Comparison of Ion-Specific Electrode and High Performance Liquid Chromatography Methods for the Determination of Iodide in Milk¹

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ABSTRACT

Two methods for the determination of I[−] in raw and processed milk were examined. A simple ion-specific electrode (ISE) method was compared against a more complex HPLC reference technique. Accuracy and precision were evaluated both within and between the 2 methods. Both methods yielded good recoveries for I[−] on spiked samples, ranging from 87 to 114% for ISE and 91 to 100% for HPLC. Within-run repeatability and between-run reproducibility were superior with the HPLC method, but were still more than acceptable with the ISE technique. Overall agreement of paired results between ISE and HPLC methods was good ($r^2 = 0.85$ on raw herd milk; $r^2 = 0.84$ on processed milk). The ISE method had a significant positive bias relative to the HPLC reference method. Both methods lend themselves well to the measurement of I[−] in raw or processed milk. Given its relatively low cost and ease of use, the ISE method is well suited as a screening method. The impressive accuracy, precision, selectivity, and limit of detection of the HPLC technique make it an ideal confirmation method.

Key words: iodine analysis, milk, ion-specific electrode, high performance liquid chromatography

INTRODUCTION

Concerns over the levels of I found in milk have emerged globally during the past 25 yr. Studies conducted in several countries suggest that there may be a public health concern caused by the presence of excessive amounts of this trace element in both raw and processed milk supplies. Some jurisdictions have, as a direct result of these concerns, established legislation that regulates the maximum allowable limit for I in milk. Iodine is required for synthesis of various thyroid

hormones that are critical for growth, reproduction, thermoregulation, calorigenesis, intermediary metabolism, protein synthesis, and neuromuscular function (Fisher and Carr, 1974).

Some methods measure total I, and others estimate I in the I[−] form. Classical microchemical approaches used for the estimation of I include mineral-distillation methods (Stolc and Nemeth, 1961) and alkaline-ashing methods (Stabel-Taucher, 1975). Although numerous modifications have been made to both methods over the years, the precision and accuracy of these procedures were often hindered by interference of fats and proteins. Other methods used to determine I in biological matrices are inductively coupled plasma mass spectrometry (Browner, 1987; Harris, 1987) and neutron activation analysis (James, 2001).

A rapid and inexpensive method for determination of I in milk is the ion-specific electrode (ISE) method. An electrode specific for the detection of the I[−] ion is coupled to a reference electrode, both of which are connected to a pH meter that is set to the potentiometric mode. Differences in the measured potential between the 2 electrodes are proportional to the concentration of the I[−] ion in a sample. The system is first calibrated against a series of aqueous I[−] standards that encompass the expected range of concentration. A Ni(NO₃)₂ matrix modifier is added to the milk samples to flood the ionic background of the sample, creating a stable environment against which the change in I[−] concentration can be measured (Richardson, 1985).

Although acceptable correlations between ISE methods and chemical reference methods have been reported, problems such as low recovery percentage, long response time at low I[−] concentrations, and instrument drift have been cited (Wheeler et al., 1980). The potential can be measured directly from the modified sample matrix, although it is generally believed that better accuracy and precision can be obtained by using a method of known addition. Known addition procedures record the potential both before and after the addition of a known quantity of I[−] standard to the matrix; the difference between the 2 measurements is then used to calculate the final concentration of the analyte.

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Methods for the determination of I by HPLC are usually based on ion chromatography. Iodine in milk is present almost exclusively in the I^- form (Underwood, 1977), and the quantitation of I^- (vs. total I) in milk using HPLC is a recognized official method (Association of Official Analytical Chemists, 1993). The separation of ionic species using HPLC is typically accomplished through the use of ion-pair chromatography (Gloor and Johnson, 1977) or ion-exchange chromatography. Ion-exchange chromatography is a form of adsorption chromatography, which involves the substitution of one ionic species with another (Hamilton and Sewell, 1977).

MATERIALS AND METHODS

Samples used in this study were selected at random from within the various milk-producing regions throughout the province of Ontario. The ages of the fresh milk samples tested were between 3 to 5 d.

An ISE method was used for the rapid determination of I^- in raw milk samples and was based, in part, on existing methods (Craven and Griffith, 1977; Richardson, 1985). The method used an Orion I^- ion selective electrode (model no. 945300, Thermo Electron Corp., Waltham, MA), a Corning model 476530 general purpose reference electrode (BDH Inc., Toronto, ON, Canada), and a Radiometer PHM84 research meter (Radiometer Analytical, Lyon, France) that was set to the potentiometric mode. A 2.0 M solution of $Ni(NO_3)_2$ was used as the matrix modifier.

