

CONTENT

- OLD AND NEW IN LIPID LOWERING THERAPY: FOCUS ON THE EMERGING DRUGS 811
DANIELA BARTOȘ, CAMELIA DIACONU, ELISABETA BĂDILĂ, ANA MARIA DARABAN
- A BRIEF OVERVIEW ON COCRYSTALS AND THEIR PHARMACEUTICAL APPLICATIONS 824
MANOJ KUMAR SARANGI
- SQUALENE – NATURAL RESOURCES AND APPLICATIONS 840
IOANA POPA, NARCISA ELENA BĂBEANU, SULTANA NIȚĂ, OVIDIU POPA
- *IN VITRO* ESTROGENIC/ANTI-ESTROGENIC EFFECTS OF CERTAIN FOOD ADDITIVES AND COSMETIC PRESERVATIVES 863
ANCA POP, BELA KISS, TUDOR DRUGAN, JULIEN CHERFAN, FELICIA LOGHIN
- COMPARATIVE STUDY ON THE VOLATILES' COMPOSITION OF *IRIS DICHOTOMA* PALL. RHIZOME EXTRACTS 874
WIRGINIA KUKULA-KOCH, KRYSZYNA SKALICKA-WOŹNIAK, ELWIRA SIENIAWSKA, JAROSLAW WIDELSKI, OTGONBATAAR URJIN, PAWEŁ GŁOWNIAK
- THE SYNERGISTIC ANTINOCICEPTIVE INTERACTION OF CODEINE AND PREGABALIN IN A SOMATIC PAIN MODEL IN MICE 882
ELIZA GRAȚIELA POPA, LILIANA MITTELU TARTAU, RAOUL VASILE LUPUȘORU, CĂTĂLINA ELENA LUPUȘORU, IULIAN STOLERIU, LĂCRĂMIOARA OCHIUZ
- ARE SSRI'S EQUALLY SAFE IN PREGNANCY? FLUOXETINE AND CITALOPRAM EXPERIMENTAL STUDY 889
BIANCA EUGENIA ŐSZ, CAMIL EUGEN VARI, MELINDA KOLCSĂR, OVIDIU SIMION COTOI, MARIUS ȘTEFAN MĂRUȘTERI, MARIA T. DOGARU
- BETADINE® IN CHEMICAL PLEURODESIS 897
CLAUDIU NISTOR, AURELIAN-EMIL RANETTI, ADRIAN CIUCHE, DANIEL PANTILE, LAURA-MARIANA CONSTANTIN, ROXANA BRÎNCOVEANU
- ANTI-TUMOR EFFECT OF *EUONYMUS EUROPAEUS* ON EHRlich TUMOR CELLS *IN VIVO* 907
BOGDAN SEVASTRE, ORSOLYA SÁRPATAKI, NELI K. OLAH, ROXANA L. STAN, MARIAN TAULESCU, IOAN MARCUS, CORNEL CĂTOI
- SPECTRAL CHARACTERIZATION OF NEW 2-((4-ETHYLPHENOXY)METHYL)-N-(ARYLCARBAMOTHIOYL) BENZAMIDES 918
MIRON TEODOR CĂPROIU, CARMEN LIMBAN, ALEXANDRU VASILE MISSIR, DIANA CAMELIA NUȚĂ, LAURENȚIU MORUȘCIAG
- PHYTOBIOLOGICAL TESTING OF SOME COMPOUNDS WITH 4(3H) - QUINAZOLONE STRUCTURE 929
MIHAELA BRATU, OCTAVIAN TUDOREL OLARU, ILEANA CORNELIA CHIRIȚĂ, MIHAELA DINU, ADRIANA IULIANA ANGHEL
- THE IMPACT OF DIFFERENCES BETWEEN REGULATORY GUIDANCE FOR *IN VITRO* TESTING ON THE DISSOLUTION PROFILES OF MYCOPHENOLATE MOFETIL 942
NAHLA NUMAN AQEL, DUMITRU LUPULEASA, MIRELA ADRIANA MITU, ANA ANDREEA STĂNESCU, CĂTĂLIN DONEA, DALIA SIMONA MIRON, FLAVIAN ȘTEFAN RĂDULESCU
- THE LEVELS OF SERUM BIOMARKERS OF INFLAMMATION IN HEMODIALYSIS PATIENTS 950
ANGELA ANTONESCU, SIMONA IOANA VICAS, ALIN C. TEUSDEA, IOANA RATIU, INA ANDREEA ANTONESCU, OTILIA MICLE, LAURA VICAS, MARIANA MURESAN, FELICIA GLIGOR
- RESEARCHES UPON INDIGENOUS HERBAL PRODUCTS FOR THERAPEUTIC VALORIFICATION IN METABOLIC DISEASES. NOTE II. POLYPHENOLS CONTENT, ANTIOXIDANT ACTIVITY AND CYTOPROTECTIVE EFFECT OF *BETULAE FOLIUM DRY EXTRACT* 961
TEODORA COSTEA, LAURIAN VLASE, VIORICA ISTUDOR, MARIA-LIDIA POPESCU, CERASELA-ELENA GÎRD
- STUDY ON THIAMPHENICOL – INDUCED SIDE EFFECTS IN HAMSTERS 971
MARIA CRIVINEANU, MANUELLA MILITARU, DENISA IOANA UDEANU, VALENTIN NICORESCU, CAMELIA PAPUC, CORINA ANA IONIȚĂ, ELENA ROTARU
- NICOTINE DETERMINATION FROM TABACCO BY GC/MS 982
DOINA ȘPAIUC, ADRIAN ȘPAC, LUMINIȚA AGOROAEI, ELENA BUTNARU
- PERCUTANEOUS PENETRATION ENHANCEMENT OF PROPRANOLOL HYDROCHLORIDE FROM HPMC-BASED HYDROETHANOLIC GELS CONTAINING TERPENES 991
LAVINIA VLAIA, IOANA OLARIU, GEORGETA CONEAC, VICENȚIU VLAIA, CĂLIN POPOIU, STĂNCIULESCU CORINA, ANA MARIA MUȚ, ZOLTAN SZABADAI, DUMITRU LUPULEASA
- PHARMACOKINETIC EVALUATION OF A NOVEL RUTHENIUM (III)-OFLOXACIN COMPLEX, AS POTENTIAL THERAPEUTIC AGENT 1009
BRUNO ȘTEFAN VELESCU, VALENTINA ANUȚĂ, VALENTINA UIVAROȘI
- RESEARCHES CONCERNING THE PROFITABILITY OF THE COMMUNITY PHARMACY WITHIN THE CONTEXT OF WORLD ECONOMIC AND FINANCIAL CRISIS 1025
ANAMARIA BOBOIA, GABRIELA CARMEN OROS, CONSTANTIN POLINICENCU, SIMONA MIREL
- THE STUDY OF PHARMACOPOLYMERAS USED IN THE ROMANIAN AREA IN XVIII-XXth CENTURIES: EVOLUTION OF PROPORTIONS FOR DRUG SUBSTANCES AND PHARMACEUTICAL PREPARATIONS 1037
EMILIA STANCU, ANA CARATA, ADRIANA-ELENA TĂEREL, VALENTINA SOROCEANU

