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Review

Ion chromatography as reference method for serum cations

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Abstract

We review our experience with the development, validation, and long-term operation of suppression-based ion chromatography reference methods for clinically important serum cations. First, we describe the accuracy and precision requirements for reference methods in clinical chemistry. Then, we present the general design of our methods and their validation with certified reference materials and method comparison with flame atomic emission and flame atomic absorption spectrometry. Additionally, we compare basic features of commercially available standard ion chromatography systems for our applications. Further, we discuss the influence of different sample preparation methods on accuracy and robustness of the methods. Among others, we describe reversed-phase clean-up and removal of anions with minicolumns or chromatographic front-cut with column-switching. Applications of the methods are presented for the field of external quality assessment and evaluation of accuracy and specificity of routine methods.

Keywords: Validation; Reference methods; Serum cations; Reviews; Metal cations

Contents

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1. Introduction

It has since long been realized that comparability of measurements in clinical chemistry, as in any other analytical discipline, can only be reached by agreement on a common metrological basis. In consequence, the International Federation of Clinical Chemistry (IFCC) recommended [1] the use of the 'Système International d'Unités' (SI) [2] in clinical chemistry. This system is shared with other physical and chemical sciences and provides an internationally accepted comprehensive and coherent system of quantities and units. However, in practice the realization of the system required the assessment of the accuracy[1] of the routine methods used and, when necessary, also their recalibration with accuracy-based reference methods and/or reference materials [7–11]. Since then, many efforts have been undertaken to harmonize measurement results in clinical chemistry based on these concepts.

One of the main obstacles for a successful implementation of the concept, however, are the matrix effects during calibration[2] of routine test systems (see also Fig. 1).

In short, calibration relates the measured analyte with the analyte defined by standards. For example, for measurement of the amount of substance concentration (mol/l) of sodium in serum, one applies a measurement procedure that gives a signal, which is transformed into a result via a measuring function. To obtain a metrologically correct result and to attribute a meaningful number to the unit, the whole measurement process needs to be calibrated. The calibrator can be a reference material, or another material for which the value has been assigned by comparison with a reference material. However, it should be stressed that the final value will only be correct when the value and the matrix of the calibrator are correct. For the classical, instrumental analytical methods for serum cations, such as flame atomic emission and flame atomic absorption spectrometry (FAES, FAAS), direct calibration was possible with weighed-in standard reference materials (SRMs), leading instantly to accurate routine measurements. For the more recently developed methods [12–21], however, direct calibration was hampered by the matrix sensitivity of the methods. This holds particularly true for methods based on measurements with ion-selective electrodes [12–14], or enzymatic determination [15–17].

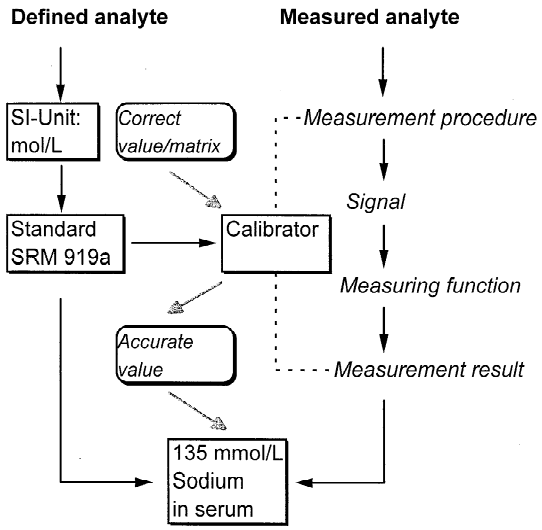

Fig. 1. Calibration of routine methods with standards.

As a solution to these problems, it was proposed to calibrate matrix-sensitive routine methods by comparison with reference methods on a representative panel of patient specimens [22] (see Fig. 2). This model requires matrix-insensitive 'validated' reference methods that can directly be calibrated with primary standards, and the accuracy of which has been assessed with matrix certified reference materials (CRMs). Such validated reference methods are then used for a split-sample method comparison with a routine method, which means parallel measurement of a sample panel with both the reference and routine method. In the case that the results of the latter

[1] Accuracy is used here in the sense of "accuracy of a method" [3,4], which refers to the systematic part of analytical error only. This is in contrast to the definition of the International Standards Organization (ISO) that addresses the "accuracy of a measurement result" [5], which includes the systematic and random part of analytical error. Our interpretation of accuracy is equivalent to the term 'trueness', which is used in other ISO documents [6]. We prefer the use of the term 'accuracy' because we expect it to be more familiar to the readers.