The electrodes were first set in approximately 100 mL of a prepared 10-mg/L standard and stirred with a magnetic stirrer; the meter was set to display readings in absolute millivolts. Once the electrodes had equilibrated to a point where they were not fluctuating >0.1 mV (typically 2 to 3 min), the output usually reached a potential of between -100 and -90 mV. The same procedure was repeated for a 1-mg/L standard solution. When the meter and electrode were operating within specifications, the anticipated changes in potential of approximately 60 mV were usually observed. Prepared standards of 100, 200, 400, 500, and 700 $\mu\text{g/L}$ were then run and recorded. Linearity of the calibration was checked externally by plotting the response (mV) vs. I^- concentration (mg/L) on a semilogarithmic scale; concentration appeared on the logarithmic axis. Calibrations with correlation coefficients of <0.95 were deemed unacceptable and were repeated. Failure to achieve the required minimum acceptance level on subsequent attempts resulted in flushing the electrolyte in the reference electrode, polishing the surface of the I^- selective-ion electrode according to the manufacturer's instructions, or replacing standards.

The reference method for this study used a Dionex DX 500 ion chromatography system consisting of a Dionex GP40 gradient pump, a Dionex AS40 autosampler, a Dionex ED40 electrochemical detector, a Dionex IonPac AS11 analytical column (4×250 mm), a Dionex IonPac AG11 guard column (4×50 mm), and a Dionex LC10 chromatography organizer (Dionex Corporation, Sunnyvale, CA). The GP40 dual pump heads and the tubing were composed of polyetherether ketone to ensure compatibility with the acidic mobile phase. Separation was isocratic, and the detector was fitted with an amperometry flow cell with Ag working electrode, AgCl reference electrode, and titanium counter electrode.

The method was based on a modified version of Dionex Application Note 37 (Dionex Corporation, 1996). The method is appropriate for use on both raw and processed milk samples, including reconstituted powdered milk samples. The analytical procedure first used a series of sample preparation steps. Samples were heated to 40°C in a Julabo model 16A constant-temperature water bath (JulaboLabortechnik, Seelbach, Germany) with Haake model D1 temperature controller (Haake, Berlin, Germany) to ensure complete melting of the milk fat. After 5 min at 40°C, the samples were inverted 8 to 10 times to ensure even distribution of fat globules, cooled to approximately 20°C in a second Julabo model 16A constant-temperature water bath, and finally mixed once again by inverting 8 to 10 times.

A 50-mL aliquot of milk was then measured in a 50-mL graduated cylinder and transferred into a 100-mL volumetric flask. This was followed by the addition of 4.0 mL of 3% acetic acid solution) using a graduated 5-mL pipette. The solution was then mixed for 5 to 10 s using a VWR Scientific Vortex-Genie 2 (model G-560). A 1-mL volumetric pipette was used to deliver 1.0 mL of concentrated nitric acid to the sample mixture, which was again mixed by vortexing. The contents of the volumetric flask were diluted to the 100-mL mark with high-purity water and mixed thoroughly by vortexing. The resultant pretreated sample mixture was then passed through an 18.5-cm Whatman 2V filter paper to prevent fouling of the Centriflo membrane (Amicon, Beverly, MA), which was used in the next step. The filtrate was collected in a 125-mL Erlenmeyer flask.

New Centriflo membrane cones were soaked in a 10% (vol/vol) ethanol solution for ≥ 1 h prior to initial use. Filter cones were then removed from the alcohol solution, drained, and placed in individual plastic conical Centriflo membrane supports, which were then placed in plastic, 50-mL centrifuge tubes filled with high-purity water and centrifuged for 10 min at $1.0 \times g$ (2,600 rpm) on a Beckman GS-6 centrifuge using a model GH 3.8, 3,750-rpm maximum rotor. The cones with supports were inverted and left to drain for 5 min, after

which a small Kimwipe was used to dab the inside surface of the cones to remove any excess traces of water. After each use, the Centriflo cones were 1) flushed with hot (~55 to 65°C) water to help remove any remaining fat and protein residues; 2) submersed for 1 h in beakers of 0.1 *N* NaOH and then rinsed 3 times with high-purity water; 3) placed in plastic conical supports filled with high-purity water and centrifugation at $1.0 \times g$ for 10 min—then repeating this process 2 more times; 4) agitated by inverting the conical supports and drained for 10 min; and finally, 5) removed from the supports and submersed overnight in beakers containing aqueous solutions of 10% (vol/vol) ethanol or, for longer-term storage, in 0.1% aqueous solutions of sodium azide.

