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Influence of the species effect on trueness of analytical results estimated by the recovery test when determining selenium by HG-AFS

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ABSTRACT

The recovery test in the "surrogate" mode is often exploited for trueness assessment when appropriate certified reference materials are not available or to support reference material studies. The species effect occurs when chemical forms of native and surrogate analytes are different. It usually results in obtaining incomparable analytical signals for both forms. Influence of the species effect on the results obtained by "surrogate" recovery test was examined. The examination was performed theoretically on the basis of a extended mathematical model and the results predicted by the model were checked experimentally. Experiments were carried out with the use of synthetic samples containing selenite ions and L-selenomethionine acting as inorganic and organic form of selenium, respectively. Moreover, real samples of thermal spring water and vitamin drink were analysed with the use of flow injection system. The system was dedicated to perform not only spiking but also UV digestion of the synthetic and real samples in order to study the species effect. The system was coupled to hydride generation atomic fluorescence spectrometer (HG-AFS) enabling to determine total amount of selenium.

1. Introduction

The analytical community employs the recovery test not only to estimate the yield of a stage of an analytical process but also – in accordance with IUPAC recommendation [1] – to assess trueness of analytical results. In the latter context, certified reference materials (CRMs) are used or – most often – a sample is spiked with a surrogate analyte [2,3]. One seems to misuse information resulting from the recovery test (*RV*), stating that trueness (*RE*) can be estimated directly from the simple relation between both values: $RE = RV - 100\%$. Theoretical and experimental considerations have proved that the obtained $RV = 100\%$ is not equivalent to achieving a result close to the real one, and – on the other hand – the true result may be obtained in the conditions which do not guarantee the complete analyte recovery [4–6].

The reasons why the relation between *RV* and *RE* is often not fulfilled are different effects which may potentially occur during the whole analytical process. These are mainly the preparative effect, i.e. a systematic loss of an analyte from a sample or an uncontrolled addition of an analyte to the sample as a contaminant, and the interference effect. Both effects may have an impact on the native and surrogate analyte changing their analytical signals. Moreover, the effects may reveal as additive or multiplicative when the signals are changed to the same extent or proportionally to the analyte concentration, respectively.

In analytical practice additional effect can be met quite often, mainly different analytical signals can be produced by different chemical forms of the same analyte. Such a phenomenon can be termed "the species effect". It has been proved that it may be a source of systematic errors at the calibration stage when the forms of an analyte in a sample and in standard solutions are different. This problem has been reported as especially important in calibration by the standard addition method [7,8]. In particular, strong difference in the analyte forms and the related analytical signals was observed for organometallic compounds analyzed with the use of atomic absorption spectrometry methods [9–11]. Kowalewska [11] reached a conclusion of her study on various organic vanadium forms that "if an analyte in a sample and in a standard are in various forms and behave differently the bias of results can be enormous. This fact should be also taken into account in the analysis of organic analytes in aqueous solutions".

Moreover, internal standards are also considered to be surrogate analytes applied for recovery estimation [2]. Utilising internal standards in quantitative bioanalyses with the use of liquid chromatography/mass spectrometry (LC/MS) enables to correct for errors of detection. Nevertheless, when a native analyte and an internal standard are not sufficiently similar in chemical structure, the ratio of the analyte and internal standard detector responses may vary as a result of different degrees of ion suppression. Therefore, internal standards in quantitative LC/MS assays are either

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structural analogues or stable isotopically labeled (SIL) analogues of the analyte. SIL internal standards are characterized by the highest level of reliability and are the recommended internal standards for MS detection since they are almost chemically identical to the native analyte [12]. However, the species effect may occur even if the SIL standards, which behavior is almost identical to the native analyte during the whole analytical procedure, are implemented in the analytical process. The fact that the standards are considered to provide better results than any other internal standards cannot be uncritically taken for granted and provide an automatic guarantee for obtaining true results, which was presented in some reports [13–15].

Regardless of the fact that the species effect has been known for years, the possibility of its occurrence is most of the time disregarded and neglected. Hence, hardly ever is the species effect a subject of research during evaluation and validation of analytical procedures. In particular, it is common not to consider it when the recovery test is used to asses trueness of analytical results.

In our previous paper, we made an attempt to evaluate influence of the preparative and interference effects on analyte recovery [6]. For this purpose a mathematical model was developed, enabling to judge usefulness of the recovery test in objective estimation of trueness. In this work continuation of the aforementioned study has been presented on the assumption that the species effect occurs in the course of the recovery procedure. The examinations were performed on the basis of extended mathematical models and the results predicted theoretically were verified experimentally. Synthetic and real samples containing selenium in inorganic (selenite) and/or organic (L-selenomethionine) forms were analyzed with the use of HG-AFS. A dedicated flow injection system, utilized in the previous work [6], was modified in order to perform not only spiking but also UV digestion of the samples. Influence of the species effect on the relation between recovery and trueness has been presented and discussed.

