ANALELE UNIVERSITATII BUCURESTI
Department of Physical Chemistry
4-12 Regina Elisabeta Blvd, District 3, Bucharest
phone: +40-21-3143508; fax: +40-21-3159249
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ARS DOCENDI PUBLISHING HOUSE
Sos. Panduri 90, District 5 Bucharest
phone/fax: +40-21-4102575
e-mail: arsdocendi@yahoo.com

VALIDATION OF AN ANALYTICAL QUANTITATIVE DETERMINATION METHOD OF CHLORIDE ANION FROM DRINKING AND SURFACE WATER, USING DIRECT POTENTIOMETRY WITH CHLORIDE-SELECTIVE ELECTRODE (II)

I.G. Tănase*, **Dana-Elena Popa, Mihaela Buleandră**

abstract In this second part of the study will be proved that the analytical method for quantitative determination of chloride anion from drinking and surface waters, using direct potentiometry at zero current, is selective, precise and accurate.

key words validation, accuracy, precision, selectivity, specificity

Introduction

Analytical method validation is made to ensure that a certain analytical methodology is accurate, specific, reproducible and robust within a specified range, where the analyte is analyzed [1].

In literature is presented a broad range of practical guidance for evaluation of the performance parameters of analytical methods [2÷7]. Additional to different approaches, the terminology and the way of results reporting varies significantly. The differences appear depending on purpose and method application, and the validation studies can become more difficult with complexity of the analysis.

It is essential that the validation study to be representative. From these reasons, this kind of studies must be realized so they prove a realistic evaluation.

In this paper it will be continued the validation study of a quantitative determination method for chloride anion, using direct potentiometry with chloride-selective electrode. After proving the existence of an appropriate working range and the linearity of the method [8], it will be shown that the method is precise, accurate and selective and that presents appropriate detection and quantification limits.

* University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, 90-92 Panduri, Bucharest, Romania, e-mail: tanase.ion@gmail.com

Experimental

All the used reagents had analytical purity quality. A 10.000 ppm chloride stock solution (WTW Germany, NIST (National Institute of Standards and Technology) traceable) was used to obtain standard solutions. KNO_3 was used to obtain the inert electrolyte solution (1 $mol \cdot L^{-1}$). For standard and working solutions preparation adequate volumes of stock standard solution were measured and then they were put in volumetric flasks. Tri-distilled water was used for all solutions preparation and for vessel cleaning.

The necessary measurements for quantitative determination of chloride ion from drinking and surface waters have been carried out using a pH/mV-metre WTW Inolab 740, with 6 point calibration; chloride-selective electrode as working electrode; R503/D-WTW reference electrode; 50, 100, 500, 1000 mL volumetric flasks (A class); 100 mL Berzelius glasses; 1, 2, 5, 10 mL pipettes (A class).

In order to prepare the standard solutions, in 50 mL volumetric flasks were put: fixed volumes (5 mL) of 1 M KNO_3 solution, increasing volumes of standard chloride solution (100 ppm) and tri-distilled water. Drinking or surface water samples were prepared as follows: in a 50 mL volumetric flask was added 25 mL from the water sample, 5 mL inert electrolyte solution (1 mol L^{-1} KNO_3) and tri-distilled water. The samples were decanted into the potentiometric cell and emf was measured.

Results and discussions

In this second part of the study, according to ICH (International Conference of Harmonization) recommendations [6,7] for analytical method validation, the following performance parameters must be taken in consideration: precision, accuracy, detection limit, quantification limit, selectivity / specificity.

1. Precision

Precision of an analytical method is showing *the closeness, matching or concordance degree of a measurement series obtained from several samples derived from the same homogenous sample under specificity conditions*.

Precision is expressed as a standard deviation (s, σ) or as a percentage relative standard deviation (RSD %).

Precision of an analytical method can be considered at three levels: *repeatability, intermediate precision and reproducibility*.

a. *Repeatability* expresses the analytical variability in the same working condition, in a short time interval (between tests/measurements and during these). Repeatability is obtained when test / measurement is realised in one laboratory, by one operator, using only one type of measuring equipment and only one method in a short time period.

Repeatability of developed method was proved on drinking and surface (Dambovita river) water samples. Analysis was done on 10 identical samples (reference material – chloride

standard solution), 10 identical samples of drinking water (Panduri) and surface water samples from Dambovita River. Experimental and calculated results are shown in Table 1.

