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ADAPTATION OF "DRIED BLOOD DROP" METHOD FOR THERAPEUTIC DRUG MONITORING

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To control the concentration of drugs with a narrow therapeutic range, and to conduct effective and safe treatments, Therapeutic Drug Monitoring (TDM) is carried out. However, to date, the implementation of TDM is associated with various difficulties, for the solution of which more convenient and less invasive methods for collecting biological material are being developed.

The aim of the study was to develop protocols for the collection and storage of "dried blood spot" (DBS) samples, as well as protocols for the validation methods for the quantitative determination of drugs in whole blood, using this technology for subsequent therapeutic drug monitoring.

Materials and methods. To analyze a "dried blood spot" method in detail and to identify the characteristic features of taking and storing biosamples, a collection and analysis of scientific literature over the past 10 years has been conducted. The search for literature materials has been carried out from open and accessible sources located in the scientific libraries of institutions, in electronic databases and search engines: Elibrary, PubMed, Scopus, Cyberleninka, Medline, ScienceDirect, Web of Science, Google Scholar. Primary protocols for taking, storing and analyzing samples of the "dried blood drop" have been prepared. To obtain the adequate quality samples, the developed protocols have been tested and optimized at the stages of selection and storage. By high-performance liquid chromatography with mass spectrometric detection (HPLC-MS/MS), using a "dried blood drop" as a sample preparation, drug validation protocols have been optimized to ensure that acceptable validation characteristics were achieved, and subsequent Therapeutic Drug Monitoring was performed.

Results. The features of the collection, storage and analysis of the "dried blood spot" samples have been revealed. Such characteristics as a spot volume effect, a hematocrit effect, a droplet uniformity, which can affect the results of a quantitative HPLC-MS/MS analysis, have been determined. For a successful use of the new methods, appropriate protocols for taking samples of "dried blood spot" from the finger of adult patients and from the heel of newborns, as well as protocols for validating methods for the quantitative determination of drugs from these samples, have been developed.

Conclusion. The application of the "dried blood spot" method using newly developed protocols for taking, storing and analyzing biological samples, relieves the existing constraints in conducting TDM, and can later become a promising method for conducting preclinical and clinical studies.

Keywords: "dried blood spot" method; therapeutic drug monitoring; bioanalysis; method validation; HPLC-MS/MS

Abbreviations: MP/D – medicinal preparation/drug; HPLC-MS/MS – high performance liquid chromatography with tandem mass spectrometry; TDM – Therapeutic Drug Monitoring; DBS – Dried Blood Spot; QCL – low quality con

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RESEARCH ARTICLE

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АДАПТАЦИЯ МЕТОДА «ВЫСУШЕННОЙ КАПЛИ КРОВИ» ДЛЯ ПРОВЕДЕНИЯ ТЕРАПЕВТИЧЕСКОГО ЛЕКАРСТВЕННОГО МОНИТОРИНГА

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Для контроля концентрации лекарственных препаратов, обладающих узким терапевтическим диапазоном, и проведения эффективных и безопасных методов лечения проводится терапевтический лекарственный мониторинг (ТЛМ). Однако на сегодняшний день проведение ТЛМ связано с различными затруднениями, для решения которых разрабатываются более удобные и менее инвазивные методы сбора биологического материла.

Цель. Разработать протоколы взятия и хранения образцов «высушенной капли крови» (Dried Blood Spot, DBS), а также протоколы валидации методов количественного определения лекарственных препаратов в цельной крови с использованием данной технологии для последующего проведения терапевтического лекарственного мониторинга.

Материалы и методы. Для детального анализа метода «высушенной капли крови» и выявления характерных особенностей взятия и хранения биообразцов, был проведен сбор и анализ научной литературы за последние 10 лет. Поиск литературных материалов проводился с помощью открытых и доступных источников, размещенных в научных библиотеках учреждений, в электронных базах данных и поисковых системах: Elibrary, PubMed, Scopus, КиберЛенинка, Medline, ScienceDirect, Web of Science, Google Scholar. Подготовили первичные протоколы взятия, хранения и анализа образцов «высушенной капли крови». На стадиях отбора и хранения проводили апробацию и оптимизацию разработанных протоколов для получения образцов надлежащего качества. Методом высокоэффективной жидкостной хроматографии с масс-спектрометрической детекцией (ВЭЖХ-МС/МС) с использованием в качестве пробоподготовки «высушенную каплю крови», оптимизировали протоколы валидации лекарственных препаратов для обеспечения достижения приемлемых валидационных характеристик и проведения последующего ТЛМ.

Результаты. Выявлены особенности сбора, хранения и анализа образцов «высушенной капли крови». Определены такие характеристики, как: эффект объёма капли, эффект гематокрита, однородность капли, которые могут оказывать влияние на результаты количественного ВЭЖХ-МС/МС анализа. Для успешного использования новой методики нами были разработаны надлежащие протоколы взятия образцов «высушенной капли крови» из пальца руки взрослых пациентов и из пятки новорожденных детей, а также протоколы валидации методов количественного определения лекарственных препаратов из данных образцов.

Заключение. Применение метода «высушенной капли крови» с использованием новых разработанных протоколов взятия, хранения и анализа биологических образцов снимает существующие ограничения при проведении ТЛМ, а также в последствии может стать перспективным методом для проведения доклинических и клинических исследований.

Ключевые слова: метод «высушенной капли крови»; терапевтический лекарственный мониторинг; биоанализ; валидация метода; ВЭЖХ-МС/МС

Список сокращений: ЛП – лекарственный препарат; ВЭЖХ-МС/МС – высокоэффективная жидкостная хроматография с масс-спектрометрической детекцией; ТЛМ – терапевтический лекарственный мониторинг; DBS – «высушенная капля крови»; QCL – низкий показатель контроля качества; QCM – средний показатель контроля качества; QCH – высокий показатель контроля качества; КК – контроль качества; НПКО – нижний предел количественного определения.

