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Ion chromatography as potential reference methodology for the determination of total sodium and potassium in human serum

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Abstract

The potential of ion chromatography to serve as a new reference method principle for the determination of total sodium and potassium in human serum was investigated. Sample pretreatment consisted of acidic dilution and filtration and detection was based on conductivity. Methods for the separate and simultaneous determination of both analytes were investigated. Further, the influence of calibration (using either a single-point calibration or a standard curve) on method imprecision, inaccuracy and analysis time was examined. The best method performance was achieved by separate analysis using single-point calibration with bracketing analysis scheme. For this variant, the mean total coefficient of variation for sodium and potassium was 1.0% and the mean method bias was −0.2% for sodium and +0.2% for potassium, as determined with three control materials from the National Institute of Standards and Technology. Our results are comparable to those of reference methods based on flame atomic emission spectrometry. Therefore, we consider ion chromatography as a valuable reference methodology for the determination of total sodium and potassium in human serum.

1. Introduction

Reference methods in laboratory medicine are used for accuracy assessment of routine methods, target-setting of internal and external quality control materials and certification of reference materials [1,2]. The currently accepted reference method principle for the determination of sodium and potassium in serum is flame atomic emission spectrometry (FAES) [3,4]. However, alternative methods are desirable since, for example, the use of two independent methods is advocated for the certification of reference materials [5]. A candidate method principle would be ion chromatography (IC), because it has been successfully applied to the determination of various serum electrolytes [6–9]. Recently, we indeed demonstrated the potential of IC to serve as a reference method principle by developing highly accurate methods for the determination of total calcium and magnesium in serum [10]. Here, we extend this application of IC to the determination of total sodium and potassium in serum. Emphasis was put on the use of standard reference materials (SRMs) from the National Institute of Standards and Technology (NIST) for calibration and ac-

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curacy assessment. Sample pretreatment consisted of dilution with the methanesulfonic acid IC eluent and filtration. Methods for the separate and simultaneous determination of sodium and potassium were compared. In addition, the influence of single-point calibration (with adapting the dilution of serum) or calibration with a standard curve on method imprecision and inaccuracy was investigated. The first results of our studies are reported here.

2. Experimental

2.1. Instrumentation

Ion chromatography was carried out with a Model DX-100 ion chromatograph from Dionex (Sunnyvale, CA, USA) equipped with a 25-μl injection loop. For chromatographic separation an Ionpac CG12 guard column (50 mm × 4 mm I.D.) coupled to an Ionpac CS12 analytical column (250 mm × 4 mm I.D.) was used. Electronic suppression was achieved with a Cation Self-Regenerating Suppressor-I system (CSRS-I, 4 mm) used in the autosuppression recycle mode. Detection was based on conductivity. All the above-mentioned equipment was from Dionex. Integration of the chromatographic signals was performed with a C-R5A integrator from Shimadzu (Kyoto, Japan). An Elgastat Maxima Analytical water purification system from Elga (Bucks, UK) was used to produce water of ultrapure quality (18.2 MΩ). Weighings were done with an electronic 100-g balance (Mettler Toledo, Greifensee, Switzerland), Type AT261 Deltarange, with a readability of 10^{-5} g. The density of serum was determined with a Model DMA 35 densitometer (accuracy 1 mg/ml) from A. Paar (Graz, Austria).

2.2. Materials

Methanesulfonic acid (purissimum quality, i.e. purity >99%) was purchased from Fluka (Buchs, Switzerland). Filtrations were done with Millex-HV_{13} filters (0.45 μm pore size) from Millipore (Bedford, MA, USA). For filtration and injection, 1-ml Plastipak syringes from Becton Dickinson (Dublin, Ireland) were used. Pipetting was done using 1-ml and 200-μl pipet tips from Eppendorf (Hamburg, Germany). To exclude external contamination at any stage of the IC analysis, no glassware was used and hand contact of vials etc. was avoided. All vials and containers used were of polypropylene or polymethylpentene from Nalgene (Hereford, UK). They were rinsed with water and dried before use.

