# UNCERTAINTY OF SPECTROMETRIC ANALYSIS

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**Abstract:** The main purpose of this paper is to present the uncertainty of spectrometric analysis considering peculiarities of chemical measurements.

Qualitative evaluation of chemical–physical transformation and impact on uncertainty in spectrometric analysis is presented.

Spectrophotometric method is used to show experimentally the influence of analysis conditions on the measurement result.

**Keywords:** uncertainty, spectrometry, sample preparation.

## 1. INTRODUCTION

Spectrometric analysis is a widely used measurement methods for qualitative and quantitative analysis.

Qualitative evaluation of spectrometric analysis result comprises influence of all possible sources and factors, including sample preparation technique, measurement principle, analyte concentration, matrix properties, etc. on measurement uncertainty [1].

Technical specifications of measuring device play a very important role in any measurement. Quite often producers as well as users are proud of high accuracy of their measuring instruments. However, while evaluating the results of spectrometric analysis, sample preparation procedures are considered insufficient. It takes place in spite of the fact that the newest, fast, automated, needed the minimum amount of sample and chemical reagents technology, and measuring equipment providing high repeatability are applied for analysis.

Uncertainty evaluation is not a simple task because of the peculiarities of chemical measurements. The measurement chemical parameter which value has to be measured always is transformed into physical parameter, and only then, the latter is measured. By such a transformation, different sample procedures, which can influence measurement result, are carried out. During the sample preparation a sample is prepared mechanically and chemically. In practice such procedures are often not assessed.

Numerous papers that have been published on the topic of uncertainty evaluation of the results of chemical measurements and uncertainty estimation guides [2, 3] Some more materials are reported in journal articles are rather general because of every chemical measurement is exclusively specific. So uncertainty can not be excluded from the working practices. It is very important to evaluate all possible parameters that effect uncertainty and all of them have to be taken into account.

This paper discusses qualitative evaluation of chemical–physical transformation and its impact on uncertainty of measurement results in spectrometric analysis.

To show experimentally the influence of chemical-physical transformation conditions on the measurement uncertainty the measurement of the concentration of iron in a water sample of spectrophotometric method was chosen.

The physical basis of spectrophotometric measurements is well known [4]. Uncertainty sources for measuring instrument (repeatability of spectrophotometer reading, spectrophotometer drift, stray light, etc) is know as well [5, 6, 7]. "Classical" uncertainty sources (weighing, volumetric operations, etc) are estimated enough [8, 9, 7]. Measurement uncertainty associated with linear least squares calibration is estimated [2,7,8,9]. However in evaluation of uncertainty of measurement results in routine laboratory attention that is paid to chemical- physical transformation conditions (sample pH, content of chemical reagent, exposure time of prepared sample, etc) are insufficient.

## 2. PECULIARITIES OF CHEMICAL MEASUREMENTS

Chemical analysis differs from other measurements. The sample preparation procedures make these measurements exclusive ones. Three main stages are required for chemical analysis:
1. Transformation of chemical parameter into physical parameter (all sample preparation procedures, auxiliary reactions, etc.);
2. Measurement of transformed parameter;
3. Evaluation of the result.

### 2.1. Chemical-physical transformation in spectrometric analysis

Spectrometric analysis involves series of methods. Parameters that have impact on the uncertainty of measurement result for various spectrometric methods are different because measured parameter differs as well. External conditions and error of measuring device have impact on every analysis. But it is not as strong as physical-chemical transformation.

Chemical-physical transformation stage comprises all mechanical and chemical sample preparation procedures. During the transformation sample is prepared in such a way that it should be a possibility to the measure physical parameter depends on concentration.

All methods of chemical instrumental analysis are based upon receiving of analytical signal and measuring of its intensity, i.e. upon chemical and physical characteristics of any material [10]. Fig. 1 illustrates procedures which have influence to uncertainty of measured concentration of analyte.
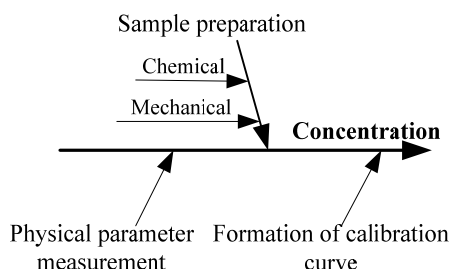


**Fig. 1. Diagram of procedures affecting the result**

Sample preparation procedures comprise all treatments or procedures from sample collection, storing, transportation conditions (vessels, duration, temperature, etc.), sample preparation for analysis (all mechanical treatments applied to the sample in order to convert it to a laboratory sample form) to measurement procedures when the final expression of the analysis is obtained by measuring a certain physical parameter. A series of preparation procedures must be performed before measuring the concentration of analyte. Amount and complexity of operations depend upon the chosen method and aim of analysis [11].

