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DETERMINATION OF LINEARITY, ACCURACY AND PRECISION OF UV-SPECTROPHOTOMETRIC METHODS OF QUANTITATIVE DETERMINATION IN FORENSIC AND TOXICOLOGICAL ANALYSIS IN THE VARIANT OF THE METHOD OF ADDITIONS

Most of international guidances on carrying out validation of bioanalytical methods [1-4] are directed to application of the method of calibration curve, it is mentioned in passing about possibility of application of the method of additions and it is not given any recommendations on the validation features of such methods are not brought [5].

ANNOTATION

The procedure of linearity, accuracy and precision determination and acceptability estimation for validation of UV-spectrophotometric methods of analytes quantitative determination in biological fluids used in forensic and toxicological analysis has been offered in the variant of the method of additions.

Keywords: linearity, accuracy, precision, UV-spectrophotometric methods, forensic and toxicological analysis, quantitative determination

INTRODUCTION

The method of calibration curve, certainly, allows to take into account and partially level the influence of matrix background absorbance on the results of determination, but demonstrates its value only when carrying out routine analyses.

In forensic and toxicological analysis we often face with single examinations, various biological liquids, organs and tissues, i.e. it is necessary to quantify an

analyte in a few different biological objects, which state can be the most various; thus the necessity of such determination carrying out can arise rarely enough. Application of the method of standard or the method of additions is considerably more effective in such situation.

In previous papers [6-11] we have offered the standardized procedures of determination of linearity, accuracy and precision for UV-spectrophotometric methods of analytes quantitative determination in biological fluids for forensic and toxicological analysis in the variant of the method of calibration curve [6-9] and the method of standard [10,11].

Depending on the work features of forensic and toxicological laboratory the necessity of carrying out of the same method of analyte quantitative determination both in the variant of the method of calibration curve and in the variant of the method of standard or the method of additions can arise. It is therefore appropriate to carry out the method validation in three variants at the same time when its developing. It is thus necessary to optimize the quantity of running experiments as much as possible.

Therefore the purpose of this paper is to study the possibility of using the method of additions when carrying out UV-spectrophotometric determination of analytes in biological fluids and to develop the procedure of determination and acceptability estimation of linearity, accuracy and precision for validation of such methods in the variant of the method of additions.

THEORETICAL PART

Using the method of additions in forensic toxicology assumes the work in two directions:

1) in the first case two samples of the same volume are selected from the specimen received for analysis; certain amount of the standard solution-addition of target analyte are added into one of them. Then both samples are subjected to the procedure of analysis according to the method and the values of absorbance A_i and A_{i+ad} are obtained respectively. The analyte concentration in the analysed specimen C_i is calculated from the ratio

$$\frac{A_i}{A_{i+ad}} = \frac{C_i}{C_i + C_{ad}} \Rightarrow C_i = C_{ad} \cdot \frac{A_i}{A_{i+ad} - A_i} \quad (1)$$

2) in the second case one sample of the fixed volume is selected from the specimen received for analysis and subjected to the procedure of analysis according to the method. On the last stage preparing the end solution to be spectrophotometric measured is carried out twice – using the solvent and the standard solution-addition of target analyte – and the values of absorbance A_i and A_{i+ad} are obtained respectively. The analyte concentration in the end solution to be spectrophotometric measured C'_i is calculated from the ratio

$$\frac{A_i}{A_{i+ad}} = \frac{C'_i}{(C_i + C_{ad})} \Rightarrow C'_i = C_{ad} \cdot \frac{A_i}{A_{i+ad} - A_i} \quad (2)$$

It is necessary to recalculation taking into account dilution K and analyte recovery from this biological matrix R [12] for calculating the analyte content in the analysed specimen C_i .

For both variants, if matrix background absorbance A_{blank} introduces the significant contribution to the absorbance of the analysed specimen A_i , it is necessary to use the updated value $A_i - A_{blank}$ [13] in calculations.

Which problems does using the method of additions allow to solve in forensic and toxicological analysis? In both variants of its application the background absorbance conditioned by matrix is the same for both solutions to be spectrophotometric measured, that it is impossible to achieve in the method of standard. Thus, if it is necessary to update the absorbance of A_i by the value of A_{blank} we considerably decrease the total uncertainty of analysis.

Using the method of additions allows us, except quantitative determination, additionally to confirm the presence of target analyte in the sample to be investigated – by the fact of absorbance increase in the maximum of absorption without significant changes in the spectrum character (absence of new maxima, widening, shift or split of the present maxima of absorption etc.).

The advantage of using just the first variant of the method of additions consists in that it allows to level the error related to the differences in influence of biological matrix on analyte (particularly on the analyte recovery from a matrix) depending on matrix state (putrid changes, time past after death coming, thermal influence and other) and source of origin (age of patient, presence of chronic diseases and other) that, as already discussed before [12], distorts the results of analysis the most.

Nevertheless, the second variant of experiment carrying out has also a right on existence, especially in those cases, when amount of the sample to be analysed come in laboratory is insufficient for selection of two parallel samples.