[2] Calibration is a "set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and corresponding values realized by standards" [5].

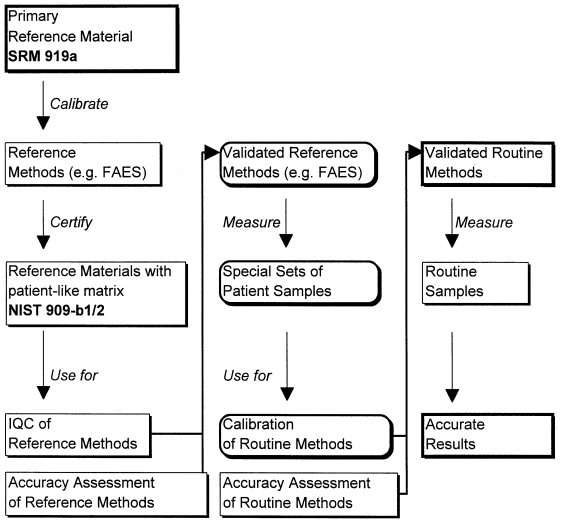

Fig. 2. Calibration of routine methods by comparison with reference methods.

measurements show that the routine method has no bias compared to the reference method, its calibration is proved to be correct. In the case of a clearly defined bias, recalibration of the routine method on the basis of the comparison is possible (provided the routine method has adequate specificity).

In view of these developments, there is an increasing demand in clinical chemistry for accurate, validated reference methods for serum analytes, amongst others for serum cations. In this context, FAES [23–25] and FAAS [26–30] are recognized as the traditional reference method principles for, respectively, serum sodium/potassium and calcium/magnesium. Our interest in the development of new reference methods for the above cations in serum was inspired by the fact that it is generally advocated that, for certification of reference materials, at least two independent measurement principles should be used to increase the reliability of the certified value [31,32]. In addition, we were looking for an analytical measurement principle for reference methods that would be applicable to a greater variety of analytes. In this perspective, we considered ion chromatography (IC) a candidate reference method principle. It had been previously applied successfully for the determination of various serum cations [33–43] and for the certification of calcium and magnesium in reference materials by the National Institute of Standards and Technology (NIST) [44,45]. In addition, the analytical flexibility of IC is such that it allows analysis of cations as well as of anions with the same basic instrumentation.

Here we review our experience with the development [46–48], validation [49], robustness [50], and application [51] of IC reference methods for serum sodium, potassium, calcium, and magnesium.

2. Discussion

2.1. General

The basis for the development of our methods was the definition of a reference method as given by the ISO: ‘‘thoroughly investigated method, clearly and exactly describing the necessary conditions and procedures, for the measurement of one or more property values that has been shown to have accuracy and precision commensurate with its intended use and that can therefore be used to assess the accuracy of other methods for the same measurement, particularly in permitting the characterization of a reference material’’ [52]. The most important element of this definition is that the accuracy and precision of a reference method ‘‘must commensurate with its intended use’’. The intended use of our methods was, for the field of clinical chemistry, the certification of control and reference materials and evaluation of routine methods by split-sample measurement. Therefore, we applied specifications for accuracy and precision that originally were recommended in connection with German guidelines for quality control in clinical chemistry [53,54] (see also Table 1). Slightly different values, intended to be used for networks of European reference laboratories, have been proposed more recently [55,56].

In order to comply with the requirements in Table 1, special protocols had to be worked out for calibration, sample pretreatment, measurement design, and assessment of performance (for details, see Refs. [46–48]). One of the most important elements was the use of primary reference materials for calibration. In our case, those were the SRMs from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), namely, sodium

Table 1
Proposed limits for the analytical error (AE), interassay R.S.D., 95% confidence interval, and bias of reference methods for serum cations[a]

Analyte	AE (%)	R.S.D. (%)	Confidence interval (%)	Bias (%)
Sodium	1.2	1.0	0.64[b]	0.56
Potassium	1.6	1.5	0.95	0.65
Calcium	2.0	2.0	1.27	0.73
Magnesium	2.4	2.0	1.27	1.13

[a]From Ref. [53,54].
[b]$n=12$ (numbers apply to target setting for EQA or certification of reference materials).

chloride (SRM 919a), potassium chloride (SRM 918), calcium carbonate (SRM 915), and magnesium gluconate dihydrate (SRM 929). All sampling and diluting steps were done under gravimetric control. Sample pretreatment and chromatographic conditions were to be optimized towards adequate selectivity and sensitivity. Last, but not least, we used the matrix SRMs 909, 909a-1, and 909a-2 from the NIST and the CRMs 303 and 304 from the Community Bureau of Reference (BCR, Brussels, Belgium) for accuracy assessment and internal quality control.

