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DETERMINATION OF VALIDATION CHARACTERISTICS OF THE UV-SPECTROPHOTOMETRIC METHOD OF DOXYLAMINE QUANTITATIVE DETERMINATION IN BLOOD IN THE VARIANT OF THE METHOD OF STANDARD

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With the purpose of improvement of carrying out the quantitative determinations in forensic and toxicological analysis the possibility of application of the method of standard for UV-spectrophotometric determination of analytes in biological fluids has been studied. The procedure for determination and acceptability estimation of linearity, accuracy and precision for validation of such methods in the variant of the method of standard tested by the example of UV-spectrophotometric method of doxylamine quantitative determination in blood has been offered. The given procedure provides application of the normalized coordinates. For normalization of the experimental data obtained two approaches have been used: 1) Approach 1: the use of the reference solution with the concentration of the analyte corresponding to its concentration in the final spectrophotometric solution measured under the condition of zero losses for the point of 100% in the normalized coordinates. 2) Approach 2: the use of the reference sample with the concentration of doxylamine corresponding to its concentration for the point of 100% in the normalized coordinates. Estimation of linearity, accuracy and repeatability of the method at the first stage has been performed using the model solutions within the approach based on assumption of insignificance of the uncertainty of the analyte quantitative determination in model solutions Δ_{As}^{model} *in comparison with the complete uncertainty of the analysis results* Δ_{As}*. At the second stage determination of linearity, accuracy, repeatability and intermediate precision of the method has been carried out on the model samples prepared using the matrix for three parallel runs. It has been shown that the method of standard can be applied for UV-spectrophotometric determination of doxylamine in blood – Approach 1 is more acceptable.*

Currently there is a number of international regulations given directive recommendations on carrying out the validation of bioanalytical methods – "Guidance for Industry: Bioanalytical method validation" (U.S. FDA, 2001) [5], "Guideline on validation of bioanalytical methods" (EMA, 2011) [7], "Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens" (UNODC, 2009) [6] and "Standard Practices for Method Validation in Forensic Toxicology" (SWGTOX, 2012) [8].

The papers mentioned are oriented to development of methods for quantitative determination of analytes in biological fluids in the variant of the method of the calibration curve. The method of the calibration curve, undoubtedly, allows to take into account and partially level the influence of the matrix background absorption on the results of determination, but proves its value only in the case of performing routine analyses. In forensic and toxicological analysis we often meet with one-time examinations, and various biological fluids, organs and tissues are sent for the examinations, i.e. it is necessary to determine the analyte quantitatively in several various biological objects, but the necessity of carrying out such determinations can arise rarely enough. In such situation plotting the calibration curve for each matrix demands rather nonrational time consumption, and to the moment of obtaining the results of analysis they can become irrelevant.

Thus, it is of interest to study the possibility of using the method of standard when carrying out UV-spectrophotometric determination of analytes in biological fluids; in this connection the procedure for determination and acceptability estimation of linearity, accuracy and precision for validation of such methods in the variant of the method of standard [3] developed according to [1] has been offered. The results of testing of the approaches offered by the example of the UV-spectrophotometric method of doxylamine quantitative determination in blood are given in this paper [2].

Materials and Methods

The process solutions: place 1000.0 mg of doxylamine succinate in a 250.0 ml volumetric flask, dissolve in distilled water and dilute to the volume with the same solvent (standard solution 1, the concentration is 4000 mcg/ml). In seven 100.0 ml volumetric flasks place 32.50; 30.00; 25.00; 20.00; 15.00; 10.00; 5.00 and 18.00 ml of doxylamine succinate standard solution 1, respectively, using a burette and dilute to the volume with distilled water (process solutions 1, 2, 3, 4, 5, 6, 7 and 8, respectively, with the concentrations of 1300, 1200, 1000, 800, 600, 400, 200 and 720 mcg/ml, respectively).

