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# Modelling the processes of sample preparation of biological objects for the subsequent determination of metronidazole

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**Abstract**

Sample preparation is the first step of toxicological screening and should provide an appropriate level of analyte recovery from biological matrices. The aim is to research the metronidazole behaviour under the conditions of a number of methods commonly used for sample preparation of biological liquids and tissues in chemical toxicological analysis and to determine the validation parameters «specificity/selectivity» and «recovery» for the most effective procedures. Taking into account the amphoteric properties of metronidazole we have proposed the extraction procedures in two variants: 1) liquid-liquid extraction with organic solvents immiscible with water (chloroform and the mixture of chloroform and 2-propanol (8:2) were used as organic solvents); 2) extraction by amphiphylic solvents followed by «salting out» with ammonium sulphate (2-propanol, acetonitrile and ethanol were used as amphiphylic solvents). The medium pH have been created with 6 M and 0.1 M solutions of hydrochloric acid, 25% ammonium hydroxide solution or 10% sodium hydroxide solution, and also using universal buffer solutions. The amount of extracted medicine has been determined by three methods. The recovery for liquid-liquid extraction of metronidazole with chloroform in the strong acid medium is too low that makes possible to recommend such processing mode for purification of aqueous extracts from coextractive substances. Processing the aqueous solutions with amphiphylic solvents followed by «salting out» with ammonium sulphate in all cases allows to extract not less than 80% of metronidazole in all media excluding application of ethanol in the strong acid and alkaline medium.

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

## INTRODUCTION

Systematic toxicological analysis carries a huge number of degrees of freedom [1, 2]. And after the sampling procedure, perhaps, the procedure of sample preparation is the most significant. It should provide an appropriate level of analyte recovery from biological matrices [3, 4]. Very often authors of articles and different guidances do not recommend to achieve 100% of the analyte recovery, most importantly that its values should be reproducible and stable at the different concentration levels and provide the required level of the method sensitivity [5 – 7]. But what about the substances presented in biological objects in trace amounts? But it is not always possible to predict the required level of the method sensitivity. We consider that at the stage of developing the bioanalytical methods it is necessary to carry out the systematic researches to choose the conditions provided the least losses of analyte in the process of isolation. Such losses are conditionally divided into two types – the losses due to performing the sample preparation operations and the losses due to interaction of analyte with matrix. It is quite difficult to reduce the losses of the second type, but it is quite possible to minimize the operational losses.

Sample preparation is in effect the first step of toxicological screening, since the creation of certain conditions makes possible at this stage to divide the substances into certain classification groups. For example, extraction in the acid medium allows to isolate compounds with acid properties, in the alkaline medium – with basic and neutral properties, and at pH 12 – 13 – compounds with marked basic properties. And further analysis we carry out in narrowed circle of target substances [1 – 4].

The situation with substances of amphoteric properties is rather more complicated because they may probably be extracted by organic solvents from aqueous solutions both at acid and alkaline pH values, and also in the neutral medium, and the recovery values may not reach significant levels.

Our work is devoted to research of the metronidazole behaviour under the conditions of a number of methods commonly used for sample preparation of biological liquids and tissues in chemical toxicological analysis.

Metronidazole belongs to the group of medicines that are the derivatives of 5-nitroimidazoles. As a result of reducing 5-$NO_2$-group and its subsequent interaction with DNA of protozoa and some anaerobes it blocks their reproduction and leads to further cells death, so the medicine is used for treatment of infectious diseases caused by Trichomonas, Lamblia, Leishmania, etc., and also for eradication of Helicobacter pylori [8 – 14].

The purpose of our work is to carry out the extraction of metronidazole from aqueous solutions using different approaches and to determine and estimate the validation parameters «specificity/selectivity» and «recovery» for the most effective procedures to choose the optimal ways of sample preparation for their further transfer to real biological objects.

## MATERIALS AND METHODS

### Reagents and chemicals

Metronidazole 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, ammonium sulphate, concentrated acetic acid, concentrated phosphoric acid, boric acid) 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» [15] was used throughout this study.

**Reference and stock solutions**

The stock solution 1 (10 mg/mL) was prepared by dissolving 1.0000 mg of metronidazole 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 metronidazole 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, or 10.00 ml of universal buffer solution with pH from 2 to 12 [16] were placed into the separation funnel, 1.00 ml of metronidazole standard solution was added and the mixture was shaken.

*Variant 1:* 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:* 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).