Sample preparation is still one of the most time-consuming, labor-intensive, and error-prone steps in the analysis cycle [12].

To solve a particular task an appropriate method of spectrometric analysis, where the quantity of analyte is determined by measurement of various material parameters, such as absorption, emission, mass-to-charge ratio, etc., is used [4].

Mechanical sample preparation.

Any treatments or procedures applied to the sample in order to convert it in to a form suitable for the chosen analytical method are attributed to sample preparation [13]. Different methods require of conformable sample preparation but there can be find common aims and preparation procedures for some spectrometrical analysis methods (atomic absorption and atomic emission spectroscopy, spectrophotometry, infrared spectroscopy). At first analyte has to be converted into a physical/chemical form compatible with chosen method of analysis (most analytical methods do not work well for solid or inhomogeneous samples) also sample for analysis must be as pure as possible (remove the maximum amount of extraneous material from the sample so that it would not interfere with subsequent measurement of the analyte of interest). Table 1 shows mechanical sample preparation procedures for different aggregative states for different spectrometric analysis methods.

**Table 1. Mechanical sample preparation procedures for different aggregation states in spectrometric analysis**

| Method of analysis | Solid sample preparation for analysis | Liquid sample preparation for analysis | Gaseous sample preparation for analysis |
|---|---|---|---|
| Atomic emission spectroscopy | Heating, dissolution, impurity separation, evaporation. | Preconcentration or dilution, impurities separation. | Impurities separation, drying. |
| Atomic absorption spectroscopy | Drying, homogenization, impurities separation, milling, sieving, extraction, filtration, atomization. | Impurities separation, filtration, preconcentration or dilution, atomization. | - |
| Spectrophotometry | Extraction, drying, preconcentration, sorption, precipitation, impurities separation, filtration, dissolution. | Preconcentration or dilution, impurities separation. | - |
| IR spectrometry | Milling, dissolution, suspendation, pressing. | Dehydration. | Filtration. |
| Mass spectroscopy | Milling, evaporation. | Direct testing. | Direct testing. |

Chemical sample preparation.

The influence of chemical sample preparation and external factors that can affect the result of spectrometric analysis on measurement of analyte concentration is presented in Table 2. Also there is given physical parameter, which has to be measured in different methods. Data in Table 2 shows that temperature of test sample is the most important factor for evaluation of results of above listed analysis methods. Significance of other components depends upon specific measurement method.

Table 2. Impact of chemical sample preparation and external factors on results of spectrometric analysis

| Methods of spectrometric analysis | Physical parameter corresponding chemical value (concentration) | Uncertainty sources | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample pH | Impact of impurities | Reaction between sample and reagents | Separation of analyte from sample matrix | Amount of chemical reagent | Volume of prepared sample | Dilution | Temperature of test sample | Stability of test sample | Retention time of prepared sample | Pressure |
| Atomic absorption spectroscopy | Absorption | | + | + | + | + | + | + | + | | + | |
| Atomic emission spectroscopy | Emission | | + | | + | + | + | + | + | + | | +* |
| Spectrophotometry | Absorption | + | + | + | | + | + | + | + | + | + | |
| IR spectroscopy | Absorption | + | + | + | | + | + | + | + | + | + | |
| Mass spectroscopy | Mass-to-charge ratio | | | | | | | | + | | | + |

* is only for gases.

# 3. UNCERTAINTY SOURCES OF SPECTROPHOTOMETRIC ANALYSIS

By force of spectrometric analysis simplicity, speed of analysis, and large selectivity, these methods are used to solve many problems of different analytes. Advanced equipment appears to be simple, but the relation between concentration of analyte in material and the signal that has been registered is rather complicated. For this reason the research of metrological characteristics becomes more complicated. In addition, an analyte signal usually depends not only on analyte concentration, but on the concentration of all other materials, material structure, sample form, environmental conditions. Furthermore, using any measurement instrument, accuracy and reliability of chemical analysis depend largely on the way the analyzed sample is prepared, because preparation mechanism is specific in chemical measurements.

