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Development and Validation of a Method for Determining Deferasirox in Human Blood Plasma by HPLC-UV

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Abstract

Introduction. Deferasirox is one of the most well-known complexing drugs chelators and is successfully used in chelating therapy for the treatment of excess iron in the human body. Deferasirox is also included in the list of vital and essential medicines, which indicates the importance of this drug for Russian healthcare. In this document, there are drugs only from a foreign manufacturer, therefore, within the framework of the trend towards import substitution, the development of deferasirox preparations of domestic production is a necessary and promising direction. In this connection, there is a need to develop a method that allows quantifying deferasirox in human blood plasma with minimal time and resource costs as part of a pharmacokinetic study.

Aim. The aim of this study is to develop a method for quantitative determination of deferasirox in human blood plasma by high performance liquid chromatography coupled with ultraviolet detection (HPLC-UV) for further bioequivalence studies.

Materials and methods. Determination of the deferasirox in human blood plasma was carried out by HPLC-UV. The method of proteins precipitation by acetonitrile was used as a sample preparation. Erlotinib solution was used as an internal standard. Mobile phase: 0.3 % solution of orthophosphoric acid in water, brought to pH 3.0 (eluent A) and 0.1 % solution of formic acid in acetonitrile (eluent B). Column was Symmetry®, 75 × 4,6 mm (Waters, США). Analytical range of the technique for deferasirox was 0.25–70.00 μg/ml in human blood plasma. Detection was carried out using a UV detector at an absorption wavelength of 299 ± 2 nm.

Results and discussion. This method was validated by selectivity, calibration curve, accuracy, precision, spike recovery, the lower limit of quantification, carry-over effect and stability.

Conclusion. A method of quantitative determination of deferasirox in human blood plasma was developed and validated by HPLC-UV. The analytical range was 0.25–70.00 μg/ml in human blood plasma. This method was used as part of a study of the pharmacokinetics and bioequivalence of deferasirox drugs.

Keywords: deferasirox, human blood plasma, iron, HPLC-UV, validation, pharmacokinetics, bioequivalence

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Timofey N. Komarov, Olga A. Archakova, Dana S. Shchelgacheva, Alexandra V. Suvorova, Polina A. Karpova have developed and validated the analytical method. Polina K. Karnakova participated in sample preparation. Natalia S. Bagaeva conducted statistical processing of the obtained results. Igor E. Shohin carried out the organization of work in this direction. All the above authors participated in the discussion of the results in the format of scientific discussion.

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Разработка и валидация методики определения деферазирокса в плазме крови человека методом ВЭЖХ-УФ

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Резюме

Введение. Деферазирокс является одним из наиболее известных комплексообразующих лекарственных средств и успешно применяется в хелатирующей терапии для лечения избытка железа в организме человека. Также деферазирокс входит в перечень жизненно необходимых и важнейших лекарственных препаратов, что свидетельствует о значимости данного лекарственного средства для российского здравоохранения. В указанном перечне присутствуют препараты только зарубежного производителя, поэтому в рамках тенденции к импортозамещению разработка препаратов деферазирокса отечественного производства является необходимым и перспективным направлением. В связи с чем возникает необходимость разработки методики, позволяющей с минимальными временными и ресурсными затратами количественно определить деферазирокс в плазме крови человека в рамках фармакокинетического исследования.

Цель. Целью исследования является разработка и валидация методики определения деферазирокса в плазме крови человека методом высокоэффективной жидкостной хроматографии с ультрафиолетовым детектированием (ВЭЖХ-УФ) для дальнейшего исследования фармакокинетики и биоэквивалентности лекарственных препаратов.

Материалы и методы. Определение деферазирокса в плазме крови человека проводили методом ВЭЖХ-УФ. В качестве пробоподготовки был использован способ осаждения белков ацетонитрилом. Раствор эрлотиниба использовался в качестве внутреннего стандарта. Подвижная фаза: 0,3%-й раствор ортофосфорной кислоты в воде, доведенный до pH 3,0 (элюент А) и 0,1%-й раствор муравьиной кислоты в ацетонитриле (элюент В). Колонка: Symmetry®, 75 × 4,6 мм (Waters, США). Аналитический диапазон методики для деферазирокса: 0,25–70,00 мкг/мл в плазме крови. Детектирование проводилось с помощью УФ-детектора при длине волны поглощения 299 ± 2 нм.

