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Research Article

Development and Validation of UV-spectrophotometric Procedures for Efavirenz Quantitative Determination

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ABSTRACT
Efavirenz is a non-nucleoside reverse transcriptase inhibitor and attributed to the group of antiretroviral medicines used for treatment of HIV infection. For efavirenz determination the method of HPLC is widely used, but efavirenz is applied in high concentration and less sensitive methods of analysis such as spectrophotometry may be useful for its quantification. The aim is to develop UV-spectrophotometric procedures of efavirenz quantification and carry out step-by-step validation of the developed procedures. UV-spectra of efavirenz in 96% ethanol and 0.1 M sodium hydroxide solution have been investigated and the absorption maximums are observed at 247 nm and 267 nm respectively. The procedures of efavirenz quantitative determination by the method of UV-spectrophotometry have been developed using the mentioned solvents and wavelengths respectively. Their validation by such parameters as stability, linearity, accuracy and precision in the variants of the method of calibration curve, method of standard and method of additions has been carried out. All procedures of efavirenz quantitative determination are acceptable for application. The best linearity, accuracy and repeatability have been fixed for the procedure with application of 0.1 M sodium hydroxide solution as a solvent in the variant of the method of additions.

Keywords*:* efavirenz, UV-spectrophotometry, validation, method of calibration curve, method of standard, method of additions

INTRODUCTION

Efavirenz is a synthetic antiretroviral medicine and attributed to the group of non-nucleoside reverse transcriptase inhibitors; it is used for treatment of HIV infection as a first-line medicine[1].

The action mechanism of efavirenz is noncompetitive suppression of reverse transcriptase (the enzyme of HIV-1 virus), at the same time efavirenz does not inhibit α-, β- and γ-DNA-polymerases. Efavirenz is active only to HIV-virus of type 1[2-4].

Efavirenz is possessed of quite a number of side effects showed by psychiatric symptoms, including insomnia, nightmares, memory loss, depression, and anxiety. Treatment with efavirenz accompanies with certain neuropsychological symptoms in 50% of cases; its neurotoxicity exceeds other antiretroviral medicines[5–12].

The studies of efavirenz showed that in 20 – 50% of cases the toxic concentrations of the medicine in blood were fixed[13–16]. There are cases of acute poisoning due to administration of efavirenz, including cases of suicide attempts[17–19].

Use of efavirenz can produce a false positive result in blood and urine tests for marijuana[20].

Chemically, efavirenz is (*S*)-6-chloro-4-cyclopropylethynyl-1,4-dihydro-4-trifluoromethyl-2*H*-3,1-benzoxazin-2-one and has the structural formula as shown on Figure 1.

For efavirenz determination the method of HPLC is widely used, it ensures high selectivity and sensitivity of analysis[21–25].

Efavirenz is applied in high concentration; the recommended single oral dose is 600 mg[4]. Thus, we may use for determination of the medicine less sensitive methods of analysis such as spectrophotometry, and chemical structure of efavirenz allows to use direct UV-spectrophotometry for its quantification.

So the purpose of our paper is to develop UV-spectrophotometric procedures of efavirenz quantification and carry out step-by-step validation of the developed procedures in the variants of the method of calibration curve (MCC), method of standard (MS) and method of additions (MA) to choose the optimal variant for further application.

MATERIALS AND METHODS

Equipment

All spectrophotometric measurements were carried out using a single beam UV/VIS spectrophotometer SPEKOL®1500 (Analytik Jena AG, Germany) with wavelength scanned from 1100 nm to 190 nm. The software was WinASPECT®Spekol 2.3. The spectral band width was 1 nm. The pair of quartz square cells S90-309Q (UNICO, USA) with 10 mm pathlength and wavelength range from

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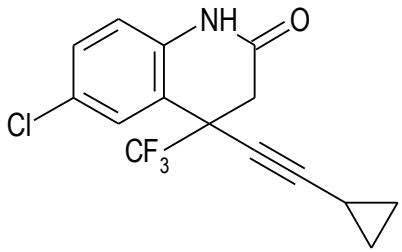


Figure 1: Chemical structure of efavirenz.

