Validation of a Capillary Electrophoresis Assay for Monitoring Iodine Nutrition in Populations for Prevention of Iodine Deficiency: An Interlaboratory Method Comparison

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Background: A capillary electrophoresis (CE) assay was recently introduced as a new method for monitoring iodine nutrition in large-scale epidemiological studies. However, further tests revealed unanticipated matrix-dependent interferences when analyzing submicromolar levels of iodide in human urine as the predominate ionic form of dietary iodine. Herein, we describe a rigorous validation study that was used to identify sources of bias and establish modifications to the original CE method to improve method accuracy.

Methods: An interlaboratory method comparison using CE with UV detection and inductively coupled plasma–mass spectrometry (ICP-MS) was performed to quantify urinary iodide concentrations (n = 71) independently at McMaster University and Hamilton General Hospital, as well as the CDC as part of their quality assurance program. A positive bias in the original CE method was indicated, and buffer conditions were subsequently optimized to overcome matrix interferences for reliable iodine status determination.

Results: Positive bias in CE was attributed to variable concentrations of sulfate, a major urinary anion interference with similar mobility to iodide under the conditions originally reported. By increasing the concentration of α-cyclodextrin in the background electrolyte, the CE method was able to tolerate urinary sulfate over its normal physiological range without loss in signal response for iodide. The optimized CE assay generated results that were consistent with ICP-MS using 2 different internal standards (^{187}Re and ^{130}Te) with a median bias under 10%.

Conclusions: CE offers a simple, selective, and cost-effective separation platform for surveillance of the iodine status of a population requiring only small volumes (<10 μL) of biobanked urine specimens, which is comparable to previously validated screening methods currently used in global health initiatives for prevention of iodine deficiency disorders.

IMPACT STATEMENT

A capillary electrophoresis (CE) method presented here offers a simple, selective, and cost-effective microseparation platform for urinary iodide determination, which is essential to support global health initiatives for prevention of iodine deficiency disorders. An interlaboratory method comparison was performed to identify and address sources of bias, resulting in an optimized CE method that generated consistent results in comparison to the reference method, inductively coupled plasma–mass spectrometry (ICP-MS). The validated CE assay allows for continuous monitoring of iodine nutrition in populations that is applicable to volume-restricted urine samples without complicated sample workup, expensive infrastructure, or high operating costs.

Iodine deficiency remains a global public health problem that is related to a spectrum of disorders, including goiter, hypothyroidism, depression, obesity, and increased risk for cardiovascular diseases and cancer in adults, as well as impaired physical and cognitive development in children (*1*–*7*). Despite well-established international salt iodization programs, dietary iodine intake patterns have changed over time in many regions due to lifestyle modifications that have contributed to increased ingestion of processed foods largely derived from noniodized salt (*8*, *9*). Arbitrarily increasing dietary iodine is not a viable solution in population health, since both chronic iodine deficiency and excess intake are associated with adverse health effects (*10*). Therefore, continuous monitoring is essential to evaluate the efficacy of global iodization programs given the reemergence of mild to moderate iodine deficiency in many countries (*7*, *11*). In this case, the median urinary iodine concentration is widely used to evaluate the iodine status of a population, since more than 90% of iodine recently ingested is excreted in the urine as iodide, the predominate ionic form of iodine that is uptaken by the thyroid gland for thyroid hormone biosynthesis (*12*). The availability of simple yet affordable methods for assessing population-based iodine nutrition is critical to guide public health policies, such as iodine supplementation of at least 150 μg daily for pregnant and breastfeeding women, now recommended by the American Academy of Pediatrics to ensure iodine adequacy during fetal growth and early childhood development (*13*).

The most common approach to analyze urinary iodine is kinetic spectrophotometry based on the Sandell–Kolthoff (S–K)[4] reaction (*14*). Despite its low operational cost, the S–K method and its variants have a number of limitations, including the need for heat digestion and oxidation of urine before the analysis, use of toxic reagents (e.g., arsenic, perchloric acid), redox interferences in urine, and changes in experimental conditions that affect reaction kinetics (*15*–*18*). As a result, inductively coupled plasma–mass spectrometry (ICP-MS) is considered the reference method for urinary iodine determination, which provides excellent precision and sensitivity without complicated sample workup (*19*). However, ICP-MS lacks specificity as it measures total iodine in urine, including iodinated contrast agents, drugs, and food additives (*20*), and also the infrastructure requires high operational costs due to argon consumption (*15*). Alternatively, capillary electrophoresis with UV absorbance detection (CE-UV) offers a simple and cost-effective microseparation platform for direct analysis of submicromolar levels of iodide in complex biological samples with adequate sensitivity when using online sample preconcentration (*21*–*23*). However, rigorous method validation studies are lacking for reliable urinary iodine determination by CE, despite the successful translation of CE-based assays for routine molecular diagnostics in some clinical laboratories (*24*).

