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Development and Validation of UV-Spectrophotometric Procedures for Secnidazole Quantitative Determination

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Abstract

Secnidazole is one of the antiprotozoal medicines from the group of 5-nitroimidazoles, which is characterized by a prolonged serum half-life. For secnidazole determination the method of HPLC is widely used, but secnidazole 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 a number of UV-spectrophotometric procedures of secnidazole quantification and carry out step-by-step validation of the developed procedures. UV-spectra of secnidazole in 0.1 M hydrochloric acid solution (A), 96% ethanol (B), 0.1 M potassium hydroxide solution in methanol (C), 0.1 M sodium hydroxide solution (D) have been investigated and it has been set that when increasing the pH value step-by-step shift of substance maximum absorption to the right is observed (277 nm → 310 nm → 314 nm → 319 nm). The procedures of secnidazole 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 and method of standard has been carried out. The procedures A, B and D of secnidazole quantitative determination are acceptable for application. The best linearity, accuracy and repeatability have been fixed for the procedure D in the variant of the method of calibration curve.

Keywords: secnidazole, UV-spectrophotometry, validation, method of calibration curve, method of standard

INTRODUCTION

5-nitroimidazoles are the group of antiprotozoal medicines widely used for treatment of infectious diseases caused by Trichomonas, Lamblia, Leishmania, etc. [1 – 8]. The action mechanism of nitroimidazoles consists in biochemical reduction of 5-nitrogroup by intracellular transport proteins of anaerobes and protozoa. Reduced nitroimidazoles interact with DNA of microorganism cells and inhibit synthesis of their nucleic acids that leads to microorganism death [6, 9 – 11].

Secnidazole is one of the medicines from the group of 5-nitroimidazoles, it is characterized by a prolonged serum half-life [12, 13]. Chemically, secnidazole is 1-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol and has the structural formula as shown on Figure 1.

The medicine has a number of side effects manifested by usual symptoms of acute intoxication (giddiness, nausea, vomiting), especially when interacting with other drugs [2, 7, 14]. And the case of taking with alcohol may be toxic for patient even when therapeutic dose is taken [14].

For secnidazole determination the method of HPLC is widely used, it ensures high selectivity and sensitivity of analysis [15 – 24].

Secnidazole is applied in high concentration; single oral dose is 1 – 2 g [7, 8, 12 – 14, 25 – 27]. Thus, we may use for determination of the medicine less sensitive methods of analysis such as spectrophotometry. Sometimes spectrophotometric methods are used with this purpose, but only in visible range after preliminary derivatization or complex formation [17, 28, 29]. But chemical structure of secnidazole allows to use direct UV-spectrophotometry for its quantification.

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

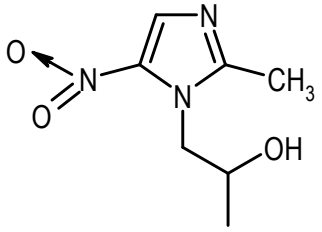


Figure 1. Chemical structure of secnidazole

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 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 – 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 – Bu-

rettes» 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» [30] was used throughout this study.

Reagents and chemicals

Secnidazole was of pharmacopoeial purity. Hydrochloric acid (≥37%, puriss. p.a., ACS reagent, fuming), methanol (≥99.8%, puriss. p.a., ACS reagent) were purchased from Sigma-Aldrich Co. LLC (USA). All other reagents (ethanol, sodium hydroxide, potassium hydroxide) were of analytical grade.

Reference and model solutions (Scheme 1)

The stock solutions 1 and 2 (250 μg/mL) were prepared by dissolving 50.0 mg of secnidazole in the solvent and the solutions were diluted to 200.0 mL with the same solvent. The reference solution (20 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 5; 10; 15; 20; 25; 30 and 35 μg/mL respectively.

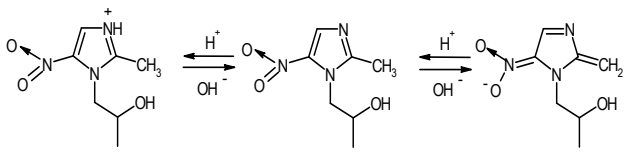
For all cases the solutions batches A, B, C and D were prepared using 4 different solvents such as 0.1 M hydrochloric acid solution, 96% ethanol, 0.1 M potassium hydroxide solution in methanol and 0.1 M sodium hydroxide solution respectively.

The absorbance of the model solutions 1 – 7 was measured 3 times with randomization of cell position. The respective solvent was used as a compensation solution.