Taking into account all mentioned above we suggest the procedure of linearity, accuracy and precision determination for UV-spectrophotometric methods of analytes quantitative determination in biological fluids in the variant of the method of additions, which fundamentals are given below.

RANGE

The procedure supposes application of the normalized coordinates; as 100% in the normalized coordinates we accept the mean toxic or lethal analyte concentration in biological fluid – depending on the purposes and tasks, for which the developed methods is intended. Taking into account that the range of toxic and lethal analyte concentrations in biological liquids can be wide enough, and the lower concentrations are fixed more often, than respective mean [14], and basing on the reasoning in relation to the value of minimal absorbance stated before [15], the lower limit of the range of method application corresponds to the point of 25% in the normalized coordinates.

As for the upper limit of the range of method application, it is necessary to divide the concepts «upper limit of the range of method linearity» and «upper limit of the range of method application» proper, or, as accepted in foreign literature [1-4], analyte «upper limit of quantification» (ULOQ), for the method of additions.

Application of the method of additions requires the linearity compliance in the range, which is as wide as possible, therefore, taking into account that UV-spectrophotometric methods can not provide the possibility of reliable analyte quantitative determination in the range of concentrations differed more, than in one order of magnitude [16], the upper limit of the range of method linearity can be accepted equal to 175% in the normalized coordinates. The number of concentration levels within the range of linearity is $g = 7$ in constant increments of 25%.

ULOQ, in turn, directly connected to the value of addition spiked into the sample and is equal to $X_{\max} = 175 - X_{ad}$.

Thus, in order to determine ULOQ it is necessary to ground the value of addition X_{ad} , i.e. to answer the question – which addition value is necessary and sufficient in order to provide the observance of requirements to the uncertainty of analysis results ($\Delta_{As} \leq 20.0\%$)?

As carrying out the analysis by the method of additions supposes absorbance measuring for two samples, it is possible to present the total uncertainty of analysis results Δ_{As} in the method of additions in such way [17]:

$$\Delta_{As} = \sqrt{\Delta_{Ai}^2 + \Delta_{Ai+ad}^2} \leq \max \Delta_{As} = 20.0\%, \quad (3)$$

where Δ_{Ai} and Δ_{Ai+ad} – are relative confidence intervals for absorbance of the sample to be analyse without addition and with addition respectively.

The equality $\Delta_{Ai} = \Delta_{Ai+ad}$ is accepted and we obtain:

$$\Delta_A = \Delta_{Ai} = \Delta_{Ai+ad} = \max \Delta_{As} / \sqrt{2} = 14.1\%. \quad (4)$$

Traditionally the advices on choice of the value of addition are following [18]:

- the addition should be of the same order as the initial content of the determined component in the sample;
- $X_i + X_{ad} = 2X_i$;
- $X_{Ad} > \frac{X_i \cdot \Delta_{Ai}}{100} + \frac{X_{i+ad} \cdot \Delta_{Ai+ad}}{100}$.

All mentioned advices suppose the increase of addition value when increasing the analyte content in the specimen X_p that is unacceptable in our case as X_i is unknown and can fluctuate in a wide range.

In order to ground the addition value theoretically, let us consider limit cases.

Case 1. The absorbance of the sample to be analysed without addition is determined at the upper limit of its confidence interval, and the absorbance of the sample to be analysed with addition – at the lower limit of its confidence interval; in this case it is possible to write down the inequation

$$\left(1 - \frac{\Delta_{As}}{100}\right) \cdot \frac{A_i}{A_{i+ad} - A_i} \leq \frac{A_i \cdot \left(1 + \frac{\Delta_A}{100}\right)}{A_{i+ad} \cdot \left(1 - \frac{\Delta_A}{100}\right) - A_i \cdot \left(1 + \frac{\Delta_A}{100}\right)} \leq \left(1 + \frac{\Delta_{As}}{100}\right) \cdot \frac{A_i}{A_{i+ad} - A_i} \quad (5)$$

When we substitute $\Delta_{As} = 20.0\%$ and $\Delta_A = 14.1\%$ in the expression (5), take into account (1) and apply the normalized coordinates, we obtain

$$0.8 \cdot \frac{X_i}{X_i + X_{ad} - X_i} \leq \frac{X_i \cdot 1.141}{(X_i + X_{ad}) \cdot 0.859 - X_i \cdot 1.141} \leq 1.2 \cdot \frac{X_i}{X_i + X_{ad} - X_i}; \quad (6a)$$

$$0.8 \cdot \frac{X_i}{X_{ad}} \leq \frac{X_i \cdot 1.141}{X_{ad} \cdot 0.859 - X_i \cdot 0.282} \leq 1.2 \cdot \frac{X_i}{X_{ad}} \quad (6b)$$

