

UDC 615.217.3:543.544.5:543.054

L. YU. KLIMENKO, V. P. MOROZ, T. A. KOSTINA

National University of Pharmacy, Kharkiv

DEVELOPMENT AND VALIDATION OF THE HPLC-PROCEDURE OF METOCLOPRAMIDE DETERMINATION IN BLOOD

The HPLC-procedure of metoclopramide quantitative determination in blood using amphiphilic solvent (acetonitrile) for analyte isolation at pH = 5 with further separation of organic layer under the conditions of aqueous phase saturation by ammonium sulphate has been developed for application in forensic and toxicological analysis. Validation of the developed procedure has been carried and the possibility of application of the method of calibration curve, method of standard and method of additions for metoclopramide quantitative determination in blood by the method of HPLC has been shown.

Key words: validation, bioanalytical methods, high-performance liquid chromatography, metoclopramide, method of calibration curve, method of standard, method of additions

THE PROBLEM STATEMENT

Metoclopramide is the specific blocker of dopamine, and also serotonin receptors, owing to this it has antiemetic action. The medicine has a number of side effects and can result in poisoning, especially for pregnant women [7, 8, 10] that is the reason of its toxicological significance.

ANALYSIS OF THE LAST RESEARCHES AND PUBLICATIONS

For metoclopramide determination in biological fluids in chemical and toxicological analysis we offered earlier the procedure of its isolation from blood based on precipitation of blood corpuscles by 0.1 mole/l hydrochloric acid solution, purification of acid aqueous extract by diethyl ether and extraction of the medicine with chloroform at pH = 10. The method allows to isolate 55 – 60 % of metoclopramide from blood [16].

ALLOCATION OF THE UNSETTLED BEFORE PARTS OF THE COMMON PROBLEM

The described procedure [16] can not provide high sensitivity of determination, besides the possibility of its application for carrying out quantitative determination of metoclopramide in blood has not been investigated. It is interesting

to develop the sensitive procedure of metoclopramide quantitative determination in blood and to study the possibilities of its application in the variants of the method of calibration curve (MCC), method of standard (MS) and method of additions (MA).

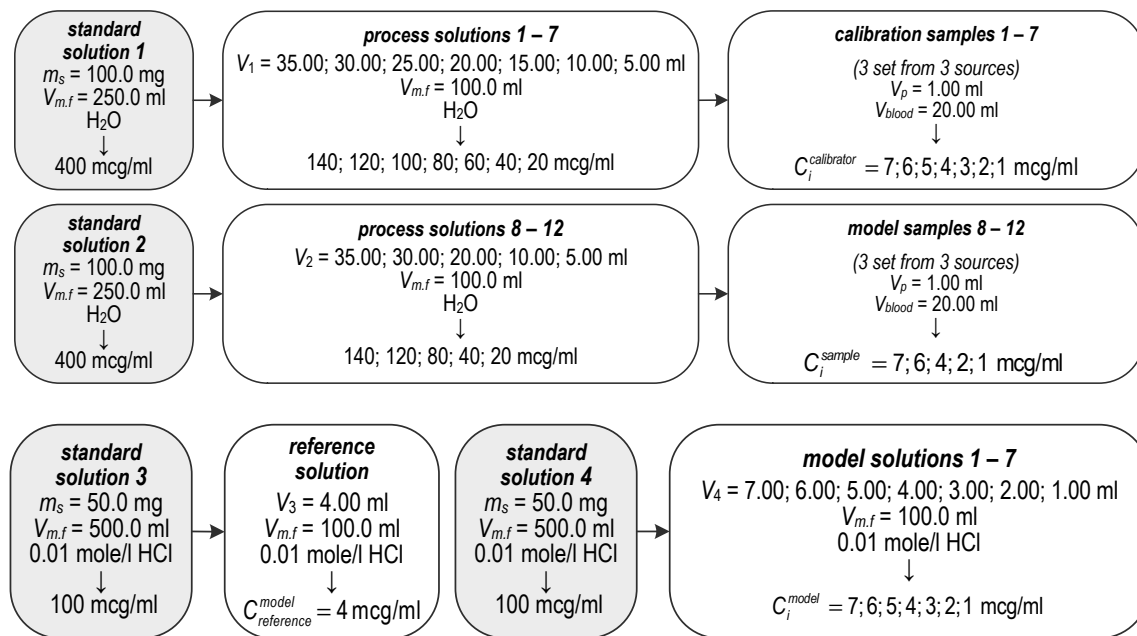
THE ARTICLE PURPOSE STATEMENT

The purpose of the paper is developing the HPLC-procedure of metoclopramide quantitative determination in blood with carrying out the sample preparation in the way of maceration with amphiphilic solvents and subsequent separation of organic layer under the conditions of aqueous phase saturation by electrolyte; carrying out validation of the developed procedure using the approaches offered by us before [2, 4–6, 9, 12–14], and also estimating the possibility of the MCC, MS and MA application for metoclopramide HPLC-determination in blood.

