THE IMPORTANCE AND ESSENCE OF THE VALIDATION PROCEDURE OF INSTRUMENTAL ANALYTICAL METHODS DEALING WITH THE DETERMINATION OF THE CHEMICAL COMPOSITION AND SPECIATION OF ENVIRONMENTAL SAMPLES

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Abstract
The validation of the instrumental analytical methods has a key importance so that the possibly most reliable chemical information could be provided. As some kind of wet chemical sample preparation is always involved in these methods, therefore the validation procedure should cover the checking both the measurement conditions and that of the sample preparation. In this paper the important points of validation of a possible analytical method using wet chemical sample preparation and ICP-AES spectrometry for the analysis of environmental samples will be discussed.

1. Introduction

The role of the measurements among them the analytical chemical measurements in the engineering is of key importance. The research, design and production are all based on measurement results. Similarly many actions and legal decisions of the local and government authorities are related to some kind of measurements. That is why it is essential that the measurement results, which form the base of so many things should be the possible most reliable and accurate. This importance is reflected in the fact that in Hungary there is a special law concerning the measurements [1]. In the European Union has a special committee, the European Committee for Standardisation which deals with measurements. Besides that EU has an Institute for Reference Materials and Measurements and an organisation, the European cooperation for Accreditation, which aims at harmonizing the activity of the testing and calibration laboratories, so that a possible uniform measurement practice could be wide-spread in the Union. The laboratory accreditation is performed according to the requirements of the MSZ EN ISO 17025 standard. This standard also declares the concept and general principles of the validation procedure of the so-called non-standardised methods. Obviously, it does not cover specific details of any given specific non-standardised method. As most of the instrumental methods applied in the chemical analysis are in the phase of rapid development, most of them have not been standardised. According the above-mentioned standard and that of the principles of the Good Laboratory Practice [2] each non-standardised measuring method has to be validated. In case of standardised methods these checks had been performed during the standardisation process.

Each measurement is eventually a comparison, i.e. comparison with the measurement unit. The different measurements can be divided into two groups. The measurement methods in the first group make a direct comparison, e.g. the comparison with the measurement unit of length. The methods that can be found in the second group perform an indirect comparison, i.e. the measured property is not directly compared, but a property, which is strongly related to the one to be measured, is being compared in the course of the measurement process. These are the so-called relative, or indirect measuring methods. Obviously, these are usually much more complicated than those in the first group.
Unfortunately, most of the instrumental methods applied in the chemical analysis belong to the second group. Because of the fact of relative measurements, the use and application of different reference materials both during calibration and checking the so-called performance characteristics of the methods have a key importance. Special care must be taken when methods with wet sample preparation procedures are considered. There are several sources of errors which have to be encountered in the course of such procedures. Complete or partial dissolution of the sample either directly or following a fusion procedure is involved in these methods. During fusion and dissolution analyte-losses may occur and/or analyte may get into the sample solution from the labware used from the chemicals applied. The wet chemical procedures applied during the sample preparation often determine the chemical form of the components to be measured. So that the most accurate measurement results could be provided, care must be taken that the analyte should be in the same chemical form both in the samples and in the standards used in the course of the calibration.

In the followings some important points of the wet chemical sample preparation procedures for ICP-AES analysis and those of the ICP-AES measurements will be discussed.

2. Important points of the wet chemical sample preparation

The wet chemical preparation of the samples is often a complicated procedure including several steps. Each of these steps has to be carefully examined in the course of the validation procedure, so that all the possible regular effects could be recognised and corrected. It must be noticed that although the key importance of the sample preparation is well-known, it is not always examined and tested according to its importance in the course of the validation procedure. Almost all the performance characteristics determined in the course of the validation process are related to the measurements. In our opinion the sample preparation is as important as the measurement therefore careful attention must be paid to that by the validation process.

The first step is – after weighing the sample using an analytical balance – taking it into solution. The dissolution often includes fusion or the application of microwave power. In most cases it is necessary to achieve the complete dissolution for reliable results. The first thing that has to be checked during the validation is whether the dissolution is complete. It must be mentioned that there are methods, where the aim is to determine the partially dissolved concentration of the analyte, in this case there is no reason to check how complete the dissolution is. The chemicals used to dissolve the samples always have some contaminants; the question is their concentration, which should be well below the concentration level expected for the analyte. The chemicals most frequently used for sample preparation are hydrochloric acid and nitric acid. In Figure 1 some contaminants of high purity hydrochloric and nitric acid can be seen. It can be noticed that both acids have a rather high Mg contents, due to the fact that they are available in bottles made of glass.