2.3. Standard materials

For calibration, standard materials of certified purity from the NIST (Gaithersburg, MD, USA) were used, i.e. sodium chloride (SRM 919a) and potassium chloride (SRM 918). For the single-point calibration approach, accurately weighed amounts of sodium chloride and potassium chloride were dissolved in water to give sodium concentrations of approximately 40 mmol/l (accurate to the second decimal) and potassium concentrations of 4.5 mmol/l (accurate to the third decimal). To the potassium chloride standard solution, pure sodium chloride was added to give a physiological concentration of approximately 130 mmol/l. Two stock solutions of each analyte were prepared and cross-checked for accuracy against each other. Deviations of less than 0.6% (on the basis of six measurements) were accepted. For the four-point calibration approach, stock solutions were prepared containing 100, 120, 140 and 160 mmol/l sodium and 2, 4, 6 and 8 mmol/l potassium, respectively. To the potassium chloride stock solutions, sodium chloride was added as described above. Solutions for the two-point calibration (used for the simultaneous determination of sodium and potassium) contained 100 (lowest calibration point) and 160 (highest calibration point) mmol/l sodium and 2 and 8 mmol/l potassium, respectively. These stock solutions were also cross-checked against each other before use. All standard solutions were stored in 500-μl portions in polypropylene vials at −20°C and discarded after two freeze–thaw cycles. The SRM 909, SRM 909a-1 and SRM 909a-2 lyophilized human

serum reference materials from NIST were reconstituted on a gravimetric basis according to the manufacturer's instructions (for SRM 909 procedure B was followed). The deviation of the volume of water used for reconstitution from the prescribed volume was taken into account and the maximum acceptable deviation was set to 0.5%. The certified concentrations of the NIST sera are listed in Tables 1 and 2. After reconstitution, the sera were aliquoted in 500-μl portions in polypropylene vials for storage at −20°C. Each aliquot was discarded after two freeze–thaw cycles.

2.4. Method

Sampling of serum and standards and further dilutions were carried out on a gravimetric basis. Volumes were chosen to give masses >25 mg to keep weighing errors below 0.1%. The pipetted volumes were calculated from the density of the solutions and the masses pipetted.

For the determination of sodium in serum using single-point calibration, a serum volume of 25 μl (containing approximately 3160–3700 nmol sodium) was sampled and diluted 200-fold with a 14 mmol/l aqueous methanesulfonic acid solution. After 1 h of equilibration, the acidified samples were filtered. The filter device was prerinsed with water three times and finally with the sample so that the first 500 μl of filtrate were discarded (care was taken to make the syringe air-free). In this way, any change in sample concentration by filtration was prevented. After filtration, a second dilution of the filtrates was done to give similar peak heights on injection of 25 μl of the diluted sample. Dependent on the serum sodium concentrations, the total dilution ranged from approximately 3500 to 4100. The final dilutions were injected without further treatment (25 μl containing approximately 0.90 nmol of sodium). The sodium standards were treated exactly in the same way as the serum samples: 25 μl of the stock solution (40 mmol/l) were diluted 1100-fold in two steps (before and after filtration) to give peak heights similar to those of the samples on injection of 25 μl of the dilution. For the determination of potassium in serum with single-point calibration, a similar approach was used: 35 μl of serum (containing approximately 125–217 nmol potassium) were sampled and further diluted 95- to 217-fold with a 14 mmol/l aqueous methanesulfonic acid solution to finally inject approximately 0.94 nmol of potassium in 25 μl of the dilution. For the standards, an aliquot of 34 μl (4.5 mmol/l) was diluted 120-fold and treated exactly in the same way as the samples.

For the quantification of sodium in serum using a four-point calibration curve, a fixed volume of 25 μl of serum was taken for analysis and diluted 6600-fold in two steps and filtered as described above (25 μl of the final dilution contained approximately 0.48–0.56 nmol sodium). Aliquots of 25 μl of each of the four sodium stock solutions (100, 120, 140 and 160 mmol/l) were treated in exactly the same way as the serum samples. For the quantification of potassium using a four-point calibration curve, 25 μl of serum were sampled and diluted 160-fold before filtration as described above (25 μl containing approximately 0.56–0.97 nmol potassium). For the standards, 25-μl aliquots of the different stock solutions (2, 4, 6 and 8 mmol/l) were treated in exactly the same way as described for the serum samples.

