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Research Article

The study of systemic exposition of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide in blood on rats

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Abstract

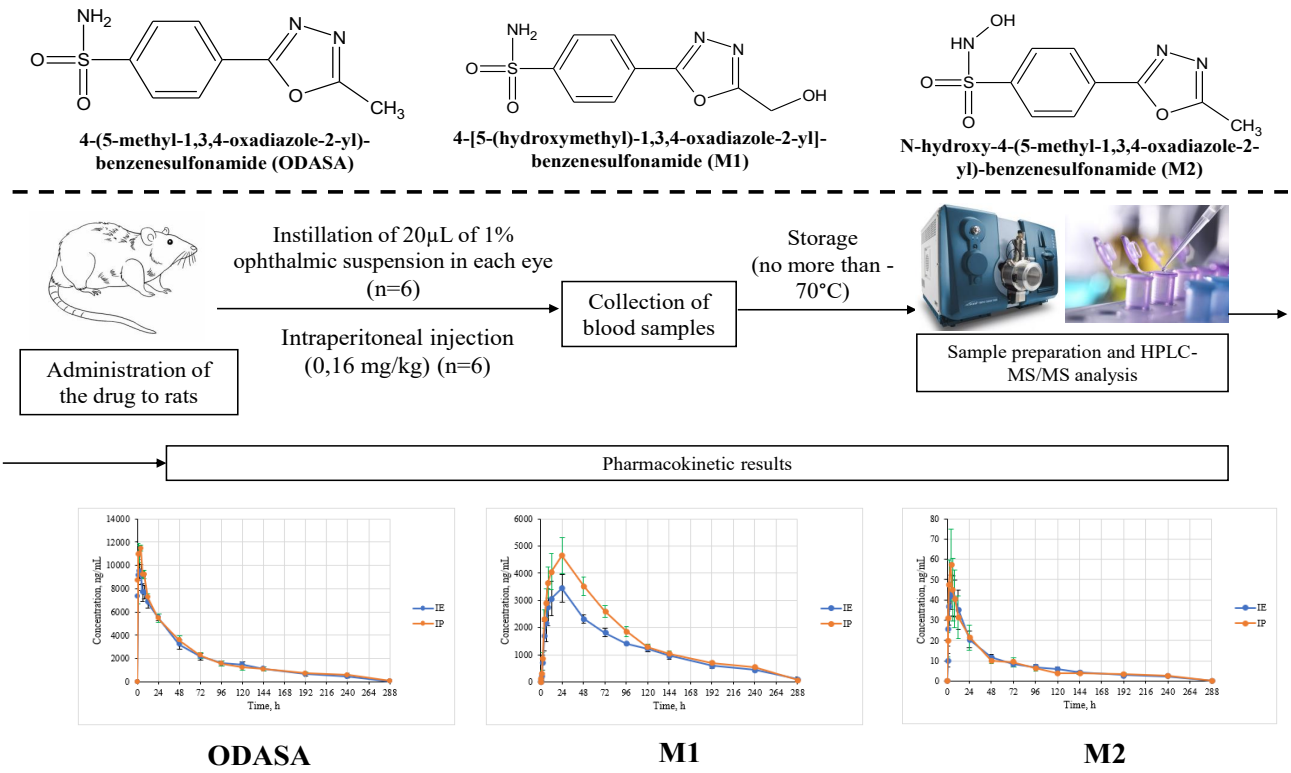
Introduction: The 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide (ODASA) is new antiglaucoma drug. The compounds similar in structure are able to accumulate in red blood cells. Therefore, it is necessary to evaluate the pharmacokinetic parameters of ODASA and its metabolite in whole blood of laboratory animals. **Aim:** Calculation of pharmacokinetic parameters of ODASA and its metabolites in rat whole blood after ocular instillation and intraperitoneal injection of the drug.

Materials and Methods: The 1% ocular suspension of ODASA was instilled into each eye in a volume of 20 µL to a group of 6 Wistar rats. The drug was also administrated by intraperitoneal injection at a dose of 1.6 mg/kg to another group of 6 Wistar rats. Sampling was carried out before administration and at 16 points for 288 hours after administration. HPLC-MS/MS was used for quantification of ODASA and its metabolites 4-[5-(hydroxymethyl)-1,3,4-oxadiazole-2-yl]-benzenesulfonamide (M1) and N-hydroxy-4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide (M2).

Results: The high concentration level of ODASA and M1 was observed: maximum blood concentration (Cmax) of ODASA after eye instillation was 10326±532 ng/mL, Cmax of M1 was 3722±505 ng/mL(M±SEM). The Cmax value of M2 was lower – 49.9±9.4 ng/mL (M±SEM). The half-life time of the studied compounds was more than 69 h. M1 has the greatest affinity for red blood cells. The value of the area under the "concentration-time" curve (AUC0-t) of M1 in blood was more than 150 times higher than AUC0-t of M1 in plasma.

Conclusion: The ODASA and its metabolites have long half-life time and high exposition in blood in comparison with that in plasma, which proves their accumulation in red blood cells.