Ключевые слова: метод «высушенной капли крови»; терапевтический лекарственный мониторинг; биоанализ; валидация метода; ВЭЖХ-МС/МС

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INTRODUCTION

"Dried blood spot" (DBS) is a relatively simple sample preparation method for collecting small volumes of blood that can eliminate plasma collection and sample freezing.

The DBS method presents a wide range of possible applications that are not feasible or difficult to perform with traditional methods of biosampling [1]. One of the main options for using such a technology is therapeutic drug monitoring (TDM).

To date, for TDM and analysis of medical preparations (MPS), it is necessary to collect a large amount of biological material – plasma or blood serum. To obtain these biosamples, it is necessary to take whole blood, which is obtained by standard venipuncture methods. However, for many groups of patients, standard methods for collecting whole blood cause significant difficulties, limiting the implementation of TDM and the adjustment of dosing regimens for drugs with a narrow therapeutic range [1, 2].

A new technology for collecting whole blood samples, is a less invasive and simple DBS method, makes it possible to overcome the significant limitations associated with standard methods, and perform TDM in the clinical situations in which it was difficult or impossible in principle [3].

However, a quantitative analysis of MPs in DBS samples require the use of highly sensitive and selective analytical technologies, such as high-performance chromatography with mass spectrometric detection/ tandem mass spectrometry (HPLC-MS/MS). The use of such a modern analytical system will optimize the analysis of biosamples for TDM and expand the possibilities of using the DBS at various stages of drug development and within the framework of pharmacokinetic studies.

However, despite a great potential and advantages of this technology, its widespread introduction into the routine practice of TDM will be possible only after a reliable confirmation of the accuracy and reproducibility of the obtained analytical data on the concentration of the studied drugs. These factors will require additional development and validation of bioanalytical methods using the DBS method at the stage of taking the biomaterial and preparing it for the study.

THE AIM of the study was to develop protocols for the collection and storage of "dried blood spot" (DBS) samples, as well as protocols for the validation methods for the quantitative determination of drugs in whole blood, using this technology for subsequent therapeutic drug monitoring.

MATERIALS AND METHODS

To analyze the DBS method and identify its characteristic features of taking and storing biosamples, a collection and analysis of scientific literature over the past 10 years have been carried out. The search for literature materials has been conducted using open and accessible sources located in the scientific libraries of institutions, in electronic databases and search engines: Elibrary, PubMed, Scopus, Cyberleninka, Medline, ScienceDirect, Web of Science, Google Scholar.

The following words and phrases were chosen as the parameters for the selection of literature: DBS; HPLC-MS; therapeutic drug monitoring; validation; sample preparation; bioanalysis. The search was also carried out using the English analogues of the keywords: DBS; HPLC-MS; therapeutic drug monitoring; validation; sample preparation; bioanalysis.

After analyzing the literature data, primary protocols for taking, storing and analyzing the DBS samples were developed, based on the detailed analysis and identification of the specific characteristics of this technique.

Then, using the HPLC-MS/MS method and a DBS as a sample preparation, the ivabradine samples were analyzed and the drug validation protocols were optimized to achieve acceptable validation characteristics and conduct subsequent TDM.

The basic equipment was an Agilent 1260 HPLC system with a binary pump and a temperature-controlled autosampler. A chromatographic separation of the components was carried out on a Poroshell 120 C18 column (4.6 x 50 mm x 2.7 µm). The analytes were determined using a Sciex QTRAP 5500 hybrid mass spectrometric system.

The mobile phase was represented by a water-acetonitrile mixture, the mobile phase modifier was a 0.1% formic acid, which was added to both the aqueous and organic mobile phases.

In the course of optimizing the conditions for a chromatographic separation, a gradient elution mode was chosen. The mobile phase ratio of water-acetonitrile (70:30) did not change at 0.6 ml/min until 0.5 minutes, after which there was a gradual change to the ratio of water-acetonitrile (0:100), which was reached at the 2nd minute. At the 3rd minute, the ratio was changed to the original, and, at the same time, the system was balanced up to the 5th minute. The mobile phase modifier was a 0.1% formic acid, which was added to both the aqueous and organic components of the mobile phase.

In this methods, ivabradine standards (Servier, France) and N-desmethylivabradine as an internal standard (Toronto Research Chemical Inc., Canada),

were used to prepare calibration standards and quality control samples.

For each analytical series, fresh standard working solutions were prepared. The final concentrations of ivabradine working solutions were 10, 20, 100, 500, 1000, 5000, 8000, 10000 ng/ml.

To obtain a calibration solution, 100 µl of whole blood was transferred into 1.5 ml microtubes and 10 µl of a working solution of the appropriate concentration was added. The concentrations of calibration solutions were 1, 2, 10, 50, 100, 500, 800, 1000 ng/ml.

Quality control (QC) samples were prepared at the following four concentration levels: 1 µg/mL (lower limit of quantitation, LLQQ), 3 µg/mL (low QC, QCL), 400 µg/mL (medium QC, QCM) and 750 mcg / ml (high QC, QCH).

Next, 20 µl of the resulting working solutions were applied to the filter paper and let them dry at room conditions for 3 hours.

To prepare the DBS samples, a disc with the diameter of 6 mm was cut from the cards with a Uni-Core marker cutter, placed in a test tube and extracted with an extraction solution. The extraction was performed with a shaker for 20 min at 25°C, then the samples were transferred to new tubes and analyzed by HPLC-MS/MS.

Statistical processing of results

The obtained data were processed using the R 3.6.1 software-statistical environment in the RStudio 1.2 program, as well as the specialized Sciex Analyst 1.6.2 software. During the system operation, the acquired data were processed in the Analyst software as full mass spectra, single or multiple ion intensities versus time, or total ion current. For a quantitative determination, a calibration curve method with a weighting factor of 1/x2 was used. The ratio of the peak areas of the analyte and the internal standard was taken as a parameter [8, 10, 12, 23].

