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Optimization of therapeutic drug monitoring of vancomycin in newborns using “Dried Blood Spot” method

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Therapeutic drug monitoring (TDM) is used to increase the individualization of pharmacotherapy, especially in patient groups with a high interindividual variability in pharmacokinetic (PK) parameters. One of these groups of patients is newborn children, for whom drug therapy, especially drugs with a narrow therapeutic range, causes a few difficulties or cannot be used in principle.

The aim of the work was to develop and validate quantitative HPLC-MS/MS methods for the determination of vancomycin in “dried blood spot” samples using new protocols and comparison of the results obtained with the results in plasma samples using standard sample preparation methods.

Materials and methods. To prepare stock and standard solutions of vancomycin and norvancomycin as an internal standard, dry portions of the corresponding certified standards of vancomycin (Servier, France) and norvancomycin (Augsburg, Germany, purity grade >95.0%) were used. A chromatographic separation of the components was carried out on a Poroshell 120 C18 column (4.6×50 mm, 2.7 µm). When developing conditions for a mass spectrometric detection of the desired substances using the multiple reaction monitoring (MRM) method, precursor ions and their corresponding product ions were determined.

Results. A quantitative HPLC-MS/MS method for the determination of vancomycin in "dried blood spot" samples was developed and validated. A comparison was made between vancomycin concentrations in "dried blood spot" samples and plasma samples. Moreover, more than 95% of the calculated average concentrations are within the limits of d-2s and d+2s, which correspond to the values of –10.2 and 12.2. That confirms the suitability of the developed method for the analysis of patient samples.

Conclusion. The results obtained make it possible for us to recommend the “dried blood spot” method for therapeutic monitoring of vancomycin, additional studies of PK in this group of patients with subsequent use of this drug in newborns and pediatric patients.

Keywords: therapeutic drug monitoring; vancomycin; HPLC/MS; validation; bioanalytics; dried blood spot method

Abbreviations: TDM – Therapeutic Drug Monitoring; PK – pharmacokinetics; PD – pharmacodynamics; HPLC – high performance liquid chromatography; MS – mass spectrometry; LLOQ – lower limit of quantitation; QC – quality control; DBS – Dried Blood Spot.

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Оптимизация терапевтического лекарственного мониторинга ванкомицина у новорожденных с применением метода «высушенной капли крови»

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Терапевтический лекарственный мониторинг (ТЛМ) используется для повышения индивидуализации фармакотерапии, особенного у групп пациентов с высокой межиндивидуальной вариабельностью фармакокинетических (ФК) параметров. Одной из таких групп пациентов являются новорожденные дети, для которых лекарственная терапия, особенного препаратами с узким терапевтическим диапазоном, вызывает ряд трудностей или не может быть применена в принципе.

Цель. Разработка и валидация методов количественного ВЭЖХ-МС/МС определения ванкомицина в образцах «высушенной капли крови» с использованием новых протоколов и сравнение полученных данных с результатами в образцах плазмы с использованием стандартных методов пробоподготовки.

Материалы и методы. Для приготовления маточных и стандартных растворов ванкомицина и норванкомицина как внутреннего стандарта использовали сухие навески соответствующих сертифицированных стандартов ванкомицина (Servier, Франция) и норванкомицина (Augsburg, Германия, степ. чистоты >95,0%). Хроматографическое разделение компонентов проводили на колонке Poroshell 120 C18 (4,6×50 мм, 2,7 мкм). При разработке условий масс-спектрометрической детекции искомых веществ методом мониторинга множественных реакций (MRM) были определены ионы-предшественники и соответствующие им ионы-продукты.

Результаты. Разработана и валидирована методика количественного ВЭЖХ-МС/МС определения ванкомицина в образцах «высушенной капли». Провели сравнение между значениями концентраций ванкомицина в образцах «высушенной капли крови» и образцах плазмы. При этом более 95% рассчитанных средних концентраций находились в пределах d-2s и d+2s, которые соответствовали значениям –10,2 и 12,2, что подтверждало пригодность разработанного метода для анализа образцов пациентов.

Заключение. Полученные результаты позволяют рекомендовать метод «высушенной капли крови» для проведения терапевтического мониторинга ванкомицина, дополнительных исследований ФК у данной группы пациентов с последующим применением данного лекарственного препарата у новорожденных и пациентов детского возраста.