2. Theoretical approach

The principle of the species effect in relation to the recovery surrogate test has been shown in Fig. 1.

Commonly, the test involves the calibration graph, ICG, prepared with the use of standard solutions containing pure analyte,

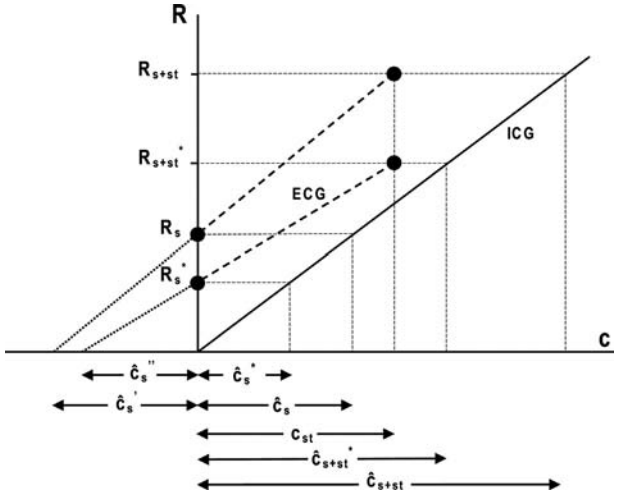

Fig. 1. Results possible to be obtained in the recovery test: in a sample before and after spiking with an analyte in concentration c_{st}, the analyte is determined in concentrations $\hat{c}_s$ and $\hat{c}_{s+st}$ (when no species effect occurs) and $\hat{c}_s^*$ and $\hat{c}_{s+st}^*$ (when the effect occurs) on the basis of the calibration graph ICG; $\hat{c}_s'$ and $\hat{c}_s''$ are the analyte concentrations in the non-spiked sample found in both cases in extrapolative way on the basis of the calibration graph ECG.

which can be expressed by the following formula

$$R = Ac \pm \varepsilon \tag{1}$$

where R stands for an analytical signal, A for a slope, c for an analyte concentration and ε represents random error following a normal distribution $N(0, \sigma)$ (since systematic errors will be exclusively taken into account, the random error ε is neglected in further considerations). A sample with native analyte of concentration c_s is spiked with a surrogate analyte of concentration c_{st}, both portions of the sample (non-spiked and spiked) are treated in accordance with a required preparative procedure and are exposed to measurements. Signals for the sample containing a native analyte, R_s, and the sample with addition of a surrogate analyte, R_{s+st}, are referred to the graph ICG in order to calculate two corresponding values of the analyte concentration, $\hat{c}_s$ and $\hat{c}_{s+st}$, in interpolative way

$$\hat{c}_s = \frac{R_s}{A} \tag{2}$$

$$\hat{c}_{s+st} = \frac{R_{s+st}}{A} \tag{3}$$

The recovery of the analyte is then calculated from the formula

$$RV = \frac{\hat{c}_{s+st} - \hat{c}_s}{c_{st}} \times 100\% \tag{4}$$

When the chemical forms of the native and the surrogate analyte are the same, they behave in the same manner during the whole analytical process. It means that R_s and R_{s+st} are proportional to the concentration of the analyte in the sample before and after spiking, respectively. Moreover, if the forms correspond to the form of the analyte used for calibration, the signals can be expressed by the following formulae [6]

$$R_s = A(1+P_1)[1+Q_1(c_m)](c_s+P_2)+Q_2(c_m)+Q_3 \tag{5}$$

$$R_{s+st} = A(1+P_1)[1+Q_1(c_m)](c_s+c_{st}+P_2)+Q_2(c_m)+Q_3 \tag{6}$$

where c_m is the total concentration of interferents in the sample, P_1 and P_2 describe preparative effect of multiplicative and additive character, respectively, and Q_1 corresponds to multiplicative interference effect, Q_2 and Q_3 stand for the factors expressing additive interference effect dependent on and independent of the concentration of interferents, respectively.

