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

The HPLC-procedure of metoclopramide quantitative determination in blood using amphiphylic 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

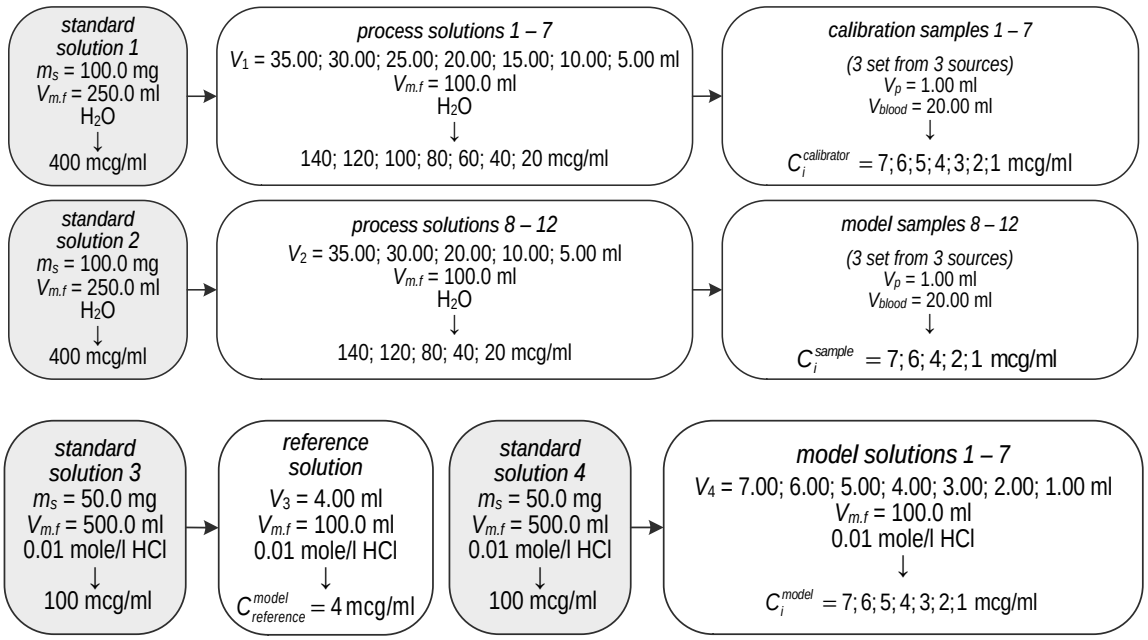
The described procedure [16] can not provide high sensitivity of determination, besides the possibility of its application for carrying out quantitative determination of metoclopramide in blood has not been investigated. It is interesting to develop the sensitive procedure of metoclopramide quantitative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