Результаты и обсуждение. Валидация разработанной методики проводилась по следующим валидационным параметрам: селективность, калибровочная кривая, точность, прецизионность, степень извлечения, нижний предел количественного определения, перенос пробы, стабильность (стабильность исходных и рабочих стандартных растворов аналита и внутреннего стандарта; краткосрочная стабильность; стабильность при трехкратной заморозке-разморозке аналита; долгосрочная стабильность аналита в матрице).

Заключение. Была разработана и валидирована методика определения лекарственного средства деферазирокс в плазме крови человека методом ВЭЖХ-УФ. Аналитический диапазон методики был подтвержден и составил 0,25–70,00 мкг/мл для деферазирокса в плазме крови. Данная методика была использована в рамках проведения исследования фармакокинетики и биоэквивалентности препаратов деферазирокса.

Ключевые слова: деферазирокс, плазма, железо, ВЭЖХ-УФ, валидация, определение, фармакокинетика, биоэквивалентность

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. Т. Н. Комаров, О. А. Арчакова, Д. С. Щелгачева, А. В. Суворова, П. А. Карпова участвовали в разработке и валидации биоаналитической методики. П. К. Карнакова занималась подготовкой проб к анализу. Н. С. Багаева отвечала за статистическую обработку данных. И. Е. Шохин отвечал за организацию исследования. Все вышеуказанные авторы принимали участие в обсуждении полученных результатов в форме научной дискуссии.

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INTRODUCTION

Deferasirox is a tridentate ligand with a high degree of affinity for ferric iron, while two drug molecules form stable complexes with ferric ion (Figure 1) [1, 2]. Owing to these properties, deferasirox has been used in chelation therapy for the treatment of β-thalassemia, sickle cell anemia due to blood transfusion, and other diseases related to excess iron in human blood [2–4]. The mechanism of such therapy is based on the binding of iron ions and the formation of chelates, which are later eliminated from the body. It should be also noted that deferasirox showed antioxidant activity, causing a significant decrease of rate of oxidative damage to human tissues induced by an excess of such essential damage as iron and copper, which are catalysts for reactive oxygen species and oxidative damage in biological systems [5, 6].

Maximum plasma concentration of deferasirox (C_{max}) and area under the pharmacokinetic curve (AUC) depend on a drug dose when taken orally, but time to reach C_{max} (T_{max}) is not dose-dependent and is about 1–2 hours. The mean half-life ($t_{1/2}$) of deferasirox is up to 19 hours [2, 7]. It is known that the main pathway of deferasirox metabolism is conjugation with glucuronic acid: acylglucuronide and 2-O-glucuronide [8]. Most (about 84 %) of deferasirox and its metabolites are excreted in bile with multidrug resistance protein 2 (MRP2), and the remaining 16 % are excreted with breast cancer resistance protein (BCRP) and with urine. Renal excretion as glucuronide is only 8 % of the administered drug dose [7, 9, 10].

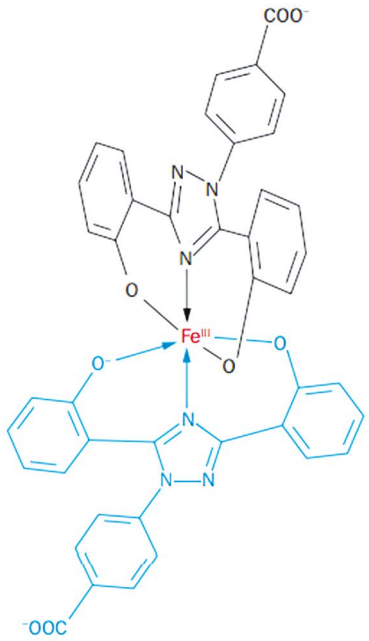


Figure 1. Chemical structure of deferasirox complex with iron

Deferasirox is included in the list of vital and essential drugs, which indicates the importance of the drug for Russian healthcare. This list contains drugs only by foreign manufacturers, therefore, as part of the trend towards import substitution, the development of domestic deferasirox products is a necessary and promising direction[1].

