Fluorimetry compared to spectrophotometry for uranium evaluation in liquid waste

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ABSTRACT
Uranium concentration levels in surface waters are of great importance because uranium presents both chemical and radiological hazard to the environment. The subject of this article is to establish that spectrophotometric method for uranium evaluation in effluents collected from liquid waste treatment generated during fabrication of nuclear fuel elements could be replaced by the optical fluorimetric technique. Both methods are briefly described. The comparison of the two methods was carried out with regard to international standards and national regulations offering from a statistical point of view a useful approach to compare two analytical measurement techniques. This methodology can be applied to any other measurement procedures. A discussion about the compliance of the fluorimetric analytical method with the mandatory discharge level of uranium concentration in surface waters is also presented.

1. Introduction
Monitoring of uranium concentration in the effluents collected from treatment of liquid waste generated during fabrication of fuel elements by Romanian Nuclear Fuel Factory is necessary to ensure that the waste is discharged into surface waters within specified uranium concentration limits in order to protect the human health and environment.

Different analytical methods for uranium assessment in water samples have been reported in the literature, such as: spectrometry (inductive coupled plasma, ICP) [1–5], photometry [3,4], fluorimetry [3,4], liquid scintillation counting (LSC) [3,4].

ICP and LSC analytical procedures imply the use of expensive equipment.

Fluorimetry seems to be a good choice, in terms of low-cost and efficiency (less working time, less human resources and reduced reagents consumption) [3].

There is apparently no detailed description of a methodology to compare two analytical procedures for uranium evaluation in waste water.

In this article, the potential of optical fluorimetry over the spectrophotometric method was studied to reduce the working time and high reagents consumption for uranium quantitative evaluation in the effluents.
The spectrophotometric method has been in routine use in the laboratory of Radioactive Waste Treatment Plant in the past 30 years. Recently, we started to analyse the effluents by fluorimetric method.

Both methods are suitable for uranium evaluation in nuclear effluents in the interest range of concentration levels (i.e. < 1 mg L\(^{-1}\)).

The main scope of this research was to provide evidence that the fluorimetric and spectrophotometric methods have not different precision and trueness. Afterwards, the decision to replace spectrophotometric method with fluorimetric technique should be made taking into account the economic factors [6].

The laboratory methods used to obtain a homogenous sample solution suitable for spectrophotometric and fluorimetric analyses are more and less laborious. The volume of taken effluent is 25 mL for both methods.

The spectrophotometric method includes the following basic steps: vaporisation of sample to dryness with concentrated nitric acid (in proportion of 2:1); dissolution of residue in 1 N nitric acid; extraction of U(VI) into an organic phase with a 5% solution of trioctylphosphine oxide (TOPO) in toluene using a separating funnel marked with code 1; back-extraction of uranium into a 0.5% oxalic acid aqueous phase (the aqueous phase with U extracted is collected into another separating funnel marked with code 2); second back-extraction of U(VI) from the organic phase into a 0.5% oxalic acid solution (this stage is performed for quantitative extraction of uranium from the organic phase) and collection of the aqueous phase into the same separating funnel no. 2 (the organic phase is discarded); treatment with toluene of aqueous phase; vaporisation up to wet salts; reduction with zinc in presence of ascorbic acid to convert U(VI) to U(IV), which forms a red coloured complex with arsenazo (III). Therefore, the spectrophotometric method is a time and reagents consuming procedure. Moreover, the appropriate calibration curve implies the same laborious preparation steps of the standard set.

The fluorimetric analysis is based on measuring the intensity of delayed fluorescence of uranyl ions after excitation by ultraviolet radiation. The luminescence of the solution is increased by addition of sodium polysilicate. The method implies vaporisation of sample with concentrated nitric acid (in proportion of 2:1), dissolution in 0.1 N nitric acid and sample measurement by mixing 1 mL sample with the solution of sodium polysilicate and double distilled water in proportion of 1:1:10. Calibration curve of the fluorimeter is achieved by measuring the luminescence of solutions with known content of uranium in the form of uranyl ions, which are mixed with sodium polysilicate and double distilled water in the same proportion of 1:1:10 as the sample.