For the evaluation of measurement result it is necessary to analyze all possible factors [14] that could influence the result.

Method of spectrophotometric analysis are commonly used group of measurement methods. Due to their simplicity, productivity of analysis, and selectivity they are widely used in laboratories of chemical analysis. However, metrological assessment of photometric measurement results is insufficient.

A diagram for evaluation of uncertainty for results obtained from the spectrophotometric analysis is provided in Figure 2. It shows that the tested object and the measurement instrument can not be separated during the evaluation process.

According to the diagram, in order to evaluate a measurement result thoroughly, all the factors that can influence the measurement results have to be analyzed.
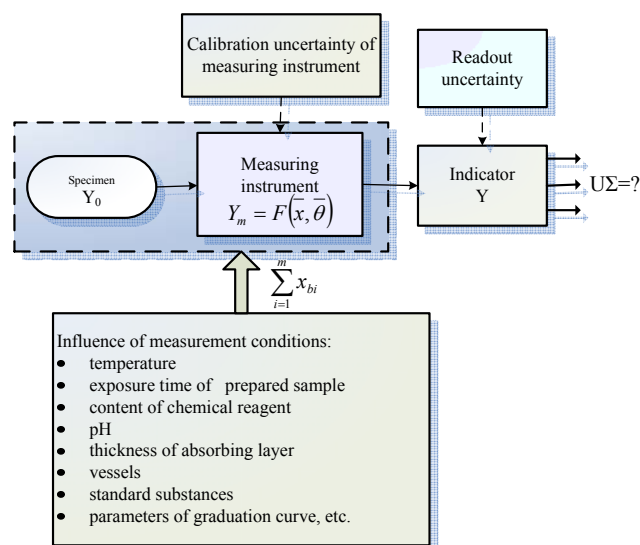


Figure 2. Diagram for evaluation of uncertainty of spectrophotometric analysis results

These factors are: technical characteristics of measuring instrument, measurement conditions, measurement matrix, sample preparation procedures, the interaction of a test item and measuring instrument, restrictions of the measurement method used, reading uncertainty, and data processing. Only after the close analysis of the above mentioned factors and the evaluation of additional conditions of a certain case, the uncertainty as accurate estimate of the measurement result can be properly provided.

# 4. METHODS AND RESULTS

4.1. Elaboration of method and identification of uncertainty sources

The measurement of the concentration of iron in a water sample was chosen for the analysis of spectrophotometric method.

A ФО-1 spectrophotometer (former Soviet Union production) was used for analysis.

The measurement of iron content by spectrophotometric method was carried out using a calibration curve. In this case, the concentration is calculated from the data obtained by the evaluation of regression equation parameters of a calibration curve and is given by:

$$c_0 = \frac{(A_0 - B_0)}{B_1} \cdot d \qquad (1)$$

where $c_0$ is the content of analyte in the sample, calculated from a calibration curve, mg/l, $A_0$ is the measured absorption of the test sample, $B_1$ is the slope of the prepared calibration curve, $B_0$ is the intercept of the prepared calibration curve, $d$ is the dilution factor.

In order to ensure the comparability among the research results two methods of measurement of iron concentration in water have been chosen:

- the ISO 6332:1988 measurement method of iron using 1,10- phenanthroline (hereinafter method A) [15];
- measurement method of iron content using sulfosalicylic acid (hereinafter method B).

A range of determination of the concentration of iron was 0,10 – 2,00 mg/l. This range covers the area of both chosen methods.