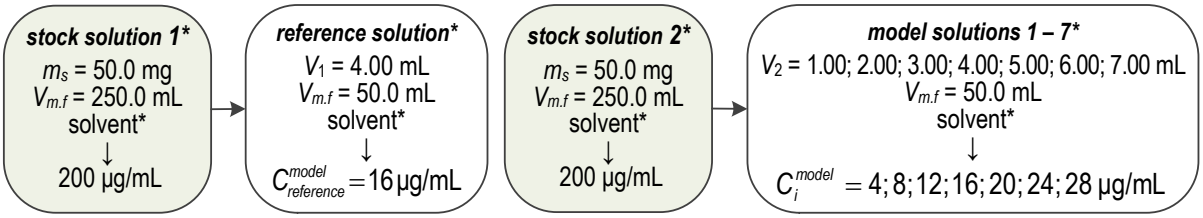
– Single-volume pipettes», ISO 1042:1998 «Laboratory glassware – One-mark volumetric flasks», ISO 4788:2005 «Laboratory glassware – Graduated measuring cylinders», ISO 385:2005 «Laboratory glassware – Burettes» and calibrated according to ISO 4787:2010 «Laboratory glassware – Volumetric instruments – Methods for testing of capacity and for use» and «Guidelines for calibration in analytical chemistry»[26] was used throughout this study.

Reagents and chemicals

Efavirenz was of pharmacopoeial purity and obtained from the pharmaceutical company «Zdorovie» Ltd. All other reagents (ethanol, sodium hydroxide) were of analytical grade.

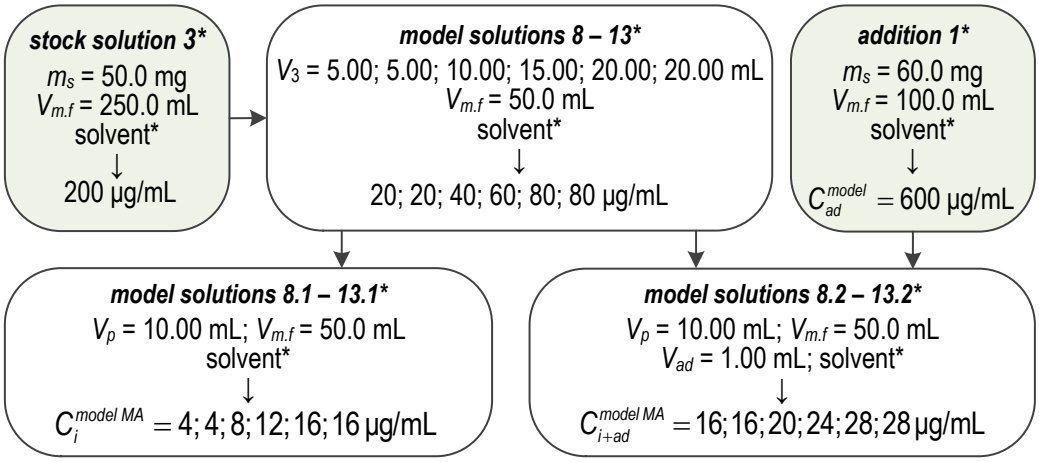
Reference and model solutions

The method of calibration curve and the method of



* ***solutions batch A:*** 96% C_2H_5OH
solutions batch B: 0.1 M NaOH

Scheme 1. The preparation procedure for reference and model solutions of efavirenz for MCC and MS.



* ***solutions batch A:*** 96% C_2H_5OH
solutions batch B: 0.1 M NaOH

Scheme 2: The preparation procedure for model solutions of efavirenz for MA.

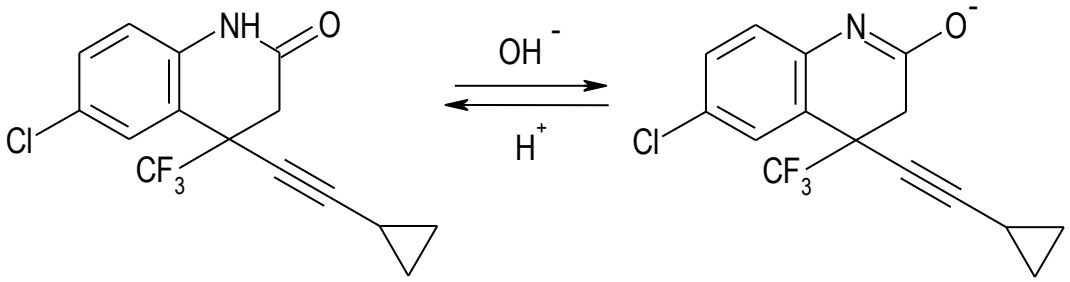


Figure 2: Possible transformations in the efavirenz solutions when changing the medium pH.

200 to 1200 nm was used throughout the whole experiment.