Table 1

Metrological characteristics of calibration straight lines $Y = b \cdot X + a$ obtained using model solutions of doxylamine succinate

Analytical range of the method	Characteristic						
	b^{model}	s_b^{model}	a^{model}		s_a^{model}	RSD_0^{model}	R_c^{model}
D = 25 – 125% (g = 5)	1.005	0.011	–0.407		1.022	0.974	0.9998
Acceptability criterion	–	–	1) $a^{model} \le 2.353 \cdot s_a^{model}$	satisfied	–	≤ 2.72%	≥ 0.9976
			if it is not satisfied 1), then 2) ≤ 2.73%	satisfied		satisfied	satisfied
D = 25 – 150% (g = 6)	1.011	0.008	–0.805		0.874	0.939	0.99987
Acceptability criterion	–	–	1) $a^{model} \le 2.132 \cdot s_a^{model}$	satisfied	–	≤ 3.00%	≥ 0.9979
			if it is not satisfied 1), then 2) ≤ 2.73%	satisfied		satisfied	satisfied
D = 25 – 175% (g = 7)	0.995	0.012	0.321		1.498	1.724	0.9996
Acceptability criterion	–	–	1) $a^{model} \le 2.015 \cdot s_a^{model}$	satisfied	–	≤ 3.18%	≥ 0.9983
			if it is not satisfied 1), then 2) ≤ 2.73%	satisfied		satisfied	satisfied

The model solutions: place 100.0 mg of doxylamine succinate in a 500.0 ml volumetric flask, dissolve in 0.1 mole/l of hydrochloric acid solution and dilute to the volume with the same solvent (standard solution 2, the concentration is 200 mcg/ml). In seven 100.0 ml volumetric flasks place 26.00; 24.00; 20.00; 16.00; 12.00; 8.00 and 4.00 ml of the doxylamine succinate standard solution 2, respectively, using a burette and dilute to the volume with 0.1 mole/l of hydrochloric acid solution (model solutions 1, 2, 3, 4, 5, 6 and 7, respectively, with the concentrations of 52, 48, 40, 32, 24, 16 and 8 mcg/ml, respectively).

The reference solution: place 400.0 mg of doxylamine succinate in a 100.0 ml volumetric flask, dissolve in the 0.1 mole/l of hydrochloric acid solution and dilute to the volume with the same solvent (standard solution 3, the concentration is 4000 mcg/ml). In a 100.0 ml volumetric flask place 18.00 ml of doxylamine succinate standard solution 3 using a burette and dilute to the volume with 0.1 mole/l of hydrochloric acid solution (standard solution 4, the concentration is 720 mcg/ml). In a 50.0 ml volumetric flask place 2.00 ml of doxylamine succinate standard solution 4 and dilute to the volume with 0.1 mole/l of hydrochloric acid solution (the reference solution, the concentration is 28.8 mcg/ml).

The model samples: 3 lines in 7 samples (20.00 ml) of the model blood (matrix) obtained from three different sources spiked with 1.00 ml of the process solutions 1-7, respectively.

The reference sample: a sample (20.00 ml) of the model blood spiked with 1.00 ml of the process solutions 8.

The solutions to be analysed: the solutions obtained by the validated method [2] for the model and reference samples.

The absorbance of the solutions to be analysed, model solutions and the reference solution was measured 3 times with taking out the cell at the wavelength of 262 nm by a SF-46 spectrophotometer in the cell with the layer thickness of 10 mm. As a compensation solution 0.1 mole/l hydrochloric acid solution was used.

Results and Discussion

Determination of validation characteristics of the UV-spectrophotometric method of doxylamine quantitative determination in blood was carried out according to the following procedure [3]:

1) the use of the normalized coordinates:

$$X_i = \frac{C_i}{C_{st}} \cdot 100\%,\ Y_i = \frac{A_i}{A_{st}} \cdot 100\%,\ Z_i = \frac{Y_i}{X_i} \cdot 100\%;$$
$$C_i = C_{sample},\quad A_i = A_{sample} - A_{blank}, \qquad (1)$$

for normalization of the experimental data obtained two approaches were used:

- *Approach 1:* the use of the reference solution with the concentration of doxylamine ($C_{reference}$) corresponding to its concentration in the final spectrophotometric solution measured under the condition of zero losses for the point of 100% in the normalized coordinates:

$$C_{st} = C_{reference},\quad A_{st} = \frac{A_{reference} \cdot R}{100}; \qquad (2)$$

- *Approach 2:* the use of the reference sample with the concentration of doxylamine ($C_{reference\ sample}$) corresponding to its concentration for the point of 100% in the normalized coordinates:

$$C_{st} = C_{reference\ sample},\quad A_{st} = A_{reference\ sample} - A_{blank}; \qquad (3)$$

