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Research Article

The evaluation of pharmacokinetic parameters of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide and its metabolites in rat plasma.

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Abstract

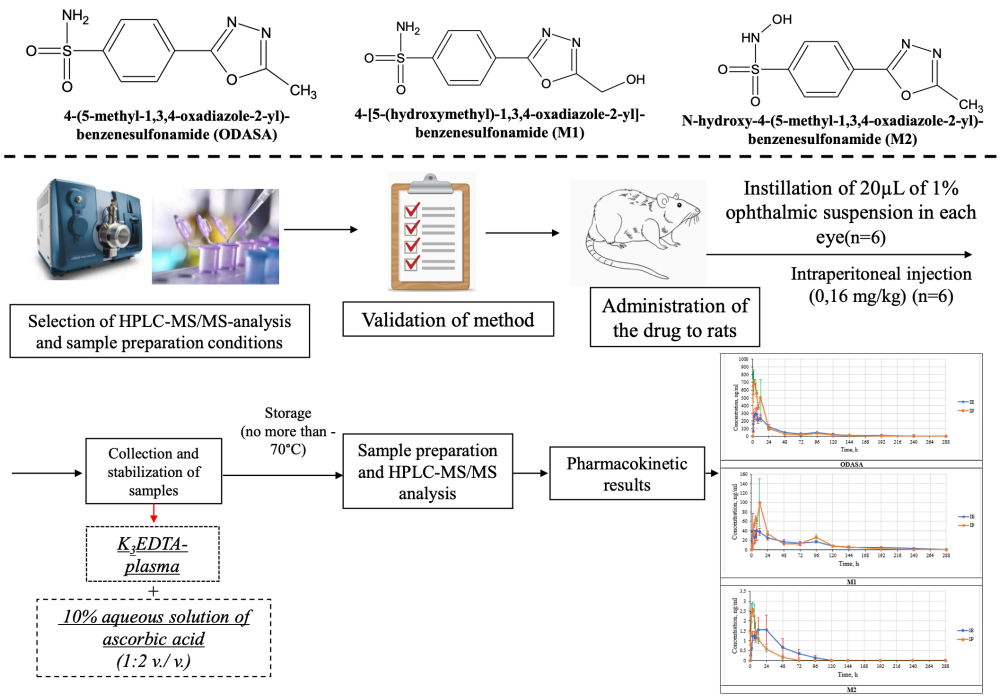
Introduction: 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide (ODASA) is a new pharmacologically active compound, which is capable of reducing intraocular pressure by inhibiting carbonic anhydrase II. It is necessary to calculate the pharmacokinetic parameters of this compound and its metabolites in blood plasma of laboratory animals during a preclinical study. **Aim:** Evaluation of pharmacokinetic parameters of ODASA and its metabolites in rat blood plasma after instillation of ocular suspension and its intraperitoneal administration.

Materials and Methods: The study was conducted on 2 groups of Wistar rats, each including 6 individuals. The first group underwent instillation of 1% ocular suspension of ODASA of 20 µL into each eye (1.6 mg/kg). The drug was injected intraperitoneally at the same dose to the second group. Blood sampling was performed before the administration, 0.5h, 1h, 2h, 4h, 6h, 8h, 12h, 24h, 48h, 72 h, 96h, 120h, 144h, 192h, 240h, 288h after the administration. Plasma was immediately stabilized by 10% solution of ascorbic acid and frozen to a temperature no higher than -70°C. The samples were analyzed using the HPLC-MS/MS method. Chromatographic separation was performed on a Kinetex Phenyl Hexyl column (50*4.6 mm, 2.6 microns) in a gradient mode. Detection was carried out in the MRM mode using electrospray ionization.

Results: The developed method was validated in the range of 2-2000 ng/mL for ODASA and 4-[5-(hydroxymethyl)-1,3,4-oxadiazole-2-yl]-benzenesulfonamide (M1) and 0.5 -500.0 ng/mL for N-hydroxy-4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide (M2). The size of the maximum plasma concentration after instillation of ODASA into eyes was 349.85±62.50 ng/mL, in M1 – 30.91±6.00 ng/mL and in M2 – 2.70±0.62 ng/mL (M±SEM). The half-life time of ODASA after ocular administration was 46.4±3.8 h, M1 – 70.0±14.3 h, and M2 - 36.5±15.2 h (M±SEM). The relative bioavailability of ODASA compared with injection was 81.03%.

Conclusion: The performed validation of the method guaranteed the accuracy of the obtained results. The active substance has a high relative bioavailability after instillation into eyes. M1 is a major metabolite, and M2 is a minor metabolite. The studied compounds had a long half-life.