Case 2. The absorbance of the sample to be analysed without addition is determined at the lower limit of its confidence interval, and the absorbance of the sample to be analysed with addition – at the upper limit of its confidence interval; in this case it is possible to write down the inequation:

$$\left(1 - \frac{\Delta_{As}}{100}\right) \cdot \frac{A_i}{A_{i+ad} - A_i} \leq \frac{A_i \cdot \left(1 - \frac{\Delta_A}{100}\right)}{A_{i+ad} \cdot \left(1 + \frac{\Delta_A}{100}\right) - A_i \cdot \left(1 - \frac{\Delta_A}{100}\right)} \leq \left(1 + \frac{\Delta_{As}}{100}\right) \cdot \frac{A_i}{A_{i+ad} - A_i};$$

and realizing the transformations as for **Case 1**, we obtain

$$0.8 \cdot \frac{X_i}{X_{ad}} \leq \frac{X_i \cdot 0.859}{X_i \cdot 0.282 + X_{ad} \cdot 1.141} \leq 1.2 \cdot \frac{X_i}{X_{ad}} \quad (8)$$

The system of inequations (6b) and (8) has not any solutions if $X_i/X_{ad} > 0$ (the graphical solution is present on Figure 1a).

Thus, if $\Delta_A = 14.1\%$ the addition X_{ad} , which allows to obtain the analysis result with the uncertainty of $\Delta_{As} \leq 20.0\%$ within the limits cases, does not exist.

On Figure 2, a the graphical solution (if $X_i/X_{ad} > 0$ and $\Delta_A > 0$) of the system of inequations (5) and (7) in relation to X_i/X_{ad} (if $\Delta_{As} = 20.0\%$) is given – under these conditions the system has the solution set (the change of X_i/X_{ad} value leads to the change of requirements to Δ_A) – and although it seems impossible to match the «universal» addition, nevertheless, it is possible to determine the maximum allowed values of Δ_A for given X_i/X_{ad} , which allow even in limit cases to keep within maximum allowed $\Delta_{As} = 20.0\%$ by means of this curve.

If we write down the inequations (5) and (7) in the less close form

$$\frac{A_i \cdot \left(1 + \frac{\Delta_A}{100}\right)}{A_{i+ad} \cdot \left(1 - \frac{\Delta_A}{100}\right) - A_i \cdot \left(1 + \frac{\Delta_A}{100}\right)} \leq \left(1 + \frac{\Delta_{As}}{100}\right) \cdot \frac{A_i}{A_{i+ad} - A_i}; \quad (9)$$

$$\left(1 - \frac{\Delta_{As}}{100}\right) \cdot \frac{A_i}{A_{i+ad} - A_i} \leq \frac{A_i \cdot \left(1 - \frac{\Delta_A}{100}\right)}{A_{i+ad} \cdot \left(1 + \frac{\Delta_A}{100}\right) - A_i \cdot \left(1 - \frac{\Delta_A}{100}\right)} < 1, \quad (10)$$

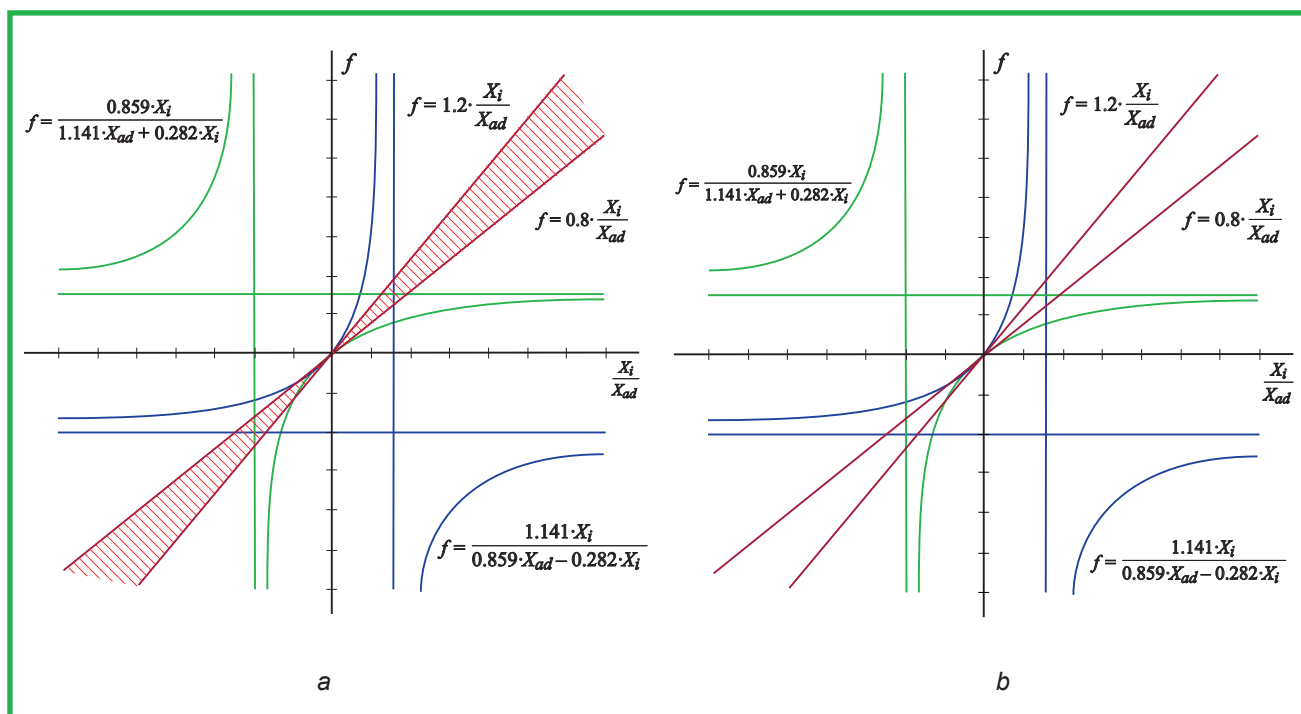


Figure 1 – Graphical solution of the system of inequations (6b) and (8)