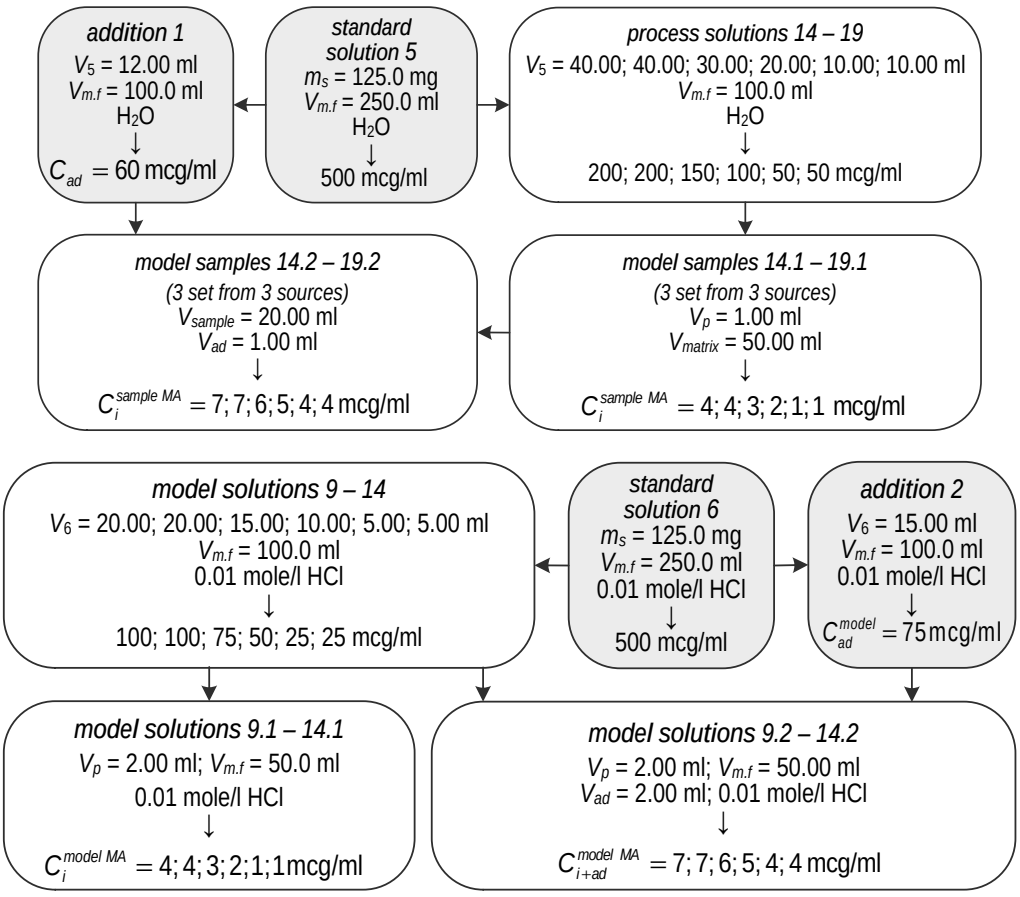
The purpose of the paper is developing the HPLC-procedure of metoclopramide quantitative determination in blood with carrying out the sample preparation in the way of maceration with amphiphylic solvents and subsequent separation of organic layer under the conditions of aqueous phase saturation by electrolyte; 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*.

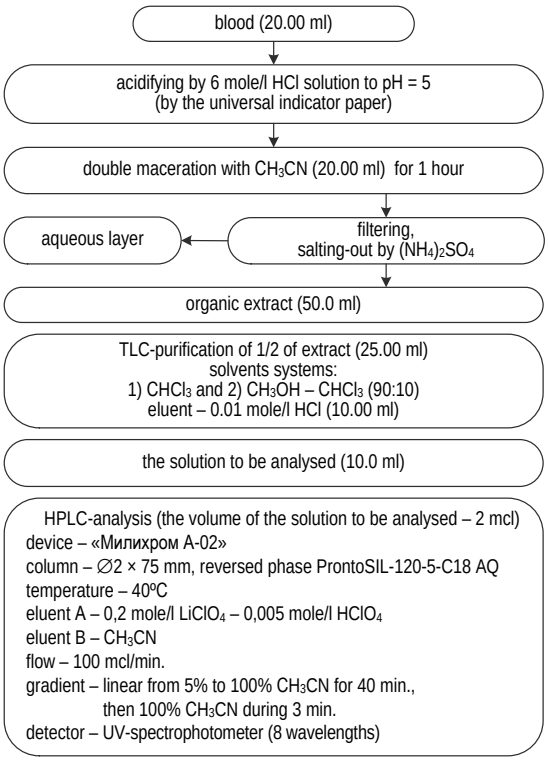


Scheme 1. *The order of solutions and samples preparation for validation of metoclopramide determination procedure in blood by the method of HPLC in the variant of MCC and MS*



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:

$$RSD_{nom} = \frac{S}{S_{nom}} \cdot 100\% \le$$

$$\le \frac{0.1 \cdot \max\Delta_{As} \cdot \sqrt{n}}{t(95\%, n-1)} = \begin{Bmatrix} 1.47\%; & n=3 \\ 1.88\%; & n=4 \\ 2.22\%; & n=5 \\ 2.52\%; & n=6 \end{Bmatrix},$$

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

where $\max\Delta_{As}$ – is the extreme relative uncertainty of the procedure of analysis, $\max\Delta_{As}$ = 20 % [3]; $\overline{S}_{25\%}$ – 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

