Oksana V. Shovkova et al /J. Pharm. Sci. & Res. Vol. 10(8), 2018, 2056-2064

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Study of secnidazole extraction from aqueous solutions

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**Abstract**

Secnidazole is a 5-nitroimidazole derivative and potential object of chemical toxicological investigations. The purpose of the work is to establish the optimal conditions of application of secnidazole extraction in the chemical toxicological analysis of biological objects for extraction purification and drug isolation. Taking into account the amphoteric properties of secnidazole we have studied the conditions of secnidazole extraction from aqueous solutions with application of liquid-liquid extraction with chloroform and the mixture of chloroform and 2-propanol (8:2); also the same experiment has been carried out after previous saturation of the solution with ammonium sulphate. Extraction procedures with application of acetonitrile, 2-propanol and ethanol have been also carried out in two variants – extraction with amphiphylic solvents followed by «salting out» with ammonium sulphate and «salting out» with ammonium sulphate followed by extraction with amphiphylic solvents. The dependences of extraction recovery of secnidazole from aqueous solutions on the medium pH have been set. Such extraction and validation parameters as «specificity/selectivity» and «recovery» for the most effective extraction procedures have been calculated. The conditions of sample preparation for biological objects (extraction purification of aqueous extracts from coextractive substances, isolation of secnidazole from aqueous extracts) for further quantitative determination of secnidazole have been proposed using both types of liquid extraction – separately and by combined scheme.

**Keywords:** secnidazole, extraction, recovery, specificity/selectivity

## INTRODUCTION

The modern pharmaceutical market is widely represented by antimicrobial drugs including medicines of the group of 5-nitroimidazole derivatives – metronidazole, ornidazole, tinidazole, etc. [1 – 4]. Secnidazole is one of the representatives of this group of medicines; it differs from other compounds of this group by prolonged serum half-life [5 – 7].

Administration of secnidazole is accompanied by a number of side effects such as unpleasant (metallic) taste and dry mouth, nausea, diarrhoea, abdominal pain, vomiting, headache, dizziness, depressive and convulsive reactions, skin itch, etc. [5 – 7].

One of the properties of secnidazole, like other derivatives of 5-nitroimidazole, is blocking the enzymes of alcohol dehydrogenase and acetaldehyde dehydrogenase, therefore when joint taking the medicines of this group with alcohol it is observed the strong intoxication syndrome manifested by intense vomiting, constant nausea, sharp headache, etc.; there is a «disulfiram-like response», when a person feels sudden blood rush to the head and upper body, feeling of difficulty in breathing, tinnitus, sharp reduction of blood pressure, tachycardia, and «death anxiety» [8 – 11]. Fatal poisonings with 5-nitroimidazole derivatives have been recorded in the case of taking with alcohol [12].

Based on the mentioned above we can make the conclusion that secnidazole is a potential object of chemical toxicological investigations.

The first stage of chemical toxicological analysis is the sample preparation of biological objects. One of the ways of such sample preparation is extraction with organic solvents [13, 14].

Since secnidazole is the substance of amphoteric nature [15], it is not possible to reliably predict its behaviour in the process of extraction with organic solvents. Therefore, it is necessary to carry out the model experiment that allows to determine the dependence of extraction recovery on the pH of aqueous medium, as well as to prove the stability of extraction recovery of the substance within certain concentration range.

The purpose of our work is to establish the optimal conditions of application of secnidazole extraction in the chemical toxicological analysis of biological objects for extraction purification and drug isolation.

## MATERIALS AND METHODS

### Reagents and chemicals

Secnidazole was of pharmacopoeial purity and obtained from the pharmaceutical company «Zdorovie» Ltd. Acetonitrile (99.8%, anhydrous), hydrochloric acid (≥37%, puriss. p.a., ACS reagent, fuming), chloroform (≥99%, anhydrous, contains 0.5 – 1.0% of ethanol as stabilizer), 2-propanol (LC-MS CHROMASOLV®), ammonium hydroxide solution (≥25% $NH_3$ in $H_2O$, puriss. p.a. plus) were purchased from Sigma-Aldrich Co. LLC (USA). All other reagents (96% ethanol, sodium hydroxide, sodium sulphate anhydrous, and ammonium sulphate) were of analytical grade.

### 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» 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. Part 2. Multispecies calibration» [16] was used throughout this study.

### Reference and stock solutions

The stock solution 1 (10 mg/mL) was prepared by dissolving 1.0000 g of secnidazole in the distilled water and the solution was diluted to 100.0 mL with the same solvent.

The stock solution 1 was diluted with the distilled water to prepare the standard solutions 1 – 3 having concentrations of 250; 500 and 1000 μg/mL respectively (correspond to the points of 25%, 50% and 100% in the normalized coordinates).

The stock solutions 2 (250 μg/mL) were prepared by dissolving 50.0 mg of secnidazole in the solvent (0.1 M hydrochloric acid solution, 96% ethanol or 0.1 M sodium hydroxide solution) and the solutions were diluted to 200.0 mL with the same solvent.

The model solutions (5, 10 and 20 μg/mL) were prepared by diluting 1.00, 2.00 or 4.00 mL of the stock solutions 2 to 50.0 mL with the respective solvent.