Weighing was carried out using digital analytical balance AN100 (AXIS, Ukraine) with d = 0.0001 g.

Glassware satisfied ISO 648:2008 «Laboratory glassware

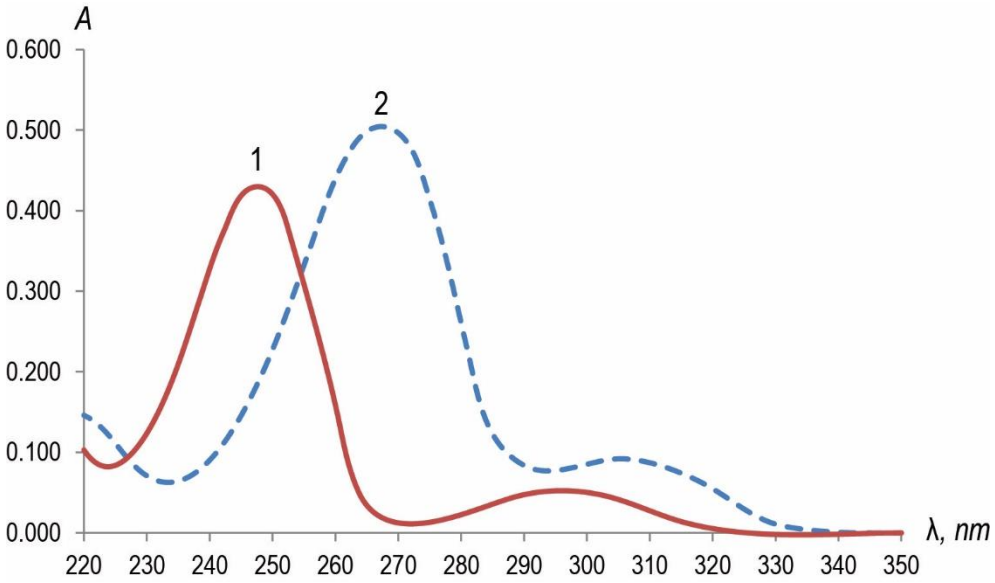


Figure 3. The UV-spectra of efavirenz (*l* = 10 mm; concentration is 10 μg/mL):
1 – solvent is 96% ethanol, λ_{max} = 247 nm ($A_{1\,cm}^{1\%}$ = 530) and 295 nm;
2 – solvent is 0.1 M sodium hydroxide solution, λ_{max} = 267 нм ($A_{1\,cm}^{1\%}$ = 610) and 307 nm