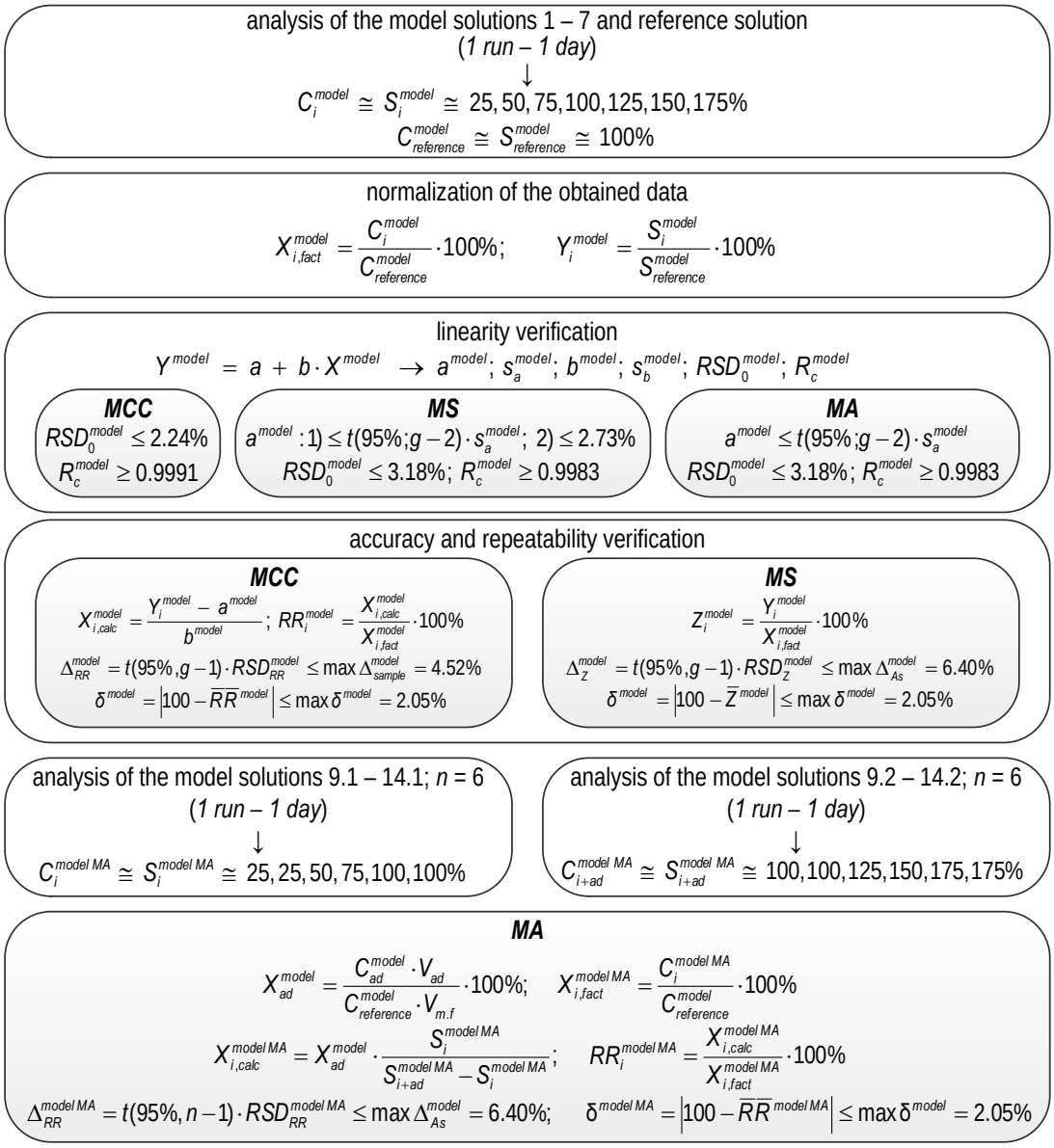
It has been suggested to carry out metoclopramide isolation from blood by its maceration with amphiphylic solvents and subsequent separation of organic layer under the conditions of aqueous phase saturation by electrolyte for increasing the efficiency; this approach enjoys wide popularity in modern forensic and toxicological analysis [1, 11, 15]. Such amphiphylic 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 amphiphylic 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 amphiphylic 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 analyte 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.