### Extraction procedures (Scheme 1)

10.00 mL of distilled water or 10.00 mL of distilled water acidified with 6 M hydrochloric acid solution to pH = 2, or 10.00

mL of distilled water acidified with 0.1 M hydrochloric acid solution to pH = 5, or 10.00 mL of distilled water alkalified with 25% ammonium hydroxide solution to pH = 9, or 10.00 mL of distilled water alkalified with 10% sodium hydroxide solution to pH = 12 were placed into the separation funnel, 1.00 ml of secnidazole standard solution was added and the mixture was shaken.

*Variant 1:* ammonium sulphate was added to the mixture till stopping its dissolution and the mixture was shaken for 15 minutes. Then the mixture was extracted with 10.00 mL of chloroform or the mixture of chloroform and 2-propanol (8:2) for 15 minutes. The obtained organic extract was separated, filtered through the paper filter with 1 g of sodium sulphate anhydrous (wetted with chloroform) into the measuring flask with the capacity of 25.0 mL, and diluted to the volume with chloroform (organic extract).

*Variant 2:* the mixture was extracted with 10.00 mL of chloroform or the mixture of chloroform and 2-propanol (8:2) for 15 minutes. The obtained organic extract was separated, filtered through the paper filter with 1 g of sodium sulphate anhydrous (wetted with chloroform) into the measuring flask with the capacity of 25.0 mL, and diluted to the volume with chloroform (organic extract).

*Variant 3:* ammonium sulphate was added to the mixture till stopping its dissolution and the mixture was shaken for 15 minutes. Then 10.00 mL of acetonitrile, 2-propanol or 96% ethanol were added to the aqueous solution and the mixture was shaken for 15 minutes. Top organic layer was separated, filtered through the paper filter with 1 g of sodium sulphate anhydrous (wetted with the organic solvent) into the measuring flask with the capacity of 25.0 mL, and diluted to the volume with the organic solvent (organic extract).

*Variant 4:* 10.00 mL of acetonitrile, 2-propanol or 96% ethanol were added to the aqueous solution and the mixture was shaken for 15 minutes. Then the mixture was «salted out» by adding ammonium sulphate till stopping its dissolution. Top organic layer was separated, filtered through the paper filter with 1 g of sodium sulphate anhydrous (wetted with the organic solvent) into the measuring flask with the capacity of 25.0 mL, and diluted to the volume with the organic solvent (organic extract).

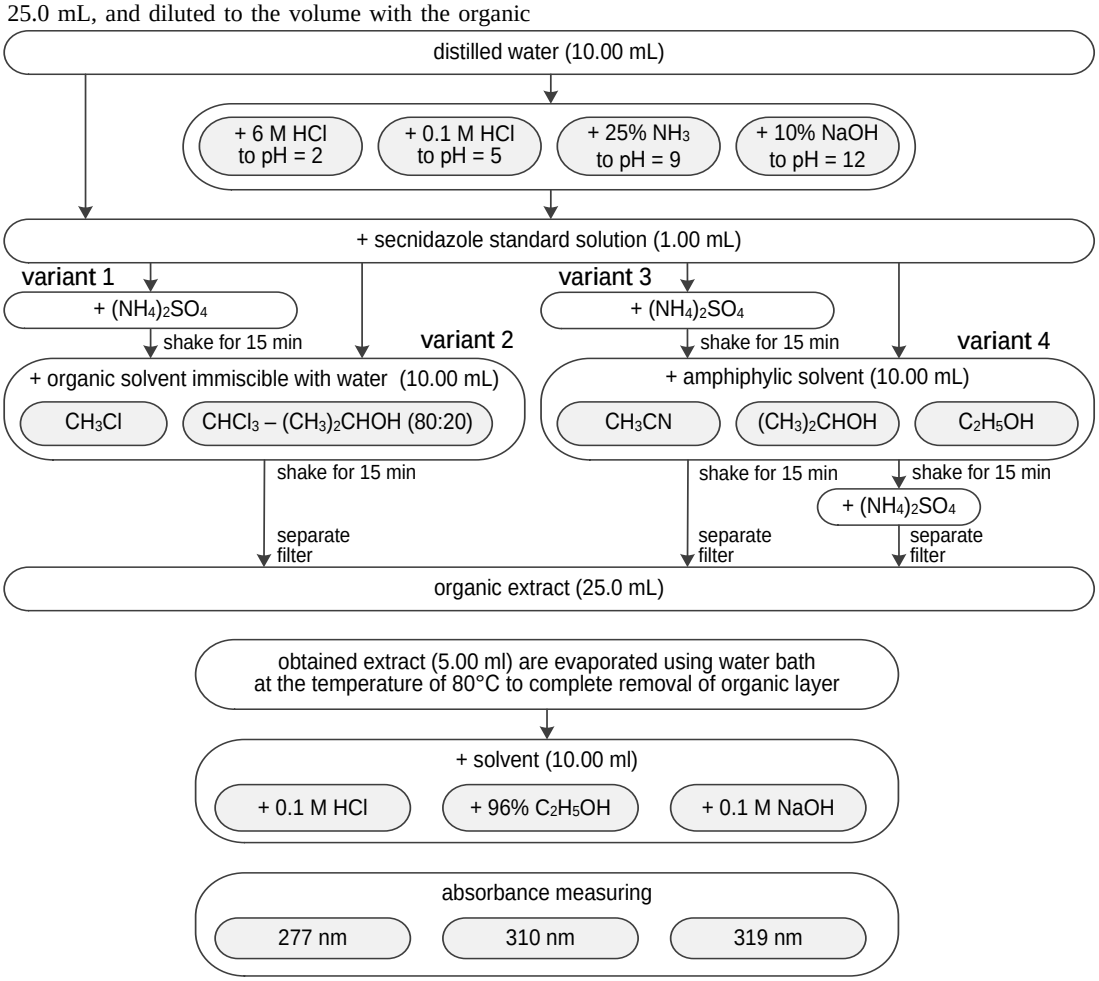
Three aliquots in 5.00 ml each of the obtained organic extract were evaporated using water-bath at the temperature of 80°C to complete removal of organic layer. The dry residues were dissolved in 10.00 ml of the respective solvents (0.1 M hydrochloric acid solution, 96% ethanol or 0.1 M sodium hydroxide solution).