RESULTS AND DISCUSSION

Analytical procedures development

Proceeding from the chemical structure the following transformations may be hypothesized for secnidazole when changing the medium pH:



Our assumptions have been confirmed by the UV-spectra of the secnidazole solutions in the different solvents with the different pH values; the UV-spectra mentioned above are presented on Figure 2.

Thus, it has been observed step-by-step shift of secnidazole absorption maximum to the right (277 nm → 310 nm → 314 nm → 319 nm) when increasing the pH value.

For each absorption maximum and solvent the values of specific absorbance have been calculated (Figure 2) for the concentration range of 5 – 35 μg/mL.

Taking into account the obtained data we have developed four UV-spectrophotometric procedures for secnidazole quantitative determination using the respective solvents – 0.1 M hydrochloric acid solution, 96% ethanol, 0.1 M potassium hydroxide solution in methanol, 0.1 M sodium hydroxide solution.

Method validation (Scheme 2)

Validation of the developed procedures has been carried out in the variants of the method of calibration curve [31 – 34, 36] and method of standard [35, 36].

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 the Scheme 2 suggested by us [36] allows to assess the suitability of the actual analytical procedure for further work.

The validation provides application of the normalized coordinates:

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

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

The secnidazole 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" [37, 38] – 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:

$$\Delta_2 \le 0.32 \cdot \Delta_1, \tag{2}$$

and proceeding from the value of extreme uncertainty Δ_{As} for the method in analytical toxicology, which equals 25% and 20% [39, 40] – 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 two approaches:

Approach 1: uncertainty of analyte quantification in model solutions Δ_{As}^{model} is equal to uncertainty of sample preparation procedure:

$$\max\Delta_{As}^{model} = \frac{\max\Delta_{As}}{\sqrt{2}} = 0.707 \cdot \max\Delta_{As} = 0.707 \cdot 20.00\% = 14.14\%;$$
$$\max\Delta_{cal}^{model} = \max\Delta_{sample}^{model} = \frac{\max\Delta_{As}^{model}}{\sqrt{2}} = 0.707 \cdot \max\Delta_{As}^{model} = 0.707 \cdot 14.14\% = 10.00\%; \quad (4)$$
$$\max\delta^{model} = 0.32 \cdot \max\Delta_{As}^{model} = 4.52\%;$$

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

Approach 2: uncertainty of analyte quantification in model solutions Δ_{As}^{model} is insignificant as compared with total uncertainty Δ_{As}:

