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Ф.Э. Сулеева

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АДРЕС РЕДАКЦИИ:
050004, РК, г. Алматы
пр. Абылай хана, 63, оф. 315
тел.: +7 (727) 273 03 73
факс: +7 (727) 273 55 00
e-mail: pharmkaz@dari.kz; pharmkaz@mail.ru

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VALIDATION OF UV-SPECTROPHOTOMETRIC METHODS OF QUANTITATIVE DETERMINATION IN FORENSIC AND TOXICOLOGICAL ANALYSIS: RECOVERY

This article is the continuation of authors research [1,2] in the field of development of the approaches to validation of methods of quantitative determination for purposes forensic and toxicological analysis and devoted to the problem of validation parameter «recovery» determination.

ANNOTATION
The theoretical approaches to determination of recovery when carrying out the validation of UV-spectrophotometric methods of quantitative determination for forensic and toxicological analysis have been formulated; the acceptability criteria for the validation parameter have been suggested and ground. The recovery for UV-spectrophotometric method of doxylamine quantitative determination in blood has been set using the offered approaches and on the basis of the obtained results the optimal stage-by-stage algorithm of the given validation parameter determination for UV-spectrophotometric methods of analytes quantitative determination in biological fluids has been developed.

Keywords: validation, UV spectrophotometric method, Governmental pharmacopeia of Ukraine, forensic and toxicological analysis.

INTRODUCTION
The validation parameter «recovery» is not used practically in pharmaceutical analysis – it is absent in the ICH guideline [3], in European Pharmacopoeia [4] and State Pharmacopoeia of Ukraine [5]. It is possible to find its mention in some papers [6] – as the ratio of «found/spiked». Such situation is explainable – the procedure of sample preparation in pharmaceutical analysis does not contain the stages, which require the extraction carrying out (result in the substance considerable losses), which efficiency is characterized by the parameter «recovery» [4,5]. Actually the parameter «found/spiked» does not characterize the substance losses due to the procedure of sample preparation, but is used for estimation of the method systematic error [6].

PURPOSE
The purpose of this paper is to analyse the approaches to determination of validation parameter «recovery» according to the requirements of the international guidances, to form the approaches to the procedure of recovery determination when carrying out the validation of UV-spectrophotometric methods of quantitative determination for forensic and toxicological analysis, to test the offered approaches to recovery determination by the example of UV-spectrophotometric method of doxylamine quantitative determination in blood and to form the step-by-step algorithm of recovery determination for UV-spectrophotometric methods of quantitative determination in forensic and toxicological analysis on the basis of the obtained results.

INVESTIGATIONS MATERIALS AND METHODS
The method to be validated: 20.00 ml of blood are coated with 10.00 ml of the 10% trichloroacetic acid aqueous solution, mixed and left for 1 hour when constant shaking. The mixture is centrifuged (during 5 minutes at 5000 rpm), the supernatant liquid is poured off and diluted to the volume of 30 ml with distilled water, its pH is checked (should be equal to 2) and the mixture is extracted with chloroform three times by portions of 10.00 ml (if stable emulsions are formed centrifugation is applied (during 5 minutes at 5000 rpm). «Alkaline» chloroform extracts are combined and filtered through the pa-
per filter («red label») with 1 g of sodium sulphate anhydrous in the measuring flask with the capacity of 50.0 ml, and diluted to the volume with chloroform. Then the investigation is carried out in two ways:

1) 2/5 of the obtained chloroform extract (20.00 ml) are 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 (>0.5 to the volume of blood taken for analysis) of the 0.1 mole/l hydrochloric acid solution.