With regard to the general approach for analysis and calibration, we opted from the beginning for single compound analysis and single point calibration. Single compound analysis is generally performed in reference method technology [55–57]. It has the advantage of better method optimization and control. For example, calibration drifts during a run can be compensated by multiple injection of standards. The disadvantage of this approach are its higher costs when several analytes are to be determined in the same sample. Single point calibration usually is done at a point that represents the mid of the reference range and sample volume is then adjusted to that point. This requires that, before IC analysis, the approximate concentrations of unknown samples have to be determined with routine methods. It should be noted that this approach requires demonstration that the method is insensitive to differences in the total sample volume taken for analysis. In our methods, different sample volumes are diluted to a final volume of 4 ml, resulting in higher dilution factors for higher concentrated samples. In this connection, we demonstrated that our methods were not influenced by sample dilution factors $>1{:}20$ [46]. However, we do not advocate dilution factors $<1{:}20$ for serum samples because this might result in an incomplete release of ions from the proteins. Further, with smaller dilution factors, the solvent displacement effect of proteins may cause problems. When proteins are removed without sample dilution (for example when ultrafiltration is applied), the remaining solution will be concentrated by approximately 4–7%, depending on the protein concentration [58]. Therefore, in the case that the expected analyte concentration spans a great range, it might be necessary to group samples according to similar concentration and perform single point calibration at different concentration levels. In our hands, single compound analysis and single point calibration proved to be superior, in terms of accuracy, to multiple compound analysis and use of complete calibration curves [47].

The development of adequate internal quality control procedures was very important for reaching the required analytical quality. These procedures concern the maximum within-day relative standard deviation ($R.S.D._{wd}$) and the maximum daily deviation from the target value of the certified accuracy control materials. We decided, for our IC methods, to set the limits in such a way that the specifications listed in Table 1 were likely to be fulfilled after the 3 measurement days in 90% of the cases. In the remaining 10% of the cases, a fourth measurement day had to be added. We thus set the limit for $R.S.D._{wd}$ to 80% of the limit for the overall reproducibility R.S.D. listed in Table 1, resulting in a limit of 0.8% for sodium, 1.2% for potassium, and 1.6% for calcium and magnesium (*note*: in the case that these limits were exceeded, outlier investigation was made before a fourth set of quadruplicates was analyzed). Accuracy control was done in such a way that sample measurements only were started pro-

vided that the deviation from the target was less than 125% of the limit for total analytical error (AE) listed in Table 1, resulting in limits of 1.5% for sodium, 2.0% for potassium, 2.5% for calcium, and 3.0% for magnesium. Otherwise, the method was checked, e.g. the standards or controls. A fourth measurement day also was added when the overall specifications listed in Table 1 were not fulfilled.

2.2. Chromatographic system

In recent years, there has been a considerable improvement in IC column technology and eluent systems for the determination of mono- and divalent cations, with the result that most of the systems that had been used before for the measurement of serum cations [33–42] became outdated. Further, most of the currently offered standard systems have incorporated these improvements. In this view, we began our method development with the standard conditions used by Dionex (Sunnyvale, CA, USA). The exception being that we slightly modified the chromatographic system for the divalent ions by using a second precolumn instead of an analytical column. This reduced analysis time considerably without adverse effect on resolution. Representative chromatograms are shown in Figs. 3–5. Later, we took the opportunity to compare several standard systems from different manufacturers [48] (see also Table 2). The comparison included systems from Dionex (DX-100 and DX-500), Alltech (Deerfield, IL, USA), and Metrohm (Herisau, Switzerland). The columns we investigated were the polymer-based Ionpac C10 and C12 (Dionex) and the silica-based Metrosep Cation 1-2 (Metrohm) and Universal Cation (Alltech). All systems were equipped with their original detection systems, i.e. based on conductivity measurement with eluent suppression in the Dionex systems, without suppression in the Alltech and Metrohm systems. The evaluation mainly focused on baseline stability, detection limit for magnesium, and specificity.