Ключевые слова: терапевтический лекарственный мониторинг; ванкомицин, ВЭЖХ/МС; валидация; биоаналитика; метод «высушенной капли крови»

Список сокращений: ТЛМ – терапевтический лекарственный мониторинг; ФК – фармакокинетика; ФД – фармакодинамика; ВЭЖХ – высокоэффективная жидкостная хроматография; МС – масс-спектрометрия; НПКО – нижний предел количественного определения; КК – контроль качества; DBS – метод «высушенной капли крови».

INTRODUCTION

Therapeutic drug monitoring (TDM) is used to increase the individualization of pharmacotherapy, especially in patient groups with high interindividual variability in pharmacokinetic (PK) parameters [1]. Such patients include newborns, for whom drug therapy, especially drugs with a narrow therapeutic range, is associated with a number of difficulties or cannot be used in principle [2, 3].

Antibiotics are especially frequently prescribed drugs for newborns. About 2.5% of full-term infants receive antibiotic therapy in the first three days of life. However, it should be taken into account that a set of various physiological and autoimmune characteristics

in this group of patients affects the PK of drugs and can lead to ineffective pharmacotherapy and the development of undesirable consequences; hidden or obvious characteristics of the body dictate the need for an individual approach with a tendency to minimize the effects (a minimum dose, a minimum number of injections). This particularly complicates the use of antibiotics, with a narrow range between minimal therapeutic and toxic concentrations. One of such drugs is vancomycin, which is prescribed to treat severe infections caused by gram-positive bacteria, such as *Staphylococcus aureus* (especially methicillin-resistant strains), coagulase-negative *Staphylococcus*, and ampicillin-resistant *Enterococcus* species [2, 4].

Some factors that determine intra- and interindividual variability, such as age, weight, and renal condition, affect the PK and, as a consequence, the pharmacodynamic (PD) parameters of vancomycin. This necessitates carrying out TDM in this group of patients, conducting a PK analysis and creating population PK models that take into account the clinically significant characteristics of newborns and their impact on the PKs of vancomycin [1, 4].

The combination of this group of patients' features does not make it possible to carry out large-scale and comprehensive studies of PK and TDM. Since standard methods for collecting biospecimens are not optimal due to objective ethical and logistical limitations in newborns and young children, there is a need to develop and test new approaches, optimize TDM, build PK models and methods for collecting biospecimens, including the ones for TDM. A promising direction in this area can be considered the "dried blood spot" method, which is widely used, for example, for screening hereditary diseases in newborns [1, 5, 6].

A method for a quantitative HPLC–MS/MS determination of vancomycin in blood plasma samples has been previously developed and validated [7], protocols for the collection, analysis and validation of "dried blood spot" samples [5] have been also prepared. In this regard, in this study, these methods have been applied.

THE AIM of the work was to develop and validate quantitative HPLC-MS/MS methods for the determination of vancomycin in "dried blood spot" samples using new protocols and comparison of the results obtained with the results in plasma samples using standard sample preparation methods.

MATERIALS AND METHODS

Ethical approval

The design and protocols of the study, samples of informed consent for approval representatives of patients were accepted and then reviewed and approved at a meeting of the Local Ethics Committee, registration number IRB00005839 IORG 0004900 (OHRP), as evidenced by an extract from protocol No. 39 dated June 28, 2022.

Chemicals and reagents

To prepare stock and standard solutions of vancomycin and norvancomycin, the following substances were used as an internal standard: highly purified water, which had been obtained in the Milli-Q system, formic acid for HPLC-MS (Scharlab, Spain), acetonitrile (Scharlab, Spain), as well as dry samples of appropriate certified vancomycin standards (Servier, France) and norvancomycin (Augsburg, Germany, purity >95.0%).

Samples of "dried blood spot"

All 15 samples of the "dried blood spot" were obtained from newborn patients who were treated with vancomycin at State Clinical Hospital No. 5 of Volgograd, the department of neontology (Russia). The samples were obtained according to standard protocols [5].

Sampling was carried out according to the following protocols:

1. The informed voluntary consent of the legal representative was formalized.
2. The card / drops with patient ID and date was/were signed.
3. The puncture site on the lateral side of the heel was chosen.
4. The foot with a warm diaper was prepared.
5. The hands were cleaned and sterile gloves were put on.
6. The heel was placed below the child's body and held without sharply bending the ankle.
7. About 5 min before the injection, a 30% glucose solution was administrated *per os* with a syringe at the dose indicated in Figure 1+non-nutritive sucking.
8. The puncture site was treated with an antiseptic solution.
9. A 30% glucose solution was re-administrated *per os* to the patient with a syringe immediately before the injection.
10. The skin was quickly pricked with a lancet and the first drop of blood was wiped off with a sterile cotton ball.
11. Immediately after the injection, a 30% glucose solution was given *per os*+nonnutritive sucking.
12. The puncture site was held down, the adjacent area was being gently pressed on and the blood was sampled a on filter paper form;
13. The map was held without touching the marked area.
14. The filter paper card was carefully touched to a drop of blood and applied to the card. The blood was let to soak in until the circle was full. The marked area was not to be touched after applying blood.
15. The blood stain was let to dry in a dark place, out of direct sunlight, for at least 4 h. The "dried blood spot" samples were not to be heated or come

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into contact with other surfaces during the drying process.