Graphical abstract



Keywords

HPLC-MS/MS, whole blood, validation, pharmacokinetics, bioavailability, rats, carbonic anhydrase II inhibitor, N-hydroxysulfonamide

Introduction

The selective inhibitors of carbonic anhydrase II (ICA II) are widely used for treatment of open-angle glaucoma (Kurysheva 2020). The 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide (Fig. 1A) (ODASA) is a new active compound of this group, being now at the stage of preclinical study. It has a local effect after eye instillation with 1% ocular suspension (Khokhlov et al 2023). ODASA is able to be absorbed by this route of administration. The 4-[5-(hydroxymethyl)-1,3,4-oxadiazole-2-yl]-benzenesulfonamide (M1) (Fig. 1B) and N-hydroxy-4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide (M2) are formed during biotransformation process of ODASA. The drug and its metabolites have long half-life time in plasma after administration to rats (Khokhlov et al 2024).

Widely-used acetazolamide (Begou et al 2020; Busardo et al 2022), dorzolamide (Lo Faro et al 2021) and brinzolamide (Naageshwaran 2021; Dhandar et al 2024) and new ICA II molecules 4-(2-methyl-1,3-oxazole-5-yl)-benzenesulfonamide and 5-[5-(trifluoromethyl)-1,2-oxazole-3-yl]-furan-2-sulfonamide (Khokhlov et al 2024; Yaichkov et al 2024) accumulate in erythrocytes. Therefore, systemic exposition of a sulfonamide derivate ODASA, which is similar to the above in structure, to blood requires a complete pharmacokinetic study.

The bioanalytical method used for plasma research (Khokhlov et 2024) can be adapted for whole blood. It is necessary to choose optimal conditions to prevent decomposition of N-hydroxymetabolite M2. Previously, N-hydroxy-4-(2-methyl-1,3-oxazole-5-yl)-benzenesulfonamide and N-hydroxy-5-[5-(trifluoromethyl)-1,2-oxazole-3-yl]-furan-2-sulfonamide (Yaichkov et al 2024) were stabilized by

acidification of the precipitate by aqueous formic acid solutions. Validation is also needed for rabbit samples because systemic exposition of the new drug should be investigated at least on two animal species (Mironov 2012).

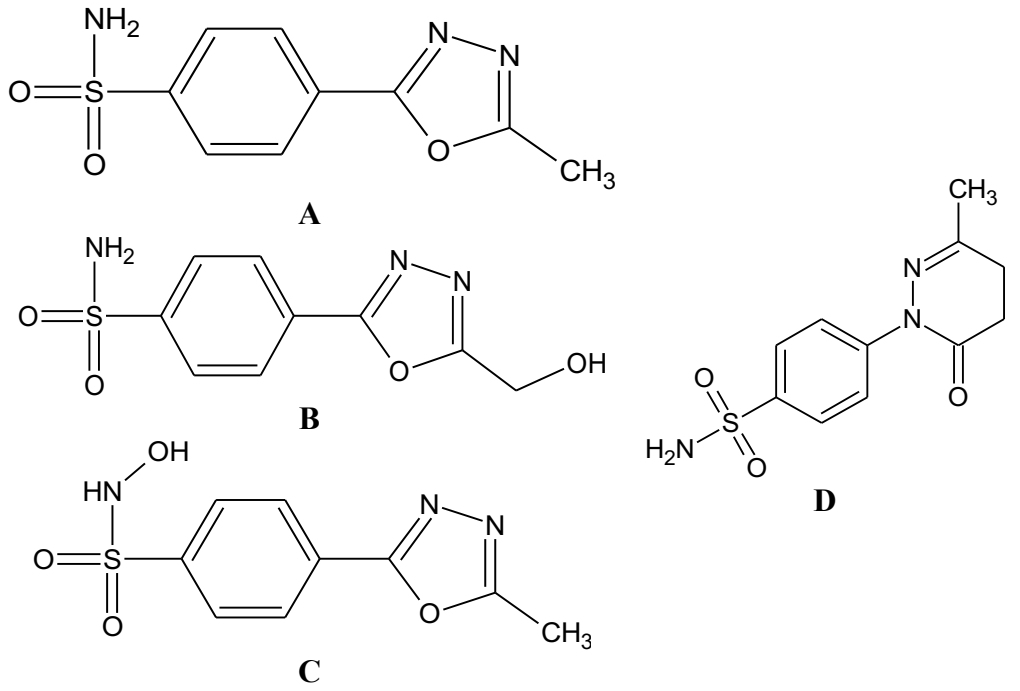


Figure 1. Structure of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide, its main metabolites and internal standard 4-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)-benzenesulfonamide. ***Note:*** **A** – 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide; **B** – 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]-benzenesulfonamide;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

The aim of the study was calculation of pharmacokinetic parameters of ODASA and its metabolites in rat whole blood after ocular instillation and intraperitoneal injection of 1% ocular suspension.

Materials and Methods

Studied compounds

The active compound 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide (99.4%) and its ophthalmic suspension were manufactured at M.V. Dorogov Pharmaceutical Technology Transfer Center (PTTC) of Yaroslavl State Pedagogical University (YSPU) named after K.D. Ushinsky. The substances of 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%) were also synthesized at that laboratory.

