DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHODS OF METRONIDAZOLE QUANTITATIVE DETERMINATION IN BLOOD

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Key words: *metronidazole, UV-spectrophotometry, blood.*

Resume: *The set of UV-spectrophotometric methods of metronidazole quantitative determination in blood using acetonitrile for analyte isolation from matrix under the conditions of aqueous phase saturation by ammonium sulphate and 0.1 mole/L HCl solution, 96% ethanol, 0.1 mole/L KOH solution in CH_3OH and 0.1 mole/L NaOH solution as solvents for spectrophotometric measurements have been developed.*

Резюме: *Разработан набор УФ-спектрофотометрических методик количественного определения метронидазола в крови с использованием ацетонитрила для изолирования аналита из матрицы в условиях насыщения водной фазы аммония сульфатом и 0,1 моль/л раствора HCl, 96% этанола, 0,1 моль/л раствора KOH в CH_3OH и 0,1 моль/л раствора NaOH в качестве растворителей для спектрофотометрирования.*

Actuality. Metronidazole is attributed to the group of antiprotozoal medicines and widely used for treatment of infectious diseases, at the same time it is possessed of quite a number of side effects showed by classic symptoms of acute intoxication, especially when interacting with other medicines and alcohol [1 – 2]. The concentrations of metronidazole in blood and urine are such high that allow to use UV-spectrophotometry for its quantitative determination [3]. All mentioned above makes actual developing UV-spectrophotometric procedures of metronidazole quantitative determination in biological liquids for application in chemical and toxicological analysis.

Purpose: to develop UV-spectrophotometric procedures of metronidazole quantitative determination in blood.

Tasks:

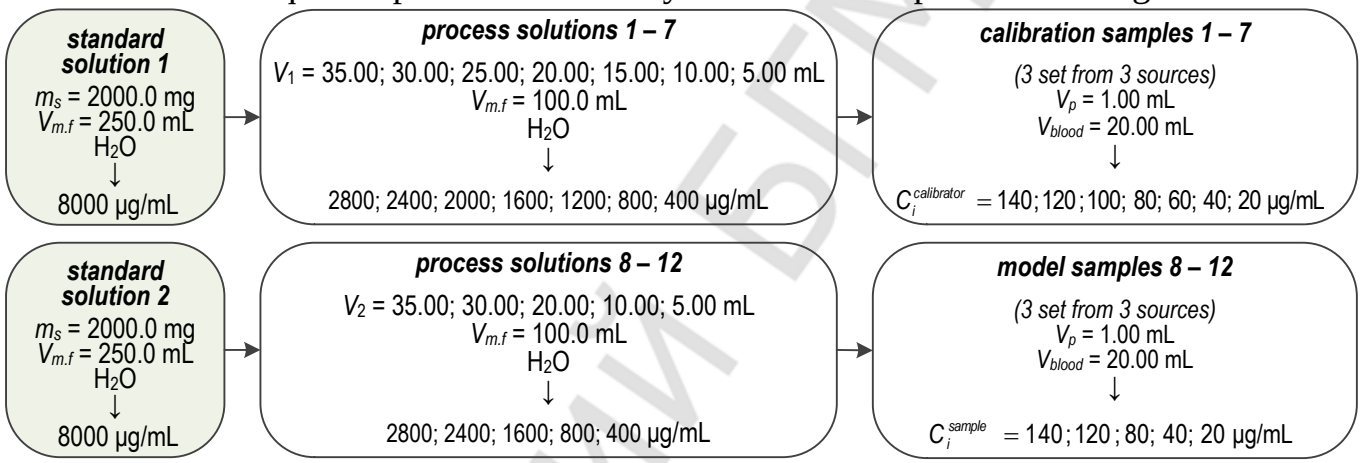
1. To offer different procedures of blood sample preparation for metronidazole quantitative determination.

2. To validate the developed procedures using the offered before approaches [4 – 8] to the determination procedure and acceptability estimation of specificity, recovery, linearity, accuracy and precision of UV-spectrophotometric methods of analytes quantitative determination in biological liquids applied in forensic and toxicological analysis.