In general, the species effect may occur when a native analyte is present in a sample in a different form than the one used for calibration and, what is more, a surrogate analyte is added to the sample in a different form then the one of the native analyte. Taking these cases into account the signals measured for the sample before and after spiking with the analyte are expressed by the formulae

$$R_s^* = A(1+P_1^*)[1+Q_1^*(c_m)](c_s+P_2^*)+Q_2^*(c_m)+Q_3 \tag{7}$$

$$R_{s+st}^* = A(1+P_1^*)[1+Q_1^*(c_m)](c_s+P_2^*)+Q_2^*(c_m) + A(1+P_1)[1+Q_1(c_m)]c_{st}+Q_2(c_m)+Q_3 \tag{8}$$

where P_1^*, Q_1^* and P_1, Q_1 are preparative (P_1) and interference (Q_1) effects of multiplicative character acting on native and surrogate analyte, respectively, P_2^* is preparative effect of additive character which influences a native analyte, Q_2^* and Q_2 are interference effects of additive character acting on a native and surrogate analyte, respectively. Finally, assuming that a sample may contain N different forms of a native analyte, Eqs. (7) and (8) take the following general formulae

$$R_s^* = \sum_{i=1}^{N} \{A(1+P_{1i}^*)[1+Q_{1i}^*(c_m)](c_s+P_{2i}^*)+Q_{2i}^*(c_m)\}+Q_3 \tag{9}$$

$$R^*_{s+st} = \sum_{i=1}^{N} \{A(1+P^*_{1i})[1+Q^*_{1i}(c_m)](c_s+P^*_{2i})+Q^*_{2i}(c_m)\} + A(1+P_1)[1+Q_1(c_m)]c_{st}+Q_2(c_m)+Q_3 \quad (10)$$

When Eqs. (9) and (10) are substituted to general Eqs. (2) and (3), respectively, the concentrations $\hat{c}_s$ and $\hat{c}_{s+st}$ found in interpolative way may be calculated according to the formulae

$$\hat{c}_s = \frac{\sum_{i=1}^{N}\{A(1+P^*_{1i})[1+Q^*_{1i}(c_m)](c_s+P^*_{2i})+Q^*_{2i}(c_m)\}+Q_3}{A} \quad (11)$$

$$\hat{c}_{s+st} = \frac{\sum_{i=1}^{N}\{A(1+P^*_{1i})[1+Q^*_{1i}(c_m)](c_s+P^*_{2i})+Q^*_{2i}(c_m)\}+A(1+P_1)[1+Q_1(c_m)]c_{st}+Q_2(c_m)+Q_3}{A} \quad (12)$$

and Eq. (4) expressing the recovery value, *RV*, takes the following form

$$RV = \frac{A(1+P_1)[1+Q_1(c_m)]c_{st}+Q_2(c_m)}{Ac_{st}} \times 100\,\% \quad (13)$$

Relative error, *RE*, used for trueness estimation, is given by

$$RE = \frac{\hat{c}_s - c_s}{c_s} \times 100\,\% \quad (14)$$

Taking Eq. (11) into account, *RE* values can be calculated from

$$RE = \left\{ \frac{\sum_{i=1}^{N}\{A(1+P^*_{1i})[1+Q^*_{1i}(c_m)]\ (c_{s_i}+P^*_{2i})+Q^*_{2i}(c_m)\}+Q_3}{A\sum_{i=1}^{N}c_{s_i}} - 1 \right\} \times 100\% \quad (15)$$

Since signals R^*_s and R^*_{s+st} can be used for construction of a calibration graph (graph ECG in Fig. 1), the analyte concentration may also be estimated in extrapolative way. In this case the concentration is calculated according to the formula

$$\hat{c}''_s = \frac{R^*_s}{A(1+P_1)[1+Q_1(c_m)]\ c_{st}+Q_2(c_m)} \times c_{st} \quad (16)$$

Basing on the formula analogous to Eq. (14)

$$RE' = \frac{\hat{c}''_s - c_s}{c_s} \times 100\,\% \quad (17)$$

The relative error of the analytical results estimated in such a way is given by

$$RE' = \left\{ \frac{\sum_{i=1}^{N}\{A(1+P^*_{1i})[1+Q^*_{1i}(c_m)]\ (c_{s_i}+P^*_{2i})+Q^*_{2i}(c_m)\}+Q_3}{\{A(1+P_1)[1+Q_1(c_m)]\ c_{st}+Q_2(c_m)\}\sum_{i=1}^{N}c_{s_i}} \times c_{st} - 1 \right\} \times 100\% \quad (18)$$

3. Experimental

3.1. Reagents and solutions

Stock solution of 1000 mg L^{-1} Se was prepared by dissolving Titrisol® standard (SeO_2 in 6.3% HNO_3, Merck, Germany) in water. Stock solution of L-selenomethionine (Se-Met) containing 1000 mg L^{-1} Se was prepared by dissolving $C_5H_{11}NO_2Se$ (≥ 98%, Sigma-Aldrich, Germany) in water. Stock solution of 1000 mg L^{-1} Cu was prepared by dissolving Titrisol® standard ($CuCl_2$ in H_2O, Merck, Germany) in water. Solution of a reducing agent containing 2% (m/v) sodium borohydride and 0.5% (m/v) sodium hydroxide was prepared directly before analysis with the use of $NaBH_4$ (≥ 98.0%, Sigma-Aldrich, Germany) and NaOH (POCh, Poland). Solutions of 3 and 6 mol L^{-1} hydrochloric acid were prepared by dilution of 37% hydrochloric acid (Merck, Germany) with water.