The absorbance of the solutions to be analysed $A_i^{extraction}$ was measured 3 times at λ = 277 nm, 310 nm and 319 nm respectively with randomization of cell position and mean values were used for calculations. The respective solvents were used as the compensation solutions.

The same series of *blank*-experiments was performed using 1.00 ml of distilled water instead of 1.00 ml of secnidazole standard solution ( $A_{procedure,i}^{extraction}$ ).

The described experiments were carried out within 3 runs.

The absorbance of the reference solutions $A_i^{model}$ was measured under the same conditions.




Scheme 1. The main stages of secnidazole extraction procedures

1 analysis of *blank*-solutions; $n = 3$, $m = 3$

$$A^{extraction}_{procedure,i} \rightarrow \overline{A}^{extraction}_{procedure}$$

$$RSD_{nom}(extraction) = \frac{S}{A_{nom}^{*}} \cdot 100\% \leq 2.68\%$$

$^{*}A_{nom} = 0.1$ ($\overline{A}^{extraction}_{procedure} / 0.32 \leq 0.1$)

$A_{nom} = \overline{A}^{extraction}_{procedure} / 0.32$ ($\overline{A}^{extraction}_{procedure} / 0.32 > 0.1$)

2 analysis of solutions; $n = 3$, $k = 3$, $m = 3$ (*3 runs / 1 day*)

$$C^{extraction}_{i} \cong A^{extraction}_{i} \cong 25, 50, 100\% \rightarrow \overline{A}^{extraction}_{i}$$

$$RSD_{nom}(solution) = \frac{S}{A_{nom}^{**}} \cdot 100\% \leq 5.93\%$$

$^{**}A_{nom} = \overline{A}^{extraction}_{25\%}$

3 analysis of model solutions; $n = 3$, $m = 3$

$$A^{model}_{i}$$

4 *extraction specificity/selectivity*

$$\delta^{extraction}_{procedure} = \frac{\overline{A}^{extraction}_{procedure}}{\overline{A}^{extraction}_{100\%}} \cdot 100\% \leq \max\delta_{procedure} = 2.56\%$$

5 *extraction recovery*

$$R^{extraction}_{i} = \frac{\overline{A}^{extraction}_{i} - \overline{A}^{extraction}_{procedure}}{A^{model}_{i}} \cdot 100\%$$

$$\overline{R}^{extraction}_{i} = \frac{\sum R^{extraction}_{i}}{n}; \quad \overline{R}^{extraction} = \frac{\sum R^{extraction}_{i}}{n \cdot m}$$

$$RSD^{extraction}_{R} = \sqrt{\frac{\sum (RSD^{extraction,m}_{R})^2}{m}}$$

$$\Delta^{extraction}_{R,r} = t(95\%; n \cdot m - 1) \cdot RSD^{extraction}_{R} \leq 10.00\%$$

Scheme 2. The main stages of calculation of extraction and validation parameters

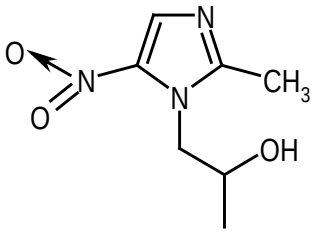



Figure 1. Chemical structure of secnidazole

## RESULTS AND DISCUSSION

Secnidazole (1-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol, Figure 1), as previously shown [15], can be described by the following transformation in aqueous solutions when changing the medium pH (Figure 2).

On the one hand, such transformations are assumed as a basis for development of the methods of secnidazole determination by the method of UV-spectrophotometry using different solvents – 0.1 M hydrochloric acid solution, 96% ethanol and 0.1 M sodium hydroxide solution [15]. On the other hand, such transformations cause amphoteric properties of secnidazole, presence of two tautomeric forms of secnidazole in different ratios at certain pH values, and also its unpredictable behaviour within the standard extraction procedures.

Taking into account all mentioned above we have studied the conditions of secnidazole extraction from aqueous solutions with application of common liquid-liquid extraction with organic solvents immiscible with water, which widely used in chemical toxicological analysis and presented at Scheme 1 (variant 1) [13, 14]. Chloroform and the mixture of chloroform and 2-propanol (8:2) have been used as organic solvents. Also the same experiment has been carried out after previous saturation of the solution with ammonium sulphate (Scheme 1, variant 2). Such type of processing is used for purification of aqueous extracts from biological objects before liquid-liquid extraction with organic solvents and can increase the extraction efficiency [17, 18].