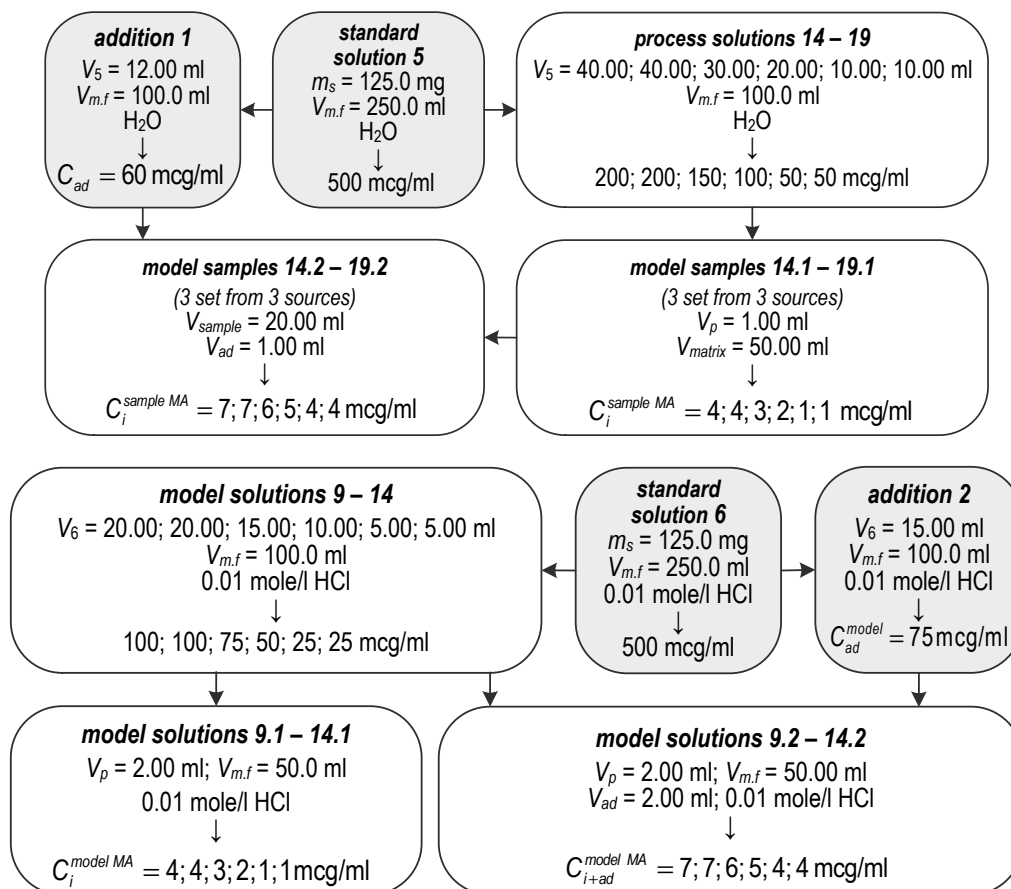
THE MAIN INFORMATION STATEMENT

Metoclopramide hydrochloride 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 *Schemes 1* and *2*.

© Authors collective, 2014

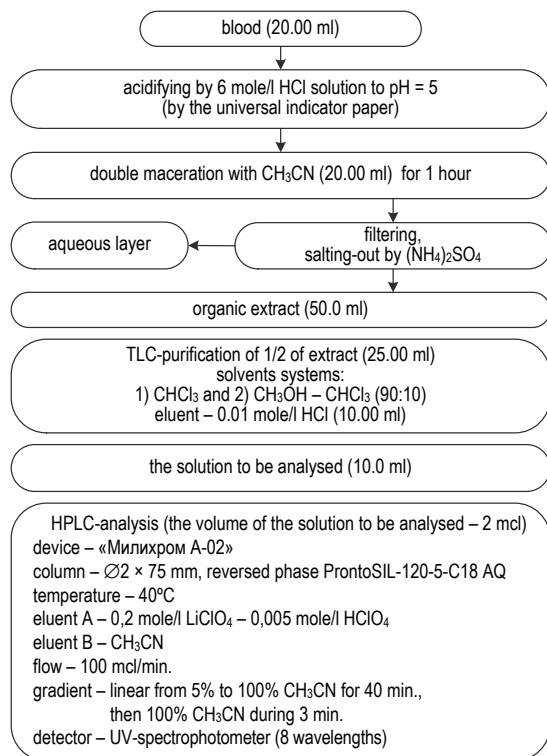


Scheme 1. The order of solutions and samples preparation for validation of metoclopramide determination procedure in blood by the method of HPLC in the variant of MCC and MS



Scheme 2. The order of solutions and samples preparation for validation of metoclopramide determination procedure in blood by the method of HPLC in the variant of MA

The main stages of the procedure of metoclopramide determination in blood by the method of HPLC are presented on *Scheme 3*.



Scheme 3. The main stages of the procedure of metoclopramide determination in blood by the method of HPLC

The calibration and model (see *Scheme 1* and *2*) and also blank-samples were analysed; the blank-samples were prepared in the following way: 5 samples (20.00 ml) of the blood obtained from the different sources, 1.00 ml of distilled water were added into them.