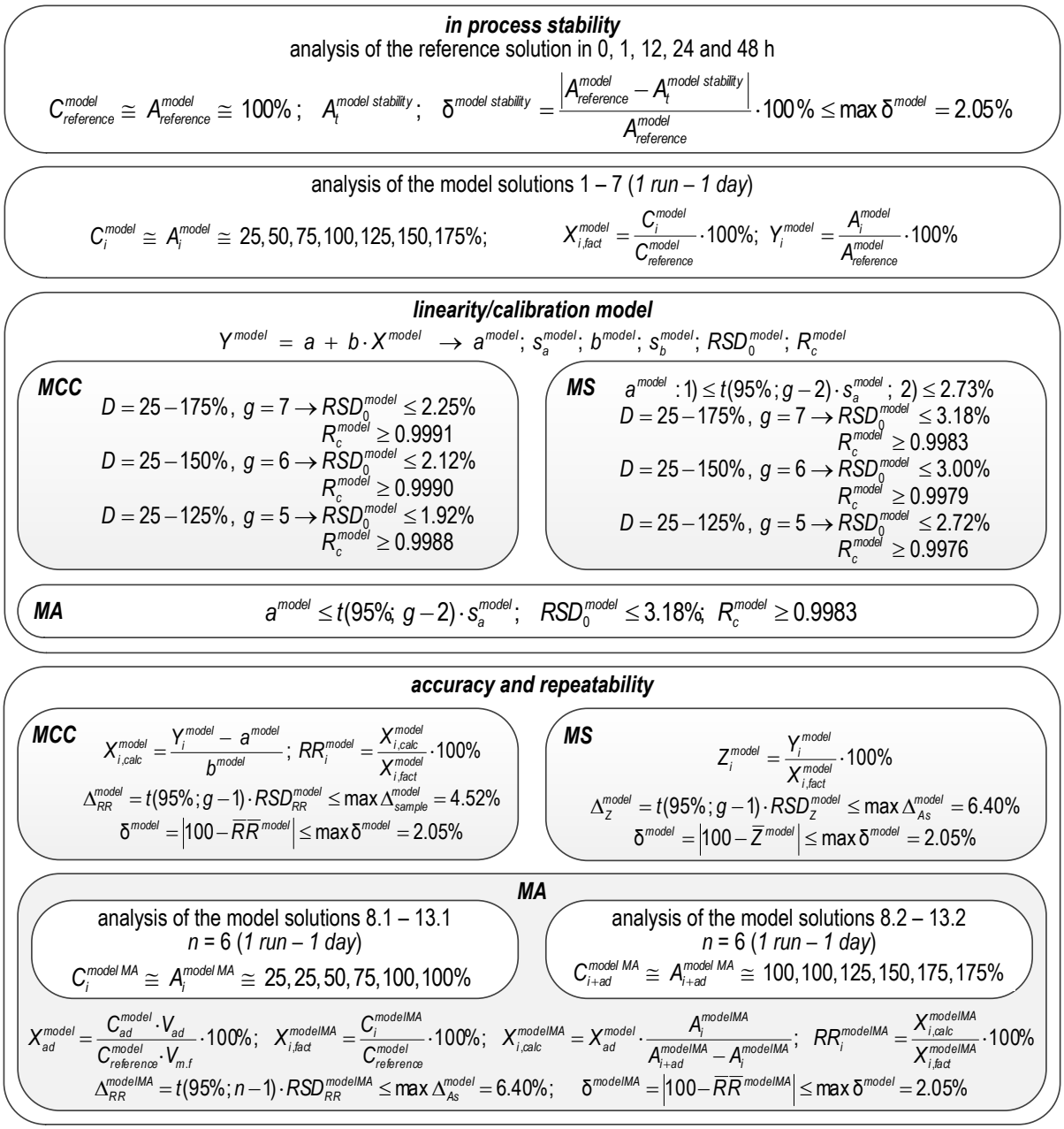
Table 1*:* The results of in process stability verification for efavirenz in model solutions.

Parameter	Values					
	0 h	1 h	12 h	24 h	36 h	48 h
	96% C_2H_5OH					
$A^{model\ stability}$	0.842	0.846	0.847	0.841	0.846	0.848
$A_0^{model\ stability} - A_t^{model\ stability}$	–	0.003	0.004	0.001	0.004	0.005
$\delta^{model\ stability}$,%	–	0.40	0.51	0.12	0.47	0.63
$\delta^{model\ stability} \le \max\delta^{model} = 2.05\%$	–	satisfied	satisfied	satisfied	satisfied	satisfied
	0.1 M NaOH					
$A^{model\ stability}$	0.968	0.966	0.967	0.962	0.964	0.964
$A_0^{model\ stability} - A_t^{model\ stability}$	–	0.003	0.001	0.007	0.004	0.005
$\delta^{model\ stability}$,%	–	0.28	0.14	0.69	0.41	0.48
$\delta^{model\ stability} \le \max\delta^{model} = 2.05\%$	–	satisfied	satisfied	satisfied	satisfied	satisfied

standard (Scheme 1)
The stock solutions 1 and 2 (200 μg/mL) were prepared by dissolving 50.0 mg of efavirenz in the solvent and the solutions were diluted to 250.0 mL with the same solvent. The reference solution (16 μg/mL) was prepared by diluting 4.00 mL of the stock solution 1 to 50.0 mL with the solvent. The stock solution 2 was diluted with the solvent to prepare the model solutions 1 – 7 having concentrations of 4; 8; 12; 16; 20; 24 and 28 μg/ml respectively.
The method of additions (Scheme 2)
The stock solution 3 (200 μg/mL) was prepared by dissolving 50.0 mg of efavirenz in the solvent and the solution was diluted to 250.0 mL with the same solvent. The addition solution 1 (600 μg/mL) was prepared by dissolving 60.0 mg of efavirenz in the solvent and the solution was diluted to 100.0 mL with the same solvent. The stock solution 3 was diluted with the solvent to prepare the model solutions 8 – 13 having concentrations of 20; 20; 40; 60; 80; 80 μg/ml respectively. The model solutions 8.1 – 13.1 were prepared by diluting 10.00 mL of the model solution 8 – 13 to 50.0 mL with the solvent. For preparing the model solutions 8.2 – 13.2 10.00 mL of the model solutions 8 – 13 were mixed with 1.00 mL of the addition solution 1 and diluted to 50.0 mL with the solvent.
For all cases the solutions batches A and B were prepared using 2 solvents such as 96% ethanol and 0.1 M sodium hydroxide solution respectively.
The absorbance of the model solutions 1 – 7, 8.1 – 13.1 and 8.2 – 13.2 was measured 3 times with randomization of cell position. The respective solvents were used as a compensation solutions.

