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DETERMINATION OF IODIDE AND BROMIDE BY ION CHROMATOGRAPHY WITH POST-COLUMN REACTION DETECTION

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SUMMARY

During an investigation into the mechanism of the biosynthesis of thyroid hormones, it became necessary to determine traces of iodide and bromide in biological matrices as well as in food. A vydac 302-IC anion-exchange column with methanesulphonic acid as the mobile phase was used for the ion chromatographic separation of iodide and bromide. A post-column reaction detector was developed based on the reaction between iodide or bromide, chloramine-T and 4,4′-bis(dimethylamino)diphenylmethane. Methods with minimal sample preparation are described for determination of iodide or bromide in serum, milk, salt and water. The detection limit is *ca.* 20 pg iodide and 15 ng bromide injected.

INTRODUCTION

Iodine is known to be an essential micronutrient, which is utilized by the thyroid gland for the biosynthesis of the thyroid hormones thyroxine and triiodothyronine. Recent epidemiological studies[1] indicate that alimentary iodine supply is still insufficient in some regions of Europe. Therefore measurements of urinary iodide excretion and iodide in serum (both may be considered to be equivalent to alimentary iodine intake), as well as investigations into the iodine content of food (such as milk products, which may be one of the main sources for iodine supply besides iodized salt) are necessary.

In addition it seemed interesting to study the bromide content of biological materials, especially of serum, since studies of the biosynthesis of thyroid hormones lead to the postulation of an eventual substitution of iodide by bromide, which would result in the formation of brominated or mixed brominated/iodinated thyronines with similar hormone activity.

Finally, attention has been drawn to the necessity for trace analysis of iodide and bromide in water[2] because of the formation of trihalomethanes during oxidative treatment of drinking water.

Methods that have been described for trace analysis of iodide in various matrices include colorimetry/catalysis[3–5], electroanalysis[6,7], neutron activation analysis[8], mass spectrometry[9], gas chromatography (GC)[2,10] and high-performance liquid chromatography (HPLC)[11–13].

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A similar variety of methods can be found in the literature for trace analysis of bromide: colorimetry[14], neutron activation analysis[15], GC[2] and HPLC[16–18].

Our own investigations dealt with the applicability of ion chromatography with selective detection to the determination of traces of bromide and iodide. In an earlier study[19] we described the determination of iodide in serum and urine by an adaptation of the catalytic photometric detection described by Feigl and Jungreis[20] [chloramine-T and 4,4′-bis-(dimethylamino)diphenylmethane] for a post-column reaction detector. In this paper we report the results of iodide determinations in human serum, as well as the application to other matrices, such as food and water. Furthermore, it is shown that this reaction detector is also useful for determinations of bromide in biological materials and water samples.

EXPERIMENTAL

Instrumentation and reagents

The chromatographic instrumentation consisted of a Waters M510 HPLC pump, a Rheodyne 7125 injection valve with a 20-μl loop or a precolumn (40 × 4 mm I.D.) filled with a Vydac anion exchanger (30 μm particle size), a Vydac 302-IC anion-exchange separation column (250 × 4.6 mm I.D.) and a Perkin-Elmer Lambda 1 spectrophotometer equipped with a 8-μl flow-through cell; the post-column reagent addition was done by a laboratory-made device consisting of a closed 2-l bottle made of polypropylene, which could be raised to an overpressure of 0.2–2 bar by nitrogen. By applying appropriate pressure the reagent was fed through a PTFE capillary to the mixing device. The reagent and the column effluent were mixed by a PTFE tee-connector (Omnifit) and a PTFE reaction coil (3 m × 0.45 mm I.D.).

The mobile phase was prepared by dissolving 3.5 g (for bromide determination) or 4.5 g (for iodide determination) of methanesulphonic acid (Fluka) in 860 ml of water, adding a solution of 0.2 g of 4,4′-bis(dimethylamino)diphenylmethane (Merck, recrystallized from ethanol) in 140 ml of ethanol and bringing the pH to 4.0 with sodium hydroxide.

For preparation of the post-column reagent, a solution of 14 g of sodium acetate trihydrate in 815 ml of water and 45 ml of glacial acetic acid were mixed with a solution of 0.15 g (for iodide determination) or 0.30 g (for bromide determination) chloramine-T trihydrate in 140 ml of ethanol.

The flow-rate of the mobile phase was 1 ml/min and that of the post-column reagent 0.6 ml/min. The detection wavelength was 600 nm.