Composition of the studied dosage form: ODASA – 50 mg, carbopol 974 – 20 mg, Tween-80 - 2.5 mg, mannitol –165 mg, 10% sodium hydroxide solution with pH 7.5-8.5, and 0.9% sodium chloride solution – up to 5 mL.

Analytical equipment

HPLC-MS/MS-method was used for quantification of ODASA and its metabolites in whole blood. The instrument included Agilent 1260 Infinity chromatograph (Agilent Technologies, Germany) and mass spectrometric detector AB Sciex QTRAP5500 (AB Sciex, Singapore) (software Analyst 1.6.2). Chromatogram integration and calculation of analyte concentrations were performed on program package MultiQuant 3.0.5 (AB Sciex, USA).

Sample preparation and HPLC-MS/MS-analysis

Individual stock solutions of ODASA, ODASA-M1, ODASA-M2, OHSA (Fig. 1D) were prepared in dimethylsulfoxide (chemically pure, Ekos-1 JSC).

Combined analytes working solutions in methanol were added to blank whole blood at a volume ratio of 1:19 for obtaining calibration (K1-K8) samples, quality control samples (LLOQ, LQC, MQC, HQC), and samples for dilution integrity (Dil) test (Table 1).

Table 1. Concentrations of studied compounds in blood spiked samples

Sample	Concentration, ng/ml		
	ODASA	**M1**	**M2**
K1 (LLOQ)	20	10	1
K2	100	50	5
K3	500	250	25
K4	2000	1000	100
K5	5000	2500	250
K6	10000	5000	500
K7	15000	7500	750
K8	20000	10000	1000
LQC	60	30	3
MQC	7500	3750	375
HQC	17500	8750	875
Dil	35000	17500	1750

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.

An aliquot of 200 µL of OHSA methanol solution was added to 20 µL of a blood sample. Then mixture was vortexed and an aliquot of 20 µL of 1% aqueous formic acid solution was added to it. Centrifugation was performed at 10000 rpm, with further cooling to 4°C after repeated stirring. The supernatant at volume of 5 µL was injected to HPLC-MS/MS-system.

The chromatographic separation was performed in gradient conditions (Table 2), which is the same for plasma method (Khokhlov et al 2024). Kinetex Phenyl-Hexyl (50*4.6 mm, 2.6 µm) column with Phenyl SecurityGuard Ultra Catridge (4.6 mm, 2,6 µm) was used. Mobile phase consisted of 0.1% aqueous formic acid (Optima LC-MS-Grade, Thermo Fisher Scientific) solution and methanol (LiChrosolv hypergrade for LCMS, Merck KGaA). The flow rate was 0.6 mL/min, column thermostat temperature was 40°C.

Table 2. The gradient elution program

Time, min.	**A, %**	**B,%**
0.0-0.5	70	30
0.5-1.5	70→5	30→95
1.5-5.0	5	95
5.0-5.1	5→70	95→30
5.1-9.0	70	30
Mobile phase: 0,1% formic acid aqueous solution - A, methanol – B		

Mass-spectrometry detection was performed on MRM-mode (Table 3). Electrospray was used for ionization of eluate in positive polarity. Internal standard method was applied for concentration calculations.

Table 3. MRM-transitions for determination of studied compounds

№	**Analyte**	**SRM- transition**	**CE, eV**
1	ODASA	240→184 m/z	45
2	ODASA (Control)	240→198 m/z	38
3	M1	256→184 m/z	40
4	M1 (Control)	256→120 m/z	40
5	M2	256→160 m/z	25
6	M2 (Control)	256→104 m/z	50
7	OHSA (Internal standard)	268→187 m/z	30
Nos 1,3,5 were used for quantification, Nos 2,4,6 were used for proof of correctness of identification			

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; OHSA – 4-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)-benzenesulfonamide.

Validation of bioanalytical method

Validation of bioanalytical method was performed using the guidelines of The Eurasian Economic Union "On Approval of the Rules for Conducting Bioequivalence Studies on Medicines in the Eurasian Economic Union" (2016). Volume of validation tests for blood of rabbit was reduced on the basis of the results of evaluation of selectivity, linearity, and matrix effect. Reinjection reproducibility was studied in accordance with the ICH M10 guideline (ICH guideline M10 on bioanalytical method validation and study sample analysis. 2022).

Animals

The research was carried out simultaneously with plasma study (Khokhlov et al 2024) on Wistar rats (SMK Stezar LLC pound, Russian Federation). There were two group consisting of 6 individuals each (3 males and 3 females): 252.0±1.3 g – for ODASA ocular instillation; 227.3±2.3 g (M±SEM) – for ODASA intraperitoneal injection.

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

Design of pharmacokinetic experiment

The instillation of ocular suspension of ODASA at a concentration of 1% was performed into each eye at a volume of 20 µL to the first group animals. The intraperitoneal injection was carried out to the second group animals at an equal dosage of 1.6 mg/kg. The animals were previously catheterized into the right jugular vein. Blood samples during both experiments were collected into K_3EDTA-tubes (0.2 mL) at 17 time points: before the administration and 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, 288 h after the administration. An aliquot of 60 µL of each sample was separated and frozen at temperature of no more than -70°C. Rats were given access to drink and feed 2 hours after beginning of the experiment, which made it possible to replenish the blood loss.

