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O. V. Shovkova, L. Yu. Klimenko, Z. V. Shovkova, M. O. Savchenko

National University of Pharmacy
53, Pushkinska str., Kharkiv, 61002, Ukraine. E-mail: lina_klimenko@nuph.edu.ua

Development and validation of GLC/FID- and GLC/MS-procedures of secnidazole determination by the methods of additions

Gas-liquid chromatography is widely used in the process of forensic toxicological examinations, but data about application of GLC with flame-ionization and mass-spectrometry detection for secnidazole determination in analytical toxicology are absent.

Aim. To develop GLC/FID- and GLC/MS-procedures for the quantitative determination of secnidazole and carry out step-by-step validation of the procedures developed in the variant of the method of additions.

Results and discussion. The chromatographic conditions has been chosen for secnidazole determination by the method of GLC in two variants of performance with flame-ionization and mass-spectrometry detection with the temperature program changing during the analysis from 70 °C to 250 °C or 320 °C. Retention times for secnidazole are 8.97 min and 11.74 min. To prove the possibility of application of the procedures proposed in further analysis their validation has been carried out in the variant of the method of additions. Such validation parameters as in-process stability, linearity, accuracy and precision have been estimated by model solutions.

Experimental part. The GLC/FID-analysis: HP 6890 Hewlett Packard; HP-1 Ø0.32 mm × 30 m, 0.25 μm; thermostat – 70 ºC (3 min), 40 ºC/min to 180 ºC (2 min), 40 ºC/min to 250 ºC (3 min); injector – 280 ºC; detector – 280 ºC; volume rate of a carrier gas (helium) – 1.5 ml/min; split mode – 1 : 2. The GLC/MS-analysis: Agilent 6890N/5973N/7683; HP-5MS Ø0.25 mm × 30 m, 0.25 μm; DB-17MS Ø0.25 mm × 30 m, 0.15 μm; columns are connected sequentially through Deans switch; thermostat – 70 ºC (2 min), 45 ºC/min. to 210 ºC, 6 ºC/min to 320 ºC (12.56 min); transfer line – 280 ºC; ion source – 230 ºC; quadrupole – 150 ºC; electron impact – 70eV; 40-750 m/z; injector – 250 ºC; splitless mode; inlet carrier gas (helium) pressure: 1st column – 26.06 psi, 2nd column – 19.30 psi.

Conclusions. New procedures for the quantitative determination of secnidazole by the method of GLC/FID and GLC/MS have been developed. Their validation has been carried out, and acceptability for application has been shown.

Key words: secnidazole; gas-liquid chromatography; validation

О. В. Шовкова, Л. Ю. Клименко, З. В. Шовкова, М. О. Савченко

Розробка та валідація ГРХ/ПІД- та ГРХ/МС-методик визначення секнідазолу методом добавок

Газо-рідинна хроматографія широко використовується в судово-токсикологічних дослідженнях, але дані про застосування ГРХ з полум'яно-іонізаційним і мас-спектрометричним детектуванням для визначення секнідазолу в аналітичній токсикології відсутні.

Мета. Розробити ГРХ/ПІД- і ГРХ/МС-методики кількісного визначення секнідазолу та провести поетапну валідацію розроблених методик у варіанті методу добавок.

Результати та їх обговорення. Хроматографічні умови були підібрані для визначення секнідазолу методом ГРХ у двох варіантах виконання з використанням полум'яно-іонізаційного і мас-спектрометричного детектування з програмованою зміною температури при аналізі від 70 °С до 250 °С або 320 °С. Час утримування для секнідазолу становить 8,97 хв і 11,74 хв. Для доказу можливості застосування пропонованих методик у подальшому аналізі було проведено їх валідацію у варіанті методу добавок. Такі валідаційні параметри, як стабільність, лінійність, правильність і прецизійність були оцінені за допомогою модельних розчинів.