A robust CE method was recently introduced for nutritional iodine status assessment with acceptable intermediate precision (CV = 12%, n = 87) over several weeks of operation, as required for large-scale epidemiological studies (*25*). The assay was optimized to provide adequate sensitivity and selectivity for quantification of iodide in human urine samples after a simple dilution step in deionized water. Dynamic complexation of iodide with

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[4] **Nonstandard abbreviations:** S–K, Sandell-Kolthoff; ICP-MS, inductively coupled plasma–mass spectrometry; CE-UV, capillary electrophoresis with UV absorbance detection; α-CD, α-cyclodextrin; BGE, background electrolyte; EQUIP, Ensuring the Quality of Urinary Iodine Procedures; PURE, Prospective Urban and Rural Epidemiological Study; T4, *L*-thyroxine.

α-cyclodextrin (α-CD) in the background electrolyte (BGE) was critical for resolving submicromolar levels of urinary iodide from up to a 10^6-fold excess of chloride, which also provided optimum conditions for online sample preconcentration via sample self-stacking (*25*). Internal method validation demonstrated good linearity (R^2 = 0.9998), sensitivity (limit of quantification = 0.20 μmol/L), and interday precision (CV <8%). However, a positive bias of up to 35% was reported in a limited set of reference urine samples from NIST that were measured by ICP-MS. This step prompted us to conduct a more extensive interlaboratory validation study to investigate the potential source(s) of bias in the original CE-UV assay relative to ICP-MS. Unexpectedly, the major UV-transparent strong anion in human urine, sulfate (*26*), was found to act as a matrix interference by impairing sample self-stacking of iodide, resulting in a loss of signal intensity exceeding 50% at high sulfate concentrations. By increasing the concentration of α-CD in the BGE, the effective mobility of iodide was shifted to avoid urinary sulfate interferences resulting in consistent results to ICP-MS, which were performed independently at Hamilton General Hospital, as well as a round-robin study with the CDC as part of their Ensuring the Quality of Urinary Iodine Procedures (EQUIP) program (*27*). Additionally, the performance of 2 commonly used internal standards for urinary iodine analysis by ICP-MS, namely ^{187}Re and ^{130}Te, was also evaluated in terms of overall method precision and accuracy due to the lack of a stable natural isotope for iodine.

MATERIALS AND METHODS

Urine samples and study design

The first method comparison was comprised of representative 24-h human urine samples (n = 50) obtained from the Prospective Urban and Rural Epidemiological (PURE) study (*28*), where CE-UV and ICP-MS analyses were conducted in a double-blinded manner at McMaster University and Hamilton General Hospital, respectively. All urine samples were labeled by a third person before analysis, with results processed by a fourth individual to keep analysts blinded during sample preparation and data processing. Spiked samples were prepared from 3 independent pooled samples, each containing 20 randomly selected 24-h urine samples from the PURE study, excluding participants who were taking *L*-thyroxine (T_4) and/or had iodine levels >0.79 μmol/L. These pooled urine samples were spiked with iodide calibrants at 4 levels that span physiologically relevant iodine concentrations (0.79, 1.58, 2.36, and 3.15 μmol/L) to compare the recovery for iodine when using CE-UV and ICP-MS. Serially diluted samples in deionized water were also prepared to evaluate potential matrix effects in urine from 3 independent pooled samples, each containing 10 randomly selected 24-h urine samples from the PURE study, excluding participants who were taking T_4 and/or had iodine <1.93 or >3.94 μmol/L to allow for multiple dilutions over an adequate dynamic range. In this case, the 3 pooled urine samples were diluted 1-, 1.5-, 2-, 4-, and 8-fold in deionized water. Five samples with high iodide (>3.94 μmol/L) were also selected randomly among the PURE study participants who were not taking T_4 to investigate potential bias in cases of excess iodine nutrition. Additionally, 10 urine samples from participants taking T_4 were randomly selected from the PURE study to evaluate potential bias from urinary iodine-containing compounds. Five samples from the CDC's EQUIP program were included in the study as well. A pooled QC was prepared by combining equal volumes from all urine samples in this study, which was analyzed intermittently (n = 9) after every 10 urine samples to evaluate method precision, whereas a blank sample (i.e., internal standard naphthalene disulfonate prepared in 2-fold diluted simulated urine matrix in deionized water) was analyzed daily to monitor for potential sample carry-over. In addition, vendor-supplied

samples for QC at levels I [mean (95% CI) = 0.944 (0.708–1.18) μmol/L) and II [3.92 (2.94–4.90) μmol/L] were obtained from RECIPE (ClinChek lyophilized urine controls, reference values determined by ICP-MS) and analyzed intermittently (n = 9) to evaluate method accuracy. A summary of the 50 urine samples in this interlaboratory validation study is described in Table 1 in the Data Supplement that accompanies the online version of this article at http://www.jalm.org/content/vol1/issue6.