Statistical analysis

The pharmacokinetic estimations were performed on software R package (v. 3.3.2) with module Bear (v. 2.7.7) using a non-compartment approach. Maximum blood concentration (C_{max}) and time for reaching it (T_{max}), area under the pharmacokinetic curve "concentration – time" from zero to the last blood sampling point (AUC_{0-t}) and to infinity ($AUC_{0-\infty}$), half-life time ($T_{1/2}$), and mean resident time (MRT) were calculated for ODASA and its metabolites.

The ratio between blood and plasma content (F_{blood}) of the studied compounds was evaluated for each animal using formula (1):

$$F_{blood} = \frac{\mathrm{AUC}_{0-t}\,(Blood)}{\mathrm{AUC}_{0-t}\,(Plasma)} \quad (1),$$

where AUC_{0-t} (Blood) – area under the pharmacokinetic curve "concentration – time" from zero to the last blood sampling point of the compound in blood; AUC_{0-t} (Plasma) – area under the pharmacokinetic curve "concentration – time" from zero to the last blood sampling point of the compound in plasma, which were calculated in plasma pharmacokinetic study (Khokhlov et al 2024).

Results and Discussion

The results of the preliminary tests of short-term stability (STS), freeze/thaw stability (FTS), and autosampler stability (ASS), which were performed on blood of rat and rabbit with K_3EDTA, heparine lithium and combination of sodium fluoride and potassium oxalate as anticoagulants, showed no significant decomposition of N-hydroxysulfonamide M2. Therefore, the most inexpensive K_3EDTA-tubes without any additional ingredients were chosen for the study. Simple precipitation of proteins by methanol, which was used for plasma research (Khokhlov et al 2024), did not prevent the decomposition of M2 in the prepared samples. Therefore, acidification of the mixture by an aqueous solution of formic acid was performed, as well as for stabilization of N-hydroxy- 4-(2-methyl-1,3-oxazole-5-yl)-benzenesulfonamide (Khokhlov et al 2023) and N-hydroxy-5-[5-(trifluoromethyl)-1,2-oxazole-3-yl]-furan-2-sulfonamide (Yaichkov et al 2024), which are similar in structure.

The selectivity was confirmed for blood of rat and rabbit: analytical signals in retention time area of ODASA, its metabolites, and OHSA was not observed on chromatograms of blank samples. Calibration dependences of the studied compounds were linear. Slopes and intercepts of calibration curves obtained on spiked samples of both species were close values (Table 4). The analytical range was 20-20000 ng/mL for ODASA, 10-10000 ng/mL – for M1, and 1-1000 ng/mL –for M2.