RESULTS AND DISCUSSION

General provisions on DBS method

Unlike standard whole blood collection methods, DBS involves collecting a minimal amount of capillary blood from the finger of an adult or the heel of a newborn child and applying the drop of blood to the marked area of filter paper. After this procedure, a drop of blood dries in air at room temperature for at least 4 hours in a dry place without direct sunlight. The dried samples are transported to the laboratory, where they are subjected to the manipulations related to cutting discs with a special device from 3 to 8 mm from the filter card. This disk is subsequently extracted with an organic solvent or a mixture of an aqueous and organic solvent. Further on, the obtained samples are quantitatively determined by various bioanalytical technologies [4].

Thus, the entire process of collecting DBS samples is considered as the distribution of a blood drop over a porous surface with a simultaneous penetration and spreading inside it. The distribution and wetting of substrate pores by blood is a complex process that depends on the physical and chemical properties of paper and blood, which must be further investigated before being used in wide clinical practice [4].

This whole mechanism of the DBS method determines a number of significant advantages of this technology over standard methods for collecting biomaterial, as well as some of its limitations.

Advantages and disadvantages of DBS technology

One of the main advantages of this technology is the collection of a sufficiently smaller amount of whole blood, which makes it possible to overcome the existing limitations of TDM for various patient groups. The DBS method also involves the process of drying samples under standard laboratory conditions and their transportation to an analytical laboratory without the use of special equipment and with a minimal risk of infection [3, 5].

It is possible to carry out the procedure for taking biomaterial at home without the involvement of special personnel. This will allow more analyzes to be performed using the DBS method and to build a more accurate concentration-time curve to optimize the dosing regimen of the drugs with a narrow therapeutic range.

All of the above advantages can have significant economic benefits in the collection and analysis of DBS samples compared to standard biosampling methods. Potential home DBS sampling would also result in significant economic benefits in patients requiring TDM [7].

However, this method is not free from shortcomings. The analysis of the DBS samples imply special highly sensitive analytical equipment and the need to train medical specialists to obtain samples of the adequate quality [8, 9].

It should be also taken into account that the currently used requirements for the validation of bioanalytical methods, which are described in the manuals for traditional matrices, do not provide all the necessary aspects of the method development, analytical and clinical validation for DBS samples and their use in TDM. Such specific parameters characteristic of the new technology are as follows: the effect of hematocrit, which can lead to different blood viscosities and a droplet distribution on paper, the homogeneity

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of the droplet and its size can affect the result obtained and, in turn, require additional validation [10, 11].

All these shortcomings cause the need to develop new protocols for taking and storing blood samples, as well as additional validation that would be characteristic of the DBS method and its use in TDM.

Analysis of current literature sources

Over the past 10 years, a large number of articles describing the development, validation of a new DBS method, as well as the possibility of using it as a part of TDM for various classes of drugs (analgesics, antibiotics, antiepileptics, antidepressants, antimalarials, antifungals, antiretrovirals, diuretics, immunosuppressants and others), have been published [12].

The existing methods for the quantitative determination of MPs using HPLC-MS/MS in the DBS samples have been analyzed (Table 1). The additional validation characteristics that are unique to the new method of collecting biosamples and have a significant impact on the results of the analysis, have been taken into account in the selected studies. These characteristics are: a droplet size, the nature of the material, a map for the DBS samples, the extraction conditions, and the hematocrit level [9, 10, 13, 15, 16, 18–20, 25–29].

Such parameters as a hematocrit effect, a drop volume effect and a droplet uniformity can have a significant impact on the result of a MP analysis in the DBS samples and can vary from sample to sample. It is these characteristics that have been identified as additional parameters requiring the development of new validation protocols for the quantitative HPLC-MS/MS analysis of drugs, as well as working out practical recommendations for medical professionals on the collection and storage of these whole blood samples to obtain acceptable analytical results. [8, 9, 17, 39]

Development of protocols for sampling, storage of samples and validation DBS

Based on the literature analysis and existing methods for collecting whole blood drops on special paper, protocols for taking DBS samples from an adult patient's finger and from a newborn's heel have developed and optimized [40–42]. The developed protocols are presented below.

Protocol for taking and storing DBS samples from newborn's heel:

1. Issue the informed voluntary consent of the legal representative;
2. Sign the drops with a patient's ID and date;
3. Choose the puncture location on the lateral side of the heel;
4. Warm up the foot with a warm diaper;
5. Clean hands and put on sterile gloves;
6. Place the heel below the baby's torso and hold it without sharp bending of the ankle;
7. Treat the puncture location with an antiseptic solution and let it dry;
8. Quickly pierce the skin laterally, in the place as shown in the picture (Fig. 1) using a lancet, and wipe off the first drop of blood with a sterile cotton ball;
9. Hold the puncture location down, gently pressing on the adjacent area, and take blood on a filter paper blank;
10. Hold the card without touching the marked area (Fig. 2);
11. Carefully, touching a drop of blood with a filter paper card, apply it to the card. Let the card absorb the blood until the circle is full. After the application, do not touch the marked area;
12. Let the blood spot dry in a dark place. Avoid direct sunlight for at least 4 hours. Do not heat or let dried spot samples come into contact with other surfaces during the drying process;
13. Seal the card (or parts of the card) in a gas-tight bag with a zipper. Store no more than one card per package in the refrigerator until sent to the laboratory at 2–8°C.

Protocol for taking and storing samples of DBS from a finger:

1. Issue the informed voluntary consent of the legal representative;
2. Sign the drops with a patient's ID and date;
3. Choose the puncture location on the index finger;
4. The phlebotamist must clean his hands and put on sterile gloves;
5. Treat the puncture location with an antiseptic solution and let it dry;
6. Quickly pierce the skin with a lancet and wipe off the first drop of blood with a sterile cotton ball;
7. Hold the puncture location down gently pressing on the adjacent area, and take blood on a filter paper blank;
8. Hold the card without touching the marked area;
9. Gently touching a drop of blood with a filter paper card, apply it to the card. Let the card absorb the blood until the circle is full. After the application, do not touch the marked area;
10. Let the blood spot dry in a dark place. Avoid direct sunlight for at least 4 hours. Do not heat or let the dried spot samples come into contact with other surfaces during the drying process;
11. Seal the card (or parts of the card) in a gas-tight bag with a zipper. Store no more than one card per package in the refrigerator until sent to the laboratory at 2–8°C.