If Mg is expected in the sample at a very low concentration, then some kind of purification of the acids to be applied (e.g. distillation using an apparatus made of silica, or ion exchange procedure etc.) is necessary before they can be used during the preparation procedure. The water used in the procedures must also be pure. The modern appliances
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utilizing the reverse osmotic technology are able to produce water, which has contaminants only at a very low concentration, in addition to that its quality can be easily monitored by measuring the electric conductivity.

![Graph of contaminants found in high-purity nitric acid and hydrochloric acid](image)

*Figure 1: Contaminants found in high-purity nitric acid and hydrochloric acid*

Besides the chemicals special care must be taken of the labware used in the fusion and dissolution processes. It is a well-known fact that most chemicals attack not only the sample to be dissolved, but the dishes, in that the processes take place, may cause contamination [3, 4, 5, 6]. Similarly the material of different crucibles applied in the course of fusion processes dissolve more or less in the fusion-mixtures. By careful selection of the labware a reasonable low contaminant concentration can be achieved. Nevertheless, in case of trace analytical methods the possible concentration of the contaminants must be determined by all means, and if it is comparable to the analyte concentration to be measured, a solution with the same contaminant levels must be applied as a blank solution. On the other hand, not only contaminants may get into the sample solution, but especially in case of volatile analytes material loss may be experienced. The material loss can be prevented by applying special equipment, which ensures that the volatile components cannot leave the apparatus. In the validation procedure the method also has to be checked for the possible analyte-loss.

### 3. Important points of the measurements

ICP-AES spectrometry requires usually simple sample preparation procedures provided only the elementary composition is needed, i.e. no speciation information is expected. After the dissolution have completed, the procedure is normally finished by making up the solution into a fixed volume in a volumetric flask. The calibration of the volumetric labware may also be relevant in some cases. As ICP spectrometry is a relative measuring method, the instrument has to be calibrated prior to the analysis. So that the calibration
could be performed a suitable series of reference solutions is needed. This solution series should be made of certified reference solutions. Regarding preparation of the calibration standard solutions the following problems might be encountered.

The best results can be achieved when the calibration standards are the solutions of certified reference materials with the possibly closest chemical composition to the samples. The sample preparation must be the same both for the standards and for the samples. Unfortunately carefully analysed certified reference materials are available just a few type of environmental samples.

Frequently one cannot find suitable standards, and in this case the standards must be prepared from one- or multi-element stock solutions, and normally the above-written principle concerning the preparation can no longer be expected to be fulfilled and in many cases somewhat poorer accuracy may be expected. Nevertheless, care must be taken that the calibration solutions should contain all the chemicals applied during the sample preparation at the same concentration they can be found in the sample solutions. If the calibration solutions are prepared from stock solutions, the main components of the samples have to be added to them at the same concentration level. That is the so-called matrix-matching. It usually requires the use of concentrated stock solutions and these solutions also have to be checked for the possible contaminants. In Figure 2 the contaminants of Cu and Fe solutions (the concentration is 20 g/dm$^3$ in both cases) can be seen.

![Figure 2: Some contaminants of concentrated high purity Cu and Fe stock solutions](image)

4. Determination of the performance characteristics

The calibration procedure is followed by the determination of the performance characteristics of the measurement. Regarding the trace analytical methods, the detection power is probably one of the most important performance characteristics. It is important to mention that the detection limit values provided by the instrument manufacturers and used
for comparison purposes in the literature have been performed in dilute aqueous solutions, containing only some little acid. In the analytical practice these values cannot be used in many cases or can only be used with caution because of the effect of the matrix. Depending on the composition and concentration of the matrix, the real detection limit values may even be 10 times higher than those of determined in aqueous solutions. Therefore, it is essential that in the course of the validation procedure the detection limit values should be measured in solutions with the same matrix composition as the samples had. In Figure 3 the spectral environment of the 220.353 nm lead line is presented using 5 mg/l concentration lead with increasing copper concentration applying ICP-AES spectrometry. Regarding the Figure it can be noticed that the spectral background is increasing with increasing copper concentration. This clearly shows the deterioration of the detection power. On the other hand, it also can be seen that beside the increasing background, the peak of the lead line decreases with higher copper concentration, which means further deterioration of the detection power. In Table 1 the calculated background equivalent concentration values are presented as the function of copper concentration.