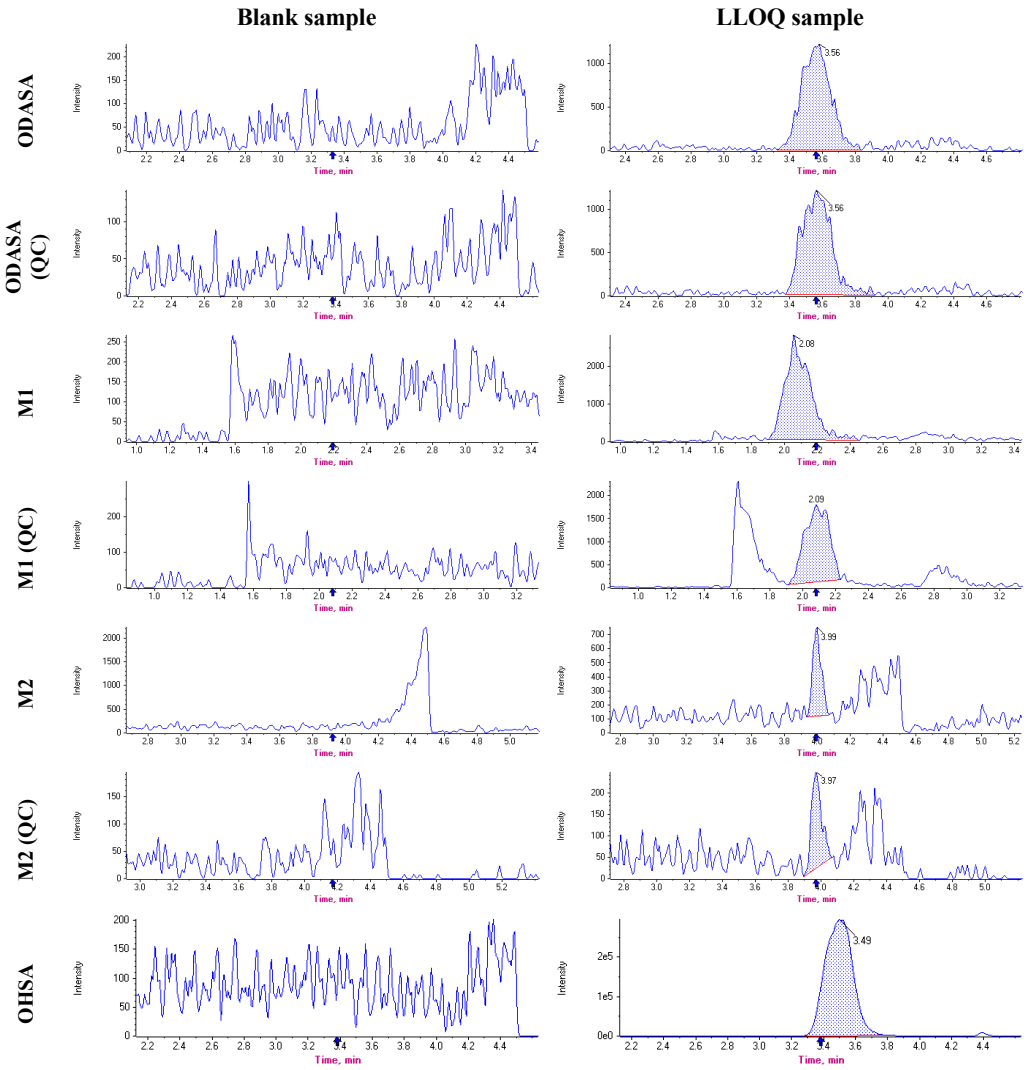


Figure 2. Examples of chromatograms of blank sample of rat blood and LLOQ blood sample. ***Note:*** ODASA – 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide; M1 – 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]-benzenesulfonamide; M2 – N-hydroxy-4-(5-methyl-1,3,4-oxadiazol-2-yl)-benzenesulfonamide; OHSA – 4-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)-benzenesulfonamide; LLOQ – lower limit of quantification.

Table 4. The evaluation of linearity of the method on whole blood of rat and rabbit

Parameter		ODASA	M1	M2
Calibration dependence		Linear	Linear	Linear
Weighting factor		1/x	1/x	1/x
Analytical range, ng/ml		20–20000	10–10000	1-1000
Slopes of calibration curves (min.-max.)	Rat (n=6)	0.000201-0.000230	0.000570-0.000726	0.001100- 0.001370
	Rabbit (n=3)	0.000203-000220	0.000582-0.000650	0.001160-0.001400
Intercepts of calibration curves (min.-max.)	Rat (n=6)	-0.00052 - -0.00123	-0.000217 - -0.000314	0.000523-0.000993
	Rabbit (n=3)	-0.00067- -0.00110	-0.000195 - -0.000252	0.000644-0.000811

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.

Matrix effects were also evaluated on quality control samples of rabbit and rats (Table 5). The coefficient of variation (CV) of the normalized matrix factor (NMF) was less than 15% for each analyte, which met the established requirements. The discrepancy of NMF values between rat and rabbit samples for ODASA, M1 and M2 did not exceed 15% (Table 5).

Table 5. The evaluation of matrix effects of the method on whole blood of rat and rabbit

Analyte	Concentration	Rat blood		Rabbit blood	
		NMF	CV (NMF), %	NMF	CV (NMF), %
ODASA	LQC	0.973	3.49	0.958	5.96
	HQC	0.980	2.86	0.935	3.40
M1	LQC	0.950	5.22	0.929	8.27
	HQC	0.980	3.81	0.960	3.05
M2	LQC	0.912	3.40	0.947	3.93
	HQC	0.972	3.59	0.967	3.09

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; NMF – normalized matrix factor.

The volume of validation tests carried out on blood of both species was optimized due to absence of significant differences between results of selectivity, linearity and matrix effects. Two batches on rat spiked samples and two batches on rabbit spiked samples were analyzed during investigation of intra-day accuracy and precision. The average value of the relative error (δ) at the concentration levels of LQC, MQC, HQC during determination of ODASA varied in the range of -5.55-6.44%, during determination of M1 – in the range of -4.97-10.39%, and during determination of M2 – in the range of -12.03- 11.45% (Table 6). The δ for LLOQ samples for ODASA did not exceed -9.18%, for M1 – -5.52%, and for M2 – 10.55%. The CV of calculated concentrations at LQC, MQC, HQC levels for ODASA was 1.44-5.38%, for M1 – 1.23-9.94%, and for M2 – 1.49-9.49% (Table 6). CV value for LLOQ samples during determination of ODASA was in the range of 5.69-8.18%, during determination of M1 – in the range of 7.49-10.74%, and during determination of M2 – in the range of 6.73-10.34% (Table 6). Combined results of evaluation of inter-day accuracy and precision also met the acceptance criteria (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). The δ was within the interval of ±15% for LQC, MQC, HQC concentration levels, and within the interval of ±20% for LLOQ concentration level. CV did not exceed 20% for LLOQ samples and 15% for other samples. Dilution integrity test (twofold dilution) was successfully performed on rat blood (Table 6). The carry-over of ODASA, M1, M2 and OHSA from a previously sample was not observed.

Short-term stability, freeze/thaw stability, and autosampler stability of analytes were confirmed on rat blood (Table 7). Long-term stability (LTS) was studied on samples of both species. The results of LTS test were acceptable: deviation from nominal value was in the range of ±15% for ODASA, M1 and M2 (Table 7). Reinjection reproducibility after 48 h also was proven (Table 6).