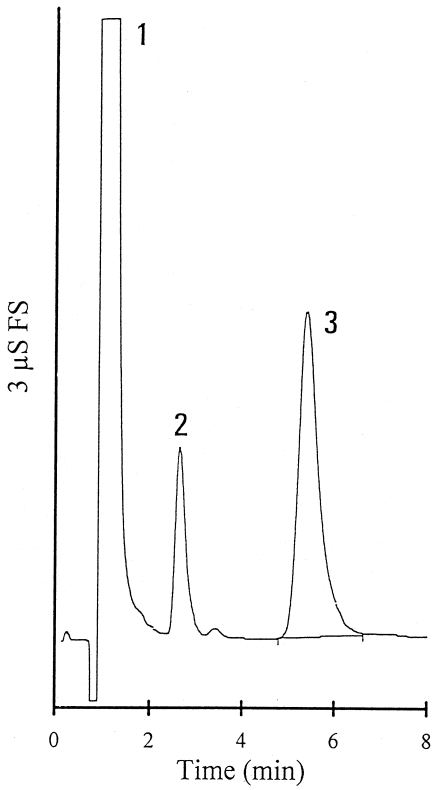

Fig. 4. Representative chromatogram for analysis of calcium (retention time 5.35 min). The designated peaks represent: (1) monovalent cations; (2) Mg^{2+}; (3) Ca^{2+}. Chromatographic conditions: DX-100; two CG10 columns; eluent, 4 mmol/l DAP/HCl and 40 mmol/l HCl; flow-rate, 1 ml/min; chemical suppression. Reproduced with permission from Ref. [46].

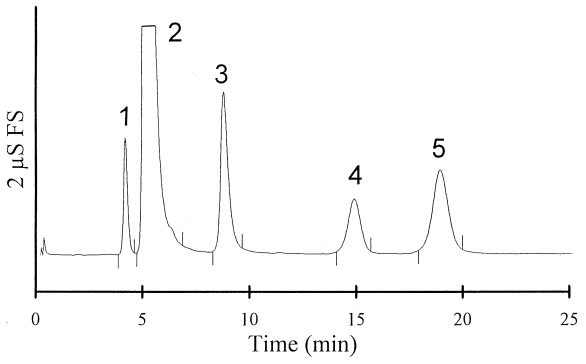

Fig. 3. Representative chromatogram for analysis of sodium or potassium (retention times 5.20 and 8.70 min). The designated peaks represent: (1) Li^+; (2) Na^+; (3) K^+; (4) Mg^{2+}; (5) Ca^{2+}. Chromatographic conditions: DX-500; CG12 and CS12 columns; eluent, 7 mmol/l H_2SO_4; flow-rate, 1 ml/min; electrochemical suppression.

Baseline registration for the systems without suppression revealed that, independent of the eluent used, the Metrohm system was superior to the Alltech system (compare Fig. 6C with Fig. 6D for

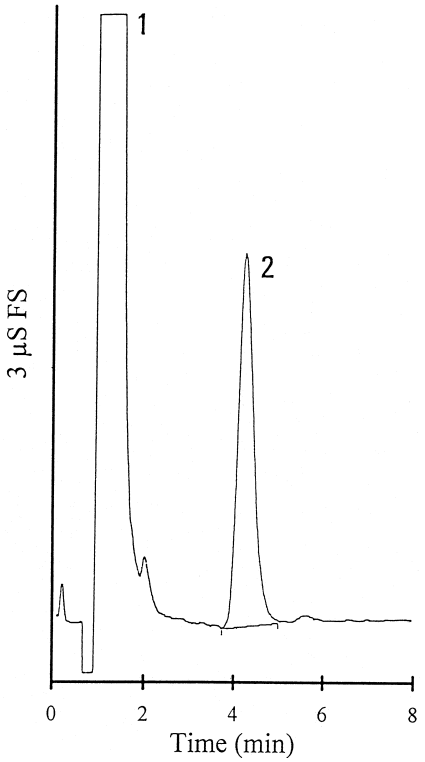

Fig. 5. Representative chromatogram for analysis of magnesium (retention time 4.2 min). The designated peaks represent: (1) monovalent cations; (2) Mg^{2+}. Chromatographic conditions: DX-100; two CG10 columns; eluent, 2 mmol/l DAP/HCl and 40 mmol/l HCl; flow-rate, 1 ml/min; chemical suppression. Reproduced with permission from Ref. [46].