Експериментальна частина. ГРХ/ПІД-аналіз: HP 6890 Hewlett Packard; HP-1 Ø0,32 мм × 30 м, 0,25 мкм; термостат – 70 ºС (3 хв), 40 ºС/хв до 180 ºС (2 хв), 40ºС/хв до 250 ºС (3 хв); інжектор – 280 ºС; детектор – 280 ºС; об'ємна швидкість газу-носія (гелію) – 1,5 мл/хв; розділення потоку – 1 : 2. ГРХ/МС-аналіз: Agilent 6890N/5973N/7683; HP-5MC Ø0,25 мм × 30 м, 0,25 мкм; DB-17MS Ø0,25 мм × 30 м, 0,15 мкм; колонки підключені послідовно через перемикач Діна; термостат – 70 ºС (2 хв), 45 ºС/хв до 210 ºС, 6 ºС/хв до 320 ºС (12,56 хв); інтерфейс мас-спектрометра – 280 ºС; джерело іонів – 230 ºС; квадруполь – 150 ºС; електронний удар – 70 еВ; 40-750 m/z; інжектор – 250 ºС; без розділення потоку; тиск газу-носія (гелію) на вході: 1-а колонка – 26,06 psi, 2-а колонка – 19,30 psi.

Висновки. Розроблені нові методики кількісного визначення секнідазолу методами ГРХ/ПІД і ГРХ/МС. Проведено їх валідацію і показано прийнятність для застосування.

Ключові слова: секнідазол; газо-рідинна хроматографія; валідація

О. В. Шовковая, Л. Ю. Клименко, З. В. Шовковая, М. А. Савченко

Разработка и валидация ГЖХ/ПИД- и ГЖХ/МС-методик определения секнидазола методом добавок

Газо-жидкостная хроматография широко используется в судебно-токсикологических исследованиях, но данные о её применении с пламенно-ионизационной и масс-спектрометрической детекцией для определения секнидазола в аналитической токсикологии отсутствуют.

Цель. Разработать ГЖХ/ПИД- и ГЖХ/МС-методики количественного определения секнидазола и провести поэтапную валидацию разработанных методик в варианте метода добавок.

Результаты. Хроматографические условия были подобраны для определения сенидазола методом ГЖХ в двух вариантах исполнения с использованием пламенно-ионизационной и масс-спектрометрической детекции с программируемым изменением температуры при анализе от 70 °С до 250 °С или 320 °С. Время удерживания для секнидазола составляет 8,97 мин и 11,74 мин. Для доказательства возможности применения предлагаемых методик в дальнейшем анализе была проведена их валидация в варианте метода добавок. Такие валидационные параметры, как стабильность, линейность, правильность и прецизионность были оценены с помощью модельных растворов.

Экспериментальная часть. ГЖХ/ПИД-анализ: HP 6890 Hewlett Packard; HP-1 Ø0,32 мм × 30 м, 0,25 мкм; термостат – 70 ºС (3 мин), 40 ºС/мин до 180 ºС (2 мин), 40 ºС/мин до 250 ºС (3 мин); инжектор – 280 ºС; детектор – 280 ºС; объемная скорость газа-носителя (гелия) – 1,5 мл/мин; разделение потока – 1 : 2. ГЖХ/МС-анализ: Agilent 6890N/5973N/7683; HP-5MC Ø0,25 мм × 30 м, 0,25 мкм; DB-17MS Ø0,25 мм × 30 м, 0,15 мкм; колонки подключены последовательно через переключатель Дина; термостат – 70 ºС (2 мин), 45 ºС/мин до 210 ºС, 6 ºС/мин до 320 ºС (12,56 мин); интерфейс масс-спектрометра – 280 ºС; источник ионов – 230 ºС; квадруполь – 150 ºС; электронный удар – 70 эВ; 40-750 m/z; инжектор – 250 ºС; без разделения потока; давление газа-носителя (гелия) на входе: 1-я колонка – 26,06 psi, 2-я колонка – 19,30 psi.

Выводы. Разработаны новые методики количественного определения секнидазола методами ГЖХ/ПИД и ГЖХ/МС. Проведена их валидация и показана приемлемость для применения.

Ключевые слова: секнидазол; газо-жидкостная хроматография; валидация

Gas-liquid chromatography (GLC) with different types of detection is widely used in the process of forensic toxicological examinations for screening and confirming investigations – with the purpose of detection, identification and determination of analytes [1, 2]. The method is applied in the analysis of 5-nitroimidazole derivatives [3-5], but data about application of GLC with flame-ionization (FID) and mass-spectrometry (MS) detection for secnidazole determination in analytical toxicology are absent.