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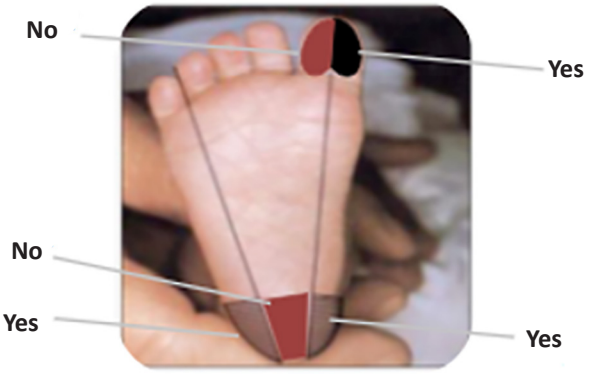

Figure 1 – Puncture location of newborn's heel

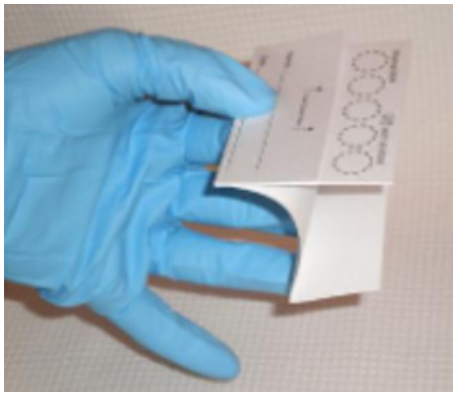

Figure 2 – Example of a map for DBS

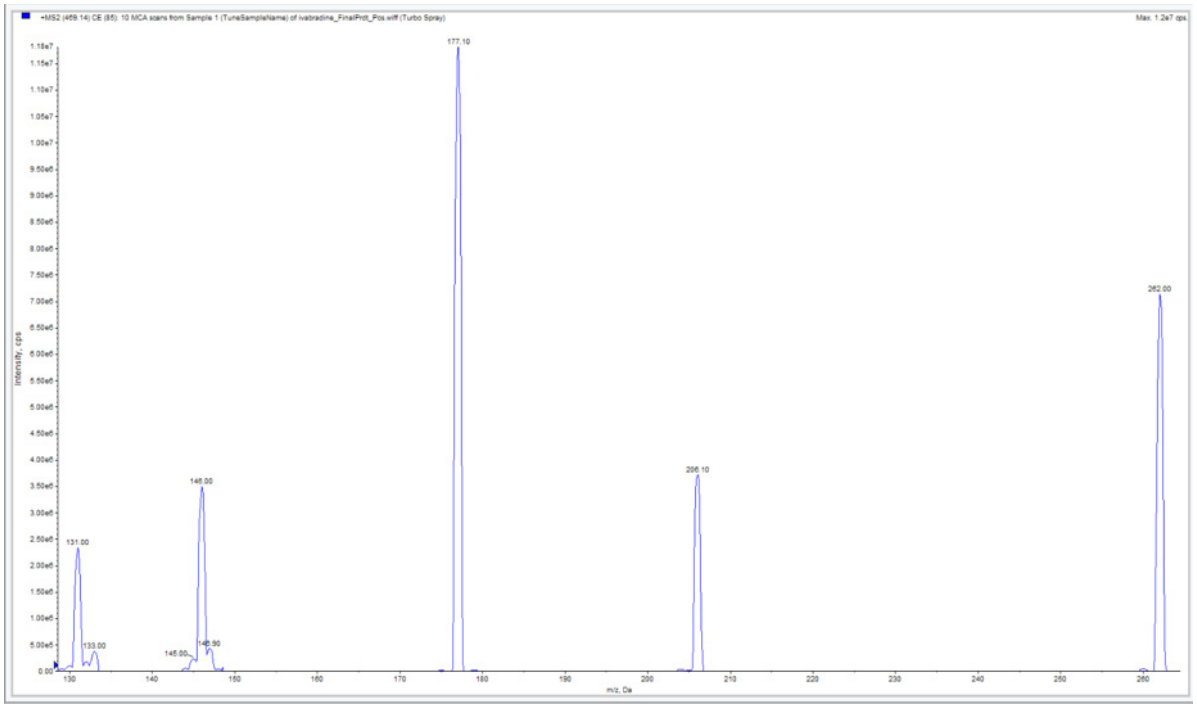

Figure 3 – Mass spectrum of ivabradine in blood plasma

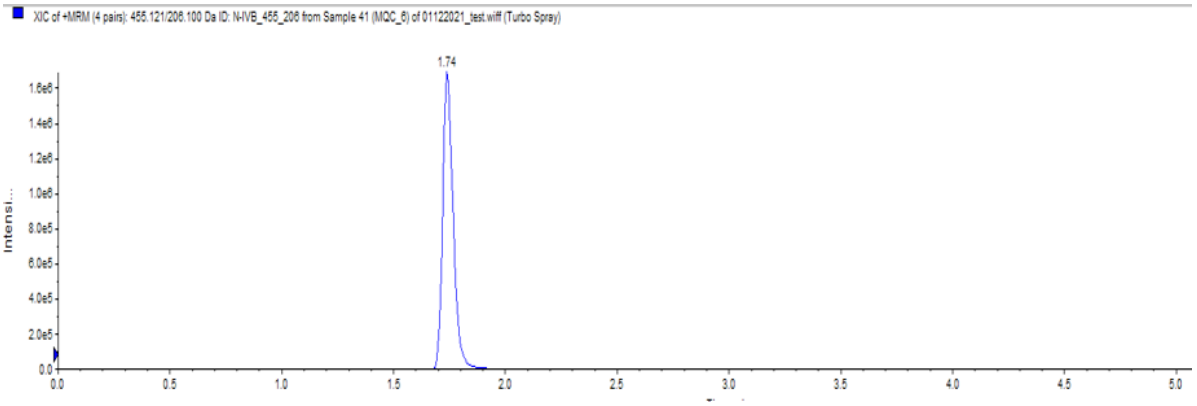

Figure 4 – Chromato-mass spectrogram of ivabradine in blood plasma
Note: on the abscissa – time (min), on the ordinates – signal intensity.