Determination of iodide or bromide in serum

Serum samples (2 ml) were ultrafiltrated using Sartorius-I tubes, and 20 μl of the filtrate were injected onto the column. In the case of elevated levels of iodide or bromide the sample can be diluted ten-fold and injected without any further pre-treatment.

Determination of iodide in milk, milk powder and non-iodized table salt

A 2-ml volume of milk (milk powder is reconstituted with an appropriate amount of water) was mixed with 4 ml of methanol and centrifuged, and 20 μl of the centrifugate were injected.

For determination of the total amount of iodine, a 50-mg sample (milk powder of lyophilized milk) was weighed into a filter paper and placed into the platinum sample holder of a 500-ml oxygen combustion flask containing 20 ml of distilled water, 20 μl of hydrazine monohydrate and 1 ml of 0.1 *N* potassium hydroxide. After burning, the flask was shaken for 20 min and 20 μl of the absorption solution were injected.

The iodide content of non-iodized table salt was determined by injection of 20 μl of a 4–10% salt solution.

Determination of iodide and bromide in water

The injection loop was replaced by the precolumn filled with anion-exchange material. Depending on the expected content up to 5 ml of the water sample were injected.

RESULTS AND DISCUSSION

Feigl and Jungreis[20] stated that their catalytic colour reaction between chloramine-T and 4,4′-bis-(dimethylamino)diphenylmethane was specific for iodide, even in the presence of an excess of bromide, as long as the proportion of 1:1 000 000 was not exceeded. In our experiments a 500–1000-fold excess of bromide yielded the same colour intensity as iodide. The sensitivity of the colour reaction for bromide could be improved to some extent by increasing the concentration of the chloramine-T solution, but the blank was also increased. The concentrations given in the experimental part were optimized with respect to a high signal-to-noise ratio. Despite the lower sensitivity for bromide this post-column reaction detector is useful for the detection of both iodide and bromide in biological material, as the bromide content is generally much higher than the iodide content.

Bromide and iodide were easily separated by ion chromatography using methanesulphonic acid as eluent. Post-column reaction detection was carried out with a hybrid reactor consisting of a pumpless system (doped mobile phase) and a conventional pumped reagent. The blue colour developed after 15–20 s; the flow-rates and reaction coil dimensions given in Experimental resulted in a residence time of *ca.* 18 s. The post-column reaction device increased the peak volume standard deviation (S.D.) from 65 μl to 217 μl. This was partly due to dilution of the mobile phase by the reagent (dilution factor 1.6), but peak dispersion might be reduced to a great extent by using a more efficient mixer and a knitted reaction tube. Nevertheless, the set-up described in this paper is adequate for routine work on various samples and is much simpler than the similar catalytic reaction detector of Lankmayr *et al.*[21] for determination of thyroid hormones, which consisted of a Technicon Autoanalyser with a special mixing device.

The detection limit (signal-to-noise ratio 3:1) of the method using iodide or bromide standards is *ca.* 20 pg iodide and 15 ng bromide injected. For iodide the S.D. of the method is 39 pg ($n = 5$) for an injection of 4.6 ng of iodide, and 16 pg ($n = 5$) for an injection of 460 pg of iodide. A linear relationship exists between the amount of iodide and the peak area from the detection limit up to at least 5 ng injected ($r = 0.9992$, $n = 5$). In the case of bromide the correlation coefficient for the calibration curve from the detection limit up to 3.2 μg injected is less favourable

TABLE I

INORGANIC IODIDE IN HUMAN SERUM AND URINE

Group A: normal diet; group B: additional daily oral intake of *ca.* 3 mg of iodide for 3 weeks

	Iodide in serum (μg/l)	*Iodide in urine (μg/g creatinine)*
Group A (n = 22)	7.2 ± 5.8	89 ± 74
Group B (n = 22)	131 ± 67.5	4003 ± 2780

(r = 0.9966, n = 6) because of a slight parabolic curvature. Nevertheless, a linear approximation is possible within small ranges. The S.D. is 5.7 ng of bromide at a level of 160 ng of bromide injected.

In continuation of previous work[19], it was now possible to measure inorganic iodide levels in plasma during normal diet and therapy with iodide-containing drugs. These data, which are listed in Table I, may be considered as a complement to results obtained by measurements of urinary iodide excretion.

Fig. 1 shows chromatograms of bromide in human serum after normal diet and in rat serum after controlled oral uptake of bromide.