The procedure for measurement of analyte by spectophotometric method according to the chosen methods is shown in Figure 3.
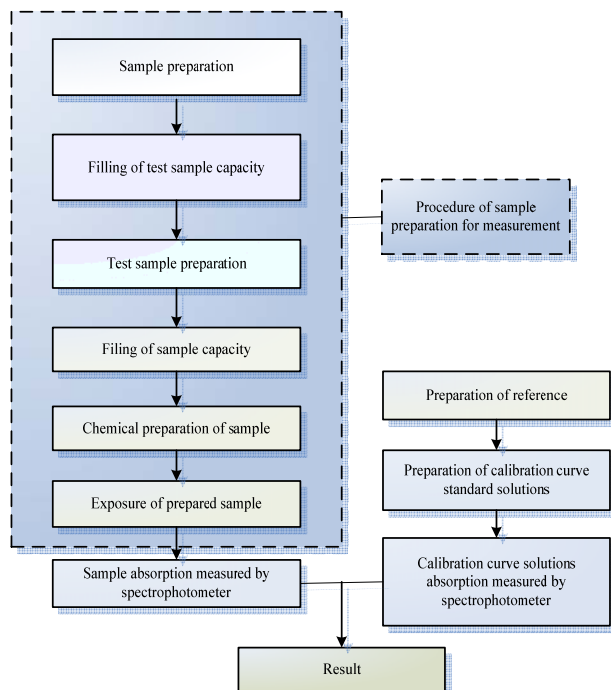


**Figure 3. Procedure for measurement of iron concentration by spectrophotometric method**

Detail analysis of both chosen methods of measurement of iron concentration in water is shown in

Figure 4. Possible factors of chemical- physical transformation in different measurement stages for A and B methods respectively are shown in the right and left of Figure 4.
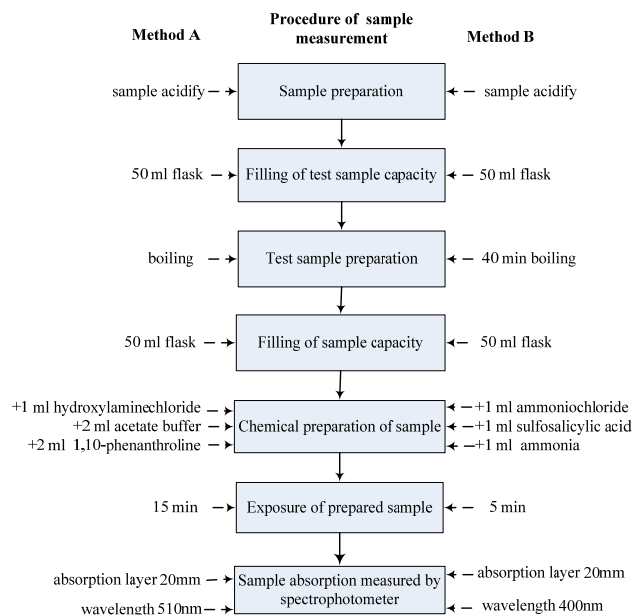


**Figure 4. Procedure for measurement of iron concentration by A and B methods**

In order to evaluate the influence of all the stages of the measurement procedure, the mathematical model for calculation of iron quantity has to be supplemented with new corrective coefficients:

$$c = c_0 \cdot d \cdot f_r \cdot f_T \cdot f_{pH} \cdot f_t \cdot f_\phi \cdot f_b \cdot f_V \qquad (2)$$

where $c$ is the concentration of iron in a test sample, mg/l, $c_0$ is the content of iron in a sample calculated from calibration curve, mg/l, $d$ is the dilution factor, $f_r$ is the correction factor relating to the content of chemical reagent, $f_T$ is the temperature correction factor, $f_{pH}$ is the correction factor relating to the solution pH, $f_t$ is the correction factor relating to the exposure time of prepared solution, $f_\phi$ is the correction factor relating to the impurities in the solution, $f_b$ is the correction factor relating to the absorption layer thickness, $f_V$ is the correction factor relating to the vessels.

In the case of measurement according to this mathematical model, the sources of uncertainty and their influence on the measurement result are given in Figure 5.
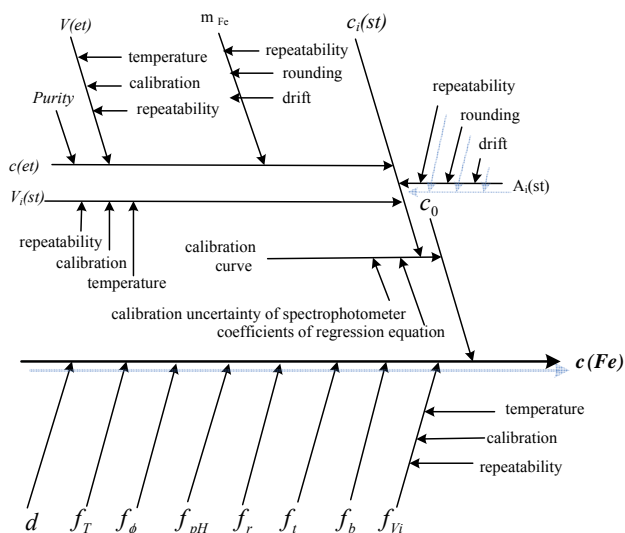
$V(et)$  $m_{Fe}$  $c_i(st)$