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Table 1 – Additional validation parameters for drug analysis by HPLC-MS/MS

MP	Class of MP	Additional DBS validation	References
Fluconazole	Antifungal	Drop volume effect	[32, 33]
Mycophenolic acid	Antitumor	–	[15, 19, 20]
Methotrexate	Antitumor	Hematocrit effect	[18]
Voriconazole	Antifungal	Drop volume effect	[14, 15]
Moxifloxacin	Antibiotic	Drop volume effect, paper type	[14, 21]
Pipyrecillin	Antibiotic	–	[18, 20]
Sirolimus	Immunosuppressant	Homogeneity, hematocrit effect, drop volume effect	[22–26]
Everolimus	Immunosuppressant	Homogeneity, hematocrit effect, drop volume	[24-26, 28, 37, 38]
Tazobactam	Antibiotic	–	[16, 31]
Vancomycin	Antibiotic	Homogeneity, hematocrit effect, drop volume effect	[16, 17]
Tacrolimus	Immunosuppressant	Homogeneity, hematocrit effect, drop volume effect	[22–30]
Cyclosporin A	Immunosuppressant	Homogeneity, hematocrit effect, drop volume effect	[26, 30, 34-36, 43]

Table 2 – Standard validation parameters

Validation parameter	Tests performed	Acceptance criteria [8, 10]
Linearity	8 calibration samples + blank sample + zero sample	≤ 15% of nominal values (≤ 20% for LLQL), not less than for 75% of samples
Selectivity	6 blank samples and 6 LLQLs	≤ 20% of LLQL
Accuracy	5 QC samples at 4 concentration levels	≤ 15% (≤ 20% for LLQL)
Precision	5 QC samples at 4 concentration levels	≤ 15% (≤ 20% for LLQL)
Matrix effect	5 QC samples at 2 concentration levels at 3 different hematocrit values	≤ 15%
Stability	5 QC samples at 2 concentration levels at room temperature at three time points (2 hours, 7 days and 14 days after applying the blood drop)	≤ 15%

Note: QC – quality control samples; LLQL – lower limit of quantitation level.

Table 3 – Specific validation parameters

Validation parameter	Tests performed	Acceptance criteria [8, 10]
Drop volume effect	5 QC samples at 3 drop volumes (10, 40, 70 µl) at 3 hematocrit levels (0.3; 0.4; 0.5), at 2 concentration levels	≤ 15% of nominal values
Hematocrit effect	5 QC samples for 3 hematocrit levels, at 2 concentration levels	≤ 15% of nominal values
Droplet uniformity	5 QC samples at 2 concentration levels at 3 hematocrit levels are compared; obtained by 2 drop cut options: from the center of the drop and at the edge.	≤ 15% of nominal values

Note: QC – quality control samples.

Table 4 – Validation parameters of DBS sample preparation methods

Parameter		Value			
		LLQL (1 ng/ml)	QCL (3 ng/ml)	QCM (400 ng/ml)	QCH (750 ng/ml)
Precision (CV %)	Inside the cycle	9.4	8.0	7.5	11.4
	Between cycles	12.5	10.1	9.2	5.8
Accuracy (%)	Inside the cycle	112.3	110.7	106.1	107.2
	Between cycles	91.2	100.7	95.9	96.3
Stability (%)		–	88.3	–	91.2
Selectivity (%)		10.4	–	–	–
Correlation coefficient		0.99			

Table 5 – Hematocrit effect on the analysis results

Hematocrit, %	QC	Nominal concentration (ng/ml)	Accuracy (%)
0.3	QCL	3	113.7
	QCH	750	109.6
0.4	QCL	3	99.4
	QCH	750	105.1
0.5	QCL	3	95.5
	QCH	750	94.7

In this case, all the obtained samples on the filter paper should be evenly distributed over the marked area and the drops should not merge with each other. In case of an incomplete distribution of blood in the marked area or the fusion of two drops, such samples are considered unacceptable and are not used for a further analysis.

Validation protocols for quantitative HPLC-MS/MS methods of analysis

To date, such organizations as the International Association for Therapeutic Drug Monitoring and Clinical Toxicology and the Food and Drug Administration (FDA) are working at developing general guidelines for the validation of dried blood spot technology, describing not only validation parameters specific to standard sample preparation methods and traditional matrices, but also specific parameters inherent only to this new technology [10, 11, 45-48, 50].

Based on the current methods analysis for the quantitative HPLC-MS/MS determination of MPs using this sample preparation method, new validation protocols using standard (Table 2) and specific validation parameters for the DBS method, were developed (Table 3).

While developing methods for the quantitative determination of ivabradine in the DBS samples, ions-ivabradine "precursors" which corresponded to the particles with m/z 469, were found out. To develop a multiple reactions monitoring (MRM) method, ion transitions corresponding to the highest intensity of the ions-"products" were used. It was found out that in the course of dissociation in the collision chamber, the most intense ions-"products" were: m/z 262.2 and 177.1 m/z (Fig. 3).

In the process of chromatographic determination under the optimized conditions, the retention time of ivabradine in blood plasma was 1.74 min (Fig. 4).

The developed methods confirmed its linearity in the concentration range from 1 to 1000 ng/ml using a weighted coefficient $1/x^2$, while >0.99. The coefficient of variation (%) calculated when determining inter- and intraday accuracy did not exceed 15% for the main range of concentrations.