Each solution to be analysed was chromatographed 3 times or, as required, more following the our offered requirements to repeatability of peaks areas S for repeated injections – the relative standard deviation of the mean RSD_{nom} calculated towards the nominal value of peak area S_{nom} should not exceed:

$$RSD_{nom} = \frac{S}{S_{nom}} \cdot 100\% \leq \frac{0.1 \cdot \max \Delta_{As} \cdot \sqrt{n}}{t(95\%, n-1)} = \begin{cases} 1.47\%; & n = 3 \\ 1.88\%; & n = 4 \\ 2.22\%; & n = 5 \\ 2.52\%; & n = 6 \end{cases}$$

$$S_{nom} = S_{min} = \bar{S}_{25\%},$$

where $\max \Delta_{As}$ – is the extreme relative uncertainty of the procedure of analysis, $\max \Delta_{As} = 20\%$ [3];

$\bar{S}_{25\%}$ – the mean peak area obtained when analysing the respective solutions with the analyte concentration corresponded to the point of 25% in the normalized coordinates (see explanations in the text).

RESULTS AND THEIR DISCUSSION

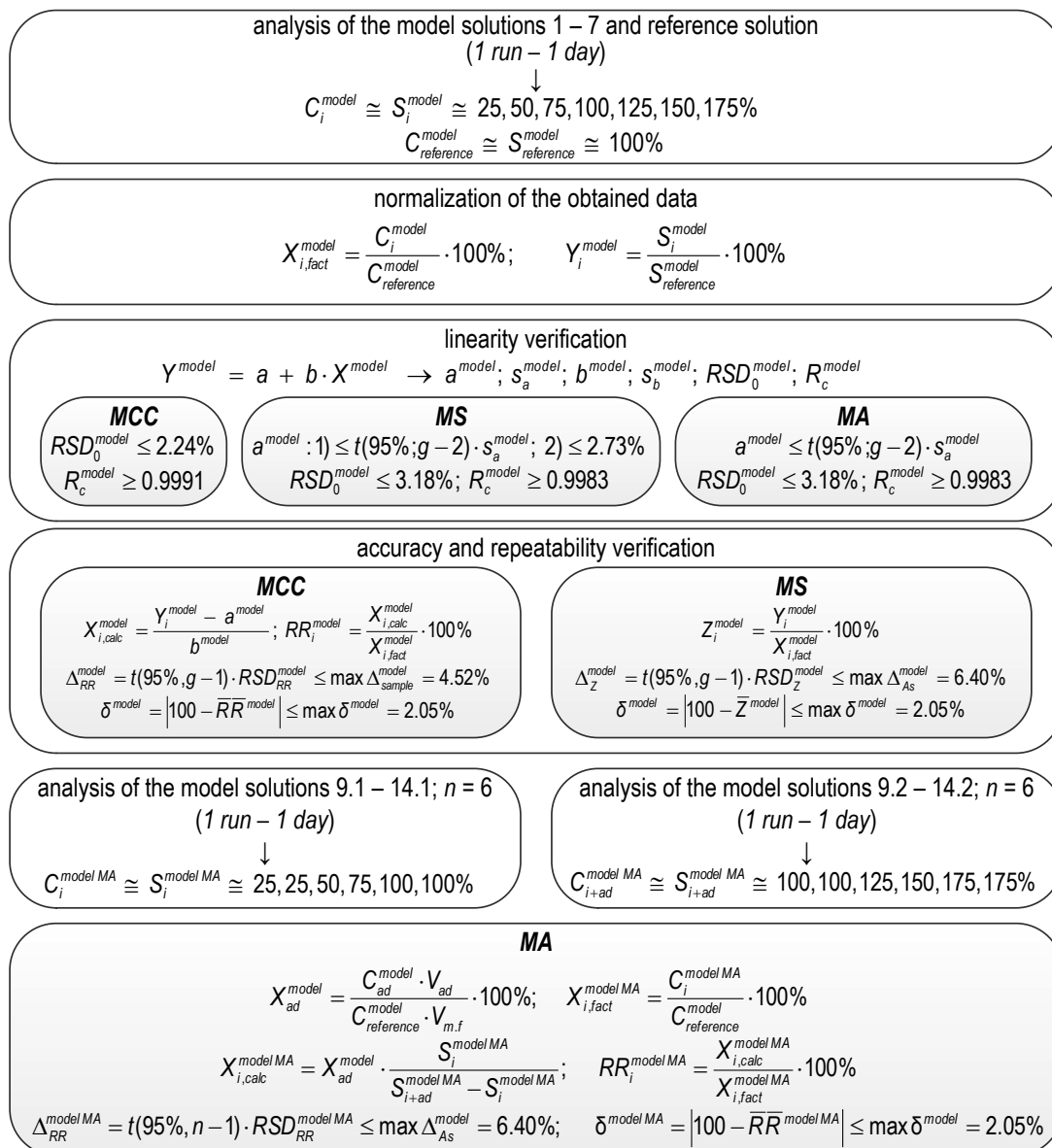
It has been suggested to carry out metoclopramide isolation from blood by its maceration with amphiphilic solvents and subsequent separation of organic layer under the conditions of aqueous phase saturation by electrolyte for increasing the efficiency; this approach enjoys wide popularity in modern forensic and toxicological analysis [1, 11, 15]. Such amphiphilic solvent as acetonitrile has been used in the experiment; ammonium sulphate has been applied as electrolyte for saturation of aqueous phase. Isolation has been carried out in the weak-acid medium (pH = 5).