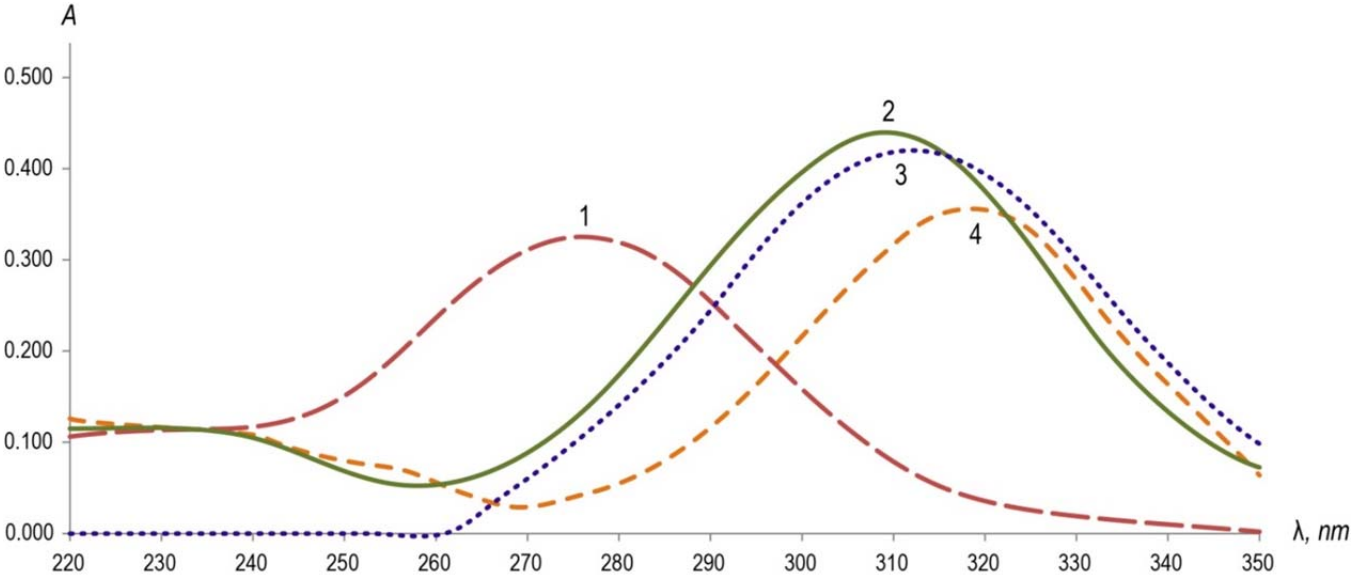


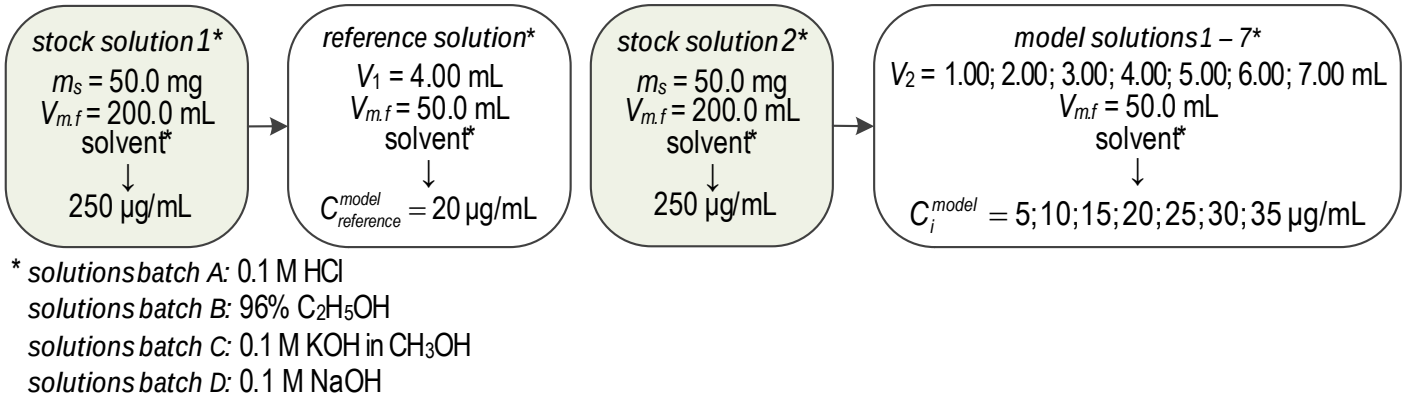
Figure 2. The UV-spectra of secnidazole (l = 10 mm; concentration is 10 µg/mL):

1 – solvent is 0.1 M hydrochloric acid solution, λ_{max} = 277 nm ($A_{1cm}^{1\%}$ = 321);

2 – solvent is 96% ethanol, λ_{max} = 310 nm ($A_{1cm}^{1\%}$ = 432);

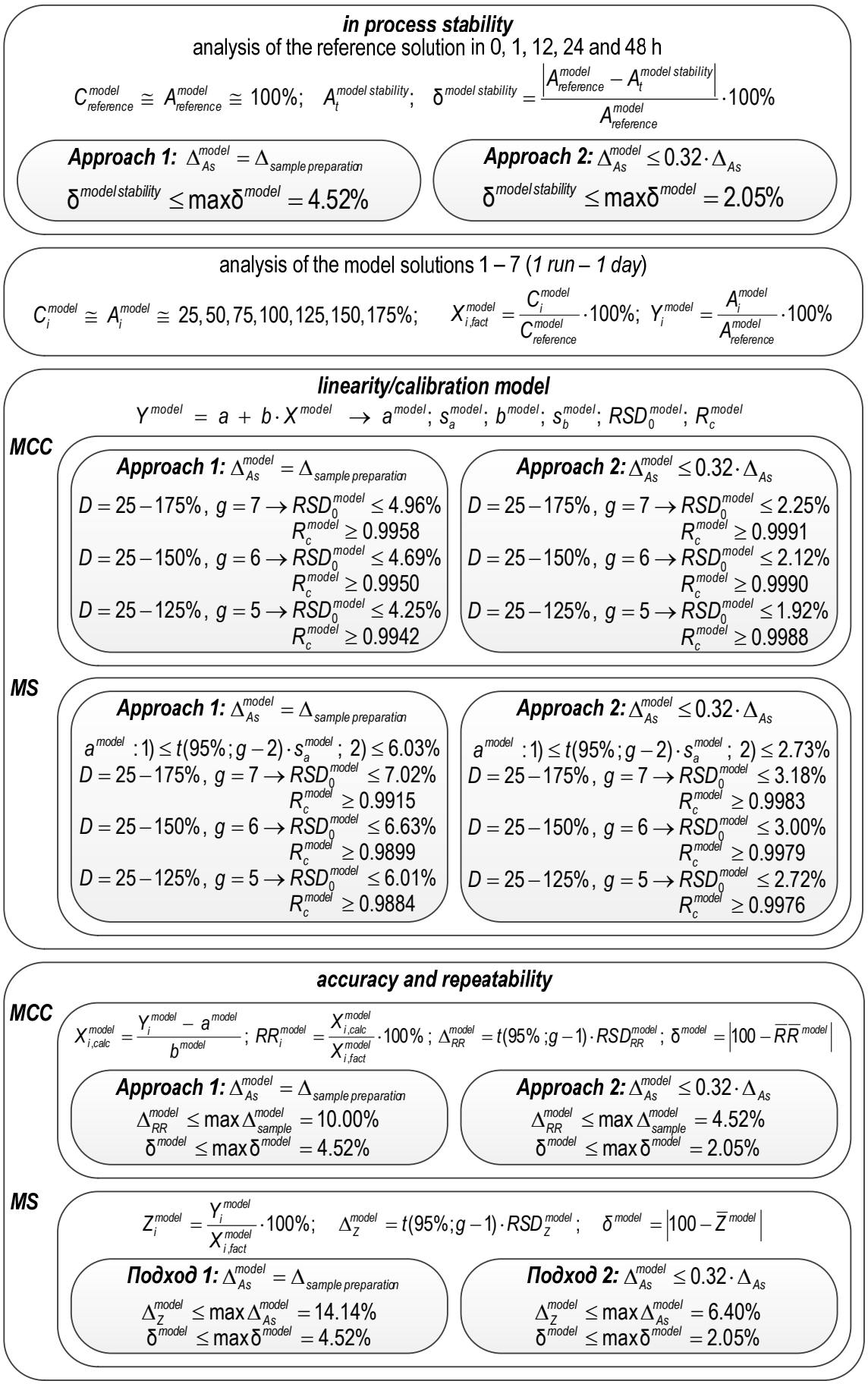
3 – solvent is 0.1 M potassium hydroxide solution in methanol, λ_{max} = 314 нм ($A_{1cm}^{1\%}$ = 418);