2. Experimental

2.1 Equipments and chemicals

A Hach® DR/3000 Spectrophotometer and a FLUORAT-02–2 M® Liquid Analyzer were used to measure uranium concentrations of the liquid waste. SRM 7115–94 standard reference material of uranium(VI) ions solution, provided by Bochvar Research Institute of Inorganic Materials, ‘Ekmets’ Ltd., was used. Other chemicals used were analytical grade reagents.
2.2 **Analysis method**

To compare the alternative fluorimetric method, called \( A \), to the reference considered spectrophotometric method, called \( R \), an experiment has to be performed to prove that the \( A \) method is as accurate as the \( R \) method. Trueness and precision are the terms that describe the accuracy of a measurement method [6–9].

The experiment (depicted in Figure 1) consists of a preceding accuracy test for the reference spectrophotometric method, followed by a bias estimation of the measurement process to ensure that the main comparison test between the two techniques fulfills the standard requirements [6].

After the primary test is completed, a supplementary validation test is performed to investigate if there are systematic deviations between the two measuring methods.

2.2.1 **Accuracy experiment for reference spectrophotometric analytical method**

Prior to develop the main experiment, an accuracy test was performed for method \( R \) using known reference materials, to accept that this method can be considered to be a reference method. The accuracy test comprises of a precision estimation of the analytical method and a trueness bias evaluation for the spectrophotometric method.

Five standards, from known reference materials, were prepared and measured carrying out the complete procedural steps of the method \( R \). Because of the complexity of the method only one measurement was employed for each level of uranium concentration. The precision for every level was accomplished taking into account the technical specifications of the apparatus as type B uncertainty evaluation of measurements [10]. The precision of the method was established by plotting the calculated uncertainty values against the values of the measured concentrations and finding a relationship between these data. After the positive check of the precision, the bias of the reference measurement method was estimated, showing that the difference between the analytical results and the known standards values used as test samples was statistically insignificant.

![Figure 1. The comparison experiment concept.](image-url)
2.2.2 **Comparison of alternative and reference measurement methods**

With the precision of the reference method well established, the main experiment was performed on 18 independent samples prepared from the different effluents resulted from the process flow of the uranium liquid waste treatment. The experiment implies a precision test and a trueness test.

Each one of the 18 samples was measured by both the spectrophotometric analytical method and the optical fluorimetric technique. The samples were measured once for method R and 10 times for method A.

All measurements were made under repeatability conditions (the same operator, the same measurement equipment, the same calibration curves, the same laboratory – with as possible the same ambient conditions, and the shortest possible time between measurements).

For precision comparison experiment, the ratios between the precision measurements of method A and method R were computed and their values were compared to a specified value considering that method R is a reference method and method A is a candidate for reference method [6]. It has been observed that the within-laboratory precision of the method A is at least as good as the precision of method R.

The trueness of the method A was compared with the trueness of the method R for each level of measurements, taking into account a confidence interval constructed around the differences between the means of the two methods. All the confidence intervals cover the zero value [8] so the bias between the trueness of the two methods is statistically insignificant. The difference between the grand means of the two methods was calculated to find if its value is statistically significant, too. Nor this test showed any evidence that the difference between the trueness of the A and R methods is unacceptable.

The confidence level for these tests was \( \alpha = 0.05 \) (the probability \( p = 1 - \alpha \) that the results of the tests to be correct is 95%).

2.2.3 **Validation procedure for fluorimetric analytical method compared to spectrophotometric analytical method**

A supplementary validation test for the lack of systematic deviation between method A and method R was accomplished using an orthogonal linear regression model [11].

The data were scrutinised by Grubbs test and the acceptance criterion for the correlation coefficient \( (r \geq 0.97) \) was fulfilled with a 0.9998 calculated value. Both computed slope and intercept parameters of the orthogonal linear relation between the precision and the trueness of the two methods conform to the procedure criteria. Therefore, the hypothesis that no systematic deviation exists between the fluorimetric and spectrophotometric methods was accepted.

2.2.4 **Compliance with upper limit of waste discharge**

Romanian regulations procedures impose a maximum uranium concentration of 1 mg L\(^{-1}\) to be discharged in surface waters [12]. Thus, the uncertainty of the fluorimetric method must be taken into account for the interpretation of the measuring procedure to meet the requirements. So, the values of the measuring results must be less than the compliance limit value minus the expanded uncertainty [13]. The expanded uncertainty [14] is calculated using a coverage factor of 3, which gives a confidence level of approximately 99.7%.
3. Results and discussion

3.1 Accuracy experiment for reference spectrophotometric analytical method

3.1.1 Calibration curve of the spectrophotometer

The evaluation of the uranium concentration in the effluents is based on the calibration curve of the spectrophotometer, displayed in Figure S1 in Supplemental data.