Carrying out isolation of analytes from biological objects in the weak-acid medium results in decreasing of co-extraction processes of biological matrix components in a number of cases [1, 11, 15]. It is necessary to note that application of amphiphilic solvents and saturated solution of ammonium sulfate allows to maintain the isolation efficiency of substances of base character in the weak-acid medium at the same level as in the alkaline medium – it is conditioned by shift of pH real value in alkaline side for mixtures of electrolytes saturated solutions with amphiphilic solvents [17].

Validation of the developed procedure has been carried out in the variant of the MCC, MS and MA [2, 4 – 6, 9, 12 – 14] using the normalized coordinates. For normalization of the obtained experimental data the reference solution with the concentration of analyte corresponded to its concentration in the end solution to be analysed under the condition of zero losses for the point of 100% in the normalized coordinates is used. The peak area for reference solution is corrected taking into account the value of recovery R , which significance and value has been showed at the preliminary stage of validation, and is used for normalization of peak areas for the calibration and model samples.

The range of the methods application is $D = 25 - 175\%$; the number of concentration levels is $g = 7$ in constant increments of 25%; as 100% the mean toxic metoclopramide concentration in blood [1] – 4 mcg/ml – is accepted.

The method validation has been carried out at the first stage using model solutions (*Scheme 4*) and proceeding from that the uncertainty of analytical quantitative determination in model solutions Δ_{As}^{model} is insignificant against the total uncertainty of analysis results Δ_{As} .



Scheme 4. The stages of validation of HPLC-method of metoclopramide determination using model solutions

The total results of validation are presented in *Table 1* and allow to point to the conclusion about acceptable linearity, accuracy and repeatability of the HPLC-procedure of metoclopramide quantitative determination in the variant of epy MCC, MS and MA that gives the possibility to recommend it to further application in forensic toxicology with the purpose of development of the methods of biological objects analysis for metoclopramide quantification.

At the second stage the method validation has been carried out using calibration and model samples – the determination procedure and acceptability criteria are presented at *Scheme 5* and *6*.

The total results of validation are presented in *Table 2*.

The results of analysis show the absence of peaks with the retention time, which is coincident with (or near to) the metoclopramide retention time, on the chromatograms of blank-samples that points to the conclusion about acceptable specificity of the developed method as for the components of biological matrix.

By results the recovery study the efficiency of metoclopramide isolation from blood is ~93%, the reproducibility of recovery values satisfies the acceptability criteria.

On the whole, the examined method is characterized by the acceptable parameters of linearity,

Table 1

**THE TOTAL RESULTS OF VALIDATION
OF METOCLOPRAMIDE DETERMINATION PROCEDURE BY THE METHOD OF HPLC,
WHICH WERE OBTAINED USING MODEL SOLUTIONS**

Parameter	MCC		MS		MA	
	value	acceptability criterion	value	acceptability criterion	value	acceptability criterion
<i>1) linearity</i>						
b^{model}	1.007	–	1.007	–	1.007	–
s_b^{model}	0.008	–	0.008	–	0.008	–
a^{model}	0.591	–	0.591	$\leq 2.73\%$	0.591	$a^{model} \leq 2.015 \cdot s_a^{model}$
s_a^{model}	0.897	–	0.897	$a^{model} \leq 2.015 \cdot s_a^{model}$	0.897	
RSD_0^{model}	1.062	$\leq 2.24\%$	1.062	$\leq 3.18\%$	1.062	$\leq 3.18\%$
RSD_C^{model}	0.9998	≥ 0.9991	0.9998	≥ 0.9983	0.9998	≥ 0.9983
<i>2) accuracy and precision</i>						
$\overline{RR}^{model} (\overline{RR}^{model MA}, \overline{Z}^{model})$	100.86	–	101.52	–	101.01	–
$\delta^{model} (\delta^{model MA})$	0.86	$\leq 2.05\%$	1.52	$\leq 2.05\%$	1.01	$\leq 2.05\%$
$RSD_{RR}^{model} (RSD_{RR}^{model MA}, RSD_Z^{model})$	1.06	–	1.07	–	2.39	–
$\Delta_{RR}^{model} (\Delta_{RR}^{model MA}, \Delta_Z^{model})$	2.06	$\leq 4.52\%$	2.08	$\leq 6.40\%$	4.81	$\leq 6.40\%$