The second method comparison was performed on human urine samples (n = 21) collected over a 2-year period after participation in the CDC's EQUIP program (*29*) over 6 rounds from May 2014 to October 2015, which is a round-robin study involving up to 120 different laboratories around the world. In this case, EQUIP samples were analyzed by CE-UV at McMaster University, whereas results for the CDC target values derived from an ICP-MS reference method, and mean results were generated from all laboratories (n = 88–102) participating in the EQUIP after exclusion of outliers. In the latter case, urinary iodine measurements were performed by 8 different analytical methods, primarily ICP-MS and various formats of the S–K method. Experimental details for urinary iodine determination using the optimized CE-UV assay and validated ICP-MS method according to the recommendations outlined in CLSI guideline EP19 are described in Methods in the online Data Supplement, including chemicals/reagents, preventative maintenance procedures, as well as tests performed to identify the source of bias in the original CE-UV assay (*25*).

Statistical analysis

Data analysis was performed using Excel (Microsoft Office) to calculate relative peak areas and determine recoveries and dilution ratios. The Statistical Package for the Social Science (SPSS) was used to perform normality tests (Shapiro–Wilk), Spearman rank correlation analysis, and statistical comparisons by a 2-tailed Student *t*-test. MedCalc (MedCalc Software) was used for Passing–Bablok regression analysis and difference or % difference Bland–Altman plots when comparing CE-UV and ICP-MS methods. Igor Pro (Wavemetrics) was used to plot electropherograms and other graphs.

RESULTS

Sources of bias and modifications in the CE-UV assay

A significant positive bias of 40%–50% was confirmed for the original CE-UV assay (*25*) in comparison with ICP-MS results. Further details from the method comparison between the original CE-UV method and ICP-MS are described in the online Data Supplement under Results and in Figs. 1–3. Briefly, calibrant solutions were prepared in 2-fold diluted simulated urine matrix, containing several major urinary electrolytes to mimic the ionic strength of human urine. To investigate the underlying cause(s) of the bias, iodide standard solutions were prepared in 2- and 5-fold diluted urine matrix, as well as in 50 mmol/L sodium chloride (Fig. 1A). The slopes of the calibration curves changed over 2-fold when comparing calibrant solutions in sodium chloride and in a 2-fold diluted urine matrix, suggesting that the source of bias was related to some other ion(s) in the simulated urine matrix. After testing binary mixtures of sodium chloride and other electrolytes, sulfate was found to be the coion causing a concentration-dependent decrease in apparent iodide signal response and lower sensitivity (Fig. 1B). Nonetheless, sulfate was also identified as an important coion affecting iodide migration time and peak shape during sample self-stacking to generate electropherograms analogous to authentic urine samples (Fig. 1C). Additional tests confirming the key role of sulfate as a matrix interference that altered the measured response (i.e., integrated peak area) of iodide are described in Results and Fig. 4 in the online Data