The curve was constructed from the standard reference material, diluted to five samples to span [0.25–2] mg L\(^{-1}\), and a blank sample using the Hach® DR/3000 Best Fit Program Mode that performs a linear regression analysis on the standards to generate the best fit calibration line.

The analytical parameters for evaluation of uranium concentration in effluents by both spectrophotometric and fluorimetric techniques are presented in (Table 1).

Very good correlation coefficients were achieved in the linearity range of concentrations for the calibration curves appropriate to both methods. The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated using the standard deviations of the regression and the slopes of the calibration curves [15]. The LOD and the LOQ for the spectrophotometric method are sensibly higher than the same parameters of the fluorimetric technique and some of the sample concentrations span these values. This means that the analytical results of these samples have a higher degree of uncertainty. The objective of the research was to estimate the uranium concentration in nuclear effluents in regard to an upper limit (1 mg L\(^{-1}\)), which is almost 100 times greater than LOD, so these results did not impeach the development of the study.

3.1.2 Uncertainty of the measurements for the spectrophotometric method

The uncertainty sources for the measuring method are the uncertainty of the calibration curve \(u(C(x))\), the uncertainty of the measuring apparatus \(u(SP)\), the uncertainty of the calibration standards \(u(CS)\), and the uncertainty of the inevitable correlations induced by successive dilutions from the same stock solution during the procedure. The uncertainties arisen from the successive dilutions are too small compared to the other uncertainties so they were not considered to the estimation of the final uncertainty of the method. The cause and effect diagram for the uncertainty sources of the spectrophotometric method is presented in Figure S2 in Supplemental data.

Table 1. Analytical parameters for spectrophotometric and fluorimetric techniques.

| Method                      | Spectrophotometry | Fluorimetry |
|-----------------------------|-------------------|-------------|
| Wavelength [nm]             | 665               | 530         |
| Linearity range [mg L\(^{-1}\)] | 0.25–2 | 0.002–1 |
| Slope, \(B1\)               | 0.505             | 8.194       |
| Standard deviation of the slope, \(SD_{B1}\) | 0.006 | 0.042 |
| Intercept, \(B0\)           | 0                 | 0           |
| Standard deviation of the intercept, \(SD_{B0}\) [mg L\(^{-1}\)] | N/A | N/A |
| Correlation coefficient, \(r\) | 0.9997 | 0.9999 |
| Standard deviation of the regression, \(S_{y/x}\) [mg L\(^{-1}\)] | 0.018 | 0.042 |
| Limit of detection, LOD [mg L\(^{-1}\)] as \(3.3S_{y/x} B1^{-1}\) [15] | 0.114 | 0.017 |
| Limit of quantitation, LOQ [mg L\(^{-1}\)] as \(10S_{y/x} B1^{-1}\) [15] | 0.347 | 0.052 |
The uncertainty of the calibration curve is calculated with Equation (1) [13].

\[
\begin{align*}
  u[C(x)] &= \frac{S}{B_1} \sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(Cx - Cst(\text{av}))^2}{S_{xx}}} \\
  S &= \sqrt{\frac{\sum_{j=1}^{n} [\text{Abs}(j)-(B_0+B_1 \cdot Ci)]^2}{n-2}} \\
  S_{xx} &= \sum_{i=1}^{n} [Ci - Cst(\text{av})]^2
\end{align*}
\]  

(1)

where:

- \(u[C(x)]\) = uncertainty of the measured concentration [mg L\(^{-1}\)];
- \(S\) = residual standard deviation;
- \(B_1\) = slope of the calibration curve;
- \(p\) = number of measurements for the concentration evaluation, \(p = 1\);
- \(n\) = number of the standards used to create the calibration curve, \(n = 5\);
- \(Cx\) = concentration value measured and displayed by the spectrophotometer;
- \(Cst(\text{av})\) = mean value of the \(Ci\) theoretical values of the \(n\) standards;
- \(S_{xx}\) = value of the non-normalised variance of the standards;
- \(\text{Abs}(j)\) = absorbance values displayed by the apparatus, when the calibration curve was constructed, for each standard;
- \(B_0\) = intercept of the calibration curve;
- \(Ci\) = theoretical values of the standards.

To compute the standard uncertainties of the spectrophotometer \(u(SP)\) and the calibration standards \(u(CS)\), the uncertainty budgets were constructed based on the technical specification provided by the apparatus producer and the passport certificate of the standard reference materials. These budgets are presented in Table S1 in Supplemental data.