Currently, several studies have been published on the determination of deferasirox in biological fluids for pharmacokinetic studies. To determine the substance in biological fluids, methods of high performance liquid chromatography – tandem mass spectrometry (HPLC/ MS), high performance liquid chromatography – fluorescence detection (HPLC-FLD) and high performance liquid chromatography – ultraviolet detection (HPLC-UV). The structure of deferasirox molecule contains several chromophore groups (see Figure 1), therefore, most of the reviewed literature sources favor HPLC-UV analysis.

[1] Decree of the Government of the Russian Federation dated October 12, 2019 № 2406r "On approval of the list of vital and essential medicinal products for 2020, the list of medicinal products including drugs prescribed by a decision of health commissions of medical organizations, a list of drugs intended for people with hemophilia, cystic fibrosis, pituitary dwarfism, Gaucher's disease, as well as the minimum range of drugs required to provide medical care" (as amended on 03/30/2022). Available at: http://www.consultant.ru/document/cons_doc_LAW_335635/ Accessed: 28.04.2022.

Many of the reviewed methods use protein precipitation with acetonitrile (ACN) or its mixture with methanol (MeOH) in various ratios for sample preparation (Table 1). As well, for sample preparation, such methods as liquid-liquid extraction with a possible subsequent derivatization to increase the fluorescence of the analyte or the addition of an additional amount of phosphate buffer to transfer deferasirox to an iron-free form are used, although the mobile phase in this case already contains a phosphate buffer with pH 3.0 [11, 12]. Several methods for the determination of deferasirox involve the addition of some ethylenediaminetetraacetate (EDTA) in order to prevent the formation of a drug-iron complex and to determine the free form of the analyte. It should be also noted that these methods use dilution in the preparation of test samples [13, 14]. Other methods consider human blood or breast milk as a research object, rather than human plasma [14, 15], or chromatographic analysis lasts for a quite long time.

The above methods for the determination of deferasirox are challenging to be implemented in practice in the conditions of daily and routine work due to labor-intensive sample preparation and duration of the analysis, in general.

This study provides the development and validation of a method for the determination of deferasirox in human plasma by HPLC with an ultraviolet detector. A simple and affordable method of protein precipitation with acetonitrile is used for sample preparation, and phosphate buffer with pH 3.0 is used as mobile phase eluent A, 0.1 % formic acid solution in acetonitrile (v/v) is used as eluent B.

MATERIALS AND METHODS

Equipment

Chromatographic separation and detection were performed on a Nexerai LC2040 high performance liquid chromatograph (Shimadzu Corporation, Japan) equipped with a column and sample thermostat, a degasser, an autosampler, and an ultraviolet detector. Source data were processed with LabSolutions Single LC software (Shimadzu Corporation, Japan).

Reagents and solutions

The following reagents were used in the study:

- acetonitrile ("UHPLC" class, PanReac, Spain);
- acetonitrile ("chemically pure" class, "Trade House HIMMED" LLC, Russia);

Table 1. **Bioanalytical methods of deferasirox quantitative determination**

Analytical method	Object	Sample preparation's notes	Analytical range, μg/ml	Reference
HPLC-UV	Human blood plasma	Protein precipitation with acetonitrile followed by addition of phosphate buffer and repeated centrifugation	0.09970–19.94	[11]
HPLC-FLD	Human blood plasma	Liquid-liquid extraction followed by dansyl chloride derivatization	0.02–2.00	[12]
LC-MS/MS	Human blood plasma	Protein precipitation with acetonitrile followed by dilution	0.04–40.00	[13]
HPLC–UV	Human blood	Dispersive liquid-liquid microextraction followed by dilution	0.20–200.00	[14]
HPLC–UV	Human breast milk	Protein precipitation with acetonitrile : methanol (70 : 30, v/v) followed by evaporation and derivatization	0.01–1.00	[15]
HPLC-UV	Human blood plasma	Protein precipitation by acetonitrile : methanol (50 : 50, v/v)	0.078125–40.00	[16]

- formic acid ("for analysis" class, PanReac, Spain);
- methanol ("chemically pure" class, "Trade House HIMMED" LLC, Russia);
- phosphoric acid ("chemically pure" class, "ComponentReaktiv" LLC, Russia);
- sodium hydroxide ("pure, pharmaceutical grade" class, PanReac, Spain);
- demineralized water (purity class I).