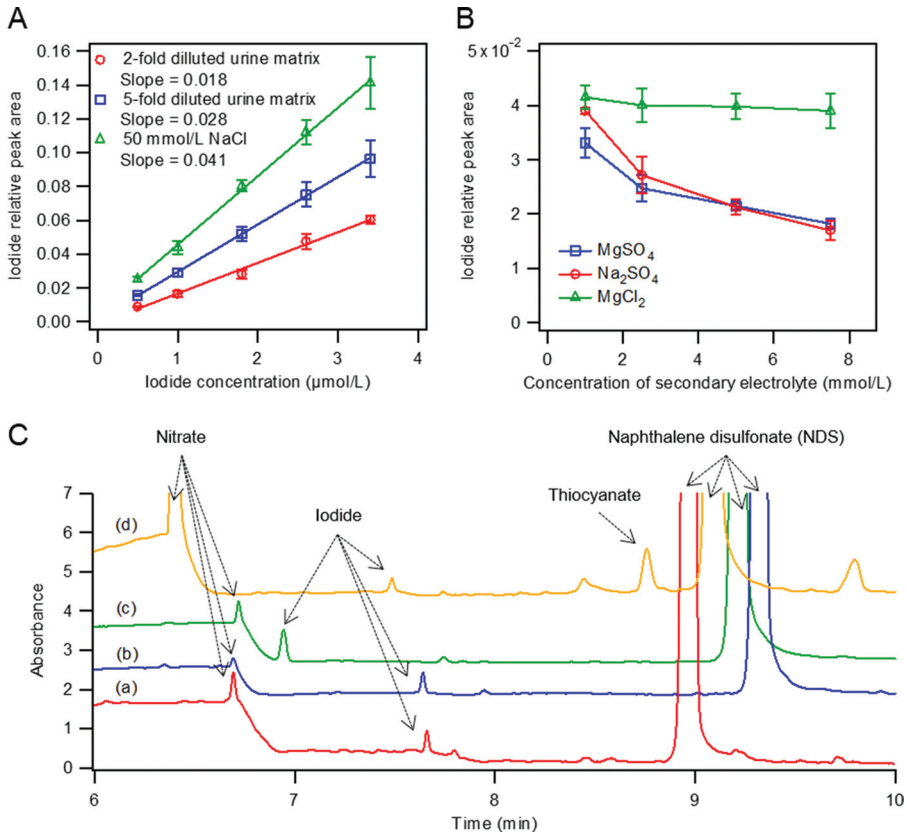

Fig. 1. Identification of source(s) of bias in the original CE-UV assay.
(A), Calibration curve for iodide in simulated urine matrix and sodium chloride. (B), Iodide response with addition of secondary electrolytes to iodide solutions in sodium chloride. (C), Electropherograms for iodide in simulated urine matrix (a), sodium chloride and sodium sulfate (b), sodium chloride (c), and pooled human urine sample (d). The sulfate content in sample or urine matrix has a major impact on both the measured signal intensity and migration time of iodide when using the original buffer conditions developed for the assay.

Supplement, such as precipitating urinary sulfate as its barium salt before CE analysis to restore the iodide response. Sulfate has an effective mobility similar to iodide under the conditions used in the original CE-UV assay when using 36 mmol/L α-CD in the BGE, which resulted in a lower apparent iodide signal than corresponding sulfate-free urine samples or matrix-matching calibrant solutions. The sulfate interference was resolved by increasing the α-CD concentration in the BGE, which selectively slows the mobility of iodide relative to sulfate due to the former ion's higher binding affinity. As shown in Fig. 2, when α-CD concentration is ≥46 mmol/L, the measured iodide response remains unaffected by changes in sulfate levels as high as 16 mmol/L, which is equivalent to 32 mmol/L in a nondiluted urine sample. Another potential source of bias for the CE-UV assay is the presence of oxidizing agents, such as nitrite or sulfite, as well as sulfamic acid (see Results in the online Data Supplement), which is used as a preservative in NIST-derived urine samples for the concomitant analysis of mercury by ICP-MS (*30*). As a result, this work

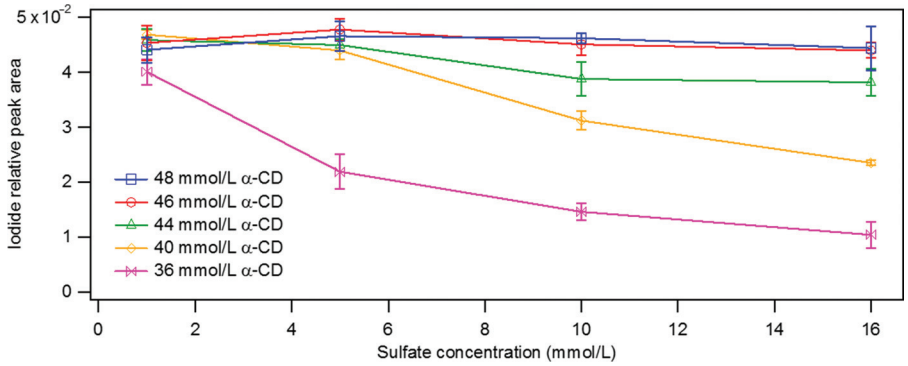

Fig. 2. Effect of α-CD concentration in the BGE on the response of iodide solutions prepared in 50 mmol/L sodium chloride containing different concentrations of sodium sulfate.
An increase in concentration of α-CD above 46 mmol/L in the background electrolyte for the CE-UV assay can tolerate high levels of sulfate in the sample matrix without loss in signal response for iodide.

highlights the need for careful selection of compatible preservatives in urine to avoid interferences when measuring iodide by CE that has been previously shown to tolerate repeat freeze-thaw cycles and delays to storage even when left at room temperature (*25*).