For each concentration value of the measured standards, the absorbance values are displayed by the spectrophotometer. Using these values in Equation (1) the uncertainty of the method \(u_{REF}\) is assessed, based on Equation (2).

\[
u_{REF} = \sqrt{u[C(x)]^2 + u(\text{Abs})^2 + u(SL)^2 + u(D)^2 + u(W)^2 + u(CS)^2}
\]

(2)

where:

- \(u_{REF}\) = uncertainty of the spectrophotometric method;
- \(u[C(x)]\) = uncertainty caused by the calibration curve of the spectrophotometer;
- \(u(\text{Abs})\) = uncertainty caused by the absorbance fluctuations;
- \(u(SL)\) = uncertainty caused by the stray light of the optical source of the apparatus;
- \(u(D)\) = uncertainty caused by the limited number of digits of the display;
- \(u(W)\) = uncertainty caused by the imperfections of the monochromator reflected in wavelength;
- \(u(CS)\) = uncertainty caused by the calibration standards of the reference materials.

The calculated values for uncertainty of the spectrophotometric method are presented in Table S2 (Supplemental data). A polynomial relation of grade 2, depicted in
Figure S3 (Supplemental data), was detected, as Equation (3), plotting the associated uncertainty values versus the concentration values (C; uREF).

\[ u_{REF} = 0.086 \times C^2 - 0.0175 \times C + 0.0536, \text{ in } [\text{mgL}^{-1}] \]  

(3)

where:

- \( u_{REF} \) = uncertainty of the spectrophotometric method;
- \( C \) = concentration value displayed by the spectrophotometer.

The relation detected is considered satisfactory (R-squared coefficient denotes a very good fit) and is chosen to calculate \( s_{Ref} \) [mg L\(^{-1}\)], the repeatability standard deviation (precision) values of the spectrophotometric method.

### 3.1.3 Trueness validation test for the spectrophotometric method

For the trueness experiment the same five standards used in repeatability test were measured as unknown samples. The uncertainty for each sample was computed using Equation (3), giving \( s_{Ref} \) values.

A single measurement \((n = 1)\) was taken for each sample, thus the statistical value \([8]\) \( A_w \), shown in Equation (4), for constructing the interval of confidence is set to 1.96.

\[ A_w = \frac{1.96}{\sqrt{n}} \]  

(4)

The raw data and the statistical evaluations are presented in (Table 2).

The confidence interval covers the zero value, for all the concentration levels of the experiment. This means that the laboratory bias of the spectrophotometric method is insignificant at the significance level of 5% for all the tested concentration levels.

### 3.2 Comparison of alternative and reference measurement methods

The statistical evaluation of the 18 process samples, measured by both methods, R (spectrophotometry) and A (fluorimetry): the means \( m_R \) and \( m_A \), standard deviations \( s_R \) and \( s_A \) and variances \( s^2_R \) and \( s^2_A \) are presented in (Table 3). The raw data are displayed in Table S3 (Supplemental data).

The standard deviations for the R method are computed using Equation (3). The standard deviations for the fluorimetric method were estimated as measurement uncertainties of type A for repetitive evaluations [10].

| Level | 1  | 2  | 3  | 4  | 5  |
|-------|----|----|----|----|----|
| Accepted reference value, \( \mu \) [mg L\(^{-1}\)] | 0.25 | 0.5 | 1  | 1.5 | 2  |
| Measured value, \( m \) [mg L\(^{-1}\)] | 0.221 | 0.472 | 1.045 | 1.501 | 1.963 |
| Standard deviation, \( s_{Ref} \) [mg L\(^{-1}\)] | 0.050152533 | 0.047255942 | 0.044703915 | 0.046708309 | 0.052386473 |
| Means difference, \( D = m - \mu \) [mg L\(^{-1}\)] | −0.029 | −0.028 | 0.045 | 0.001 | −0.037 |
| Inferior limit of the confidence interval, \( D - A_w \times s_{Ref} \) [mg L\(^{-1}\)] | −0.12729896 | −0.120621647 | −0.042619673 | −0.09054828 | −0.13967749 |
| Superior limit of the confidence interval, \( D + A_w \times s_{Ref} \) [mg L\(^{-1}\)] | 0.069298964 | 0.064621647 | 0.132619673 | 0.092548285 | 0.065677488 |
Each measurement value of raw data for the fluorimetric method was scrutinised for one outlying observation, applying the Grubbs test – Equation (5), and comparing the test value with the critical value \( 7 \) of 2.29 (10 measurements for a 5% significance level) for each concentration level.

\[
G = \frac{|m_A - x|}{s_A}
\]  

(5)

If the computed value is greater than the critical value, then the measured value is considered an outlier and must be excluded from the series of data. New statistical parameters (mean and standard deviation) must be calculated according to the new raw data and the Grubbs test must be rechecked.