The lower limit of the procedure quantification was determined based on the data of linearity, accuracy, and precision. The minimum concentration of ivabradine in the samples of DBS in the analytical range for which it is possible to quantify ivabradine with values of the relative standard deviation of no more than 20%, was taken as the lower limit of quantification in these methods, and it was 1 ng/mL (Table 4).

For the DBS method, the influence of specific parameters of the new method on the results of the analysis was also evaluated.

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The hematocrit effect was evaluated at 3 levels (0.3; 0.4; 0.5) for QCL and QCH, the concentrations obtained were in the range from 95.5 to 107.1% of the nominal (Table 5).

To validate the volume effect, 3 volumes (10, 40, 70 µl) (30, 40 and 55 µl) were analyzed at the average hematocrit level (0.4), at 2 concentration levels in 5 series. At the average volume, the relative error of the calculated concentrations did not exceed 15% of the obtained values.

When validating the droplet homogeneity, the results of the QC samples at QCL and QCH, obtained by 2 drop cut options – from the center of the droplet, and from the edge, were compared. The analysis was carried out in 5 series. In this case, the relative error in comparing the concentrations of the samples obtained from the central and edge notches did not exceed 15% of the nominal values.

The DBS samples at QCL and QCH levels were used to assess stability, and the samples were analyzed at three time points of 1, 7 and 14 days along with freshly prepared samples as a part of the same analytical series. The calculated concentrations of the samples after storage were compared with the average concentrations of freshly prepared quality control samples. At the same time, the values obtained after 14 days of storage were in the ranged of 87.2-93.8%.

Thermal stability was evaluated when storing the DBS samples for 14 days at 22°C and 45°C, as potential temperatures during their storage and transportation.

The developed protocols turned out to be suitable for validating the methods for the ivabradine quantitative determination and are acceptable for a further use in research and TDM.

CONCLUSION

It should be noted that the DBS method is the latest method of biological sampling, which has a number of significant advantages over the standard methods.

With the use of the optimized sample preparation and the new protocols that have been developed for taking, storing, validating, and methods for the quantitative analysis of DBS samples, the technology is widely used in preclinical and clinical studies, therapeutic and toxicological drug monitoring, as well as in large epidemiological studies. At the same time, this technology can provide a more cost-effective model for the analysis of drugs, as well as provide much-needed pharmacokinetic results in a fairly efficient and reliable way.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

ISA, TEZ, AMD – research concept, article planning, review of literature sources, materials collection, article writing and editing; VIP, AVS – development of the study design, editing and final approval of the article.

REFERENCES

1. Abaimov DA, Sariev AK, Noskova TYu, Shvedkov VV, Shiryaeva MV, Styrova EYu, Prokhorov DI, Seyfulla RD. modern technologies in therapeutic drug monitoring (review). Epilepsy and paroxysmal conditions. 2013;5(2):31–41. Russian
2. Déglon J, Thomas A, Mangin P, Staub C. Direct analysis of dried blood spots coupled with mass spectrometry: concepts and biomedical applications. Anal Bioanal Chem. 2012 Mar;402(8):2485–98. DOI: 10.1007/s00216-011-5161-6
3. Demirev PA. Dried blood spots: analysis and applications. Anal Chem. 2013 Jan 15;85(2):779–89. DOI: 10.1021/ac303205m
4. Edelbroek PM, van der Heijden J, Stolk LM. Dried blood spot methods in therapeutic drug monitoring: methods, assays, and pitfalls. Ther Drug Monit. 2009 Jun;31(3):327–36. DOI: 10.1097/FTD.0b013e31819e91ce
5. Antunes MV, Charão MF, Linden R. Dried blood spots analysis with mass spectrometry: Potentials and pitfalls in therapeutic drug monitoring. Clin Biochem. 2016 Sep;49(13–14):1035–46. DOI: 10.1016/j.clinbiochem.2016.05.004
6. Amsterdam PV, Waldrop C. The application of dried blood spot sampling in global clinical trials. Bioanalysis. 2010 Nov;2(11):1783–6. DOI: 10.4155/bio.10.158
7. Jimmerson LC, Zheng JH, Bushman LR, MacBrayne CE, Anderson PL, Kiser JJ. Development and validation of a dried blood spot assay for the quantification of ribavirin using liquid chromatography coupled to mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2014 Jan 1;944:18–24. DOI: 10.1016/j.jchromb.2013.10.035