16. The card (or parts of the card) was to be sealed in a gas-tight zip-lock bag. No more than one card per package should be stored in the refrigerator at 2–8°C until being sent to the laboratory.

Equipment

The components were separated using an Agilent 1260 HPLC system (Agilent, USA). The analytes were detected using a Sciex QTRAP 5500 hybrid mass spectrometry system (AB Sciex Pte. Ltd., Singapore). To weigh dry substances, semi-microanalytical balances Ohaus Explorer EX225/AD (Ohaus, USA) and centrifuge 5427 R (Eppendorf, USA) were used. GE Whatman FTA WB129242 DMPK-B (USA) card was used for archiving "dried blood spots". While the system was on, the acquired data was sent to the Analyst Software.

Preparation of stock solution of substance and internal standard

To prepare standard solutions of vancomycin and an internal standard with a concentration of 1.00 mg/ml, dry samples (weighing 25 mg) were weighed and then placed in a 25-ml volumetric flask and diluted to the mark with ultrapure water.

Preparation of solution for extracting “dried blood spot” samples

Separately, an extraction solution was prepared; that was a mixture of water and methanol in a 1:1 ratio with 0.1% formic acid.

Preparation of working standards solutions

For each analytical series, fresh working standards solutions were prepared. The final concentrations of working solutions were: 10, 20, 50, 100, 200, 500 and 1000 µg/ml.

Preparation of calibration standards and quality control samples in “dried blood spot” samples

100 µl of the whole blood was transferred into 1.5 ml microtubes, and 10 µl of a working solution of the appropriate concentration was added to obtain calibration solutions: 1, 2, 5, 10, 20, 50 and 100 µg/ml for vancomycin. For quality control (QC) samples 1 (a lower limit of quantitation – LLOQ); 7.5 (low QC); 35 (average QC); 75 (high QC) µg/ml for vancomycin. Next, 20 µl of working solutions were applied to filter paper and dried for 3 h.

Analyzed samples preparation

To prepare the samples of “dried blood spot”, a 6 mm diameter card disk was used, onto which the samples were applied using a special device and which was subsequently analyzed.

Extraction of “dried blood spot” samples

400 µl of the extraction solution was added to the test tube with a cut out disk. The tube was stirred at 1000 rpm for 30 min at 25°C. After 10 min of centrifugation at 10 000 g, a 350 µl aliquot of the supernatant was evaporated at 45°C in a vacuum centrifuge. The dried extract was collected in 100 µl of mobile phase A, centrifuged for 10 min at 10 000 g, and 20 µl of the supernatant was injected into a high-performance liquid chromatography with a mass spectrometric detection – an HPLC-MS/MS system.

Chromatographic and mass spectrometric conditions

Thus, based on the data sources available and the authors’ research, the authors have developed and validated a bioanalytical method for vancomycin in human plasma using an HPLC-MS/MS system that meets the requirements of the research protocol and is validated in accordance with the FDA guidance for enterprises “Bioanalytical Method” Validation” and the EMEA “Guideline on bioanalytical method validation”.

When developing the conditions for a mass spectrometric detection of the sought substances, “precursor ions” (725.3 m/z) and their corresponding “product” ions (88.1 and 387.9 m/z) were used.

Statistical analyses

Validation of the bioanalytical method was carried out in accordance with the “Guide for the Evaluation of Drugs” (Vol. I), as well as with the guidelines of the FDA (U.S. Food and Drug Administration) and EMA (European Medicines Agency) for the following indicators: stability, selectivity, linearity, accuracy, precision, a lower limit of quantitation. Experimentally calculated concentrations of calibration standards should be within ±15% of the nominal values (with the exception of LLOQ, for which these values may be within ±20%) and also according to specific indicators, such as the effect of a drop volume, the effect of hematocrit, drop uniformity according to the already developed protocols [8–10]. The obtained data were processed using the statistical Software environment R 3.6.1 in the RStudio 1.2 program, as well as specialized Software Sciex Analyst 1.6.2.