The high values of extraction recovery can be reached when applying amphiphylic solvents and separation of organic layer with electrolyte [18]. This type of extraction procedures has been also carried out by us in two variants – extraction with amphiphylic solvent followed by «salting out» with electrolyte (Scheme 1, variant 3) and «salting out» with electrolyte followed by extraction with amphiphylic solvent (Scheme 1, variant 4). Acetonitrile, 2-propanol and ethanol have been used as amphiphylic solvents; ammonium sulphate has been applied as an electrolyte.

Usually the dependence of extraction recovery of analyte from aqueous solutions on the medium pH is studied experimentally using buffer solutions with certain pH values [19]. Also the dependence of extraction recovery on pH can be investigated using acids and alkalis [20], as it is accepted for sample preparation in chemical toxicological analysis [13, 14]. As previously shown [20], the differences in the results of determination of extraction recovery by different methods of analysis are observed in the case of application of acids and alkalis for pH creation, and also higher values of *blank*-absorbances are fixed that worsens the specificity of procedure.

Therefore, in our experiments the necessary pH values were created with 6 M and 0.1 M solutions of hydrochloric acid (to pH = 2 and 5 respectively), 25% ammonium hydroxide solution (to pH = 9) or 10% sodium hydroxide solution (to pH = 12).

In all cases, extraction recovery was determined for a single processing of aqueous solution with an organic solvent for 15 minutes at the concentration levels of secnidazole corresponded to the points of 25%, 50% and 100% in the normalized coordinates. For each extraction procedure the experiments were carried out within three runs ($k = 3$) during one day.

The amount of extracted medicine was determined by three methods ($m = 3$) using 0.1 M hydrochloric acid solution, 96% ethanol and 0.1 M sodium hydroxide solution and measuring the absorbance at $\lambda = 277$ nm, 310 nm and 319 nm respectively.

Such extraction and validation parameters as «specificity/selectivity» and «recovery» have been calculated according to Scheme 2 supposed by us taking into account the recommendations [21 – 24].

At the *Stage 1* the *blank*-experiment (processing of *blank*-solutions) is carried out by three methods of determination ($m = 3$). The mean *blank*-absorbances $\overline{A}^{extraction}_{procedure}$ for each method of determination are calculated using the obtained values of $A^{extraction}_{procedure,i}$, and repeatability of individual values of $A^{extraction}_{procedure,i}$ is checked.

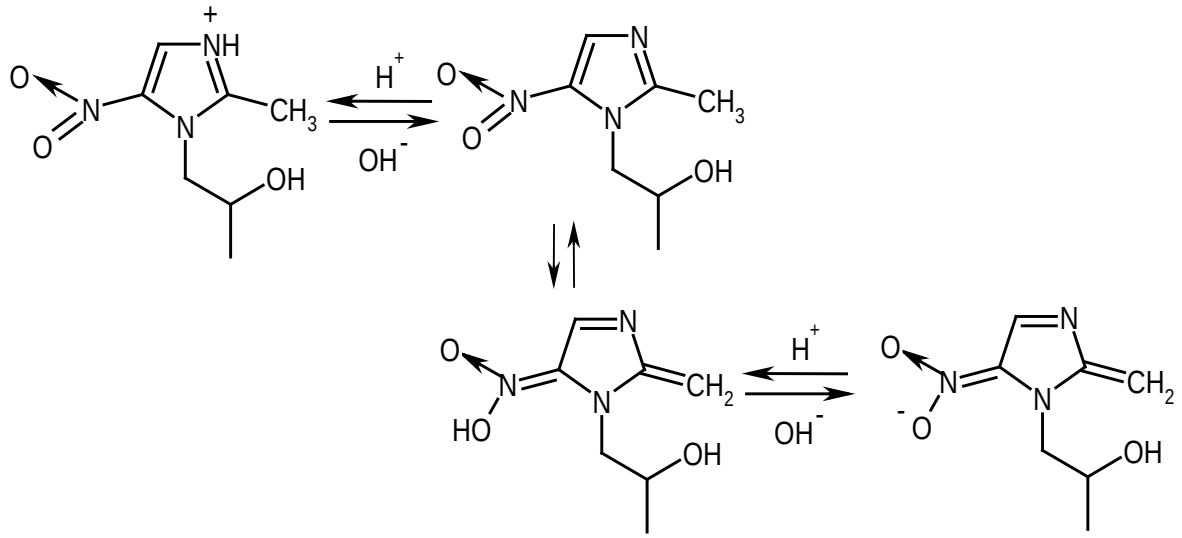


Figure 2. Possible transformations in the secnidazole solutions when changing the medium pH