Graphical abstract



Keywords

HPLC-MS/MS, plasma, stabilization, validation, pharmacokinetics, bioavailability, rats, carbonic anhydrase II inhibitor, N-hydroxysulfonamide

Introduction

The pharmacokinetic study is mandatory for every drug, including ocular dosage forms. Evaluation of bioavailability for local action compounds is necessary to predict possible systemic therapeutic effects and side effects. 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide (ODASA) (Fig. 1A) is a new compound which selectively inhibits carbonic anhydrase II and decreases intraocular pressure, which is administered in form of 1% ocular suspension. ODASA is metabolized by hydroxylation of methyl group of oxadiazole cycle with formation of 4-[5-(hydroxymethyl)-1,3,4-oxadiazole-2-yl]-benzenesulfonamide (M1) (Fig. 1B) and by hydroxylation of a sulfonamide group with formation of N-hydroxy-4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide (M2) (Fig. 1C) (Khokhlov et al. 2024). Study of pharmacokinetic parameters and bioavailability of this drug in plasma or whole blood has not been carried out yet.

It is known that carbonic anhydrase II inhibitors, which similar in structure, are able to accumulate in red blood cells and have a half-life time of more than 2 days (Begou et al. 2020; Lo Faro et al. 2021; Dhandar et al. 2022). Therefore, long period of sampling after administration of the drug is necessary for correct evaluation of the pharmacokinetic parameters of ODASA and its metabolites in the plasma of laboratory animals. The substance of ODASA is not soluble in water. Therefore, relative bioavailability has been studied in comparison with intraperitoneal administration due to impossibility of intravenous injection.

The bioanalytical method for ODASA metabolite profiling is too long for routine sample analysis (Khohklov et al. 2024). Therefore, more rapid chromatographic program will be developed. It is also necessary to optimize the ratio of plasma and stabilizer solution and to choose an anticoagulant for accurate determination of M2. Previously, aqueous solutions of ascorbic acid were applied to prevent the decomposition of N-hydroxysulfonamides, and K_3EDTA and mixture of sodium fluoride and potassium oxalate were used as anticoagulants (Khohklov et al. 2023; Yaichkov et al. 2024). Assessment of the pharmacokinetic parameters of the drug in preclinical study is required on at least two animal species, one of which does not belong to rodents (Mironov 2012). Rabbits are commonly used for this aim (Malygin et al. 2020; Xiong et al. 2020; Wilson et al. 2023). Therefore, a new bioanalytical method will be developed for analysis of plasma of both animal species.

The aim of study is evaluation of pharmacokinetic parameters of ODASA and its metabolites in rat blood plasma after instillation of ocular suspension and its intraperitoneal administration.

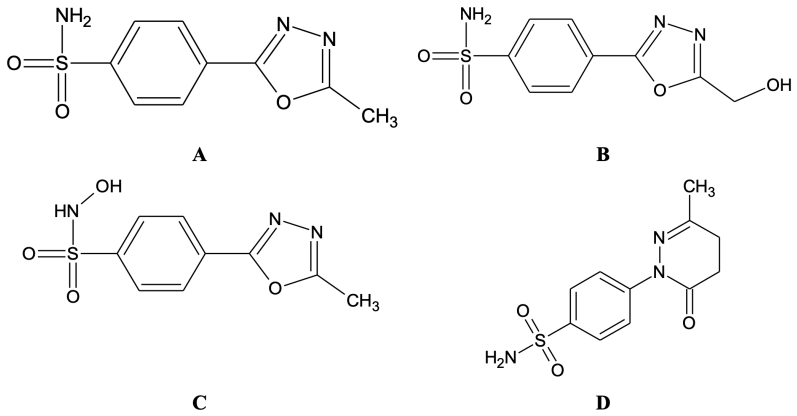


Figure 1. Structure of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide, its main metabolites and internal standard. Note: **A** – 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide; **B** – 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]-benzenesulfonamide; **C** – N-hydroxy-4-(5-methyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide; **D** – 4-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)-benzenesulfonamide.

Materials and Methods

Equipment and reagents

The study was carried out using a HPLC-MS/MS system, which includes tandem mass spectrometric detector AB Sciex QTRAP5500 (AB Sciex, Singapore) and an Agilent 1260 Infinity chromatograph (Agilent Technologies, Germany) with a binary pump G1312B, autosampler G1329B with an external thermostat G1330B, a column thermostat G1316A. The software Analyst 1.6.2 was used to control the device, and MultiQuant 3.0.5 (AB Sciex) was used to integrate the obtained chromatograms.

