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THE GAS–LIQUID CHROMATOGRAPHIC DETERMINATION OF INORGANIC IODINE, IODIDE AND TIGHTLY BOUND IODINE IN MILK

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SUMMARY

Methods are described whereby the content of inorganic iodine, iodide and tightly bound organic iodine in milk can be determined by gas–liquid chromatography, either separately or in combination. The procedure can also be used for other biological specimens.

INTRODUCTION

The iodine content in milk is proportional to the iodine intake and reflects the degree of contamination of the milk. The use of iodophors as disinfectants in sanitation programmes for mastitis control, feeding with mineral supplements containing high iodine concentrations, and administration of potassium iodide as a therapeutic in reproduction disorders may result in higher iodine concentrations[1–3]. Milk constitutes a significant source of iodine for humans: it is beneficial in connection with its antigoitrogenic properties, but may be detrimental in some instances owing to toxic effects caused by overdosing. From these points of view and in order to study the function of iodine as an essential trace element in normal metabolic processes in man and animals, a highly sensitive and specific analytical method is needed.

Very sensitive micro-scale methods for the determination of iodine are based on Sandell and Kolthoff's reaction[4,5]. The method is satisfactory for samples that contain iodine in the 0.4–10.0 ng range, with an overall error of less than 6%, but some divalent cations (Cu, Ni, Co, Fe, Zn, Hg), Cr(III) and Ag(I) can interfere[5]. Recently, Hasty[6,7] described a gas chromatographic procedure based on the derivatization of iodine with ketones and measuring their response to an electron capture detector. The method is relatively simple and rapid, free from serious interferences and permits the determination of iodine and iodide separately and together. The detailed procedure for the various iodine compounds was not described, however. Moreover, the liquid phase proposed (5% SE-30 on Varaport) is unsuitable because of tailing peaks, low column efficiency and short retention times.

This paper describes an improved gas–liquid chromatographic (GLC) method for the routine determination of inorganic iodine, iodide and tightly bound (C–I) organic iodine in milk, which can be easily applied to various biological materials.

PROCEDURE

Reagents

All chemicals used were of analytical-reagent grade. The filter paper was the type for analytical purposes, retaining fine-grained precipitates. Water was glass-distilled from 0.05% sodium hydroxide solution.

Sulphuric acid (5.0 *M*), hydrochloric acid (2.0 *M*), potassium carbonate (2.0 *M*) and acetone (5.0 *M*) were used. Potassium iodate was a 100 ppm solution in water and *n*-hexane was of "Nanograde" reagent purity.

Iodide standard. Potassium iodide, dried in a dark desiccator, was used to prepare the standard solutions. A stock iodide solution (100 ppm of I^-) was made by dissolving 32.7 mg of potassium iodide in deionized water and diluting to 250 ml. A working iodide standard solution (1 ppm of I^-) was prepared by diluting the stock iodide solution 1:100 with deionized water. As iodide is sensitive to light, the iodide solutions should be stored in the cold in dark bottles.

Iodine standard. To prepare a 1 ppm iodine solution, about 10 mg of resublimed iodine were dissolved in hot (50°) deionized water, cooled to room temperature and diluted with water so as to give an absorbance of 0.00588 in a 1-cm cell at 460 nm (ref. 6).

A 0.01 ppm working iodine standard solution was prepared by diluting the 1 ppm solution 1:100 with distilled water. The iodine standards were stored under the same conditions as the iodide solutions.

Derivatization

Iodide. Samples of 12 ml of milk were mixed well with 12 ml of water and 3 ml of 5 *M* sulphuric acid, allowed to stand for 15 min at room temperature for protein precipitation and then filtered. Portions of 4.5 ml of the clear filtrates (some may be opalescent) were placed in glass-stoppered 25- or 50-ml flasks, mixed with 0.5 ml of 5 *M* acetone and 0.05 ml of 100 ppm potassium iodate solution, allowed to stand for 30 min at room temperature for conversion of iodine into iodoacetone and shaken vigorously for 5 min with 5 ml of *n*-hexane. The mixtures were transferred into test-tubes (16 × 100 mm) and centrifuged for 3 min at 5000 rpm. The upper organic phase was decanted into a small test-tube (12 × 100 mm), stoppered with tin-foil and 0.5-μl aliquots of the *n*-hexane extract were injected into the gas chromatograph.