When validating the homogeneity of the drop, the results of two concentration levels of QC samples at three different hematocrit levels, obtained with 2 options for cutting the drop – from the center of the drop and at its edge, were compared. The analysis was carried out in 5 replicates. In this case, when comparing concentrations from the samples obtained from the central and marginal cutouts, the relative error should not exceed 15%.

To assess the influence of the drop volume effect, it was necessary to analyze 3 different volumes (10, 40, 70 µl) at an average hematocrit level (0.4), at 2 concentration levels in 5 replicates. In this case, the relative error should not exceed 15%.

The effect of hematocrit was also assessed at 3 levels (0.3; 0.4; 0.5), at 2 concentration levels in 5 replicates. The relative error of the calculated concentrations should not exceed 15% of the obtained values at an average hematocrit level.

The level of agreement between the developed HPLC-MS/MS method for the "dried blood spot" method and the traditional HPLC-MS/MS method with a protein precipitation as a sample preparation was examined by the Bland-Altman method.

The method makes it possible to compare the results of measurements performed in two different ways. Its essence is that for each pair of measurements the difference and the average are calculated. The average difference calculated for all pairs of characteristics in the data set under study characterizes the systematic discrepancy of indicators, the presence of which indicates an incomplete correspondence of the results obtained by different methods, and the standard deviation of the differences reflects the dispersion degree of the results.

The mean values and standard deviations were calculated using Microsoft Excel 2010 (Microsoft Corporation, USA); the statistical processing of the study results was carried out using GraphPad Prism 6 (USA).

The mean (M) and standard deviation (SD) of the difference between the two matching readings were calculated to determine the equivalence of the two methods. To confirm the suitability of the DBS analytical method for the analysis of patient samples, however, more than 95% of the calculated mean concentrations between the two methods must fall within the d-2s and d+2s limits.

RESULTS AND DISCUSSION

The development of a quantitative HPLC-MS/MS method for the determination of vancomycin included the determination of optimal parameters for a chromatographic separation, as well as a subsequent mass spectrometric detection.

Using the previously accumulated experience, optimal conditions for the chromatographic separation had been selected. The separation was carried out on a Poroshell 120 C18 column (4.6×50 mm, 2.7 µm). The mobile phase was an 80/20 acetonitrile and water mix at a flow rate of 0.3 ml/min. A 0.1% solution of formic acid was added to both the aqueous and organic components of the mobile phase. When optimizing the chromatographic separation conditions, an isocratic elution mode was chosen [5, 8, 11, 12].

In the isocratic elution mode, the retention time of vancomycin was 1.63 min (Fig. 1).

The electrospray ionization (ESI) was used as the ionization method. The ion detection was carried out in a positive polarity mode.

When developing the conditions for the mass spectrometric detection of the sought substances, "precursor ions" (725.3 m/z) and their corresponding "product" ions (88.1 and 387.9 m/z) were used.

The developed method confirmed its linearity in the concentration range from 1 to 100 µg/ml (K1 – 1 µg/ml, K2 – 3 µg/ml, K3 – 5 µg/ml, K4 – 10 µg/ml, K5 – 25 µg/ml, K6 – 50 µg/ml, K7 – 80 µg/ml, K8 – 100 µg/ml) when a weighted coefficient of *1/x^2*, with R^2 >0.99 was used. The coefficient of variation (%) calculated when determining an inter- and intraday accuracy did not exceed 15% for the main concentration range.

The lower level of quantification (LLOQ) of the methods was determined based on the linearity, accuracy and precision data given in Table 1. The selectivity was validated by analyzing 6 blank samples and 6 lower level of quantification (LLOQ) samples. The peak area in the analyte retention time region did not exceed 20% of the LLOQ (Table 1).

For the "dried blood spot" method, the influence of hematocrit, drop volume and drop homogeneity was also assessed.

The effect of hematocrit was assessed at 3 hematocrit levels (0.3; 0.4; 0.5), for QCL and QCH, and the resulting concentrations ranged from 94.3 to 105.8% of nominal ones (Table 2).

To validate the volume effect, 3 volumes (10, 40, 70 µl) at an average hematocrit level (0.4), at 2 concentration levels in 5 replicates were analyzed. The relative error of the calculated concentrations did not exceed 15% of the obtained values at an average volume.