Standards substances of analytes and internal standard were produced at M.V. Dorogov Pharmaceutical Technology Transfer Center of Yaroslavl State Pedagogical University (YSPU) named after K.D. Ushinsky:

- 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide – 99.4%
- 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]-benzenesulfonamide – 98.3%
- N-hydroxy-4-(5-methyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide – 98.1%
- 4-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)-benzenesulfonamide (OHSA) (Fig. 1D) – 97.4%.

Methanol (LiChrosolv hypergrade for LCMS, Merck KGaA) and formic acid (Optima LC-MS-Grade, Thermo Fisher Scientific), which are suitable for HPLC-MS analysis, were used for preparation of a mobile phase. Water purification was performed using a Sartorius Arium Mini (Sartorius AG, Germany). Ascorbic acid (chemically pure, JSC Lenreactive) was applied as a stabilizer of M2. Dimethyl Sulfoxide (chemically pure, Ekos-1 JSC) was used for preparation of stock and working solutions of analytes and OHSA.

Sample preparation and HPLC-MS/MS-analysis

Spiked samples were prepared by mixing of 95 µL of blank plasma, 50 µL of 10% aqueous ascorbic acid solution and 5 µL of combined working solution of analytes. Concentration of calibration samples (K1-K8), quality control samples of low (LQC), middle (MQC) and high (HQC) concentration levels, samples for dilution integrity evaluation (Dil) are shown in Table 1.

Extraction of the studied compounds from biological matrix was performed by addition of 100 µL of OHSA methanol solution to 20 µL of stabilized plasma. The mixture was vortexed and centrifugated (5 min, 10000 rpm, cooling to 4°C). The supernatant was analyzed using HPLC-MS/MS.

Table 1. Concentrations of analytes in model plasma samples

Analyte	*Concentration, ng/mL*							
	Calibration samples							
	K1 (LLOQ)	**K2**	**K3**	**K4**	**K5**	**K6**	**K7**	**K8**
ODASA and M1	2	10	50	200	500	1000	1500	2000
M2	0.5	2.5	12.5	50.0	125.0	250.0	375.0	500.0
	Quality control samples							
	LQC		**MQC**		**HQC**		**Dil**	
ODASA and M1	6		750		1750		3500	
M2	1.5		187.5		437.5		875.0	

Note: ODASA – 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide, M1 – 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]-benzenesulfonamide, M2 – N-hydroxy-4-(5-methyl-1,3,4-oxadiazol-2-yl)-benzenesulfonamide, K1-K8 – calibration samples; LLOQ – lower limit of quantification; LQC, MQC, HQC – quality control samples of the lower, middle and upper levels; Dil – samples for dilution integrity test.

The chromatographic separation was carried out on a Kinetex Phenyl-Hexyl (50*4.6 mm, 2.6 µm) column with Phenyl Security Guard Ultra Cartridge (4.6 mm, 2,6 µm). Gradient elution was performed at a flow rate of 600 µL/min and a temperature of 40°C using 0.1% aqueous formic acid solution and methanol (Table 2).

Table 2. The parameters of gradient elution of the method

Time, min.	A, %	B, %
0.0	70	30
0.5	70	30
1.5	5	95
5.0	5	95
5.1	70	30
9.0	70	30

Mobile phase: 0.1% formic acid aqueous solution - A, methanol – B

Electrospray ionization (ESI) (positive polarity) and MRM-mode were used for mass-spectrometry detection. ESI voltage was 5000V, source temperature was 600°C. Quantitative and qualitative MRM-transitions are represented in able 3. Calibration curves for calculation of concentration were created using of ratio of chromatographic peak area of analyte and internal standard of OHSA.

Validation of bioanalytical method

Validation was performed in accordance with the requirements of Russian guidelines (On Approval of the Rules for Conducting Bioequivalence Studies on Medicines in the Eurasian Economic Union. Decision of the Council of the Eurasian Economic Commission № 85. 2016) and ICH M10 guideline (ICH Guideline M10 on Bioanalytical Method Validation and Study Sample Analysis 2022). The scope of validation tests was optimized (Yaichkov et al. 2024). Selectivity, matrix effects, calibration curve, long-term stability (LTS) were evaluated on plasma of both species. Two batches on rat plasma and two batches on rabbit plasma were analyzed for a study of precision and accuracy. Other tests were performed on rat plasma.