4 – solvent is 0.1 M sodium hydroxide solution, λ_{max} = 319 нм ($A_{1cm}^{1\%}$ = 362)



* *solutions batch A:* 0.1 M HCl
solutions batch B: 96% C_2H_5OH
solutions batch C: 0.1 M KOH in CH_3OH
solutions batch D: 0.1 M NaOH

Scheme 1. The preparation procedure for reference and model solutions of secnidazole



Scheme 2. The validation stages of UV-spectrophotometric procedures for secnidazole determination

Validation results

In process stability of secnidazole 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 secnidazole in model solutions is satisfied the acceptability criteria for all periods of time only when using 0.1 M hydrochloric acid solution as a solvent (both for *Approach 1* and *Approach 2*).

The solutions of secnidazole in 96% ethanol are stable during 36 hours after their preparation within the Approach 1 and during 48 hours within the Approach 2.

0.1 M sodium hydroxide solution may be used as a solvent for measuring only during 12 – 24 hours after solutions preparation.

Measuring the absorbance of the secnidazole solutions in 0.1 M potassium hydroxide solution in methanol should be carried out only immediately after their preparation.

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 confidence interval Δ_{RR}^{model} and the systematic error δ^{model} respectively (Table 3);
- *MS:* the ratios Z_i^{model} for the model solutions 1 – 7 were calculated and used to determine the confidence interval Δ_Z^{model} and the systematic error δ^{model} respectively (Table 4).

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

Table 1 **The results of in process stability verification for secnidazole in model solutions**