2) 2/5 of the obtained chloroform extract (20.00 ml) are evaporated using water-bath at the temperature of 80°C to complete removal of organic layer; the dry residue is dissolved in >0.5 ml of chloroform and applied quantitatively on the start line of the «Sorbfil» PTLC-IIB chromatographic plate (the plates have been processed preliminary with the 0.1 mole/l potassium hydroxide solution in methanol and then dried out at 110°C for 30 minutes) in the form of stripe 2 cm wide. Near 10 ml of the doxylamine succinate standard chloroform solution (concentration is 1 mg/ml) are applied in point («testifier»). The plate is eluted in chloroform twice. After drying the plate is eluted using the mixture of chloroform and methanol (90:10) as a mobile phase, dried out, and the «testifier» stripe is developed with the Dragendorff reagent and the spot of brown colour in the area of Rf = 0.5 – 0.7 is observed. The sorbent is carefully removed from the plate part with area of 3 cm × 1 cm opposite the spot of «testifier» by scalpel in the glass bottle. 10.00 ml of the 0.1 mole/l hydrochloric acid solution are added into the bottle and the bottle content is shaken during 5 minutes, then filter-tered in the measuring flask with the capacity of 10.0 ml (× 0.5 to the volume of blood taken for analysis) and diluted to the volume through the filter («red label») with the same solvent.

The process solutions: 400.0 mg of doxylamine succinate were placed in the measuring flask with the capacity of 100.0 ml, dissolved in distilled water and the solution was diluted to the volume with the same solvent (the standard solution 1, the concentration was 4000 mcg/ml); 32.50; 20.00; 10.00 and 5.00 ml respectively of the doxylamine succinate standard solution 1 were placed using burette in four measuring flasks with the capacity of 100.0 ml and the solutions were diluted to the volume with distilled water (the process solutions 1, 2, 3 and 4 respectively, the concentrations were 1300, 800, 400 and 200 mcg/ml respectively).

The reference solutions: 100.0 mg of doxylamine succinate were placed in the measuring flask with the capacity of 500.0 ml, dissolved in the 0.1 mole/l hydrochloric acid solution and the solution was diluted to the volume with the same solvent (the standard solution 2, the concentration was 200 mcg/ml); 26.00; 16.00; 8.00 and 4.00 ml respectively of the doxylamine succinate standard solution 2 were placed using burette in four measuring flasks with the capacity of 100.0 ml and the solutions were diluted to the volume with the 0.1 mole/l hydrochloric acid solution (the reference solutions 1, 2, 3 and 4 respectively, the concentrations were 52, 32, 16 and 8 mcg/ml respectively).

The samples to be analysed: 1-4) 4 lines in 3 samples (20.00 ml) of model blood (matrix) obtained from the different sources, which were spiked with 1.00 ml of the process solutions 1-4 respectively; 5-8) 4 lines in 3 samples (20.00 ml) of model blood obtained from the different sources, which were spiked with 1.00 ml of distilled water; 9) 5 samples (20.00 ml) of model blood obtained from the different sources, which were spiked with 1.00 ml of distilled water (blank-samples).

The solutions to be analysed: the solutions obtained by the method mentioned above for the samples to be analysed 1-4 and 9; the solutions obtained by the method mentioned above for the samples to be analysed 5-8 using in the last stage the reference solutions 1-4 respectively as the solvent instead of the 0.1 mole/l hydrochloric acid solution.

The absorbance of the solutions to be analysed 1-8 and the reference solutions were measured 3 times with taking out the cell at the wavelength of 262 nm by the spectrophotometer СФ-46 in the cell with the layer thickness of 10 mm. The 0.1 mole/l hydrochloric acid solution was used as the compensation solution.

The absorption UV-spectrum was recorded for the solutions to be analysed 9 by the spectrophotometer СФ-46 in the wavelength range of 220-350 nm in the cell with the layer thickness of 10 mm. The 0.1 mole/l hydrochloric acid solution was used as the compensation solution.

INVESTIGATIONS RESULTS AND THEIR DISCUSSION

«Guidance for Industry: Bioanalytical method validation» (U.S. FDA, 2001) [7] and «Standard Practices for Method Validation in Forensic Toxicology» (SWGTOX, 2012) [8] define recovery as «the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method». The glossary of «Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens» (UNODC, 2009) [9] give the following definition for parameter «recovery» – «the percentage of the drug, metabolite, or internal standard originally in the specimen that reaches the end of the procedure».