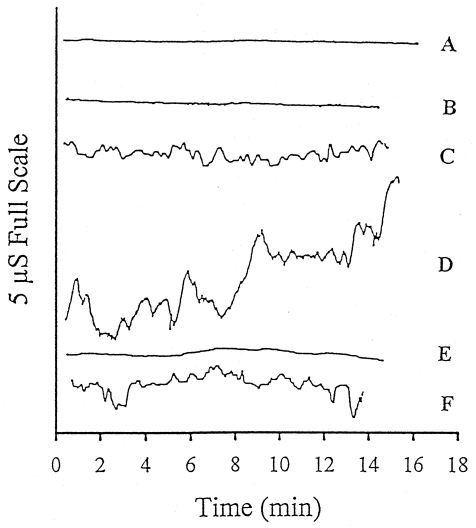

Fig. 6. Representative baseline registrations obtained with different ion chromatography systems, columns, and eluents under identical data acquisition conditions. (A) Dionex DX-500, electrochemical suppression. (B) Dionex DX-100, electrochemical suppression. (C) Metrohm, unsuppressed. (D) Alltech, unsuppressed. (A–D) Column, Alltech Universal Cation; eluent, 3 mmol/l MSA; flow-rate, 1.5 ml/min. (E) Metrohm system; Column: Metrosep Cation 1-2; eluent, 4 mmol/l TA+1 mmol/l DPA, unsuppressed; flow-rate, 1.5 ml/min. (F) Dionex DX-100; Column: 2× CG10; eluent, 4 mmol/l DAP+40 mmol/l HCl, chemically suppressed; flow-rate, 1 ml/min.

the methanesulfonic acid (MSA) eluent; data for the tartaric acid/dipicolinic acid (TA/DPA) eluent are not shown). The reason for this might be that the Metrohm system used a double-piston pump, while the Alltech system used a single-piston one. However, for the same MSA eluent, the best baseline (stability and signal-to-noise ratio) was achieved with the Dionex systems using electrochemical suppression (see Fig. 6A and B). This confirms the advan-

Table 2
Standard IC systems from various manufacturers

System-component	Manufacturer/System name			
	Dionex DX-100	Dionex DX-500	Alltech	Metrohm IC 690
Pump	Single piston	Dual piston	Single piston	Dual piston
Column	Polymer/sulfonate (C10) Polymer/carboxylate (C12)		Silica (polymer coated)/ carboxylate	Silica (polymer coated)/ carboxylate
Eluent	HCl/DAP (C10) MSA (C12)		MSA or citric acid	TA/DPA
Suppression	Chemical or electrochemical		None (electronic zero)[a]	None (electronic zero)[b]
Detection	Conductivity		Conductivity	Conductivity

MSA, methanesulfonic acid; TA, tartaric acid; DPA, dipicolinic acid; DAP, DL-2,3-diaminopropionic acid monohydrochloride.
[a]Electrochemical suppression now available.
[b]Chemical suppression available only for anions.

tage of suppression for eluents with a high background conductivity (*note:* in the meanwhile, Alltech also offers a suppression system for cation analysis; the suppressor system from Metrohm is restricted to anion analysis). Interestingly, the baselines were similar for the two Dionex systems, despite the fact that the DX-100 is equipped with a single-piston pump while the DX-500 had a dual-piston one. Therefore, it seemed that the pump type is not of great importance under conditions of suppressed conductivity. This was confirmed by the fact that use of the DX-100 with the Dionex dual-piston pump did not improve the quality of the baseline. It is worth noting that the baseline of the DX-100 with the DL-2,3-diaminopropionic acid monohydrochloride/hydrochloric acid (DAP/HCl) eluent in the chemical suppression mode was worse than that of the Metrohm system with the MSA eluent (compare Fig. 6F with Fig. 6C). In addition, the baseline of the Metrohm system with the TA/DPA eluent was nearly as good as those of the Dionex systems using electrochemical suppression (compare Fig. 6E with Fig. 6A+B). This means that, in terms of baseline stability and signal-to-noise ratio, a non-suppressed eluent with a low conductivity may be superior to a suppressed eluent with a high conductivity. In this context it has to be mentioned that, unfortunately, electrochemical suppression cannot be applied to eluents based upon HCl or nitric acid.

