Journal of Chromatography, 431 (1988) 309–316
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4323

EXCRETION OF IODIDE IN 24-h URINE AS DETERMINED BY ION-PAIR REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

J. ODINK*, J.J.P. BOGAARDS and H. SANDMAN

Department of Clinical Biochemistry, TNO-CIVO Toxicology and Nutrition Institute, P.O. Box 360, 3700 AJ Zeist (The Netherlands)

R.J. EGGER

Department of Nutrition, TNO-CIVO Toxicology and Nutrition Institute, P.O. Box 360, 3700 AJ Zeist (The Netherlands)

and

G.A. ARKESTEYN and P. DE JONG

Department of Analytical Chemistry, TNO Division of Technology for Society, P.O. Box 217, 2600 AE Delft (The Netherlands)

(First received April 7th, 1988; revised manuscript received May 31st, 1988)

SUMMARY

A simple method is presented for the routine analysis of iodide in urine. After a one-step sample clean-up, iodide was separated by ion-pair reversed-phase liquid chromatography and detected electrochemically with a silver electrode. The coefficient of variation of a single analysis of iodide in a pooled urine sample (530 nmol/l) was 7.6%. The detection limit, derived from a signal-to-noise ratio of 3, was 3 pmol, corresponding to 0.06 μmol/l. The recovery of iodide added to urine was 96 ± 7%. The accuracy of the method was assessed by analysing ten different samples with neutron activation analysis. The data obtained with the two methods showed a high correlation ($r = 0.991$) and did not differ significantly. Excretion of iodide in samples of 24-h urine from a free-living population was shown to have a log-normal distribution and to be higher in men than in women. The iodide/creatinine ratio was independent of sex and increased with age.

INTRODUCTION

To eradicate the problem of endemic goitre, iodization programmes have been instituted in many iodine-deficient areas of the world. According to the World Health Organization 43 countries were using iodized salt in 1976 [1]. In the Neth-

0378-4347/88/$03.50

erlands, iodization of salt started in 1942 [2]. Measurement of urinary excretion of iodide has been shown to provide a reliable estimate of daily iodine intake and hence of the need for and effect of iodization programmes [3–5].

Several methods have been presented for the determination of iodide in biological fluids, among which the most common are the colorimetric ceric–arsenic assay [6–10] and methods involving neutron activation analysis (NAA) [11–13].

The former method is based on a redox reaction between cerium(IV) and arsenic(III), which is catalysed by iodide [14]. The reduction in the yellow cerium(IV) is measured spectrophotometrically. However, this method has been reported to suffer from several potential sources of error; any contamination leads to reduction of cerium(IV) and decolorization of the solution [7]. Moreover, the catalytic nature of the assay requires strict adherence to procedures to prevent erroneous results.

Methods with NAA have been demonstrated to possess the required sensitivity and accuracy, but are not suitable for routine practice because (i) specialized facilities are needed, (ii) the relatively short half-life (25 min) of the ^{128}I formed limits the number of samples that can be analysed in one run, and (iii) quantitation in urine requires a time-consuming separation procedure to remove the interfering radioisotopes of chlorine, sodium and bromine.

Recently, Hurst et al. [15] described a high-performance liquid chromatographic (HPLC) method for the determination of iodide in serum. Detection was electrochemical, with a silver electrode as working electrode on which silver iodide is formed. This reaction requires a relatively low potential and thus increases the specificity and reduces the occurrence of peaks of other components in the HPLC elution profile. Therefore, we decided to investigate whether this method was also suitable for the determination of iodide in urine.

EXPERIMENTAL

Apparatus

A modular HPLC system was assembled with a Perkin-Elmer Series 10 solvent-delivery system (Gouda, The Netherlands), a Perkin-Elmer ISS 100 autoinjector and a Bioanalytical Systems amperometric detector (West Lafayette, IN, U.S.A.), consisting of a Model LC-4B control unit equipped with a Model RE-1 Ag/AgCl reference electrode, a Model TG-5M 0.127 mm gasket, a Model TL-9A silver working electrode and a stainless-steel auxiliary electrode. The injector and detector were connected on-line to a Perkin-Elmer 3230 Labdata system operating with LIMS/CLAS 2000 software.