All reagents were of analytical grade. Working solutions were made up directly before analysis by diluting the stock solutions with water. Deionized water obtained from HLP5sp system (Hydrolab, Poland) was used throughout the work.

Samples of thermal spring water (La Roche-Posay, France) and vitamin drink (Oshee, Poland) were degassed for 15 min by ultrasonication prior to analysis. They were not diluted before measurements.

3.2. Instrumentation

A flow injection manifold, presented in Fig. 2, was designed to perform examinations. It consisted of three peristaltic pumps Minipuls 3 (Gilson, France), a fully rotary eight-port valve (Zhaofa, China), a two-positional eight-port valve (PerkinElmer, USA) equipped with 1000 μL loop volume, and a flow-through digestion unit (MLE GmbH, Germany) composed of a UV lamp (5 W) and

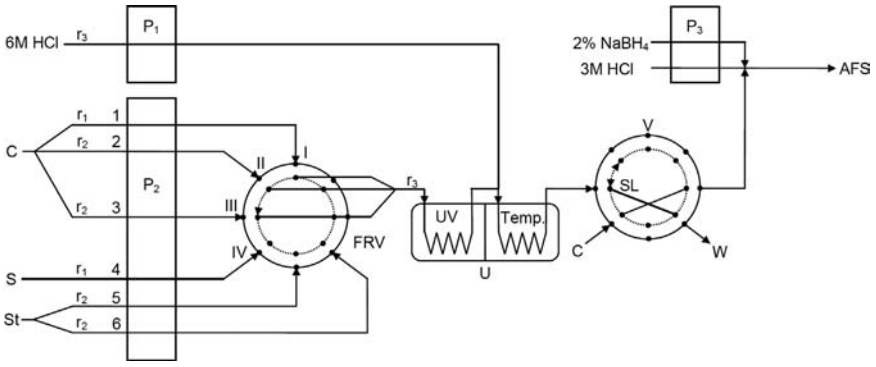

Fig. 2. Scheme of the exploited flow-injection system: P_1–P_3: peristaltic pumps, FRV: fully rotary valve, U: UV/Temp. digestion unit, V: two-positional injection valve, SL: sample loop, C: carrier, S: sample, St: standard solution, W: waste, AFS: atomic fluorescence spectrometer, 1–6: pump tubes, r_1–r_3: flow rates, I–IV: working positions of FRV.

thermoreactor (105 °C). Tygon® pump tubes of 0.76 or 1.52 mm id (Cole-Palmer, USA) were used. The flow system was operated with software created in our laboratory.

The manifold was coupled to an atomic fluorescence spectrometer AFS 830 (Beijing Titan Instruments Co., China) equipped with a flow hydride-generation system (HG-AFS) and employed for measurements of total concentration of selenium. Operating current (pulsed value) of a selenium hollow cathode lamp (HCL) was 100 mA. Argon was used as a shielded gas and a carrier gas at the flow rate of 800 and 300 mL min^{-1}, respectively. The readout time was set at 16 s in order to obtain entirely emerged peaks. Signals were processed in peak area mode.

3.3. Operation of flow system

Sample (S), standard (St) and carrier (C) solutions were delivered to the fully rotary valve (FRV) through tubes 1–6 as presented in Fig. 2. FRV was operated in four positions (I–IV) changed by counter-clockwise gradual rotation of FRV moving part of 45°. In each position three streams of solutions, one propelled with the flow rate r_1 and the other two propelled with the flow rate r_2, were selected from six streams introduced to FRV and were merged with each other with the total flow rate of $r_3=r_1+2r_2$. Since $r_1=3r_2$, in each step of the procedure the sample and the standard solutions were diluted in constant degrees of 3/5 and 1/5, respectively. Afterwards, thus prepared sample was introduced into the flow-through digestion unit and exposed to UV radiation and elevated temperature for 7 min. in flow conditions (if needed, UV lamp was turned off). In order to change oxidative conditions into reductive ones, in which reduction of Se(VI) to Se(IV) and subsequent formation of hydrogen selenide is the most efficient, 6 mol L^{-1} HCl was introduced to the thermoreactor at the same flow rate as the sample, r_3, and merged with the sample stream in high temperature. Then, a loop (SL) of a two-positional injection valve (V) was filled with the processed sample. The loop was subsequently filled with 3 mol L^{-1} HCl carrier stream after changing the position of the valve into *injection* and finally the sample was subjected to hydride generation followed with signal measurement.