For the simultaneous quantification of sodium and potassium in serum using a two-point calibration curve, a fixed volume of 25 μl of serum was sampled, diluted 160-fold and filtered as described above (25 μl containing approximately 0.56–0.97 nmol of potassium and 19.77–23.20 nmol of sodium, respectively). The standards, containing 100–160 mmol/l sodium and 2–8 mmol/l potassium, again were treated in the same way as the samples.

The conditions for ion-exchange chromatography of sodium and potassium were elution at a flow-rate of 1 ml/min with an eluent of 14 mmol/l aqueous methanesulfonic acid [11]. In both methods, suppression was performed in the autosuppression recycle mode with the current setting at approximately 300 mA. Conductivity was measured with a detector range of 3 μS, except in the method for the simultaneous determination of sodium and potassium, in which

during the run (after elution of sodium and before elution of potassium) the range was switched from 100 to 3 μS.

2.5. Analysis and measurement protocol

For the quantification of total sodium and potassium in the lyophilized SRMs, three different vials were used to compensate for the variation in the dry-mass content in each vial. The concentration measured for each SRM serum was always calculated from twelve independent measurements performed on three different days. This means that every day four serum aliquots (A–D) from each SRM were analyzed. Measurements were done in the bracketing mode, i.e. injection of serum samples in between the standards. In the methods for the separate determination of sodium or potassium with single-point calibration, three independently weighed-in standards (I–III) were used. The bracketing injection protocol was as follows: standards I–III; samples 1A–D; second injection of standards I–III; samples 2A–D; etc. Calculation of the sodium or potassium concentrations in the serum samples was based on the results for the duplicate injections of the standards encompassing the samples.

In the methods for the separate determination of sodium or potassium using a four-point calibration curve, four different standards with increasing concentration (I–IV) were used. Injections of serum samples and standards were done according to the following scheme: standards I–IV; samples 1A–D; second injection of standards IV–I; samples 2A–D; etc. The concentrations of the serum samples were derived by interpolation on the linear regression curve of the standards encompassing the samples.

In the method for the simultaneous determination of sodium and potassium using a two-point calibration curve, a low-concentration (I), and a high-concentration (II) standard for both sodium and potassium were used. According to the bracketing measurement scheme, standards and serum samples were injected as follows: standards I, II; samples 1A–D; second injection of standards II, I; samples 2A–D; etc. Linear regression of the results for the standards encompassing a series of serum samples was applied for calculation of their concentrations.

2.6. Estimation of analytical performance

The precision and total error of the IC methods for the determination of total sodium and potassium in serum were estimated from replicate analyses of the three human serum SRMs from NIST according to the following measurement design: quadruplicate analysis of each serum in three independent series. The overall precision was estimated from the coefficient of variation (C.V., %) calculated for the twelve measurement results, the total error by the deviation of the mean of the twelve measurement results from the certified value.

2.7. Safety considerations

The method demands no specific safety precautions. Lyophilized control sera of human origin have to be handled as potentially infectious. General guidelines for work with acids have to be respected.

3. Results and discussions

The above described methods were intended for use as reference methods for target-setting in external quality assessment (EQA). We aimed at reaching the requirements for the German EQA scheme [12], imposing a maximum total error (T.E.) of 1.2% for sodium and 1.6% for potassium (T.E. includes method bias and 95% confidence interval). From these limits, specifications for maximum method bias, C.V., and number of measurements can be derived. For example, with a maximum method C.V. of 1.0% (1.5%) and twelve measurements, the 95% confidence interval is ± 0.64% (±0.95%), resulting in a bias limit of 0.56% for sodium and 0.65% for potassium [12]. To comply with this concept, every reference method value had to comprise twelve independent measurements and the limits for the C.V.s calculated from those twelve measurements

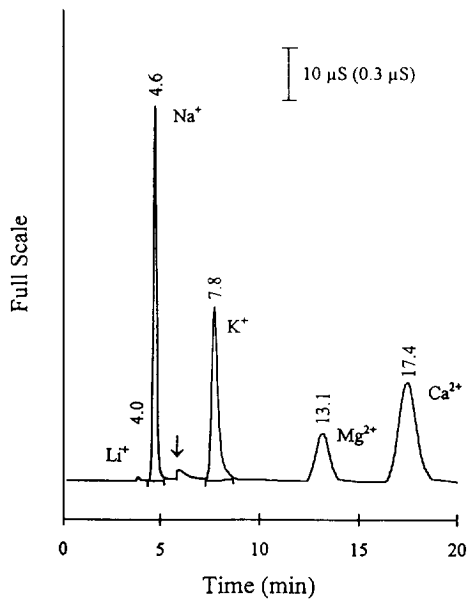

Fig. 1. Representative chromatogram of a serum sample processed for the simultaneous determination of sodium and potassium. The detector range was initially 100 μS and switched to 3 μS after the sodium peak (indicated by the arrow). For chromatographic conditions, see Experimental section.