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PHARMACOKINETIC EVALUATION OF A NOVEL RUTHENIUM (III)-OFLOXACIN COMPLEX, AS POTENTIAL THERAPEUTIC AGENT

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Abstract

The aim of the study was to investigate the pharmacokinetic profile of a novel ruthenium (III)-ofloxacin complex, with the general formula $[\text{Ru}(\text{oflo})_2\text{Cl}_3(\text{DMSO})]$ and the released ofloxacin in an animal experimental model, following a single dose of 100 mg/kg b.w., intraperitoneally administered. A HPLC method for the simultaneous quantification of the ruthenium-ofloxacin complex and ofloxacin from rat plasma was developed and validated according to the international guidelines. The method was applied in the pharmacokinetic study. The sample analyses allowed estimating both complex and released ofloxacin pharmacokinetic profile. Both complex and released ofloxacin kinetics are described by a bicompartimental model. The obtained results suggest a pharmacokinetic profile of the complex suitable for a distribution in the animal organism.

Rezumat

Scopul prezentului studiu a fost investigarea profilului farmacocinetic al unui complex nou al ruteniului cu ofloxacina, cu formula generală $[\text{Ru}(\text{oflo})_2\text{Cl}_3(\text{DMSO})]$ și a ofloxacinei eliberate, într-un studiu experimental pe animale, după administrarea în doză unică de 100 mg/kg corp, pe cale intraperitoneală. S-a dezvoltat și validat în conformitate cu reglementările internaționale o metodă HPLC, pentru dozarea simultană a complexului și ofloxacinei din plasma de șobolan. Analiza probelor biologice a permis estimarea profilului farmacocinetic pentru complex și ofloxacina eliberată. Atât complexul cât și ofloxacina eliberată urmează un model farmacocinetic bicompartimental. Datele obținute au indicat o distribuție a complexului în compartimentele profunde ale organismului.

Keywords: Ruthenium-ofloxacin complex, HPLC assay, rat plasma, pharmacokinetics

Introduction

Cancer is a widespread disease targeting different population groups. Cancer treatment is difficult to tolerate and the complete eradication of the disease is impossible to achieve with the available drugs. In the researches undertaken for identifying new entities able to treat cancer, some transitional metals complexes seem to be a viable option. Platinum complexes cisplatin carboplatin and oxaliplatin are among the most important chemotherapeutics used against a wide variety of cancers [1]. Different transitional metals (ruthenium, vanadium, cobalt, gadolinium, gold, rhodium, iridium, etc.) complexes have been investigated for their potential to treat cancer [2-5]. Ruthenium complexes appear to be promising anticancer agents and a phase I clinical study was conducted to evaluate the maximum-tolerated dose, profile of adverse events, and dose-limiting toxicity of NAMI-A (imidazolium-*trans*-DMSO-imidazole-tetrachlororuthenate) in patients with solid tumours [6].