2) the application range is 25 – 125%, 25 – 150%, 25 – 175%; the mean lethal doxylamine concentration in blood [4] – 25 mg/l (that corresponds to 36 mg/l of doxylamine succinate) was accepted as 100%; the number of concentration levels is g = 5, 6 or 7 (depending on the application range chosen) in constant increments of 25%;

3) estimation of linearity, accuracy and repeatability of the method at the first stage was performed using the model solutions within the approach based on assump-

Table 2

Results of accuracy and repeatability determination for the UV-spectrophotometric method of doxylamine succinate quantitative determination by model solutions

Concentration of doxylamine succinate in the model solution (C_{st} = 28.8 mcg/ml)		Absorbance (A_{st} = 0.801)	Found in % to standard absorbance Y_i^{model}, %	$Z_i^{model} = \frac{Y_i^{model}}{X_i^{model}} \cdot 100\%$
C_i^{model}, mcg/ml	X_i^{model}, %			
8.00	27.78	0.226	28.21	101.55
16.00	55.56	0.444	55.43	99.77
24.00	83.33	0.657	82.02	98.43
32.00	111.11	0.890	111.11	100.00
40.00	138.89	1.121	139.95	100.76
48.00	166.67	1.348	168.29	100.97
52.00	180.56	1.421	177.40	98.25
		D = 25 – 175% (*g* = 7)	*D* = 25 – 150% (*g* = 6)	*D* = 25 – 125% (*g* = 5)
$\overline{Z}^{model}$, %		99.96	100.25	100.10
RSD_Z^{model},%		1.26	1.10	1.17
$\Delta_{As}^{model},\% = \Delta_Z^{model} = RSD_Z^{model} \cdot t(95\%, g-1) \le 6.40\%$		2.45	2.22	2.49
		satisfied	satisfied	satisfied
$\delta^{model} = \left\|100 - \overline{Z}^{model}\right\| \le 2.05\%$		0.04	0.25	0.10
		satisfied	satisfied	satisfied

Table 3

Metrological characteristics of calibration straight lines $Y = b \cdot X + a$ for the UV-spectrophotometric method of doxylamine quantitative determination in blood without preliminary TLC-purification

Characteristic		*Approach 1*			*Approach 2*		
		25 – 125% (*g* = 5)	25 – 150% (*g* = 6)	25 – 175% (*g* = 7)	25 – 125% (*g* = 5)	25 – 150% (*g* = 6)	25 – 175% (*g* = 7)
b	1st day	0.975	1.009	0.993	1.007	1.042	1,026
	2nd day	0.991	1.023	1.007	1.024	1.056	1.041
	3d day	0.987	1.021	1.005	1.019	1.055	1.038
	mean	0.985	1.017	1.002	1.017	1.051	1.035
s_b	1st day	0.038	0.032	0.026	0.039	0.033	0.027
	2nd day	0.040	0.032	0.026	0.041	0.033	0.027
	3d day	0.045	0.036	0.028	0.047	0.037	0.029
	mean	0.041	0.033	0.026	0.042	0.034	0.027
a	1st day	4.956	2.729	3.825	5.127	2.822	3.954
	2nd day	–0.243	–2.266	–1.193	–0.256	–2.345	–1.234
	3d day	1.657	–0.560	0.534	1.709	–0.582	0.548
	mean	2.080	–0.042	1.028	2.152	–0.039	1.064
Acceptability criterion		$a \le t(95\%, g-2) \cdot s_a$ if it is not satisfied 1), then 2) ≤ 8.53%					
		satisfied	satisfied	satisfied	satisfied	satisfied	satisfied
s_a	1st day	3.512	3.449	3.149	3.622	3.561	3.251
	2nd day	3.685	3.439	3.130	3.813	3.556	3.237
	3d day	4.159	3.845	3.453	4.296	3.972	3.567
	mean	3.766	3.544	3.210	3.895	3.664	3.316
RSD_0	1st day	3.349	3.705	3.624	3.453	3.826	3.742
	2nd day	3.514	3.694	3.602	3.636	3.819	3.725
	3d day	3.966	4.131	3.974	4.096	4.267	4.105
	mean	3.591	3.807	3.694	3.714	3.935	3.817
Acceptability criterion		≤ 8,50%	≤ 9.38%	≤ 9.93%	≤ 8.50%	≤ 9.38%	≤ 9.93%
		satisfied	satisfied	satisfied	satisfied	satisfied	satisfied
R_c	1st day	0.9977	0.9980	0.9983	0.9977	0.9980	0.9983
	2nd day	0.9976	0.9981	0.9984	0.9976	0.9981	0.9984
	3d day	0.9969	0.9976	0.9980	0.9969	0.9976	0.9980
	mean	0.9974	0.9979	0.9983	0.9974	0.9979	0.9983
Acceptability criterion		≥ 0.9766	≥ 0.9797	≥ 0.9830	≥ 0.9766	≥ 0.9797	≥ 0.9830
		satisfied	satisfied	satisfied	satisfied	satisfied	satisfied