were set to 1.0% for sodium and 1.5% for potassium. We investigated whether IC principally was able to achieve those specifications. We used the NIST SRMs 909, 909a-1 and 909a-2 for evaluation of method accuracy because, at the moment, they are the only available reference materials for serum total sodium and potassium. Chromatographic conditions were adopted from the recommendations for use of the CS12 column [11] and were identical for all methods. Before collecting data, the reliability of the chromatographic system (column head pressure, stability of baseline and background suppression) was verified. A representative chromatogram of a serum sample processed for the simultaneous determination of sodium and potassium is shown in Fig. 1. Good separation and high signal intensities were obtained for reliable quantification. For all methods, bracketing injection was chosen and similar sample preparation could be applied. The methods were different with respect to the mode of calibration (see Experimental).

For the separate determination of sodium and potassium two different calibration modes were used, i.e. single- and multiple-point calibration. In the single-point calibration mode, the same absolute amount of the analyte in both the standards and the serum samples was injected into the chromatographic system. For this reason, each serum sample had to be diluted as a function of its individual concentration which had to be roughly determined before IC analysis. Using this calibration mode, good method accuracy was achieved (see column A in Tables 1 and 2). The maximum deviation from the certified value was −0.4% for sodium and +0.4% for potassium. Method precision for potassium satisfied the preset requirement, but for sodium it was slightly higher than required. For sodium the maximum C.V. was 1.3% (required 1.0%). For potassium the maximum CV was 1.2% (required 1.5%). It should be noted that the

Table 1
Analytical performance of the proposed IC methods for the measurement of sodium according to different analytical approaches and derived from analysis of three certified human serum reference materials

Serum	A			B		C	
	Concentration (mmol/l)	Δ (%) Target	C.V. (%) (n = 12)	Δ (%) Target	C.V. (%) (n = 12)	Δ (%) Target	C.V. (%) (n = 12)
SRM 909	133.9	−0.4	0.7	+0.9	0.8	+0.2	1.3
SRM 909a-1	148.5	−0.2	1.3	+1.1	1.5	+1.8	1.4
SRM 909a-2	126.5	+0.1	0.9	+1.3	0.8	+1.2	0.9

A: Separate determination of sodium in serum using single-point calibration (bracketing). B: Separate determination of sodium using a four-point calibration curve. C: Simultaneous determination of sodium and potassium using a two-point calibration curve.

Table 2
Analytical performance of the proposed IC methods for the measurement of potassium according to different analytical approaches and derived from analysis of three certified human serum reference materials

Serum	A			B		C	
	Concentration (mmol/l)	Δ (%) Target	C.V. (%) ($n = 12$)	Δ (%) Target	C.V. (%) ($n = 12$)	Δ (%) Target	C.V. (%) ($n = 12$)
SRM 909	3.567	+0.4	1.0	−1.5	1.5	−1.3	1.5
SRM 909a-1	3.656	+0.3	0.7	−1.6	1.2	−1.3	1.4
SRM 909a-2	6.210	± 0.0	1.2	−1.5	0.7	−0.1	0.9

A: Separate determination of potassium in serum using single-point calibration (bracketing). B: Separate determination of potassium using a four-point calibration curve. C: Simultaneous determination of sodium and potassium using a two-point calibration curve.

precision data include the within- and between-day errors of weighing, variations introduced during sample pretreatment, errors during IC measurements and the variation in dry mass of the lyophilized controls.