The described differences in baseline quality were directly reflected in the detection limits reached for magnesium. The detection limit for magnesium (defined as a signal-to-noise ratio >3), determined with an aqueous standard, was 1.3 pmol using the DX-100 system in the chemical suppression mode. With the Metrohm system and using the TA/DPA eluent, it was 0.4 pmol, whilst with the DX-500 system used in the electrochemical suppression mode, even 0.2 pmol was reached. In the Alltech system, the baseline drift was so high that the detection limit was >4 pmol.

The standard chromatograms (not shown) as provided by the manufacturers demonstrated that, generally, all systems are applicable for the simultaneous determination of sodium, potassium, calcium, and magnesium. However, with some serum samples, chromatographic interferences at the elution site of potassium have been observed with the Alltech Universal Cation column, and in the region of the divalent ions with the Ionpac C12 and the Metrosep 1-2 column (unpublished observations). These interferences might be resolved for specific sera by optimization of the respective eluents, however, other ones might then occur in other sera. In contrast to this, we did not experience any interference problem with the Ionpac C10 column when used for the analysis of calcium and magnesium, and with the Ionpac C12 column when used for sodium and potassium. From the observations described here, it is obvious that the specificity of each chromatographic system should be evaluated with a sufficiently high number of serum samples.

2.3. Robustness

We have applied our IC methods now for more than 4 years. During that time, unfortunately, we have been faced with a variety of practical operating problems [50]. On the long term, we were faced with suppressor problems, for example, increased backpressure and decreased resolution and sensitivity. We first assumed that the problems were caused by incomplete removal of proteins and/or organics. Therefore, we changed sample pretreatment from simple acidic dilution and filtration, into acidic dilution, additional heating at 70°C for 2 h, and reversed-phase purification [49]. In spite of this, the problems persisted, particularly with calcium and magnesium analysis which applied a DAP/HCl eluent and chemical suppression. Therefore, with respect to the suppression mode, we looked for a substitute for the DAP/HCl eluent that could be electrochemically suppressed. This, in particular, since it would have allowed us to use the same suppression mode for mono- and divalent ions and, therefore, would have facilitated system operation. Such an eluent was indeed found by combining sulfuric acid (H_2SO_4) with histidine [59]. The substitution of DAP/HCl eluents with histidine/H_2SO_4 eluents had no adverse effect on retention time, baseline stability or chromatographic resolution.

Unfortunately, in spite of the aforementioned modifications, the suppressor problems persisted. This drew our attention to the fact that anionic species might be responsible for it. Removal of

anions by application of anion-exchange columns was not possible because it affected method accuracy in an unpredictable way [50]. Therefore, we removed the anions by 'front-cut', which had no adverse effect on accuracy. However, this required the installation of an additional pump and switching valve to the system [50]. These modifications made it possible to return to our original sample preparation procedure applying simple acidic dilution and filtration [46,47]. However, with this procedure we observed decrease of response after 1 week of operation due to column contamination. It is noteworthy that the performance could be restored by organic column rinsing. Consequently, we added 10% acetonitrile to the eluent, which, since then, has resulted in a trouble-free operation of the modified system. Because of the positive experiences with the calcium and magnesium methods, we decided to adopt the 'front-cut' also for the analysis of sodium and potassium. Addition of acetonitrile, however, was not favourable because it led to a high background and instable baseline. The reasons for the latter are unclear to us. *Note*: additional baseline stabilization can be achieved by pumping the suppression fluid through the suppressor, instead of using a pressurized bottle for delivery.

Further, during potassium analysis with new columns we observed a different behaviour of standards and samples (diluted and filtrated) (unpublished observations). After some days of measurement, samples showed an increased response (up to 7%) compared to standards. We were not able to find an explanation for this strange behaviour. Neither additional sample purification (anion-exchange or reversed-phase) nor reduction of injection volume (from 25 to 15 μl) were able to solve the problem. However, this phenomenon disappeared after organic rinsing of the column as prescribed by the manufacturer. Similar, unexplained effects, also were observed by others [60].