When validating the drop homogeneity, the results of QC samples with QCL and QCH, obtained with 2 options for cutting the drop – from the center of the drop and from the edge, were compared. The analysis was carried out in 5 replicates. At the same time, in the comparison of the concentrations from the samples obtained from the central and marginal cutouts, the relative error did not exceed 15% of the nominal values.

The thermal stability was assessed by storing the "dried blood spot" samples for 14 days at 22 and 45°C, as potential temperatures for storing and transporting samples. To assess the stability, ''dried blood spot'' samples were used at the QCL and QCH levels, herewith, the samples were analyzed at three time points of 1, 7 and 14 days along with freshly prepared samples in one analytical batch. The calculated concentrations of the samples after the storage were compared with the mean concentrations of freshly prepared quality control samples. After 14 days of storage, the values were in the range of 85.4–87.5%.

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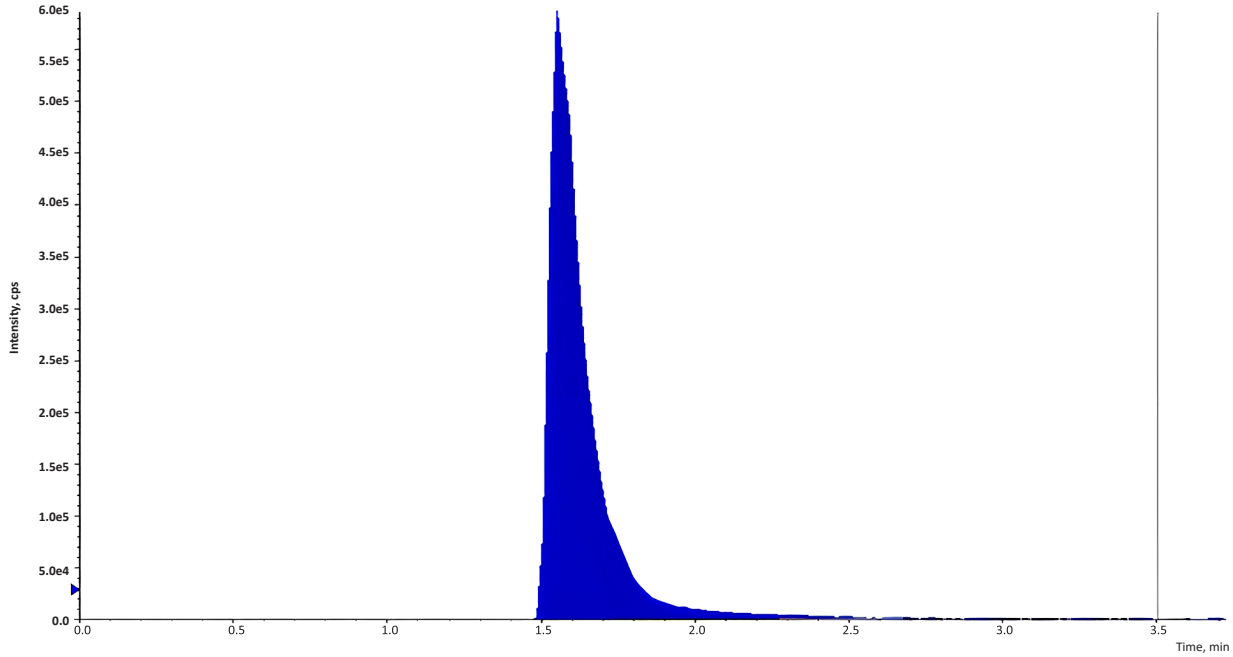