RESULTS AND DISCUSSION

Analytical procedures development
Proceeding from the chemical structure the following transformations may be hypothesized for efavirenz when



Scheme 3. The validation stages of UV-spectrophotometric procedures for efavirenz determination

changing the medium pH (Figure 2).

Our assumptions have been confirmed by the UV-spectra of the efavirenz solutions in the different solvents with the different pH values; the UV-spectra mentioned above are presented on Figure 3.

Thus, it has been observed the shift of efavirenz absorption maximum to the right (247 nm → 267 nm) when increasing the pH from neutral to alkaline values.

For each absorption maximum and solvent the values of specific absorbance have been calculated (Figure 3) for the concentration range of 4 – 28 μg/mL.

Taking into account the obtained data we have developed two UV-spectrophotometric procedures for efavirenz quantitative determination using the respective solvents – 96% ethanol and 0.1 M sodium hydroxide solution.

Method validation (Scheme 3)

Validation of the developed procedures has been carried out in the variants of the method of calibration curve[27–31], method of standard[27,32] and method of additions[27,33].

Such validation parameters as in process stability, linearity/calibration model, accuracy and precision (repeatability) have been estimated by model solutions.

Method validation by model solutions according to Scheme 3 suggested by us[27] allows to assess the suitability of the actual analytical procedure for further work.

The validation provides application of the normalized coordinates:

$$X_i = \frac{C_i}{C_{st}} \cdot 100\%; \qquad Y_i = \frac{A_i}{A_{st}} \cdot 100\% \qquad (1)$$

,

i. e. transition from the equation $A_i = b_1 \cdot C_i + a_1$ to the equation $Y_i = b_2 \cdot X_i + a_2$, that allows to calculate the

Table 2: The results of linearity verification of efavirenz determination procedures by the method of UV-spectrophotometry.

Parameter	Values		Acceptability criterion		
	96% C_2H_5OH	0.1 M NaOH	*MCC*	*MS*	*MA*
			D = 25 – 175% (g = 7)		
b^{model}	0.989	1.021	–	–	
s_b^{model}	0.014	0.008	–	–	
a^{model}	2.030	–0.733	–	≤ 2.73%	
s_a^{model}	1.514	0.857	–	$a^{model} \le 2.015 \cdot s_a^{model}$	
RSD_0^{model}	1.791	1.014	≤ 2.25%	≤ 3.18%	
R_c^{model}	0.9995	0.9999	≥ 0.9991	≥ 0.9983	
			D = 25 – 150% (g = 6)		
b^{model}	1.001	1.019	–	–	–
s_b^{model}	0.016	0.011	–	–	–
a^{model}	1.185	–0.654	–	≤ 2.73%	–
s_a^{model}	1.568	1.051	–	$a^{model} \le 2.015 \cdot s_a^{model}$	–
RSD_0^{model}	1.684	1.129	≤ 2.12%	≤ 3.00%	–
R_c^{model}	0.9995	0.9998	≥ 0.9990	≥ 0.9979	–
			D = 25 – 125% (g = 5)		
b^{model}	1.026	1.011	–	–	–
s_b^{model}	0.011	0.015	–	–	–
a^{model}	–0.261	–0.189	–	≤ 2.73%	–
s_a^{model}	0.937	1.237	–	$a^{model} \le 2.015 \cdot s_a^{model}$	–
RSD_0^{model}	0.893	1.179	≤ 1.92%	≤ 2.72%	–
R_c^{model}	0.9998	0.9997	≥ 0.9988	≥ 0.9976	–

$$\max\Delta_{As}^{model} = 0.32 \cdot \max\Delta_{As} = 0.32 \cdot 20.00\% = 6.40\%;$$
$$\max\Delta_{cal}^{model} = \max\Delta_{sample}^{model} = \frac{\max\Delta_{As}^{model}}{\sqrt{2}} = 0.707 \cdot \max\Delta_{As}^{model} = 0.707 \cdot 6.40\% = 4.52\%; \quad \ldots(5)$$
$$\max\delta^{model} = 0.32 \cdot \max\Delta_{As}^{model} = 0.32 \cdot 6.40\% = 2.05\%.$$

Equation 5: uncertainty of analyte quantification in model solutions

validation characteristics, which do not depend on the analyte and features of the method of analysis.