A blank and standard were prepared by using 12 ml of water or 10.8 ml of water plus 1.2 ml of working iodide standard instead of milk throughout the entire procedure. Portions of 0.2 μl of standard and 0.5 μl of blank were injected on to the GLC column.

Inorganic iodine. Portions of about 5 ml of defatted and deproteinized filtrates obtained as described for iodide were placed in a 5-ml Cornwall syringe fitted with a 25-mm filter holder and filtered through a GS 0.22-μm Millipore membrane filter.

Then 4.5-ml samples of clear aqueous filtrates were transferred into glass-stoppered flasks, mixed with 0.5 ml of 5 *M* acetone, allowed to stand for 30 min at room temperature and extracted into 5 ml of *n*-hexane. The procedure was then continued as for iodide.

For every set of analyses, a blank and standard, in which 12 ml of milk was replaced by 12 ml of distilled water or 12 ml of 0.01 ppm iodine standard, were carried

through the tests in exactly the same way as the unknown. Portions of 3 μl of unknown and blank and 0.3 μl of a standard were injected on to the GLC column.

Total iodine. A 0.4-ml volume of fresh milk was placed in a 35-ml "Rasotherm" Kjeldahl long-necked flask, mixed with 1.0 ml of 2 *M* potassium carbonate solution, dried overnight at 105° and then dry ashed in a muffle furnace at 600–610° for exactly 2.0 h. On the following day, the white residue was dissolved in 2 ml of 2 *M* hydrochloric acid, diluted to 4 ml with distilled water, mixed with 0.5 ml of 5 *M* sulphuric acid, 0.5 ml of 5 *M* acetone and 0.05 ml of 100 ppm potassium iodate solution. After 30 min, the iodoacetone formed was extracted with 5 ml of *n*-hexane, the upper organic layer was decanted into test-tubes and 0.2-μl aliquots were injected into the GLC column. The blank, consisting of 1 ml 2 *M* sodium carbonate solution, was run in the same way as the unknown and the standard used was the same as described for iodide. A 0.2-μl sample of blank and 0.8 μl of standard were injected into the chromatograph.

Determination of iodoacetone by GLC

A Pye Series 104 chromatograph fitted with a nickel-63 electron capture detector was used.

The column was 1.5 m long and 6 mm O.D. and was filled with 80–100 mesh Varaport 30 coated with 5% of 1,5-pentanediol succinate. The liquid phase was prepared in our laboratory as follows. An 11.0-ml volume of 1,5-pentanediol and 11.8 g of succinic acid were heated in an oil-bath to 160° and 32 g of anhydrous copper(II) sulphate were added to the clear solution. After submersion for 2 h in the oil-bath at 160° with continuous stirring, the mixture was left to cool. The cold re

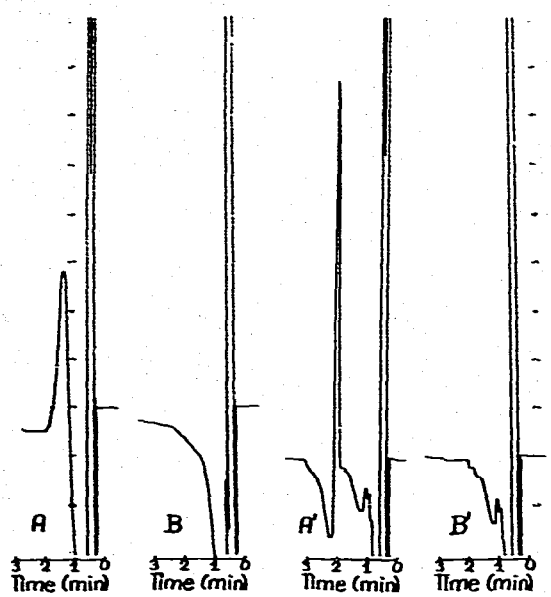

Fig. 1. Chromatograms of iodoacetone standard (A, A') and blank (B, B') using a nickel-63 electron capture detector. Columns: 1.5 m packed with 5% SE-30 (A, B) and 5% 1,5-pentanediol succinate (A', B') on 80–100 mesh Varaport 30. Column temperature, 125°; detector temperature, 150°; argon flow-rate, 100 ml/min.