Parameter		Values					
		0 h	1 h	12 h	24 h	36 h	48 h
0.1 M HCl							
$A^{model\ stability}$		0.632	0.630	0.634	0.635	0.626	0.625
$A_0^{model\ stability} - A_t^{model\ stability}$		–	0.002	0.002	0.003	0.006	0.008
$\delta^{model\ stability}, \% \le \max\delta^{model}$		–	0.32	0.26	0.47	1.00	1.21
Approach 1	≤ 4.52%	–	satisfied	satisfied	satisfied	satisfied	satisfied
Approach 2	≤ 2.05%	–	satisfied	satisfied	satisfied	satisfied	satisfied
96% C_2H_5OH							
$A^{model\ stability}$		0.854	0.853	0.854	0.860	0.866	0.875
$A_0^{model\ stability} - A_t^{model\ stability}$		–	0.002	0.000	0.006	0.012	0.020
$\delta^{model\ stability}, \% \le \max\delta^{model}$		–	0.20	0.04	0.70	1.37	2.38
Approach 1	≤ 4.52%	–	satisfied	satisfied	satisfied	satisfied	satisfied
Approach 2	≤ 2.05%	–	satisfied	satisfied	satisfied	satisfied	unsatisfied
0.1 M KOH in CH_3OH							
$A^{model\ stability}$		0.815	0.854	0.836	0.742	0.786	0.848
$A_0^{model\ stability} - A_t^{model\ stability}$		–	0.039	0.020	0.074	0.030	0.032
$\delta^{model\ stability}, \% \le \max\delta^{model}$		–	4.78	2.49	9.04	3.64	3.97
Approach 1	≤ 4.52%	–	unsatisfied	satisfied	unsatisfied	satisfied	satisfied
Approach 2	≤ 2.05%	–	unsatisfied	unsatisfied	unsatisfied	unsatisfied	unsatisfied
0.1 M NaOH							
$A^{model\ stability}$		0.734	0.738	0.742	0.754	0.780	0.789
$A_0^{model\ stability} - A_t^{model\ stability}$		–	0.004	0.008	0.020	0.046	0.055
$\delta^{model\ stability}, \% \le \max\delta^{model}$		–	0.59	1.14	2.77	6.31	7.45
Approach 1	≤ 4.52%	–	satisfied	satisfied	satisfied	unsatisfied	unsatisfied
Approach 2	≤ 2.05%	–	satisfied	satisfied	unsatisfied	unsatisfied	unsatisfied

Table 2 **The results of linearity verification of secnidazole determination procedures by the method of UV-spectrophotometry**

Parameter	Values				Acceptability criterion			
	0.1 M HCl	**96% C_2H_5OH**	**0.1 M KOH in CH_3OH**	**0.1 M NaOH**	*MCC*		*MS*	
					Approach 1	*Approach 2*	*Approach 1*	*Approach 2*
D = 25 – 175% (g = 7)								
b^{model}	0.976	1.002	1.017	1.002	–	–	–	–
s_b^{model}	0.017	0.006	0.014	0.010	–	–	–	–
a^{model}	2.621	0.702	0.403	–0.740	–	–	≤ 2.73%	≤ 6.03%
s_a^{model}	1.928	0.707	1.598	1.163	–	–	$a^{model} \le 2.015 \cdot s_a^{model}$	
RSD_0^{model}	2.282	0.836	1.890	1.377	≤ 2.25%	≤ 4.96%	≤ 3.18%	≤ 7.02%
R_c^{model}	0.9992	0.9999	0.9995	0.9997	≥ 0.9991	≥ 0.9958	≥ 0.9983	≥ 0.9915
D = 25 – 150% (g = 6)								
b^{model}	1.002	0.998	1.025	0.988	–	–	–	–
s_b^{model}	0.012	0.009	0.019	0.009	–	–	–	–
a^{model}	0.879	0.921	-0.101	0.182	–	–	≤ 2.73%	≤ 6.03%
s_a^{model}	1.151	0.831	1.874	0.920	–	–	$a^{model} \le 2.015 \cdot s_a^{model}$	
RSD_0^{model}	1.236	0.892	2.013	0.988	≤ 2.12%	≤ 4.69%	≤ 3.00%	≤ 6.63%
R_c^{model}	0.9997	0.9999	0.9993	0.9998	≥ 0.9990	≥ 0.9950	≥ 0.9979	≥ 0.9899
D = 25 – 125% (g = 5)								
b^{model}	1.003	0.984	1.045	0.980	–	–	–	–
s_b^{model}	0.018	0.004	0.023	0.013	–	–	–	–
a^{model}	0.833	1.748	–1.292	0.658	–	–	≤ 2.73%	≤ 6.03%
s_a^{model}	1.496	0.304	1.927	1.037	–	–	$a^{model} \le 2.015 \cdot s_a^{model}$	
RSD_0^{model}	1.426	0.290	1.837	0.989	≤ 1.92%	≤ 4.25%	≤ 2.72%	≤ 6.01%
R_c^{model}	0.9995	1.0000	0.9993	0.9998	≥ 0.9988	≥ 0.9942	≥ 0.9976	≥ 0.9884

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

Factual concentration of secnidazole in model solution ($C_{reference}^{model}$ = 20 μg/mL)		Absorbance A_i^{model}	Found in % to standard absorbance Y_i^{model},%	Calculated concentration of secnidazole 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%
0.1 M HCl									
5	25	0.164	25.99	23.93	25.05	25.07	95.73	100.19	100.29
10	50	0.328	51.92	50.50	50.92	50.92	100.99	101.84	101.85
15	75	0.477	75.38	74.52	74.32	74.31	99.36	99.09	99.08
20	100	0.629	99.47	99.20	98.35	98.32	99.20	98.35	98.32
25	125	0.807	127.62	128.03	126.43	126.38	102.42	101.14	101.10
30	150	0.956	151.19	152.16	149.93	–	101.44	99.96	–
35	175	1.076	170.22	171.65	–	–	98.09	–	–
$A_{reference}^{model}$ = 0.632			$\overline{RR}^{model}$,%				99.61	100.10	100.13

