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Journal of Chromatography B, 803 (2004) 363–366
JOURNAL OF CHROMATOGRAPHY B
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Short communication

Ion chromatographic determination of thyroxine in urine

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Received 24 June 2003; received in revised form 29 December 2003; accepted 5 January 2004

Abstract

Thyroxine (3,5,3′,5′-tetraiodo thyronine) is administered to patients suffering from endemic goiter as also in cases of non-iodine deficient ethiology and hypothyroidism. It is suggested that the uptake of thyroxine can be monitored by assessing the levels of the same in the urine of patients under treatment. For the purpose, a highly sensitive and selective ion chromatographic procedure is developed. The sample of urine is treated with sodium hydroxide and UV irradiated to convert iodine in thyroxine to iodide. Subsequently, iodide is separated on an anion exchanger AS 4A column using 50 mM NaOH as the eluent and determined spectrophotometrically at 226 nm.

Keywords: Thyroxine

1. Introduction

Deficiency of iodine is associated with the occurrence of endemic goiter in humans [1]. For treatment of this type of goiter and also to simple goiter of non-iodine deficient ethiology, and hypothyroidism, thyroxine is administered. It would be useful to monitor the measurement of the thyroxine uptake of patients. In this context, it is suggested that the thyroxine in urine, would be a good indicator.

Thyroxine, 3,5,3′,5′-tetraiodo thyronine, has four iodine atoms in its structure (Fig. 1). Hence, it is possible to develop a sensitive method for its determination by the measurement of iodine, present therein. The first step is to convert the organic iodine to inorganic iodide, which can then be determined by suitable methods. For the former, dry ashing or wet digestion methods are suggested. In dry ashing, there is always a possibility of losses of iodine and hence it is preferable to go for wet digestion of samples. Similarly for the determination of iodide, various methods based on spectrophotometry, ICP-AES, gas phase molecular absorption spectrometry, catalytic spectrophotometry, kinetic triple wavelength spectrophotometry, flourimetry, membrane permeation flow injection analysis, catalytic spectrophotometry–flourimetry, ion selective electrode, chemiluminescence [2–11] are reported. In addition, ion chromatography (IC) has been suggested as it combines in itself, separation as well as determination of iodide [12,13]. Chandramouleeswaran et al. [12] in their paper have reviewed these IC methods. Immuno assay methods are becoming popular for the analysis of thyroxine in biological fluids especially serum [14].

Preliminary experiments indicated that fusion with sodium hydroxide or UV irradiation of a sodium hydroxide solution of thyroxine in the presence of H_2O_2, could convert organic iodine in thyroxine to inorganic iodide. Dissolution of thyroxine in sodium hydroxide and irradiation with UV in the presence of H_2O_2 has been adopted in the present case, due to its easy adaptability. This is followed by ion chromatographic separation following the 'heart cut method' on an anion exchange column AS 4A and spectrophotometric determination of iodide at 226 nm.

2. Experimental

2.1. Instrumentation

The ion chromatograph used in these experiments is a Dionex model DX 300, equipped with UV-Vis variable wavelength detector. The separator column is AS 4A-SC, 4 × 250 mm, a strong base anion-exchange resin. A guard column, AG 4A-SC, 4 × 50 mm, is also used.

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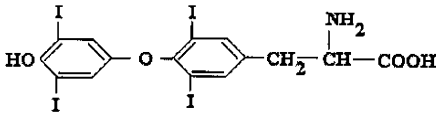
1570-0232/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2004.01.001



3,5,3', 5'- tetraiodo thyronine (Thyroxine)

Fig. 1. Structure of thyroxine.

Table 1
Retention time and peak areas of the chromatograms for different concentrations of iodide

Conc. of iodide (ppm)	Retention time (min)	Peak area (arbitrary units)
5	4.06	5206.0 ± 208.0[a]
2	4.06	2382.4 ± 95.3[a]
1	4.05	1120.1 ± 44.8[a]
0.1	4.05	112.8 ± 4.5[a]

[a] Standard deviation based on six measurements.

2.2. Reagents

For preparation of solutions, use Millipore water, which has a specific conductivity of $<1\,\mu s\,cm^{-1}$. Filter all solutions through 0.45 μm Millipore filter before injection into the Ion Chromatograph.

2.2.1. Standard iodide solution

Prepare a 1000 mg/l stock solution of iodide by dissolving 0.131 g of AR grade potassium iodide in 100 ml water. Prepare working standards of 5–0.1 ppm iodide by appropriate dilution of the stock solution.