Table 4

Results of accuracy and repeatability determination for UV-spectrophotometric method of doxylamine quantitative determination in blood without preliminary TLC-purification

Concentration of doxylamine succinate in blood (C_{st} = 36 mcg/ml)		Absorbance A_i (A_{blank} = 0.060)			Approach 1						Approach 2					
					found in % to standard absorbance Y_i, % ($A_{st} = \frac{A_{reference} \cdot R}{100} = 0.532$)			$Z_i = \frac{Y_i}{X_i} \cdot 100\%$			found in % to standard absorbance Y_i, % ($A_{st} = A_{reference\ sample} - A_{blank} = 0.515$)			$Z_i = \frac{Y_i}{X_i} \cdot 100\%$		
C_i, mcg/ml	X_i, %	1st day	2nd day	3d day	1st day	2nd day	3d day	1st day	2nd day	3d day	1st day	2nd day	3d day	1st day	2nd day	3d day
10.00	27.78	0.222	0.195	0.205	30.45	25.38	27.26	109.61	91.36	98.13	31.46	26.21	28.16	113.25	94.35	101.37
20.00	55.56	0.368	0.347	0.352	57.89	53.95	54.89	104.19	97.10	98.79	59.81	55.73	56.70	107.65	100.31	102.05
30.00	83.33	0.545	0.526	0.537	91.17	87.59	89.66	109.41	105.11	107.60	94.17	90.49	92.62	113.01	108.59	111.15
40.00	111.11	0.662	0.644	0.654	113.16	109.77	111.65	101.85	98.79	100.49	116.89	113.40	115.34	105.20	102.06	103.81
50.00	138.89	0.795	0.779	0.783	138.16	135.15	135.90	99.47	97.31	97.85	142.72	139.61	140.39	102.76	100.52	101.08
60.00	166.67	0.986	0.970	0.979	174.06	171.05	172.74	104.43	102.63	103.64	179.81	176.70	178.45	107.88	106.02	107.07
65.00	180.56	1.021	1.008	1.015	180.64	178.20	179.51	100.04	98.69	99.42	186.60	184.08	185.44	103.35	101.95	102.70
D = 25 – 175% (g = 7)					$\overline{Z}$, %			104.14	98.71	100.85	$\overline{Z}$, %			107.59	101.97	104.18
					RSD_Z, %			4.12	4.38	3.56	RSD_Z, %			4.25	4.53	3.68
					$\Delta_{As}, \% = \Delta_Z = RSD_Z \cdot 1.943 \le 20\%$			8.01	8.51	6.92	$\Delta_{As}, \% = \Delta_Z = RSD_Z \cdot 1.943 \le 20\%$			8.26	8.80	7.15
								satisfied	satisfied	satisfied				satisfied	satisfied	satisfied
					$\delta = \lvert 100 - \overline{Z} \rvert \le 6.40\%$			4.14	1.29	0.85	$\delta = \lvert 100 - \overline{Z} \rvert \le 6.40\%$			7.59	1.97	4.18
								satisfied	satisfied	satisfied				no	satisfied	satisfied
D = 25 – 150% (g = 6)					$\overline{Z}$, %			104.83	98.72	101.08	$\overline{Z}$, %			108.29	101.98	104.42
					RSD_Z, %			4.05	4.79	3.84	RSD_Z, %			4.19	4.96	3.97
					$\Delta_{As}, \% = \Delta_Z = RSD_Z \cdot 2.015 \le 20\%$			8.16	9.65	7.74	$\Delta_{As}, \% = \Delta_Z = RSD_Z \cdot 2.015 \le 20\%$			8.44	9.99	8.00
								satisfied	satisfied	satisfied				satisfied	satisfied	satisfied
					$\delta = \lvert 100 - \overline{Z} \rvert \le 6.40\%$			4.83	1.28	1.08	$\delta = \lvert 100 - \overline{Z} \rvert \le 6.40\%$			8.29	1.98	4.42
								satisfied	satisfied	satisfied				no	satisfied	satisfied
D = 25 – 125% (g = 5)					$\overline{Z}$, %			104.91	97.93	100.57	$\overline{Z}$, %			108.37	101.17	103.89
					RSD_Z, %			4.52	4.91	4.06	RSD_Z, %			4.67	5.09	4.19
					$\Delta_{As}, \% = \Delta_Z = RSD_Z \cdot 2.132 \le 20\%$			9.64	10.47	8.66	$\Delta_{As}, \% = \Delta_Z = RSD_Z \cdot 2.132 \le 20\%$			9.96	10.85	8.93
								satisfied	satisfied	satisfied				satisfied	satisfied	satisfied
					$\delta = \lvert 100 - \overline{Z} \rvert \le 6.40\%$			4.91	2.07	0.57	$\delta = \lvert 100 - \overline{Z} \rvert \le 6.40\%$			8.37	1.17	3.89
								satisfied	satisfied	satisfied				no	satisfied	satisfied