temperature — repeatability
calibration — rounding
Purity — repeatability — drift

repeatability
rounding
drift

$c(et)$ ——————————————→  $A_i(st)$
$V_i(st)$ ——————————————→  $c_0$

repeatability
calibration — calibration curve
temperature
calibration uncertainty of spectrophotometer
coefficients of regression equation

——————————————→ $c\,(Fe)$

temperature
calibration
repeatability

$d$  $f_T$  $f_\phi$  $f_{pH}$  $f_r$  $f_t$  $f_b$  $f_{Vi}$

**Fig. 5. Diagram of factors affecting the result**

To evaluate the changes of analysis conditions a cycle of experimental measurements has been conducted. The analysis was carried out using different variants, changing measurement conditions. The matrix of measurement procedure used for experiments is displayed in Table 3.

**Table 3. Matrix of experiments for iron concentration measurement procedure**

| PARAMETER | POSSIBLE VARIANTS | | | | | |
|---|---|---|---|---|---|---|
| | **J** | **K** | **L** | **M** | **N** | **O** |
| C/c | c | C | C | C | C | C |
| D/d | D | d | D | D | D | D |
| E/e | E | E | e | E | E | E |
| F/f | F | F | F | f | F | F |
| G/g | G | G | G | G | g | G |
| H/h | H | H | H | H | H | h |

Parameters given in Table 3: C is the normal temperature of test solution, c is the temperature of a solution under measurement that is lower or higher than normal temperature, D is the normal exposure time of a prepared sample, d is the exposure time of a prepared sample that is lower or higher than the normal time, E is the normal amount of a chemical reagent, e is the amount of a chemical reagent that is lower or higher than the normal amount, F is the normal thickness of an absorption layer, f is the thickness of an absorption layer that is lower or higher than the normal thickness, G is the normal pH value in a test solution, g is the pH value in a test solution that is lower or higher than the normal value, H is a test sample without impurities, h is a test sample with chemical substances as impurities added to it.

Limits in experimental conditions of possible variants for iron concentration measurement procedure are shown in Table 4.

**Table 4. Limits of experimental conditions**

| Measurement condition | Method | Experimental conditions | | |
|---|---|---|---|---|
| | | Low limit | Normal conditions | High limit |
| Temperature, $^0$C | A | 10 | 20 | 30 |
| | B | 10 | 20 | 30 |
| Exposure time of a prepared sample, min | A | 5 | 15 | 20 |
| | B | 3 | 5 | 10 |
| Amount of a chemical reagent, ml | A | 1 | 2 | 4 |
| | B | 0,5 | 1 | 2,5 |
| Absorption layer, mm | A | 5 | 20 | 30 |
| | B | 5 | 20 | 30 |
| pH | A | 1 | 4,5 | 9 |
| | B | 4 | ≥9 | 10 |
| Impurities: Fe:Zn Fe:Cu Fe:Co | A and B | - | - | 1:10 |

Experiments to estimate the influence of variations of measurement conditions on the results demonstrated that the deviations of temperature have a significant influence on the results of both methods while measuring in the range of small concentrations (up to 0.5 mg/l). However, in the range of concentration over 0.5 mg/l, deviations of temperature are significant as well, as deviations in particular points can reach up to 20% of the nominal value. When calculating expanded uncertainty in the range of concentration up to 1 mg/l with the test temperature of $20\pm3^0C$, both methods require to include the uncertainty component associated with the temperature.

Deviations in the amount of chemical reagents from the normal value are important for the results of both methods, particularly in the concentration over 1 mg/l, where the deviations of result gain up to 40% of the nominal value. In the range of smaller concentration, deviations reach up to 10% from of the nominal value. Analysis of measurement results showed that the influence of component associated with chemical reagent differs with the different concentration of a test analyte.