Animals

Pharmacokinetic experiment was performed on 2 groups Wistar rats (SMK Stezar LLC, Russian Federation). Every group included 3 males and 3 females. Weight of rats for ocular instillation was 252.0±1.3 g, for intraperitoneal injection it was 227.3±2.3 g (M±SEM).

The study was approved by the Ethics Committee of YSPU named after K.D. Ushinsky (Minutes №2 of 06 March 2024).

Design of pharmacokinetic part

The administration of ODASA ocular suspension at a concentration of 1% was carried out in each eye at a volume of 20 µL, which corresponded to a dosage of 1.6 mg/kg. This dosage was injected intraperitoneally to the second group of rats. Blood sampling was performed into K_3EDTA-tubes (0.2 mL) using a catheter which was inserted into the right jugular vein. The following time points was chosen for both experiments: 0 h (before administration) 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 48, h, 72 h, 96 h, 120 h, 144 h, 192 h, 240 h, and 288 h after administration. The part of each sample at a volume of 60 µL was separated for a pharmacokinetic study in whole blood. Plasma was immediately obtained using centrifugation (5 min, 3000 rpm, cooled to 4°C), stabilized by addition of 10% ascorbic acid solution at a volume ratio of 1:2 and frozen before analysis. The storage temperature was no higher than –70°C.

Statistical analysis

The software R package (v. 3.3.2) with module Bear (v. 2.7.7) was applied for non-compartment pharmacokinetic analysis. The following pharmacokinetic parameters were evaluated:

- maximum concentration in plasma – C_{max};
- time-to-peak concentration in plasma and blood – T_{max};
- area under the pharmacokinetic ”concentration – time” curve from zero to the last blood sampling point – AUC_{0-t};
- area under the pharmacokinetic curve from time zero to infinity – $AUC_{0-\infty}$;
- half-life time – $T_{1/2}$;
- average drug retention time in plasma (mean resident time) – MRT.

The software Statsoft Statistica 10.0.1011 was used for calculation of descriptive statistics (mean, standard deviation (SD), coefficient of variation (CV), standard error of the mean (SEM)).

Table 3. The parameters of mass-spectrometry detection of analytes

№	Analyte	SRM-transition		CE, eV
		Q1	Q3	
1	ODASA	240	184	35
2	ODASA (Control)	240	198	30
3	M1	256	184	30
4	M1 (Control)	256	120	40
5	M2	256	160	25
6	M2 (Control)	256	104	50
7	OHSA	268	187	30

№1,3,5 were used for quantification, №2,4,6 - were used for proof of correctness of identification

Results and Discussions

Selection of an anticoagulant and stabilizer for M2 was performed using the results of preliminary tests: short-term stability (STS), freeze/thaw stability (FTS), and autosampler stability (ASS) (Khokhlov et al. 2023; Yaichkov et al. 2024). Aqueous solutions of sodium sulfite, sodium thiosulfate, sodium metabisulfite, ammonium acetate and ammonium formate could not stop decomposition of N-hydroxymetabolite. Addition of 10% ascorbic acid solution to rat and rabbit plasma in a volume ratio of 1:2 (stabilizer:plasma) allowed preventing degradation of M2. Anticoagulants K_3EDTA, heparine lithium and combination of sodium fluoride and potassium oxalate did not affect stability of M2. Therefore, K_3EDTA was selected as the most commercially available.

The validation was performed after the stabilizer selection on plasma with addition of 10% ascorbic acid solution. There were not any chromatographic peaks at retention time area of analyte and internal standard of blank samples of both species on chromatograms (Fig. 2).

Linearity of the method was confirmed in the range of 2.0-2000.0 ng/mL for ODASA and M1, 0.5-500.0 ng/mL for M2. Coefficient of variation of normalized matrix factor (NMF) in experiments on rabbit and rat plasma did not exceed 15% (Table 4). There was no significant difference (>15%) between NMF values of both species.

Successful results of the study of selectivity, linearity and matrix effect allowed optimizing the scope of validation tests (Yaichkov et al. 2024). Intraday and interday accuracy and precision of the method were confirmed. Relative accuracy (δ) of the calculated concentration of all analytes did not exceed ±15% for LQC, MQC and HQC samples and did not exceed ±20% for LLOQ samples (Table 4). Coefficient of variation (CV) was less than 15% for LQC, MQC and HQC samples and less than 20% for LLOQ samples (Table 4). Results of dilution integrity test were acceptable (On Approval of the Rules for Conducting Bioequivalence Studies on Medicines in the Eurasian Economic Union. Decision of the Council of the Eurasian Economic Commission № 85. 2016): twofold dilution of plasma did not affect the accuracy and precision of determination of ODASA, M1 and M2.

