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.

REFERENCES

1. Brook I. Spectrum and treatment of anaerobic infections. *J Infect Chemother*, 2016, *22(1)*, 1–13.
2. Lamp KC, Freeman CD, Klutman NE, Lacy MK. Pharmacokinetics and pharmacodynamics of the nitroimidazole antimicrobials. *Clin Pharmacokinet*, 1999, *36(5)*, 353–373.
3. Jarrad AM, Debnath A, Miyamoto Y, Hansford KA, Pelingon R, Butler MS, Bains T, Karoli T, Blaskovich MA, Eckmann L, Cooper MA. Nitroimidazole carboxamides as antiparasitic agents targeting Giardia lamblia, Entamoeba histolytica and Trichomonas vaginalis. *Eur J Med Chem*, 2016, *120*, 353–362.
4. Sobel R, Sobel JD. Metronidazole for the treatment of vaginal infections. *Expert Opin Pharmacother*, 2015, *16(7)*, 1109–1115.
5. Castelli M, Malagoli M, Ruberto AI, Baggio A, Casolari C, Cermelli C, Bossa MR, Rossi T, Paolucci F, Roffia S. In-vitro studies of two 5-nitroimidazole derivatives. *J Antimicrob Chemother*, 1997, *40(1)*, 19–25.
6. Mandalapu D, Kushwaha B, Gupta S, Singh N, Shukla M, Kumar J, Tanpula DK, Sankhwar SN, Maikhuri JP, Siddiqi MI, Lal J, Gupta G, Sharma VL. 2-Methyl-4/5-nitroimidazole derivatives potentiated against sexually transmitted Trichomonas: Design, synthesis, biology and 3D-QSAR study. *Eur J Med Chem*, 2016, *124*, 820–839.
7. Pasupuleti V, Escobedo AA, Deshpande A, Thota P, Roman Y, Hernandez AV. Efficacy of 5-nitroimidazoles for the treatment of giardiasis: a systematic review of randomized controlled trials. *PLoS Negl Trop Dis*, 2014, *8(3)*, e2733.
8. Thulkar J, Kriplani A, Agarwal N. A comparative study of oral single dose of metronidazole, tinidazole, secnidazole and ornidazole in bacterial vaginosis. *Indian J Pharmacol*, 2012, *44(2)*, 243–245.
9. Upcroft P, Upcroft JA. Drug targets and mechanisms of resistance in the anaerobic protozoa. *Clin Microbiol Rev*, 2001, *14(1)*, 150–164.
10. Church DL, Rabin HR, Laishley EJ. Reduction of 2-, 4- and 5-nitroimidazole drugs by hydrogenase 1 in Clostridium pasteurianum. *J Antimicrob Chemother*, 1990, *25(1)*, 15–23.
11. Kedderis GL, Argenbright LS, Miwa GT. Covalent interaction of 5-nitroimidazoles with DNA and protein in vitro: mechanism of reductive activation. *Chem Res Toxicol*, 1989, *2(3)*, 146–149.
12. Videau D, Niel G, Siboulet A, Catalan F. Secnidazole. A 5-nitroimidazole derivative with a long half-life. *Br J Vener Dis*, 1978, *54(2)*, 77–80.
13. Symonds J. Secnidazole – a nitroimidazole with a prolonged serum half-life. *J Antimicrob Chemother*, 1979, *5(4)*, 484–486.
14. Gillis JC, Wiseman LR. Secnidazole. A review of its antimicrobial activity, pharmacokinetic properties and therapeutic use in the management of protozoal infections and bacterial vaginosis. *Drugs*, 1996, *51(4)*, 621–638.
15. Li X, Sun J, Wang G, Zheng Y, Yan B, Xie H, Gu Y, Ren H. Determination of secnidazole in human plasma by high-performance