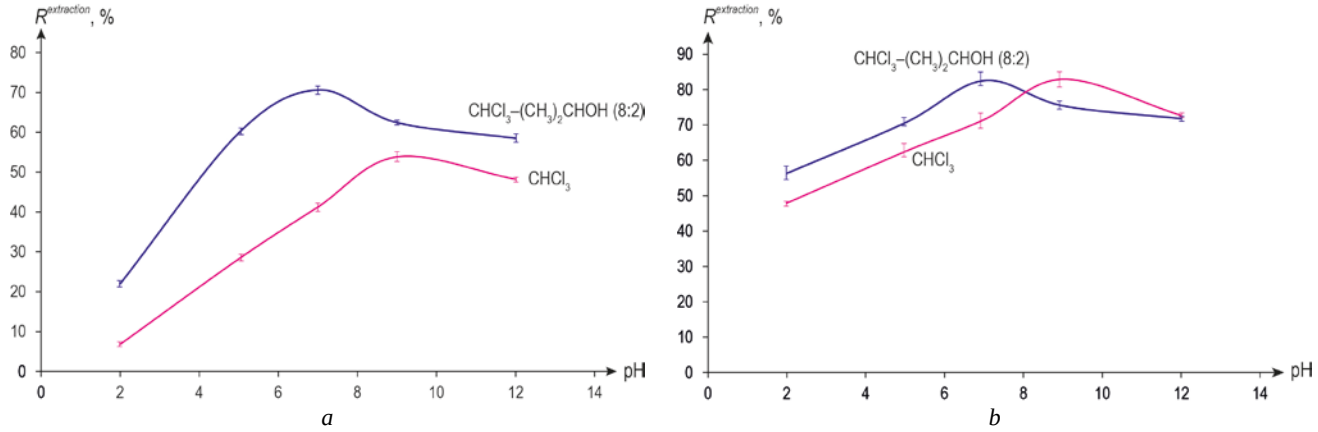



Figure 3. Dependence of the secnidazole extraction recovery from the medium pH:
*a* – liquid-liquid extraction with organic solvents immiscible with water;
*b* – «salting out» with ammonium sulphate followed by liquid-liquid extraction with organic solvents immiscible with water

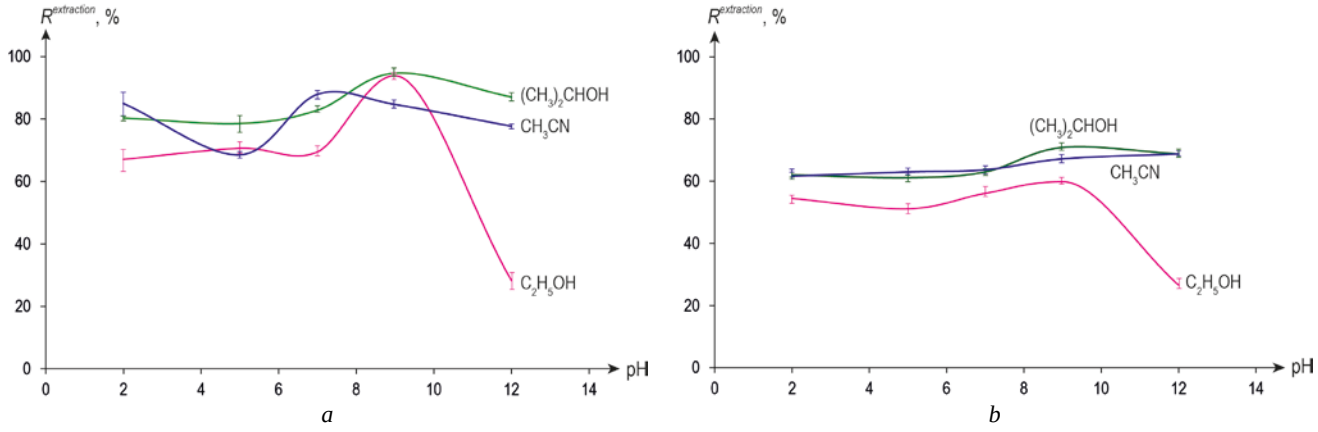



Figure 4. Dependence of the secnidazole extraction recovery from the medium pH:
*a* – extraction with amphiphylic solvents followed by «salting out» with ammonium sulphate;
*b* – «salting out» with ammonium sulphate followed by extraction with amphiphylic solvents

*Table 1*

**Results of determination of recovery and specificity for the procedure of secnidazole extraction with chloroform**

| **without previous «salting out» with ammonium sulphate (pH = 9)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration point | $\overline{A}_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
| | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm |
| 25% | 0.103 | 0.129 | 0.108 | 0.164 | 0.225 | 0.187 | 56.73 | 53.45 | 52.55 |
| 50% | 0.187 | 0.246 | 0.203 | 0.328 | 0.436 | 0.360 | 54.01 | 54.42 | 53.69 |
| 100% | 0.378 | 0.463 | 0.403 | 0.629 | 0.858 | 0.734 | 58.52 | 53.00 | 53.59 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 56.42 | 53.62 | 53.28 |
| | 0.010 | 0.008 | 0.010 | $\overline{R}^{extraction}$, % | | | 54.44 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 4.03 | 1.35 | 1.18 |
| | 2.56 | 1.82 | 2.45 | $RSD_R^{extraction}$, % | | | 2.55 | | |
| | satisfied | satisfied | satisfied | $\Delta_{R,r}^{extraction}$, % | | | 4.74 | ≤ 10.00% | satisfied |
| **with previous «salting out» with ammonium sulphate (pH = 9)** | | | | | | | | | |
| Concentration point | $\overline{A}_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
| | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm |
| 25% | 0.147 | 0.202 | 0.171 | 0.164 | 0.225 | 0.187 | 83.57 | 84.52 | 85.88 |
| 50% | 0.276 | 0.381 | 0.322 | 0.328 | 0.436 | 0.360 | 81.08 | 84.72 | 86.69 |
| 100% | 0.514 | 0.734 | 0.627 | 0.627 | 0.854 | 0.739 | 80.43 | 84.63 | 83.45 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 81.69 | 84.62 | 85.34 |
| | 0.010 | 0.012 | 0.010 | $\overline{R}^{extraction}$, % | | | 83.89 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 2.03 | 0.12 | 1.97 |
| | 1.92 | 1.57 | 1.58 | $RSD_R^{extraction}$, % | | | 1.64 | | |
| | satisfied | satisfied | satisfied | $\Delta_{R,r}^{extraction}$, % | | | 3.04 | ≤ 10.00% | satisfied |