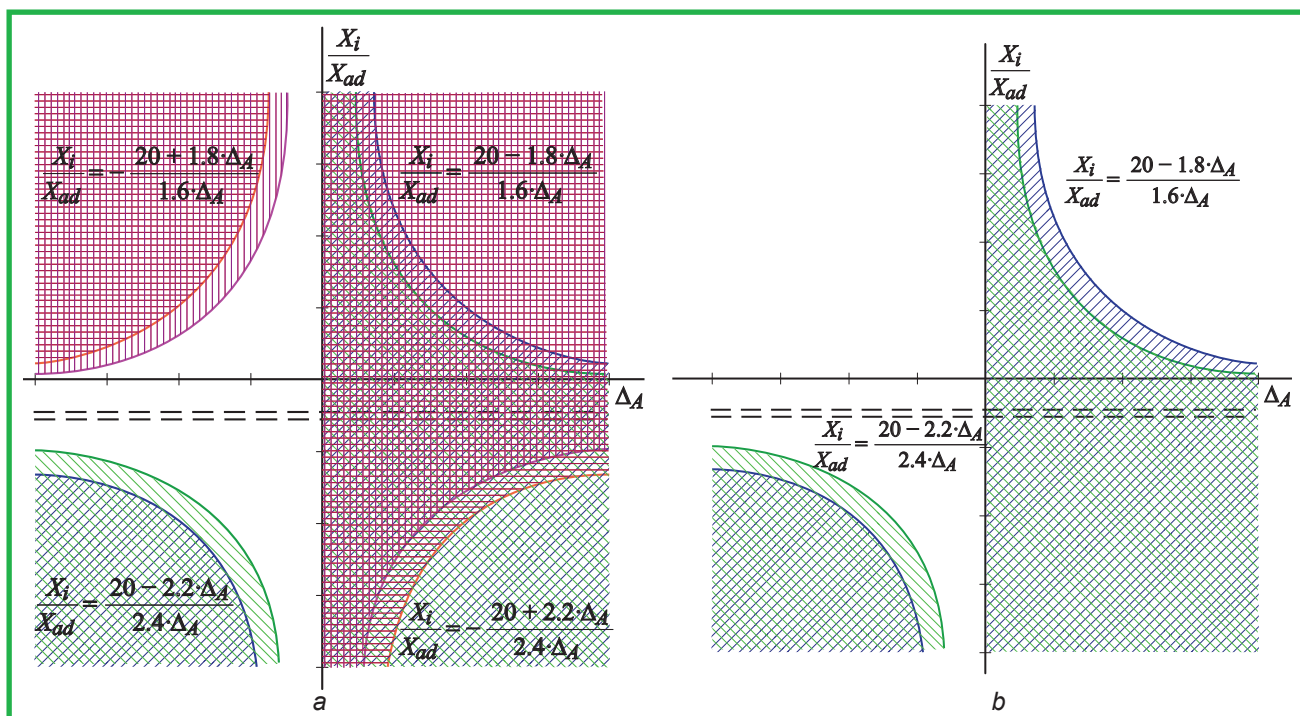


Figure 2 – Graphical solution in relation to X_i/X_{ad} of the system of inequations: a – (5) and (7); b – (9) and (10)

that system of the obtained inequations (9) and (10) has the same solution set (Figure 2b).

On Figure 3, a the graphical solution of the system of inequations (5) and (7) in relation to Δ_{As} (if $\Delta_A = 14.1\%$, $X_i/X_{ad} > 0$ and $\Delta_{As} > 0$) is given – if the relative confidence interval for absorbance is equal to 14.1%, the end result can be obtain only with the uncertainty $\Delta_{As} > 32.83\%$ if $X_i/X_{ad} \rightarrow 3.0461$; if $\Delta_{As} > 20.0\%$, the system has not any solutions.

Solving the given inequations in the light form as given above does not change the situation (Figure 3b). Writing down the inequations (6) and (8) in the similar light form does not allow to find the solution for their system (Figure 1b).

