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RESEARCH ARTICLE

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Ion chromatography as candidate reference method for the determination of chloride in human serum

Min Shen[1,2] | Minmin Tu[1] | Wei Zhang[1] | Jihua Zou[1] | Man Zhang[2] | Zheng Cao[3] | Bingde Zou[1]

[1]Reference Laboratory, MedicalSystem Biotechnology Co., Ltd., Ningbo, China

[2]Department of Laboratory Medicine, Beijing Shijitan Hospital, Beijing, China

[3]Department of Laboratory Medicine, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, China

Correspondence
Jihua Zou, Reference Laboratory, MedicalSystem Biotechnology Co., Ltd., Ningbo, China.
Email: jihua.zou@nbmedicalsystem.com

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Abstract

Background: We developed an ion chromatography (IC) method for measurement of chloride in human serum which was regarded as a simple, rapid, accurate, and sensitive technique. The method will be hopefully selected as a candidate reference method.

Method: Serum aliquots of 0.1 mL were diluted 500 times with Milli-Q water, and chloride in serum samples was measured by IC with a gradient elution procedure using a KOH eluent generator.

Results: Based on the data, chloride in human serum was well detected by IC. The calibration curve for chloride was linear in the concentration range from 0 to 0.42 mmol/L with a correlation coefficient of .99995 under the optimum experimental conditions. The chloride concentration had a good linear relationship with the peak areas of chloride. This method was sensitive because of the low limit of detection (LOD) and the low limit of quantification (LOQ) 9.87×10^{-5} mmol/L and 3.27×10^{-4} mmol/L, respectively. Besides, the method was highly precise with the within-run coefficient of variations (CVs) for the measurement of low, medium, and high concentration level samples 0.32%, 0.73%, and 0.50%. As for the evaluation of accuracy, the biases were less than ±1% and 2% by comparing with National Institute of Science and Technology (NIST) standard material SRM 956d and 2013-2018 IFCC-RELA samples, respectively. Finally, the biases between IC method and the inductively coupled plasma mass spectrometry (ICP-MS) method were less than 1% which showed good agreement.

Conclusion: Ion chromatography is a simple sample treatment procedure for the determination of chloride in human serum with high sensitivity and specificity. The proposed method could be recommended as a candidate reference method for the determination of serum chloride in human serum.

KEYWORDS

candidate reference method, ion chromatography, serum chloride

Min Shen and Minmin Tu contributed equally to this work.

1 | INTRODUCTION

Serum chloride is the major anion in human body which has to be kept within narrow limit to ensure the maintenance of electrolyte homeostasis in both intra- and extracellular compartments of the organism. The reference range in serum of normal individuals is in a range of 98-106 mmol/L. Some disorders, such as hypernatremia, impaired renal function, hypertonic dehydration, nephritis, respiratory alkalosis, severe vomiting, and diarrhea are related to the chloride concentration which has deviated the normal range in human body. Therefore, standardizing the measurement of chloride is important in order to ensure the accuracy and traceability of conventional system detection as well as accurately judgment the health of the patient.

Now, the routine methods which are used to determine the serum chloride were mercury thiocyanate colorimetric method, mercury nitrate titration, ion selective electrode method,[1] and enzyme coupling method. They are detected based on fully automatic biochemical analyzer[2] which can determine the concentration of chloride in serum efficiently. However, the accuracy and stability of the measurement results by routine detection system need to be improved for more accurate diagnosis. Therefore, the study of traceability and standardization of serum chloride should be on the agenda so that the accuracy of routine methods can be improved.

