_3$, $NaNO_2$, $NaNO_3$, KSCN and disodium oxalate were purchased from Sigma-Aldrich (Steinheim, Germany), with KIO_3 being obtained from Merck (Darmstadt, Germany).

All solutions were prepared using deionised water 18 MΩ cm from a resin Aqualab system (VWR, Leuven, Belgium).

2.2. Chromatographic system

The HPLC system (Shimadzu, Kyoto, Japan) consisted of the following components which were connected in series: dual-plunger parallel-flow solvent delivery module LC-20AD, on-line degasser DGU-20A5, auto-sampler SIL-20A, oven CTO-20AC and system controller CBM-20A. The signal was recorded with an electrochemical detector including an Ag/AgCl reference electrode, a 50 μm gasket defining an analytical cell volume of 2.5 μL, and a silver working electrode (Antec Leyden, Netherlands). The system controller and the detector were connected on-line to a Dell computer operating with the LC Solution software from Shimadzu (Kyoto, Japan). The chromatographic column was an X Terra® MS C18 reversed phase column, 3.9×150 mm^2, 9 nm, 5 μm (Waters Corporation, Milford, MA, USA) which was kept at 35 °C in the column heater.

The silver working electrode was treated as necessary with a polishing cloth and diamond slurry and then rinsed with deionised water.

The mobile phase consisted of water–methanol (95:5, v/v), pH 6.8 containing 2.5 mmol/L $Na_2HPO_4 \cdot 12H_2O$, 0.5 mmol/L $EDTA\text{-}Na_2H_2$, 2.5 mmol/L TBAP and 3 mmol/L di-*n*-butylamine.

2.3. Electrochemical detection and optimization

The electrochemical reaction occurred in a three-electrode analytical system where the potential of the working electrode (Ag) was measured and compared to that of a stable reference electrode (Ag/AgCl) through which no current flowed. A power supply maintained the working electrode at a potential of 0.1 V in comparison with the reference electrode potential.

The following tri-potential waveform was applied to the silver-working electrode vs. the Ag/AgCl reference electrode: $E_1 = -0.15$ V (t_1=0–1.9 s, t_d=700 ms), $E_2 = -1.15$ V (t_2=1.9–1.97 s) and $E_3 = -0.3$ V (t_3=1.97–2 s). This potential waveform was characterized by a detection potential of −0.15 V for 1.9 s (t_1) with current integrated between 1.2 and 1.9 s (time interval t_d), where the time difference of 0–1.2 s was the electrode stabilization time. Then, a cleaning pulse of −1.15 V during the time interval t_2, when Ag^+ in AgI precipitate was reduced to Ag^0, facilitated the removal of the AgI layer from the electrode surface. Finally, a rest potential of −0.3 V was applied for 0.03 s (t_3) before commencing a new cycle. The slow waveform time of 2 s was found to be necessary in order to completely stabilize the electrode during use.

The full-scale integrator sensitivity was 1.0 V and the detector sensitivity was 50 nA.

To select the optimum operating potential for determining iodide in the selected mobile phase, we generated a current–voltage curve by repeatedly injecting a calibration solution (100 μg/L potassium iodide) at various potentials. Increasing the potential in 0.05 V increments resulted in no further increase in signal response at a working potential of −0.15 V. We therefore used this potential under routine conditions as it was shown to be the lowest potential in the plateau region of the current–voltage curve, along with minimized noise.

2.4. Iodide calibrators

Working solutions of 20 μg/L, 50 μg/L, 100 μg/L, 200 μg/L and 250 μg/L were prepared from 100 mg/L iodide solution containing 130.8 mg of potassium iodide in 1 L of deionised water.

2.5. Collection of urine samples

Urine samples were analyzed using both HPLC and AA, with the samples originating from 490 patients for whom urinary iodine measurements were requested by their treating physicians. The samples were kept frozen until assayed.

2.6. Analytical procedure

Three millilitres of each urine sample were poured onto C18 Sep-Pak (Waters) extraction columns which were rinsed prior to use with 5 mL methanol and 10 mL deionised water. While the first 2 mL of eluate were discarded, the third millilitre was collected. A semi-automated Vacelut® (Agilent Vacuum Products Division, Middelburg, Netherlands) low pressure manifold was used for elution.

Five hundred microliters of the eluate or calibrator were collected into a clean tube, with 500 μL of aqueous TBAP solution (0.1 M) added. The formed ion-pairs were extracted using 5 mL dichloromethane after mixing at 2–8 °C for 30 min. Following 5 min centrifugation at 3500*g*, 4 mL of the organic phase was withdrawn and evaporated to dryness at 40 °C in a water bath. The dry residue was then mixed with 500 μL mobile phase.

All separations were carried out at 1 mL/min flow rate using an isocratic mobile phase, with an injection volume of 30 μL and analysis temperature of 35 °C. Concentrations were calculated based on peak areas using the automatic integrator LC-Solution. The calibration curve was plotted by linear regression.

Samples extracts with iodide concentrations exceeding the linearity limit (500 μg/L) were diluted with the HPLC buffer and injected directly into the system.

After use, a methanol/water (30:70, v/v) wash solution was passed through the HPLC setup at 0.3 mL/min for 45 min for rinsing, with the solution being kept in the system.

2.7. Statistical analysis

For comparison purposes, iodine concentrations of 490 random samples were determined using both the HPLC and AA methods. The AA method for iodine concentration measurements