The results of the Grubbs test are shown in Table S4 (Supplemental data). None of the computed values exceeded the critical value so all the tested items were accepted as truthful. Therefore, no exclusion from the raw data was performed, and the values of the statistical parameters (means, standard deviations and variances) are kept according to (Table 3) for further estimates.

### 3.2.1 Precision comparison experiment

Within-laboratory precision test for the fluorimetric method \( A \) is performed, considering the \( R \) method a reference method. The test is applied for each measured concentration level.

The test consists in comparing the ratio between the alternative method variance and reference method variance with the critical value \( 6 \) of 1.88 computed for 9 degrees of freedom (10 measurements for each level of concentration for the alternative method).

If the critical value is greater than the computed value, then the precision of the alternative method is as good as the precision of the reference method. If the critical value is less than the computed value, the precision of the \( A \) method is poorer than the precision of the \( R \) method.

The results of the statistical test are given in (Table 3).

None of the computed values of the test exceeded the critical value, so there was no evidence that the within-laboratory precision of the fluorimetric method was not as good as the precision of the spectrophotometric method.

| Level | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-------|---|---|---|---|---|---|---|---|---|
| \( m_R \) [mg L\(^{-1}\)] | 0.7700 | 0.5800 | 0.0900 | 0.3080 | 0.1360 | 0.3720 | 0.2500 | 0.2640 | 0.0600 |
| \( m_A \) [mg L\(^{-1}\)] | 0.7704 | 0.5694 | 0.0870 | 0.2998 | 0.1371 | 0.3717 | 0.2490 | 0.2630 | 0.0587 |
| \( s_R \) [mg L\(^{-1}\)] | 4.52E-02 | 4.63E-02 | 5.21E-02 | 4.90E-02 | 5.14E-02 | 4.83E-02 | 4.98E-02 | 4.96E-02 | 5.26E-02 |
| \( s_A \) [mg L\(^{-1}\)] | 4.14E-03 | 2.13E-02 | 3.59E-03 | 2.86E-03 | 3.57E-03 | 2.21E-03 | 2.87E-03 | 2.71E-03 | 4.32E-03 |
| \( s_R^2 \) [mg\(^2\) L\(^{-2}\)] | 2.05E-03 | 2.15E-03 | 2.71E-03 | 2.40E-03 | 2.64E-03 | 2.33E-03 | 2.48E-03 | 2.46E-03 | 2.77E-03 |
| \( s_A^2 \) [mg\(^2\) L\(^{-2}\)] | 1.72E-05 | 4.55E-04 | 1.29E-05 | 8.18E-05 | 1.28E-05 | 8.22E-05 | 7.33E-05 | 1.87E-05 | 7.33E-05 |
| \( s_{A^2}/s_R^2 \) | 8.39E-03 | 2.12E-01 | 4.75E-03 | 3.40E-03 | 4.84E-03 | 2.10E-03 | 3.32E-03 | 2.98E-03 | 6.76E-03 |