Milk samples (fresh milk or reconstituted milk powder) were injected after protein precipitation by addition of methanol. Though this sample preparation does not remove fats, no interferences were observed, but as a precaution the column was flushed with methanol after *ca.* 25 injections. Recovery experiments were carried out by spiking a milk sample containing 20 μg iodide/l with 23 μg iodide/l. The average recovery (n = 5) was 106.5 ± 2.3% (S.D.). Fig. 2 shows the chromatogram of a BCR reference material of spiked skim milk powder with a certified iodine content of 5.35 μg/g. An amount of 5.60 μg/g was found by our method. It is also possible to differentiate between iodide and other iodine species, if the total amount of iodine is determined after oxygen flask combustion of dried or lyophilized samples as proposed by Hurst *et al.*[12], who used HPLC with UV detection, which is inferior to the detection principle presented here with respect to selectivity and sensitivity.

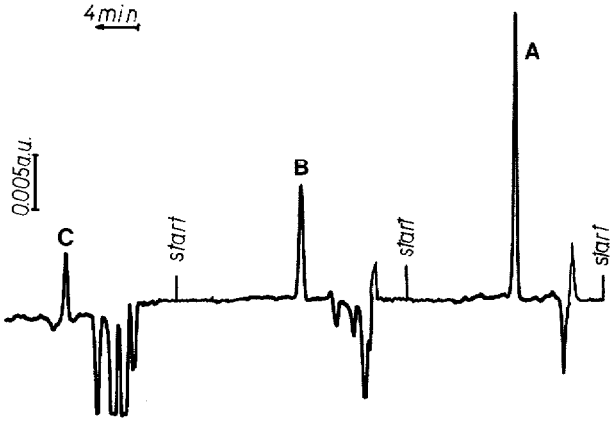

Fig. 1. Typical chromatograms for bromide in serum. (A) Standard 8 mg bromide/l; (B) ten-fold diluted serum of a rat with a daily oral intake of 0.4 mg of bromide for 3 weeks; (C) human serum, ultrafiltrated.

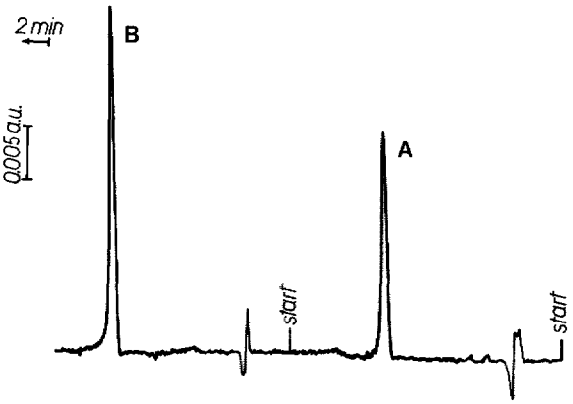

Fig. 2. Determination of iodide in milk. (A) Skimmed milk powder; (B) standard containing 26.9 μg iodide/l.

The natural iodide content of non-iodized table salts may be lower than 50 μg/kg[13]. Fig. 3 presents the chromatogram of a 20-μl injection of a 4% sodium chloride solution, the salt containing 0.36 mg iodide/kg. Since 10% salt solutions may be injected without loss of chromatographic resolution, the detection limit is at least 10 μg/kg.

Luckas[13] proposed a scheme for differentiating between iodide, elemental iodine and iodate in salt using HPLC with UV detection: the iodide content is determined by a first injection; then the sample is reduced by hydrazinium sulphate and injected a second time to yield the sum of iodide and elemental iodine; the sum of iodide, elemental iodine and iodate is determined by a third injection after reduction with tin(II) chloride. Nevertheless, this scheme may lead to erroneous results because our experiments indicated that under HPLC conditions (high pressure, stainless-steel columns and tubing) an on-column reduction of elemental iodine takes place. Fig. 4 shows the results of our experiments concerning on-column reduction of iodine; these experiments were done with ion-pair chromatography [RP-18 as stationary

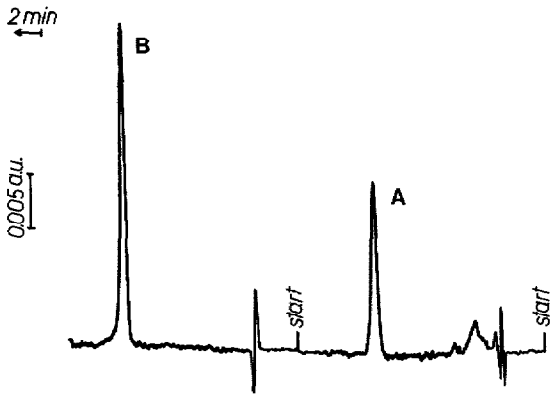

Fig. 3. Determination of iodide in sodium chloride. (A) 4% sodium chloride solution; (B) standard containing 26.9 μg iodide/l.