In the body text of the FDA [7] and UNODC [9] guidelines it is said be-sides that the recovery of an analyte in an assay is the detector response ob-tained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the pure authentic standard. Whereas the SWGTOX paper [8] says that the recovery can be done as a percentage of the detector response obtained from an amount of the analyte added to the matrix before extraction compared to the detector response obtained from the same concentration of the analyte added after extraction.

Thus, all three international papers coordinate, one way or another, the validation parameter «recovery» with
The extraction procedure – the main stage of the procedure of sample preparation that allows to apply to this parameter the «extraction degree» term.

The FDA [7] and UNODC [9] guidances suggest to carry out the recovery experiments by comparing the analytical results for extracted samples with unextracted standards that represent 100% recovery. At the same time using the blanks of the biological matrix once the final extracts have been obtained, which have been spiked with the true concentration of the pure authentic standard corresponded to 100% recovery, are provided for special cases by the UNODC [9] paper; and the SWGTOX [8] guidance suggests to determine the recovery only in this way.

According to the advices of FDA [7] and UNODC [9] it is necessary to study the recovery for three concentrations (low, medium and high), the UNODC [9] guidance accentuates that the samples intended for evaluation of the method precision and accuracy are used for this purpose. The SWGTOX [8] guidance limits itself for this purpose to applying the solutions of two concentration levels – low and high. Concerning the samples quantity subjected to the analysis at each concentration level, there are five samples in the UNODC [9] guidance, no less than six – in the SWGTOX [8] paper (the samples quantity can be increased for postmortem materials), and FDA [7] does not standardize this parameter at all. The SWGTOX [8] guidance accentuates that all samples of matrix should be obtained from different sources.

For calculation of recovery value no calculation formulae are recommended in the FDA [7] paper, the SWGTOX [8] guidance suggests to use the following formula (S1 and S2 are the peaks areas of analyte for blank-samples fortified before and after extraction respectively):

\[ R = \frac{S_1}{S_2} \cdot 100\% \]  (1.1)

that implies using the chromatographic methods of analysis. When calculating the mean values of peaks areas are used for each set of samples.

The UNODC [9] guidance recommends to use the following formula for the optical methods of analysis (A1 and A2 are the absorbance of analyte in the extracted sample and reference solution respectively):

\[ R = \frac{A_1}{A_2} \cdot 100\% \]  (1.2)

or – for the chromatographic methods of analysis – the following formula (S1 and S3 are the peaks areas of analyte and internal standard in the extracted sample respectively; S2 and S4 are the peaks areas of analyte and internal for standard reference solution (blank-sample fortified after extraction) respectively):

\[ R = \frac{S_1}{S_3} \cdot \frac{S_4}{S_2} \cdot 100\% \]  (1.3)

It should be noted that the conception «correction for recovery» are included in the glossary the UNODC [9] guidance, where it is said, if there is no internal standard (which automatically compensates for incomplete recovery) then the results of analysis must be multiplied by a correction factor to obtain the values which would have been produced if the recovery had been 100%.

This sentence seems to us disputable – in the first place, work with the calibration curve plotted using the fortified samples of biological matrix allows to compensate incomplete analyte extraction from matrix, whether the method supposes using internal standard or not. Whereas application of internal standard can level incomplete extraction only in the case when the values of recovery target analyte and internal standard are identical, moreover, if the sensitivity of recovery values to the change of the analyte concentration and biological matrix state is identical. It is necessary to correct the result of analysis for recovery value only in the case when the calibration curve is plotted using the reference solutions.