In the step of calibration graph preparation, a set of standards solutions were directed to FRV in position II through tube 4, allowing each standard to be merged with each other and mixed with two streams of the carrier solution. For the purpose of recovery examination, tube 4 was filled with a sample (as seen in Fig. 2) and the position of FRV was changed from I to IV, hence the following solutions were prepared: C+C+C (in position I), C+C+S (II), C+S+St (III) and S+St+St (IV).

4. Results

4.1. Examination of synthetic samples

Formulae (13), (15) and (18) were verified experimentally with the use of twelve synthetic samples containing two forms of selenium: inorganic (Se) and organic (Se-Met). Concentrations of selenium present in each sample (native analyte), c_s, and added to each sample (surrogate analyte), c_{st}, were stated at the level of 20 μg L^{-1}. For the sample containing both inorganic and organic form of the native analyte, each form was established in the concentration of 20 μg L^{-1}). Copper was present in some samples in concentration c_m equal to 20 mg L^{-1}. Inorganic selenium standards of 20, 60 and 90 μg L^{-1} were used to prepare calibration graph ICG (see Fig. 1).

The following effects were considered: multiplicative preparative effect (P_1^*, P_1) and multiplicative interference effect (Q_1^*, Q_1). The additive effects (i.e. P_2^*, Q_2^* (c_m), Q_2 (c_m), Q_3) were not taken into account (i.e. they were assumed as equal zero). The values of parameters P_1^*, P_1, Q_1^*, Q_1 were obtained experimentally.

The preparative effect was provoked in the presence of organic form of selenium (Se-Met) in a sample. When UV lamp was turned on, Se-Met was totally digested to inorganic form and then $P_1^*=P_1=0$. By turning UV lamp off incomplete digestion of Se-Met (caused by high temperature in strongly acidic environment only) was observed at the level of ca. 50%. Consequently $P_1^*=P_1=-0.5$.

Negative interference effect of multiplicative character was achieved by the presence of Cu^{2+} in a sample. It took place during generation of selenium hydride and its mechanism is based on formation of Cu^0 in a form of a dispersed metal colloid (selenium hydride is captured and decomposed in an irreversible way by the colloid [16]). The interferent in concentration of 20 mg L^{-1}, resulted in ca. 50% decrease in fluorescence intensity, hence $Q_1^*=Q_1=-0.5$ (when Cu^{2+} was not present in a sample, $Q_1^*=Q_1=0$). The effect occurred regardless of the form of the native and the surrogate analyte. The values of P_1^*, P_1, Q_1^*, Q_1 for each analyzed system have been shown in Table 1.

Theoretical values of recovery, *RV*, and relative errors, *RE* and *RE′*, relating to interpolative and extrapolative analytical results, were calculated from Eqs. (13), (15) and (18), respectively, for given values of parameters P_1^*, P_1, Q_1^*, Q_1.

Calibration graphs ICG and ECG (see Fig. 1) were constructed by fitting linear function to experimental points with the use of the least squares method and analyte concentrations in the samples were estimated in interpolative ($\hat{c}_s$, $\hat{c}_{s+st}$) and extrapolative ($\hat{c}_s'$) way, respectively. Since two values of $\hat{c}_{s+st}$ could be found for each sample

Table 1
Comparison of theoretical and experimental values of recovery (*RV*) and relative errors obtained in interpolative (*RE*) and extrapolative (*RE′*) ways for synthetic samples containing inorganic selenium (Se) and/or selenomethionine (Se-Met) and digested (+) or not (−) by using UV lamp with or without addition of interferent (Cu); P_1^*, P_1, Q_1^* and Q_1 are the parameters in Eqs. (13), (15) and (18) (see text).