| Level | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|-------|----|----|----|----|----|----|----|----|----|
| \( m_R \) [mg L\(^{-1}\)] | 0.1400 | 0.1140 | 0.9800 | 0.5420 | 0.4360 | 0.8940 | 0.6400 | 0.9000 | 0.6140 |
| \( m_A \) [mg L\(^{-1}\)] | 0.1411 | 0.1145 | 0.9945 | 0.5431 | 0.4309 | 0.8997 | 0.6556 | 0.9234 | 0.6194 |
| \( s_R \) [mg L\(^{-1}\)] | 5.13E-02 | 5.17E-02 | 4.47E-02 | 4.66E-02 | 4.76E-02 | 4.48E-02 | 4.59E-02 | 4.48E-02 | 4.61E-02 |
| \( s_A \) [mg L\(^{-1}\)] | 5.38E-03 | 1.51E-03 | 4.24E-03 | 4.82E-03 | 9.94E-04 | 2.41E-03 | 5.74E-03 | 7.04E-03 | 2.41E-03 |
| \( s_R^2 \) [mg\(^2\) L\(^{-2}\)] | 2.63E-03 | 2.67E-03 | 2.00E-03 | 2.18E-03 | 2.27E-03 | 2.01E-03 | 2.11E-03 | 2.01E-03 | 2.13E-03 |
| \( s_A^2 \) [mg\(^2\) L\(^{-2}\)] | 2.90E-05 | 2.28E-06 | 5.83E-06 | 2.32E-05 | 9.89E-07 | 5.79E-06 | 3.29E-05 | 4.96E-05 | 5.82E-06 |
| \( s_{A^2}/s_R^2 \) | 1.10E-02 | 8.52E-04 | 2.92E-03 | 1.07E-02 | 4.36E-04 | 2.88E-03 | 1.56E-02 | 2.47E-02 | 2.74E-03 |
3.2.2 Trueness comparison experiment

To construct the confidence interval around the differences between the means of both methods, the 0.62 value for $A_w$ parameter was computed by Equation (4), taking into account that $n = 10$ measurements are assessed for each concentration level of the method A.

The trueness test results are offered in (Table 4). The values from (Table 3) were used for $m_A$, $m_R$ and $s_R$.

Because the confidence interval covers the zero value, for all the concentration levels of the experiment, the bias between the fluorimetric trueness and spectrophotometric trueness is considered statistically insignificant, at the significance level of 5%.

Plotting the uncertainty values for the fluorimetric method versus the average values of each concentration level no linear, polynomial or logarithmic relation could be found, so the Equation (6) was used to compute the final value of the repeatability standard deviation of the alternative method [7].

$$s_{RA} = \sum_{i=1}^{q} \frac{s_i}{q}$$

where:

$s_{RA}$ = value of repeatability standard deviation for the alternative method;

$s_i$ = value of standard deviation $s_A$ from (Table 3), for each $q$ level of the 18 concentration levels.

The grand mean of each method is computed, that is the average of the means from the (Table 3) for all the 18 concentration levels, then repeatability $s_{R}$ of the method $R$ is estimated with Equation (3) and repeatability $s_{RA}$ of the method $A$ is evaluated with Equation (6).

### Table 4. The trueness of fluorimetric method compared to the trueness of spectrophotometric method.

| Level | Means difference, $D = m_A - m_R$ [mg L$^{-1}$] | Inferior limit of the confidence interval, $D - A_w s_R$ [mg L$^{-1}$] | Superior limit of the confidence interval, $D + A_w s_R$ [mg L$^{-1}$] |
|-------|-----------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| 1     | -0.0004                                       | -0.0284                                         | 0.0276                                          |
| 2     | 0.0106                                        | -0.0181                                         | 0.0393                                          |
| 3     | 0.0030                                        | -0.0293                                         | 0.0353                                          |
| 4     | 0.0082                                        | -0.0222                                         | 0.0386                                          |
| 5     | -0.0011                                       | -0.0329                                         | 0.0307                                          |
| 6     | 0.0003                                        | -0.0296                                         | 0.0302                                          |
| 7     | 0.0010                                        | -0.0298                                         | 0.0318                                          |
| 8     | 0.0010                                        | -0.0297                                         | 0.0317                                          |
| 9     | 0.0013                                        | -0.0313                                         | 0.0339                                          |
| 10    | -0.0011                                       | -0.0329                                         | 0.0307                                          |
| 11    | -0.0005                                       | -0.0326                                         | 0.0316                                          |
| 12    | -0.0145                                       | -0.0422                                         | 0.0132                                          |
| 13    | -0.0011                                       | -0.0300                                         | 0.0278                                          |
| 14    | 0.0051                                        | -0.0244                                         | 0.0346                                          |
| 15    | -0.0057                                       | -0.0335                                         | 0.0221                                          |
| 16    | -0.0156                                       | -0.0441                                         | 0.0129                                          |
| 17    | -0.0234                                       | -0.0512                                         | 0.0044                                          |
| 18    | -0.0054                                       | -0.0340                                         | 0.0232                                          |
To perform the statistical test for comparison between the means of the two methods, the critical value from Equation (7) is used [6].

\[
Cr = \left| \frac{\bar{m}_A - \bar{m}_R}{s} \right|
\]  

(7)

where:

\( Cr \) = critic value for the statistical test of comparison between the grand means;
\( \bar{m}_A \) = grand mean of the fluorimetric method (alternative method);
\( \bar{m}_R \) = grand mean of the spectrophotometric method (reference method);
\( s = \sqrt{s_{\text{rA}}^2 + s_{\text{rR}}^2} \); \( s_{\text{rA}} \) is the repeatability of the method \( A \), estimated with Equation (6), and \( s_{\text{rR}} \) is the repeatability of the method \( R \), computed with Equation (3) for \( \bar{m}_R \).