The efavirenz concentration in the model solution for the point of 100% in the normalized coordinates $C_{100\%}^{model}$ has been chosen as the concentration provided the absorbance at the level of 0.7 – 0.9.

For normalization of the obtained experimental data the reference solution with the analyte concentration of $C_{reference}^{model} = C_{100\%}^{model}$ is used.

The analytical ranges D of the methods application are 25 – 125%, 25 – 150% and 25 – 175%; the number of concentration levels g equals 5, 6 or 7 respectively in constant increments of 25%.

Acceptability criteria for validation parameters have been formed on the basis of systematic application of "insignificance concept"[34,35] – the confidence interval Δ_2 is insignificant as compared with the confidence interval Δ_1 at the conventional level p = 95%, if the following inequality is correct:

$$\Delta_2 \le 0.32 \cdot \Delta_1, \qquad (2)$$

and proceeding from the value of extreme uncertainty Δ_{As} for the method in analytical toxicology, which equals 25% and 20%[36,37] – for the lowest point of the analytical range of the methods application and for the rest of range.

In the *MCC* acceptability criteria for linear dependence and precision have been found proceeding from the equality of uncertainty of plotting the calibration curve Δ_{cal} and uncertainty of analysis of the sample to be analysed Δ_{sample}.

Acceptability criteria for validation parameters have been calculated proceeding from the assumption that

Table 3: The results of accuracy and precision verification (MCC) of efavirenz determination procedures by the method of UV-spectrophotometry.

Factual concentration of efavirenz in model solution ($C_{reference}^{model} = 16$ µg/mL)		Absorbance A_i^{model}	Found in % to standard absorbance Y_i^{model},%	Calculated concentration of efavirenz in model solution $X_{i,calc}^{model}$,%			RR_i^{model},%		
C_i^{model}, µg/mL	$X_{i,fact}^{model}$,%			25 – 175%	25 – 150%	25 – 125%	25 – 175%	25 – 150%	25 – 125%
96% C_2H_5OH									
4	25.0	0.219	25.96	24.21	24.74	25.56	96.83	98.98	102.22
8	50.0	0.427	50.73	49.27	49.49	49.70	98.53	98.97	99.40
12	75.0	0.645	76.53	75.36	75.25	74.85	100.49	100.34	99.79
16	100.0	0.853	101.31	100.42	100.00	98.99	100.42	100.00	98.99
20	125.0	1.086	128.93	128.36	127.58	125.91	102.69	102.07	100.73
24	150.0	1.258	149.31	148.98	147.94	–	99.32	98.62	–
28	175.0	1.461	173.45	173.40	–	–	99.08	–	–
$A_{reference}^{model} = 0.842$						$\overline{RR}^{model}$,%	99.62	99.83	100.23
						$\delta^{model},\% = \lvert 100 - \overline{RR}^{model} \rvert$	0.38	0.17	0.23
						$\delta^{model} \le \max\delta^{model} = 2.05\%$	satisfied	satisfied	satisfied
						RSD_{RR}^{model},%	1.83	1.28	1.29
						$\Delta_{RR}^{model},\% = RSD_{RR}^{model} \cdot t(95\%; g-1)$	3.57	2.58	2.75
						$\Delta_{RR}^{model} \le \max\Delta_{sample}^{model} = 4.52\%$	satisfied	satisfied	satisfied
0.1 M NaOH									
4	25.0	0.250	25.85	26.05	26.00	25.75	104.20	104.01	103.00
8	50.0	0.484	49.98	49.70	49.68	49.61	99.39	99.35	99.22
12	75.0	0.730	75.42	74.62	74.63	74.76	99.50	99.51	99.68
16	100.0	0.964	99.59	98.30	98.34	98.66	98.30	98.34	98.66
20	125.0	1.234	127.47	125.63	125.69	126.23	100.50	100.56	100.98
24	150.0	1.481	152.91	150.55	150.65	–	100.37	100.43	–
28	175.0	1.724	178.00	175.14	–	–	100.08	–	–
$A_{reference}^{model} = 0.968$						$\overline{RR}^{model}$,%	100.34	100.37	100.31
						$\delta^{model},\% = \lvert 100 - \overline{RR}^{model} \rvert$	0.34	0.37	0.31
						$\delta^{model} \le \max\delta^{model} = 2.05\%$	satisfied	satisfied	satisfied
						RSD_{RR}^{model},%	1.86	1.96	1.73
						$\Delta_{RR}^{model},\% = RSD_{RR}^{model} \cdot t(95\%; g-1)$	3.61	3.95	3.69
						$\Delta_{RR}^{model} \le \max\Delta_{sample}^{model} = 4.52\%$	satisfied	satisfied	satisfied

uncertainty of analyte quantification in model solutions Δ_{As}^{model} is insignificant as compared with total uncertainty Δ_{As}:

Validation results

In process stability of efavirenz in the model solution was verified in the way of measuring the absorbance for the reference solution immediately and in 1, 12, 24 and 48 hours after its preparation, and the systematic error $\delta^{model\ stability}$ was calculated and assessed (Table 1).

In process stability of efavirenz in model solutions is satisfied the acceptability criteria for all periods of time and for both solvents.

These results have been taken into account when determining all validation parameters.

To determine *linearity/calibration model* the model solutions 1 – 7 were analysed within 1 run, correlation coefficient R_c^{model}, rest standard deviation RSD_0^{model} and also absolute term a^{model} (if it is necessary) were calculated and assessed (Table 2).

To estimate *precision (repeatability) and accuracy:*

MCC: the model solutions 1 – 7 concentrations were calculated using the linear dependence obtained and the values «found/given» RR_i^{model} were used to determine the

Table 4*:* The results of accuracy and precision verification (MS) of efavirenz determination procedures by the method of UV-spectrophotometry.

Factual concentration of efavirenz in model solution ($C^{model}_{reference} = 16$ µg/mL)		Absorbance A^{model}_i	Found in % to standard absorbance Y^{model}_i,%	Z^{model}_i,%		
C^{model}_i, µg/mL	$X^{model}_{i,fact}$,%			25 – 175%	25 – 150%	25 – 125%
			96% C_2H_5OH			
4	25.0	0.219	25.96	103.84	103.84	103.84
8	50.0	0.427	50.73	101.46	101.46	101.46
12	75.0	0.645	76.53	102.04	102.04	102.04
16	100.0	0.853	101.31	101.31	101.31	101.31
20	125.0	1.086	128.93	103.14	103.14	103.14
24	150.0	1.258	149.31	99.54	99.54	–
28	175.0	1.461	173.45	99.11	–	–
$A^{model}_{reference} = 0.842$			$\bar{Z}^{model}$,%	101.49	101.89	102.36
			$\delta^{model},\% = \lvert 100 - \bar{Z}^{model} \rvert$	1.49	1.89	2.36
			$\delta^{model} \le \max\delta^{model} = 2.05\%$	satisfied	satisfied	satisfied
			RSD^{model}_Z,%	1.73	1.51	1.10
			$\Delta^{model}_Z,\% = RSD^{model}_Z \cdot t(95\%; g-1)$	3.37	3.05	2.34
			$\Delta^{model}_Z \le \max\Delta^{model}_{As} = 6.40\%$	satisfied	satisfied	satisfied
			0.1 M NaOH			
4	25.0	0.250	25.85	103.41	103.41	103.41
8	50.0	0.484	49.98	99.97	99.97	99.97
12	75.0	0.730	75.42	100.56	100.56	100.56
16	100.0	0.964	99.59	99.59	99.59	99.59
20	125.0	1.234	127.47	101.98	101.98	101.98
24	150.0	1.481	152.91	101.94	101.94	–
28	175.0	1.724	178.00	101.72	–	–
$A^{model}_{reference} = 0.968$			$\bar{Z}^{model}$,%	101.31	101.24	101.10
			$\delta^{model},\% = \lvert 100 - \bar{Z}^{model} \rvert$	1.31	1.24	1.10
			$\delta^{model} \le \max\delta^{model} = 2.05\%$	satisfied	satisfied	satisfied
			RSD^{model}_Z,%	1.34	1.45	1.58
			$\Delta^{model}_Z,\% = RSD^{model}_Z \cdot t(95\%; g-1)$	2.60	2.93	3.36
			$\Delta^{model}_Z \le \max\Delta^{model}_{As} = 6.40\%$	satisfied	satisfied	satisfied

confidence interval Δ^{model}_{RR} and the systematic error δ^{model} respectively (Table 3);

MS: the ratios Z^{model}_i for the model solutions 1 – 7 were calculated and used to determine the confidence interval Δ^{model}_Z and the systematic error δ^{model} respectively (Table 4);

MA: the model solutions 8.1 – 13.1 and 8.2 – 13.2 were analysed within 1 run, the model solutions 8.1 – 13.1 concentrations were recalculated and the values «found/given» $RR^{model\ MA}_i$ were used to determine the confidence interval $\Delta^{model\ MA}_{RR}$ and the systematic error $\delta^{model\ MA}$ respectively.