Stainless-steel Hyperchrome HPLC columns (7.5 cm × 4.6 mm I.D.) (Salm & Kipp, Breukelen, The Netherlands) were packed with ODS-Hypersil 3 μm (Shandon, Zeist, The Netherlands) at 63 MPa by the balanced-density slurry technique, on a column-packing installation designed at the TNO-CIVO Toxicology and Nutrition Institute, using a Haskel pump Type DSTV-150 (Amman Technik, Stuttgart, F.R.G.). The slurry and packing solvents were 2-propanol and methanol, respectively (Merck, Darmstadt, F.R.G.).

Chemicals and reagents

Tetrabutylammonium phosphate was purchased from Waters Assoc. (Etten-Leur, The Netherlands) and *n*-octylamine from Janssen Chimica (Goirle, The Netherlands). Analytical-grade methanol, orthophosphoric acid and Suprapure sodium iodide were obtained from Merck (Meppel, The Netherlands). ODS, 40 μm, 3-ml disposable extraction columns were purchased from Baker (Deventer, The Netherlands).

HPLC analysis with electrochemical detection

After thawing, urine samples were centrifuged for 10 min at 1700 *g* and room temperature. Before use, the extraction columns were rinsed with 6 ml of methanol and 3 ml of distilled deionized water. Then two 1-ml aliquots of urine were poured onto each column. The first millilitre of eluate was discarded and the second one was collected.

Analyses were carried out by injecting 50-μl aliquots (aqueous solutions containing the reference compound (1 μmol/l) or eluates of the extraction columns) onto the HPLC column. Isocratic HPLC was achieved at a flow-rate of 1.8 ml/min with a solution containing 10 m*M* *n*-octylamine and 5 m*M* tetrabutylammonium phosphate, adjusted to pH 7.0 with orthophosphoric acid. Electrochemical detection (ED) was at a potential difference of +10 mV vs. Ag/AgCl. The full-scale detector sensitivity was 10 nA, corresponding to an integrator sensitivity of 10^5 counts. Concentrations were calculated from peak heights.

Neutron activation analysis

Determination of iodide in urine with NAA was performed essentially as described by Heurtebise and Ross [11]. In short, the pH of urine was adjusted to 8–9 with ammonia. Potassium bisulphite was added, to a final concentration of 22.5 m*M*. To calculate the chemical recovery, ca. 3.7 kBq of ^{131}I were added. The samples were then irradiated in the "Hoger Onderwijs Reactor" of the IRI (Interuniversitair Reactor Instituut) at Delft, The Netherlands, at a thermal neutron flux of ca. 10^{13} cm^{-2} s^{-1} for 20 min. Part of the irradiated sample was poured onto an iodonated resin, which was subsequently washed with water and a solution of sodium chloride (343 m*M*). The resin was then counted with a germanium (lithium) semiconductor. The peak intensity at 443 keV (^{128}I) was compared with the activity of a reference solution of iodide to calculate the iodide concentration of the sample. The peak intensity at 365 keV (^{131}I) showed that the recovery was over 97%, therefore no corrections were made.

Collection and analysis of 24-h urine

In 1981, 380 male and 444 female, apparently healthy volunteers (mean age 45 years; range 18—92 years), all inhabitants of Brielle (The Netherlands), collected their urine for 24 h. Volumes were measured and aliquots (10 ml) were stored at −20°C. Each sample was analysed in duplicate with HPLC–ED.