8. Timmerman P, White S, Globig S, Lüdtke S, Brunet L, Smeraglia J. EBF recommendation on the validation of bioanalytical methods for dried blood spots. Bioanalysis. 2011 Jul;3(14):1567–75. DOI: 10.4155/bio.11.132
9. Jager NG, Rosing H, Schellens JH, Beijnen JH. Procedures and practices for the validation of bioanalytical methods using dried blood spots: a review. Bioanalysis. 2014 Sep;6(18):2481–514. DOI: 10.4155/bio.14.185.
10. Capiau S, Veenhof H, Koster RA, Bergqvist Y, Boettcher M, Halmingh O, Keevil BG, Koch BCP, Linden R, Pistos C, Stolk LM, Touw DJ, Stove CP, Alffenaar JC. Official International Association for Therapeutic Drug Monitoring and Clinical Toxicology Guideline: Development and Validation of Dried Blood Spot-Based Methods for Therapeutic Drug Monitoring. Ther Drug Monit. 2019 Aug;41(4):409–30. DOI: 10.1097/FTD.0000000000000643
11. Martial LC, Aarnoutse RE, Schreuder MF, Henriet SS, Brüggemann RJ, Joore MA. Cost Evaluation of Dried Blood Spot Home Sampling as Compared to Conventional Sampling for Therapeutic Drug Monitoring in Children. PLoS One. 2016 Dec 12;11(12):e0167433. DOI: 10.1371/journal.pone.0167433
12. Barfield M. The Application of Dried Blood Spots in Toxicokinetic and Pharmacokinetic Studies. Thesis submitted in partial fulfilment of the requirements of the University of Lincoln. 2017: 188 p.
13. Vu DH, Koster RA, Alffenaar JW, Brouwers JR, Uges DR. Determination of moxifloxacin in dried blood spots using LC-MS/MS and the impact of the hematocrit and blood volume. J Chromatogr B Analyt Technol Biomed Life Sci. 2011 May 1;879(15–16):1063–70. DOI: 10.1016/j.jchromb.2011.03.017
14. Arpini J, Antunes MV, Pacheco LS, Gnatta D, Rodrigues MF, Keitel E, Linden R. Clinical evaluation of a dried blood spot method for determination of mycophenolic acid in renal transplant patients. Clin Biochem. 2013 Dec;46(18):1905–8. DOI: 10.1016/j.clinbiochem.2013.10.011
15. Barco S, Risso FM, Bruschettini M, Bandettini R, Ramenghi LA, Tripodi G, Castagnola E, Cangemi G. A validated LC-MS/MS method for the quantification of piperacillin/tazobactam on dried blood spot. Bioanalysis. 2014;6(21):2795–802. DOI: 10.4155/bio.14.205
16. Al-Ghazawi M, Khaled Daoud Noor EH, Hadidi K, Alzweiri M, Aburuz S. Determination of vancomycin content in dried blood spots for therapeutic drug monitoring. Acta Poloniae Pharmaceutica. 2021;78(1): 3–10. DOI:10.32383/appdr/132021
17. Hawwa AF, Albawab A, Rooney M, Wedderburn LR, Beresford MW, McElnay JC. A novel dried blood spot-LCMS method for the quantification of methotrexate polyglutamates as a potential marker for methotrexate use in children. PLoS One. 2014 Feb 25;9(2):e89908. DOI: 10.1371/journal.pone.0089908
18. Wilhelm AJ, den Burger JC, Chahbouni A, Vos RM, Sinjewel A. Analysis of mycophenolic acid in dried blood spots using reversed phase high performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2009 Nov 15;877(30):3916–9. DOI: 10.1016/j.jchromb.2009.09.037
19. Heinig K, Bucheli F, Hartenbach R, Gajate-Perez A. Determination of mycophenolic acid and its phenyl glucuronide in human plasma, ultrafiltrate, blood, DBS and dried plasma spots. Bioanalysis. 2010 Aug;2(8): 1423–35. DOI: 10.4155/bio.10.99
20. Scribel L, Zavascki AP, Matos D, Silveira F, Peralta T, Gonçalves Landgraf N, Lamb Wink P, Cezimbra da Silva AC, Bordin Andriguetti N, Loss Lisboa L, Venzon Antunes M, Linden R. Vancomycin and creatinine determination in dried blood spots: Analytical validation and clinical assessment. J Chromatogr B Analyt Technol Biomed Life Sci. 2020 Jan 15;1137:121897. DOI: 10.1016/j.jchromb.2019.121897
21. Koster RA, Alffenaar JW, Greijdanus B, Uges DR. Fast LC-MS/MS analysis of tacrolimus, sirolimus, everolimus and cyclosporin A in dried blood spots and the influence of the hematocrit and immunosuppressant concentration on recovery. Talanta. 2013 Oct 15;115:47–54. DOI: 10.1016/j.talanta.2013.04.027
22. Sadilkova K, Busby B, Dickerson JA, Rutledge JC, Jack RM. Clinical validation and implementation of a multiplexed immunosuppressant assay in dried blood spots by LC-MS/MS. Clin Chim Acta. 2013 Jun 5;421:152–6. DOI: 10.1016/j.cca.2013.02.009
23. Li Q, Cao D, Huang Y, Xu H, Yu C, Li Z. Development and validation of a sensitive LC-MS/MS method for determination of tacrolimus on dried blood spots. Biomed Chromatogr. 2013 Mar;27(3):327–34. DOI: 10.1002/bmc.2795
24. Egas AC, Van Maarseveen EM, Kwakkel-Van Erp JM. Rapid and combined measurement of cyclosporin a, tacrolimus, sirolimus and everolimus in whole blood and dried blood spot with LC-MSMS. J Heart Lung Transplant. 2014; 33 (Issue 4): S68. DOI: 10.1016/j.healun.2014.01.217
25. Koop DR, Bleyle LA, Munar M, Cherala G, Al-Uzri A. Analysis of tacrolimus and creatinine from a single dried blood spot using liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2013 May 1;926:54–61. DOI: 10.1016/j.jchromb.2013.02.035
26. Shokati T, Bodenberger N, Gadpaille H, Schniedewind B, Vinks AA, Jiang W, Alloway RR, Christians U. Quantification of the Immunosuppressant Tacrolimus on Dried Blood Spots Using LC-MS/MS. J Vis Exp. 2015 Nov 8;(105):e52424. DOI: 10.3791/52424
27. Zwart TC, Gokoel SRM, van der Boog PJM, de Fijter JW, Kweekel DM, Swen JJ, Guchelaar HJ, Moes DJAR. Therapeutic drug monitoring of tacrolimus and mycophenolic acid in outpatient renal transplant recipients using a volumetric dried blood spot sampling device. Br J Clin Pharmacol. 2018 Dec;84(12):2889–902. DOI: 10.1111/bcp.13755