Sample	Sample		UV lamp	P_1^*	P_1	Q_1^*	Q_1	*RE* [%]		*RE′* [%]		*RV* [%]	
	Native analyte	Surrogate analyte						Theoret.	Experim.	Theoret.	Experim.	Theoret.	Experim.
1	Se	Se	−	0	0	0	0	0	−1.7	0	−1.9	100	97.5
2	Se-Met	Se	+	0	0	0	0	0	−0.1	0	1.4	100	97.8
3	Se-Met	Se	−	−0.5	0	0	0	−50	−48.7	−50	−47.7	100	100.1
4	Se-Met	Se-Met	+	0	0	0	0	0	−2.0	0	−12.2	100	102.2
5	Se-Met	Se-Met	−	−0.5	−0.5	0	0	−50	−48.3	0	−4.5	50	51.3
6	Se+Cu	Se	+	0	0	−0.5	−0.5	−50	−45.0	0	3.7	50	52.3
7	Se-Met+Cu	Se	+	0	0	−0.5	−0.5	−50	−41.9	0	−6.1	50	57.7
8	Se-Met+Cu	Se	−	−0.5	0	−0.5	−0.5	−75	−84.3	−50	−70.8	50	54.2
9	Se	Se-Met	+	0	0	0	0	0	−1.0	0	−5.4	100	98.2
10	Se	Se-Met	−	0	−0.5	0	0	0	−1.0	100	59.3	50	55.7
11	Se-Met+Se	Se-Met	+	0	0	0	0	0	0.8	0	−3.7	100	103.7
12	Se-Met+Se	Se-Met	−	−0.5	−0.5	0	0	−25	−25.8	50	85.3	50	53.7

(as in the flow system the analyte was added to each sample twice), two values of *RV* were calculated and the mean value of *RV* can be assumed as the final one. Experimental values *RV*, *RE* and *RE′* were calculated from Eqs. (4), (14) and (17), respectively.

The results obtained theoretically and experimentally for synthetic samples 1–12 have been collected in Table 1. In most cases they are comparable with each other to such extent that the differences can be considered as caused by both inaccuracy in experimental evaluation of parameters P_1^*, P_1, Q_1^*, Q_1 and random fluctuations of analytical signals. Greater differences between some values obtained for samples 8 and 12 can be explained by strong decrease in sensitivity due to incomplete digestion of organic form of both the native and the surrogate analyte (sample 12) or of the native analyte only but influenced by the interferent (sample 8). In general, it can be assumed that the obtained results indicate correctness of the models (9) and (10). Consequently, Eqs. (13), (15) and (18) were derived properly and can be utilized to draw conclusions on recovery in the context of trueness when the species effect occurs during analytical procedure.

The results obtained for *RE* and *RV* shown in Table 1 indicate that true analytical result (*RE*=0%) could be predicted on the basis of complete recovery (*RV*=100%) in two cases: (a) when the species effect (as well as the preparative and interference effects) did not exist, i.e. when both the native and the surrogate analyte were originally present in an inorganic form (sample 1), and (b) when the species effect was prevented by complete digestion of the organic form of the native and/or the surrogate analyte to an inorganic form (samples 2, 4, 9, and 11). If the multiplicative effect additionally occurred (samples 6 and 7), the analytical results obtained in interpolative way were not true, nevertheless their true values could be estimated from the relation *RE*=*RV*−100% (as expected on the basis of the examinations presented previously [6]).

Incomplete digestion of a sample containing an organic form of selenium (different from the form used in the standard solutions) provoked the species effect and, consequently, a risk of incorrect estimation of the analytical result basing on *RV* value. Only in the specific case, when both the native and the surrogate analyte were in pure organic form (sample 5), relation *RE*=*RV*−100% was valid. However, if the native analyte was different from the added one completely (samples 3 and 10) or even partly (sample 12), the analyte was determined with different systematic error and trueness, *RE*, could not be predicted by *RV* values. It was also observed that true results were obtained in the case of incomplete recovery (sample 10), while complete recovery did not assure true result (sample 3). The interference effect made such situation even more complicated (sample 8).

The results estimated in extrapolative way (*RE′*) corresponded to the interpolative results (*RE*) when the species effect did not originally occur (sample 1) or was eliminated by digestion (samples 2, 4, 9, and 11). If, in addition, the analyte was multiplicatively influenced by an interferent (samples 6 and 7), the interference effect was compensated and extrapolative estimation of the analyte concentration gave true analytical result (*RE′*=0%). What is interesting, the same result was observed when digestion was incomplete but the native and the surrogate analyte were in the same organic forms (sample 5).

However, when the native and the surrogate analyte were in different forms and the sample was not digested (samples 3, 8, 10, and 12), the results estimated in extrapolative way (*RE′*) could not to be true due to the species effect. In comparison with the corresponding *RE* values they might be the same (sample 3) or different (samples 8, 10, and 12), but, in general, they could not be predicted by *RV* values from the equation *RE′*=*RV*−100%. (in the case of sample 8 such compatibility of *RE′* and *RV* values was accidental as both values were increased by the species and interference effects, respectively, to the same extent).