Tree 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 residue is dissolved in 10.00 ml of the solvent (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 solvent was used as a compensation solution.

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

The described experiment was carried out within 3 runs/days.

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

## RESULTS AND DISCUSSION

Chemically, metronidazole is 2-methyl-5-nitroimidazole-1-ethanol and has the structural formula as shown on Figure 1.

As previously shown [17, 18], the transformation of 5-nitroimidazoles in aqueous solutions when changing the medium pH can be described by the following scheme (Figure 2):

The presence of such transformations effects on the nature of the metronidazole UV-spectra at the different pH values that has been used to develop the methods of its 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 [18].

Since the presented transformations are equilibrium and, respectively, are characterized by certain equilibrium constants, in aqueous solutions at certain pH values it is possible simultaneously the presence of two tautomeric forms of metronidazole in different ratios that, in turn, may effect on the extraction recovery of the medicine from aqueous solutions when changing the medium pH. That is, metronidazole is the typical ampholyte by its properties and it is not possible to reliably predict its behaviour within the standard extraction procedures.

Taking into account the amphoteric properties of metronidazole we proposed the procedure of extraction studies presented at Scheme 1.

Variant 1 – commonly used liquid-liquid extraction with organic solvents immiscible with water (chloroform and the mixture of chloroform and 2-propanol (8:2) were used as organic solvents).

Variant 2 – extraction by amphiphylic solvents followed by the separation of the organic layer under the conditions of aqueous phase saturation with electrolyte (2-propanol, acetonitrile and ethanol were used as amphiphylic solvents; ammonium sulphate has been applied as an electrolyte).

Usually the dependence of the extraction recovery of analyte from aqueous solutions on the medium pH is studied experimentally using buffer solutions with certain pH values [19]. And such experiments were performed by us at the first stage of investigations.

At the same time, in chemical toxicological analysis the necessary pH value is created using acids, ammonium hydroxide solution and alkalis [1 – 4]. And such experiments were performed by us at the second stage of investigations. The medium pH were created with 6 M and 0.1 M solutions of hydrochloric acid, 25% ammonium hydroxide solution or 10% sodium hydroxide solution.

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 metronidazole corresponded to the points of 25%, 50% and 100% in the normalized coordinates.

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

To confirm specificity/selectivity for each extraction procedure processing of *blank*-solutions was carried out.

The calculations of the extraction parameters and validation parameters «specificity/selectivity» and «recovery» was carried out according to the following scheme supposed by us taking into account the recommendations [20, 21].

***Stage 1.*** Calculate the mean *blank*-absorbance $\overline{A}^{extraction}_{procedure,i}$ using the absorbances of the solutions to be analysed $A^{extraction}_{procedure,i}$ obtained in *blank*-experiments and check the repeatability of individual values of $A^{extraction}_{procedure,i}$:

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

$$^{*}A_{nom} = 0.1 \ (\overline{A}^{extraction}_{procedure,i} / 0.32 \le 0.1);\ A_{nom} = \overline{A}^{extraction}_{procedure,i} / 0.32 \ (\overline{A}^{extraction}_{procedure,i} / 0.32 > 0.1)$$

***Stage 2.*** Calculate the mean absorbance $\overline{A}^{extraction}_{i}$ using the absorbances of the solutions to be analysed $A^{extraction}_{i}$ obtained in the main experiments and check the repeatability of individual values of $A^{extraction}_{i}$:

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

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

***Stage 3.*** Calculate the value of $\delta^{extraction}_{procedure}, \%$ relative to the solution to be analysed corresponding to the point of 100% in the normalized coordinates and check its acceptability:

$$\delta^{extraction}_{procedure(100\%)} = \frac{\overline{A}^{extraction}_{procedure,i}}{\overline{A}^{extraction}_{100\%}} \cdot 100\% \le \max\delta_{procedure} = 2.56\%.$$

***Stage 4***. Calculate the values of extraction recovery $R^{extraction}_{i}, \%$ for each individual experiment:

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

Calculate the mean values $\overline{R}^{extraction}_{i}, \%$ for each concentration level and method of determination within all runs ($n$ = 3). Calculate the total mean value $\overline{R}^{extraction}, \%$ for all concentration levels ($k$ = 3) and for all methods of determination ($m$ = 3), and check the reproducibility of the recovery values – calculate the total relative standard deviation $RSD^{extraction}_{R}, \%$ and the total relative confidence interval $\Delta^{extraction}_{R,r}, \%$:

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

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

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

The total results of our experiments are presented on Figure 3 – 4. The curves illustrate the set of recovery values obtained in all respective experiments.