According to the list of reference measurement methods/procedures for serum chloride from Joint Committee for Traceability in Laboratory Medicine (JCTLM), reference measurement procedures have been described for the determination of chloride in serum using coulometry,[3] isotope dilution mass spectrometry (ID/TIMS),[4] inductively coupled plasma mass spectrometry (ICP-MS)[5] and inductively coupled plasma optical emission spectrometry (ICP-OES).[6] However, the methods are not suitable for extension. First of all, coulometry is known as a suitable method because of the relatively high precision of the measurement. Nevertheless, the solubility of silver chloride is the key influencing factor of coulometric titration. Methanol, ethanol, acetone, acetic acid, and trifluoroacetic acid are added to reduce the solubility of silver chloride. Due to the addition of organic solvents, harmful effects will be emerged.[7] Secondly, the ID/TIMS which can accurately determine the concentration of chloride as the definitive method is too expensive to apply for the general laboratory. Once more, ICP-MS has high accuracy and sensitivity, but the high operating cost makes it difficult for general laboratories to complete. At last, ICP-OES can be measured without pre-dilution of samples, but more susceptible to instrument instability. In the light of the problem, a more suitable measurement procedure should be developed to apply to more laboratories. According to the use of ion chromatography (IC) reference measurement procedures to determine cations in human serum reported in the previous literature,[8-11] the measurement precisions obtained are comparable to those achieved in clinical chemistry using other reference procedures and are approximately in the range of 1%.[12]

Herein, IC is applied to determine the concentration of chloride in serum and evaluate this method. IC can simultaneously analyze a variety of ions with good selectivity, high sensitivity, good stability, and low operating cost. A novel measurement procedure can be applied to be as a candidate reference method for the determination of chloride in human serum.

2 | EXPERIMENTAL

2.1 | Instrumentation

A model Dionex-2000 ion chromatography (Thermo Fisher Scientific Inc.) equipped with an AS40 automated sampler operator and an EG50 automated eluent generator was used. For chromatographic separation, an Ionpac AG 19HC guard column (50 mm × 5 mm i.d.) coupled to an Ionpac AS 19HC analytical column (250 mm × 5 mm i.d.) was used. Electronic suppression was achieved with an anion Self-Regenerating Suppressor system (ASRS-II, 4 mm) used in the auto-suppression recycle mode. Detection was based on conductivity. The chromatographic signals were performed with a chameleon chromatography workstation (Chromeleon 7.1.2). A water purification system (Millipore) was used to provide Milli-Q water of ultra-pure quality (18.2 MΩ). Weighing procedures were done with a model of XS205 Dual Range electronic balance (Mettler Toledo) which has a readability of 0.01 mg. A rotator PTR-35 (Grant-bio) was used to redissolve the lyophilized serum. Pipetting was done using 5 and 1 mL pipet tips from Eppendorf (Eppendorf AG). A total of 50 and 100-mL volumetric flasks (Asone) were used to deal with constant volume.

2.2 | Materials

Human serum samples were collected from different patients. IFCC-RELA (IFCC External Quality assessment scheme for Reference Laboratories in Laboratory Medicine) specimens were obtained from Referenzinstitut für Bioanalytik [RfB]. The standard reference material (SRM 919b, SRM 956d) was purchased from National Institute of Standards and Technology (NIST). The water was dealed with Millex-LH syringe filters (0.45 μm pore size, Hydrophilic, PTFE, 13 mm, IC-certified) from Millipore. For methodological studies, 27 fresh serum samples were collected from the leftovers of patient samples in Medical system Diagnostics. SRM 919b (pure sodium chloride) were used for the preparation of standard curve. Three different serum samples were detected for analysis of precision. For analysis of accuracy, the results of IFCC-RELA samples from 2013 to 2018 and SRM 956d were used as reference standards.

2.3 | Ion chromatography

Chloride was separated using the isocratic conditions with an Ion pac AS 19HC analytical column. The eluent used was KOH. The gradient setting procedure of KOH was as follows: 0-12 minutes, the concentration of KOH was 10 mmol/L; 25 minutes, the concentration of KOH was upped to 45 mmol/L; 25-30 minutes, the concentration of KOH was downed to 10 mmol/L for the next injection. The injection volume was 25 μL, and the eluent flow-rate was 1.5 mL/min. Anions

were detected with suppressed conductivity detection. The results were determined by external standard method of peak areas.

2.4 | Preparation of calibrators and calibration curves

A calibration graph was constructed by plotting the peak areas against the concentrations of the standard injection for the chloride. For the accurate results, the stock solution of chloride was prepared by dissolving the standard reference material (0.24799 g SRM 919b) in Milli-Q water using a 100 mL volumetric flask. The working standard solutions were prepared fresh daily at six levels (0 mmol/L, 0.08 mmol/L, 0.17 mmol/L, 0.25 mmol/L, 0.34 mmol/L, 0.42 mmol/L) by stepwise dilution of the stock with Milli-Q water, and the density measurement was similar to Zhang's report.[13] Peak areas were collected against chloride concentrations and used for construction of the calibration graph.