From our point of view it is necessary radically to change the attitude to the validation parameter «recovery» – it should be labeled as the main and compulsory and its determination should be carried out at the preliminary stages of validation simultaneously with confirmation of specificity/selectivity of the method. The extraction degree (both its value and reproducibility and consistency within the limits of analytical range of the method) is the critical factor for decision making about that whether it is necessary to continue development of the method at all, as the extraction de-
degree is often of low value when little amount of analyte in
the sample and increases sharply with the increase of its
concentration, besides it can be greatly changed depending
on the state of biological matrix (putrefaction, chronic disea-
ses et al.). What are offered to us by all studied internation-
apapers [7-9] – to check linearity, precision and accuracy,
analysing at least 39 samples of biological matrix, and only
thereafter, obtaining unsatisfactory results, to make the con-
clusion about unfitness of the method for decision of the as-
signed tasks. Whereas we offer at the stage of verification
of specificity/selectivity of the method, increasing the num-
ber of the samples to be analysed to 3, at once to deter-
dine the parameter «recovery», to check its reproducibil-
ity within the limits of analytical range of the method, and at
this stage, obtaining negative results, to say about unreas-
onability of subsequent work with this method.

Confirmation of specificity/selectivity of UV-spectro-
photometric method of analytes quantitative determination
in biological fluids is carried out in the points of 25%,
50% and 175% (the normalized coordinates are used)
according to the approaches offered by us previously [2].
For recovery determination of these methods we suggest
to enter additionally the analysis carrying out in the point
of 100%. In this way we provide fulfillment of internatio-
nal requirements (three concentrations levels – low, me-
dium and high) and control additionally the most critical
part of the method analytical range – near LLOQ, where
differences in recovery values are often observed.

For confirmation of acceptability of the recovery va-
alue reproducibility we suggest to check fulfillment of two
criteria simultaneous:

• the slope for linear dependence \( R = f(c) \) should sta-
tistically insignificantly differ from zero on conditions the
significance of absolute term (the linear dependence \( R =
bc + a \) goes over \( R = a \) in ideal situation) that is it is
necessary to prove that the value of b less, and the value of
a more than the confidence interval of its uncertainty [11]:

\[
b \leq \Delta_b; \quad a \geq \Delta_a; \quad (1.4)
\]

• the relative confidence interval should not exceed
the ex-
treme uncertainty of analysis \( \Delta A_s \) by the value [6]:

\[
\Delta_{R,\%} \leq \text{max} \Delta; \quad (1.5)
\]

The questions requiring the confirmation in the pro-
cess of experiment performance are left:

1) How many samples should be analysed at each
concentration level?

2) Which solution should be used for presentation of
100% recovery – directly the reference solution or the
blank-extract fortified with the reference solution?

3) Which formula should be used for calculation of
recovery?

4) Should the mean values of absorbance obtained
for each concentration level be used for calculations, or
should we process all array of the separate values ob-
tained experimentally?

The normalized coordinates [2,6] have been applied
for carrying out the validation of UV-spectrophotomet-
ric method of doxylamine quantitative determination in
blood; the analytical range of the method is 25-175% [2];
the lethal doxylamine concentration in blood [12] – 25
mg/l (that corresponded to 36 mg/l of doxylamine succi-
nate) has been accepted as 100%.

The step-by-step algorithm of experiment perfor-
ance for determination of recovery of UV-spectropho-
tometric method of analytes quantitative determination
in biological fluids has been formed on the basis of the
approaches offered above. Its main stages have been
resulted on Scheme 1 and illustrated by the obtained
experimental data for UV-spectrophotometric method of
doxylamine quantitative determination in blood.

The measurements and calculations recommended
in the sequel to compulsory implementation when carry-
out the validation of UV-spectrophotometric methods
of analytes quantitative determination in biological fluids
have been set off in bold on Scheme 1, – the rest of
measurements and calculations is of secondary nature
and has been performed for the purpose of answering
the questions raised above.