Thus, we did not succeed in theoretical way to ground the value of X_{ad} leveler all limit cases, therefore we suggest to estimate X_{ad} for the most sensible to its value X_i ,

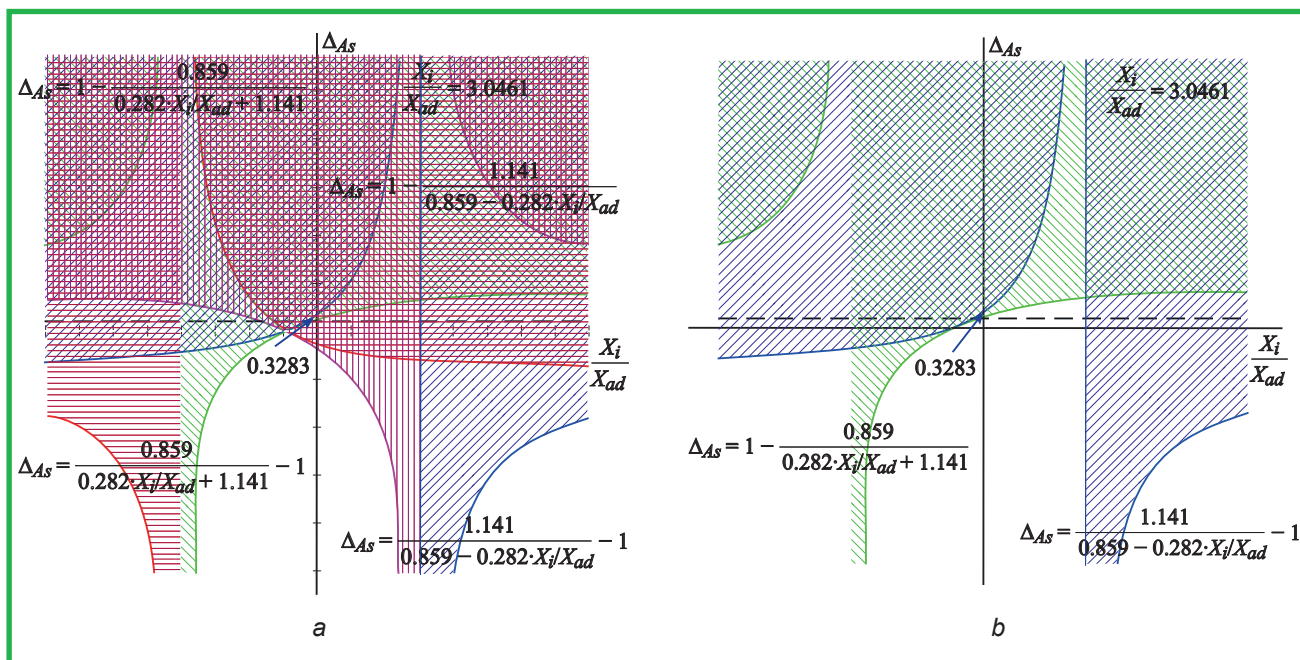


Figure 3 – Graphical solution in relation to Δ_{As} of the system of inequations: a – (5) and (7); b – (9) and (10)

– the lower limit of the range of method linearity $X_{\min} = 25\%$ and $ULOQ - X_{\max} = 175 - X_{ad}$, proceeding from the following limitations:

$$1) (X_{\min} + X_{ad}) \cdot 0.859 - X_{\min} \cdot 1.141 > (X_{\min} + X_{ad}) \cdot \frac{\Delta_A}{100} + X_{\min} \cdot \frac{\Delta_A}{100}; \quad (11)$$

$$2) (X_{\min} + X_{ad}) \cdot 1.141 - X_{\min} \cdot 0.859 < 3 \cdot (X_{\min} + X_{ad}) \cdot \frac{\Delta_A}{100} + 3 \cdot X_{\min} \cdot \frac{\Delta_A}{100}; \quad (12)$$

$$3) (X_{\max} + X_{ad}) \cdot 0.859 - X_{\max} \cdot 1.141 > (X_{\max} + X_{ad}) \cdot \frac{\Delta_A}{100} + X_{\max} \cdot \frac{\Delta_A}{100}; \quad (13)$$

$$4) (X_{\max} + X_{ad}) \cdot 1.141 - X_{\max} \cdot 0.859 < 3 \cdot (X_{\max} + X_{ad}) \cdot \frac{\Delta_A}{100} + 3 \cdot X_{\max} \cdot \frac{\Delta_A}{100}; \quad (14)$$

i.e. we consider the difference between measurands for Case 1 should be more than sum of their absolute confidence intervals, and for Case 2 – less than triple sum of their absolute confidence intervals.

Solving these inequations we obtain that for X_{\min} the value of addition should be 19%, for $X_{\max} - 78\%$.

It should be noted that taking into account advantages of the method of additions (see higher), appearance of errors with different sign in practice when carrying out two sequential experiments using the same matrix is seemed improbable, it is therefore necessary to study the possibility of using different additions – 25%, 50%, 75% and 100%, in the process of validation in experimental way and only after that to make the final decision in relation to the value of X_{ad} recommended to further application.

LINEARITY

The linearity of method in the variant of the method of additions is determined, as well as in the variant of the method of calibration curve or the method of standard, in two stages – by model solutions (without matrix) and by calibration samples respectively [6-11]. The procedure of determination proper almost completely coincides with carrying out this experiment in the variant of the method of calibration curve [6-9]. Differences touch some moments of normalization of absorbances values and acceptability criteria for linear dependence.