2.5 | Method for the determination of chloride in serum

Firstly, the samples and water were kept at room temperature. After 30 minutes of equilibration, 0.1 mL sample was accurately pipetted in 50 mL volumetric flask and then the sample was dissolved in Milli-Q water and analyzed by IC method as 2.3 showed. The concentration of chloride was calculated from the calibration graph for the anion separately. The volumetric flask was first washed with Milli-Q water, then rinsed with chromic acid and finally washed with Milli-Q water for later use.

3 | RESULT AND DISCUSSION

The chromatogram in Figure 1 clearly showed the advantages of using the IC to determine the concentration of chloride in human serum. Serum chloride is the primary anion in body, and other anions are in low rate of total anions.[14] In consideration of this case, the sample was diluted 500-fold with Milli-Q water. In the chromatogram of serum sample, the peak was well separated from other anions (organic acids, phosphate, sulfate, etc,) and most ions did not cause any interference in the determination. The method can remove the interference of other anions for the chloride which can not only simplify the process, but also get more accurate results. So the sample processing was fit for this research.

3.1 | Standard curve and sensitivity of the IC method

Ion chromatography as a candidate reference method for the determination of chloride in human serum needed to be studied on

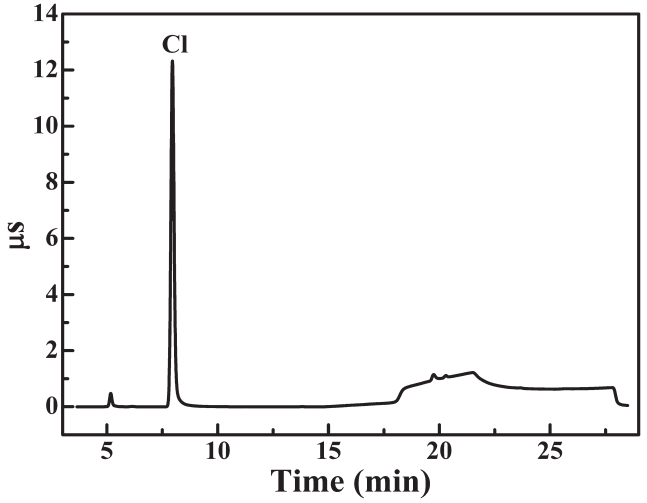

FIGURE 1 Serum chromatogram after dilution

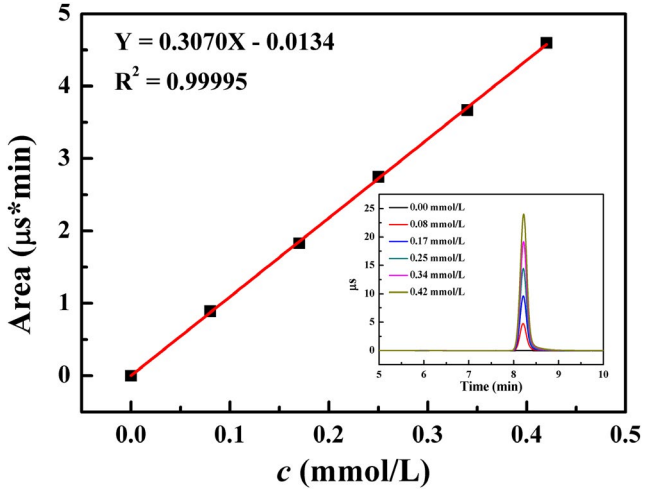

FIGURE 2 The standard curve of chloride

the performance evaluation. In this study, the peaks were evaluated on the basis of their areas. Unfortunately, the results could not be calculated just through the evaluation software. The standard solutions were needed so that the concentrations of chloride could be gotten by comparing with the areas of peaks. The standard material was detected, and then the linearity data and coefficient constant for determination of chloride were calculated. In Figure 2 and Table 1, the linearity between peak areas and chloride concentration was obtained in the range 0-0.42 mmol/L. In order to get the most accurate result, the sodium chloride which purity is 99.999% was chosen as the standard, and it was important to have the accurate weighing and constant volume. The linear equation was $Y = 0.3070X - 0.0134$, $R^2 = .99995$. The concentration of chloride in serum specimens was determined by the linear equation. The limit of detection (LOD) for chloride was determined at three times the noises, and the limit of quantification (LOQ) for chloride was determined at ten times the noises. LOD and LOQ values were 9.87×10^{-5} mmol/L and 3.27×10^{-4} mmol/L, respectively.