At the *Stage 2* the main experiment is carried out for three concentration level ($n$ = 3) within three runs ($k$ = 3) by three methods of determination ($m$ = 3). The mean *blank*-absorbances $\overline{A}_i^{extraction}$ within all runs for each concentration level and method of determination are calculated using the obtained values of $A_i^{extraction}$, and repeatability of individual values of $A_i^{extraction}$ is checked.

At the *Stage 3* the absorbances of model solutions $A_i^{model}$ are measured for three concentration level ($n$ = 3) by three methods of determination ($m$ = 3).

At the *Stage 4* the values of $\delta_{procedure}^{extraction}$, % , for each method of determination are calculated relative to the solution to be analysed corresponding to the point of 100% in the normalized coordinates, and its acceptability is checked.

At the *Stage 5* the values of extraction recovery $R_i^{extraction}$, % , for each concentration level ($n$ = 3) and method of determination ($m$ = 3) within all runs ($k$ = 3) are calculated. Then the mean values of $\overline{R}_i^{extraction}$, % , for all concentration levels ($n$ = 3) by each method of determination ($m$ = 3) are calculated. The total mean value of $\overline{R}^{extraction}$, % , for all concentration levels ($n$ = 3) and for all methods of determination ($m$ = 3), and also the total relative standard deviation $RSD_R^{extraction}$, % , and total relative confidence interval $\Delta_{R,r}^{extraction}$, % , are calculated; reproducibility of the recovery values is checked.

The total results of our experiments are presented on Figure 3 – 4. The curves illustrate the dependence of the secnidazole extraction recovery on the medium pH.

Thus, we may see that in all cases the liquid-liquid extraction from aqueous solutions in the neutral and weak alkaline medium provides the sufficiently high levels of secnidazole isolation – both in the standard liquid-liquid extraction procedure and after previous «salting out» with ammonium sulphate.

It should be noted that variant 2 of extraction procedure (after previous «salting out» with ammonium sulphate) gives a higher values of recovery (by 10 – 30%) than for respective liquid-liquid extraction procedures without «salting out».

*Table 2*

**Results of determination of recovery and specificity for the procedure of secnidazole extraction with the mixture of chloroform and 2-propanol (8:2)**

| **without previous «salting out» with ammonium sulphate (pH = 7)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration point | $\overline{A}_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
| | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm |
| 25% | 0.128 | 0.176 | 0.146 | 0.164 | 0.225 | 0.187 | 72.95 | 71.75 | 71.17 |
| 50% | 0.242 | 0.330 | 0.274 | 0.328 | 0.436 | 0.360 | 71.24 | 72.32 | 72.75 |
| 100% | 0.449 | 0.620 | 0.534 | 0.629 | 0.858 | 0.734 | 70.11 | 70.55 | 71.15 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 71.43 | 71.54 | 71.69 |
| | 0.008 | 0.014 | 0.012 | $\overline{R}^{extraction}$, % | | | 71.55 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 2.00 | 1.26 | 1.28 |
| | 1.73 | 2.28 | 2.29 | $RSD_R^{extraction}$, % | | | 1.56 | | |
| | satisfied | satisfied | satisfied | $\Delta_{R,r}^{extraction}$, % | | | 2.89 | ≤ 10.00% | satisfied |
| **with previous «salting out» with ammonium sulphate (pH = 7)** | | | | | | | | | |
| Concentration point | $\overline{A}_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
| | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm |
| 25% | 0.151 | 0.192 | 0.161 | 0.164 | 0.225 | 0.187 | 85.13 | 80.03 | 80.31 |
| 50% | 0.282 | 0.365 | 0.304 | 0.328 | 0.436 | 0.360 | 82.71 | 80.83 | 81.56 |
| 100% | 0.517 | 0.713 | 0.638 | 0.627 | 0.854 | 0.739 | 80.73 | 82.08 | 84.76 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 82.85 | 80.98 | 82.21 |
| | 0.011 | 0.012 | 0.011 | $\overline{R}^{extraction}$, % | | | 82.01 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 2.66 | 1.27 | 2.79 |
| | 2.11 | 1.70 | 1.71 | $RSD_R^{extraction}$, % | | | 2.35 | | |
| | satisfied | satisfied | satisfied | $\Delta_{R,r}^{extraction}$, % | | | 4.36 | ≤ 10.00% | satisfied |

The maximum recovery of liquid-liquid extraction of secnidazole with chloroform is observed at pH = 9 (variant 1 – ≈55%, variant 2 – ≈85%). Addition of 2-propanol to chloroform increases the extraction recovery of secnidazole from aqueous solutions (variant 1 – ≈70%, variant 2 – ≈80%), but shifts the extraction maximum to neutral medium.