4.2. Examination of real samples

Samples of thermal spring water (La Roche-Posay, France) and vitamin drink (Oshee, Poland) were analysed in the same way as the synthetic samples. According to the manufacturer's declaration selenium was present in the samples in concentrations 60 and 200 μg L^{-1}, respectively. Selenium was added to the samples in inorganic (Se) or organic (Se-Met) forms in concentration of 20 μg L^{-1}. The inorganic selenium standards of 20, 60 and 90 μg L^{-1} were used to prepare a calibration graph. The results of the study are presented in Table 2.

In both samples the analyte was found in much lower concentration than the declared values. Since in most cases selenium could be fully recovered, a strong additive preparative effect was diagnosed [6]. The most likely reason was a loss of selenium as a result of inappropriate storage of the samples. It should be noted that selenium, as a highly volatile element, is particularly unstable in samples with neutral pH and stored at room temperatures for a long period of time (i.e. from production to selling). Due to great differences between the found and the declared selenium concentrations *RE* and *RE′* values were also high and similar to each other, hence their comparison with recoveries RV_1 and RV_2 (obtained for one- and twofold addition of the analyte) and detection of the species effect was difficult and unreliable. Therefore, the preparative effect was excluded from considerations and the expected analytical results were assumed to be close to the concentrations obtained for completely digested samples. Concentration of selenium in the real samples were thus estimated at the level of 14 and 11 μg L^{-1} for the thermal spring water and the vitamin drink, respectively (these concentrations and the adequate *RE* and *RE′* values have been given in parentheses in Table 2).

The form of selenium in the sample of thermal spring water was not specified but it was expected to be inorganic only. This supposition was confirmed by the obtained results: if the form of selenium added to this sample was inorganic (originally or due

Table 2

Results of examinations of real samples: expected concentrations of selenium (c_s) compared with concentrations found in interpolative ($\hat{c}_s$), and extrapolative ($\hat{c}_s'$) ways with corresponding relative errors (*RE*, *RE′*) and recovery values obtained for one- (RV_1) and twofold (RV_2) addition of surrogate analyte to the sample (in parentheses the values not biased by the additive preparative effects are given; see text).

Sample	Surrogate analyte	UV lamp	c_s [μg L^{-1}]	$\hat{c}_s$ [μg L^{-1}]	$\hat{c}_s'$ [μg L^{-1}]	*RE* [%]	*RE′* [%]	RV_1 [%]	RV_2 [%]
Thermal spring water	Se	+	60 (14)	14.76	12.59	−75.4 (5.4)	−79.0 (−10.1)	96.9	97.6
		−		13.96	12.23	−76.7 (−0.3)	−79.6 (−12.6)	87.1	95.3
	Se-Met	+		14.76	13.40	−75.4 (5.4)	−77.7 (−4.2)	96.2	104.6
		−		13.96	22.36	−76.7 (−0.3)	−62.7 (59.6)	47.4	54.0
Vitamin drink	Se	+	200 (11)	10.92	9.52	−94.5 (−0.7)	−99.9 (−13.5)	92.9	101.4
		−		2.16	0.29	−98.9 (−80.3)	−96.1 (−97.4)	99.5	118.2
	Se-Met	+		10.92	16.95	−94.5 (−0.7)	−99.1 (54.1)	104.5	64.3
		−		2.16	1.74	−98.9 (−80.3)	−99.4 (−84.2)	47.2	53.9

to complete digestion), RE and RE' were close to 0% and RV_1 and RV_2 values were adequately close to 100%. However, if selenium was added to the sample in an organic form and the sample was not digested completely, RE value (still close to 0%) could not be predicted by the recovery values and it was different from RE' value. This evidently indicated the presence of the species effect (compare with the results obtained for sample 10 in Table 1).

In the case of the sample of vitamin drink the situation appeared to be even more complicated. When the sample was spiked with selenium in an inorganic form and exposed to UV radiation, it was digested totally and the analyte was recovered completely, i.e. $RV_1 \approx RV_2 \approx 100\%$. However, if selenium was added in an organic form, the RV_2 value was different from 100% (and different from RV_1). Apparently, the sample enriched with greater addition of the analyte contained too much organic selenium to be digested completely. This assumption was confirmed by the observed species effect, when the sample spiked with both inorganic and inorganic selenium was not exposed to UV radiation. The results obtained in the last case ($RE \approx RE' \neq 0\%$, $RV_1 = RV_2 \neq 100\%$, but $RE \neq RV - 100\%$) may indicate presence of many forms of selenium in the sample (compare with the results obtained for sample 12 in Table 1).