Figure 1 – Chromatography-mass spectra of vancomycin in DBS samples

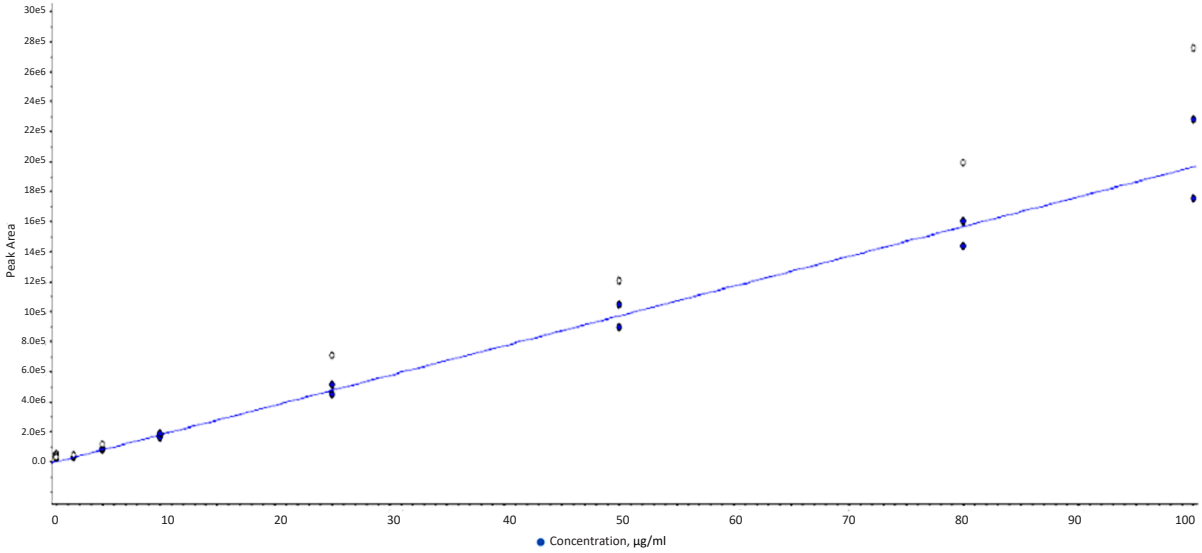

Figure 2 – Calibration curve of vancomycin in blood plasma

Table 1 – Table of validation parameters for the method using a "dried blood spot" as a sample preparation

Parameter		Value			
		LLOQ (1 µg/ml)	Lower QC (7,5 µg/ml)	Mean QC (35 µg/ml)	Top QC (75 µg/ml)
Precision (CV%)	Within a cycle	2.7	2.1	2.7	3.7
	Between cycles	7.6	4.7	6.9	7.0
Correctness (%)	Within a cycle	104.6	94.4	101.6	101.8
	Between cycles	91.3	95.1	103.2	107.6
Stability (%)		–	85.3	–	87.5
Selectivity (%)		2.7	–	–	–
Correlation coefficient			0.99		

Note: QC – quality qontrol.

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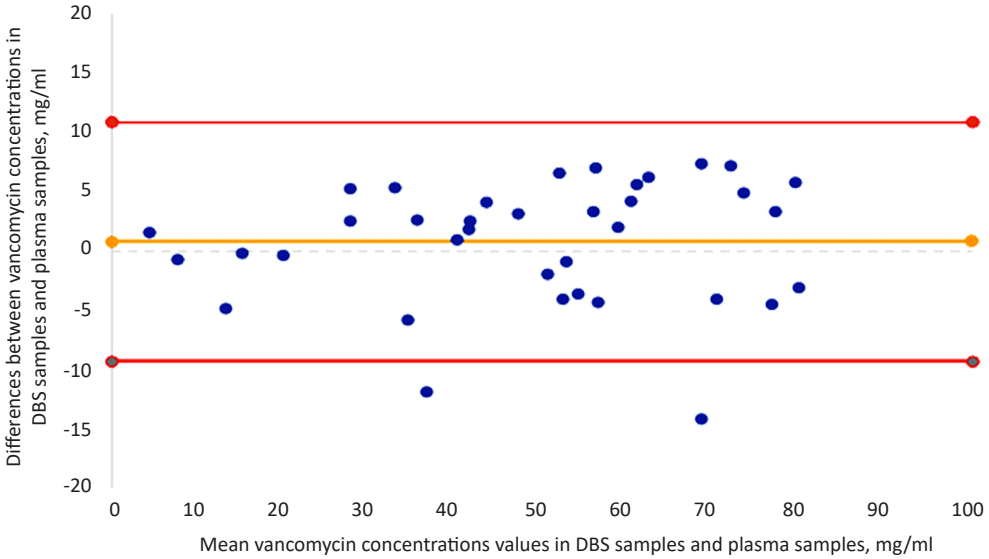

Figure 3 – Comparison of two Bland-Altman sample preparation methods for vancomycin
Note: DBS – Dried Blood Spot.