Pharmacokinetic parameters of the studied compound were calculated (Table 8) and pharmacokinetic curves were plotted after analysis of rat samples. There were high blood concentrations of ODASA and M1. C_{max} of the drug and the main metabolite after eye instillation (IE) was 10326±532 ng/mL and 3722±505 ng/mL (M±SEM), respectively. Content of M2 was lower than ODASA and M1. Parameter $AUC_{0\text{-}t}$ of M1 was higher after intraperitoneal injection (IP). The ratio between blood and plasma content (F_{Blood}) of M1 was 179.39±16.97 during IE and 155.47±6.50 during IP, which is about 3 times higher than F_{Blood} of ODASA (Table 8). This difference indicates a greater affinity of M1 for ligand binding sites and the ability to competitively displace ODASA and M2 from erythrocytes. It explains close values of $AUC_{0\text{-}t}$ of ODASA during both administration routes. Higher amount of the main metabolite after IP was faster superseded by the active compound from red blood cells and accelerate the decreasing ODASA concentrations (Fig. 3A). The relative bioavailability (RBA) of the active compound in comparison with that in intraperitoneal injection (IP) was 94.1%. It is by 13% higher than RBA of ODASA, which was calculated in plasma (Khokhlov et al 2024). Similar-to-structure 5-[5-(trifluoromethyl)-1,2-oxazole-3-yl]-furan-2-sulfonamide also have RBA in blood more than 90% (Yaichkov et al 2024).

Table 6. The results of confirmation of accuracy and precision of the method

Test		Concentration	ODASA		M1		M2	
			δ, %	CV, %	δ, %	CV,%	δ, %	CV,%
Intra-day accuracy and precision	Batch 1 (n=6)	LLOQ	-9.18	6.64	-0.40	8.94	10.55	6.73
		LQC	2.84	5.38	6.21	4.74	-9.38	4.92
		MQC	-3.63	2.06	-2.28	2.40	-9.67	2.66
		HQC	6.44	1.44	9.05	3.78	11.45	2.46
	Batch 2 (n=6)	LLOQ	-8.79	7.42	-0.12	7.49	8.80	7.32
		LQC	2.41	5.11	5.41	5.56	-2.48	9.49
		MQC	-3.62	1.71	-4.97	1.23	-8.42	2.78
		HQC	6.03	2.14	7.64	1.33	10.21	2.87
	Batch 3 (n=6)	LLOQ	-2.87	5.69	-5.52	10.74	7.27	10.34
		LQC	3.81	5.27	10.39	3.48	2.80	8.15
		MQC	-5.55	1.74	-4.67	1.54	-10.94	3.02
		HQC	4.40	2.21	7.49	1.79	7.53	1.95
	Batch 4 (n=6)	LLOQ	-7.08	8.18	-2.53	8.31	10.08	7.12
		LQC	3.26	4.16	5.53	9.94	-1.39	8.26
		MQC	-3.59	2.38	-3.20	3.32	-12.03	2.93
		HQC	5.42	1.44	3.64	3.02	7.86	1.49
Inter-day accuracy and precision (n=24)		LLOQ	-6.98	7.10	-2.15	8.60	9.18	7.53
		LQC	3.08	4.69	6.89	6.24	-2.61	8.75
		MQC	-4.10	2.06	-3.78	2.43	-10.26	3.07
		HQC	5.60	1.80	6.96	3.14	9.26	2.60
Reinjection reproducibility (48 h) (n=6)		LLOQ	-6.08	5.31	-2.90	4.83	7.48	7.53
		LQC	2.38	3.13	3.48	8.39	1.26	5.18
		MQC	-1.38	1.99	-0.55	2.31	-7.34	3.99
		HQC	4.34	1.79	2.81	2.19	5.07	2.58
Dilution integrity (n=6) (twofold)		Dil	4.89	4.33	1.39	2.86	1.77	2.70

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; n – number of samples on each concentration level

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; LQC, MQC, HQC – quality control samples of the lower, middle and upper levels; Dil – samples for dilution integrity test.

Table 7. The results of stability evaluation of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide and its metabolites in whole blood

Test		Concentration	ODASA		M1		M2	
			δ, %	CV, %	δ, %	CV, %	δ, %	CV, %
Short-term stability at room temperature (24 h)		LQC (n=6)	1.87	3.65	1.21	5.67	-12.90	8.44
		HQC (n=6)	4.05	3.09	3.23	2.83	12.43	3.31
Stability after 3 cycles of freeze (12 h) and thaw (4h)		LQC (n=6)	5.94	6.84	0.67	14.66	-6.52	8.54
		HQC (n=6)	4.71	2.49	2.70	3.10	7.50	2.07
Autosampler stability (48 h at +4°C)		LQC (n=6)	1.87	5.92	-1.81	7.20	-13.39	10.62
		HQC (n=6)	4.36	2.21	6.51	2.69	11.06	4.51
Long-term stability at freezer (no higher than -70°C, 30 days)	Rat blood	LQC (n=6)	2.27	5.93	-4.23	11.94	-4.51	10.28
		HQC (n=6)	5.08	2.22	6.51	3.01	4.04	2.48
	Rabbit blood	LQC (n=6)	3.30	2.95	4.75	5.67	-1.27	6.66
		HQC (n=6)	4.58	1.73	2.65	2.14	6.73	1.79

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; LQC, HQC – quality control samples of the lower and upper levels.