Thus, in the case of application of procedure of liquid-liquid extraction with chloroform or the mixture of chloroform and 2-propanol (8:2) the increase of metronidazole extraction recovery is observed when increasing the value of the medium pH; and jump increasing for R is fixed within the pH range from 4 to 5, and then gradual increment takes place to pH = 12. Addition of 2-propanol to chloroform leads to significant increase of the extraction recovery (on average of 30 – 40%) within the pH range from 7 to 12 (to 60 – 75% respectively).

It should be noted that the recovery for liquid-liquid extraction of metronidazole with chloroform in the strong acid medium is too low that makes possible to recommend such processing mode for purification of aqueous extracts from biological material from coextractive substances.

Processing the aqueous solutions with amphiphylic solvents followed by «salting out» with ammonium sulphate in all cases allows to extract sufficiently high amounts of metronidazole in all media (not less than 80%) excluding application of ethanol in the strong acid and strong alkaline medium. For application of

acetonitrile we may not determine the extraction maximum, because under all conditions the recovery exceeds 95%. For application of 2-propanol the highest value of recovery is observed in the strong acid medium (≈98%) and then gradually decreases to 85% (at pH = 5) and the same value is fixed in the neutral and alkaline medium. In the case of ethanol application low values are fixed at the ends of the pH range and the gradual increase in the extraction recovery is observed to the middle of the pH range (the maximum is 90% at pH = 9).

Application of liquid-liquid extraction provides the high specificity/selectivity of the procedure (*blank*-absorbance does not exceed 0.010); in the case of extraction with amphiphylic solvents the values of *blank*-absorbance are higher in 2 – 3 times.

We may see that in the case of buffer solutions application it is observed the distinct consistent pattern in dependence of the extraction recovery on the medium pH – gradual R increasing / decreasing without sharp jumps or valleys. In the case of application of acids and bases for creating the medium pH the picture is not so regular that can be explained by interaction of the analyte or organic solvent with free acid or base presented in the solution.

Application of acids and bases also increases the absorbance values in *blank*-experiments and, respectively, worsens the specificity of the procedures, however, for the best variants these values do not exceed the acceptability criteria in most cases.

It should be noted that when adding acids and bases to the extraction medium followed by processing with amphiphylic solvents leads to significant difference in the results of metronidazole quantification by different methods. The highest difference is for application of 0.1 M hydrochloric acid solution as an solvent in UV-spectrophotometry, and also the highest values of *blank*-absorbance are fixed in these cases. When carrying out determinations in 0.1 M sodium hydroxide solutions the *blank*-absorbance is low enough, and the dependence «recovery – pH» has the most pattern character. This solvent we recommend as the most useful for further application.

The high extraction efficiency in the strong acid medium allow to develop the procedures of sample preparation of biological objects with processing at pH < 2 that provide obtaining of the purer extracts than in the neutral and alkaline meduim.

Detailed data on the values of recovery and specificity for the variants of extraction procedures with the best results are presented in Table 1 – 4.

**Table 1-Results of determination of recovery and specificity for the extraction procedure with application of 10% sodium hydroxide solution (pH = 12) and the mixture of chloroform and 2-propanol (8:2) as an solvent**

| Concentration point | $A_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
|---|---|---|---|---|---|---|---|---|---|
| | 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% | 0.138 | 0.211 | 0.205 | 0.189 | 0.255 | 0.261 | 68.90 | 77.21 | 74.61 |
| 50% | 0.269 | 0.385 | 0.386 | 0.369 | 0.506 | 0.515 | 70.79 | 73.30 | 73.01 |
| 100% | 0.544 | 0.771 | 0.785 | 0.731 | 1.005 | 1.026 | 73.37 | 75.29 | 75.56 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 71.02 | 75.27 | 74.39 |
| | 0.008 | 0.014 | 0.010 | $\overline{R}^{extraction}$, % | | | 73.56 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$,% | | | 3.16 | 2.60 | 1.73 |
| | 1.43 | 1.83 | 1.28 | $RSD_R^{extraction}$,% | | | 2.57 | | |
| | satisfied | satisfied | satisfied | $\Delta_{R,r}^{extraction}$,% | | | 4.77 | ≤ 10.00% | satisfied |