Table 2 – Effect of hematocrit on analysis results

Hematocrit, %	QC	Nominal concentration, mg/ml	Accuracy, %
0,3	LQC	7.5	94.3
	HQC	75	97.6
0,4	LQC	7.5	95.8
	HQC	75	98.1
0,5	LQC	7.5	95.6
	HQC	75	105.8

Table 3 – Effect of drop volume on analysis results

Drop volume, µl	QC	Nominal concentration, mg/ml	Accuracy, %
10	LQC	7.5	105.7
	HQC	75	108.9
40	LQC	7.5	96.4
	HQC	75	104.5
70	LQC	7.5	91.5
	HQC	75	96.4

Table 4 – Influence of cut location on analysis results

Cutout location	QC	Nominal concentration, mg/ml	Accuracy, %
Center cutout	LQC	7.5	98.3
	HQC	75	103.7
Edge cutout	LQC	7.5	93.3
	HQC	75	96.5

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For a comparative study, the samples of a "dried blood spot" were used; they had been obtained from newborns undergoing vancomycin therapy at the State Healthcare Institution of Clinical Hospital No. 5. Vancomycin concentrations were compared between dried blood spot and plasma samples (Fig. 3).

M and SD of the difference between the two matching readings were calculated to determine the equivalence of the two methods. The results of the study showed that more than 95% of the calculated mean concentrations are within the limits of d–2s and d+2s, which correspond to the values of –10.2 and 12.2. This result confirms the developed method suitability for the analysis.

DISCUSSION

TDM is an algorithm for measuring the amount of specific drugs at specific intervals to maintain constant concentrations in the patient's bloodstream in order to optimize and individualize dosing regimens. This approach is used to monitor drugs with a narrow therapeutic index, significant PK variability, or those whose target concentrations are difficult to control. For drugs with a clear dose-effect relationship, TDM can significantly increase the effectiveness and safety of treatment and reduce costs in the social and medical sphere.

Currently, TDM in newborns is not a routine practice. Large clinical centers that have the capacity to conduct it to assess the target exposure, are most often limited to the determination of C_{trough} vancomycin [4].

As defined by C_{trough}, the generally accepted therapeutic aim of vancomycin monitoring is to assess a systemic exposure as the residual concentration before the next dose of the drug is administered. According to the international recommendations, the target vancomycin C_{trough} should be at a level of 10–20 mg/l and be above the MIC (minimum inhibitory concentration) [1]. The recommended C_{trough} range makes it possible to achieve the required PD parameters – AUC24 / MIC [4].

However, the main problem facing physicians is the need to assess the possibility and accuracy of the extrapolation of target PK and PD parameters (C_{trough}=10–20 mg/l and AUC24 / MIC >400) [1].

According to the international recommendations, the target C_{trough} level is currently 15-20 mg/l and the AUC24 / MIC ratio is >400. A number of studies in individual populations indicate a high variability in PK parameters, with target C_{trough} values of 15–20 mg/l and the ratio AUC24 / MIC >400 did not reach an average of 30–33% of the study population [13, 14].

Today, TDM of vancomycin involves, in addition to measuring steady-state residual concentrations (C_{trough}), calculating the area under the PK curve over 24 h (AUC24), followed by the assessment of the target PK/PD ratio, which is expressed as the ratio of AUC24 to the minimum inhibitory concentration (MIC) AUC24 / MIC >400 [13, 15, 16].

Newborns and pediatric patients are not only sensitive to certain xenobiotics, but also exposed to various risks of viral, bacterial or fungal invasions, many of which significantly alter functioning of the metabolic and excretory systems. The neonatal age may be a risk factor for the development of nephrotoxicity, the significance of which increases in the proportion to prematurity.

The previous studies with the use of mathematical modeling and TDM in patients with an impaired renal function and infectious complications have shown that their presence significantly affects PK parameters. Thus, it was demonstrated that with a standard approach to dosing, in 65% of cases it is not possible to achieve the target values for C_{trough} 15–20 μg/ml after 48 h from the start of therapy. This indicates a high variability of PK vancomycin parameters, especially in the group of patients with an impaired renal function [17].

In a study by Kim J. et al. it has been shown that in neonates with an adequate renal function, a dosing regimen of 9 to 12 mg/kg IV every 8 h is less likely to achieve the target vancomycin concentrations [18].

To evaluate all these parameters and optimize TDM, various mathematical models that make possible the prediction of unknown PK parameters, are available. There are known both simple one-compartment models – "medical calculators", and more complex ones – "dose calculators", the use of which allows you to adjust the dosage regimen to achieve C_{trough} in the range of 15–20 μg/ml [1, 19].

It is worth highlighting that international recommendations for vancomycin dosage regimens are not always optimal. Thus, in premature patients, these recommendations are insufficient to obtain minimum serum concentrations of 10 to 20 μg/ml [20].

PK / PD studies for antibiotics already used in clinical practice are necessary to optimize dosage regimens, including preventing the development of resistance. Since the results of studies conducted on adults are often not applicable to newborns, local TDM is not only a useful tool for optimizing therapy, but also a valuable source of the data that can be not only of the applied importance, but also of a fundamental nature. Separate issues that need to be addressed are methods of collecting biospecimens and optimizing their quantity, which is associated with the age and size of patients [21].