Secnidazole is one of 5-nitroimidazole derivatives, which is characterized by a prolonged serum half-life [6, 7] and widely used for treatment of protozoal diseases [8, 9]. Secnidazole is 1-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol and has the structural formula as shown on Fig. 1.

The aim of our paper was to develop GLC/FID- and GLC/MS-procedures for the quantitative determination of secnidazole and carry out step-by-step validation of the procedures developed in the variant of the method of additions [10, 11] in order to confirm their acceptability for further application in analytical toxicology.

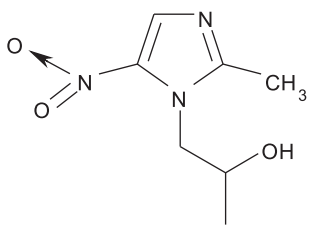


Fig. 1. The chemical structure of secnidazole

Results and discussion

Since secnidazole was readily soluble and stable in aqueous solutions, distilled water was proposed by us for preparation of the reference and model solutions in developing GLC/FID- and GLC/MS-procedures for the quantitative determination of secnidazole.

Previously, the chromatographic conditions were chosen for secnidazole determination by the method of gas-liquid chromatography in two variants of performance with flame-ionization and mass-spectrometry detection with the temperature program changing during the analysis from 70 °C to 250 °C or 320 °C, respectively. The typical chromatograms of secnidazole are presented in Fig. 2 and 3.

The mass-spectrum of secnidazole obtained under the GLC/MS-conditions proposed is presented in Fig. 4.

To prove the possibility of application of the procedures proposed in further analysis their validation was in the variant of the method of additions [10, 11].

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

The validation provides application of the normalized coordinates:

$$X_i = \frac{C_i}{C_{st}} \cdot 100\ \%;\ \ Y_i = \frac{S_i}{S_{st}} \cdot 100\ \%, \qquad (1)$$

were: i. e. transition from the equation $S_i = b_i \cdot C_i + a_i$ to the equation $Y_i = b_2 \cdot X_i + a_2$, it allows to calculate the validation characteristics, which do not depend on the analyte and features of the method of analysis [12, 13].

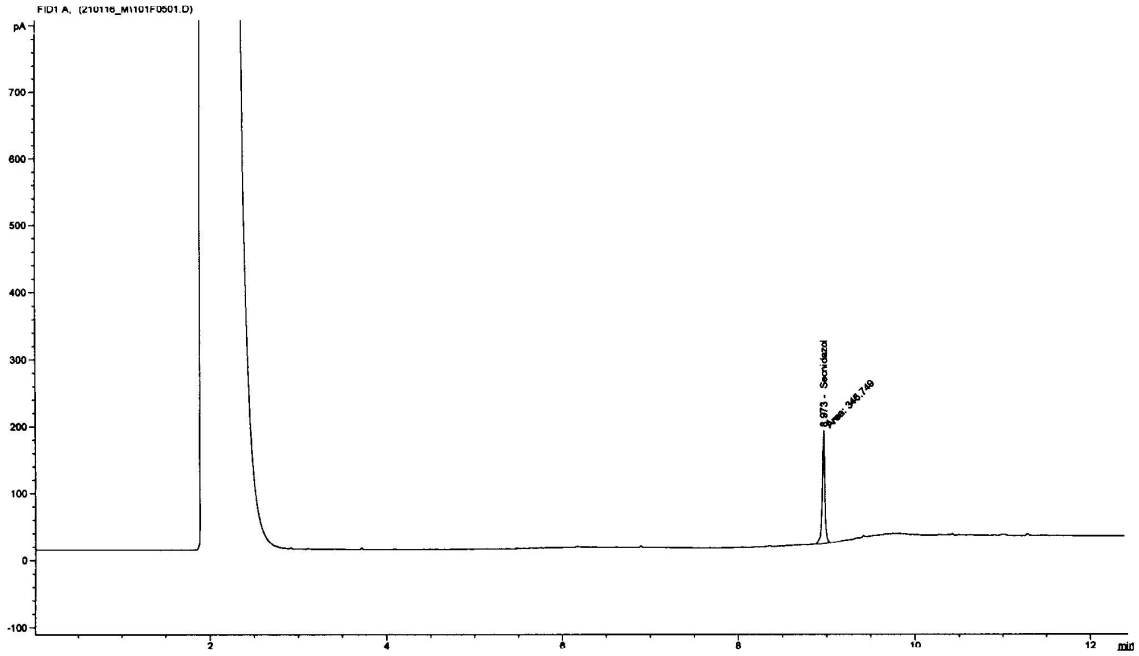


Fig. 2. The typical chromatogram of secnidazole under the proposed GLC/FID-conditions