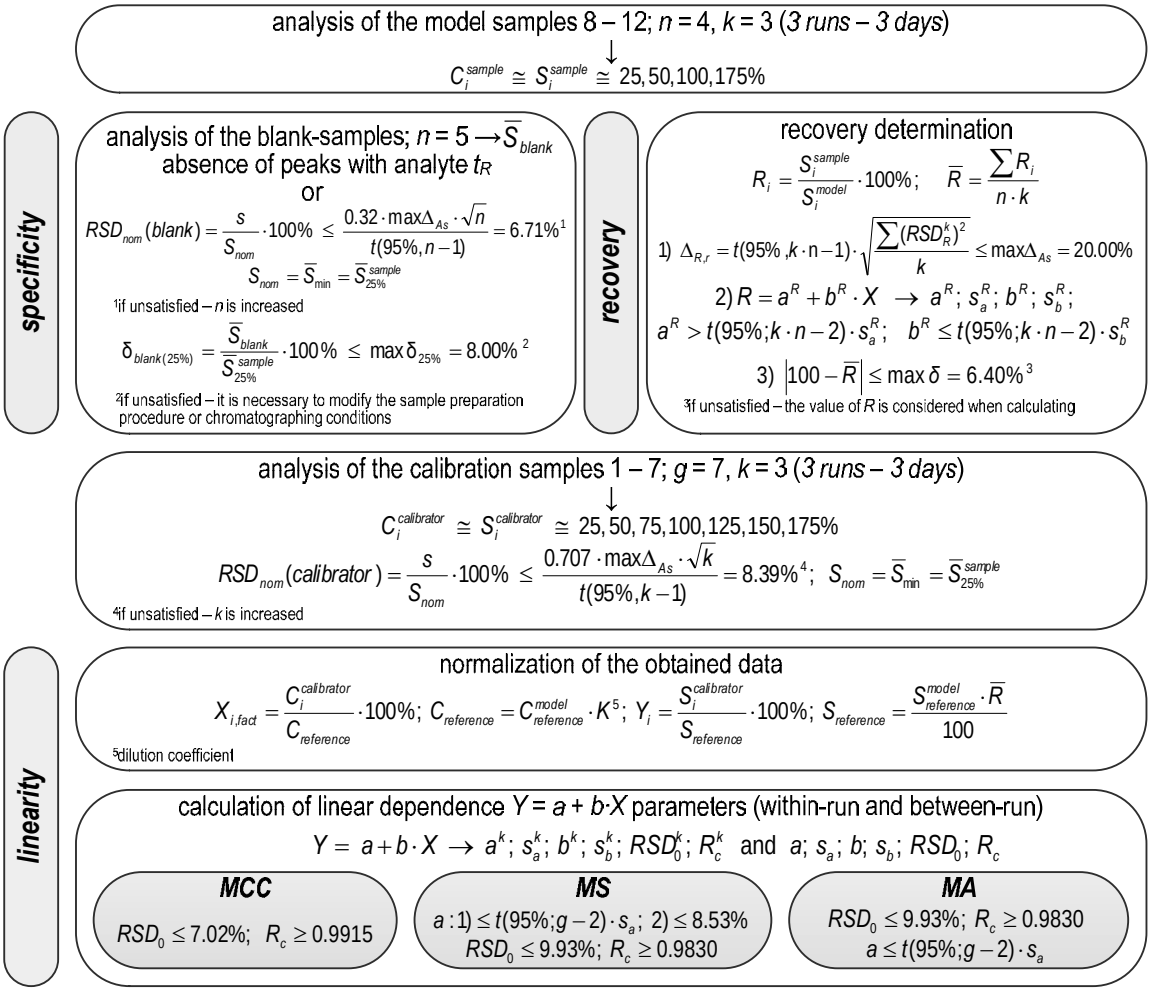
By results the recovery study the efficiency of metoclopramide isolation from blood is ~93%, the reproducibility of recovery values satisfies the acceptability criteria.