Data analysis

The inter-assay and intra-assay standard deviations ($S.D._b$ and $S.W._w$, respectively) were calculated with analysis of variance (ANOVA) from the data ob-

tained for a pooled urine sample, analysed in quadruplicate on 32 days over a period of 3 months under routine conditions. The overall S.D. ($S.D._o$), i.e. the S.D. of a single analysis, was calculated as follows:

$$(S.D._o)^2 = (S.D._b)^2 + (S.D._w)^2.$$

Differences between slopes obtained by spiking different urine samples with increasing amounts of sodium iodide were investigated with ANOVA. Differences between the data obtained with NAA and the method presented were investigated with regression analysis according to the method of Deming [16].

The influence of age, sex and their interaction (i.e. the difference in age dependency between men and women) on the volume of 24-h urine and on the excretion of iodide and creatinine were investigated with regression analysis.

RESULTS AND DISCUSSION

Applicability of the assay

Typical HPLC–ED profiles of a reference solution, a pooled urine sample and the same sample spiked with sodium iodide are shown in Fig. 1. The retention time of iodide is 6 min. Owing to the presence of the peak of an unknown component at 13 min, the total analysis time for one sample is 15 min. Per hour, fifteen samples can be handled for sample pretreatment. Under routine conditions the HPLC column can be used for ca. 600 injections.

The electrochemical response was linear up to 75 pmol (1.5 μmol/l). Samples with higher iodide concentrations were diluted prior to injection. If a signal-to-noise ratio of at least 3 is assumed, the detection limit of the assay is 3 pmol. This

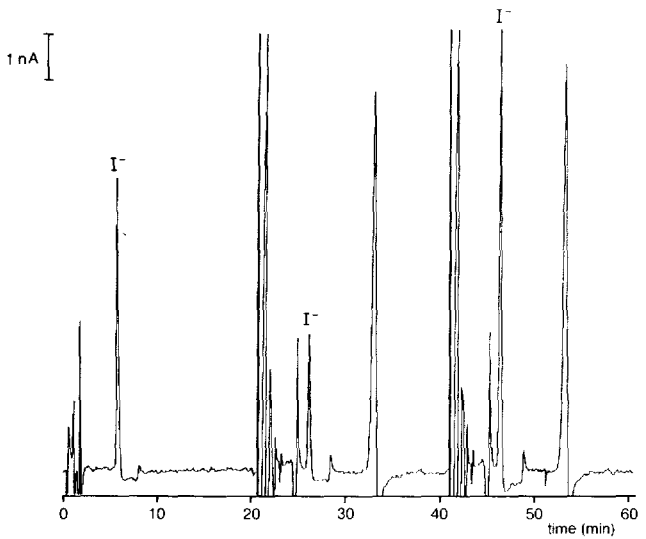

Fig. 1. Chromatograms of (left) 50 μl of a standard solution containing 50 pmol of sodium iodide, (middle) 50 μl of a pooled urine sample and (right) 50 μl of a pooled urine sample spiked with 50 pmol of sodium iodide.

corresponds to 0.06 μmol/l, which is below the concentrations we have observed in 24-h urine samples of more than a thousand apparently healthy volunteers (range 0.11–26.5 μmol/l; unpublished observations).

Precision, recovery and accuracy

For a pooled urine sample (530 nmol/l), the $S.D._b$, $S.D._w$ and $S.D._o$ of the assay under routine conditions were 31, 26 and 40 nmol/l, respectively, corresponding to a coefficient of variation of a single analysis of 7.6%.

To investigate possible interferences by compounds in urine, ten samples with iodide contents ranging from 0.30 to 2.83 μmol/l were spiked with increasing amounts of sodium iodide and analysed. For each sample the slope between the amount added and the amount measured was calculated. The results obtained are shown in Fig. 2. There was no significant difference between the slopes obtained for the different urine samples ($P=0.271$). The mean slope was 0.97. This suggests the absence of interference by compounds in urine and indicates an almost complete recovery of iodide, which is in agreement with the recovery (96 ± 7%)