Table 1 Experimental results and statistical calculated results obtained when demonstrating repeatability on 10 chloride standard solution samples, drinking water samples and surface water samples

Calculated statistical parameter	Chloride standard solution samples	Surface water samples	Drinking water samples
Number of replicates samples	10	10	10
Average value, $\bar{x}$	12.9164 mg/mL	6.6348 mg/mL	9.7706 mg/mL
Standard deviation, s	0.2470 mg/mL	0.1230 mg/mL	0.1669 mg/mL
Average standard deviation, $s_{\bar{x}}$	0.0781 mg/mL	0.0388 mg/mL	0.0528 mg/mL
Relative standard deviation, RSD	0.0191	0.0185	0.0170
Percentage relative standard deviation, RSD%	1.91 %	1.85 %	1.70 %
Limit repeatability, r	0.4064 mg/mL	0.2023 mg/mL	0.2747 mg/mL
$r/\sqrt{n}$	0.1285 mg/mL	0.0639 mg/mL	0.0868 mg/mL
Individual confidence interval	x ±0.4064 mg/mL	x ±0.2023 mg/mL	x ±0.2747 mg/mL
Confidence interval for average value	$\bar{x}$ ± 0.1285 mg/mL	$\bar{x}$ ± 0.0639 mg/mL	$\bar{x}$ ± 0.0868 mg/mL

b. *Intermediate precision*, according to ICH [6,7] represents "long term variability of the measurement process when identical samples are analyzed/measured using the same method, in the same laboratory, but on two or more different instruments, by different operators, on a longer time period and is determined by comparing results obtained for one laboratory for a certain number of weeks".

The intermediate precision of an analytical method may reflect discrepancies between results obtained by different operators, on different instruments, with standard solutions and reagents from different producers.

In this case study, for proving intermediate precision were measured reference materials samples (chloride standard solutions) and real samples (drinking and surface water samples), in the same laboratory, using different operators, different days, different chloride – selective electrodes and the developed method. Measurements results are presented in Table 2, for every working day and every analyst, on 6 identical drinking water samples.

The results (standard deviation and RSD %) for every working day and every operator (analyst), but also those obtained for different days and analyst grouping indicated a good intermediate precision. This is proved by standard deviation and RSD % values, which are much smaller then those predicted by Horwitz equation for concentration level that was working for (1÷100 ppm Cl^-), for which RSD % must lie between 8% and 16%, or, according to AOAC PVM, between 5.3% and 11%. Combining data obtained for more working days and more analysts it was established that the confidence in measurement precision increases with the increase of number of samples; a small deterioration of RSD % was noticed when analytical variability is increasing.

2. Accuracy

Accuracy of an analytical procedure is showing „*the closeness*" of the value accepted as conventional true value or as reference value and the measured value [6,7].

In this case study the accuracy was proved using recovery method, consisting in the addition of a known amount of pure active constituent to the blank sample or real sample, the analysis of the resulting mixture and comparison of the obtained and the expected results.

Table 2 Statistical calculation results for proving the intermediate precision of the developed method for quantitative determination of chloride ion from drinking water samples

	Analyst 1 Day 1	Analyst 2 Day 2	Analyst 3 Day 3	Analyst 1+2 Day 1+2	Analyst 2+3 Day 2+3	Analyst 1+3 Day 1+3	Analyst 1+2+3 Day 1+2+3
Average value, $\bar{x}$ (mg/L)	6.0503	6.6920	6.8231	6.3711	6.7576	6.4367	6.5218
Standard deviation, s (mg/L)	0.0897	0.1032	0.0849	0.3475	0.1131	0.4121	0.3582
Average standard deviation, $s_{\bar{x}}$ (mg/L)	0.0366	0.0421	0.0346	0.1003	0.0326	0.1189	0.0844
Relative standard deviation, RSD	0.0148	0.0154	0.0124	0.05455	0.0167	0.0640	0.0549
Percentage relative standard deviation, RSD%	1.48 %	1.54 %	1.24 %	5.45 %	1.67 %	6.40 %	5.49 %
Limit repeatability, r (mg/L)	0.1476	0.1698	0.1396	0.5717	0.1861	0.6779	0.5893
$r/\sqrt{n}$ (mg/L)	0.0602	0.0693	0.0570	0.1650	0.0537	0.1957	0.1389
Individual confidence interval (mg/L)	$\bar{x} \pm 0.1476$	$\bar{x} \pm 0.1698$	$\bar{x} \pm 0.1396$	$\bar{x} \pm 0.5717$	$\bar{x} \pm 0.1861$	$\bar{x} \pm 0.6779$	$\bar{x} \pm 0.5893$
Confidence interval for average value (mg/L)	$\bar{x} \pm 0.0602$	$\bar{x} \pm 0.0693$	$\bar{x} \pm 0.0570$	$\bar{x} \pm 0.1650$	$\bar{x} \pm 0.0537$	$\bar{x} \pm 0.1957$	$\bar{x} \pm 0.1389$
Number of determinations	6	6	6	12	12	12	18

In this case study were used drinking water samples fortified with known amounts of chloride: for initial drinking water sample (50 mL) was measured th e emf, then were made 3 successive additions (2 mL each) of standard chloride solution (100 ppm), electromotive force being measured after each addition. Experimental results obtained for proving method accuracy are presented in Table 3.

The recovery degree obtained in this study being between 85.26 % and 93.26 %, it lies between imposed limits (85÷110%) for this concentration level, given by Horwitz equation.

3. Detection limit and quantification limit

The detection limit (LD) is, as formulated by ICH [6, 7], the most used term in chemical analysis and represents „*the smallest analyte concentration in a sample, that can be*

detected with reasonable statistical certainty, but not necessary quantifiable as an accurate value under the established test conditions"

The quantification limit (LC) is "*the lowest concentration or amount of substance that can be quantitatively determined with an acceptable repeatability and accuracy level*".