2.2.2. 0.10 M and 0.05 M NaOH

Dissolve 4.1 g of NaOH in the minimum amount of water and make up to 1000 ml. Standardize by titration with acid. Pipette out an appropriate aliquot and dilute to volumes to give 0.10 and 0.05 M NaOH.

2.2.3. Thyroxine (1.53 mg/ml)

Transfer 0.153 g of thyroxine to a 100 ml beaker containing 50 ml of 1 M NaOH. Irradiate with UV of 250 W for 4 h. Make up to 100 ml. Prepare by suitable dilution,

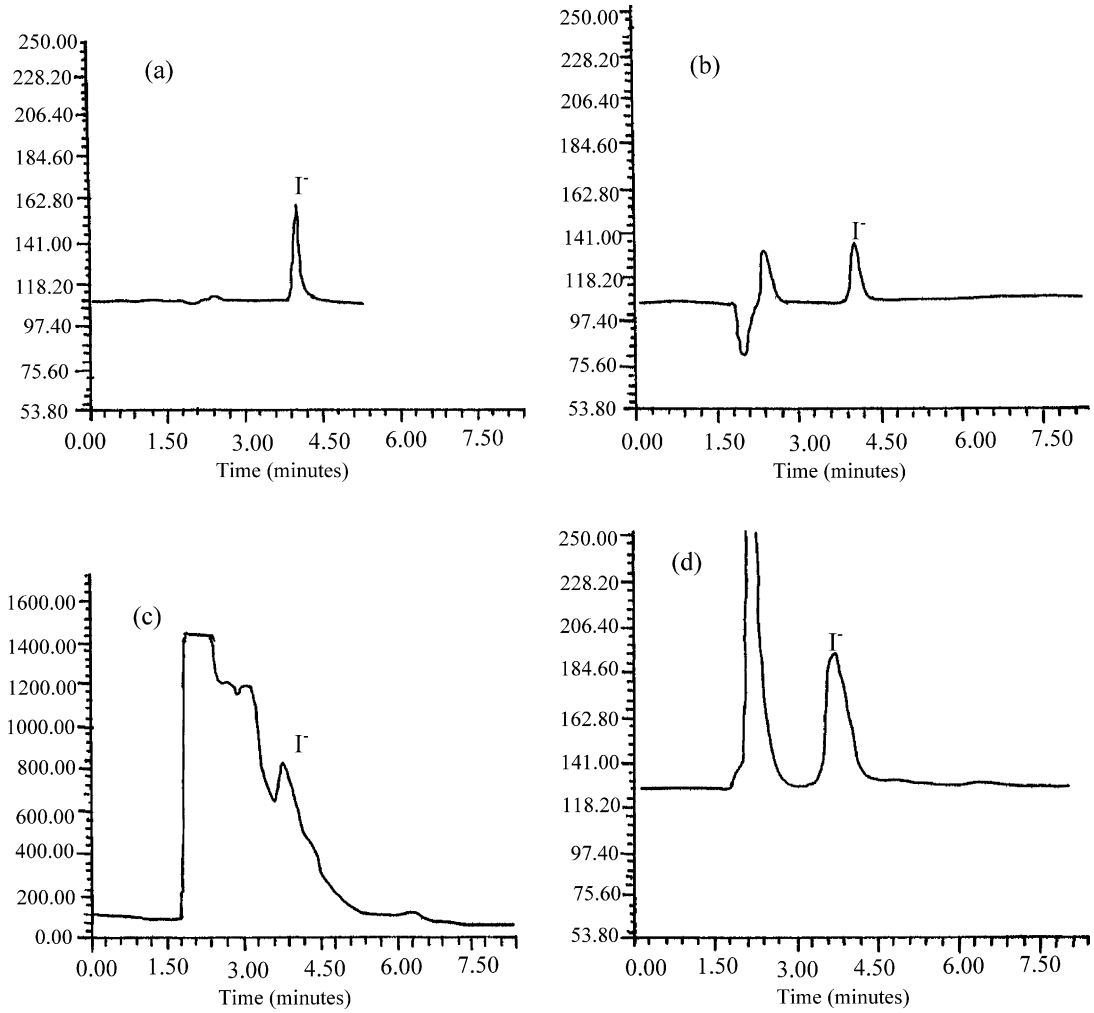


Fig. 2. Chromatograms of: (a) potassium iodide (b) thyroxine (c) urine (d) urine after 'heart cut method'.

solutions containing 7.65–0.15 ppm thyroxine (equivalent to 5–0.1 ppm iodide).