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

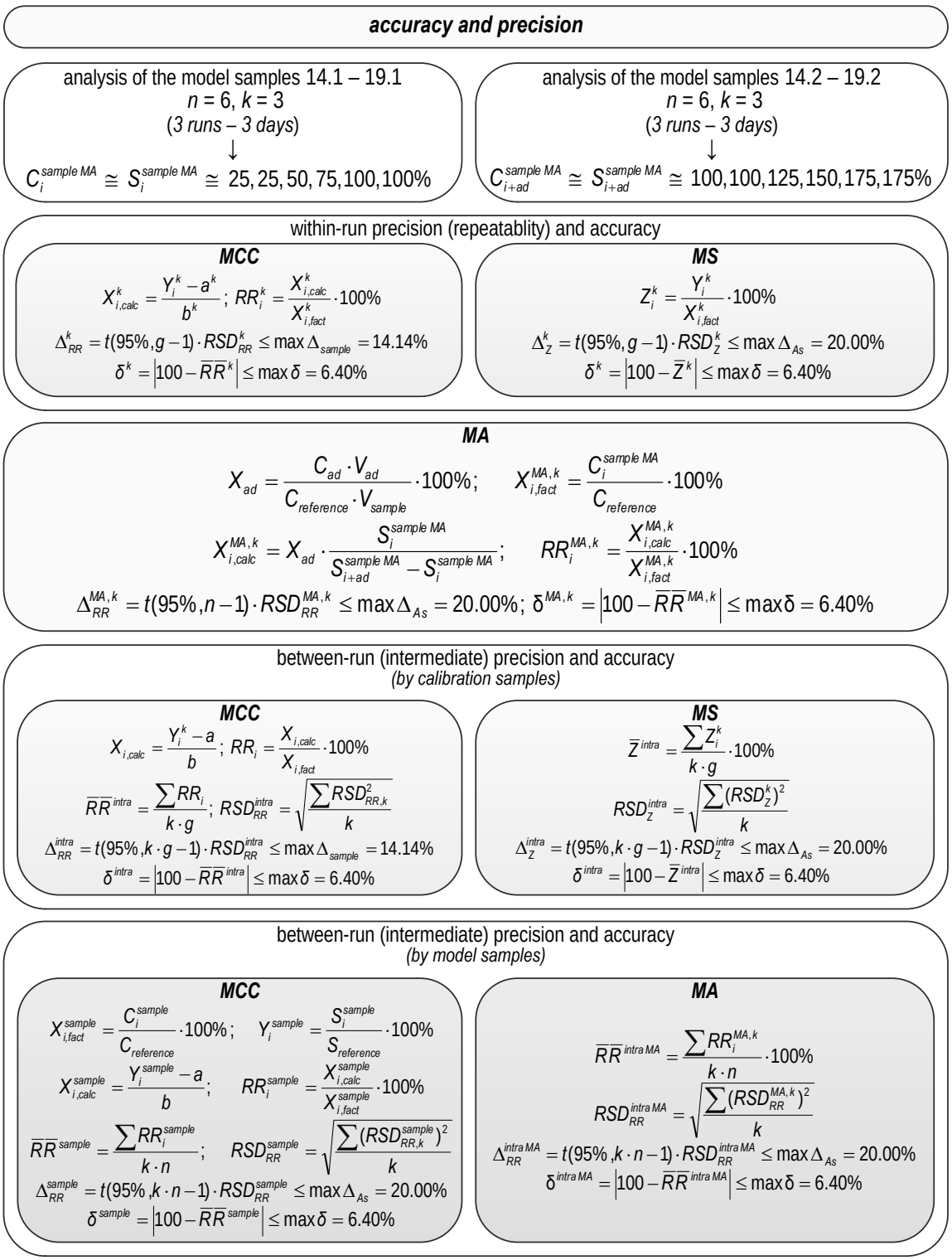
Table 1

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
1) linearity						
b^{model}	1.007	–	1.007	–	1.007	–
S_b^{model}	0.008	–	0.008	–	0.008	–
a^{model}	0.591	–	0.591	≤ 2.73%	0.591	$a^{model} \le 2.015 \cdot S_a^{model}$
S_a^{model}	0.897	–	0.897	$a^{model} \le 2.015 \cdot S_a^{model}$	0.897	
RSD_0^{model}	1.062	≤ 2.24%	1.062	≤ 3.18%	1.062	≤ 3.18%
RSD_C^{model}	0.9998	≥ 0.9991	0.9998	≥ 0.9983	0.9998	≥ 0.9983
2) accuracy and precision						
$\overline{RR}^{model}(\overline{RR}^{model\,MA}, \overline{Z}^{model})$	100.86	–	101.52	–	101.01	–
$\delta^{model}(\delta^{model\,MA})$	0.86	≤ 2.05%	1.52	≤ 2.05%	1.01	≤ 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	≤ 4.52%	2.08	≤ 6.40%	4.81	≤ 6.40%



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



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			acceptability criterion	value			acceptability criterion
1) linearity												
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	–1.990	–4.449	–	–2.423	–1.990	–4.449	≤ 8.53%	–2.423	–1.990	–4.449	$a \le 2.015 \cdot s_a$
s_a	1.834	2.483	1.012	–	1.834	2.483	1.012	$a \le 2.015 \cdot s_a$	1.834	2.483	1.012	
RSD_0	2.170	2.938	1.198	≤ 7.02%	2.170	2.938	1.198	≤ 9.93 %	2.170	2.938	1.198	≤ 9.93%
R_C	0.9994	0.9989	0.9998	≥ 0.9915	0.9994	0.9989	0.9998	≥ 0.9830	0.9994	0.9989	0.9998	≥ 0.9830
2) accuracy and precision												
$\overline{\overline{RR}}^k (\overline{\overline{RR}}^{MA,k}, \overline{Z}^k)$	100.72	100.47	100.31	–	102.58	102.95	101,55	–	99.52	98.99	100.32	–
$\delta^k (\delta^{MA,k})$	0.72	0.47	0.31	≤ 6.40%	2.58	2.95	1,55	≤ 6,40%	0.48	1.01	0.32	≤ 6.40%