liquid chromatography with UV detection and its application to the bioequivalence studies. *Biomed Chromatogr*, 2007, *21(3)*, 304–309.
16. Ravi SK, Naidu MU, Sekhar EC, Rao TR, Shobha JC, Rani PU, Surya KJ. Rapid and selective analysis of secnidazole in human plasma using high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl*, 1997, *691(1)*, 208–211.
17. El Wallily AF, Abdine HH, Razak OA, Zamel S. Spectrophotometric and HPLC determination of secnidazole in pharmaceutical tablets. *J Pharm Biomed Anal*, 2000, *22(6)*, 887–897.
18. Mitrowska K, Antczak M. Development and validation of a liquid chromatography with tandem mass spectrometry method for the determination of nitroimidazole residues in beeswax. *J Sep Sci*, 2017, doi: 10.1002/jssc.201600928.
19. Hernández-Mesa M, D'Orazio G, Rocco A, García-Campaña AM, Blanco CC, Fanali S. Capillary electrochromatography-mass spectrometry for the determination of 5-nitroimidazole antibiotics in urine samples. *Electrophoresis*, 2015, *36(20)*, 2606–2615.
20. Rúbies A, Sans G, Kumar P, Granados M, Companyó R, Centrich F. High-throughput method for the determination of nitroimidazoles in muscle samples by liquid chromatography coupled to mass spectrometry. *Anal Bioanal Chem*, 2015, *407(15)*, 4411–4421.
21. Du J, Zhang Y, Chen Y, Liu D, Chen X, Zhong D. Enantioselective HPLC determination and pharmacokinetic study of secnidazole enantiomers in rats. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2014, *965*, 224–230.
22. Sun H, Wang F, Ai L, Guo C, Chen R. Validated method for determination of eight banned nitroimidazole residues in natural casings by LC/MS/MS with solid-phase extraction. *J AOAC Int*, 2009, *92(2)*, 612–621.
23. Sun HW, Wang FC, Ai LF. Simultaneous determination of seven nitroimidazole residues in meat by using HPLC-UV detection with solid-phase extraction. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2007, *857(2)*, 296–300.
24. Bakshi M, Singh S. ICH guidance in practice: establishment of inherent stability of secnidazole and development of a validated stability-indicating high-performance liquid chromatographic assay method. *J Pharm Biomed Anal*, 2004, *36(4)*, 769–775.
25. Tenenbaum H, Cuisinier FJ, Le Liboux A, Pichard E, Montay G, Frydman A. Secnidazole concentrations in plasma and crevicular fluid after a single oral dose. *J Clin Periodontol*, 1993, *20(7)*, 505–508.
26. Zhu DQ, Hu KL, Tao WX, Feng L, Duan H, Jiang XG, Chen J. Evaluation of the bioequivalence and pharmacokinetics of two formulations of secnidazole after single oral administration in healthy volunteers. *Arzneimittelforschung*, 2007, *57(11)*, 723–726.
27. Khan S, Haseeb M, Baig MH, Bagga PS, Siddiqui HH, Kamal MA, Khan MS. Improved efficiency and stability of secnidazole – An ideal delivery system. *Saudi J Biol Sci*, 2015, *22(1)*, 42–49.
28. Darwish KM, Salama I, Mostafa S, El-Sadek M. Extractional spectrophotometric analysis of metronidazole, tinidazole, ornidazole and secnidazole bases through acid-dye complexation using bromothymol blue dye. *Pak J Pharm Sci*, 2012, *25(1)*, 207–217.
29. Saffaj T, Charrouf M, Abourriche A, Abboud Y, Bennamara A, Berrada M. Spectrophotometric determination of metronidazole and secnidazole in pharmaceutical preparations. *Farmaco*, 2004, *59(10)*, 843–846.
30. Danzer K, Otto M, Currie LA. Guidelines for calibration in analytical chemistry. Part 2. Multispecies calibration. *Pure Appl Chem*, 2004, *76(6)*, 1215–1225.
31. Klimenko LYu, Petyunin GP. Development of approaches to validation of UV-spectrophotometric methods of quantitative determination in forensic and toxicological analysis: linearity and application range. *Farmatsevtychnyi chasopys*, 2014, *2(30)*, 46–51.
32. Klimenko LYu, Petyunin GP, Trut SM, Moroz VP. [Acceptability criteria for linear dependence when validating UV-spectrophotometric methods of quantitative determination in forensic and toxicological analysis]. *Current issues in pharmacy and medicine: science and practice*, 2014, *2(15)*, 15–22 [Article in Russian].
33. Klimenko LYu, Trut SM, Petyunin GP, Kostina TA. Determining accuracy in validation of UV-spectrophotometric methods of quantitative measurement in forensic toxicological analysis. *Ukrainian Biopharmaceutical Journal*, 2014, *2(31)*, 55–67.
34. Klimenko LYu, Trut SM, Mykytenko OYe. Approaches to determination of precision for UV-spectrophotometric methods of quantitative determination in forensic and toxicological analysis. *Farmatsyia Kazakhstana*, 2014, *3(154)*, 44–48.
35. Klimenko LYu. [Development of approaches to determination of linearity, accuracy and precision of UV-spectrophotometric methods of quantitative determination by the method of standard in forensic and toxicological analysis]. *Farmatsyia Kazakhstana*, 2014, *4(155)*, 31–35 [Article in Russian].
36. Klimenko LYu. The integrated approach to development and validation of the procedures of analytes quantification in biological fluids for chemical and toxicological analysis, DSc thesis, National University of Pharmacy (Kharkiv, Ukraine, 2016) [in Russian].
37. State Pharmacopoeia of Ukraine, SE «Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines», Kharkiv, 2016, 2nd ed.
38. Gryzodub OI. Standardized validation procedures for methods of medicines quality control, SE «Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines», Kharkiv, 2016.
39. Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens, United Nations Office on Drugs and Crime, Laboratory and Scientific Section, New York, 2009.
40. Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem material, Pharmaceutical Press, London, 2011, 4th ed.