2.4. Short description of current methods

In view of the above-described experience, we would recommend the following methods for the analysis of serum cations by IC.

2.4.1. Preparation of samples

Dilution with 2 mmol/l HCl (dilution factors >1:20); 1 h equilibration, and filtration over 0.45-μm filters. 'Front-cut' (time to be adapted to analyte).

2.4.2. Chromatographic system

Sodium and potassium: CG12 and CS12 column (Dionex); eluent 7 mmol/l H_2SO_4; flow, 1 ml/min; electrochemical suppression. Calcium (magnesium): two CG10 columns (Dionex); eluent, 17 mmol/l H_2SO_4+2 mmol/l histidine (0.7 mmol/l histidine)+ 10% (v/v) acetonitrile; flow, 1 ml/min; electrochemical suppression. Pump: single- or double-piston pump (Dionex). Detector: conductivity detector CDX-500: all concentrations can be covered without scale switching.

2.5. Accuracy and precision

The initial evaluation of our methods for determination of serum cations with matrix SRMs/CRMs showed a generally good accuracy and precision of the IC methodology [46,47]. In addition to this, we had the opportunity to measure, over a period of more than 2 years, unknown serum samples in parallel with laboratories that applied the classical FAES/FAAS reference methods [49]. Usually, samples were measured in batches of four to six and within a period of 6 weeks. During these distinct measurement campaigns, internal quality control was performed with SRMs/CRMs. In this way, we were able to investigate the long-term performance of our methods. In Table 3, typical data for precision and accuracy obtained over the 2-year period are presented. The precision is expressed as overall R.S.D., since it consists of the combination of the between-day measurement R.S.D. (12 measurements performed as quadruplicates on 3 days), the sample

Table 3
Precision (overall R.S.D., $n=12$) and accuracy data obtained for internal quality control with certified SRMs/CRMs in different measurement campaigns

Analyte	n[a]	Mean R.S.D. (%)	Bias (%)
Sodium	9	0.7	+0.1
Potassium	8	0.9	−0.3
Calcium	10	1.3	−0.1
Magnesium	10	1.0	±0.0

[a]Number of measurement campaigns performed over a period of 2 years.

preparation R.S.D., and the dry-mass variability of the lyophilized samples. The mean R.S.D.s achieved with the IC methods were 0.7% for sodium, 0.9% for potassium, 1.3% for calcium, and 1.0% for magnesium. For comparison, the classical reference methods based on FAES/FAAS typically achieve R.S.D.s of <1% for sodium, <1.5% for potassium, <1.3% for calcium, and <1.1% for magnesium [24–26,30,55]. These data thus demonstrate that IC can reach a precision similar to the classical reference methods.

The bias of the methods (Table 3) was judged from the mean deviations of the IC results for the SRMs/CRMs from their respective target values. It was +0.1% for sodium, −0.3% for potassium, −0.1% for calcium, and ±0.0% for magnesium.

The above data show that the limits of overall R.S.D. and bias, as presented in Table 1, were in no case exceeded. They allowed us thus to conclude that also the long-term performance of the IC methods is well commensurate with the preset specifications.

Currently, we are investigating the use of internal standards (rubidium for sodium and potassium analysis and strontium for calcium and magnesium analysis) for improvement of measurement precision. They are intended for compensation of short-term fluctuations of detector response, however, not for calibration. The latter is due to possible inaccuracies stemming from the fact that, because of the protein matrix, the internal standard and analyte might behave differently during sample preparation, as observed by us when we applied ultrafiltration for deproteinization [46].

2.6. Validation of the IC methods by comparison with FAES/FAAS

As mentioned before, we compared our IC methods with FAES/FAAS over a period of more than 2 years by measurement of >25 unknown samples [49]. Table 4 shows the mean deviations observed during this method comparison. They were +0.9% for sodium, +1.0% for potassium, ±0.0% for calcium, and +0.1% for magnesium. Additionally, with the exception of two values each for sodium (−2.9 and +4.3%) and potassium (−4.5 and +3.3%), all deviations for single samples were within the limit of two-times the AE stated in Table 1 (*note:* two-times AE because FAES/FAAS were operated under the same specifications as IC).