Method comparison between ICP-MS and optimized CE-UV assay

Iodide concentrations in 24-h urine samples ranged from approximately 0.20 to 11.50 μmol/L and were not normally distributed (Shapiro–Wilk, P <0.001, n = 50). The optimized CE-UV assay using 46 mmol/L α-CD in the BGE provided results consistent with ICP-MS, with a median bias of 2.1% and −4.8% when using the internal standards ^{187}Re and ^{130}Te, respectively. In this case, a strong correlation between the methods was observed with Spearman rank correlation coefficients of 0.959 and 0.963 (P <0.001) for ^{187}Re and ^{130}Te, respectively. No evidence of systematic bias was observed between the optimized CE-UV assay and ICP-MS using ^{187}Re as internal standard, as indicated by a slope and y-intercept that were not significantly different from 1 and 0, respectively, after Passing–Bablok regression (Fig. 3A), whereas a small negative bias was detected when using ^{130}Te as internal standard (Fig. 3B). Overall, the CE-UV assay had good agreement with ICP-MS, with 95% of the differences within −0.66 to 0.79 μmol/L for ^{187}Re (Fig. 4A) and −0.87 to 0.71 μmol/L for ^{130}Te (Fig. 4C). Similarly, the mean percentage differences between the methods were 6.3% and −0.4% for ^{187}Re and ^{130}Te when using % difference Bland–Altman plots, respectively (Fig. 4, B and D). Excellent accuracy was also indicated by recoveries in the CE-UV method ranging from 99.5% to 102.7% (Table 1) and a mean slope for the spike-recovery studies not significantly different from unity (Fig. 5A). Also, data points in the serial dilution were very close to the ideal line, as shown in Fig. 5B [mean y-intercept (95% CI) = 0.05 (0.02–0.08)]. Among the samples with very high iodine, only 2 remained for comparison after exclusion of an outlier and reclassification of 2 other samples as having normal iodine levels when using ICP-MS and the revised CE-UV method, which resulted in bias under 10%. Samples from subjects taking T_4 did not reveal a clear trend when comparing CE-UV and ICP-MS methods with an average bias of −2.3% and −7.2% for ^{187}Re and ^{130}Te, respectively. Additionally, the median bias for the EQUIP samples was reduced

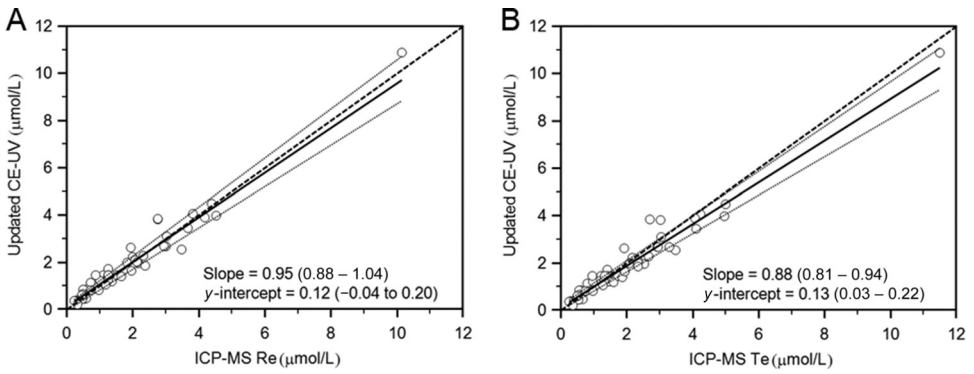

Fig. 3. Passing–Bablok regression comparing the optimized CE-UV assay with ICP-MS using the internal standards ^{187}Re (A) and ^{130}Te (B).
Plots show slopes and *y*-intercepts followed by their 95% CI. Regression, 95% CI, and equality line are represented by solid, dotted, and dashed lines, respectively.

to 10.4% and 15.6% compared to the CDC target using ICP-MS and the mean from all laboratories (S–K methods and ICP-MS), respectively (see Fig. 5 in the online Data Supplement). The precision of the CE-UV assay was under 12% when using a single nonisotope internal standard based on intermittent analysis of pooled QC and vendor-derived reference QC samples (see Table 2 in the online Data Supplement). Additionally, the calibration curve [$y = (0.0454 \pm 0.0006)\,x$, for iodide concentrations in μmol/L] showed good linearity (R^2 = 0.9989) over the concentration range of 0.15 to 4.00 μmol/L (see Fig. 6 in the online Data Supplement) as required for assessment of the iodine nutritional status on a population level.