**Table 2- Results of determination of recovery and specificity for the extraction procedure with application of 6 M hydrochloric acid solution (pH = 2) and acetonitrile as an solvent**

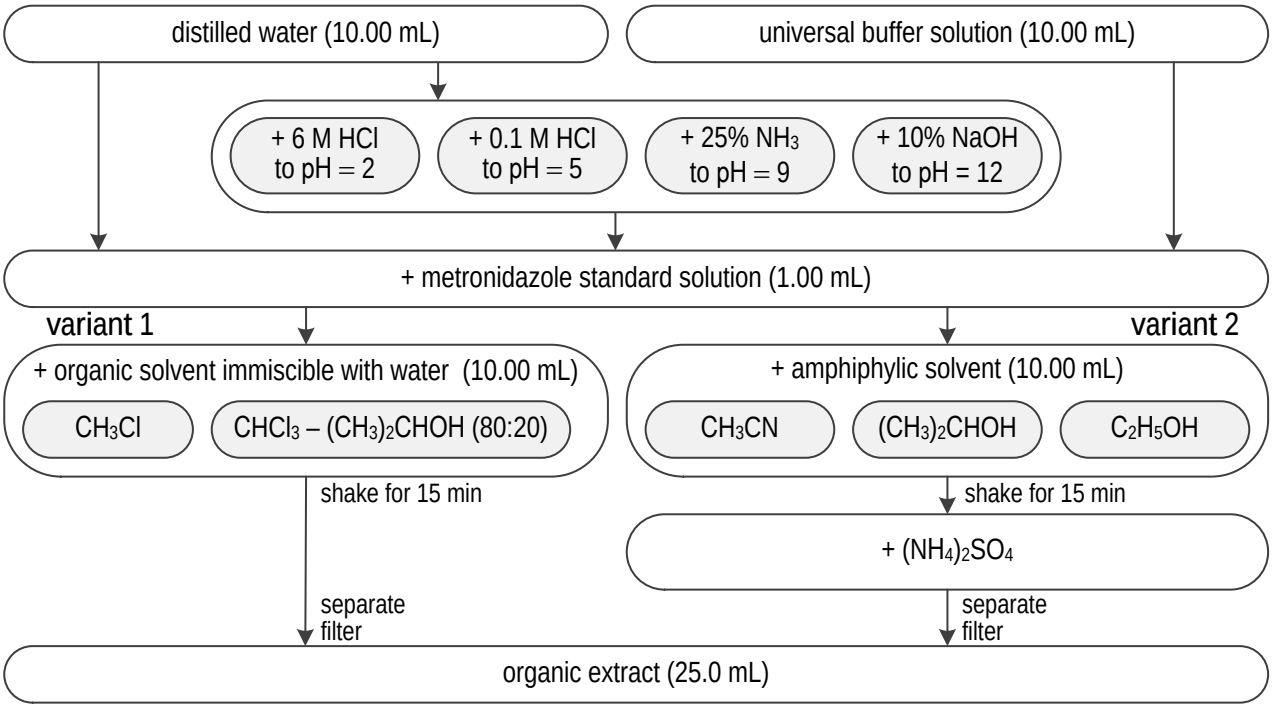
| Concentration point | $A_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm | 0.1 M HCl, 277 nm | 96% $C_2H_5OH$, 310 nm | 0.1 M NaOH, 319 nm |
| 25% | 0.197 | 0.275 | 0.281 | 0.189 | 0.255 | 0.261 | 89.74 | 99.22 | 101.38 |
| 50% | 0.349 | 0.516 | 0.525 | 0.369 | 0.506 | 0.515 | 87.27 | 97.64 | 98.76 |
| 100% | 0.672 | 1.010 | 1.019 | 0.731 | 1.005 | 1.026 | 88.18 | 98.29 | 97.75 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 88.40 | 98.38 | 99.29 |
| | 0.027 | 0.022 | 0.016 | $\overline{R}^{extraction}$, % | | | 95.36 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$,% | | | 1.41 | 0.81 | 1.89 |
| | 4.01 | 2.24 | 1.61 | $RSD_R^{extraction}$,% | | | 1.44 | | |
| | unsatisfied | satisfied | satisfied | $\Delta_{R,r}^{extraction}$,% | | | 2.67 | ≤ 10.00% | satisfied |