To prepare stock and working solutions, substances deferasirox (CTX Life Science Pvt. Ltd., India, content 100.20 %) and erlotinib hydrochloride (USP reference standard, content 99.80 %) were used.

The stock standard solution of deferasirox was prepared by dissolving an accurate weigh of the substance in "chemically pure" methanol. Working standard solutions were prepared by diluting an aliquot of the stock standard solution with the same solvent according to Table 2.

To prepare a working standard solution of the internal standard (IS) of erlotinib, an accurate weigh of the standard sample of erlotinib hydrochloride was quantitatively transferred into a volumetric flask and dissolved in chemically pure acetonitrile.

Intact plasma samples, stock and working standard solutions were stored in a freezer at –50°C to –35 °C.

Table 2. **Concentrations of deferasirox and IS at calibration levels and quality control samples**

Level	Deferasirox concentration, μg/ml	IS concentration, μg/ml	Notes
1	0.25	20,00	LLOQ – lower limit of quantification L- low level of concentration M1 and M2 – middle levels of concentration H – high level of concentration
2	0.50		
3	1.00		
4	5.00		
5	10.00		
6	30.00		
7	60.00		
8	70.00		
LLOQ	0.25		
L	0.75		
M1	21.00		
M2	42.00		
H	56.00		

Sample preparation

To 200 µl of the sample (calibration sample, quality control sample and intact plasma sample) placed in Eppendorf centrifuge microtubes 2 ml, 10 µl of the IS working solution was added, then 400 µl of acetonitrile was added, mixed on a Vortex shaker for 5–10 seconds, then centrifuged for 15 minutes at an acceleration of 15 000 g. Next, the supernatant was transferred into chromatographic vials and placed into the chromatography autosampler trays.

Conditions for chromatographic separation and detection

- Column: Symmetry®, 75 × 4.6 mm (Waters, USA).
- Pre-column: Phenomenex SecurityGuard™. Cartridges Widepore C18 4 × 3.0 mm.
- Thermostat temperature: 40 °C.
- Mobile phase: 0.3 % phosphoric acid in water adjusted to pH 3.0 (eluent A); 0.1 % formic acid in acetonitrile (v/v) (eluent B).
- Flow rate of the mobile phase: 1.0 ml/min.
- Composition gradient of the mobile phase is shown in Figure 2.
- Injection volume: 10 µl.
- Retention time of Deferasirox: approx. 3.8 min.
- Retention time of Erlotinib: approx. 3.2 min.
- Run time: 0.0–7.0 min.
- Detection: UV detector at an absorption wavelength of 299 ± 2 nm.
- Frequency of the detector signal recording: 5 Hz.

RESULTS AND DISCUSSION

Method development

During the method development, a number of features arose: the effect of metals in the chromatographic system on the study results, difference in the drug determination in plasma between its free and chelated forms, the effect of hydrolysis of deferasirox chelate with iron exposed to acidic and alkaline agents, the selection of a mobile phase and a suitable IS. After a series of experiments, it was found out that systemic iron from the equipment did not have any effect on the study result. The use of a 0.3 % phosphoric acid solution in water adjusted to pH 3.0 as the mobile phase provided acidic hydrolysis of the drug chelate with iron and led to the possible determination of total deferasirox in the free form. As well, due to the presence of an acidic center in the molecule in this system, deferasirox was in a non-ionized form, which made it possible to increase its retention on the chromatographic column and obtain a narrow and symmetrical peak on the chromatogram.

Acetonitrile was used as a precipitant, since it provided the most complete precipitation of plasma proteins and the optimal shape of chromatographic peaks. Despite the fact that other methods of sample preparation are given in the literature, the selected method made it possible to simplify the process and obtain a method that meets the current requirements of regulatory documentation. To determine the study drug and IS, an ultraviolet detector was used, which made it possible to obtain the peaks of deferasirox and erlotinib due to the presence of chromophore groups in their structure (Table 3). While developing the method, based on physicochemical properties of the analytes, a Symmetry® chromatographic column, 75 × 4.6 mm (Waters, USA) was selected.