Comparison of internal standards in ICP-MS method

In the method comparison, ^{187}Re and ^{130}Te were both used as internal standards for ICP-MS, since other isotopes of iodine are radioactive. Based on intermittently analyzed QC samples used to evaluate system stability and sample carry-over during analysis, ^{187}Re was significantly (P <0.05, Student t-test) more precise and accurate (mean CV = 1.78%, bias <6%) than ^{130}Te (mean CV = 5.63%, bias <12%; see Table 2 in the online Data Supplement). Similarly, better accuracy in ICP-MS was achieved in spike-recovery studies (Table 1) when using ^{187}Re as an internal standard with average recoveries ranging from 100.5% to 103.0%, whereas ^{130}Te had a higher bias ranging from 104.5% to 115.0%. As expected, a comparison between the 2 internal standards showed a strong correlation with a Spearman rank correlation coefficient of 0.996 (P <0.001), but results obtained with ^{130}Te were on average 6.8% higher than with ^{187}Re. This result is a small but significant difference given the high precision of the ICP-MS method (see Fig. 7 in the online Data Supplement).

DISCUSSION

The CE-UV assay reported here offers a simple, selective, and cost-effective method for urinary iodine determination in population studies as compared to the classic S–K kinetic spectrophotometric assay and ICP-MS. The original CE-UV method (*25*) was determined to be susceptible to bias due to the dependence of measured iodine responses on sulfate present in

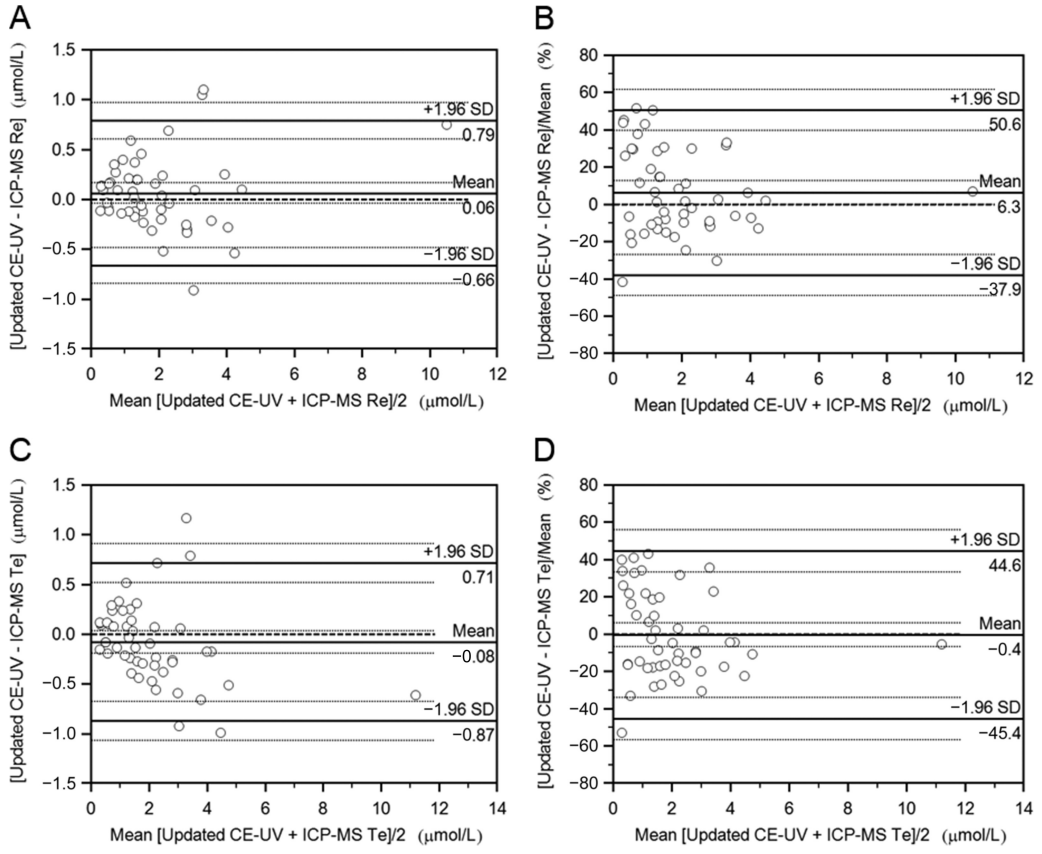

Fig. 4. Bland–Altman plots of differences (A, C) and % differences (B, D) between the updated CE-UV assay and ICP-MS using the internal standards ^{187}Re and ^{130}Te, respectively.
The mean difference/% difference and limits of agreement (± 1.96 SD) are represented as solid lines, while their 95% CI and the zero are indicated by dotted and dashed lines, respectively.