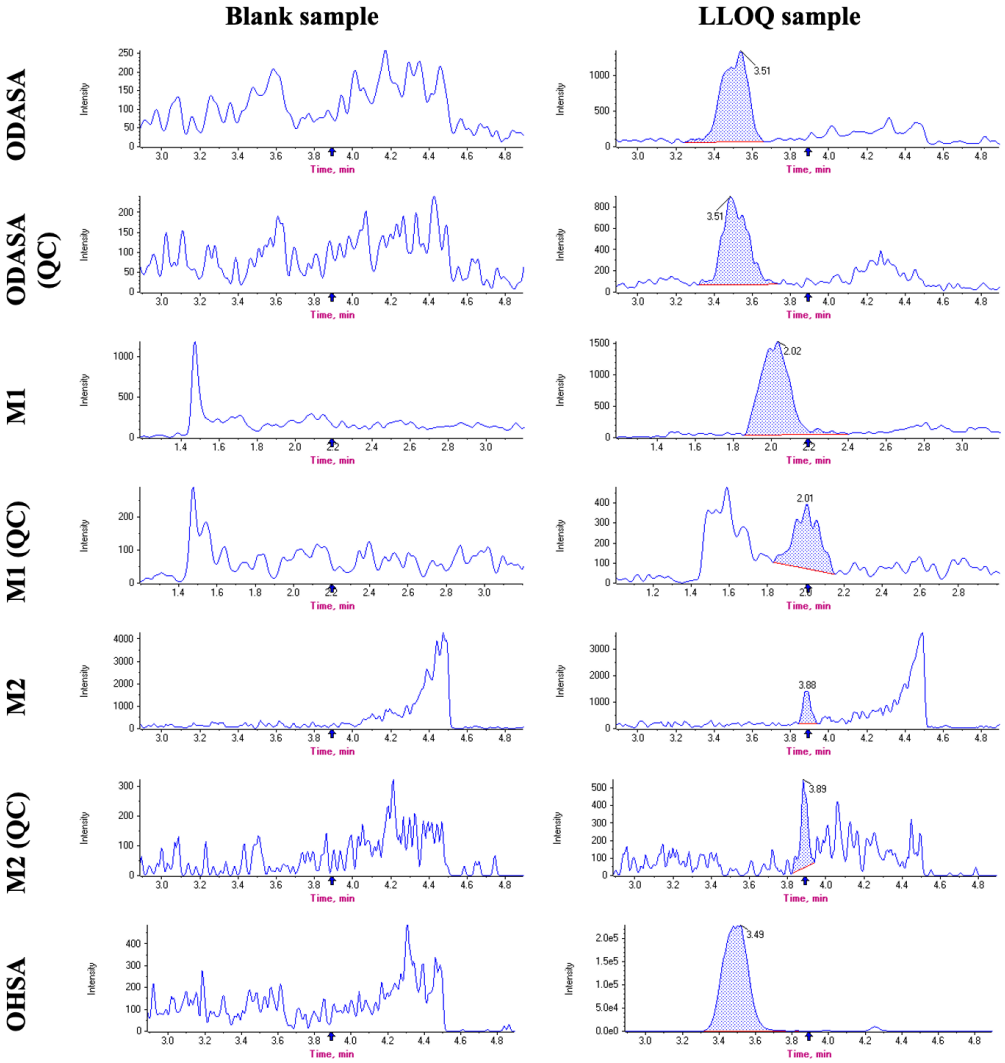


Figure 2. Examples of chromatograms of blank sample of rat plasma and LLOQ plasma sample. ***Note:*** ODASA – 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide, M1 – 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]-benzenesulfonamide, M2 – N-hydroxy-4-(5-methyl-1,3,4-oxadiazol-2-yl)-benzenesulfonamide, LLOQ – lower limit of quantification.