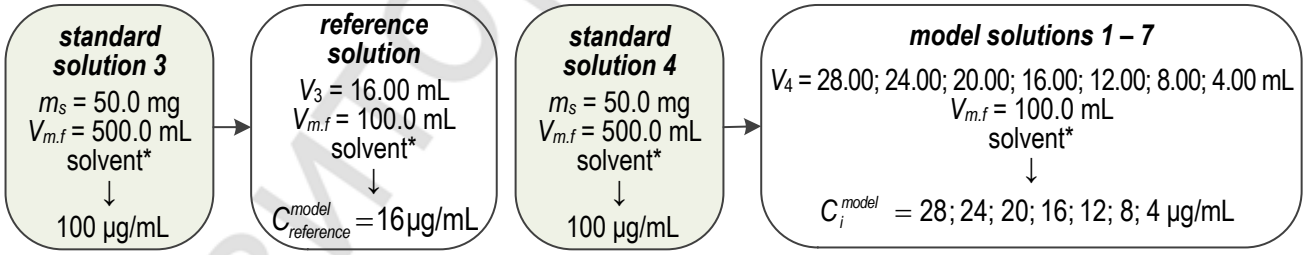
Materials and methods. Metronidazole of pharmacopoeial purity was used in the experiment. The procedure of preparation of standard, process and model solutions, and also model and calibration samples is presented on Picture1 and 2.

The design of experiment on development of procedures of metronidazole determination in blood is presented on Picture3.

Results and their discussion. It has been suggested to carry out metronidazole isolation from blood using acetonitrile with subsequent separation of organic layer under the conditions of aqueous phase saturation by ammonium sulphate according to Picture3.



Picture 1 – The order of samples preparation for validation of procedures of metronidazole determination in blood



* 1) 0.1 mole/L HCl (λ_{max} = 277 nm);
2) 96% C_2H_5OH (λ_{max} = 310 nm);
3) 0.1 mole/L KOH in CH_3OH (λ_{max} = 314 nm);
4) 0.1 mole/L NaOH (λ_{max} = 319 nm)

Pic. 2 – The order of solutions preparation for validation of procedures of metronidazole determination in blood

Isolation has been carried out in the acid (pH = 2), weak-acid (pH = 5) and alkaline medium (pH = 11).

The development and validation of procedures of metronidazole quantitative determination was carried out according to the following scheme [5]: application of the normalized coordinates (normalization by the reference solution); the application ranges is 25 – 175%; the number of concentration levels is g = 7 in constant increments of 25%.