The permissible deviation of thickness of the absorbing layer, which influence is not significant for both methods used, is (-0.05÷ +0.05). More considerable deviations of the absorbing layer make an influence on measurement results over full range of concentrations. Particularly large deviations of results are met in the range of small concentrations.

Deviations of the sample pH from the normal value influence the measurement results of both methods in full range of concentration. The more different from the required the pH value is, the bigger the result deviations are.

Deviations from the specified conditions do not influence measurement results if measurements are carried out using the method B and exposure time of prepared

sample is kept (-1; +2) min. The deviations of exposure time of prepared sample have a significant influence on results of method A in the range of concentration from 1 mg/l up to 2 mg/l, when the exposure time for a prepared sample is less than required according to the methodics.

If a solution contains impurities, they reduce the accuracy and reliability of measurement results. Experiments proved that the influence of impurities decreases if the concentration of test material increases. When measuring iron concentration in polluted samples or the ones of unknown composition, it is necessary to include the component of sample pollution in the calculations of combined relative standard uncertainty.

4.2. Evaluation of Uncertainty Components and Combined Uncertainty

The uncertainty of measurement for iron content in water is calculated by combining all standard uncertainties of input values $c_0, f_r, f_T, f_{pH}, f_t, f_\varphi, f_b, f_V$. Uncertainties have been combined using the errors propagation rule [2,3].

Relation between the measurement result and input parameters is expressed by the following model:

$$c = f(x_1, x_2, \ldots, x_i \ldots x_N) \qquad (3)$$

where $x_1 \ldots x_i, \ldots, x_N$ expresses input parameters $(c_0, f_r, f_T, f_{pH}, f_t, f_\varphi, f_b, f_V)$.

The combined standard uncertainty of the measured analyte in full range of measured concentrations is calculated as:

$$u(c_{Fe})^2 = \sum_{i=1}^{N} \left( \frac{\partial c_{Fe}}{\partial x_i} \right)^2 \cdot u(x_i)^2 \qquad (4)$$

where $u(x_i)$ are the standard uncertainties of input parameters, $(\partial c_{Fe} / \partial x_i)$ are the sensitivity coefficients.

For calculating the uncertainty of calibration curve $u(KC)$ the standard uncertainty of regression equation for calibration curve, standard uncertainty of concentration of standard solutions for calibration curve, calibration uncertainty of measuring instrument have been evaluated.

The standard uncertainty of regression equation for calibration curve $u(reg.kr)$ is calculated by the least squares method.

The purity of reference material, volume of an reference solution, volume of standard solutions, weighing of reference material, preparation of a reference solution in a flask of the volume $V$ influence the uncertainty of standard solutions concentration $u(st.t)$.

Calibration of measuring glass vessels, temperature, and repeatability of readings has an influence on the volume of uncertainty in preparing reference and standard solutions. Such calculations are carried out by evaluating the dependence of water mass on temperature and including the correction factor of the atmospherical pressure.

Standard uncertainties of measuring glass vessels are stated as the uncertainty of volume with the given evaluation of the standard uncertainty for calibration of glass vessel, standard uncertainty of volume associated with the temperature, and repeatability uncertainty.

The components of uncertainty evaluated according to the experiments are the following:

- the component evaluating the amount of chemical reagent;
- the component of the temperature of the test solution;
- the pH component of the test solution;
- the component evaluating the time-exposure of the prepared solution;
- the component of the impurities in the test solution;
- the component of the absorbing layer thickness.

The standard uncertainties of the above mentioned components are calculated analyzing experimental curves of each uncertainty component and taking into account possible real conditions of the analysis.

The expanded uncertainty of iron measurement $U(c)$ is obtained by multiplying the combined uncertainty by a coverage factor $k = 2$. A level of confidence is 95 %.