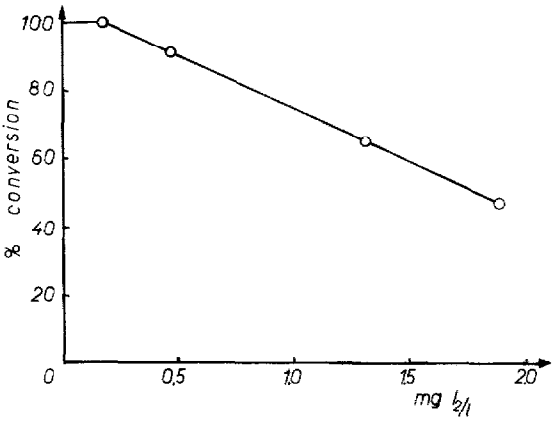

Fig. 4. On-column reduction of elemental iodine: percentage conversion as a function of sample concentration.

phase, 0.01 *M* octylammonium phosphate (pH 4) as mobile phase] and UV detection at 226 nm. The same results hold true for ion-exchange chromatography. This means that generally only the sum of iodide and elemental iodine can be determined.

When analysing water samples it is possible to inject sample volumes up to 5 ml (larger volumes were not tried), if the sample loop is replaced by a short anion-exchange column. This means that the detection limit can be lowered to a sample concentration of *ca.* 4 ng/l (4 ppt) for iodide and 3 μg/l (3 ppb) for bromide. Nevertheless, it should be stressed that only free bromide and iodide can be measured and the possibility cannot be excluded that these ions form complexes with certain cations and are therefore not detectable.

On the whole, ion chromatography with selective post-column reaction detection as described is this paper seems to be a good alternative to existing methods for determination of iodide and bromide in complex biological matrices, as well as in water at ppt and ppb levels. Sample preparation is minimal, which reduces the risks of contamination and is advantageous with respect to automation.

REFERENCES

1 P. C. Scriba, in R. Hall and J. Köbberling (Editors), *Thyroid Disorders Associated With Iodine Deficiency and Excess*, Raven Press, New York, 1985, p. 7.
2 M. Grandet, L. Weil and K. E. Quentin, *Z. Wasser Abwasser Forsch.*, 16 (1983) 66.
3 G. Aumont and J. C. Tressol, *Analyst (London)*, 112 (1987) 875.
4 P. W. F. Fischer, M. R. L'Abbe and A. Giroux, *J. Assoc. Off. Anal. Chem.*, 69 (1986) 687.
5 A. Wiechen and B. Kock, *Fresenius' Z. Anal. Chem.*, 319 (1984) 569.
6 W. Holek, *Anal. Chem.*, 59 (1987) 2218.
7 D. Thompson, S. Lee and R. Allen, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 1380.
8 J. J. Fardy and G. D. McOrist, *J. Radioanal. Nucl. Chem. Lett.*, 87 (1984) 239.
9 W. Schindlmeier and K. G. Heumann, *Fresenius' Z. Anal. Chem.*, 320 (1985) 745.
10 D. J. Doedens, *J. Anal. Toxicol.*, 9 (1985) 109.
11 W. J. Hurst, J. W. Stefovic and W. J. White, *J. Liq. Chromatogr.*, 7 (1984) 2021.
12 W. J. Hurst, K. P. Snyder and R. A. Martin, *J. Liq. Chromatogr.*, 6 (1983) 2067.
13 B. Luckas, *Dtsch. Lebensm. Rdsch.*, 82 (1986) 357.

14 T. Anfält and S. Twengström, *Anal. Chim. Acta*, 179 (1986) 453.
15 Z. B. Alfassl and N. Lavl, *Anal. Chem.*, 55 (1983) 796.
16 C. E. Goewie and E. A. Hogendoorn, *J. Chromatogr.*, 344 (1985) 157.
17 M. E. Miller and C. J. Cappon, *Clin. Chem.*, 30 (1984) 781.
18 P. de Jong and M. Burggraaf, *Clin. Chim. Acta*, 132 (1983) 63.
19 W. Buchberger and K. Winsauer, *Mikrochim. Acta Wien*, III (1985) 347.
20 F. Feigl and E. Jungreis, *Fresenius' Z. Anal. Chem.*, 161 (1958) 87.
21 E. P. Lankmayr, B. Maichin, G. Knapp and F. Nachtmann, *J. Chromatogr.*, 224 (1981) 239.