If the critical value \( Cr \) is less than 2, then the difference between the means of the two methods is statistically insignificant. If the critical value is greater than 2, then it is evident that the difference between the biases of the two methods is unacceptable.

The evaluation and result of the test are presented in (Table 5), emphasising the statistical insignificance between the trueness of the fluorimetric and spectrophotometric analytical methods.

### 3.3 Validation procedure for fluorimetric analytical method compared to spectrophotometric analytical method

To implement the orthogonal linear regression model, statistical parameters \( m_A \) and \( m_R \) from (Table 3) are used as raw data. The single Grubbs test is applied to these data to inspect for outliers. For a number of 18 levels, the Grubbs critical value [7] at a significance level of 5% is 2.651. The construction and the results of the test are shown in Table 6.

In Table 6, the \( G \) values are the Grubbs test results, \( \bar{D}_i \) represents the grand mean of the \( D_i \) values and \( s_{Dr} \) is the standard deviation of the \( D_i \) values. The grand means for the two methods \( (\bar{m}_A, \bar{m}_R) \) and the standard deviations of the means for the method \( A \) and for the method \( R \) \( (s_{mA}, s_{mR}) \) are also presented. For all the 18 concentration levels of the experiment, the Grubbs results are less than the critical value, so all \( m_A \) and \( m_R \) values are kept for the assessments of the validation procedure.

The parameters of the orthogonal linear regression [11] are calculated according to Equation (8).
Table 6. The statistical evaluation of the means and Grubbs test results for the orthogonal linear regression model.

| Level indicative | $m_B$  | $m_A$  | $M = \frac{m_B + m_A}{2}$ | $D = m_B - m_A$ | $D_r = \frac{D}{M}$ | $100$ | $G = \frac{|m_B - m_A|}{s_{mR}}$ |
|------------------|--------|--------|---------------------------|----------------|------------------|------|-------------------------|
| U1               | 0.7700 | 0.7704 | 0.7702                    | -0.0004        | -0.0519          | 0.0969 |
| U2               | 0.5800 | 0.5694 | 0.5747                    | 0.0106         | 1.8444           | 1.0600 |
| U3               | 0.0900 | 0.0870 | 0.0885                    | 0.0030         | 3.3898           | 2.0029 |
| U4               | 0.3080 | 0.2998 | 0.3039                    | 0.0082         | 2.6983           | 1.5809 |
| U5               | 0.1360 | 0.1371 | 0.1366                    | -0.0011        | -0.8056          | 0.5567 |
| U6               | 0.3720 | 0.3717 | 0.3719                    | 0.0003         | 0.0807           | 0.0160 |
| U7               | 0.2500 | 0.2490 | 0.2495                    | 0.0010         | 0.4008           | 0.1793 |
| U8               | 0.2640 | 0.2630 | 0.2635                    | 0.0010         | 0.3795           | 0.1663 |
| U9               | 0.0600 | 0.0587 | 0.0594                    | 0.0013         | 2.1904           | 1.2711 |
| U10              | 0.1400 | 0.1411 | 0.1406                    | -0.0011        | -0.7826          | 0.5427 |
| U11              | 0.1140 | 0.1145 | 0.1143                    | -0.0005        | -0.4376          | 0.3323 |
| U12              | 0.9800 | 0.9945 | 0.9873                    | -0.0145        | -1.4687          | 0.9613 |
| U13              | 0.5420 | 0.5431 | 0.5426                    | -0.0011        | -0.2027          | 0.1890 |
| U14              | 0.4360 | 0.4309 | 0.4335                    | 0.0051         | 1.1766           | 0.6526 |
| U15              | 0.8940 | 0.8997 | 0.8969                    | -0.0057        | -0.6356          | 0.4530 |
| U16              | 0.6400 | 0.6556 | 0.6478                    | -0.0156        | -2.4082          | 1.5345 |
| U17              | 0.9000 | 0.9234 | 0.9117                    | -0.0234        | -2.5666          | 1.6311 |
| U18              | 0.6140 | 0.6194 | 0.6167                    | -0.0054        | -0.8756          | 0.5995 |
| **Grand means for** $m_B$, $m_A$, $D_r$ | 0.4494 | 0.4516 | 0.4494                    | 0.1070         | 0.1070           | 0.1070 |
| **Standard deviations for** $m_B$, $m_A$, $D_r$ | 0.3031 | 0.3082 | 1.6391                    |                |                  |        |