Table 4. The results of validation of the bioanalytical method

Test			ODASA		M1		M2	
Calibration curve		Range	2-2000		2-2000		0.5-500.0	
		Function	linear		linear		linear	
		Weighting factor	1/x		1/x		1/x	
Intraday accuracy and precision		Concentration	δ, %	CV, %	δ, %	CV, %	δ, %	CV, %
Batch 1 (n=6)*		LLOQ	-0.52	8.98	6.49	14.85	7.47	18.20
		LQC	-4.66	3.26	-6.54	11.77	-7.03	12.37
		MQC	2.20	2.96	-10.09	7.28	8.90	4.54
		HQC	-3.78	5.69	-10.96	7.16	5.85	7.03
Batch 2 (n=6)		LLOQ	7.10	12.39	2.44	16.47	-11.10	9.92
		LQC	7.87	5.20	2.83	10.56	7.46	4.68
		MQC	8.31	6.53	0.25	8.11	8.38	7.85
		HQC	-0.41	10.78	-3.25	14.65	-0.56	14.28
Batch 3 (n=6)		LLOQ	4.64	11.78	-4.27	10.65	-2.03	14.14
		LQC	4.89	5.76	-0.69	3.90	-1.03	9.71
		MQC	3.67	6.44	9.94	6.75	5.85	6.52
		HQC	3.06	7.89	6.63	8.12	10.41	7.71
Batch 4 (n=6)		LLOQ	2.40	7.92	1.10	11.80	5.20	9.22
		LQC	4.67	5.29	-3.98	7.45	-3.53	7.93
		MQC	3.96	6.96	10.70	6.71	3.82	8.00
		HQC	2.97	7.27	5.90	7.37	8.60	8.21
Interday accuracy and precision		LLOQ	3.41	10.20	1.44	13.41	-0.12	14.75
		LQC	3.19	6.62	-2.10	9.10	-1.04	9.95
		MQC	4.53	6.00	2.70	10.77	6.74	6.67
		HQC	0.39	7.85	-0.42	11.73	6.07	9.75
Reinjection reproducibility (48 h)		LLOQ	11.38	10.51	4.50	12.18	16.97	19.35
		LQC	4.76	6.93	1.03	9.73	6.28	6.56
		MQC	2.44	5.79	2.38	2.35	-5.13	9.00
		HQC	-1.61	8.19	-2.14	7.73	-3.91	10.47
Dilution integrity		Dil	4.69	7.79	7.33	7.88	7.94	11.00
Matrix effects	Plasma species	Concentration	NMF	CV (NMF), %	NMF	CV (NMF), %	NMF	CV (NMF), %
	Rat	LQC	0.976	9.17	0.897	5.27	0.977	8.25
	(n=6)	HQC	0.956	5.80	0.825	6.98	1.031	9.13
	Rabbit	LQC	0.962	11.15	0.908	10.28	0.970	9.71
	(n=6)	HQC	0.956	10.54	0.843	9.84	0.988	7.41

Note: series 1 and 2 were performed on rat plasma, series 3 and 4 were performed on rabbit plasma; selectivity of the method was studied within series 2 and 4; * – number of samples on each concentration value. ODASA – 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide, M1 – 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]benzenesulfonamide, M2 – N-hydroxy-4-(5-methyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide, LLOQ – lower limit of quantification; LQC, MQC, HQC – quality control samples of the lower, middle and upper levels; Dil – samples for dilution integrity test, NMF – normalized matrix factor.

Validation tests of STS, FTS and ASS were performed on stabilized rat plasma. ODASA and its metabolites were stable for 24 h of storage at room temperature, 3 cycles of freezing and thawing, 48 h of storage of prepared samples in an autosampler. The Analytes were also preserved in whole blood during 24 h. The study of LTS was carried out on the plasma of rats and rabbits. Interspecific differences in the activity of enzyme systems did not affect the stability of ODASA, M1 and M2. The reproducibility was also preserved after reinjection of analytical batch after 48 h. All these details can be found in Table 5.

The maximum concentration of ODASA, as well as its metabolites after intraperitoneal administration (IP) occurs earlier than after instillation into the eyes (IE). The C_{max} value of ODASA after IP is almost 3 times higher than after IE, which is associated with a faster penetration of the active substance into the systemic circulation. The half-life of the studied compounds after injection is also faster (Table 6). The value of T ½ of ODASA is 46.4 ± 3.8 h, of M1 – 70.0 ± 14.3 h, and of M2 – 36.5 ± 15.2 h (M ± SEM) in case of IE. Half-life time comparable in duration is characteristic of structurally similar 5-[5-(trifluoromethyl)-1,2-oxazole-3-yl]-furan-2-sulfonamide (Yaichkov et al 2024). This is indicated to the accumulation of these compounds in red blood cells. The relative bioavailability of the active substance after instillation into eyes is high. It was 81.03%. A second peak of a concentration increase was observed after 96 hours of the experiment on the pharmacokinetic profiles of ODASA, as well as M1 (Fig. 3). It was caused by the enterohepatic recirculation of these compounds.