The sample filtrates were poured into the prepared Centriflo cones, filling them to within 5 mm of the top using 14.6-cm Pasteur pipettes. Samples were then centrifuged at $1.0 \times g$ for 30 min, after which the resulting supernatant was passed through prepared Chromosep-RP C18 cartridges. The first approximately 3 mL of supernatant was discarded, and the remainder was collected into two 0.5-mL plastic autosampler vials and capped. Two 0.5-mL aliquots were then created for each milk sample, thus allowing for duplicate determinations on each milk sample. The injection volume for each final prepared sample was 100 μ L.

Potassium iodide calibration standards were prepared using serial dilutions of a stock standard solution. A 100-mL aqueous stock solution with a concentration of 10,000 mg/L of KI was prepared by weighing out 1.307 g of reagent-grade KI; transferring the weighed reagent quantitatively to a 100-mL volumetric flask, making its volume rise to the mark with high-purity water; and allowing the solution to equilibrate for 1 to 2 d. Intermediate standard solutions of 1,000, 100, 10, and 1 μ g/L were made by serial dilution. Working standards of 50, 100, 200, and 400 μ g/L were prepared. A water and nitric acid mobile phase was prepared, resulting in a final required concentration of 50 mM nitric acid. The normal flow rate used during analytical runs was 1.5 mL/min.

The electrochemical detector was configured to operate in direct current amperometry mode. A Ag working electrode was used along with a Ag/AgCl reference electrode. The applied voltage during analysis was +0.05 V. Reversal of the current to -0.10 V was used between samples to help clean AgI residues from the surface of the Ag target. This compound forms as a byproduct of the detection reaction between I^- ions from the sample and the Ag target while under the influence of a positive applied voltage. So, reversal of the applied voltage helps reduce fouling of the Ag electrode. Despite this precaution, AgI eventually does form a molecular “skin” over

the active surface of the Ag working electrode, and periodic polishing (about every 400 samples) of the Ag target was required. The system was typically left under low-flow (0.1 mL/min) conditions with no voltage applied between runs, so as to maintain the equilibrated state of the columns and the reference electrode of the detector.

Calibration of the system was required prior to the start of each sample run, once a stable baseline was achieved. A 4-point calibration using 50, 100, 200, and 400 μ g of KI/L standards was used. A minimum correlation coefficient of 0.997 was consistently attained and, hence, became the minimum acceptance criterion for calibrations prior to each run, although values of 0.999+ were more typical.

A maximum of 20 samples were prepared and analyzed within a discrete sample run; 2 aliquots for analysis were prepared from each. Control standards were included, in duplicate, in each run and were evenly distributed among the start, middle, and end of the run. The performance of these standards was used to evaluate the success of each run throughout the active testing range (between 50 and 400 μ g/L); maximum deviations of up to $\pm 10\%$ were accepted. Data from sample runs in which these control limits were exceeded were not included in the database. Sample concentrations greater than the active calibration range after the initial run were appropriately diluted with high-purity water and were rerun to bring them within the required testing range.

RESULTS AND DISCUSSION

Mean ISE recoveries for 50-, 100-, 200-, 400-, and 1,000- μ g/L standard spikes on 25 milk samples were 114 ± 18 , 99 ± 7 , 108 ± 10 , 96 ± 8 , and 87 ± 6 μ g/L, respectively. Because average levels of recovery for near trace-level work typically vary from 80 to 120%, the observed levels of recovery for this method are excellent. Overall levels of recovery were, on average, higher for the 50-, 100-, and 200- μ g/L standard spikes vs. those for the 400- and 1,000- μ g/L standard spikes. This could have been due to differences in instrument linearity throughout the test range.

Mean HPLC recoveries for 100-, 200-, 400-, and 1,000- μ g/L standard spikes on 25 milk samples each were 94 ± 6 , 94 ± 6 , 100 ± 6 , and 91 ± 5 μ g/L, respectively. Overall, levels of recovery were very similar for all 4 sets of data, indicating that the response of the method was similar regardless of the I^- concentration. The limit of detection of the HPLC method (calculated as $3 \times$ SD of analyte blank) is 6 μ g/L, and the limit of quantitation (calculated as $10 \times$ SD of analyte blank) is 20 μ g/L.