The recovery for liquid-liquid extraction (variant 1) of secnidazole with chloroform in the strong acid medium is too low (≈7%) that makes possible to recommend such processing mode for purification of aqueous extracts from biological material. After previous «salting out» with ammonium sulphate the value of extraction recovery at pH = 2 is increased till ≈45%, therefore we cannot carry out extraction purification in the strong acid medium after saturation of aqueous solution with ammonium sulphate.

Processing the aqueous solutions with amphiphylic solvents followed by «salting out» with ammonium sulphate in all cases allows to extract sufficiently high amounts of secnidazole (>80%) in all media excluding application of ethanol in the strong alkaline medium (≈30%). However, the extraction maximum is also fixed in the neutral and weak alkaline medium.

The high extraction efficiency with amphiphylic solvents (variant 3) in the strong acid medium allows to develop the procedures of sample preparation of biological objects with analyte isolation at pH < 2 that provide obtaining of the purer extracts than in the neutral and alkaline medium.

In the case of application of amphiphylic solvents with previous processing by ammonium sulphate (variant 4) the values of extraction recovery are decreased by 10 – 40% for all points of medium pH.

It should be noted that application of liquid-liquid extraction (both variants 1 and 2) and amphiphylic solvents with previous processing by ammonium sulphate (variant 4) provides the high specificity/selectivity of the procedure (*blank*-absorbance does not exceed 0.014); in the case of extraction with amphiphylic solvents followed by «salting out» with ammonium sulphate (variant 3) the values of *blank*-absorbance are higher by 2 – 3 times.

Detailed data of the values of recovery and specificity for the variants of extraction procedures with the best results are presented in Table 1 – 5.

Thus, in the case of choosing the procedure of sample preparation for biological objects with application of liquid-liquid extraction the following sequences of operations are optimal:

1) Acidifying to pH = 2 with 6 M solution of hydrochloric acid; extraction purification with chloroform at pH = 2; «salting out» with ammonium sulphate; neutralization to pH = 7 with 25% ammonium hydroxide solution; extraction with the mixture of chloroform and 2-propanol (8:2) at pH = 7;
2) Acidifying to pH = 2 with 6 M solution of hydrochloric acid; extraction purification with chloroform at pH = 2; «salting out» with ammonium sulphate; alkalifying to pH = 9 with 25% ammonium hydroxide solution; extraction with chloroform at pH = 9.

In the case of choosing the procedure of sample preparation for biological objects with amphiphylic solvents the following sequences of operations are optimal:
1) Acidifying to pH = 2 with 6 M solution of hydrochloric acid; extraction with acetonitrile or 2-propanol at pH = 2 followed by «salting out» with ammonium sulphate;
2) Acidifying to pH = 2 with 6 M solution of hydrochloric acid; alkalifying to pH = 9 with 25% ammonium hydroxide solution; extraction with ethanol or 2-propanol at pH = 9 followed by «salting out» with ammonium sulphate;
3) Acidifying to pH = 2 with 6 M solution of hydrochloric acid; neutralization to pH = 7 with 25% ammonium hydroxide solution; extraction with acetonitrile at pH = 7 followed by «salting out» with ammonium sulphate.

And also combined schemes of sample preparation are possible:
1) Acidifying to pH = 2 with 6 M solution of hydrochloric acid; extraction purification with chloroform at pH = 2; extraction with acetonitrile or 2-propanol at pH = 2 followed by «salting out» with ammonium sulphate;
2) Acidifying to pH = 2 with 6 M solution of hydrochloric acid; extraction purification with chloroform at pH = 2; alkalifying to pH = 9 with 25% ammonium hydroxide solution; extraction with ethanol or 2-propanol at pH = 9 followed by «salting out» with ammonium sulphate;
3) Acidifying to pH = 2 with 6 M solution of hydrochloric acid; extraction purification with chloroform at pH = 2; neutralization to pH = 7 with 25% ammonium hydroxide solution; extraction with acetonitrile at pH = 7 followed by «salting out» with ammonium sulphate.

*Table 3*

**Results of determination of recovery and specificity for the procedure of secnidazole extraction with 2-propanol followed by «salting out» with ammonium sulphate**