The pharmacokinetic profile of the M2 metabolite after instillation of the suspension into the eyes was characterized by a slow increase in the concentration of this analyte to a value of about 50 ng/mL by 5.2±1.1 h (M±SEM) after administration and thenits slow decrease (Fig. 3C). Higher C_{max} level of this metabolite of about 59 ng/mL was detected after IP. It was achieved faster – after 2.8±0.6 h after injection (Table 8). There were close values of AUC_{0-t} of M2 with both routes of administration. It was also connected with lower F_{blood} in comparison with F_{blood} of M1 (Table 8). The main metabolite was faster superseded from erythrocytes by the minor metabolite. Similarity of the parameter of AUC_{0-t} was also characteristic of N-hydroxy-5-[5-(trifluoromethyl)-1,2-oxazole-3-yl]-furan-2-sulfonamide after IE and IP (Yaichkov et al 2024).

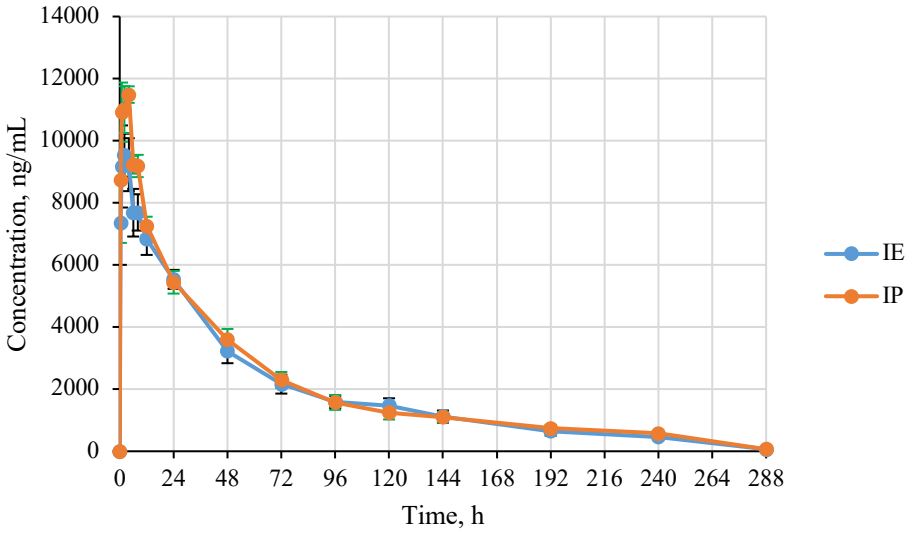
Table 8. Pharmacokinetic parameters of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide and its metabolites in rat blood after eyes instillation and intraperitoneal injection

Substance		ODASA (n=6)		M1 (n=6)		M2 (n=6)	
Route of administration		IE	IP	IE	IP	IE	IP
C_{max}, ng /mL	M± SEM	10326± 532	12391± 351	3722± 505	4704± 536	49.9± 9.4	58.4± 19.6
	RSD	12.62	6.94	33.26	27.92	46.04	82.14
T_{max}, h	M± SEM	3.1± 1.4	2.3± 0.7	18.4± 3.2	22.0± 2.2	5.2±1.1	2.8±0.6
	RSD	112.11	73.03	42.38	24.39	50.47	49.91
AUC_{0-t}, ng*h/mL	M± SEM	519022± 56392	551575± 41724	357026± 12675	446637± 34668	2125± 184	2122± 538
	RSD	26.61	18.53	8.70	19.01	21.21	62.04
$AUC_{0-\infty}$, ng*h/mL	M± SEM	557760± 58977	620242± 45550	402909± 19292	510483± 33809	2493± 219	2544± 515
	RSD	25.90	17.99	11.73	16.22	21.50	49.55
$T_{1/2}$, h	M± SEM	69.7± 8.0	92.8± 10.4	76.7±7.0	89.5±10.3	107.6± 14.9	120.0± 32.1
	RSD	28.10	27.48	22.40	28.12	33.97	65.61
MRT, h	M± SEM	64.6± 4.9	64.2± 3.6	82.8± 9.0	80.2± 5.7	66.2± 5.4	68.3± 4.6
	RSD	18.39	13.61	26.70	17.38	20.13	16.43
F_{Blood}	M± SEM	56.25± 10.23	45.19± 7.47	179.39± 16.97	155.47± 6.50	76.98± 19.88	62.26± 6.34
	RSD	44.53	40.49	23.16	10.24	63.27	24.95