5. Conclusions

The performed theoretical and experimental examinations have proved that the species effect is a serious obstacle in reliable estimation of trueness of an analytical result on the basis of the recovery test. As revealed, the relation $RE = RV - 100\%$, commonly used in analytical practice, can only be exploited in the following cases:

- when the species effect does not exist, i.e. when both the native and the surrogate analytes are present in the same form as the analyte in the standard solutions used for calibration,
- when the species effect is eliminated by complete conversion of the native analyte to the form of the analyte added to the sample and used for calibration,
- when the species effect exists in such a mode that both the native and the surrogate analyte are used in the same form but different from the form of the analyte used for calibration.

In the latter case the species effect is manifested as the multiplicative interference effect, i.e. the form of the native and the surrogate analyte in a sample plays a role of an interferent in relation to another form of the same analyte in the standard solutions. However, such situation is difficult to be met in practice, as the form of native analyte in a sample has to be well known or well recognized (in order to know exactly what the form of the surrogate analyte should be).

Comparison between extrapolative and interpolative estimations of the analytical result is not very useful in diagnosis of the species effect. They are equal to each other when the species effect does not occur and they are unpredictably different when the species effect occurs. However, one should be aware of the fact that the differences between both estimations can also be caused by preparative and interference effects [6]. When the occurred effects (including the species effect) are recognized as being of pure multiplicative character, not only is the relation $RE = RV - 100\%$ valid but also extrapolative estimation indicates true analytical result. However, it has to be kept in mind that in any case the extrapolative results should be interpreted more carefully then the interpolative ones, as they are obtained, as a rule, with greater errors (especially when the analytical signals are very low e.g. due to the species effect).

Taking into account the examinations shown previously [6] and in the present paper it may be concluded that the aspect of recovery, which plays an important role in the production and interpretation of results (e.g. for correction of raw data to produce the final results), can be influenced by various effects (including the species effect) especially when an analytical method is dependent on many stages of transfer of a compound from a complex matrix into a simple solution. Considering complexity of the sample preparation process and the subsequent measurement, estimation of recovery tends to be an inevitable step in order to obtain true analytical results.

It is commonly recognized that analytical methods must be validated and constantly tested with the use of quality control procedures. Recovery is an essential component of validation and testing processes that a laboratory should implement in order to produce reliable analytical data and thereafter be accredited to international standards to assure reliability. However, the lack of awareness of far-reaching consequences of the presence of the species effect as well as other effects during analytical procedure may lead to adopting an uncritical approach to interpretation of the recovery data and thus contribute to draw incorrect conclusions on trueness of analytical results on the basis of recovery estimation.

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References

[1] M. Thompson, S.L.R. Ellison, R. Wood, Pure Appl. Chem. 74 (2002) 835–855.
[2] M. Thompson, S.L.R. Ellison, A. Fajgelj, P. Willetts, R. Wood, Pure Appl. Chem. 71 (1999) 337–348.
[3] D.T. Burns, K. Danzer, A. Townshend, Pure Appl. Chem. 74 (2002) 2201–2205.
[4] P. Kościelniak, Analusis 27 (1999) 381–382.
[5] P. Kościelniak, Anal. Lett. 37 (2004) 2625–2640.
[6] M. Stafiński, M. Wieczorek, P. Janicki, P. Kościelniak, Talanta 96 (2012) 39–43.
[7] B. Welz, Z. Fresenius, Anal. Chem. 325 (1986) 95–101.
[8] P. Kościelniak, J. Kozak, Anal. Chim. Acta. 460 (2002) 235–245.
[9] M. Kashiki, S. Yamazoe, S. Oshima, Anal. Chim. Acta 54 (1971) 533–537.
[10] V.I. Slaveykova, F. Rastegar, M.J.F. Leroy, J. Anal. At. Spectrom. 11 (1996) 997–1002.
[11] Z. Kowalewska, Spectrochim. Acta, Part B 62 (2007) 273–282.
[12] E. Stokvis, H. Rosing, J.H. Beijnen, Rapid Commun. Mass Spectrom. 19 (2005) 401–407.
[13] I. Fu, E.J. Woolf, B.K. Matuszewski, J. Pharm. Biomed. Anal. 18 (1998) 347–357.
[14] C.M. Chavez-Eng, M.L. Constanzer, B.K. Matuszewski, J. Chromatogr. B 767 (2002) 117–129.
[15] L. Yang, N. Wu, R.P. Clement, P.J. Rudewicz, J. Chromatogr. B 799 (2004) 271–280.
[16] A. D'Ulivo, L. Gianfranceschi, L. Lampugnani, R. Zamboni, Spectrochim. Acta, Part B 57 (2002) 2081–2094.