4.3. Results and Discussion

In carrying out measurements by method A different influence of uncertainty components to expanded uncertainty has been determined for the following concentration ranges: up to 0.2 mg/l and from 0.2 mg/l to 2 mg/l. This influence is illustrated in Figure 6. Relative combined standard uncertainty is up to 0.12 in the range of small concentrations up to 0.2 mg/l and up to 0.07 in the range of concentrations from 0.2 mg/l up to 2 mg/l. The analysis of uncertainty components has shown that in the range of small concentration the components depending on the temperature, the pH of test solution, the sample matrix, and the calibration curve have the most significant influence on the measurement uncertainty.
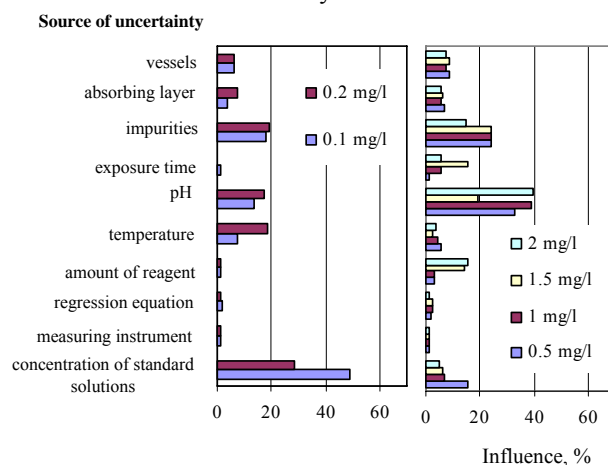


Fig. 6. The influence of uncertainty components on expanded uncertainty in the measurement of iron concentration using method A.

In the range of concentrations over 0.2 mg/l the components of the amount of chemical reagent, the pH of test solution, the sample matrix, and the concentration of standard solutions of graduation curve have a dominant influence on the measurement uncertainty of analyte.

In carrying out measurements at specified conditions of analysis using method B, the influence of uncertainty components on expanded uncertainty has been determined in the range of concentrations up to 0.5 mg/l and from 0.5 mg/l to 2 mg/l. This influence is shown in Figure 7.
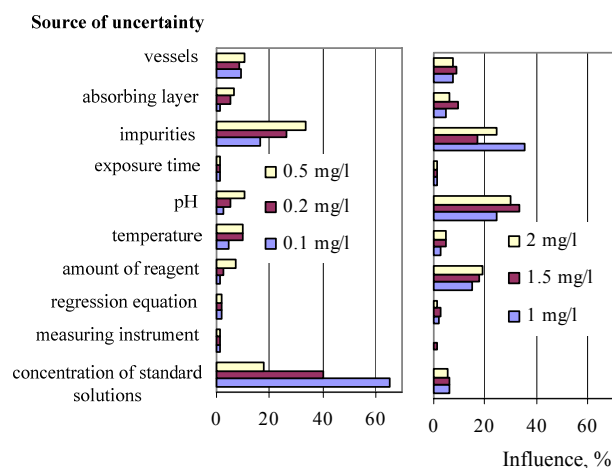


**Figure 7. The influence of uncertainty components on expanded uncertainty in measurement of iron concentration by method B**

Relative combined standard uncertainty is up to 0.17 in the range of concentrations up to 0.5 mg/l and up to 0.07 in the range of concentrations from 0.5 mg/l up to 2 mg/l.

Research results on uncertainty components demonstrate that the component of solution exposure time has not any significant influence on measurement results in full range of tested concentrations. Predominant components in particular concentrations are different. In the range of small concentrations, components of the calibration curve and the matrix of a tested sample have the most significant influence on the measurement uncertainty of analyte. In the range of concentration over 0.5 mg/l, the biggest uncertainty components are due to the amount of chemical reagent, the pH of test solution, and the sample matrix.

Histograms in Figures 6 and 7 confirm the statement that characteristics of measuring instrument are not sufficient to evaluate analyte measurement uncertainty. Therefore it is very important to ensure the conditions of sample preparation and measurement process, and it is necessary to take into account correction factors in the analyte measurement model.

## 5. CONCLUSION

To make the final chemical measurement model it is necessary to evaluate parameters that appear in three main phases: physical-chemical transformation, measurement and estimation of the result.

The influence of separate sample preparation procedures and quality of their performance has direct impact on uncertainty of spectrometric analysis results. Most of the hints are not new. They should already be considered in standard procedures in well-organized professional laboratories. It is a new challenge to put them in the context of measurement uncertainty, which arises from chemical and mechanical sample preparation. The knowledge of measurement uncertainty is an additional value to any analytical result.

The analysis of measurement of iron content in water by photometric method was performed and showed that uncertainty was basically formed by the first chemical analysis phases (measurement conditions, the standard solutions, and the parameters of calibration curve). Therefore, the total measurement uncertainty of material concentration is insignificantly sensitive to the parameters of the used measuring instrument.

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