The secnidazole concentration in the model solution for the point of 100 % in the normalized coordinates $C_{100\,\%}^{model}$ was chosen as the concentration provided the "signal/noise" ratio at the level of 40 [10].

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

The analytical range D of the method application was 25-175 %; the number of concentration levels g equaled 7 in constant increments of 25 %.

Acceptability criteria for validation parameters were formed on the basis of systematic application of the "insignificance concept" [12, 13] and proceeding from the value of the extreme uncertainty Δ_{As}, which equaled 20 % for the method in analytical toxicology [1, 14].

Acceptability criteria for validation parameters were calculated proceeding from the assumption that uncertainty of the analyte quantification in model solutions Δ_{As}^{model} was insignificant as compared with the total uncertainty Δ_{As}:

$$\begin{aligned} \max\Delta_{As}^{model} &= 0.32 \cdot \max_{model}\Delta_{As} = 0.32 \cdot 20.00\,\% = 6.40\,\%; \\ \max\delta^{model} &= 0.32 \cdot \max\Delta_{As}^{model} = 0.32 \cdot 6.40\,\% = 2.05\,\%. \end{aligned} \quad (2)$$

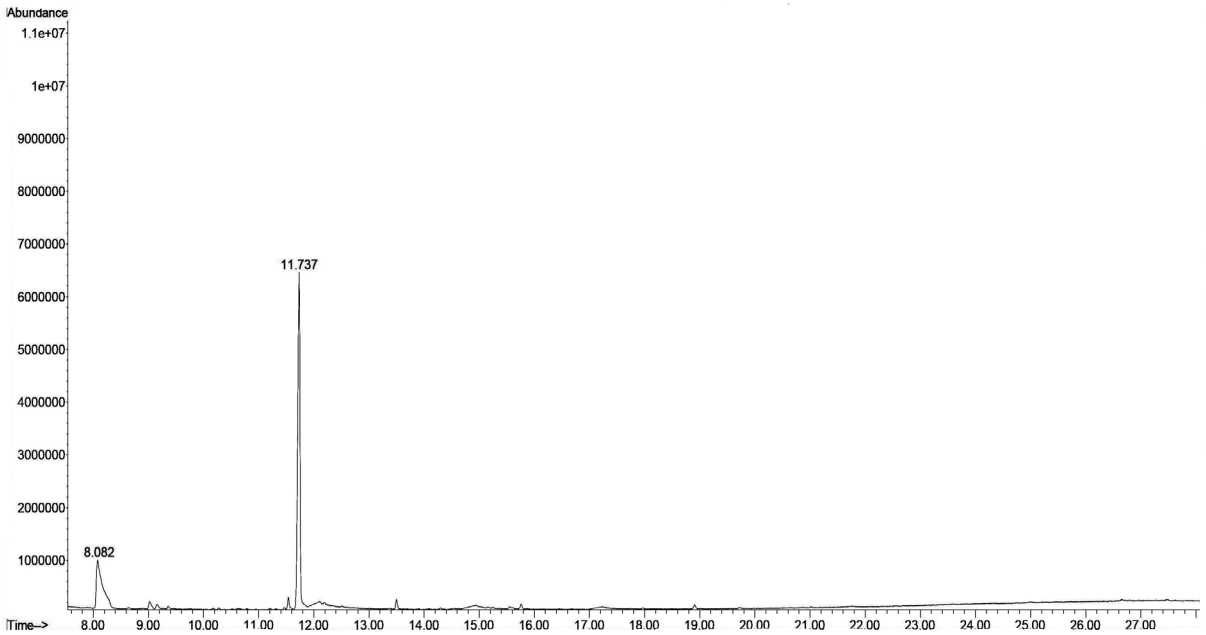


Fig. 3. The typical chromatogram of secnidazole under the proposed GLC/MS-conditions