2.3. Standardisation with iodide

Inject 25 μl of standard KI solution of different concentrations ranging from 5–0.1 ppm iodide into the AS 4A column of the DX 300 Dionex Chromatograph. Elute at a flow rate of 1 ml/min with 0.05 M NaOH. Carry out the measurement of the absorbance at 226 nm. The iodide peak appears at 4.07 min (Fig. 2a). Measure the areas of the peak corresponding to different concentrations of iodide (Table 1).

2.4. Standardisation with thyroxine

Inject 25 μl of fused thyroxine solution of concentrations ranging from 0.15 to 7.65 ppm into the column and elute with 0.05 M NaOH at a flow rate of 1 ml/min. Carry out the measurement of the absorbance at 226 nm (Fig. 2b). Compute the area of the chromatogram elution peak for iodide (retention time: 4.07 min) (Table 2).

2.5. Determination of thyroxine in urine sample

Take a representative sample of urine by collecting it over a 24 h period [15]. Mix, take 5 ml aliquot, add 2.5 ml of 1 M NaOH and 0.1 ml H_2O_2 (30%). Irradiate for 4 h under UV. Make up to 10 ml. Inject 25 μl of the above solution into the ion chromatograph and proceed as above. Initially, a broad peak in the range of 1.5–3.2 min is observed, followed by the iodide peak with retention time at 4.07 min (Fig. 2c). In order to eliminate the former, follow the 'heart cut method'. Inject the sample of urine and start the eluent flow. Collect the eluent flowing between 3.5 and 4.5 min. Carry out the injection and collection four times, thus yielding 4 ml. Evaporate and make up to 1 ml. Inject 25 μl of the evaporated solution. Measure the area of the peak with peak maximum at 4.07 min (Fig. 2d).

2.6. Standard addition method

In order to validate the method, in the absence of reference standards, the standard addition technique has been used. Take two separate aliquots of 5 ml of urine each. To one, add 1 ml of 15.3 ppm of thyroxine, 2.5 ml of 1 M sodium hydroxide and 0.1 ml of hydrogen peroxide. Irradiate, make up to 10 ml and follow the procedure as in Section 2.4. Compute the peak area. Similarly, to another aliquot, add 2 ml of 15.3 ppm thyroxine and determine the thyroxine content by the above procedure.

Table 2
Retention time and peak areas for different concentrations of thyroxine

Conc. of thyroxine (ppm)	Equivalent iodine (ppm)	Retention time	Peak area
7.65	5	4.05	5084.4 ± 355.8[a]
3.06	2	4.06	2310.1 ± 161.7[a]
1.53	1	4.05	1180.5 ± 82.6[a]
0.15	0.1	4.06	118.5 ± 8.3[a]

[a] Standard deviation based on six measurements.

Table 3
Analytical results of thyroxine in urine by the present method, the WHO method and by standard addition

Sample	Present method (ppm)	WHO method (ppm)
Urine	0.19 ± 0.018[b]	0.21
Urine + 0.15[a]	0.33 ± 0.034[b]	0.34
Urine + 0.31[a]	0.49 ± 0.052[b]	0.53

[a] Standard additions of thyroxine at 0.15 and 0.31 ppm.
[b] Standard deviation on the basis of $n = 6$.

2.7. Comparison with WHO method

The urine samples analyzed by the present method are also compared with the WHO method [16,17]. This data is also included in Table 3.

3. Interference studies

Interference studies of several constituents present in urine, especially anions were carried out. These include urea, ammonium, chloride, sulphate, phosphate and thiocyanate. These were added to the urine sample to a concentration 5 mg/l of each. There was no interference.

4. Results and discussion

The monitoring of thyroxine in urine is important in assessing the uptake of the thyroxine, especially in the case of patients administered with it. Though dual column with the conductivity method of determination was initially attempted, this could not be pursued as the urea in the urine sample gets converted to NH_4^+ ions and saturate the suppressor column. Therefore, the single separator column with photometric determination at 226 nm for iodide was adopted. Iodide peak appears at 4.07 min. There is linearity between the peak area and concentration in the range of 0.1–5 ppm of iodide.

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