matrix matching solutions used for calibrants and found endogenously in authentic human urine samples. Sulfate is a highly abundant yet variable inorganic electrolyte in human urine reflecting dietary protein intake with concentration levels varying from 0.5 to 32 mmol/L (*26*, *31*). Sulfate is a stable and UV-transparent strong anion; thus, it does not present a direct spectral interference to iodide for which absorbance is optimally monitored at 226 nm. However, since sulfate has an apparent mobility similar to iodide under the original CE method conditions, it can contribute to interferences during sample self-stacking that is used as an online sample preconcentration technique to enhance concentration sensitivity in CE (*32*, *33*). This effect was previously reported in a CE-UV assay for iodide analysis in complex biological samples (*22*), where sulfate was found to affect the apparent signal and migration time for iodide during sample self-stacking. In our case, this interference was resolved by increasing the concentration of α-CD in the BGE to slow down the effective mobility of iodide relative to sulfate, due to larger fraction of iodide:α-CD complex, which generated equivalent separation performance in both authentic

Table 1. Recoveries for 3 independent pooled 24-h urine samples (n = 20) spiked at four different iodide concentration levels and analyzed by CE-UV and ICP-MS.

Iodide spiked, μmol/L	Mean recovery (SD), %			
	Original CE-UV	Updated CE-UV	ICP-MS Re	ICP-MS Te
0.79	136.9 (36.0)	100.0 (4.8)	100.6 (2.8)	110.8 (11.1)
1.58	136.2 (15.0)	99.5 (5.9)	100.5 (3.4)	115.0 (2.1)
2.36	139.1 (8.6)	102.5 (6.7)	103.0 (1.2)	104.5 (10.4)
3.15	135.7 (9.8)	102.7 (9.5)	101.5 (0.9)[a]	113.3 (0.3)[a]

[a] An outlier was excluded from this calculation for ICP-MS.

urine samples and calibrant solutions containing sulfate. Importantly, the CE-UV assay tolerates high concentration levels of urinary sulfate while requiring up to a 50-fold lower sample volume (10 μL) of urine than conventional ICP-MS and kinetic spectrophotometric assays.

The interlaboratory method comparison conducted independently at McMaster University and Hamilton General Hospital together with participation in 6 rounds of the CDC's EQUIP initiative over 2 years provides strong evidence that results obtained by the CE-UV assay are in good agreement with previously validated methods widely used for urinary iodine determination in large-scale epidemiological studies, including the CDC's ICP-MS reference method. In fact, measured differences between the methods [mean (95% CI) = 0.06 (−0.66 to 0.79 μmol/L) for optimized CE-UV vs ICP-MS using ^{187}Re] are comparable with differences previously reported for a comparison between ICP-MS and the S–K assay [0.03 (−0.55 to 0.49 μmol/L)] (*34*). Concentration-dependent differences between the methods reported for ICP-MS vs S–K assay (*34*) are also consistent with our results when comparing ICP-MS vs CE-UV. Furthermore, a much better agreement was also obtained for EQUIP urine samples used in an international round-robin study, including both

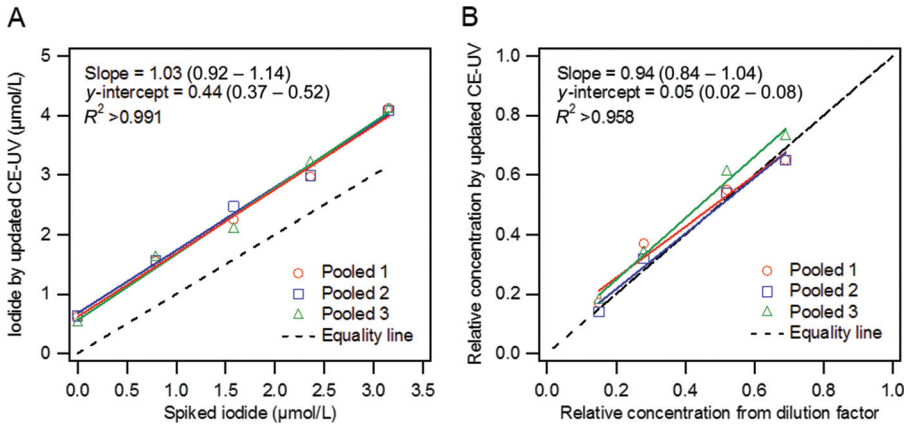

Fig. 5. Spike recovery (A) and serial dilution (B) for 3 independent pooled human urine samples analyzed by the optimized CE-UV assay.
Solid lines represent the least-squares linear regression, and slopes and *y*-intercepts are shown in the graphs, followed by their 95% CI.