1. Procedure of determination. Determination of linearity by model solutions is carried out for one run, measuring the absorbance of each solution 3 times with taking out the cell; normalization of the obtained mean values of absorbance is carried out by the reference solution with the concentration of analyte corresponded to the point of 100% in the normalized coordinates.

When verifying the linearity by calibration samples their number for each concentration level is no less than three and determined by the results of calculation of $s_{nom,r}$ value, which acceptability estimation is carried out according to the following criterion:

$$s_{nom,r}(sample) \leq \max s_{nom,r} = 0,707 \cdot \max \Delta_{As} \cdot \sqrt{n}/t(95\%, n-1). \quad (15)$$

Each replicate experiment is carried out within individual run/day using the matrix samples obtained from

the same source; calculation of the parameters of linear dependence is carried out for each run (within-run (within-day) linearity) and by the mean values of replicate experiments (between-run (between-day) linearity).

For normalization of the obtained experimental data it is suggested to use the reference solution with the concentration of analyte ($C_{reference}$) corresponded to its concentration in the end solution to be spectrophotometric measured under the condition of zero losses for the point of 100% in the normalized coordinates; the absorbance of such reference solution ($A_{reference}$) is corrected by the value of recovery R obtained at the preliminary stage of validation [12] and is used for normalization of absorbance values – the expressions for the normalized coordinates have such appearance:

$$X_i = \frac{C_i}{C_{st}} \cdot 100\%, \quad Y_i = \frac{A_i}{A_{st}} \cdot 100\%; \\ C_i > C_{sample}, \quad C_{st} = C_{reference}; \quad (16) \\ A_i = A_{sample} - A_{blank}, \quad A_{st} = \frac{A_{reference} \cdot R}{100}.$$

The absorbance values of calibration samples, and also model samples used for verifying accuracy and precision, were updated by the value of A_{blank} , but only in the case when its significance were confirmed at the preliminary stage of validation [13]. Such approach is needed for decline of influence of the systematic error introduced by the components of blank-sample.

2. Acceptability criteria. For development of acceptability criteria for linear dependence by model solutions we proceed from the possibility of presentation the total uncertainty of analysis results Δ_{As} for methods of analyte quantitative determination in biological fluids by way of two components:

- the uncertainty of analyte quantitative determination in model solutions Δ_{As}^{model} ;
- the uncertainty of sample preparation procedure $\Delta_{sample\ preparation}$;

For evaluation of Δ_{As}^{model} value we suggest to proceed from insignificance of the uncertainty of analyte quantitative determination in model solutions Δ_{As}^{model} in comparison with the complete uncertainty of analysis results Δ_{As} , i.e.:

$$\Delta_{As}^{model} \leq \max \Delta_{As}^{model} = 0.32 \cdot \max \Delta_{As} = 0.32 \cdot 20.0\% = 6.40\%, \quad (17)$$

the requirements to the systematic error:

$$\delta^{model} \leq \max \delta^{model} = 0.32 \cdot \max \Delta_{As}^{model} = 0.32 \cdot 6.40\% = 2.05\%. \quad (18)$$

According to [17]:

$$\Delta_{As}^{model} \leq t(95\%, g-2) \cdot RSD_0^{model}, \quad (19)$$

hence it is possible to obtain the requirements to RSD_0^{model} :

$$RSD_0^{model} \leq \max RSD_0^{model} = \frac{0.32 \cdot \max \Delta_{As}}{t(95\%, g-2)} = \frac{0.32 \cdot 20.0}{2.0150} = 3.18\%. \quad (20)$$

Knowing the range of method application we may calculate RSD_{range} [17]:

$$RSD_{range} = \sqrt{\frac{\sum_{i=1}^g (X_i - \bar{X})^2}{g-1}} = 54.01\%, \quad (21)$$

and, when we substitute obtained value of RSD_{range} and RSD_0^{model} into formula [17]:

$$R_c^{model} = \sqrt{1 - \frac{(RSD_0^{model})^2}{RSD_{range}^2}} = 0.9983, \quad (22)$$

we obtain the requirements to the value of correlation coefficient R_c^{model} .

According to [17], the segment cut off from y-axis (absolute term a) characterizes the systematic error when analysis by the method of additions, and requirements to it are built on two levels:

- statistically insignificant difference from zero:

$$a^{model} \leq t(95\%, g-2) \cdot s_a^{model}, \quad (23)$$

- practically insignificant difference from zero – if proceed from that

$$Y_i = a + b \cdot X_i; \quad Y_{i+ad} = a + b \cdot (X_i + X_{ad}), \quad (24)$$

then it is possible to present the systematic error δ_a , introduced by absolute term in calculation of analyte content in the sample to be analysed, in following way (taking into account the closeness of slope b to one in the normalized coordinates and smallness of absolute term a):

$$\delta_a, \% = 100 \cdot \left(\frac{a + b \cdot X_i}{a + b \cdot (X_i + X_{ad})} - \frac{X_i}{X_i + X_{ad}} \right). \quad (25)$$

$$\frac{X_i + X_{ad}}{X_i} = \frac{a \cdot X_{ad} \cdot 100}{X_i \cdot (X_i + X_{ad})} \leq \max \delta.$$

It is obvious from the ratio (25) that δ_a decreases if the analyte concentration in the sample to be analysed X_i increased, but increases if the value of addition X_{ad} increases.