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-4-331-342

28. Hinchliffe E, Adaway J, Fildes J, Rowan A, Keevil BG. Therapeutic drug monitoring of ciclosporin A and tacrolimus in heart lung transplant patients using dried blood spots. Ann Clin Biochem. 2014 Jan;51(Pt 1):106–9. DOI: 10.1177/0004563213488759
29. Cohen-Wolkowiez M, Watt KM, Zhou C, Bloom BT, Poindexter B, Castro L, Gao J, Capparelli EV, Benjamin DK Jr, Smith PB. Developmental pharmacokinetics of piperacillin and tazobactam using plasma and dried blood spots from infants. Antimicrob Agents Chemother. 2014 May;58(5):2856–65. DOI: 10.1128/AAC.02139-13
30. van der Elst KC, Span LF, van Hateren K, Vermeulen KM, van der Werf TS, Greijdanus B, Kosterink JG, Uges DR, Alffenaar JW. Dried blood spot analysis suitable for therapeutic drug monitoring of voriconazole, fluconazole, and posaconazole. Antimicrob Agents Chemother. 2013 Oct;57(10):4999–5004. DOI: 10.1128/AAC.00707-13
31. Wilhelm AJ, den Burger JC, Vos RM, Chahbouni A, Sinjewel A. Analysis of cyclosporin A in dried blood spots using liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2009 May 15;877(14-15):1595–8. DOI: 10.1016/j.jchromb.2009.03.024.
32. Wilhelm AJ, Klijn A, den Burger JC, Visser OJ, Veldkamp AI, Janssen JJ, Swart EL. Clinical validation of dried blood spot sampling in therapeutic drug monitoring of ciclosporin A in allogeneic stem cell transplant recipients: direct comparison between capillary and venous sampling. Ther Drug Monit. 2013 Feb;35(1):92–5. DOI: 10.1097/FTD.0b013e31827d76ce
33. Hinchliffe E, Adaway JE, Keevil BG. Simultaneous measurement of cyclosporin A and tacrolimus from dried blood spots by ultra high performance liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2012 Feb 1;883–4:102-7. DOI: 10.1016/j.jchromb.2011.05.016
34. Antunes MV, Charão MF, Linden R. Dried blood spots analysis with mass spectrometry: Potentials and pitfalls in therapeutic drug monitoring. Clin Biochem. 2016 Sep;49(13–14):1035–46. DOI: 10.1016/j.clinbiochem.2016.05.004
35. Hoffman JT, Rossi SS, Espina-Quinto R, Letendre S, Capparelli EV. Determination of efavirenz in human dried blood spots by reversed-phase high-performance liquid chromatography with UV detection. Ther Drug Monit. 2013 Apr;35(2):203–8. DOI: 10.1097/FTD.0b013e31827fb72b.
36. Li W, Lee MS. Dried Blood Spots: Applications and Techniques; Wiley, 2014: 376 p.
37. Chermont AG, Falcão LF, de Souza Silva EH, de Cássia Xavier Balda R, Guinsburg R. Skin-to-skin contact and/or oral 25% dextrose for procedural pain relief for term newborn infants. Pediatrics. 2009 Dec;124(6):e1101–7. DOI: 10.1542/peds.2009-0993
38. Hummel P, Puchalski M, Creech SD, Weiss MG. Clinical reliability and validity of the N-PASS: neonatal pain, agitation and sedation scale with prolonged pain. J Perinatol. 2008 Jan;28(1):55–60. DOI: 10.1038/sj.jp.7211861
39. Kireev SS. Pain and stress in the newborns (literature review). Journal of New Medical Technologies. 2016;23(4):328–42. DOI 10.12737/issn.1609-2163
40. Streit F, William Armstrong V, Oellerich M. Rapid Liquid Chromatography–Tandem Mass Spectrometry Routine Method for Simultaneous Determination of Sirolimus, Everolimus, Tacrolimus, and Cyclosporin A in Whole Blood. Clinical Chemistry. 2002;48 (Issue 6):955–8. DOI: 10.1093/clinchem/48.6.955
41. Tsao JC, Evans S, Meldrum M, Altman T, Zeltzer LK. A Review of CAM for Procedural Pain in Infancy: Part I. Sucrose and Non-nutritive Sucking. Evid Based Complement Alternat Med. 2008 Dec;5(4):371–81. DOI: 10.1093/ecam/nem084
42. Freeman JD, Rosman LM, Ratcliff JD, Strickland PT, Graham DR, Silbergeld EK. State of the Science in Dried Blood Spots. Clin Chem. 2018 Apr;64(4):656–79. DOI: 10.1373/clinchem.2017.275966
43. Zakaria R, Allen KJ, Koplin JJ, Roche P, Greaves RF. Advantages and Challenges of Dried Blood Spot Analysis by Mass Spectrometry Across the Total Testing Process. EJIFCC. 2016 Dec 1;27(4):288–317.
44. Linder C. Possibilities of Dried Blood Spots As a Matrix in Therapeutic Drug Monitoring of Antiepileptic Drugs in Children. Karolinska Institutet, Stockholm, Sweden. 2019: 61 p.
45. Murphy SC, Daza G, Chang M, Coombs R. Laser cutting eliminates nucleic acid cross-contamination in dried-blood-spot processing. J Clin Microbiol. 2012 Dec;50(12):4128–30. DOI: 10.1128/JCM.02549-12.
46. Li Y, Henion J, Abbott R, Wang P. Dried blood spots as a sampling technique for the quantitative determination of guanfacine in clinical studies. Bioanalysis. 2011 Nov;3(22):2501–14. DOI: 10.4155/bio.11.262.
47. Klak A, Pauwels S, Vermeersch P. Preanalytical considerations in therapeutic drug monitoring of immunosuppressants with dried blood spots. Diagnosis (Berl). 2019 Mar 26;6(1):57–68. DOI: 10.1515/dx-2018-0034.
48. Knapen LM, Beera Y, Brüggemannc RM, Stolkab LM, Vries F, Vivianne CG, et al. Development and validation of an analytical method using UPLC–MS/MS to quantify everolimus in dried blood spots in the oncology setting. Journal of Pharmaceutical and Biomedical Analysis. 2018;149:106–13. DOI: 10.1016/j.jpba.2017.10.039
49. Börsch-Supan A, Börsch-Supan M, Weiss LM. Dried blood spot samples and their validation. Health and socio-economic status over the life course. 2019: 349–58. DOI:10.1515/9783110617245-036

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