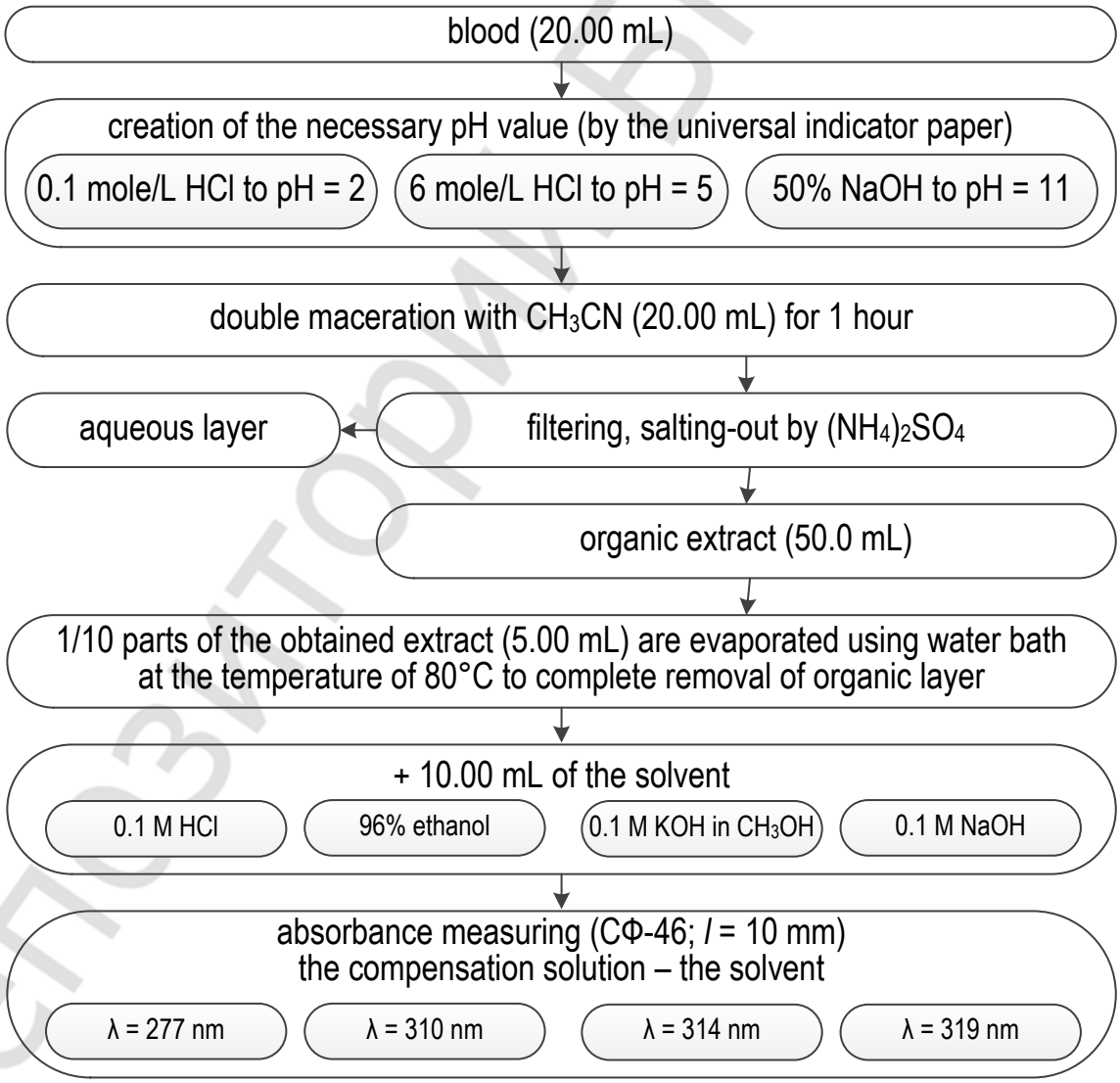
The metronidazole concentration in blood corresponding to the point of 100% in the normalized coordinates – 80 mcg/ml – was chosen as the mean metronidazole concentration in blood for acute poisoning.

Validation of the developed procedures by model solutions was carried out earlier and their acceptability for further application in forensic toxicology was shown.

Validation by matrix samples has been carried out by such parameters as

«specificity/selectivity», «recovery», «linearity», «accuracy» and «precision» [4 – 8].

The results of specificity study show that carrying out metronidazole isolation from blood using acetonitrile provides low contribution of biological matrix components into the absorbance of the sample to be analysed for all variants of the solvents used for analysis, and the lowest value was observed when carrying out the experiment in the weak-acid medium.



Picture 3 – The main stages of the procedures of metronidazole determination in blood by the method of UV-spectrophotometry

It is possible to point to the conclusion about high efficiency of metronidazole isolation from blood under suggested conditions – not less than 90% – by the results of recovery study. The method with acetonitrile application in the weak-acid medium is characterized by the best extraction efficiency.

The values of reproducibility for recovery (≤ 20.00%) and blank-samples absorbance (≤ 6.71%) satisfy the acceptability criteria for all variants of the methods.

All examined methods are characterized by the acceptable parameters of linearity (

$RSD_0 \leq 6.01\%$; $R_c \geq 0.9884$), accuracy ($\delta \leq 6.40\%$) and precision (within-run ≤ 14.14%; between-run ≤ 20.00%), and the obtained data are the evidence of application possibility of the developed methods for metronidazole spectrophotometric determination in blood.

Conclusions:

1. We have developed the set of UV-spectrophotometric methods of metronidazole quantitative determination in blood using acetonitrile for analyte isolation from matrix under the conditions of aqueous phase saturation by ammonium sulphate and 0.1 mole/L HCl solution, 96% ethanol, 0.1 mole/L KOH solution in CH_3OH and 0.1 mole/L NaOH solution as solvents for spectrophotometric measurements.

2. Acetonitrile application in the weak-acid medium (pH = 5) is optimal – contribution of matrix components into the absorbance of the sample to be analysed does not exceed 10%, extraction efficiency is ~95%.

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