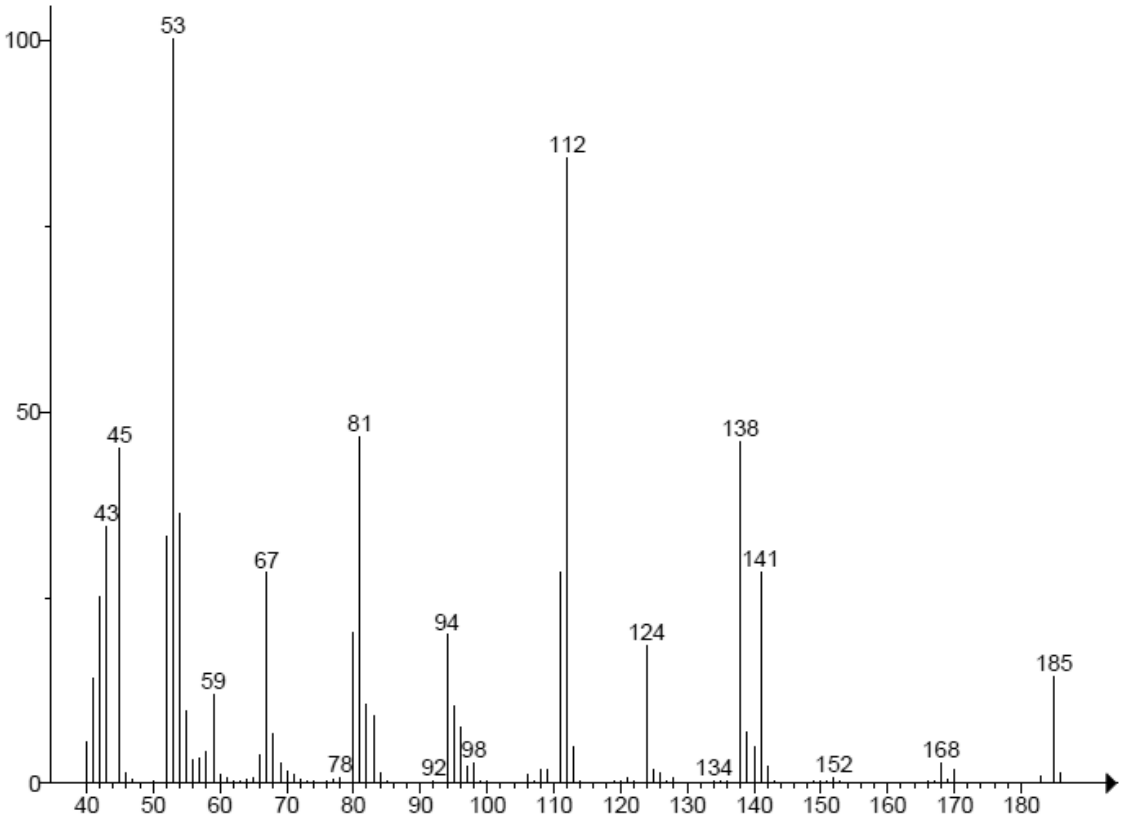


Fig. 4. The mass-spectrum of secnidazole

Validation results. *In-process stability* of secnidazole in the model solution was verified by chromatographing the reference solution immediately and in 1, 12, 24 and 48 hours after its preparation, and the systematic error $\sigma^{model\ stability}$ was calculated and assessed (Tab. 1). *In-process stability* of secnidazole in model solutions satisfied the acceptability criteria for all periods of time.

To determine *linearity/calibration model* the model solutions 1-7 were analyzed within 1 run, the correlation coefficient R_c^{model}, the rest standard deviation RSD_0^{model}, as well as the absolute term a^{model} were calculated and assessed (Tab. 2).