TABLE 1 Linear range, the limit of detection, and the limit of quantitation of chloride

Program	Linear range (mmol/L)	Regression equation	Correlation coefficient	LOD (mmol/L)	LOQ (mmol/L)
Cl	0-0.42	$Y = 0.3070X - 0.0134$	$R^2 = .99995$	9.87×10^{-5}	3.29×10^{-4}

Abbreviations: LOD, low limit of detection; LOQ, low limit of quantification.

3.2 | Precision and accuracy of IC method

To test the precision of this method, three different concentration levels of serum samples including high, medium, and low concentration serum samples were chosen. For each concentration, six samples were pretreated in parallel and then detected by IC method. Chloride concentration, within-run CVs, and with-day CVs are presented in Table 2. The within-run CVs for the measurement were less than 0.8% and the with-day CVs ranged from 0.58% to 0.97%. The result can be proved that the method was stable for the determination of serum chloride.

To test the accuracy of this method, the results obtained from serum samples were used to compare with SRM 956d and IFCC-RELA samples. The data were listed in Table 3 and 4. In Table 3, the results obtained by the proposed method were compared with those results obtained using a Joint Committee for Traceability in Laboratory Medicine (JCTLM) listed method as reference measurement procedures, that is, the value detected using the IC method was compared with the certified value of the SRM 956d. The results were in good agreement by calculating the bias. The bias of the proposed IC method was also monitored by calculation of the difference between the IC results and the mean results set as target values from the IFCC External Quality assessment scheme for Reference Laboratories in Laboratory Medicine. As the results were showed in Table 4, the deviations of 2013-2018 IFCC-RELA samples were less than 1.15% which was in the request of ± 2%. The fact that IC can determine the accurate concentration of chloride in human serum has been revealed.

3.3 | Method comparison

Inductively coupled plasma mass spectrometry is accepted as reference methods which were used by many reference laboratories. This method has been proved to be the correct measurement of chloride in human serum. The concentration of chloride determined by accepted reference method was regarded as the standard. In Figure 3, the result of determination by proposed method showed that IC and ICP-MS had the good comparability because of the good linear correlation coefficient ($R^2 = .998$), slope (a = 1.0229), and intercept (b = 0.0538). So IC method has good accuracy. Simultaneously, the sample pretreatment procedure was very simple and the detection process was easy. The low concentration of chloride also can be detected even at 9.87×10^{-5} mmol/L. Therefore, IC can be recommended as the candidate reference method for the determination of chloride in human serum.

The cost of using the IC as the reference method was lower than other reference methods. Besides, the accuracy was good which reached the request. In a word, IC method can be applied to the standardization and traceability of serum chloride, which can provide effective way for the determination of routine testing to the reference method.

3.4 | Measurement uncertainty

The measurement uncertainty of the proposed method is strictly evaluated according to the evaluation procedure of measurement uncertainty which were established on Guide to the Expression of Uncertainty in Measurement (GUM, 1999). When evaluating the calibration and measurement capability (CMC), the different mole concentrations of samples were detected, and their measurement uncertainty was evaluated. With the mole concentration from low to high, the uncertainty of measurement was descending. According to the China National Accreditation Service for Conformity Assessment standard CNAS-GL 37 "Guidance on Expression of Calibration and Measurement Capability (CMC)," the measurement range of chloride was 78.9-129.4 mmol/L, which corresponds to the expanded uncertainty was 0.70%-0.50%, respectively.