The statistical evaluation of the means and Grubbs test results for the orthogonal linear regression model.

\[
\begin{aligned}
C_1 &= \frac{s_{mA}}{s_{mR}} \\
C_0 &= \bar{m}_A - \frac{s_{mA}}{s_{mR}} \bar{m}_R \\
\end{aligned}
\]

where:

- $C_1 = \text{slope of the orthogonal linear curve}$;
- $C_0 = \text{intercept of the linear relation between the A and R variables}$;
- $\bar{m}_A = \text{grand mean of the fluorimetric method (alternative method)}$;
- $\bar{m}_R = \text{grand mean of the spectrophotometric method (reference method)}$;
- $s_{mA} = \text{standard deviation value of the series of means for the alternative method}$;
- $s_{mR} = \text{standard deviation value of the series of means for the reference method}$.

Figure 2. The orthogonal relation between the data of the spectrophotometric ($R$) and fluorimetric ($A$) methods.
The $C_1$ and $C_0$ parameters were computed using the values from (Table 6).

The orthogonal linear regression relation between the means of each concentration level for the two methods is depicted in (Figure 2).

The fluorimetric method can be accepted if the correlation coefficient $r$ between the two series of means for the A and R methods [11] is greater than 0.97.

To accept the hypothesis that no systematic deviation exists between the fluorimetric and spectrophotometric methods, the criteria in the set of Equation (9) must be fulfilled [11].

$$
\begin{align*}
1 - \frac{s_{\text{fr}}}{m_R} & \leq C_1 \leq 1 + \frac{s_{\text{fr}}}{m_R} \\
|C_0| & \leq s_{\text{fr}}
\end{align*}
$$

where:
- $C_1 =$ slope of the orthogonal linear curve;
- $C_0 =$ intercept of the linear relation between the A and R variables;
- $m_R =$ grand mean of the spectrophotometric method (reference method);
- $s_{\text{fr}} =$ repeatability of the reference method for $m_R$ value.

The results of the validation test are presented in (Table 7). The values for the grand mean and the repeatability standard deviation for method R are extracted from the (Table 5).

The data shown in (Table 7) reveal that all the compulsory criteria of the validation procedure are achieved, so it is accepted that no systematic deviation exists between the fluorimetric alternative method and the reference spectrophotometric method, therefore the verification test is validated.

### 3.4 Compliance with upper limit of waste discharge

With a value of 0.00446 mg L$^{-1}$ for repeatability standard deviation $s_{\text{frA}}$ – see Equation (6) and (Table 5) – the expanded uncertainty of the fluorimetric analytical method is set to 0.01338 mg L$^{-1}$ for a coverage factor of 3 to give a 99.7% confidence level. This means that no more than $(1.0000 - 0.01338)$ mg L$^{-1}$ values of uranium concentration can be accepted to be discharged in surface waters, i.e. 0.987 mg L$^{-1}$ (Figure 3).

For uranium concentration values greater than 0.987 mg L$^{-1}$ a supplementary dilution must be performed to accomplish the compliance limit. This was the case of the process sample no. 12 from (Table 3).

### Conclusion

The assessments to compare the laborious and time consuming reference spectrophotometric method, used for uranium estimation in effluents collected from treatment of
liquid waste resulted from fuel elements fabrication by Romanian Nuclear Fuel Factory, against an easier and rapid alternative fluorimetric technique, proved that the last one is as accurate as the reference routine. The results demonstrate the operational reliability of the fluorimetric method for the designed purpose.

Therefore, the fluorimetric method can replace the spectrophotometric technique to measure the uranium concentration in the aqueous wastes. Care must be taken if an upper limit of compliance must be fulfilled. The value of the $3\sigma$ extended standard deviation of the alternative method must be taken into account such that the measured waste concentration values do not exceed the compliance upper limit minus the extended uncertainty. In a contrary case a supplementary dilution must be performed.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported by the Romanian Institute for Nuclear Research [grant number 3672].

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