Most recommendations are developed for adult patients [1, 3, 4, 11]. As for newborns, standardization of the process is hampered by a smaller number of studies, which means a significantly smaller amount of data for the analysis, variability of PK parameters, more complex modeling with the need to take into account more covariates [4].

Modern development of guidelines, instructions and protocols requires additional PK studies, construction of mathematical models and implementation of TDM algorithms. This is what causes difficulties, since research and TDM, in addition to financing and going through a large number of bureaucratic procedures, require solving many technical problems [6]. For example, the issue of determining the optimal number of biological samples has not been resolved; the blood from which plasma or serum is subsequently obtained is taken from a vein, but its quantity and technical complexity significantly complicate the process of developing regulatory documentation [1]. TDM of antibiotics in newborns and premature infants is rarely practiced precisely because of difficulties with blood sampling [1].

The above points to the need to develop efficient and less invasive methods for obtaining biospecimens from newborns and pediatric patients. One of them is the "dried blood spot" method (DBS), which is the most convenient way to obtain biomaterial compared to the standard ones [6]. It is based on the use of a small amount of blood, which is applied to special paper and dried under certain conditions. The resulting biological samples are more stable and more convenient to transport and store [4, 22]. The indicated advantages not only reveal the feasibility of using the "dried blood spot" method for TDM in cases with newborns or pediatric patients, but also determine the need for development processes in this direction, for example, when using vancomycin in the treatment of diseases in newborns and pediatric patients [1].

Obtaining biological samples for a sample preparation and analysis using the "dried blood spot" method requires additional optimization. Currently used bioanalytical method validation protocols, which are described in manuals for traditional samples, do not take into account all the necessary aspects of the new "dried blood spot" technology and complicate the subsequent application of the method in TDM. Specific characteristics such as hematocrit, droplet homogeneity, and droplet size may influence assay results, requiring the identification of new approaches to the development and validation of qualitative and quantitative assays [4, 23].

The information content and predictive value of TDM when conducting well-founded, from a PKs point of view, individualization when selecting a dosage regimen is determined by the reliability of the analytical methods used for the quantitative determination of drugs in biological samples [24]. For these purposes, it is necessary to use selective and highly sensitive analytical methods, such as high-performance liquid chromatography with a mass spectrometric detection (HPLC-MS/MS) [25, 26].

PK studies and TDM require large numbers of biospecimens, which is difficult to implement when working with neonates [27]. As a result, there is a need to use less invasive methods of a sample preparation for their subsequent implementation in algorithms for TDM. One of the promising approaches for carrying out such monitoring in newborns may be the "dried blood spot" method. A necessary direction, without which the introduction of new methods of monitoring and optimization of pharmacotherapy in newborns will be difficult, is the development of highly selective and specific methods for the qualitative and quantitative determination of vancomycin using the "dried blood spot" method [28].

CONCLUSION

One of the important results of this study was the determination of the optimal conditions for a quantitative analysis method using high-performance liquid chromatography and mass spectrometric detection for the determination of vancomycin in "dried blood spot" samples. As a result of the study, it was possible to validate the quantitative HPLC-MS/MS method for the determination of vancomycin in "dried blood spot" samples. The identity of the results of a quantitative vancomycin determination with standard and proposed sample preparation options has been proven.

The above makes it possible to recommend the "dried blood spot" method for TDM of vancomycin and additional studies of PKs in this group of patients, which can optimize and bring the pharmacotherapy of newborns closer to the ideals of personalized medicine. The development of such methods makes it possible to solve an important practical problem in the field of studying the PKs of newborns, which is important for clinical pharmacology since conducting clinical studies in young patients is complicated, among other things, by these reasons. The development of methods for the qualitative and quantitative determination of various substances in the blood using biological samples of minimal size will significantly expand the possibilities of conducting clinical studies, which will certainly contribute to the development of not only general and clinical pharmacology, but will also significantly enhance pharmacotherapy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHORS' CONTRIBUTION

Ivan S. Anikeev, Tatyana E. Zayachnikova, Yulia S. Kazmina – concept, planning of the article, review of literary sources, collection of materials, article writing and editing; Vladimir I. Petrov, Andrey V. Strygin, Denis V. Kurkin – development of the study design, editing and final approval of the article.
All authors made equivalent and equal contributions to the preparation of the publication. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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