| **pH = 2** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration point | $\overline{A}_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
| | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm |
| 25% | 0.164 | 0.221 | 0.173 | 0.164 | 0.225 | 0.187 | 81.00 | 83.28 | 81.79 |
| 50% | 0.303 | 0.391 | 0.312 | 0.328 | 0.436 | 0.360 | 83.08 | 82.00 | 81.50 |
| 100% | 0.538 | 0.721 | 0.609 | 0.629 | 0.858 | 0.734 | 80.67 | 80.08 | 80.29 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 81.58 | 81.79 | 81.19 |
| | 0.030 | 0.033 | 0.019 | $\overline{R}^{extraction}$, % | | | 81.52 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 1.60 | 1.97 | 0.98 |
| | 5.66 | 4.61 | 3.18 | $RSD_R^{extraction}$, % | | | 1.57 | | |
| | unsatisfied | unsatisfied | unsatisfied | $\Delta_{R,r}^{extraction}$, % | | | 2.92 | ≤ 10.00% | satisfied |
| **pH = 9** | | | | | | | | | |
| Concentration point | $\overline{A}_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
| | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm |
| 25% | 0.170 | 0.242 | 0.197 | 0.164 | 0.225 | 0.187 | 93.64 | 93.44 | 95.85 |
| 50% | 0.327 | 0.451 | 0.356 | 0.328 | 0.436 | 0.360 | 94.52 | 96.08 | 94.13 |
| 100% | 0.605 | 0.855 | 0.712 | 0.629 | 0.858 | 0.734 | 93.61 | 95.96 | 94.63 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 93.92 | 95.16 | 94.87 |
| | 0.016 | 0.031 | 0.017 | $\overline{R}^{extraction}$, % | | | 94.65 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 0.55 | 1.57 | 0.93 |
| | 2.70 | 3.66 | 2.43 | $RSD_R^{extraction}$, % | | | 1.10 | | |
| | unsatisfied | unsatisfied | satisfied | $\Delta_{R,r}^{extraction}$, % | | | 2.04 | ≤ 10.00% | satisfied |

*Table 4*

**Results of determination of recovery and specificity for the procedure of secnidazole extraction with acetonitrile followed by «salting out» with ammonium sulphate**

| **pH = 2** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration point | $\overline{A}_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
| | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm |
| 25% | 0.165 | 0.227 | 0.169 | 0.164 | 0.225 | 0.187 | 83.91 | 90.98 | 81.67 |
| 50% | 0.295 | 0.415 | 0.322 | 0.328 | 0.436 | 0.360 | 81.76 | 89.94 | 84.92 |
| 100% | 0.544 | 0.784 | 0.637 | 0.629 | 0.858 | 0.734 | 82.12 | 88.67 | 84.62 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 82.60 | 89.86 | 83.74 |
| | 0.027 | 0.022 | 0.016 | $\overline{R}^{extraction}$, % | | | 85.40 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 1.39 | 1.28 | 2.14 |
| | 4.97 | 2.86 | 2.55 | $RSD_R^{extraction}$, % | | | 1.65 | | |
| | unsatisfied | unsatisfied | satisfied | $\Delta_{R,r}^{extraction}$, % | | | 3.07 | ≤ 10.00% | satisfied |
| **pH = 7** | | | | | | | | | |
| Concentration point | $\overline{A}_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
| | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm |
| 25% | 0.161 | 0.241 | 0.191 | 0.164 | 0.225 | 0.187 | 79.78 | 94.38 | 91.52 |
| 50% | 0.309 | 0.421 | 0.343 | 0.328 | 0.436 | 0.360 | 84.84 | 89.99 | 89.99 |
| 100% | 0.577 | 0.788 | 0.668 | 0.629 | 0.858 | 0.734 | 87.00 | 88.48 | 88.28 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 83.87 | 90.95 | 89.93 |
| | 0.030 | 0.028 | 0.020 | $\overline{R}^{extraction}$, % | | | 88.25 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 4.42 | 3.37 | 1.80 |
| | 5.22 | 3.58 | 2.96 | $RSD_R^{extraction}$, % | | | 3.37 | | |
| | unsatisfied | unsatisfied | unsatisfied | $\Delta_{R,r}^{extraction}$, % | | | 6.27 | ≤ 10.00% | satisfied |

*Table 5*

**Results of determination of recovery and specificity for the procedure of secnidazole extraction with 96% ethanol followed by «salting out» with ammonium sulphate**

| **pH = 2** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration point | $\overline{A}_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
| | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm |
| 25% | 0.195 | 0.263 | 0.206 | 0.164 | 0.225 | 0.187 | 90.06 | 88.86 | 88.97 |
| 50% | 0.356 | 0.465 | 0.370 | 0.328 | 0.436 | 0.360 | 94.01 | 92.11 | 91.78 |
| 100% | 0.633 | 0.859 | 0.716 | 0.629 | 0.858 | 0.734 | 93.08 | 92.69 | 92.16 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 92.38 | 91.22 | 90.97 |
| | 0.047 | 0.063 | 0.040 | $\overline{R}^{extraction}$, % | | | 91.52 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 2.23 | 2.26 | 1.92 |
| | 7.50 | 7.35 | 5.55 | $RSD_R^{extraction}$, % | | | 2.14 | | |
| | unsatisfied | unsatisfied | unsatisfied | $\Delta_{R,r}^{extraction}$, % | | | 3.99 | ≤ 10.00% | satisfied |

## CONCLUSIONS

The dependences of the liquid-liquid extraction recovery of secnidazole from aqueous solutions on the medium pH using chloroform and the mixture of chloroform and 2-propanol (8:2) have been set in two variants – after previous saturation of the solution with ammonium sulphate and without such processing. And also the dependences of the extraction recovery of secnidazole on the medium pH using acetonitrile, 2-propanol and ethanol have been set in two variants – after previous saturation of the solution with ammonium sulphate and with subsequent «salting out» with ammonium sulphate. Such extraction and validation parameters as «specificity/selectivity» and «recovery» for the most effective extraction procedures have been calculated. The conditions of sample preparation for biological objects (extraction purification of aqueous extracts from coextractive substances, isolation of secnidazole from aqueous extracts) for further quantitative determination of secnidazole have been proposed using both types of liquid extraction – separately and by combined scheme.

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