Table 5

Results of intermediate precision determination of the UV-spectrophotometric method of doxylamine quantitative determination in blood without preliminary TLC-purification

Characteristic	*Approach 1*			*Approach 2*		
	25 – 125% ($g = 5$)	25 – 150% ($g = 6$)	25 – 175% ($g = 7$)	25 – 125% ($g = 5$)	25 – 150% ($g = 6$)	25 – 175% ($g = 7$)
$\overline{Z}^{intra}$, %	101.14	101.54	101.23	104.48	104.90	104.58
RSD_Z^{intra},%	4.51	4.25	4.03	4.67	4.39	4.17
$\Delta_Z^{intra},\% = t(95\%, 3g-1) \cdot RSD_Z^{intra} \le 20\%$	7.94	7.39	6.95	8.23	7.64	7.19
	satisfied	satisfied	satisfied	satisfied	satisfied	satisfied

tion of insignificance of the uncertainty of the analyte quantitative determination in model solutions Δ_{As}^{model} in comparison with the complete uncertainty of the analysis results Δ_{As}, according to the acceptability criteria calculated for the residual standard deviation RSD_0^{model}, absolute term a^{model} and correlation coefficient R_c^{model} for the variants of the method application range offered, as well as to the requirements to accuracy and repeatability ($\delta^{model} \le 2.05\%$ and $\Delta_{As}^{model} \le 6.40\%$);

4) at the second stage determination of linearity, accuracy, repeatability and intermediate precision of the method was performed on the model samples prepared using the matrix for three parallel runs; within each run the values of the linearity parameters, values δ and Δ_Z were determined and compared with the calculated critical values – maxRSD_0, max$a$, minR_c, maxδ and maxΔ_Z;

for verification of intermediate precision the pooled mean value $\overline{Z}^{intra}$, the pooled relative standard deviation RSD_Z^{intra},% and the relative confidence interval Δ_Z^{intra},% were calculated for three runs obtained; the value Δ_Z^{intra},% should not exceed the extreme uncertainty of analysis maxΔ_{As}:

$$\Delta_Z^{intra} = t(95\%, 3g-1) \cdot RSD_Z^{intra} \le \max \Delta_{As}. \quad (4)$$

The results of linearity, accuracy and repeatability determination of the UV-spectrophotometric method of doxylamine quantitative determination using the model solutions are given in Tab. 1 and 2.

The results of linearity, accuracy, repeatability and intermediate precision determination of the UV-spectrophotometric method of doxylamine quantitative determination using the model samples are given in Tab. 3, 4 and 5.

The data from Tab. 1-5 are the evidence that the method of standard can be applied for UV-spectrophotometric determination of doxylamine in blood; thus, the requirements offered to the validation parameters are completely performed only in the case of using *Approach 1*.