analysis of the model samples 8 – 12; $n = 4, k = 3$ (3 runs – 3 days)

$$C_i^{sample} \cong S_i^{sample} \cong 25, 50, 100, 175\%$$

specificity

analysis of the blank-samples; $n = 5 \rightarrow \overline{S}_{blank}$
absence of peaks with analyte t_R

$$RSD_{nom}(blank) = \frac{s}{S_{nom}} \cdot 100\% \leq \frac{0.32 \cdot \max \Delta_{As} \cdot \sqrt{n}}{t(95\%, n-1)} = 6.71\%^1$$

$$S_{nom} = \overline{S}_{min} = \overline{S}_{25\%}^{sample}$$

¹if unsatisfied – n is increased

$$\delta_{blank(25\%)} = \frac{\overline{S}_{blank}}{S_{25\%}^{sample}} \cdot 100\% \leq \max \delta_{25\%} = 8.00\%^2$$

²if unsatisfied – it is necessary to modify the sample preparation procedure or chromatographing conditions

recovery

recovery determination

$$R_i = \frac{S_i^{sample}}{S_i^{model}} \cdot 100\%; \quad \overline{R} = \frac{\sum R_i}{n \cdot k}$$

$$1) \Delta_{R,f} = t(95\%; k \cdot n - 1) \cdot \sqrt{\frac{\sum (RSD_R)^2}{k}} \leq \max \Delta_{As} = 20.00\%$$

$$2) R = a^R + b^R \cdot X \rightarrow a^R; s_a^R; b^R; s_b^R;$$

$$a^R > t(95\%; k \cdot n - 2) \cdot s_a^R; \quad b^R \leq t(95\%; k \cdot n - 2) \cdot s_b^R$$

$$3) |100 - \overline{R}| \leq \max \delta = 6.40\%^3$$

³if unsatisfied – the value of R is considered when calculating

analysis of the calibration samples 1 – 7; $g = 7, k = 3$ (3 runs – 3 days)

$$C_i^{calibrator} \cong S_i^{calibrator} \cong 25, 50, 75, 100, 125, 150, 175\%$$

$$RSD_{nom}(calibrator) = \frac{s}{S_{nom}} \cdot 100\% \leq \frac{0.707 \cdot \max \Delta_{As} \cdot \sqrt{k}}{t(95\%, k-1)} = 8.39\%^4; \quad S_{nom} = \overline{S}_{min} = \overline{S}_{25\%}^{sample}$$

⁴if unsatisfied – k is increased

linearity

normalization of the obtained data

$$X_{i, fact} = \frac{C_i^{calibrator}}{C_{reference}} \cdot 100\%; \quad C_{reference} = C_{reference}^{model} \cdot K^5; \quad Y_i = \frac{S_i^{calibrator}}{S_{reference}} \cdot 100\%; \quad S_{reference} = \frac{S_{reference}^{model} \cdot \overline{R}}{100}$$

⁵dilution coefficient

calculation of linear dependence $Y = a + b \cdot X$ parameters (within-run and between-run)

$$Y = a + b \cdot X \rightarrow a^k; s_a^k; b^k; s_b^k; RSD_0^k; R_c^k \text{ and } a; s_a; b; s_b; RSD_0; R_c$$

MCC

$$RSD_0 \leq 7.02\%; \quad R_c \geq 0.9915$$

MS

$$a : 1) \leq t(95\%; g-2) \cdot s_a; \quad 2) \leq 8.53\%$$

$$RSD_0 \leq 9.93\%; \quad R_c \geq 0.9830$$

MA

$$RSD_0 \leq 9.93\%; \quad R_c \geq 0.9830$$

$$a \leq t(95\%; g-2) \cdot s_a$$

Scheme 5. The scheme of specificity, recovery and linearity verification of HPLC-method of metoclopramide determination in blood using calibration and model samples

accuracy and precision

 analysis of the model samples 14.1 – 19.1
 $n = 6, k = 3$
 (3 runs – 3 days)

$$C_i^{\text{sample MA}} \cong S_i^{\text{sample MA}} \cong 25, 25, 50, 75, 100, 100\%$$

 analysis of the model samples 14.2 – 19.2
 $n = 6, k = 3$
 (3 runs – 3 days)

$$C_{i+ad}^{\text{sample MA}} \cong S_{i+ad}^{\text{sample MA}} \cong 100, 100, 125, 150, 175, 175\%$$

within-run precision (repeatability) and accuracy

MCC

$$X_{i,\text{calc}}^k = \frac{Y_i^k - a^k}{b^k}; RR_i^k = \frac{X_{i,\text{calc}}^k}{X_{i,\text{fact}}^k} \cdot 100\%$$

$$\Delta_{RR}^k = t(95\%, g-1) \cdot RSD_{RR}^k \leq \max \Delta_{\text{sample}} = 14.14\%$$