Let us solve the equation (25) in graphical form (Figure 4):

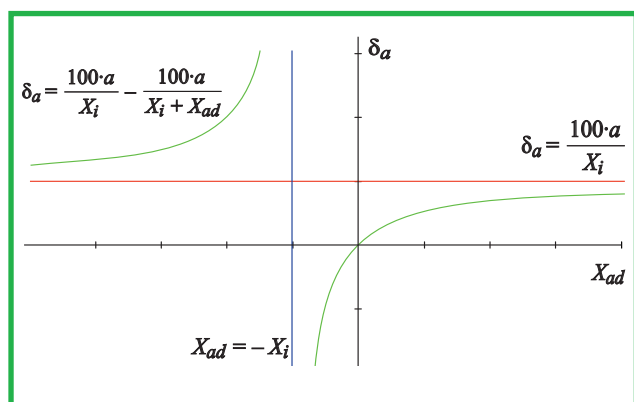


Figure 4 – Graphical solution of the equation (25)

Thus

$$\delta_a \rightarrow \frac{100 \cdot a}{X_i} \leq \max \delta. \quad (26)$$

We may come to the same solution from the expression

$$\delta_a, \% = \frac{100 \cdot \left(\frac{(a + b \cdot X_i) \cdot X_{ad}}{a + b \cdot (X_i + X_{ad})} - \frac{X_i \cdot X_{ad}}{(X_i + X_{ad}) - X_i} \right)}{\frac{X_i \cdot X_{ad}}{(X_i + X_{ad}) - X_i}} = \quad (27)$$

$$\frac{100 \cdot a}{X_i} \leq \max \delta.$$

δ_a reaches the maximum value if $X_i = X_{min}$.

Thus

$$a^{model} \leq \max a^{model} = \frac{0.32 \cdot \max \Delta_{As}^{model} \cdot X_{min}}{100} = \frac{0.32 \cdot 6.40 \cdot 25}{100} = 0.51\%. \quad (28)$$

Similarly we calculate the acceptability criteria for the linear dependence parameters obtained by calibration samples:

$$RSD_0 \leq \max RSD_0 = \frac{\max \Delta_{As}}{t(95\%, g-2)} = \frac{20.0}{2.0150} = 9.93\%; \quad (29)$$

$$R_c = \sqrt{1 - \frac{RSD_0^2}{RSD_{range}^2}} = 0.9830; \quad (30)$$

$$a \leq t(95\%, g-2) \cdot s_a; \quad (31)$$

$$a \leq \max a = \frac{0.32 \cdot \max \Delta_{As} \cdot X_{min}}{100} = \quad (32)$$

$$\frac{0.32 \cdot 20.0 \cdot 25}{100} = 1.60\%.$$

ACCURACY AND PRECISION

Determination of accuracy and precision for methods at the first stage is carried out using the batch of model solution ($n = 6$):

- for $X_{ad} = 25\% - 25\%, 50\%, 75\%, 100\%, 125\%, 150\%$;
- for $X_{ad} = 50\% - 25\%, 25\%, 50\%, 75\%, 100\%, 125\%$;
- for $X_{ad} = 75\% - 25\%, 25\%, 50\%, 75\%, 100\%, 100\%$;
- for $X_{ad} = 100\% - 25\%, 25\%, 50\%, 50\%, 75\%, 75\%$;

each solution is analysed twice – without and with spiking the addition respectively. Based on the data obtained we calculate $X_{calc}^{model}, \%$ and $RR^{model}, \%$:

$$X_{calc}^{model}, \% = X_{ad} \cdot \frac{A_i^{model}}{A_{i+ad}^{model} - A_i^{model}}; \quad (33)$$

$$RR^{model}, \% = \frac{X_{calc}^{model}}{X_{fact}^{model}} \cdot 100. \quad (34)$$

The obtained values are used for calculation of

$\overline{RR}^{model}, \%, \delta^{model}, \%$ and $\Delta_{As}^{model}, \%$:

$$\delta^{model}, \% = \left| 100 - \overline{RR}^{model} \right| \leq \max \delta^{model} = \quad (35)$$

$$0.32 \cdot \max \Delta_{As}^{model} = 0.32 \cdot 6.40\% = 2.05\%;$$

$$\Delta_{RR}^{model}, \% = \Delta_{AS}^{model} = t(95\%, n-1) \cdot RSD_{RR}^{model} \leq \max \Delta_{AS}^{model} = 6.40\%.$$

At the second stage accuracy and repeatability of methods are determined by the model samples prepared using appropriate matrix.