The values of confidence interval and systematic error were compared with the respective acceptability criteria.

Validation of the procedures has been carried out within 3 different analytical runs using different batches of reagents and different glassware; experiments have been performed by three different analysts. The results obtained within one analytical run are presented in Tables 1 – 5, but results of other analytical runs are at the same range of values.

The total results of validation allow to point to the conclusion about acceptable *linearity*, *accuracy* and *precision* of both UV-spectrophotometric procedures of

Table 5*:* The results of accuracy and precision verification (MA) of efavirenz determination procedures by the method of UV-spectrophotometry

Factual concentration of efavirenz in model solution ($C_{reference}^{model} = 16$ μg/mL)		Absorbance		Calculated concentration of efavirenz in model solution	$RR_i^{model\,MA}$,%
$C_i^{model\,MA}$, μg/mL	$X_{i,fact}^{model\,MA}$,%	$A_i^{model\,MA}$	$A_{i+ad}^{model\,MA}$	$X_{i,calc}^{model\,MA}$,%	
96% C_2H_5OH					
4	25	0.211	0.849	24.80	99.22
4	25	0.209	0.826	25.41	101.62
8	50	0.421	1.061	49.34	98.67
12	75	0.631	1.251	76.33	101.77
16	100	0.837	1.471	99.01	99.01
16	100	0.851	1.481	101.31	101.31
				$\overline{RR}^{model\,MA\,Д}$,%	100.27
				$\delta^{model\,MA},\% = \left\|100 - \overline{RR}^{model\,MA}\right\|$	0.27
				$\delta^{model\,MA} \le \max\delta^{model} = 2.05\%$	satisfied
				$RSD_{RR}^{model\,MA}$,%	1.44
				$\Delta_{RR}^{model\,MA} = t(95\%; n-1) \cdot RSD_{RR}^{model\,MA}$	2.91
				$\Delta_{RR}^{model\,MA} \le \max\Delta_{As}^{model} = 6.40\%$	satisfied
0.1 M NaOH					
8	50	0.242	0.964	50.28	100.55
8	50	0.244	0.962	50.97	101.95
16	100	0.489	1.224	99.80	99.80
24	150	0.738	1.480	149.19	99.46
32	200	0.973	1.718	195.91	97.95
32	200	0.978	1.717	198.51	99.26
				$\overline{RR}^{model\,MA\,Д}$,%	99.83
				$\delta^{model\,MA},\% = \left\|100 - \overline{RR}^{model\,MA}\right\|$	0.17
				$\delta^{model\,MA} \le \max\delta^{model} = 2.05\%$	satisfied
				$RSD_{RR}^{model\,MA}$,%	1.34
				$\Delta_{RR}^{model\,MA} = t(95\%; n-1) \cdot RSD_{RR}^{model\,MA}$	2.70
				$\Delta_{RR}^{model\,MA} \le \max\Delta_{As}^{model} = 6.40\%$	satisfied

efavirenz quantitative determination in the variant of the MCC, MS and MA for all ranges of the method application. It gives us the possibility to recommend these procedures for further application in forensic toxicology with the purpose of development of the methods of biological liquids analysis for efavirenz quantification.

For the most cases the procedures in the variant of MA are characterized by the best values of accuracy and the middle level of precision. In turn, the procedures in the variant of MS are characterized by the best values of precision and the worst values of accuracy. For the variant of MCC the middle accuracy and the worst precision are observed. Thus application of the method of additions is optimal for analysis.

As for the solvents used in analysis, it should be noted that the best linearity, accuracy and repeatability have been fixed for the procedure with application of 0.1 M sodium hydroxide solution as a solvent.

CONCLUSIONS

Two new procedures of efavirenz quantitative determination by the method of UV-spectrophotometry have been developed using 96% ethanol and 0.1 M sodium hydroxide solution as the solvents (wavelengths λ_{max} are 247 nm and 267 nm respectively). Their validation by such parameters as stability, linearity, accuracy and precision in the variants of the method of calibration curve, method of standard and method of additions has been carried out and acceptability for application has been shown.

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