Method validation

The method for determination of deferasirox was validated as part of a pharmacokinetic study in human plasma, based on the Rules for conducting bioequivalence studies of medicinal products within the Eurasian

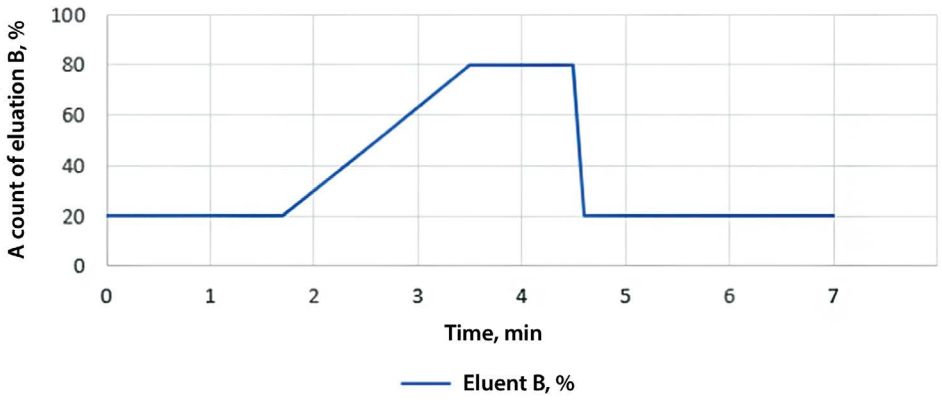
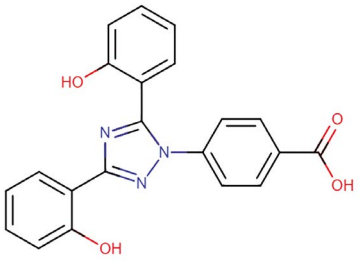
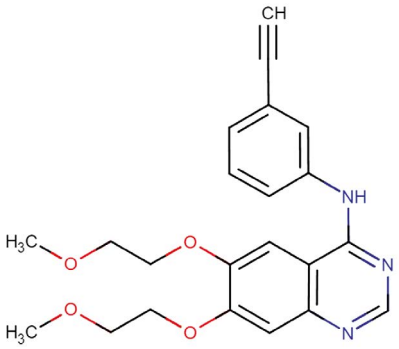


Figure 2. **Elution's gradient**

Table 3. **Chemical and physical characteristics of the analyte and of the IS**

Name of the substance	Molecular mass, g/mol	pKa	log P	Chemical formula
Deferasirox[1]	373.3615	4.55	4.74	
Erlotinib[2]	393.4357	16.14	3.2	

Note. [1] Deferasirox. Drugbank. Available to: https://go.drugbank.com/drugs/DB01609. Assessed: 24.02.2022.
[2] Erlotinib. Drugbank. Available to: https://go.drugbank.com/drugs/DB00530. Assessed: 24.02.2022.

Economic Union[1], as well as the FDA[2] and EMA[3] guidelines by such parameters as inter- and intercycle selectivity, calibration curve, accuracy and precision at LLOQ levels, L, M1, M2, H (see Table 2), recovery, sample carryover, lower limit of quantification (LLOQ) and detection limit, stability [stability of stock and working solutions of the analyte. and IS standards; short-term stability ("bench-top" and "post-preparative"); stability in triple freezing-thawing of the analyte; long-term stability of the analyte in the matrix].

[1] Rules for conducting bioequivalence studies of medicinal products within the Eurasian Economic Union (approved by decision № 85 of the Council of the Eurasian Economic Commission of 03.11.2016). Available at: https://docs.cntd.ru/document/456026107. Accessed: 18.03.2022.

[2] Food and Drug Administration. Available at: https://www.fda.gov/regulatory-information/search-fda-guidancedocuments/bioanalytical-method-validation-guidanceindustry. Assessed: 18.03.2022.

[3] European Medicines Agency. Available at: https://www.ema.europa.eu/en/bioanalytical-method-validation. Assessed: 18.03.2022.