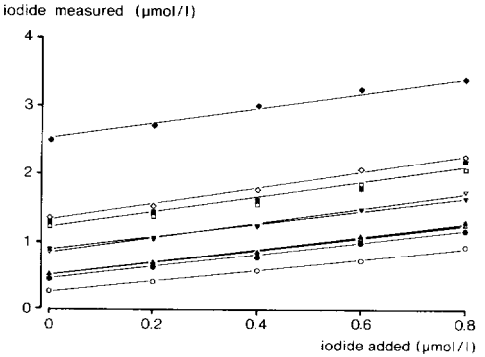

Fig. 2. Standard addition curves of iodide to different urine samples. The results are the mean of three experiments.

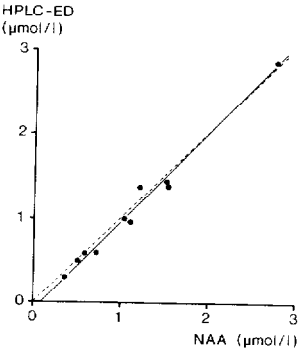

Fig. 3. Comparison of the mean iodide contents of ten urine samples determined with HPLC-ED and NAA. Values obtained with NAA are the mean of single analyses on two days; values obtained with the proposed method are the mean of single analyses on three days. (- - -) Line of identity; (—) regression line estimated according to the method of Deming [16].

obtained by analysing a pooled urine sample spiked with sodium iodide (1 μmol/l) on eighteen different days.

To obtain insight into the accuracy of the proposed method the iodide content of ten different urine samples was also determined with NAA. The results are shown in Fig. 3. The estimated regression coefficient (1.037 ± 0.045) and intercept (0.087 ± 0.058) did not differ significantly from 1 and 0, respectively. The correlation coefficient was high ($r = 0.991$).

The results obtained for the recovery and for the comparison with NAA are indicative of the accuracy of the method presented. It may be argued that differences in sample pretreatment between the two methods have introduced a potential source of error in method comparison, owing to the extraction of different forms of iodine. However, we consider this very unlikely, because, under physiological conditions, iodine is almost completely excreted in urine as iodide [18].

Optimization

In the method proposed by Hurst et al. [15] for the analysis of iodide in serum, hexadecyltrimethylammonium chloride was used as modifier in the HPLC mobile phase. We initially used the bromide salt of this modifier. However, better results were obtained with *n*-octylamine as used by De Kleijn [17]; it reduced the dip observed just after the iodide peak and resulted in a more stable baseline. Furthermore, the use of acetonitrile in the mobile phase could be avoided by addition of tetrabutylammonium phosphate.

To investigate the redox potential to be used for detection, a reference solution and a urine sample were injected at potentials ranging from −140 to +40 mV. At +10 mV, plateau values of the current for both solutions were found. Therefore, this potential was used under routine conditions.

The use of extraction columns for sample pretreatment resulted in clear and colourless samples and in extended life of the HPLC column. It did not cause a visible improvement of the chromatograms. This was not unexpected, since both extraction and HPLC columns were packed with ODS.

Possible interferences

To investigate possible interferences by compounds that form silver salts or can be oxidized at a low potential, solutions of hydrochloric acid, oxalic acid, sodium bromide, fluoride, ascorbate, nitrite and sulphite were injected. The halogens (5 μmol) gave a small response just after the void volume. Sodium ascorbate (2.5 nmol), oxalic acid (15 nmol) and sodium nitrite (0.5 μmol) did not respond at the applied potential. Sodium sulphite (0.5 μmol), however, gave a response at 13 min. Its retention time was equal to that of the unknown component mentioned. However, it is unlikely that the peak in urine can be attributed to sulphite since the signal was very stable, whereas the signal of sodium sulphite added to urine declined with time.

Effect of age and sex

The data regarding the influence of age and sex on the excretion in 24-h urine are summarized in Table I. Both iodide and the iodide/creatinine ratio showed a log–normal distribution. Five outliers were excluded from the analysis.