Table 4
Mean deviation of the IC results from the FAES/FAAS values and mean overall R.S.D.s obtained during method comparison

Analyte	n[a]	Mean deviation (%)	Mean R.S.D.s (%)
Sodium	27	+0.9	0.8
Potassium	27	+1.0	1.2
Calcium	31	±0.0	1.4
Magnesium	31	+0.1	1.3

[a]Number of samples used for the comparison.

Also listed in Table 4 are the precisions of the IC measurements as observed with the samples used for the comparison of IC with FAES/FAAS. The mean R.S.D.s amounted to 0.8% (n=27) for sodium, 1.2% (n=27) for potassium, 1.4% (n=31) for calcium, and 1.3% (n=31) for magnesium. These R.S.D. values were in agreement with the R.S.D.s obtained for the certified SRMs/CRMs used for internal quality control. In this way, the method comparison confirmed the precision and accuracy data observed with the quality control materials.

We want to note, however, the slight positive bias of approximately 1% observed for sodium and potassium. Up to now, we have not been able to investigate whether it was due to the IC methods, the FAES methods, or both.

3. Applications

3.1. Method comparisons with a panel of patient samples

As already addressed before, reference methods are particularly useful in clinical chemistry for accuracy assessment of routine methods. In this regard, the assessment of routine methods for calcium is most interesting. Measurement of serum calcium is still considered a challenge for the routine laboratory, because its low biological variation requires highly accurate and precise routine methods [61]. Therefore, we evaluated four frequently used routine test systems for serum calcium with our IC reference method by split-sample measurement of a panel of 88 patient samples [51]. The emphasis of the study was assessment of the overall bias of the test systems and their sensitivity to common sample

matrices. It revealed that the *intrinsic quality* of commonly used test systems for serum calcium satisfies the criteria for method bias. However, the study was undertaken under within-run and rigorous internal quality control conditions, and in the application laboratories of the respective manufacturers. Therefore, it is to be expected that the same test systems will not perform with the same quality in the routine laboratory. Errors of two- to three-fold of those observed in the study may have to be taken into account. In this respect, we can conclude that routine measurements of serum calcium are still a challenge for the average routine laboratory. This holds especially true for appropriate installation of a test system as well as the efforts that have to be invested for internal quality control. In particular, laboratories should use stringent internal quality control criteria and control materials with highly accurate target values, and should take special care to reduce between-run variability.

Currently, we are preparing a similar study for potassium. This time, besides investigation of the intrinsic quality as offered by the manufacturer, we intend also to investigate the quality of potassium analysis in the routine clinical laboratory. For this reason, we included several routine laboratories in the study.

Such applications will become more and more important in the future, since European [62] and international [63] regulations currently are elaborated that will require comparison of routine test systems with reference methods.

3.2. External quality assessment

Reference method values are important in external quality assessment (EQA) for the following reasons. In clinical chemistry, EQA usually applies multianalyte, spiked, delipidized, and lyophilized serum-based samples. As already addressed, many routine methods are sensitive to samples with artificial matrices. Therefore, such highly processed materials often give the wrong impression of method accuracy, and reference methods are needed to test their suitability for accuracy assessment of routine methods. Further, EQA results often are interpreted in terms of deviation from the consensus means. This approach, however, may be misleading because the consensus mean might be influenced too much by the results from laboratories that apply the method of the market leader. Therefore, it is recommended to use native materials and reference method target values when EQA is used for investigating accuracy of routine methods [64]. By comparison of native and processed sera with IC and FAES reference method target values for sodium and potassium in Czech EQA-surveys, we demonstrated that processed sera exhibit matrix effects, in particular, for routine methods that use ion-selective electrodes for measurement (unpublished results).

4. Conclusions

We have developed IC methods which enable us to reliably determine sodium, potassium, calcium, and magnesium in serum, with simple sample pretreatment procedures and in reasonable overall analysis time. From the analytical properties in terms of precision and accuracy we conclude that the IC methods are well suited to serve as reference methods in clinical chemistry. The latter was proven by validation of the methods with CRMs/SRMs and method comparison with the traditional reference methods based on FAES/FAAS. However, according to our experience over a number of years, it is obvious that performance of standard IC systems is sensitive to samples with complex matrices. Therefore, for successful long-term application with these kinds of samples, the robustness of standard IC systems generally has to be improved, e.g. by instrumental alterations such as the described 'front-cut'. In addition, it should be realized that the availability of certified accuracy control materials for method evaluation and continuous performance control is indispensable.

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