Selectivity

To evaluate this parameter, we analyzed 6 samples of blank plasma, 2 samples of hyperlipidemic blank plasma and 2 samples of hemolytic blank plasma obtained from different sources, as well as, samples with the addition of the working standard solution of deferasirox № 1 and IS working standard solution (see Table 2).

On the chromatograms of blank plasma samples, the signals of the peaks corresponding to the retention times of deferasirox and erlotinib did not exceed 20 % of the signal at the level of LLQL and 5 % of the IS signal, respectively. The chromatogram of a blank plasma sample is shown in Figure 3.

Calibration curve

Eight intact plasma samples were analyzed with the addition of erlotinib working standard solution and deferasirox working standard solutions to obtain analyte and IS concentrations corresponding to levels 1–8 (see Table 2).

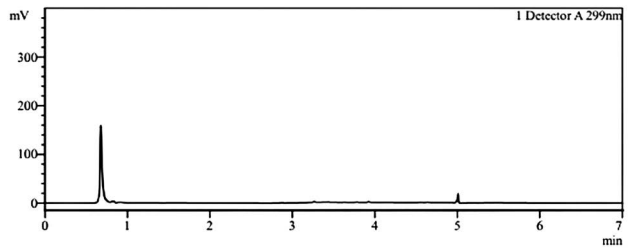


Figure 3. **Blank human blood plasma sample chromatogram**

According to the obtained values, calibration graphs were plotted in coordinates of "the ratio between deferasirox peak area to erlotinib peak area and the ratio of deferasirox concentration to erlotinib concentration in plasma".

Calibration plots had a linear relationship (Figure 4). The obtained correlation coefficients correspond to the normal values (not less than 0.99). The concentration deviations of calibration samples, calculated by the equation of linear dependence on the nominal values, did not exceed the normal value, no more than 20 % for level 1 of the calibration curve and no more than 15 % for levels 2–8.

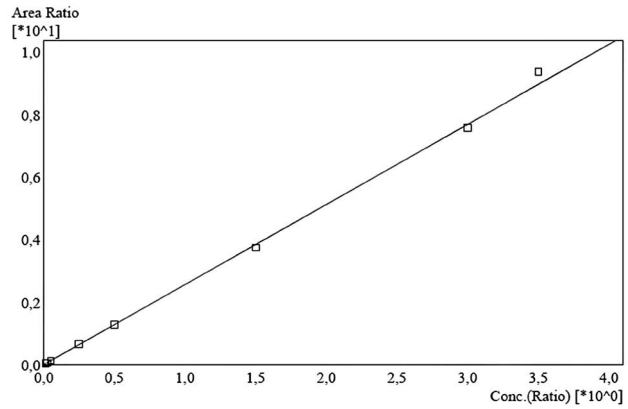


Figure 4. **Calibration curve**

Accuracy and Precision

Calibration samples of blood plasma corresponding to the levels of LLOQ, L, M1, M2, H were analyzed (see table 2). Analyzes were performed in 3 sequences of 5 sample injections for each of the 5 levels of deferasirox concentrations.

The parameter was evaluated within a cycle, between two and three cycles. For the obtained concentration values, the relative standard deviation (RSD, %) and relative error (E, %) were calculated. The data corresponded to the normal values (table 4).

Table 4. **Accuracy and precision of deferasirox determination procedure (inter-day, intra-day 1, intra-day 2)**

Injected (μg/ml)	RSD, %			*E*, %		
	Inter-day (*n* = 5)	Intra-day 1 (*n* = 10)	Intra-day 2 (*n* = 15)	Inter-day (*n* = 5)	Intra-day 1 (*n* = 10)	Intra-day 2 (*n* = 15)
0.25	0.74	4.60	5.05	7.53	3.19	0.98
0.75	0.26	9.42	8.06	2.17	-6.21	–4.14
21.00	0.34	2.44	3.26	3.77	1.43	3.28
42.00	0.18	0.78	1.90	–1.01	–1.72	–0.50
56.00	0.12	0.43	2.08	–0.22	–0.61	0.80

Recovery

To evaluate recovery (RI), 3 samples prepared from intact, hemolytic and hyperlipidemic plasma were analyzed without the effect of recovery at levels L, M1, M2 and H (see Table 2), as well as, quality control samples prepared on various intact matrices, to assess recovery. The data are presented in Table 5. The recovery should not be 100 %, but it was necessary to ensure efficient and reproducible recovery of substances from the biological matrix. The RSD of the calculated recovery values of the analyte from biological matrices did not exceed 15 %.