On the basis of information resulted on Scheme 1 it
is possible to formulate the followings conditions of ex-
periment performance on determination of validation pa-
rameter «recovery» for UV-spectrophotometric method
of analytes quantitative determination in biological fluids:

• determination of recovery should be carried out in
the points of 25%, 50%, 100% and 175%, analysing in 3
matrix samples obtained from different sources for each
concentration level

• directly the reference solution can be used as the
solution corresponding to 100% recovery (the results of
recovery determination with using the blank-extracts for-
tified with the reference solution are differed insignif-
antly from the results obtained with using the reference so-
lutions, but they require fulfillment of considerably more
cumbersome experiment at the same time);

• the formula (2.2) should be used for calculation of
recovery, at the same time an alternative is proposed to
laboratory – to use the mean value of Ablank obtained
under the analytical wavelength or the mean maximal
fixed value of Ablank max in the range of wavelength
of 220-350 nm;

• when checking the reproducibility of the recovery
value we recommend to use all array of the separate va-
ues (12) obtained experimentally in calculations.

Taking into account the offered conditions determina-
tion of parameter «recovery» for UV-spectrophotometric
method of doxylamine quantitative determination in blood
with preliminary TLC-purification has been carried out;
the obtained results have been resulted on Scheme 2.

According to the obtained results UV-spectropho-
tometric method of doxylamine quantitative determina-
tion in blood without preliminary TLC-purification is char-
acterized by rather higher extraction degree, than the
same method, but with fulfilment of TLC-purification; at
the same time it should be noted that the indices of recovery reproducibility for the method with TLC-purification are some better, than for the method without TLC-purification.

**Scheme 1** – The algorithm of experiment performance on determination of recovery by the example of UV-spectrophotometric method of doxylamine quantitative determination in blood without preliminary TLC-purification