To estimate *precision and accuracy* the model solutions 8.1-13.1 and 8.2-13.2 were analyzed within 1 run, concentrations of the model solutions 8.1-13.1 were recalculated:

$$X_{ad}^{model}=\frac{C_{ad}^{model}\cdot V_{ad}}{C_{reference}^{model}\cdot V_{m.f}}\cdot 100\ \%;\ X_{i,fact}^{model\ MA}=\frac{C_i^{model\ MA}}{C_{reference}^{model}}\cdot 100\ \%;$$
$$X_{i,\ calc}^{model\ MA}=X_{ad}^{model}\cdot\frac{S_i^{model\ MA}}{S_{i+ad}^{model\ MA}-S_i^{model\ MA}}. \quad (3)$$

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
GLC/FID						
$S^{model\ stability}$	346	344	346	347	344	341
$S_0^{model\ stability}-S_t^{model\ stability}$	–	2	0	1	2	5
$\sigma^{model\ stability}\le 2.05\ \%$	–	0.58	0.00	0.29	0.58	1.45
		satisfied	satisfied	satisfied	satisfied	satisfied
GLC/MS						
$S^{model\ stability}$	147270	148145	148747	147869	147235	148102
$S_0^{model\ stability}-S_t^{model\ stability}$	–	875	1477	599	35	832
$\sigma^{model\ stability}\le 2.05\ \%$	–	0.59	1.00	0.41	0.02	0.56
		satisfied	satisfied	satisfied	satisfied	satisfied

Table 2

The results of linearity verification of secnidazole determination procedures by the method of gas-liquid chromatography in the variant of the method of additions

Parameter	Values		Acceptability criterion
	GLC/FID	**GLC/MS**	
b^{model}	1.020	0.998	–
s_b^{model}	0.014	0.012	–
a^{model}	–2.353	1.622	≤ 2.73 %
s_a^{model}	1.514	1.369	$a^{model} \le 2.015 \cdot s_a^{model}$
RSD_0^{model}	1.792	1.620	≤ 3.18 %
R_c^{model}	0.9996	0.9996	≥ 0.9983

The values "found/given" $RR_i^{model\ MA}$, % were calculated and used to determine the confidence interval $\Delta_{RR}^{model\ MA}$ and the systematic error $\sigma^{model\ MA}$, respectively:

$$RR_i^{model\ MA} = \frac{X_{i,\,calc}^{model\ MA}}{X_{i,\,fact}^{model\ MA}} \cdot 100\ \%;$$

$$\Delta_{RR}^{model\ MA} = t(95\,\%;\ n-1) \cdot RSD_{RR}^{model\ MA} \le \max \Delta_{As}^{model} = 6.40\,\%; \quad (4)$$

$$\delta^{model\ MA} = \left|100 - \overline{RR}^{model\ MA}\right| \le \max \delta^{model} = 2.05\ \%.$$

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

The results obtained within one analytical run are presented in Tab. 3-4.

The total results of validation allow making the conclusion about acceptable *linearity*, *accuracy* and *precision* of GLC/FID- and GLC/MS-procedures for the quantitative determination of secnidazole in the variant of the method of additions. It gives us the possibility to recommend this procedure for further application in analytical toxicology with the purpose of development of methods of biological liquids analysis for the quantitative determination of secnidazole.

Experimental part

Secnidazole was of pharmacopoeial purity.

Instrumentation and chromatographic conditions. The GLC/FID-analysis conditions were as follows:

- device – HP 6890 Hewlett Packard;
- column – HP-1 Ø0.32 mm × 30 m, 0.25 μm, 100 % dimethylpolysiloxane;

Table 3

The results of accuracy and precision verification of GLC/FID-procedure for secnidazole determination in the variant of the method of additions

Factual concentration of secnidazole in the model solution ($C_{reference}^{model}$ = 8 μg/mL)		Peak area		Calculated concentration of secnidazole in the model solution $X_{i,\,calc}^{model\ MA}$,%	$RR_i^{model\ MA}$, %
$C_i^{model\ MA}$, μg/mL	$X_{i,\,fact}^{model\ MA}$, %	$S_i^{model\ MA}$	$S_{i+ad}^{model\ MA}$		
2	25	84	345	24.14	96.55
2	25	88	347	25.48	101.93
4	50	174	436	49.81	99.62
6	75	254	518	72.16	96.21
8	100	351	615	99.72	99.72
8	100	348	609	100.00	100.00
				$\overline{RR}^{model\ MA}$, %	99.00
				$\sigma^{model\ MA}$, % = $\left\| 100 - \overline{RR}^{model\ MA} \right\|$	1.00
				$\sigma^{model\ MA} \le \max\sigma^{model}$ = 2.05 %	satisfied
				$RSD_{RR}^{model\ MA}$,%	2.20
				$\Delta_{RR}^{model\ MA} = t(95\,\%;\ n-1) \cdot RSD_{RR}^{model\ MA}$	4.44
				$\Delta_{RR}^{model\ MA} \le \max \Delta_{As}^{model}$ = 6.40 %	satisfied