**Table 3-Results of determination of recovery and specificity for the extraction procedure with application of 6 M hydrochloric acid solution (pH = 2) and 2-propanol as an solvent**

| Concentration point | $A_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
|---|---|---|---|---|---|---|---|---|---|
| | 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% | 0.217 | 0.280 | 0.279 | 0.189 | 0.255 | 0.261 | 98.94 | 96.87 | 99.64 |
| 50% | 0.385 | 0.515 | 0.516 | 0.369 | 0.506 | 0.515 | 96.02 | 95.22 | 96.37 |
| 100% | 0.725 | 0.986 | 0.993 | 0.731 | 1.005 | 1.026 | 94.97 | 94.79 | 94.91 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 96.64 | 95.62 | 96.97 |
| | 0.030 | 0.033 | 0.019 | $\overline{R}^{extraction}$, % | | | 96.41 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 2.13 | 1.15 | 2.50 |
| | 4.13 | 3.32 | 1.96 | $RSD_R^{extraction}$, % | | | 2.01 | | |
| | unsatisfied | unsatisfied | satisfied | $\Delta_{R,r}^{extraction}$, % | | | 3.73 | ≤ 10.00% | satisfied |

**Table 4-Results of determination of recovery and specificity for the extraction procedure with application of 25% ammonium hydroxide solution (pH = 9) and 96% ethanol as an solvent**

| Concentration point | $A_i^{extraction}$ | | | $A_i^{model}$ | | | $R_i^{extraction}$, % | | |
|---|---|---|---|---|---|---|---|---|---|
| | 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% | 0.200 | 0.272 | 0.284 | 0.189 | 0.255 | 0.261 | 80.87 | 81.84 | 93.47 |
| 50% | 0.345 | 0.490 | 0.515 | 0.369 | 0.506 | 0.515 | 80.50 | 84.29 | 92.35 |
| 100% | 0.633 | 0.922 | 0.983 | 0.731 | 1.005 | 1.026 | 80.05 | 85.46 | 91.99 |
| | $\overline{A}_{procedure,i}^{extraction}$ | | | $\overline{R}_i^{extraction}$, % | | | 80.47 | 83.86 | 92.60 |
| | 0.047 | 0.063 | 0.040 | $\overline{R}^{extraction}$, % | | | 85.65 | | |
| | $\delta_{procedure}^{extraction}$, % ≤ 2.56% | | | $RSD_R^{extraction,m}$, % | | | 0.51 | 2.20 | 0.83 |
| | 7.47 | 6.70 | 4.09 | $RSD_R^{extraction}$, % | | | 1.39 | | |
| | unsatisfied | unsatisfied | unsatisfied | $\Delta_{R,r}^{extraction}$, % | | | 2.59 | ≤ 10.00% | satisfied |




**Scheme 1. The main stages of metronidazole extraction procedures**

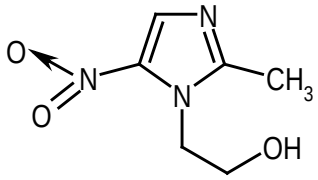


**Figure 1. Chemical structure of metronidazole**

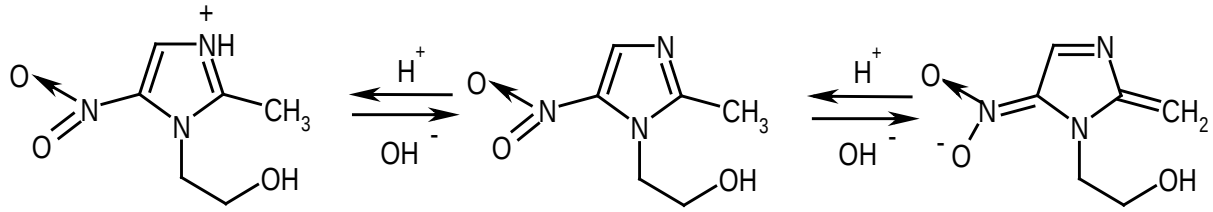
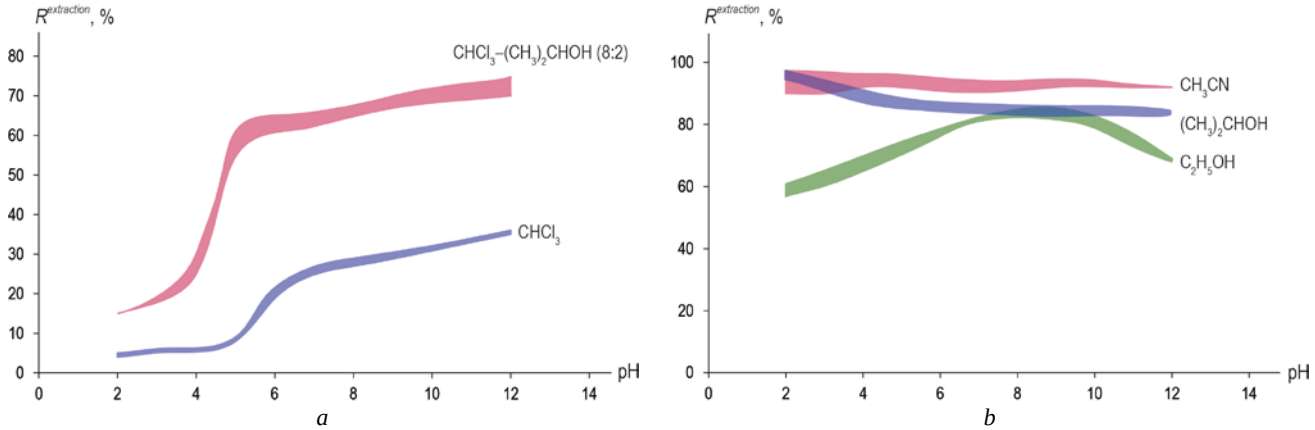


