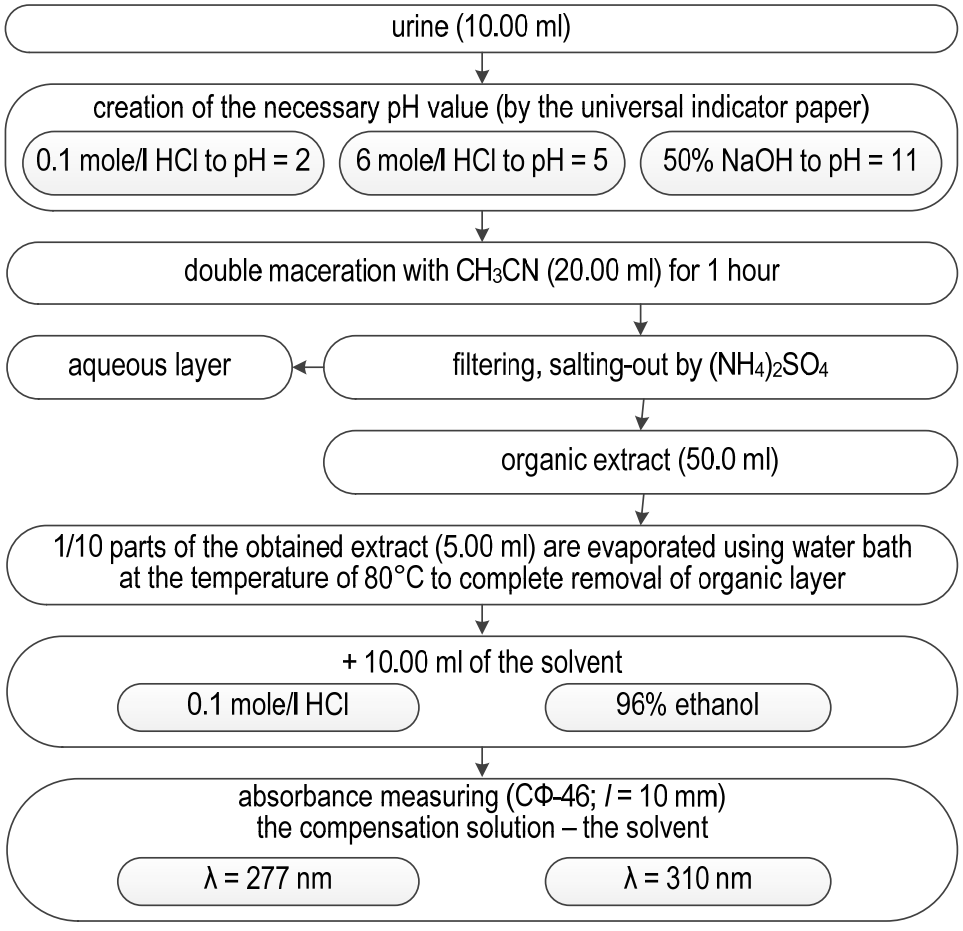
# DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHODS OF METRONIDAZOLE QUANTITATIVE DETERMINATION IN URINE

Shkarlat G. L., Zhuravel I. O., Klimenko L. Yu.
The National University of Pharmacy, Kharkiv, Ukraine
toxchem@ukrfa.kharkov.ua

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.

The purpose of this paper is to develop UV-spectrophotometric procedures of metronidazole quantitative determination in urine and to validate the developed procedures using the offered before approaches 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.

It has been suggested to carry out metronidazole isolation from urine using acetonitrile with subsequent separation of organic layer under the conditions of aqueous phase saturation by ammonium sulphate according to the scheme.



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: 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 urine corresponding to the point of 100% in the normalized coordinates – 80 mcg/ml – was chosen as the mean metronidazole concentration in urine 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».

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

It is possible to point to the conclusion about high efficiency of metronidazole isolation from urine 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 urine.

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