$RSD_{RR}^k (RSD_{RR}^{MA,k}, RSD_Z^k)$	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	≤ 14.14%	3.50	7.15	8,14	≤ 20,00%	7.03	8.18	7.46	≤ 20.00%
$\overline{\overline{RR}}^{intra} (\overline{\overline{RR}}^{intra\,MA}, \overline{Z}^{intra})$	100.50			–	102.36			–	99.61			–
$\delta^{intra} (\delta^{intra\,MA})$	0.50			≤ 6.40%	2.36			≤ 6.40%	0.39			≤ 6.40%
$RSD_{RR}^{intra} (RSD_{RR}^{intra\,MA}, RSD_Z^{intra})$	3.44			–	3.39			–	3.76			–
$\Delta_{RR}^{intra} (\Delta_{RR}^{intra\,MA}, \Delta_Z^{intra})$	5.93			≤ 14.14%	5.85			≤ 20.00%	6.54			≤ 20.00%
$\overline{\overline{RR}}^{sample}$	101.28			–	–			–	–			–
δ^{sample}	1.28			≤ 6.40%	–			–	–			–
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 amphiphylic 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.

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РОЗРОБКА ТА ВАЛІДАЦІЯ ВЕРХ-МЕТОДИКИ ВИЗНАЧЕННЯ МЕТОКЛОПРАМІДУ В КРОВІ

Розроблено ВЕРХ-методику кількісного визначення метоклопраміду в крові з використанням для виділення аналіту амфіфільного розчинника (ацетонітрилу) за pH = 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.

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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 pH = 5 с последующим отделением органического слоя в условиях насыщения водной фазы аммония сульфатом для применения в судебно-токсикологическом анализе. Проведена валидация разработанной методики и показана возможность применения метода калибровочного графика, метода стандарта и метода добавок для количественного определения метоклопрамида в крови методом ВЭЖХ.

Ключевые слова: валидация, биоаналитические методики, высокоэффективная жидкостная хроматография, метоклопрамид, метод калибровочного графика, метод стандарта, метод добавок.

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