Table 5. The results of study of stability of analytes in plasma samples

Test	Temperature mode	% of deviation from nominal value					
		ODASA		M1		M2	
		LQC (n=6)	HQC (n=6)	LQC (n=6)	HQC (n=6)	LQC (n=6)	HQC (n=6)
Short-term stability	room temperature (24 h)	12.36	9.15	-1.66	10.12	5.10	10.01
Freeze-thaw stability	no higher than -70˚C (12 h) / room temperature (4 h)	0.44	-0.85	-5.21	4.98	6.64	1.17
Autosampler stability	no higher than +4˚C (48 h)	-3.21	0.39	-0.78	2.39	0.52	3.85
Long-term stability (rat plasma)	no higher than -70˚C (29 days)	11.09	-5.87	1.88	-4.68	5.29	-7.07
Long-term stability (rabbit plasma)	no higher than -70˚C (29 days)	1.87	-1.72	-0.72	4.71	-0.78	4.43
Stability in whole blood	room temperature (24 h)	2.00	4.05	1.21	3.23	-12.90	12.43

Table 6. Pharmacokinetic parameters of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide and its metabolites after eyes instillation and intraperitoneal injection

Compound	Route of administ-ration	Parameter	C_{max}, ng /ml	T_{max}, h	AUC_{0-t}, ng*h/ml	$AUC_{0-\infty}$, ng*h/ml	$T_{1/2}$, h	MRT, h
ODASA (n=6)	Instillation into eyes	M±SEM	349.85±62.50	7.0±1.7	10584±1555	11110±1627	46.4±3.8	51.7±4.0
		RSD	43.76	59.25	35.98	35.88	20.18	19.12
	Intraperitoneal administration	M±SEM	1041.91±149.49	4.2±1.8	13061±1673	13480±1626	38.2±8.3	29.2±2.8
		RSD	35.14	104.52	31.38	29.55	53.02	23.34
M1 (n=6)	Instillation into eyes	M±SEM	30.91±6.00	13.3±2.2	2008±250	2315±311	70.0±14.3	77.7±5.6
		RSD	47.59	40.99	30.46	32.88	49.93	17.54
	Intraperitoneal administration	M±SEM	67.54±6.78	5.3±0.7	2890±247	3385±355	44.0±6.8	54.6±2.7
		RSD	24.58	30.62	20.96	25.73	37.74	12.27
M2 (n=6)	Instillation into eyes	M±SEM	2.70±0.62	9.3±3.2	68±33	104±35	36.5±15.2	18.6±5.7
		RSD	55.78	83.18	119.33	81.85	102.21	75.26
	Intraperitoneal administration	M±SEM	3.46±0.38	3.3±0.7	36±8	49±9	16.6±3.5	11.1±2.3
		RSD	27.14	48.99	54.30	44.20	51.34	50.72

M1 is the main metabolite of ODASA. The time to peak concentration of this analyte comes later than for the active substance (Table 6). The half-life time of M1 is also longer than such of ODASA. M2 is a minor metabolite. Lower plasma exposure of this compound was observed compared with those of ODASA and M1 (Fig. 3). Thus, the AUC_{0-t} value of M2 after IE was 68 ±33 ng*h/ml, and after IP – 36 ±8 ng*h/ml (Table 6) (M±SEM). A lower AUC_{0-t} value after the injection is caused by the absence of M2 in plasma at points 72 and 96 h and other later time points. This is associated with longer absorption and excretion of ODASA after IE. It provides maintaining a higher concentration level of M2 during the period of 12-96 hours of the experiment.

Thus, the active substance has a high plasma concentration after instillation into eyes of rats, like the compounds similar to it in structure – 4-(2-Methyl-1,3-oxazol-5-yl) benzenesulfonamide (Khokhlov et al. 2023) and 5-[5-(trifluoromethyl)-1,2-oxazole-3-yl]-furan-2-sulfonamide (Yaichkov et al. 2024). Therefore, it is relevant to conduct a pharmacokinetic study of ODASA on rabbits to identify interspecific differences in the degree of absorption and intensity of metabolism. The long half-life time of ODASA and its metabolites indicates their accumulation in erythrocytes, as well as other representatives of carbonic anhydrase II inhibitors (Lo Faro et al. 2021; Dhanda et al. 2022). Therefore, it is necessary to evaluate the pharmacokinetic parameters of these compounds in whole blood. Also, full study of excretion, distribution and accumulation of ODASA after IE will be carried out.