Table 3 Results obtained when proving accuracy

Sample	E_i (V) vs. ESC	Additions (mL) Cl^- solution (100 ppm)	E (V) vs ESC after additions	Concentration (mg L^{-1})	Recovery (%)
Sample 1 (5 mL 1 M KNO_3 + 25 mL drinking water + tri-distilled water in 50 mL volumetric flask)	0.2522	-	-	11.92065	
		+ 2 mL	0.2451	15.56812	91.18
		+ 2 mL more	0.2394	19.16847	90.59
		+ 2 mL more	0.2352	22.15631	85.27
Sample 2 (5 mL 1 M KNO_3 + 25 mL drinking water + tri-distilled water in 50 mL volumetric flask)	0.2521	-	-	11.97246	
		+ 2 mL	0.2449	15.70236	93.26
		+ 2 mL more	0.2393	19.25131	90.98
		+ 2 mL more	0.2350	22.34508	86.44
Sample 3 (5 mL 1 M KNO_3 + 25 mL drinking water + tri-distilled water in 50 mL volumetric flask)	0.2518	-	-	12.12834	
		+ 2 mL	0.2448	15.77082	91.06
		+ 2 mL more	0.2392	19.33449	90.07
		+ 2 mL more	0.2394	22.44206	85.34

To determine in practice *the lowest detectable signal* more several methods can be applied:

a) 10 independent blank samples, measured once each:

$$x_{LD} = x_{m(blank)} + 3\sigma_{blank}, \quad (1)$$

where σ_{blank} is the standard deviation of the blank sample;

b) 10 independent blank samples fortified to the lowest accepted concentration, measured once each:

$$x_{LD} = 0 + 3\sigma_{sample} \quad (2)$$

c) from calibration data.

This study has used the (b) method because the smallest concentration accepted as lower limit of working range is known; for this concentration were already realized measurements on 10 independent samples and σ_{sample} was calculated as $s_1 = 0.00059264$.

When calculating detection limit was obtained: $x_{LD} = 0 + 3 \cdot 0.00059264 = 0.00177$ mg/L.

Two methods can also be used for the practical determination of the *lowest quantifiable signal*:

a) 10 independent blank samples measured once each:

$$x_{LQ} = x_{m(blank)} + 10\sigma_{blank}, \quad (3)$$

where σ_{blank} is the standard deviation of blank sample;

b) 10 independent blank samples fortified to the lowest accepted concentration, measured once each:

$$x_{LQ} = 0 + 10\sigma_{sample} = 10 \cdot 0.00059264 = 0.0059264 \text{ mg/L} \quad (4)$$

4. Selectivity / specificity

Both selectivity and specificity are performance parameters which characterize the performance of the analytical method, providing an idea about the soundness of the analytical method. Certain authors are providing different definitions for these terms; other authors are considering them to be identical. In some cases, the terms „selectivity" and „specificity" are regarded as interchangeable, and in other cases the term „specificity" is considered to be 100% „selectivity" [5]. Before using a developed analytical method for a quantitative analytical measurement, the specificity of that method has to be proven.

Selectivity is defined as ability of the analytical or bioanalytical method to differentiate and measure the analytes in presence of those components expected to be in a sample. *Specificity* is defined as ability of the analytical method to evaluate unequivocally the analyte in presence of those components expected to be in a sample [5].

In this case, the selectivity of the quantitative determination method can be appreciated by taking in consideration the potentiometric selectivity coefficients values of the chloride-selective electrode in comparison with other possible interfering ions in chloride determination. In quality certificate of the electrode the producer supplies the selectivity coefficients values and the interfering ions concentrations ratios values and those of the measured ion that produce a 10% error (Table 4).

Table 4 $c_{ion.interf}/c_{Cl^-}$ **ratio values that produce a 10% measurement error and potentiometric selectivity coefficients values of some interfering ions [6,7].**

Interfering ion	Br^-	I^-	S^{2-}	CN^-	NH_3	$S_2O_4^{2-}$	HO^-
Ratio $c_{ion\ interf.}/c_{Cl^-}$	$3 \cdot 10^{-3}$	$5 \cdot 10^{-7}$	$1 \cdot 10^{-6}$	$2 \cdot 10^{-7}$	0,12	0,01	-
$K^{pot}_{Cl^-/X^-}$	100	$1 \cdot 10^{6}$	must be absent	$1 \cdot 10^{-4}$	0,1	60	0,01

Potentiometric selectivity coefficients characterize an ion-selective electrode from the most important point of view for the analytical applications. Knowing the values is necessary when the adequate electrode for a certain sample is selected. These selectivity coefficients can take values between $1 \cdot 10^{-4}$ and $1 \cdot 10^{4}$; we can say that an electrode is selective when $K^{pot}_{i/j} < 1$. Following the data from Table 4, it is clear that the specified chemical species (that represents disturbing interferences when quantitative determination of chloride is realized) must be strictly controlled for the method to have a certain degree of selectivity.

Conclusions

The developed analytical method was proved to be fit for the purpose, is precise, accurate and selective and presents an appropriate detection limit and quantification limit.

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