$$\delta^k = |100 - \overline{RR}^k| \leq \max \delta = 6.40\%$$

MS

$$Z_i^k = \frac{Y_i^k}{X_{i,\text{fact}}^k} \cdot 100\%$$

$$\Delta_Z^k = t(95\%, g-1) \cdot RSD_Z^k \leq \max \Delta_{As} = 20.00\%$$

$$\delta^k = |100 - \overline{Z}^k| \leq \max \delta = 6.40\%$$

MA

$$X_{ad} = \frac{C_{ad} \cdot V_{ad}}{C_{\text{reference}} \cdot V_{\text{sample}}} \cdot 100\%; X_{i,\text{fact}}^{MA,k} = \frac{C_i^{\text{sample MA}}}{C_{\text{reference}}} \cdot 100\%$$

$$X_{i,\text{calc}}^{MA,k} = X_{ad} \cdot \frac{S_i^{\text{sample MA}}}{S_{i+ad}^{\text{sample MA}} - S_i^{\text{sample MA}}}; RR_i^{MA,k} = \frac{X_{i,\text{calc}}^{MA,k}}{X_{i,\text{fact}}^{MA,k}} \cdot 100\%$$

$$\Delta_{RR}^{MA,k} = t(95\%, n-1) \cdot RSD_{RR}^{MA,k} \leq \max \Delta_{As} = 20.00\%; \delta^{MA,k} = |100 - \overline{RR}^{MA,k}| \leq \max \delta = 6.40\%$$

 between-run (intermediate) precision and accuracy
 (by calibration samples)

MCC

$$X_{i,\text{calc}} = \frac{Y_i^k - a}{b}; RR_i = \frac{X_{i,\text{calc}}}{X_{i,\text{fact}}} \cdot 100\%$$

$$\overline{RR}^{\text{intra}} = \frac{\sum RR_i}{k \cdot g}; RSD_{RR}^{\text{intra}} = \sqrt{\frac{\sum RSD_{RR,k}^2}{k}}$$

$$\Delta_{RR}^{\text{intra}} = t(95\%, k \cdot g - 1) \cdot RSD_{RR}^{\text{intra}} \leq \max \Delta_{\text{sample}} = 14.14\%$$

$$\delta^{\text{intra}} = |100 - \overline{RR}^{\text{intra}}| \leq \max \delta = 6.40\%$$

MS

$$\overline{Z}^{\text{intra}} = \frac{\sum Z_i^k}{k \cdot g} \cdot 100\%$$

$$RSD_Z^{\text{intra}} = \sqrt{\frac{\sum (RSD_Z^k)^2}{k}}$$

$$\Delta_Z^{\text{intra}} = t(95\%, k \cdot g - 1) \cdot RSD_Z^{\text{intra}} \leq \max \Delta_{As} = 20.00\%$$

$$\delta^{\text{intra}} = |100 - \overline{Z}^{\text{intra}}| \leq \max \delta = 6.40\%$$

 between-run (intermediate) precision and accuracy
 (by model samples)

MCC

$$X_{i,\text{fact}}^{\text{sample}} = \frac{C_i^{\text{sample}}}{C_{\text{reference}}} \cdot 100\%; Y_i^{\text{sample}} = \frac{S_i^{\text{sample}}}{S_{\text{reference}}} \cdot 100\%$$

$$X_{i,\text{calc}}^{\text{sample}} = \frac{Y_i^{\text{sample}} - a}{b}; RR_i^{\text{sample}} = \frac{X_{i,\text{calc}}^{\text{sample}}}{X_{i,\text{fact}}^{\text{sample}}} \cdot 100\%$$

$$\overline{RR}^{\text{sample}} = \frac{\sum RR_i^{\text{sample}}}{k \cdot n}; RSD_{RR}^{\text{sample}} = \sqrt{\frac{\sum (RSD_{RR,k}^{\text{sample}})^2}{k}}$$

$$\Delta_{RR}^{\text{sample}} = t(95\%, k \cdot n - 1) \cdot RSD_{RR}^{\text{sample}} \leq \max \Delta_{As} = 20.00\%$$

$$\delta^{\text{sample}} = |100 - \overline{RR}^{\text{sample}}| \leq \max \delta = 6.40\%$$

MA

$$\overline{RR}^{\text{intra MA}} = \frac{\sum RR_i^{MA,k}}{k \cdot n} \cdot 100\%$$

$$RSD_{RR}^{\text{intra MA}} = \sqrt{\frac{\sum (RSD_{RR}^{MA,k})^2}{k}}$$

$$\Delta_{RR}^{\text{intra MA}} = t(95\%, k \cdot n - 1) \cdot RSD_{RR}^{\text{intra MA}} \leq \max \Delta_{As} = 20.00\%$$

$$\delta^{\text{intra MA}} = |100 - \overline{RR}^{\text{intra MA}}| \leq \max \delta = 6.40\%$$

Scheme 6. The scheme of precision and accuracy verification of HPLC-method of metoclopramide determination in blood using calibration and model samples

Table 2

THE TOTAL RESULTS OF VALIDATION OF METOCLOPRAMIDE DETERMINATION PROCEDURE IN BLOOD BY THE METHOD OF HPLC, WHICH WERE OBTAINED USING MODEL AND CALIBRATION SAMPLES