![Figure 3: The spectral environment of the 220.353 nm lead line using 5 mg/l lead solution and ICP spectrometry](image)

<table>
<thead>
<tr>
<th>Copper [mg/l]</th>
<th>BEQ for Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.714</td>
</tr>
<tr>
<td>1000</td>
<td>1.125</td>
</tr>
<tr>
<td>5000</td>
<td>2.78</td>
</tr>
<tr>
<td>20000</td>
<td>8.00</td>
</tr>
</tbody>
</table>

*Table 1* 
Background equivalent values measured on the 220.353 nm lead line by ICP spectrometry, as the function of the copper concentration
The data of Table 1 also can be seen in Figure 4. Looking at this figure it can be stated that an excellent linear function can be established between the rising copper concentration and that of the BEQ values. It also can be noticed, that the detection power is eight times poorer at 20 000 mg/l copper concentration than in copper-free aqueous solution.

\[ y = 0.0004x + 0.8026 \]

\[ R^2 = 0.9988 \]

**Figure 4:** The background equivalent concentration measured on the 220.353 nm lead line using ICP-AES spectrometry as the function of copper concentration in the solution

Another important performance characteristic of an analytical method is the repeatability, which may have different interpretations in case of a method with wet chemical sample preparation. On one hand it may mean the repeatability of the measurement, which means the measurement of the same sample solution. This characterizes rather the instrument – i.e. the ICP-spectrometer – than the method. On the other hand repeatability may also mean the repeatability of the measurement, when more than one sample solution – usually 3 or 5 – prepared from the same sample have been measured. In both cases the repeatability is characterized by the standard deviation calculated from the measurements. In the latter case the random errors of both the sample preparation and the measurements are included in the resulting value, while in the first case only the effect of the measurement determines the value. In our opinion only the latter case provides reliable values, which may characterise the method being validated.

The concept of reproducibility is rather close to that of the repeatability. The main difference is that repeatability applies to the case when almost all conditions are unchanged, while reproducibility covers the cases when one or more conditions have been changed. As it involves those important cases when samples taken from the same lot of the material being tested by different laboratories and in that way it provides information what extent of deviations in the results can be expected, it has to be determined during the validation by all means.
One of the questions with key importance is that how close the measuring results may be to the – unknown – real value of the analyte concentration. It is the accuracy that can answer this question. In the course of the validation it is determined by measuring at least one or more certified reference materials, with chemical composition close to the samples going to be analysed. In lack of certified reference materials, real information can be provided only with big difficulties.

According to the principles of the Good laboratory Practice, all the regular effects have to be recognized and corrected in the course of the method development, so that only the so-called random effects could influence the measurement results. It is the measurement uncertainty that gives information about the extent of the random effects therefore it also has to be determined. When the measurement uncertainty is determined in the course of the validation, all the possible sources of random errors must be estimated and taken into account.

The resulting total uncertainty must be calculated and it is usually converted into the form of confidence intervals.

Last but not least, the possible interference effects also have to be checked. In case of ICP-AES spectrometry mainly spectral, physical and sometimes chemical interferences have to be encountered.

There are some other performance characteristics playing only minor role such as linearity, analytical working range etc. Because of their minor importance they are not discussed here now.

5. Summary

In this paper the specific features and requirements of the validation procedure in case of instrumental analytical chemical methods has been discussed. Special emphasis was laid to the role and importance of the wet chemical sample preparation, as most of the environmental samples are analysed by methods using such preparation procedures. It was mentioned that special care has to be taken of checking the completeness of the dissolution, the possible contaminants originating partially from the labware, partially from the water and chemicals applied. The role of the certified reference materials was also discussed. As for as the determination of the performance characteristics of the methods, the importance of determining the real detection power was also emphasised. The specific feature of the repeatability was also discussed. From the above information it can be seen that validation of an analytical chemical method is a complex procedure and it involves a tedious amount of work, but as it has a key importance, it should be performed in case of each non-standardised method.
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