Factual concentration of secnidazole in model solution ($C^{model}_{reference}$ = 20 μg/mL)		Absorbance A_i^{model}	Found in % to standard absorbance Y_i^{model},%	Calculated concentration of secnidazole in model solution $X^{model}_{i,calc}$,%			RR_i^{model},%		
C_i^{model}, μg/mL	$X^{model}_{i,fact}$,%			25 – 175%	25 – 150%	25 – 125%	25 – 175%	25 – 150%	25 – 125%
$\delta^{model},\% = \left\|100 - \overline{RR}^{model}\right\| \le \max\ \delta^{model}$							0.39	0.10	0.13
				Approach 1		≤ 4.52%	satisfied	satisfied	satisfied
				Approach 2		≤ 2.05%	satisfied	satisfied	satisfied
$RSD^{model}_{RR},\%$							2.26	1.28	1.44
$\Delta^{model}_{RR},\% = RSD^{model}_{RR} \cdot t(95\%; g-1) \le \max\Delta^{model}_{sample}$							4.40	2.58	3.07
				Approach 1		≤ 10.00%	satisfied	satisfied	satisfied
				Approach 2		≤ 4.52%	satisfied	satisfied	satisfied
96% C_2H_5OH									
5	25	0.225	26.38	25.63	25.50	25.02	102.53	101.99	100.10
10	50	0.436	51.07	50.29	50.24	50.12	100.58	100.47	100.24
15	75	0.642	75.19	74.36	74.39	74.62	99.15	99.18	99.49
20	100	0.858	100.47	99.60	99.71	100.31	99.60	99.71	100.31
25	125	1.065	124.70	123.80	123.98	124.93	99.04	99.19	99.94
30	150	1.297	151.85	150.91	151.18	–	100.60	100.79	–
35	175	1.507	176.39	175.41	–	–	100.23	–	–
$A^{model}_{reference} = 0.854$			$\overline{RR}^{model},\%$				100.25	100.22	100.02
$\delta^{model},\% = \left\|100 - \overline{RR}^{model}\right\| \le \max\delta^{model}$							0.25	0.22	0.02
				Approach 1		≤ 4.52%	satisfied	satisfied	satisfied
				Approach 2		≤ 2.05%	satisfied	satisfied	satisfied
$RSD^{model}_{RR},\%$							1.19	1.09	0.32
$\Delta^{model}_{RR},\% = RSD^{model}_{RR} \cdot t(95\%; g-1) \le \max\Delta^{model}_{sample}$							2.31	2.19	0.69
				Approach 1		≤ 10.00%	satisfied	satisfied	satisfied
				Approach 2		≤ 4.52%	satisfied	satisfied	satisfied
0.1 M KOH in CH_3OH									
5	25	0.194	23.75	22.96	23.28	23.97	91.84	93.13	95.87
10	50	0.422	51.76	50.50	50.62	50.77	100.99	101.23	101.53
15	75	0.645	79.07	77.35	77.27	76.90	103.13	103.03	102.54
20	100	0.825	101.14	99.06	98.82	98.03	99.06	98.82	98.03
25	125	1.057	129.68	127.12	126.67	125.34	101.69	101.34	100.27
30	150	1.238	151.88	148.95	148.34	–	99.30	98.89	–
35	175	1.447	177.43	174.07	–	–	99.47	–	–
$A^{model}_{reference} = 0.815$			$\overline{RR}^{model},\%$				99.36	99.41	99.65
$\delta^{model},\% = \left\|100 - \overline{RR}^{model}\right\| \le \max\delta^{model}$							0.64	0.59	0.35
				Approach 1		≤ 4.52%	satisfied	satisfied	satisfied
				Approach 2		≤ 2.05%	satisfied	satisfied	satisfied
$RSD^{model}_{RR},\%$							3.63	3.47	2.70
$\Delta^{model}_{RR},\% = RSD^{model}_{RR} \cdot t(95\%; g-1) \le \max\Delta^{model}_{sample}$							7.05	6.99	5.76
				Approach 1		≤ 10.00%	satisfied	satisfied	satisfied