Parameter	MCC			MS			MA		
	value	acceptability criterion	value	value	acceptability criterion	value	acceptability criterion	value	acceptability criterion
<i>1) linearity</i>									
b	1.054	1.054	1.078	1.054	1.054	1.078	1.054	1.054	1.078
s_b	0.016	0.022	0.009	0.016	0.022	0.009	0.016	0.022	0.009
a	-2.423	-4.449	-	-2.423	-1.990	-4.449	-2.423	-1.990	-4.449
s_a	1.834	2.483	1.012	1.834	2.483	1.012	1.834	2.483	1.012
RSD₀	2.170	2.938	1.198	2.170	2.938	1.198	2.170	2.938	1.198
R_c	0.9994	0.9989	0.9998	0.9994	0.9989	0.9998	0.9994	0.9989	0.9998
<i>2) accuracy and precision</i>									
$\overline{RR}^k (\overline{RR}^{MA,k}, \overline{Z}^k)$	100.72	100.47	100.31	102.58	102.95	101.55	99.52	98.99	100.32
$\delta^k (\sigma^{MA,k})$	0.72	0.47	0.31	2.58	2.95	1.55	0.48	1.01	0.32
$RSD_{RR}^k (RSD_{RR}^{MA,k}, RSD_Z^k)$	3.22	3.82	2.09	1.80	3.68	4.19	3.49	4.06	3.70
$\Delta_{RR}^k (\Delta_{RR}^{MA,k}, \Delta_Z^k)$	6.26	7.42	4.06	3.50	7.15	8.14	7.03	8.18	7.46
$\overline{RR}_{intra} (\overline{RR}_{intra}^{MA}, \overline{Z}_{intra})$	100.50			102.36			99.61		
$\delta_{intra}^k (\sigma_{intra}^{MA})$	0.50			2.36			0.39		
$RSD_{RR}^{intra} (RSD_{RR}^{intra MA}, RSD_Z^{intra})$	3.44			3.39			3.76		
$\Delta_{RR}^{intra} (\Delta_{RR}^{intra MA}, \Delta_Z^{intra})$	5.93			5.85			6.54		
RR_{sample}	101.28			-			-		
σ_{sample}	1.28			-			-		
RSD_{RR}^{sample}	3.41			-			-		
Δ_{RR}^{sample}	6.12			≤ 20.00%			-		

accuracy and precision both in the variant of the MCC, MS and MA.

In the case of analysis by the MS and MA it is noted the worsening of repeatability and intermediate precision of the analysis results as compared with the MCC. It is necessary to note that the MA provides the highest acceptability of the method accuracy, while in the MS the systematic error is maximal.

CONCLUSIONS AND FURTHER RESEARCHES OUTLOOK

1. The HPLC-procedure of metoclopramide quantitative determination in blood using amphiphilic solvent (acetonitrile) for analyte isolation at pH = 5 with further separation of organic layer under the conditions of aqueous phase saturation by ammonium sulphate has been developed for application in forensic and toxicological analysis.

2. Validation of the developed procedure has been carried out using model solutions, and also calibration and model samples by such parameters as specificity, recovery, linearity, accuracy and precision.

3. The possibility of application of the MCC, MS and MA for metoclopramide quantitative determination in blood using the offered HPLC-procedure has been shown.

LIST OF THE USED INFORMATION SOURCES

- Clarke's analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem material: 4th ed. / edited by A. C. Moffat, M. D. Osselson, B. Widdop. – London: Pharmaceutical Press, 2011. – 2609 p.
- Determination of accuracy when validating UV-spectrophotometric methods of quantitative determination in forensic and toxicological analysis / L. Yu. Klimenko, S. M. Trut, G. P. Petyunin, T. A. Kostina // Український біофармацевтичний журнал. – 2014. – №2 (29). – С.55–67.
- Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens / United Nations Office on Drugs and Crime, Laboratory and Scientific Section. – New York: United Nations, 2009. – 70 p.
- Klimenko L. Yu. Development of approaches to validation of UV-spectrophotometric methods of quantitative determination in forensic and toxicological analysis: linearity and application range / L. Yu. Klimenko, G. P. Petyunin // Фармацевтичний часопис. – 2014. – №2 (30). – С. 46 – 51.