Table 4

The results of accuracy and precision verification of GLC/MS-procedure for secnidazole determination in the variant of the method of additions

Factual concentration of secnidazole in the model solution ($C_{reference}^{model}$ = 8 μg/mL)		Peak area		Calculated concentration of secnidazole in the model solution $X_{i,calc}^{model\ MA}$,%	$RR_i^{model\ MA}$, %
$C_i^{model\ MA}$, μg/mL	$X_{i,fact}^{model\ MA}$, %	$S_i^{model\ MA}$	$S_{i+ad}^{model\ MA}$		
2	25	37965	149587	25.51	102.04
2	25	38654	152326	25.50	102.01
4	50	74458	184457	50.77	101.53
6	75	112365	225478	74.50	99.34
8	100	147789	258458	100.16	100.16
8	100	148754	263256	97.44	97.44
$\overline{RR}^{model\ MA}$, %					100.42
$\sigma^{model\ MA}, \% = \lvert 100 - \overline{RR}^{model\ MA} \rvert$					0.42
$\sigma^{model\ MA} \le \max\sigma^{model} = 2.05\ \%$					satisfied
$RSD_{RR}^{model\ MA}$,%					1.82
$\Delta_{RR}^{model\ MA} = t(95\ \%; n-1) \cdot RSD_{RR}^{model\ MA}$					3.67
$\Delta_{RR}^{model\ MA} \le \max \Delta_{As}^{model} = 6.40\ \%$					satisfied

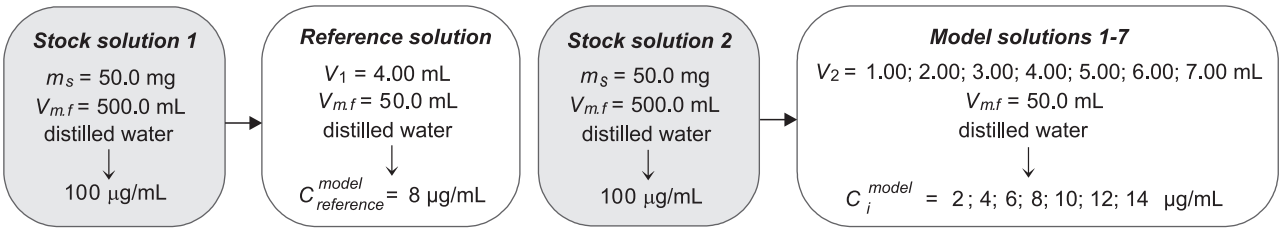
- temperature of the column thermostat – 70 °C (3 min), increasing the temperature with the rate of 40 °C/min to 180 °C (keeping for 2 min), increasing the temperature with the rate of 40 °C/min to 250 °C (keeping for 3 min);
- injector temperature – 280 °C;
- detector – flame-ionization;
- detector temperature – 280 °C;
- volume rate of a carrier gas (helium) – 1.5 ml/min;
- split mode – 1 : 2;
- the volume of injection – 2 μL.

The GLC/MS-analysis conditions were as follows:

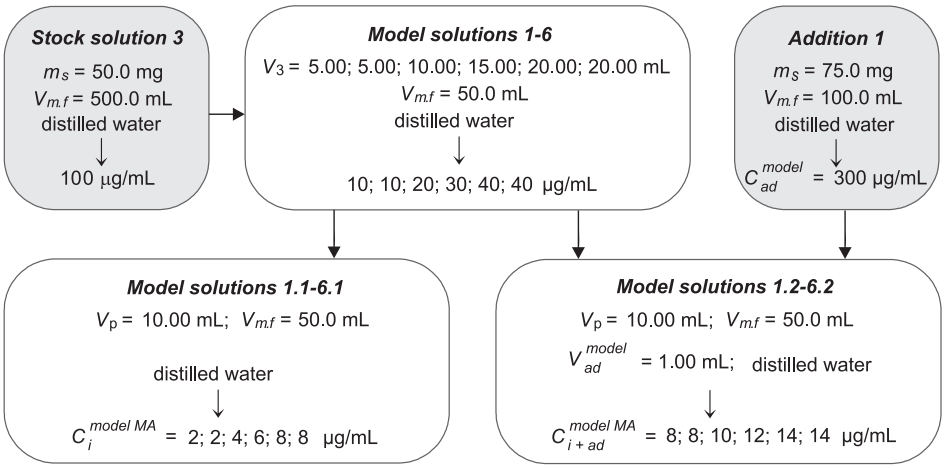
- device – Agilent 6890N Gas Chromatograph;
- columns – 1) HP-5MS ø0.25 mm × 30 m, 0.25 μm, 5 % diphenyllpolysiloxan/95 % dimethylpolysiloxan; 2) DB-17MS Ø0.25 mm × 30 m, 0.15 μm, 5 % diphenyllpolysiloxan/50 % dimethylpolysiloxan; columns were connected sequentially through Deans switch;
- temperature of the column thermostat – 70 °C (2 min), increasing the temperature with the rate of 45 °C/min to 210 °C, increasing the temperature with the rate of 6 °C/min to 320 °C (keeping for 12.56 min);
- detector – mass spectrometer Agilent 5973N MSD with a turbo pump;
- transfer line temperature – 280 °C; ion source temperature – 230 °C; quadrupole temperature – 150 °C;
- ionization mode – electron impact; electron energy – 70eV;
- scanning range – 40-750 m/z; threshold – 110;
- injector – Agilent 7683 Injector/Autosampler;
- injector temperature – 250 °C;
- splitless mode;
- inlet carrier gas (helium) pressure: 1st column – 26.06 psi, 2nd column – 19.30 psi;
- the volume of injection – 1 μL.

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

The 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 glas-



Scheme 1. The preparation procedure of reference and model solutions of secnidazole for the "linearity/calibration model" study



Scheme 2. The preparation procedure of model solutions of secnidazole for the accuracy and precision study

sware – 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" [15] was used throughout this study.

Reference and model solutions (Scheme 1 and 2)**.** The stock solutions 1, 2 and 3 (100 μg/mL) were prepared by dissolving 50.0 mg of secnidazole in distilled water, and the solutions were diluted to 500.0 mL with the same solvent. The reference solution (8 μg/mL) was prepared by diluting 4.00 mL of the stock solution 1 to 50.0 mL with distilled water. The stock solution 2 was diluted with distilled water to prepare the model solutions 1-7 with the concentrations of 2; 4; 6; 8; 10; 12 and 14 μg/mL, respectively.

The addition solution 1 (300 μg/mL) was prepa red by dissolving 60.0 mg of secnidazole in distilled water, and the solution was diluted to 200.0 mL with the same solvent. The stock solution 3 was diluted with distilled water to prepare the model solutions 8 – 13 with the concentrations of 10; 10; 20; 30; 40; 40 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 distilled water. To prepare 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.

When carrying out experiments each solution (except the in-process stability study) was chromatographed 3 times or more, as required, following the requirements to repeatability of peak areas *S* for replicate injections offered by us [10] – the relative standard deviation of the mean RSD_{nom} calculated towards the nominal value of the peak area S_{nom} should not exceed:

$$RSD_{nom} = \frac{s}{S_{nom}} \cdot 100\ \% \le \max RSD_{nom} =$$

$$= \frac{0.1 \cdot \max \Delta_{As} \cdot \sqrt{n}}{t(95\ \%;\ n-1)} = \left\{ \begin{matrix} 1.21\ \%;\ n = 3 \\ 1.74\ \%;\ n = 4 \\ 2.15\ \%;\ n = 5 \\ 2.49\ \%;\ n = 6 \end{matrix} \right\}, \quad (5)$$

where: S_{nom} – is the mean peak area obtained when analyzing the model solution 1. The mean values were used in further calculations.

Conclusions

New procedures for the quantitative determination of secnidazole by the method of GLC/FID and GLC/MS have been developed. Their validation by such parameters as stability, linearity, accuracy and precision in the variants of the method of additions has been carried out, and acceptability for application has been shown.

Conflict of interests: authors have no conflict of interests to declare.

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