With this purpose we suggest to carry out researches for three parallel runs, each run consists of 6 (the concentrations see higher) samples of biological matrix obtained from the same source and spiked with analyte, i. e. for analysis of each run the individual source of biological matrix is used. Each sample is analysed twice – without and with spiking the addition respectively – and using the first and the second variant of experiment carrying out (see higher). Based on the data obtained we calculate $X_{calc}, \%$ и $RR, \%$:

$$X_{calc}, \% = X_{ad} \cdot \frac{A_i}{A_{i+ad} - A_i};$$

$$X_{calc}, \% = X_{ad} \cdot \frac{A_i}{A_{i+ad} - A_i} \cdot \frac{K \cdot 100}{R}$$

$$RR, \% = \frac{X_{calc}}{X_{fact}} \cdot 100.$$

The obtained values are used for calculation of $\overline{RR}, \%, \delta, \%$ and $\Delta_{AS}, \%$:

$$\delta, \% = |100 - \overline{RR}| \leq \max \delta = 0.32 \cdot \max \Delta_{AS} = 0.32 \cdot 20.0\% = 6.40\%;$$

$$\Delta_{RR}, \% = \Delta_{AS} = t(95\%, n-1) \cdot RSD_{RR} \leq \max \Delta_{AS} = 20.0\%$$

For verification of intermediate (between-run) precision the pooled mean value \overline{RR}^{intra} , pooled relative standard deviation $RSD_{RR}^{intra}, \%$ and relative confidence interval are calculated for three runs obtained when repeatability verifying [19]. The value Δ_{RR}^{intra} should not exceed the extreme uncertainty of analysis $\max \Delta_{AS}$:

$$\Delta_{RR}^{intra} = t(95\%, 3n-1) \cdot RSD_{RR}^{intra} \leq \max \Delta_{AS}.$$

CONCLUSIONS

Thus, we have offered and grounded the procedure of linearity, accuracy and precision determination and acceptability estimation for validation of UV-spectrophotometric methods of analytes quantitative determination in biological fluids used in forensic and toxicological analysis in the variant of the method of additions.

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СОТТЫҚ-ТОКСИКОЛОГИЯЛЫҚ ТАЛДАУ КЕЗІНДЕ САНДЫҚ АНЫҚТАУДАҒЫ УФ- СПЕКТРОФОТОМЕТРИЯЛЫҚ ӘДІСТЕРІНІҢ СЫЗЫҚТЫЛЫҒЫН, ДҰРЫСТЫҒЫН ЖӘНЕ ПРЕЦИЗИОНДЫЛЫҒЫН ҚОСУ ТӘСІЛІМЕН АНЫҚТАУ

Қосу тәсілімен соттық-токсикологиялық талдау кезінде қолданылатын биологиялық сұйықтықтағы аналиттерді сандық анықтаудағы уф-спектрофотометриялық әдістерін валидациялауда сызықтылық, дұрыстық және прецизиондылық тиімділігін анықтау және бағалау тәсілі ұсынылды.

Түйін сөздер: сызықтылық, дұрыстық, прецизиондылық, уф-спектрофотометриялық әдістер, соттық-токсикологиялық талдау, сандық анықтау.

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ОПРЕДЕЛЕНИЕ ЛИНЕЙНОСТИ, ПРАВИЛЬНОСТИ И ПРЕЦИЗИОННОСТИ УФ-СПЕКТРОФОТОМЕТРИЧЕСКИХ МЕТОДИК КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ В СУДЕБНО- ТОКСИКОЛОГИЧЕСКОМ АНАЛИЗЕ В ВАРИАНТЕ МЕТОДА ДОБАВОК

Предложена процедура определения и оценки приемлемости линейности, правильности и прецизионности для валидации УФ-спектрофотометрических методик количественного определения аналитов в биологических жидкостях, применяемых в судебно-токсикологическом анализе, в варианте метода добавок.

Ключевые слова: линейность, правильность, прецизионность, УФ-спектрофотометрические методики, судебно-токсикологический анализ, количественное определение. ■

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БЕЗОПАСНОСТЬ ЛЕКАРСТВ

Риск при лечении пневмонии дорипенемом

FDA сообщает, что применение дорипенема (антибактериальное средство из группы карбапенемов) для лечения пневмонии, связанное с использованием искусственной вентиляции легких (ИВЛ), имеет повышенный риск смертности и меньшую клиническую эффективность по сравнению с применением комбинации «имипенем+циластатин». На основе анализа данных трехлетнего клинического исследования, которое было преждевременно остановлено в 2011 г. в связи с проблемами безопасности, FDA одобрило изменения в инструкции дорипенема, сообщающие об этих рисках. Пересмотренная инструкция включает в себя предупреждение о том, что в настоящее время дорипенем не одобрен для лечения пневмонии любого типа.

В клиническом исследовании, которое было приостановлено, пациенты с ИВЛ-ассоциированной бактериальной пневмонией получали либо 7-дневное лечение дорипенемом, либо 10-дневное лечение имипенемом/циластатином. По результатам оценки смертность от всех причин была выше в группе больных, получавших дорипенем (23%; n=31/135), чем в группе, получавшей имипенем+циластатин (16,7% n=22/132).

FDA сообщает, что дорипенем по-прежнему считается безопасным и эффективным для применения по другим утвержденным FDA показаниям: лечение осложненных интраабдоминальных инфекций и осложненных инфекций мочевыводящих путей, включая инфекции почек (пиелонефрит).

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