Table 1. Summary of precision statistics for determination of I^- by ion-specific electrode (ISE) and HPLC methods

	ISE	HPLC
	(μg/L)	
Repeatability (within runs)		
Mean absolute difference	14	2
SD	12	2
Number of samples	25	51
Reproducibility (between runs)		
Mean difference	10	-4
SD	17	16
Number of samples	20	24

Table 1 is a summary of the precision statistics for both the ISE and HPLC methods. Results that differed by $>5 \times SD$ of differences were considered outliers and removed from the data set. There were 2 such outliers at 469 and 503 μg/L.

A comparison of the relative accuracy of the ISE vs. HPLC methods based on the paired analytical data from 134 herd milk samples yielded overall means of 276 ± 155 and 231 ± 132 μg/L, respectively. Results reported using the ISE method were approximately 19% higher, on average, than those reported using the HPLC method for the same samples. The overall mean difference on the paired data for ISE vs. HPLC was 45 ± 89 μg/L. A correlation coefficient of 0.85 was calculated on the outlier-corrected data set for ISE vs. HPLC. The data, therefore, suggest reasonably strong correlations between results from both of these analytical methods.

The overall means for the ISE method data and the HPLC method data on 67 processed milks were 343 ± 85 and 307 ± 87 μg/L, respectively. Results reported by ISE were, again, higher on average (by approximately 12%) than those reported by HPLC on the same samples. The overall mean difference on the paired data for ISE vs. HPLC was 36 ± 48 μg/L. A correlation coefficient of 0.84 was calculated on the uncorrected raw data set, because no extreme outliers were observed within the differences between individual data pairs. The paired results for processed milk again suggest that there is a fairly strong correlation between the data produced by both ISE and HPLC methods.

CONCLUSIONS

Within-method precision and accuracy was good for the ISE method and excellent for the HPLC method. Agreement between ISE and HPLC methods was acceptable. A significant positive bias of the ISE method

relative to HPLC may be due, in part, to interfering ions because the method is not exclusively selective for I^- . Therefore, both methods are suitable for measuring I^- in raw or processed milk. Low cost and simplicity of the ISE method make it well suited as a rapid screening method. In jurisdictions where maximum residue limits are established for I^- , the HPLC method is well suited as a confirmation method for ISE results that are close to the maximum residue limits.

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REFERENCES

- Association of Official Analytical Chemists. 1993. Method 992.22-Iodine (as iodide) in pasteurized liquid milk and skim milk powder. 4th Suppl. to Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.
- Browner, R. F. 1987. Pages 244–288 in *Inductively Coupled Plasma Emission Spectroscopy, Part II Applications and Fundamentals*. P. W. J. M. Boumans, ed. Wiley, New York, NY.
- Craven, G. S., and M. C. Griffith. 1977. Iodine determination in milk by iodide specific ion electrode and X-ray fluorescence spectrometry. *Aust. J. Dairy Technol.* 32:75–82.
- Dionex Corporation. 1996. The Determination of Iodide in Milk Products, Application Note 37. Dionex Corp., Sunnyvale, CA.
- Fisher, K. D., and C. J. Carr. 1974. Iodine in Foods: Chemical Methodology and Sources of Iodine in the Human Diet. FDA-71-294. Life Sci. Res. Office, FASEB, Bethesda, MD.
- Gloor, R., and E. L. Johnson. 1977. Practical aspects of reverse phase ion pair chromatography. *J. Chromatogr. Sci.* 15:413–423.
- Hamilton, R. J., and P. A. Sewell. 1977. *Introduction to High Performance Liquid Chromatography*. John Wiley and Sons, New York, NY.
- Harris, D. C. 1987. *Quantitative Chemical Analysis*. 2nd ed. W. H. Freeman and Company, New York, NY.
- James, W. D. 2001. Neutron activation analysis. In *Neutron Activation Analysis Laboratory, Center for Chemical Characterization and Analysis*, Texas A&M website. Available: www.chem.tamu.edu/services/naa/naa.htm. Accessed Aug. 2005.
- Richardson, G. H. 1985. *Standard Methods for the Examination of Dairy Products*. 15th ed. Am. Public Health Assoc., Washington, DC.
- Stabel-Taucher, R. 1975. Determination of iodine in milk. *Finn. Chem. Letters* 1:27–30.
- Stolc, V., and S. Nemeth. 1961. Microestimation of iodine in milk. *J. Dairy Sci.* 44:2187–2193.
- Underwood, E. J. 1977. *Trace Elements in Human and Animal Nutrition*. 4th ed. Acad. Press, New York, NY.
- Wheeler, S. M., L. R. Fell, G. H. Fleet, and R. J. Ashley. 1980. The evaluation of two brands of ion-selective electrode used to measure added iodide and iodophor in milk. *Aust. J. Dairy Technol.* 35:26–29.