Table 5. **Calculation of deferasirox recovery at L, M1, M2, H levels from the different biological matrix**

Biological matrix	Deferasirox recovery %			
	L	M1	M2	H
Blank human blood plasma	104.07	96.15	97.86	91.90
	104.37	96.53	98.02	92.34
	104.16	96.28	97.50	91.54
Hemolyzed blank human blood plasma	110.43	97.35	102.62	92.60
	111.17	98.07	102.46	92,74
	104.28	97.85	102.80	92.43
Lipemic blank human blood plasma	104.52	101.70	100.29	97.94
	101.09	101.70	100.42	97.51
	106.04	101.41	100.03	97.32
Average	99.60			
S.D.	4.88			
RSD, %	4.90			

Lower limit of quantitation

LLOQ of the method was determined based on calibration curve data, accuracy, and precision. The minimum concentrations of deferasirox in blood plasma in the corresponding analytical ranges for deferasirox may be quantified with RSD and E values of not more than 20 % were taken as LLOQ of the method. The lower limit of quantification of the method was 0.25 μg/mL.

The chromatogram of blood plasma containing deferasirox at the level of LLOQ is shown in Figure 5.

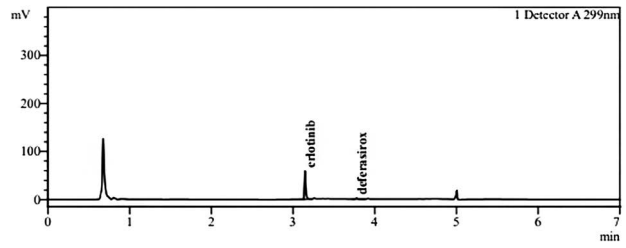


Figure 5. Chromatogram of human blood plasma with deferasirox content at the level of LLOQ

Stability

The following types of stability were confirmed at the lower (L level) and upper (H level) levels of deferasirox concentrations: short-term stability ("bench-top" and "post-preparative"), stability during three freeze-thaws, stability of stock and working standard solutions of deferasirox and erlotinib (when stored for 57 days at temperatures –50 °C to –35 °C). The long-term stability of deferasirox in plasma was evaluated within 57 days when stored at –50°C to –35°C.

Sample carryover

In the sequential analysis of calibration samples with the highest concentration and intact plasma samples, the chromatograms of intact plasma samples did not show peaks with retention times corresponding to the peaks of deferasirox and IS.

Application of the developed method

On the basis of the developed method, an analytical stage was carried out to investigate the pharmacokinetics and bioequivalence of the domestic drug in capsules 360 mg and reference drug Jadenu®, film-coated tablets 360 mg (Novartis Pharma AG, Switzerland). Figure 6 shows a representative chromatogram of a plasma sample from a volunteer who took part in this study.

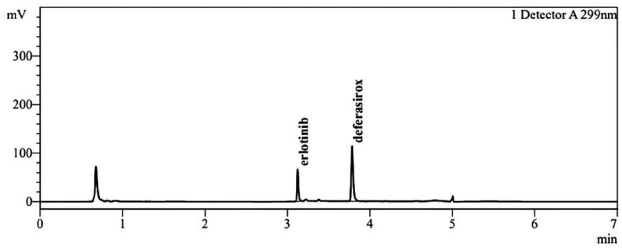


Figure 6. Chromatogram of volunteer human blood plasma sample

Individual profiles of changes in the values of deferasirox concentrations in human plasma over the time (*t*), recorded after administration of the study drug and the reference drug, were characterized by the maximum concentration of the drug substance, the area under the "concentration-time" curve from the dosing to the last estimated concentration in time point *t*, above the LLOQ (AUC_{0-t}), the area under the "concentration – time" curve from the dosing to infinity ($AUC_{0-\infty}$).

Table 6 shows the arithmetic and geometric mean values for pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of the study drugs.