<table>
<thead>
<tr>
<th>Stage 1. Analysis of the samples 1-9 according to the method mentioned above</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>Measuring the absorbance of the solutions to be analysed 9</td>
</tr>
<tr>
<td>A 262 нм: 0.051; 0.077; 0.064; 0.045; 0.062</td>
</tr>
<tr>
<td>Аblank = 0.060</td>
</tr>
<tr>
<td>A max: 0.058; 0.077; 0.064; 0.052; 0.062</td>
</tr>
<tr>
<td>Аblank max = 0.063</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Measuring the absorbance of the solutions to be analysed 1-4</td>
</tr>
<tr>
<td>A 262 нм: 0.222; 0.195; 0.205</td>
</tr>
<tr>
<td>Аsample (~25%) = 0.207</td>
</tr>
<tr>
<td>A 262 нм: 0.368; 0.347; 0.352</td>
</tr>
<tr>
<td>Аsample (~50%) = 0.356</td>
</tr>
<tr>
<td>A 262 нм: 0.679; 0.644; 0.654</td>
</tr>
<tr>
<td>Аsample (~100%) = 0.659</td>
</tr>
<tr>
<td>A 262 нм: 1.021; 1.008; 1.015</td>
</tr>
<tr>
<td>Аsample (~175%) = 1.015</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Measuring the absorbance of the solutions to be analysed 5-8</td>
</tr>
<tr>
<td>A 262 нм: 0.303; 0.277; 0.290</td>
</tr>
<tr>
<td>Аblank standard (~25%) = 0.290</td>
</tr>
<tr>
<td>A 262 нм: 0.521; 0.494; 0.508</td>
</tr>
<tr>
<td>Аblank standard (~50%) = 0.507</td>
</tr>
<tr>
<td>A 262 нм: 0.967; 0.941; 0.954</td>
</tr>
<tr>
<td>Аblank standard (~100%) = 0.954</td>
</tr>
<tr>
<td>A 262 нм: 1.498; 1.470; 1.485</td>
</tr>
<tr>
<td>Аblank standard (~175%) = 1.484</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Measuring the absorbance of the reference solutions</td>
</tr>
<tr>
<td>Аreference (~25%) = 0.226</td>
</tr>
<tr>
<td>Аreference (~50%) = 0.444</td>
</tr>
<tr>
<td>Аreference (~100%) = 0.890</td>
</tr>
<tr>
<td>Аreference (~175%) = 1.421</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage 2. Calculation of the recovery value and verification of its reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>Rsample</td>
</tr>
<tr>
<td>Rreference</td>
</tr>
<tr>
<td>=</td>
</tr>
<tr>
<td>(2.1)</td>
</tr>
<tr>
<td>R~25%: 98.23; 86.28; 90.71</td>
</tr>
<tr>
<td>R~50%: 82.95; 78.21; 79.34</td>
</tr>
<tr>
<td>R~100%: 76.30; 72.33; 73.46</td>
</tr>
<tr>
<td>R~175%: 71.87; 70.91; 71.45</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Rsample – Ablank</td>
</tr>
<tr>
<td>Rreference</td>
</tr>
<tr>
<td>=</td>
</tr>
<tr>
<td>(2.2a)</td>
</tr>
<tr>
<td>R~25%: 71.77; 59.82; 64.25</td>
</tr>
<tr>
<td>R~50%: 69.47; 64.73; 65.86</td>
</tr>
<tr>
<td>R~100%: 69.58; 65.62; 66.74</td>
</tr>
<tr>
<td>R~175%: 67.87; 66.70; 67.24</td>
</tr>
<tr>
<td>b = 0.01; ~b = 0.04; 0.01 ~ 0.04</td>
</tr>
<tr>
<td>a = 65.68; ~a = 3.66; 65.68 ~ 3.66</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Rsample – Ablank max</td>
</tr>
<tr>
<td>Rreference</td>
</tr>
<tr>
<td>=</td>
</tr>
<tr>
<td>(2.2b)</td>
</tr>
<tr>
<td>R~25%: 73.27; 70.40; 70.69</td>
</tr>
<tr>
<td>R~50%: 68.83; 64.10; 65.23</td>
</tr>
<tr>
<td>R~100%: 69.23; 65.30; 66.42</td>
</tr>
<tr>
<td>R~175%: 67.48; 66.51; 67.05</td>
</tr>
<tr>
<td>b = 0.02; ~b = 0.04; 0.02 ~ 0.04</td>
</tr>
<tr>
<td>a = 64.55; ~a = 3.72; 64.55 ~ 3.72</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Rsample</td>
</tr>
<tr>
<td>Ablank standart</td>
</tr>
<tr>
<td>=</td>
</tr>
<tr>
<td>(2.3)</td>
</tr>
<tr>
<td>R~25%: 73.27; 70.40; 70.69</td>
</tr>
<tr>
<td>R~50%: 70.68; 67.24; 69.34</td>
</tr>
<tr>
<td>R~100%: 70.23; 68.46; 68.53</td>
</tr>
<tr>
<td>R~175%: 68.18; 68.55; 68.37</td>
</tr>
</tbody>
</table>
In conclusion, the following should be mentioned – in spite of that the value of recovery is not critical for methods used in forensic and toxicological analysis it is necessary to try to obtain the maximum possible extraction degree when developing the method. Carrying out the parallel experiments on determination of specificity/selectivity and recovery makes possible changing the volumes of extractants, extraction multiplicity et al. for developers of the method with the purpose of providing the acceptable values of the validation parameters to be investigated.

CONCLUSIONS

Thus, the theoretical approaches to determination of recovery when carrying out the validation of UV-spectrophotometric methods of quantitative determination for forensic and toxicological analysis have been formulated; the acceptability criteria for the validation parameter have been suggested and ground. The recovery for UV-spectrophotometric method of doxylamine quantitative determination in blood has been set using the offered approaches and on the basis of the obtained results the optimal stage-by-stage algorithm of the given validation parameter determination for UV-spectrophotometric methods of analytes quantitative determination in biological fluids has been developed.