action product was extracted with two 100-ml portions of dichloromethane and clarified by means of filter paper. The filtrate obtained was allowed to stand overnight in a glass-stoppered bottle in a cold room (4°) and filtered again. The clear solution was concentrated by evaporation of dichloromethane in a water-bath at 90°. Then the solvent-free ester was heated in an oil-bath at 250° for 1 h and cooled to room temperature. The dark brown residue was dissolved in 60 ml of chloroform, shaken twice with 5 g of activated charcoal and filtered through a filter paper. The clear, pale yellow filtrate was mixed with an equal volume of methanol and shaken twice with 50 ml of four-times-distilled water. The upper methanolic–water phase was discarded and the lower phase was filtered through a filter paper and a G-5 sintered glass filter. After vacuum evaporation of chloroform at 100°, 14 g of a pale yellow, oily substance were obtained, which, after several days, became solid and white. It should be noted

TABLE I

PRECISION OF ANALYSIS OF DIFFERENT IODINE FORMS IN COW'S MILK TAKEN BEFORE AND AFTER INCOSAN W TEAT DIPPING

Day before or after teat dipping	*Inorganic iodine (ppb)* *	*Iodide (ppm)*		*Total iodine (ppm)*	
		Series 1	*Series 2*	*Series 1*	*Series 2*
Before					
8	0.91	0.037	0.037	1.993	2.105
7	1.03	0.032	0.036	2.183	2.292
6	0.93	0.034	0.035	2.323	2.336
5	1.42	0.036	0.036	2.221	2.416
3	0.75	0.032	0.032	1.901	1.990
2	1.17	0.043	0.042	2.171	2.291
1	1.18	0.038	0.038	1.920	2.016
Average of series	1.06	0.036	0.036	2.093	2.192
Average value of two series ± standard deviation	± 0.20	0.0362 ± 0.0032		2.143 ± 0.167	
After					
1	1.03	0.040	0.039	2.195	2.308
2	0.86	0.045	0.048	2.277	2.355
3	1.00	0.044	0.046	2.059	2.176
5	1.26	0.043	0.041	2.022	2.061
6	0.91	0.034	0.033	2.330	2.323
7	0.96	0.038	0.039	1.976	2.232
8	0.98	0.035	0.034	2.138	1.786
9	1.18	0.050	0.047	2.042	2.278
10	1.32	0.037	0.034	2.150	2.208
12	1.10	0.037	0.035	2.006	2.090
13	1.13	0.041	0.041	2.137	2.167
14	1.33	0.032	0.033	1.966	2.412
22	1.24	0.042	0.043	2.227	2.266
28	0.85	0.026	0.028	2.304	2.362
Average of series	1.09	0.039	0.039	2.131	2.216
Average value of two series ± standard deviation	± 0.16	0.0388 ± 0.0058		2.173 ± 0.144	

* Throughout this article, the American billion (10^9) is meant.

that all glassware, filter papers and charcoal should be purified by washing with chloroform, and the reagents used should be of the highest purity possible.

The flow-rate of argon used as carrier and scavenger gas was 100 ml/min.

The most suitable column and detector temperatures were found to be 125° and 150°, respectively. The detector was operated in the pulsed (500 μsec) mode. The amplifier controls were set as follows: backing of $\times$ 100, attenuator $2 \cdot 10^2$ for free iodine, $5 \cdot 10^2$ for iodide and $10 \cdot 10^2$ for total iodine determinations.

RESULTS

Fig. 1 shows typical chromatograms obtained with iodine standard and blank by the use of the above column packing and that proposed by Hasty[6]. Table I gives the results of inorganic iodine, iodide and total iodine determinations before and after iodophor Incosan W (Inco, Warsaw, Poland) teat dipping. The total iodine concentration found by the recommended method significantly exceeded the levels (0.050–0.150 ppm) which were considered to be normal by Iwarsson and co-workers[2,3], and resembled that (1.9 ppm) obtained by Iwarsson[2] on cows which had received minerals with extremely high iodine concentrations.