Factual concentration of secnidazole in model solution ($C^{model}_{reference}$ = 20 μg/mL)		Absorbance A_i^{model}	Found in % to standard absorbance Y_i^{model},%	Calculated concentration of secnidazole in model solution $X^{model}_{i,calc}$,%			RR_i^{model},%		
C_i^{model}, μg/mL	$X^{model}_{i,fact}$,%			25 – 175%	25 – 150%	25 – 125%	25 – 175%	25 – 150%	25 – 125%
				Approach 2		≤ 4.52%	unsatisfied	unsatisfied	unsatisfied
0.1 M NaOH									
5	25	0.187	25.52	26.21	25.64	25.37	104.84	102.57	101.48
10	50	0.360	49.00	49.64	49.40	49.33	99.28	98.81	98.66
15	75	0.541	73.75	74.34	74.45	74.58	99.12	99.26	99.44
20	100	0.734	100.00	100.54	101.01	101.37	100.54	101.01	101.37
25	125	0.899	122.52	123.02	123.81	124.35	98.41	99.04	99.48
30	150	1.094	149.09	149.53	150.69	–	99.69	100.46	–
35	175	1.294	176.34	176.72	–	–	100.99	–	–
$A^{model}_{reference} = 0.734$			$\overline{RR}^{model}$,%				100.41	100.19	100.09
$\delta^{model},\% = \left\|100 - \overline{RR}^{model}\right\| \le \max\delta^{model}$							0.41	0.19	0.09
				Approach 1		≤ 4.52%	satisfied	satisfied	satisfied
				Approach 2		≤ 2.05%	satisfied	satisfied	satisfied
$RSD^{model}_{RR},\%$							2.14	1.45	1.27
$\Delta^{model}_{RR},\% = RSD^{model}_{RR} \cdot t(95\%; g-1) \le \max\Delta^{model}_{sample}$							4.15	2.92	2.70
				Approach 1		≤ 10.00%	satisfied	satisfied	satisfied
				Approach 2		≤ 4.52%	satisfied	satisfied	satisfied

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

Factual concentration of secnidazole in model solution ($C^{model}_{reference}$ = 20 μg/mL)		Absorbance A_i^{model}	Found in % to standard absorbance Y_i^{model},%	Z_i^{model},%		
C_i^{model}, μg/mL	$X^{model}_{i,fact}$,%			25 – 175%	25 – 150%	25 – 125%
0.1 M HCl						
5	25	0.164	25.99	103.95	103.95	103.95
10	50	0.328	51.92	103.85	103.85	103.85
15	75	0.477	75.38	100.51	100.51	100.51
20	100	0.629	99.47	99.47	99.47	99.47
25	125	0.807	127.62	102.10	102.10	102.10
30	150	0.956	151.19	100.79	100.79	–
35	175	1.076	170.22	97.27	–	–
$A^{model}_{reference} = 0.632$		$\overline{Z}^{model}$,%		101.13	101.78	101.98
$\delta^{model},\% = \left\|100 - \overline{Z}^{model}\right\| \le \max\delta^{model}$				1.13	1.78	1.98
		Approach 1	≤ 4.52%	satisfied	satisfied	satisfied
		Approach 2	≤ 2.05%	satisfied	satisfied	satisfied
$RSD^{model}_{Z},\%$				2.40	1.84	1.99
$\Delta^{model}_{Z},\% = RSD^{model}_{Z} \cdot t(95\%; g-1) \le \max\Delta^{model}_{As}$				4.66	3.72	4.24
		Approach 1	≤ 14.14%	satisfied	satisfied	satisfied