Table 6. Arithmetic means and geometric means values of pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of drugs

Parameters	Mean (GMean)	
	Test	Ref
C_{max}, ng/ml	15470.69 (14835.09)	17007.17 (16156.61)
AUC_{0-t}, ng · hr/ ml	126573.68 (117373.66)	122832.27 (116930.07)
$AUC_{0-\infty}$, ng · hr/ ml	138394.34 (128355.91)	134336.92 (127641.14)

Note. The values are presented as Mean (GMean) format; Mean is arithmetic mean; GMean is geometric mean. Test drug (Test), Reference drug (Ref).

The pharmacokinetic profiles of the domestically produced study drug and reference drug Jadenu® are shown in Figure 7.

CONCLUSION

A method for the quantification of deferasirox in human plasma by the HPLCV method was developed and validated. The confirmed analytical range of the method was 0.25–70.00 μg/ml in blood plasma, which allows using the developed method for the analytical part of pharmacokinetic studies of deferasirox products. This method was successfully used in the pharmacokinetics

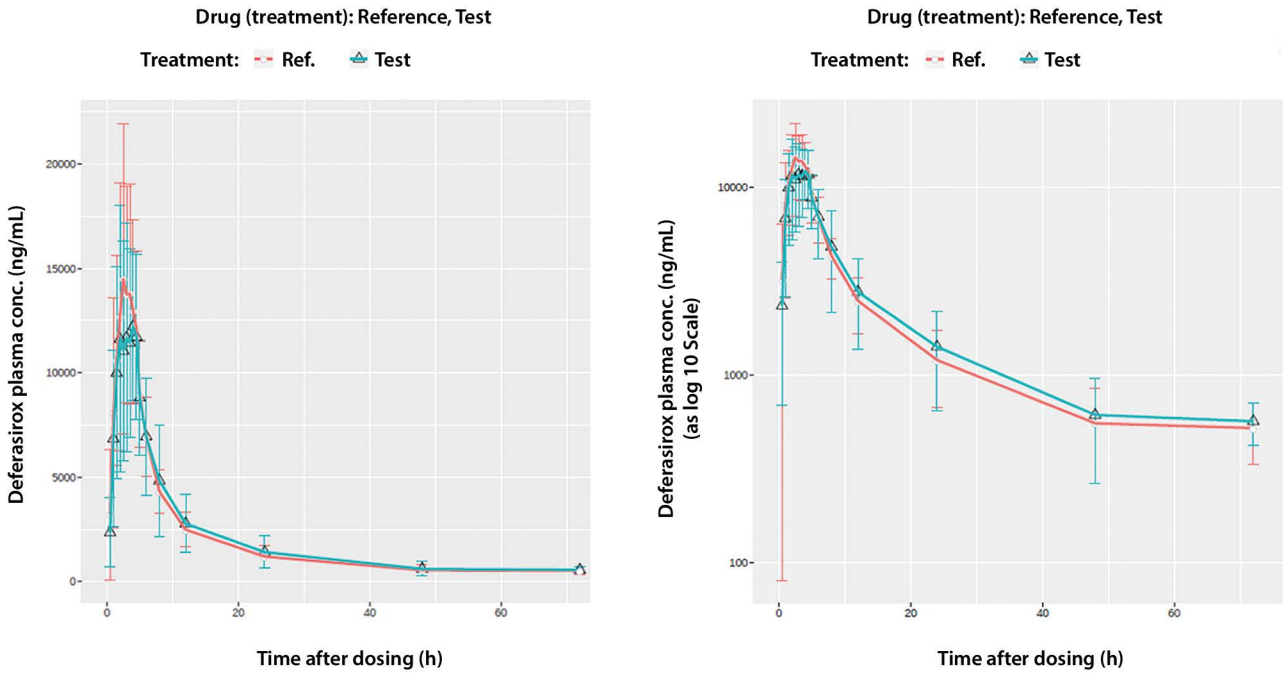


Figure 7. Average pharmacokinetic profiles of deferasirox (in linear and log-linear scales, with standard deviations). Test drug (Test), Reference drug (Ref)

and bioequivalence study of the domestically produced drug, the active ingredient of which is deferasirox, compared to Jadenu®.

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