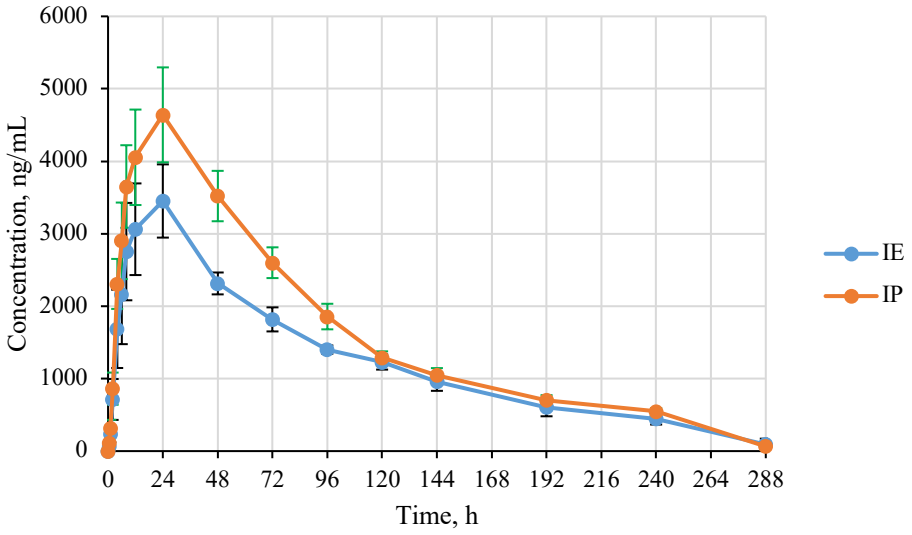
Note: ODASA – 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide; M1 – 4-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]benzenesulfonamide; M2 – N-hydroxy-4-(5-methyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide; IE – eye instillation; IP – intraperitoneal administration.

The half-life time of the studied compounds in blood was longer than its half-life time in rat plasma (Table 8) (Khokhlov et al. 2024). Thus, $T_{1/2}$ of ODASA after IE was 69.7± 8.0 h, $T_{1/2}$ of M1 – 76.7±7.0 h, and $T_{1/2}$ of M2 – 107.6± 14.9 h. It is also typical for ICAII such as 4-(2-methyl-1,3-oxazole-5-yl)-benzenesulfonamide (Khokhlov et al. 2023) and 5-[5-(trifluoromethyl)-1,2-oxazole-3-yl]-furan-2-sulfonamide (Yaichkov et al. 2024), which is caused by erythrocyte accumulation of these drugs. $T_{1/2}$ of ODASA, M1 and M2 in case of IE was shorter than $T_{1/2}$ in case of IP. It was also observed in pharmacokinetic study of 5-[5-(trifluoromethyl)-1,2-oxazole-3-yl]-furan-2-sulfonamide (Yaichkov et al. 2024).

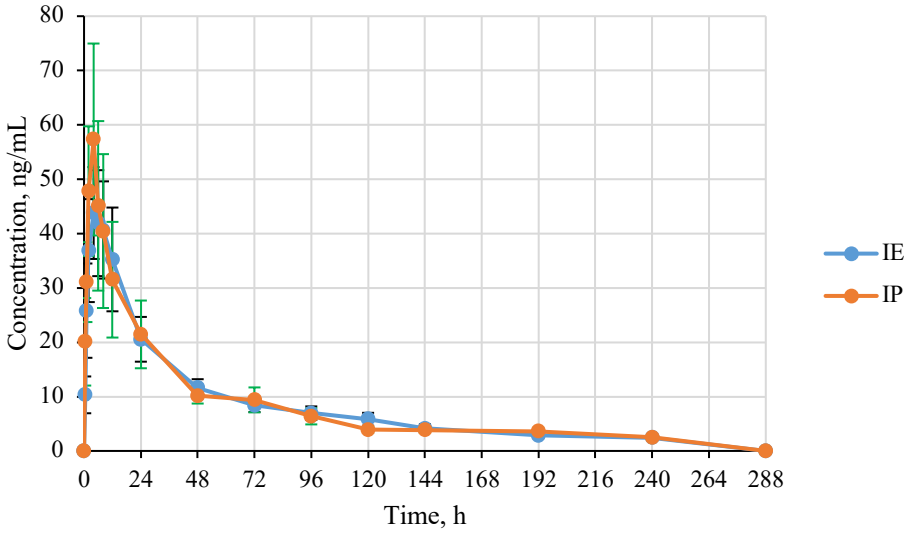
Thus, the accumulation of ODASA and its metabolites in erythrocytes was observed in the course of the study. Data obtained through research in plasma should be used for a correct assessment of the relative bioavailability of the drug. It was caused by competitive displacement of ODASA from erythrocytes by its main metabolite M1, which is formed in greater quantities during intraperitoneal administration. This reduces the AUC_{0-t} of ODASA at IP and leads to RBA difference of 13 % in comparison with plasma.



A



B



C

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 blood (error intervals: ±SEM). ***Note:*** IE – instillation into eyes; IP – intraperitoneal injection.

Conclusion

Systemic exposition of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide in rat blood was successfully studied using validated HPLC-MS/MS -method. The content of 4-(5-methyl-1,3,4-oxadiazole-2-yl)-benzenesulfonamide and its metabolites in the blood of rats was more than 40 times higher than their plasma content. These compounds have a long half-life time in range of 2.5 to 5 days. The greatest affinity for red blood cells was shown by 4-[5-(hydroxymethyl)-1,3,4-oxadiazole-2-yl]-benzenesulfonamide. This metabolite can competitively displace the active compound and N-hydroxy-4-(5-methyl-1,3,4-oxadiazol-2-yl)-benzenesulfonamide from the ligand binding sites.

Additional information

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.

Ethics statements

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

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