In the four-point calibration mode, standard curves covering the whole pathophysiological concentration ranges were used, allowing a fixed dilution of all samples. The results were calculated by linear interpolation on the standard curves encompassing the sample measurements (see Experimental). According to the bracketing injection scheme, sixteen standards had to be analyzed in total for the analysis of the three NIST serum samples. The reason for injecting the first series of four standards in sequence of increasing concentration, and the second in reverse sequence was to outweigh eventual drifts in the system. Overall, this approach was more time-consuming than single-point calibration and gave less accurate results during our first experiments (see column B in Tables 1 and 2). For sodium a positive bias of approximately +1.0% was observed, while it was approximately −1.5% for potassium. Further studies (repeated measurement campaigns) have to be performed in order to clarify whether this bias was really due to the calibration mode or due to problems related to the actual measurement campaign. Method precision was not significantly different from the single-point calibration mode (see Tables 1 and 2).

For the simultaneous determination of sodium and potassium, a two-point calibration was performed, using the extremes of the four-point calibration curves. Again, all serum samples could equally be diluted. Results were calculated by linear interpolation on the standard curves. Because of the differences in absolute concentration between sodium and potassium (25–35-fold) and of fixed dilution, measurements had to be done at different detector ranges (100 μS for sodium and 3 μS for potassium, see Fig. 1). In order to increase the number of standards, double injection of the two standards encompassing a series of serum samples was performed, as shown in Fig. 2. This resulted in a total of eight standards for the analysis of the three NIST sera. Again, standards were injected in increasing and decreasing concentration sequence. Method accuracy and precision were similar to the four-point calibration approach (see column C in Tables 1 and 2). The same remark as above holds true for elucidating the source of increased inaccuracy as compared to single-point calibration. Nevertheless, because of time saving, simultaneous determination of sodium and potassium would be of particular interest for further method improvement.

The accuracy and precision reached for the IC methods for total sodium and potassium, in particular when using single-point calibration, are comparable to those of the proposed candidate reference methods based on FAES. For the FAES method of sodium, a mean C.V. of 0.5% and a maximum deviation of +0.7% from the

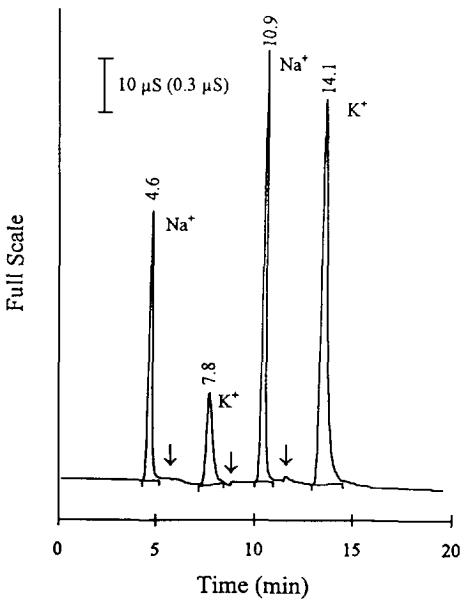

Fig. 2. Chromatogram for a double injection of standards for the simultaneous determination of sodium and potassium. The detector range was 100 μS for sodium and 3 μS for potassium (switching indicated by the three arrows). The second injection was done after elution of sodium as indicated by the first arrow.

certified target value was reported [3] (in our IC method, using single-point calibration, the mean C.V. was 1.0%; the maximum deviation was −0.4%). For the FAES method of potasssium, a mean C.V. of 1.3% and a deviation of +1.1% from the certified value was reported [4] (in our IC method, using single-point calibration, the mean C.V. was 1.0%; the maximum deviation was +0.4%).

4. Conclusion

We have developed IC methods which enable us to reliably determine total sodium and potassium in human serum. The sample pretreatment procedure is very simple, and the overall analysis time is reasonable. From the precision and accuracy obtained here, we suggest that IC principally may be considered as a valuable reference methodology, comparable to FAES. At this stage of the work, the results with single-point calibration fulfilled better the preset analytical requirements than those obtained with the calibration curve approach. However, further investigations should be performed to improve the precision and accuracy of the time-saving simultaneous determination of total sodium and potassium with the two-point calibration approach. The perspective of the use of IC as reference methodology is particularly attractive because of its great flexibility. Also clinically important divalent cations like calcium and magnesium or anions like chloride or phosphate can be analyzed with principally the same equipment. Further, IC might be an alternative to FAES when the latter is not available in laboratories relying predominantly on chromatographic techniques.

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