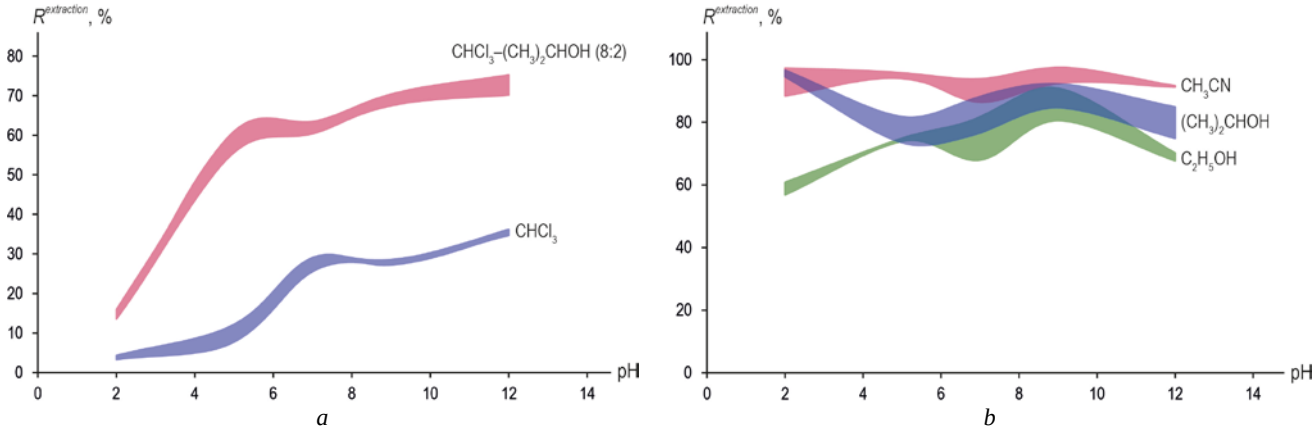
**Figure 2. Possible transformations in the metronidazole solutions when changing the medium pH**




*a* *b*

**Figure 3. Dependence of the metronidazole extraction recovery from the pH medium (buffer solutions were used for pH creating):**

*a* – liquid-liquid extraction with organic solvents immiscible with water;
*b* – extraction by amphiphylic solvents followed by «salting out» with ammonium sulphate




*a* *b*

**Figure 4. Dependence of the metronidazole extraction recovery from the pH medium (acids and bases were used for pH creating):**

*a* – liquid-liquid extraction with organic solvents immiscible with water;
*b* – extraction by amphiphylic solvents followed by «salting out» with ammonium sulphate

## CONCLUSIONS

The dependences of the extraction recovery of metronidazole from aqueous solutions on the medium pH using chloroform and the mixture of chloroform and 2-propanol (8:2), as well as isopropanol, acetonitrile and ethanol followed by the separation of the organic layer under the conditions of aqueous phase saturation with ammonium sulphate have been set. The conditions for extraction purification of aqueous extracts from biological objects from coextractive substances for further quantitative determination of metronidazole have been proposed, as well as optimal conditions for isolation of metronidazole from biological objects by liquid extraction with organic solvents have been determined.

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