CONCLUSIONS

The procedure of determination and acceptability estimation of linearity, accuracy and precision offered previously [3] for validation of UV-spectrophotometric methods of quantitative determination of analytes in biological fluids used in forensic and toxicological analysis in the variant of the method of standard has been tested by the example of the UV-spectrophotometric method of doxylamine quantitative determination in blood. It has been shown that *Approach 1* based on the use of the reference sample with the concentration of doxylamine corresponding to its concentration for the point of 100% in the normalized coordinates for normalization of absorbance values is more acceptable.

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ВИЗНАЧЕННЯ ВАЛІДАЦІЙНИХ ХАРАКТЕРИСТИК УФ-СПЕКТРОФОТОМЕТРИЧНОЇ МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ ДОКСИЛАМІНУ В КРОВІ У ВАРІАНТІ МЕТОДУ СТАНДАРТУ

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Ключові слова: *валідація; біоаналітичні методики; УФ-спектрофотометрія; доксиламін; метод стандарту*

З метою раціоналізації проведення кількісних визначень в судово-токсикологічному аналізі вивчена можливість використання методу стандарту при проведенні УФ-спектрофотометричного визначення аналітів у біологічних рідинах. Запропоновано процедуру визначення та оцінки прийнятності лінійності, правильності та прецизійності для валідації таких методик у варіанті методу стандарту, що апробовано на прикладі УФ-спектрофотометричної методики кількісного визначення доксиламіну в крові. Запропонована процедура передбачає використання нормалізованих координат – для нормалізації отриманих експериментальних даних використано два підходи. 1) Підхід 1: використання розчину порівняння з концентрацією аналіту, що відповідає його концентрації в кінцевому спектрофотометрованому розчині за умови нульових втрат для точки 100% в нормалізованих координатах. 2) Підхід 2: використання зразка порівняння з концентрацією аналіту, що відповідає його концентрації для точки 100% в нормалізованих координатах. Оцінку лінійності, правильності і збіжності методики на першому етапі виконували з використанням модельних розчинів у рамках підходу, що ґрунтується на припущенні незначущості невизначеності кількісного визначення аналіту в модельних розчинах Δ_{As}^{model} у порівнянні з повною невизначеністю результатів аналізу Δ_{As}. На другому етапі проводили визначення лінійності, правильності, збіжності і внутрішньолабораторної прецизійності методики на модельних зразках, приготованих з використанням матриці, для трьох паралельних послідовностей. Показано, що для УФ-спектрофотометричного визначення доксиламіну в крові можна застосовувати метод стандарту – при цьому більш прийнятним є Підхід 1.

ОПРЕДЕЛЕНИЕ ВАЛИДАЦИОННЫХ ХАРАКТЕРИСТИК УФ-СПЕКТРОФОТОМЕТРИЧЕСКОЙ МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ДОКСИЛАМИНА В КРОВИ В ВАРИАНТЕ МЕТОДА СТАНДАРТА

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Ключевые слова: *валидация; биоаналитические методики; УФ-спектрофотометрия; доксиламин, метод стандарта*

С целью рационализации проведения количественных определений в судебно-токсикологическом анализе изучена возможность использования метода стандарта при проведении УФ-спектрофотометрического определения аналитов в биологических жидкостях. Предложена процедура определения и оценки приемлемости линейности, правильности и прецизионности для валидации таких методик в варианте метода стандарта, которая апробирована на примере УФ-спектрофотометрической методики количественного определения доксиламина в крови. Предложенная процедура предполагает использование нормализованных координат – для нормализации полученных экспериментальных данных использованы два подхода. 1) Подход 1: использование раствора сравнения с концентрацией аналита, соответствующей его концентрации в конечном спектрофотометрируемом растворе при условии нулевых потерь для точки 100% в нормализованных координатах. 2) Подход 2: использование образца сравнения с концентрацией аналита, соответствующей его концентрации для точки 100% в нормализованных координатах. Оценку линейности, правильности и сходимости методики на первом этапе выполняли с использованием модельных растворов в рамках подхода, основанного на предположении незначимости неопределенности количественного определения аналита в модельных растворах Δ_{As}^{model} по сравнению с полной неопределенностью результатов анализа Δ_{As}. На втором этапе проводили определение линейности, правильности, сходимости и внутрилабораторной прецизионности методики на модельных образцах, приготовленных с использованием матрицы, для трех параллельных последовательностей. Показано, что для УФ-спектрофотометрического определения доксиламина в крови можно применять метод стандарта – при этом более приемлемым является Подход 1.