- Klimenko, L. Yu. Approaches to determination of precision for UV-spectrophotometric methods of quantitative determination in forensic and toxicological analysis / L. Yu. Klimenko, S. M. Trut, O. Ye. Mykytenko // Фармація Казахстана. – 2014. – №3. – С. 43 – 50.
- Klimenko L. Yu. Determination of linearity, accuracy and precision of UV-spectrophotometric methods of quantification in forensic and toxicological analysis in the variant of the method of additions / L. Yu. Klimenko // Фармація Казахстана. – 2014. – №7. – С. 51–58.
- Metoclopramide in pregnancy and risk of major congenital malformations and fetal death / B. Pasternak, H. Svanström, D. Mølgaard-Nielsen, M. Melbye, A. Hviid // JAMA. – Vol. 310 (15). – P. 1601 – 1611.
- Supariwala, A. Neuroleptic malignant syndrome with metoclopramide overdose coexisting with Clostridium difficile diarrhea / A. Supariwala, G. Kant, R. E. Jean // Intensive Care Med. – 2011. – V. 37 (10). – P. 1706 – 1708.
- Validation of UV-spectrophotometric methods of quantitative determination in forensic and toxicological analysis: recovery / L. Yu. Klimenko, S. M. Trut, G. P. Petyunin, I. M. Ivanchuk // Фармація Казахстана. – 2013. – №12. – С. 42 – 48.
- Vrachnis, N. Fetal outcomes associated with metoclopramide use in pregnancy / N. Vrachnis, D. Zygouris, Z. Iliodromiti // JAMA. – 2014. – V. 311 (6). – P. 623 – 624.
- Герасимов Д. А. Химико-токсикологическое исследование нимесулида и близких по структуре соединений: дис. ... канд. фарм. наук / Д. А. Герасимов. – Курск, 2014. – 326 с.
- Клименко Л. Ю. Подходы к определению специфичности / селективности при валидации УФ-спектрофотометрических методик количественного определения в судебно-токсикологическом анализе / Л. Ю. Клименко, Г. П. Петюнин, Т. А. Костина // Фармація Казахстана. – 2013. – №8. – С. 53 – 56.
- Клименко Л. Ю. Разработка подходов к определению линейности, правильности и прецизионности УФ-спектрофотометрических методик количественного определения методом стандарта в судебно-токсикологическом анализе / Л. Ю. Клименко // Фармація Казахстана. – 2014. – № 4. – С. 31–35.
- Критерии приемлемости линейной зависимости при проведении валидации УФ-спектрофотометрических методик количественного определения в судебно-токсикологическом анализе / Л. Ю. Клименко, Г. П. Пе-

- тюнин, С. Н. Трут, В. П. Мороз // Актуальні питання фармацевтичної і медичної науки та практики. – 2014. – №2 (15). – С. 15 – 22.
15. Маміна О. О. Розробка та удосконалення методів аналізу органічних лікарських речовин загального дослідження при проведенні судово-медичних експертиз: дис. ... д-ра фарм. наук / О. О. Маміна. – Х., 2008. – 294 с.
16. Мороз В. П. Хіміко-токсикологічне дослідження метоклопраміду: автореф. дис. ... канд. фарм. наук / В. П. Мороз. – Х., 2000. – 19 с.
17. Смотров М. П. Топологическая трансформация фазовых диаграмм тройных систем соль-бинарный растворитель с всаливанием-высаливанием / М. П. Смотров. – Саратов, 2012. – 281 с.

УДК 615.217.3:543.544.5:543.054

Л. Ю. Клименко, В. П. Мороз, Т. А. Костина

РОЗРОБКА ТА ВАЛІДАЦІЯ ВЕРХ-МЕТОДИКИ ВИЗНАЧЕННЯ МЕТОКЛОПРАМІДУ В КРОВІ

Розроблено ВЕРХ-методику кількісного визначення метоклопраміду в крові з використанням для виділення аналіту амфифільного розчинника (ацетонітрилу) за рН = 5 з подальшим відділенням органічного шару в умовах насичення водної фази амонію сульфатом для застосування в судово-токсикологічному аналізі. Проведено валідацію розробленої методики та показано можливість застосування методу калібрувального графіку, методу стандарту і методу добавок для кількісного визначення метоклопраміду в крові з використанням запропонованої методики.

Ключові слова: валідація, біоаналітичні методики, високоефективна рідинна хроматографія, метоклопрамід, метод калібрувального графіку, метод стандарту, метод добавок.

УДК 615.217.3:543.544.5:543.054

Л. Ю. Клименко, В. П. Мороз, Т. А. Костина

РАЗРАБОТКА И ВАЛИДАЦИЯ ВЭЖХ-МЕТОДИКИ ОПРЕДЕЛЕНИЯ МЕТОКЛОПРАМИДА В КРОВИ

Разработана ВЭЖХ-методика количественного определения метоклопрамида в крови с использованием для выделения аналита амфифильного растворителя (ацетонитрила) при рН = 5 с последующим отделением органического слоя в условиях насыщения водной фазы аммония сульфатом для применения в судебно-токсикологическом анализе. Проведена валідація розробленої методики і показано можливість застосування методу калібрувального графіку, методу стандарту і методу добавок для кількісного визначення метоклопраміду в крові методом ВЭЖХ.

Ключевые слова: валідація, біоаналітичні методики, високоефективна жидкостная хроматографія, метоклопрамід, метод калібрувального графіку, метод стандарту, метод добавок.

Адреса для листування:

61168, м. Харков, ул. Блюхера, 4
Кафедра аналитической химии НФаУ
Тел.: (0572) 67-94-24, 67-91-93
E-mail: lynnne2@ukr.net

Надійшла до редакції:

14.10.2014