3.5 | Robustness

The proposed IC method has been applied to the determination of chloride in human serum for more than 5 years. During that time, more than 1000 serum specimens have been determined by ion

TABLE 2 Precision of Ion chromatography analysis of chloride in serum

Concentration level	Mean (mmol/L)	Within-run CVs (%)	Mean (mmol/L)	Between-run CVs (%)
Low	90.46	0.32	90.70	0.58
Medium	108.05	0.73	108.86	0.97
High	127.37	0.50	128.39	0.96

TABLE 3 Comparison of values between serum samples and SRM 956d

Name	Measured value (mmol/L)	CV (%)	Standard value (mmol/L)	Bias (%)	IFCC Equivalent limit
NIST SRM 956d L1	93.98	0.79	94.53 ± 0.21	−0.58	±2.00%
NIST SRM 956d L2	107.55	0.28	108.5 ± 0.2	−0.88	
NIST SRM 956d L3	121.56	0.43	122.6 ± 0.3	−0.85	

TABLE 4 Comparison of values between serum samples and IFCC External Quality assessment scheme for Reference Laboratories in Laboratory Medicine (IFCC-RELA) samples

Name	Measured value (mmol/L)	CV (%)	Extended uncertainty (mmol/L)	Target value (mmol/L)	Bias (%)	IFCC equivalent limit
2013RELA-A	110.66	0.44	0.83	109.88	0.71	±2.00%
2013RELA-B	113.76	0.81	0.77	112.98	0.70	
2014RELA-A	120.35	0.42	0.75	119.80	0.46	
2014RELA-B	125.62	0.59	0.91	124.88	0.59	
2015RELA-A	125.40	0.67	0.64	124.30	0.88	
2015RELA-B	129.60	0.42	0.73	128.15	1.13	
2016RELA-A	118.57	0.37	1.10	118.03	0.49	
2016RELA-B	138.40	0.80	0.61	137.43	0.71	
2017RELA-A	127.40	0.70	1.30	127.46	−0.05	
2017RELA-B	122.80	0.81	1.30	123.11	−0.25	
2018RELA-A	118.15	0.72	0.93	118.16	−0.55	
2018RELA-B	143.76	0.43	0.93	144.60	−0.55	

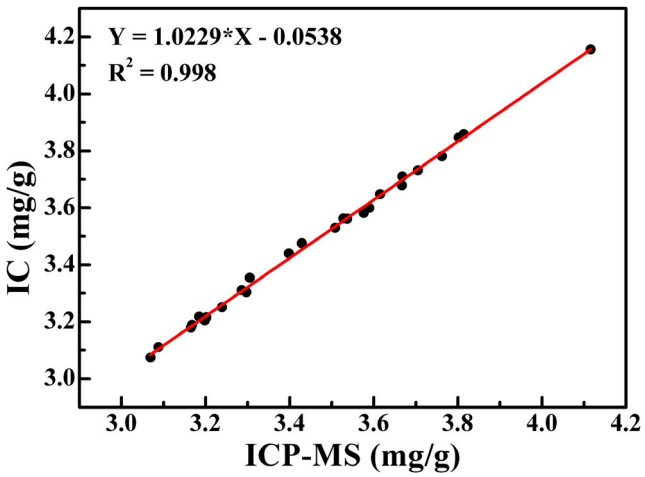

FIGURE 3 The comparison of ICP-MS and IC

chromatography, fortunately, we have not encountered obvious suppressor injury or column contamination, which leads to the decrease of chromatographic resolutions, and the results are inaccuracy. This phenomenon could be related to a dilution of 500 times with Milli-Q water makes the organic content in the serum specimen very low, which is not enough to cause suppressor injury and column contamination. In fact, the same ion chromatography system was used throughout this work without replacing the suppressor or analytical column since year 2013.

4 | CONCLUSION

The IC method can precisely measure the concentration of chloride in human serum. The sample pretreatment procedure is simple, the detection is sensitive, and the analysis process is easy. From the precision, accuracy and recovery experiments mentioned above, IC has more practical value in traceability process and principally considered as a candidate reference methodology, comparable to ICP-MS or ICP-OES. At the stage of the work, the good results revealed that IC can be recommended as the candidate reference method for the determination of chloride in human serum.

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ORCID

Min Shen https://orcid.org/0000-0001-9050-3768
Zheng Cao https://orcid.org/0000-0002-0968-1864

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