ТУЙІН

Л.Ю. Клименко1, С.Н. Трут2, Г.П. Петюнін3, И.М. Иванчук4,
фармацевтикағылымдарның кандидаты, доцент, талдамалы химия кафедрасының доценті,
Україна Денсаулық сақтау министрлігінің «Укрвакцина» мемлекеттік кәсіпорнаның бас директорының орынбасары2,
фармацевтика ғылымдарының докторы, профессор, дипломнан кейінгі білім беретін Харьков медицина академиясының клиникалық биохимия, сот-медициналық токсикология және фармация кафедрасының менгерушісі3,
фармацевтика ғылымдарының кандидаты, доцент, Ивано-Франківського університета фармацетика кафедрасының доценті4.
СОТ-ТОКСИКОЛОГИЯЛЫҚ ТАЛДАУ ЖАСАУ БАРЫСЫНДА МӨЛШЕРДІ БЕЛГІЛЕЙТІН УФ-СПЕКТРОГРАФИЯЛЫҚ әДІСТІҢ СЕНИМДІЛІГІН ТЕКСЕРУ: ШЫҒАРЫП АЛУ ДЕҢГЕІ

Сот-токсикологиялық талдау жасау барысында мөлшерді белгілейтін УФ-спектрографиялық әдістің сенімділігін тексеру кезінде оны шығарып алу деңгейі теориялық тәсілі тұжырымдалды. Аталған сенімділікі тексеру көрсеткіштеріне жарамдылық ұсынылып, дәлелденді.

Қанның құрамындағы доксиламин мөлшерін белгілейтін УФ-спектрографиялық әдісі үшін шығарып алу деңгейі белгіленді. Ұсынылған тәсілдерді пайдаланып және алынған нәтижелердің негізінде биологиялық сұйықтығардыға аналиттердің мөлшерін белгілейтін УФ-спектрографиялық әдіс үшін аталған параметрлердің сенімділігі тексеруді анықтаудың оңтайлы алгоритмі даярланды.

Кілт сөздер: сенімділікті тексеру, УФ-спектрофотометриялық әдіс, Украинаның мемлекеттік фармакеу, сот-токсикологиялық талдау.

РЕЗЮМЕ

Л.Ю. КЛИМЕНКО¹, С.Н. ТРУТ², Г.П. ПЕТЮНИН³, И.М. ИВАНЧУК⁴, кандидат фармацевтических наук, доцент, доцент кафедры аналитической химии, Национальный фармацевтический университет, г. Харьков, Украина¹; заместитель генерального директора государственного предприятия «Укрвакцина» Министерства здравоохранения Украины²; доктор фармацевтических наук, профессор, заведующий кафедрой клинической биохимии, судебно-медицинской токсикологии и фармации Харьковской медицинской академии последипломного образования³; кандидат фармацевтических наук, доцент, доцент кафедры фармации Ивано-Франковского национального медицинского университета⁴.

ВАЛИДАЦИЯ УФ-СПЕКТРОФОТОМЕТРИЧЕСКИХ МЕТОДИК КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ В СУДЕБНО-ТОКСИКОЛОГИЧЕСКОМ АНАЛИЗЕ: СТЕПЕНЬ ИЗВЛЕЧЕНИЯ

Сформулированы теоретические подходы к определению степени извлечения при проведении валидации УФ-спектрофотометрических методик количественного определения для судебно-токсикологического анализа. Предложены и обоснованы критерии приемлемости для данного валидационного параметра.

Установлена степень извлечения для УФ-спектрофотометрической методики количественного определения доксиламина в крови. С использованием предложенных подходов и на основании полученных результатов разработан оптимальный поэтапный алгоритм определения данного валидационного параметра для УФ-спектрофотометрической методики количественного определения аналитов в биологических жидкостях.

Ключевые слова: валидация, УФ-спектрофотометрический метод, Государственная фармакеу Украины, судебно-токсикологический анализ.

Literature:
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