DISCUSSION

As shown in Fig. 1, the form of the peak, the retention time and the sensitivity were better when 1,5-pentanediol succinate was used instead of SE-30.

According to the findings of Hasty[6], only monoiodoacetone was found as the reaction product. 1,1-Diiodoacetone appeared if the reaction medium was less acidic than that proposed by Hasty[6] (0.5 *M* sulphuric acid).

The clean-up and extraction procedures used in the present work gave excellent peaks at 3-min intervals. Moreover, no decrease in the sensitivity of the detector during continuous operation over a 7-h period was observed, although Hasty[6,7] noted a gradual deterioration in the detector response. In order to prevent the gradual build-up of deposits on the column or detector, they should be regenerated overnight using temperatures 50% higher than during the operating conditions.

Although all halogens react equally well with ketones, nevertheless the effects of fluorine, chlorine and bromine can be disregarded because of the negligible amounts connected with strong oxidative properties and high toxicity for living organisms. On the other hand, halides other than iodide cannot be oxidized to halogens by iodate. Therefore, it seems that the method described here is relatively free from interferences.

The use of the same amounts and chemical forms of the standard and the unknown enabled convenient calculations to be made, because the effects of different sample sizes and distribution coefficients between *n*-hexane and the aqueous layer, and of the amplification during the derivatization, could be omitted. The standard and unknown did not vary other than in the aliquots injected into the column, and only in the case of total iodine should a factor 5 (0.4 ml of milk and 2.0 ml of standard) be used. Moreover, owing to the linearity of detector response found by Hasty[6], no calibration graphs need to be checked daily.

In order to evaluate the precision, duplicate analyses of iodide and total iodine

in 21 average milk samples (each from ten cows) were performed. The relative standard deviation calculated on the basis of results listed in Table I was 2.46% for iodide and 2.64% for total iodine. The precision of the procedure depends to a great extent on the sample injection technique and quality of the syringe, and on the shaking technique. The time of contact of two phases should be not less than 5 min. Derivatization of iodine with 2-butanone or preferably 3-pentanone instead of propanone improves the extraction, because the products yielded are extracted more completely[7]. Additionally, in the case of the determination of total iodine, care should be taken with dry ashing. Only a high-quality muffle furnace with a constant temperature over the whole of the chamber will give good reproducibility.

The accuracy of every analytical method as well as the detection limit also depend on the blank value. For ten determinations, the mean blank values ± standard deviations were as follows: inorganic iodine, 0.06 ± 0.016 ppb; iodide, 0.014 ± 0.0005 ppm; total iodine, 0.055 ± 0.002 ppm. Thus the detection limit, defined as the amount of an element that gives a reading of twice the standard deviation, is 0.03 ppb for inorganic iodine, 0.001 ppm for iodide and 0.004 ppm for total iodine.

In order to check the iodine losses during alkaline mineralization, the recovery of 1 ppm of iodide added to five milk samples was tested. The average value obtained of 80.5% agreed well with the 17% total iodine loss found by Matthes *et al.*[4] for grass samples with iodine-131 as tracer.

It seems, therefore, that the proposed procedure possesses satisfactory sensitivity, selectivity, precision and accuracy for application to the study of iodine metabolism in physiological and pathological conditions, as well as of iodine residues in foodstuffs.

Finally it should be noted that the values given in Table I can be compared with other published values in only a few cases. Most workers determined the total iodine concentration in milk by means of the technique described for PBI* in blood serum, employing bromide–bromate solution in a mixture of about 2 *N* acetic acid and 0.05 *N* sulphuric acid to liberate the bound iodine. The acidity in this reaction system corresponds to that (1 *N* sulphuric acid) used in our method for the liberation of inorganic iodine and iodide from complexes. Iodide was then oxidized to iodine in the reaction with iodate. The remaining tightly bound iodine represents the compounds in which C–I bonds occur. The energy of this bond (53 kcal/mole) is similar to those of C–P and C–S bonds, which are resistant to acid hydrolysis and can be disrupted only by wet or dry ashing. Therefore, the values obtained by the PBI method represent only the fractions of associated iodine and iodide. In order to determine total iodine, a digestion procedure should be used.

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* PBI = Protein bound iodine.