the CDC target (ICP-MS) and the mean of all laboratories (S–K assay and ICP-MS). Although ICP-MS has better precision and lower limit of quantification, the CE-UV method offers acceptable precision (CV ≤12%) and adequate sensitivity to measure urinary iodine levels associated with moderate deficiency (0.16–0.38 μmol/L) according to the WHO categories (*12*). Both techniques require a simple dilution step for urine before analysis unlike the S–K method. As a high-efficiency microseparation technique for ions based on differences in their electrophoretic mobility (i.e., effective charge density), the CE-UV assay offers highly selective determination of iodide, the predominate chemical form of iodine that is bioavailable to the thyroid gland after gastrointestinal absorption from food and water sources (*35*). On another hand, ICP-MS quantifies total iodine after nebulization and atomization of all iodine-containing compounds from urine in a high-temperature plasma source, which includes iodinated drugs, radiologic contrasts, and food additives that are not used as a source of iodine for thyroid hormone biosynthesis. Also, our previous study demonstrated that CE offers excellent robustness (*25*) when analyzing iodine in pooled 24-h urine samples (n = 87) over 5 weeks of continuous operation while applying standard operating protocols and daily preventative maintenance on the instrument (refer to Methods in the online Data Supplement). Although direct comparison of measured results using different methods should always be interpreted with caution, this validation study provides compelling evidence that CE-UV reliably measures urinary iodine nutritional status that is in close agreement with the reference method, ICP-MS.

A small but significant difference was observed for ICP-MS results normalized to ^{187}Re and ^{130}Te. The choice of internal standard in ICP-MS is essential to correct for urine matrix effects, which affect quantification due to variable sample components that influence ionization efficiency and ion transmission over time. Isotope dilution analysis provides the most accurate and precise results in ICP-MS, as an isotope internal standard has very similar *m/z* and identical first ionization potential energies. Iodine, however, is a monoisotopic element with a single radioisotope that is substantially stable (^{129}I, $t_{1/2}$ = 15.7 million year). Although ^{129}I has been reported as the optimum internal standard for urinary iodine analysis (*36*, *37*), other nonisotopic internal standards are generally used because of concerns related to potential radiological hazards and special safety measures required for handling ^{129}I. Our study has demonstrated that ^{187}Re provides more precise and accurate results than ^{130}Te, which was unexpected considering that ^{130}Te has an *m/z* and first ionization potential (9.01 eV) that better matches iodine (*m/z* 127, 10.45 eV) relative to ^{187}Re (7.83 eV) based on data from the NIST atomic spectra database. Therefore, other factors aside from the mass gain region and the ionization potential play an important role in correcting for ion suppression effects in ICP-MS that requires further investigation. Indeed, the internal standard used in CDC's ICP-MS reference method was changed in 2014 from ^{130}Te to ^{187}Re due to better performance that also allows for simultaneous analysis of iodine and mercury (*38*, *39*). In this work, better agreement was also found for measured urinary iodine concentrations between CE-UV assay and ICP-MS using ^{187}Re.

In summary, a validated CE-UV assay presented here offers laboratories a simple method for urinary iodide determination with adequate sensitivity, selectivity, and precision with minimal sample handling. Importantly, this method has excellent accuracy that is consistent with both ICP-MS and S–K methods widely used for iodine status monitoring and risk assessment for the prevention of iodine deficiency disorders in populations rather than individual diagnosis of thyroid dysfunction in a clinical setting. CE-UV also provides a more cost-effective platform for reliable estimation of recent dietary iodine intake in certain regions, where ICP-MS is not feasible due to high infrastructure

and long-term operating costs. Additionally, CE offers better selectivity for iodide (i.e., the active iodine species for thyroid uptake) than ICP-MS, while allowing for analysis of volume-restricted biobanked urine specimens. Urinary thiocyanate and nitrate levels can also be measured by the same CE assay (*25*), which are relevant environmental iodine-uptake inhibitors contributing to thyroid-related health problems in populations with adequate dietary iodine (*40*). Interference caused by urinary sulfate was resolved by increasing the concentration of α-CD in the BGE. Samples containing high levels of oxidizing agents could still contribute to incidental bias with signal loss of iodide in CE via in situ reduction to molecular iodine that is electrically neutral. Nevertheless, nitrite and sulfite are reactive anions, present at low levels in cases of bacterial infection/unpreserved stored urine samples or in rare cases of sulfite oxidase deficiency (*26*), whereas sulfamic acid is a preservative that can be avoided for samples intended for iodine quantification. Future work will evaluate the iodine nutritional status of the Canadian population that is at higher risk for iodine deficiency due to recent changes in habitual diet and lifestyle.

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