TABLE I

EXCRETION OF IODIDE IN 24-h URINE FROM 377 MEN AND 442 WOMEN AGED FROM 18 TO 92 YEARS

Regression model: parameter = intercept$_i$ + slope × age + residual (i = men, women). Differences between slopes for men and women were not significant ($P > 0.05$). N.S. = not significant.

Parameter	Value at mean age (44.5 years)			Slope (unit/year)			$S_{residual}$
	Men	Women	P value	Mean	S.E.	P	
Volume (ml)	1500	1400	<0.005	1.25	1.08	N.S.	500
Creatinine (mmol)	17.6	13.0	<0.001	-0.068	0.007	<0.001	3.24
Iodide (nmol)	1001	797	<0.001*	-0.45	1.18	N.S.*	542
Iodide/creatinine (μmol/mol)	58.1	62.1	N.S.*	0.258	0.081	<0.001*	37.0

*P value calculated from regression analysis on log-transformed data.

For none of the parameters investigated were any differences in age dependency between men and women observed. Iodide excretion was higher in men, owing to a higher intake of iodine-containing food products (unpublished observations). When expressed per mol of creatinine, it was independent of sex and increased with age, owing to the dependency of creatinine excretion on both sex and age.

CONCLUSION

The method presented is an alternative to the current methods of iodide determination in urine. It is easy to perform and accurate, while its precision and detection limits are sufficient for the evaluation of the effects of iodization programmes. The relatively low potential difference of +10 mV vs. Ag/AgCl which can be used for the formation of silver iodide, increases the specificity and reduces the occurrence of other peaks in the HPLC profile.

REFERENCES

1 E.M. Demayer, F.M. Lowenstein and C.H. Thilly, The Control of Endemic Goiter, World Health Organization, Geneva, 1979, p. 49.
2 R.J. Egger and W.J. van Ommen, Ned. Tijdschr. Diëtisten, 37 (1982) 237.
3 J.B. Stanbury, A.M. Ermans, B.S. Hetzel, E.A. Pretell and A. Querido, WHO Chronicle, 28 (1974) 220.
4 H.E. Sauberlich, Laboratory Tests for the Assessment of Nutritional Status, CRC Press, Cleveland, OH, 1977, p. 124.
5 M.M. van Rees-Wortelboer, J.P. Schröder-van der Elst, A. Lycklama and D. van der Heide, Ned. Tijdschr. Geneesk., 131 (1987) 1821.
6 M.C. Sanz, T. Brechbuhler and L.J. Green, Clin. Chim. Acta, 1 (1956) 570.
7 H. Hoch, S.L. Sinett and T.H. McGavack, Clin. Chem., 10 (1964) 799.
8 H. Hoch and C.G. Lewallen, Clin. Chem., 15 (1969) 204.
9 P.J. Garry, D.W. Lashley and G.M. Owen, Clin. Chem., 19 (1973) 950.
10 G. Aumont and J.C. Tressol, Analyst, 112 (1987) 875.
11 M. Heurtebise and W.J. Ross, Anal. Chem., 43 (1971) 1438.

12 R. Cornelis and J. Hoste, J. Radioanal. Chem., 13 (1973) 419.
13 R. Cornelis, A. Speecke and J. Hoste, Anal. Chim. Acta, 78 (1975) 317.
14 E.B. Sandell and I.M. Kolthoff, Mikrochim. Acta, 1 (1937) 9.
15 W.J. Hurst, J.W. Stefovic and W.J. White, J. Liq. Chromatogr., 7 (1984) 2021.
16 P.J. Cornbleet and N. Gochman, Clin. Chem., 25 (1979) 432.
17 J.P. De Kleijn, Deutsche Lebensmittel-Rundschau, 79 (1983) 184.
18 E.J. Wayne, D.A. Koutras and W.D. Alexander, Clinical Aspects of Iodine Metabolism, Blackwell Scientific Publications, Oxford, 1964, p. 73.