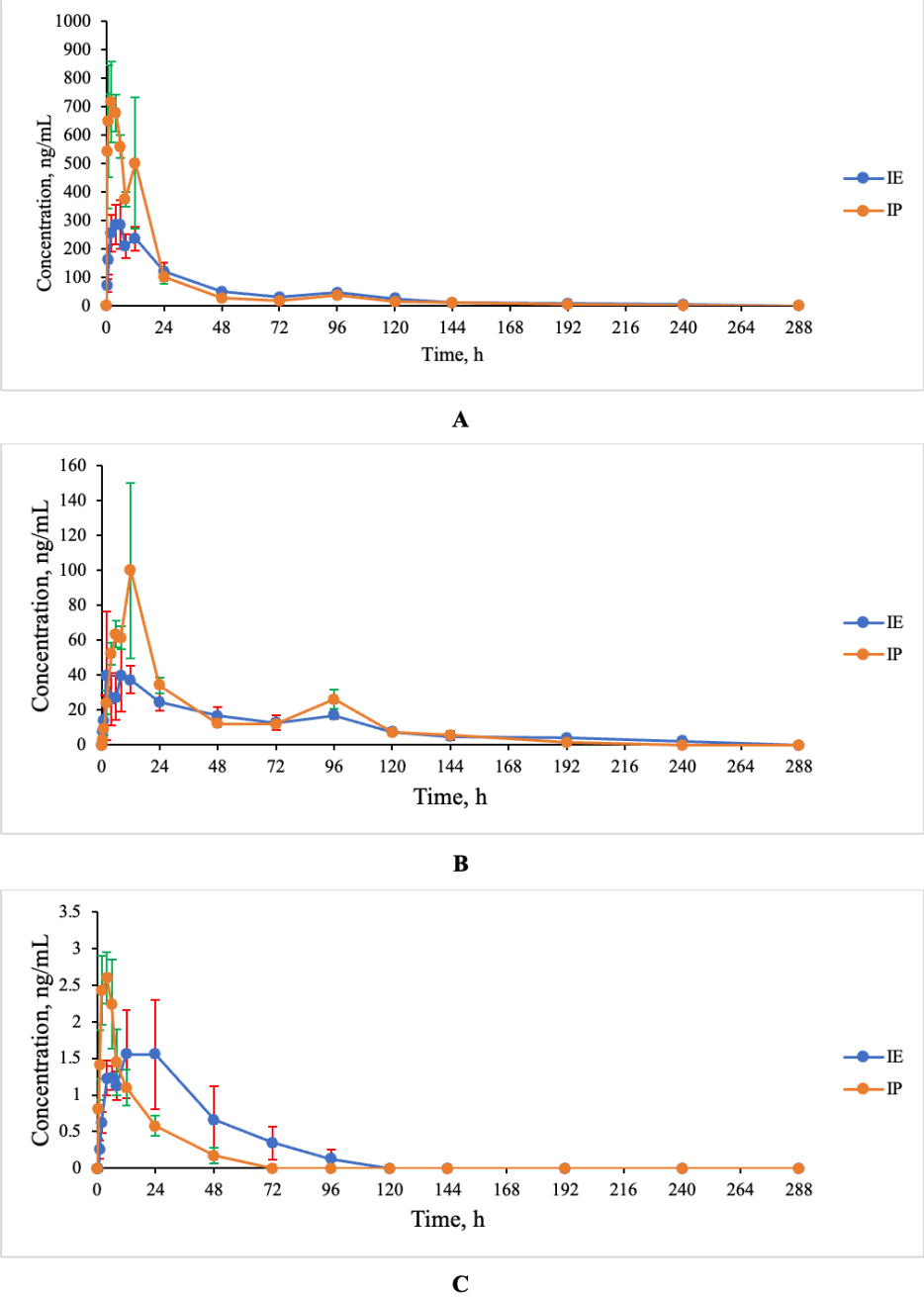


Figure 3. Pharmacokinetic profiles of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide (A), 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]benzenesulfonamide (B), N-hydroxy-4-(5-methyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide (C) in rat plasma. ***Note:*** IE – instillation into eyes; IP – intraperitoneal injection.

Conclusion

The new fast and sensitive method of determination of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide and its metabolites in animal plasma has been developed, validated and applied for the pharmacokinetic study. The drug after its instillation into eyes has high plasma concentrations and relative bioavailability more than 81%. The 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]-benzenesulfonamide is the main metabolite of the studied compound. The content of N-hydroxy-4-(5-methyl-1,3,4-oxadiazol-2-yl)-benzenesulfonamide in rat plasma is significantly lower than the active substance and its C-hydroxyderivate. The 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide and its hydroxylation products have long half-life time due to possible erythrocyte accumulation. Enterohepatic recirculation is observed in the drug and 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]-benzenesulfonamide.

Conflict of interest

The authors declare the absence of a conflict of interests.

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Data availability

All of the data that support the findings of this study are available in the main text.

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