Factual concentration of secnidazole in model solution ($C^{model}_{reference}$ = 20 μg/mL)		Absorbance A_i^{model}	Found in % to standard absorbance Y_i^{model},%	Z_i^{model},%		
C_i^{model}, μg/mL	$X^{model}_{i,fact}$,%			25 – 175%	25 – 150%	25 – 125%
		Approach 2	≤ 6.40%	satisfied	satisfied	satisfied
96% C_2H_5OH						
5	25	0.225	26.38	105.50	105.50	105.50
10	50	0.436	51.07	102.15	102.15	102.15
15	75	0.642	75.19	100.25	100.25	100.25
20	100	0.858	100.47	100.47	100.47	100.47
25	125	1.065	124.70	99.76	99.76	99.76
30	150	1.297	151.85	101.24	101.24	–
35	175	1.507	176.39	100.80	–	–
$A^{model}_{reference} = 0.854$		$\overline{Z}^{model}$,%		101.45	101.56	101.62
$\delta^{model},\% = \left\|100 - \overline{Z}^{model}\right\| \le \max\delta^{model}$				1.45	1.56	1.62
		Approach 1	≤ 4.52%	satisfied	satisfied	satisfied
		Approach 2	≤ 2.05%	satisfied	satisfied	satisfied
$RSD_Z^{model},\%$				1.94	2.10	2.35
$\Delta_Z^{model},\% = RSD_Z^{model} \cdot t(95\%; g-1) \le \max\Delta_{As}^{model}$				3.77	4.24	5.00
		Approach 1	≤ 14.14%	satisfied	satisfied	satisfied
		Approach 2	≤ 6.40%	satisfied	satisfied	satisfied
0.1 M KOH in CH_3OH						
5	25	0.194	23.75	95.01	95.01	95.01
10	50	0.422	51.76	103.52	103.52	103.52
15	75	0.645	79.07	105.42	105.42	105.42
20	100	0.825	101.14	101.14	101.14	101.14
25	125	1.057	129.68	103.74	103.74	103.74
30	150	1.238	151.88	101.25	101.25	–
35	175	1.447	177.43	101.39	–	–
$A^{model}_{reference} = 0.815$		$\overline{Z}^{model}$,%		101.64	101.68	101.77
$\delta^{model},\% = \left\|100 - \overline{Z}^{model}\right\| \le \max\delta^{model}$				1.64	1.68	1.77
		Approach 1	≤ 4.52%	satisfied	satisfied	satisfied
		Approach 2	≤ 2.05%	satisfied	satisfied	satisfied
$RSD_Z^{model},\%$				3.33	3.65	4.07
$\Delta_Z^{model},\% = RSD_Z^{model} \cdot t(95\%; g-1) \le \max\Delta_{As}^{model}$				6.48	7.35	8.68
		Approach 1	≤ 14.14%	satisfied	satisfied	satisfied
		Approach 2	≤ 6.40%	unsatisfied	unsatisfied	unsatisfied
0.1 M NaOH						
5	25	0.187	25.52	102.09	102.09	102.09
10	50	0.360	49.00	98.00	98.00	98.00
15	75	0.541	73.75	98.33	98.33	98.33
20	100	0.734	100.00	100.00	100.00	100.00
25	125	0.899	122.52	98.02	98.02	98.02
30	150	1.094	149.09	99.39	99.39	–

Factual concentration of secnidazole in model solution ($C^{model}_{reference}$ = 20 μg/mL)		Absorbance A_i^{model}	Found in % to standard absorbance Y_i^{model},%	Z_i^{model},%		
C_i^{model}, μg/mL	$X^{model}_{i,fact}$,%			25 – 175%	25 – 150%	25 – 125%
35	175	1.294	176.34	100.77	–	–
$A^{model}_{reference} = 0.734$		$\overline{Z}^{model}$,%		99.52	99.31	99.29
$\delta^{model},\% = \left\|100 - \overline{Z}^{model}\right\| \le \max \delta^{model}$				0.48	0.69	0.71
		Approach 1	≤ 4.52%	satisfied	satisfied	satisfied
		Approach 2	≤ 2.05%	satisfied	satisfied	satisfied
$RSD_Z^{model},\%$				1.55	1.58	1.77
$\Delta_Z^{model},\% = RSD_Z^{model} \cdot t(95\%; g-1) \le \max \Delta_{As}^{model}$				3.01	3.19	3.77
		Approach 1	≤ 14.14%	satisfied	satisfied	satisfied
		Approach 2	≤ 6.40%	satisfied	satisfied	satisfied

The total results of validation allow to point to the conclusion about acceptable *linearity*, *accuracy* and *precision* of three UV-spectrophotometric procedures (batches A, B and D) of secnidazole quantitative determination in the variant of the MCC and MS for all ranges of the method application and for both approaches to acceptability estimation. 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 secnidazole quantification.

The UV-spectrophotometric procedure C (solvent is 0.1 M potassium hydroxide solution in methanol) is characterized by the worst values of precision and accuracy, which are acceptable only within *Approach 1*). Taking into account the results of stability verification the procedure C should not be used for secnidazole quantitative determination.

For the most cases the procedures in the variant of MCC are characterized by the better values of precision and accuracy than for the variant of MS. That makes the variant of MCC 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 D (0.1 M sodium hydroxide solution is used as s solvent), the worst ones – for the procedure C (0.1 M potassium hydroxide solution in methanol is used as s solvent). The reason of the phenomenon is apparently the existence of the most stable form of secnidazole in aqueous alkali and its borderline state in methanol alkali.

CONCLUSIONS

Three new procedures of secnidazole quantitative determination by the method of UV-spectrophotometry have been developed using 0.1 M hydrochloric acid solution, 96% ethanol and 0.1 M sodium hydroxide solution as the solvents (wavelengths λ_{max} are 277 nm, 310 nm and 319 nm respectively). Their validation by such parameters as stability, linearity, accuracy and precision in the variants of the method of calibration curve and method of standard has been carried out and acceptability for application has been shown.

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