Ruthenium organometallic complexes like Ru-polypyridyl [7] $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]^+$ (arene = e.g., biphenyl, dihydrophenanthrene, tetrahydroanthracene) [8], $[(\eta(6)\text{-p-cymene})\text{Ru}(2\text{-anthracen-9-ylmethylene-N-ethylhydrazinecarbothioamide})\text{Cl}]\text{Cl}$ are able to interact with DNA substrate and exert their action as anticancer agents [9].

A series of Ru(III) complexes with ligands belonging to the quinolone antibiotic class was designed, synthesized and physico-chemically characterized [10].

In vitro experiments were conducted to evaluate the Ru-quinolones complexes interaction with DNA and the results are promising for further testing for ruthenium(III)-ofloxacin complex (Figure 1), with the general formula $[\text{Ru}(\text{oflo})_2\text{Cl}_3(\text{DMSO})]$ [11].

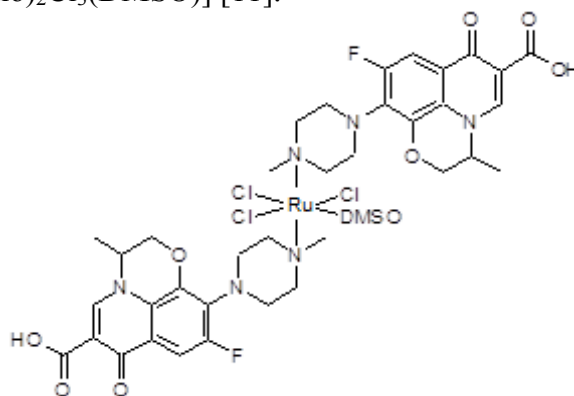


Figure 1.

Chemical structure of $[\text{Ru}(\text{oflo})_2\text{Cl}_3(\text{DMSO})]$ complex (Ru-oflo)

The present study was designed to evaluate the pharmacokinetic (PK) properties of a ruthenium-ofloxacin complex in rats after a single i.p. dose of 100 mg/kg b.w.. The aim of the study was to evaluate the pharmacokinetic profile of Ru-oflo complex and the released amount of ofloxacin from metallic complex in a rat experimental model.

Both complex and released ofloxacin kinetics described by a bicompartamental model. The obtained results suggest a PK profile of the complex suitable for a profound distribution in the animal organism.

Materials and Methods

Materials

Ruthenium-ofloxacin complex was synthesized and physico-chemical characterized according to a method described in a previous paper [10].

Ofloxacin USP Reference Standard was used for the preparation of the standard solutions and of spiked plasma samples.

The solvents used (acetonitrile and methanol) of HPLC grade were purchased from Sigma-Aldrich.

Analytical grade trifluoroacetic acid was purchased from Scharlau while methylene chloride was manufactured by Merck KGaA (Darmstadt, Germany).

Polyethylene glycol 400 (PEG 400) was purchased from Fluka (St. Louis, MO, USA). HPLC grade water was purified by a TKA-Genpure UV system.

Experimental animals

Female Wistar rats weighing (135 ± 11 g) from the Cantacuzino Institute, Bucharest were used. The rats were housed in plastic cages in an air-conditioned animal room and fed on granulated food with free access to water.

The temperature and relative humidity were continuously monitored using a thermohygrometer. The temperature was between 20°C and 22°C and the relative humidity was generally maintained at 35-45%.

All procedures were carried out in accordance with the Directive 86/609/EEC of 24th November 1986, on the protection of animals used for experimental and other scientific purposes.

Pharmacokinetics and Study design

28 female Wistar rats were randomly distributed in 5 groups of 5 animals each and 3 animals were used as blank.

The animals received 100 mg/b.w. (5 mg/mL in DMSO) Ruthenium-ofloxacin complex, by intraperitoneal injection, using an adapted procedure [12].

The administered dose represented 1/10 of the maximum administered dose that proved no lethal effect.

Following the administration, the animals were sacrificed at 30 min, 1 h, 2 h, 6 h and 24 h respectively. The blood was collected on Na₂EDTA and was centrifuged; plasma samples were stored at -20°C for HPLC analyses.

Pharmacokinetic analysis was performed with Kinetica 2000 software.

HPLC assay

The analyses were carried out using a Waters Liquid Chromatograph (600E Multisolvant Delivery System, 717 Autosampler, 486 Tunable Absorbance Detector, Waters, Milford, MA, USA). Empower Software package (Waters, Milford, MA, USA) was used to control instrument, data acquisition and data analysis. The UV detector was set at 282 nm.

The chromatographic separation was achieved on a Hypersil Gold, 5 µm 150 x 4 mm column (Thermo Scientific) using as mobile phase an isocratic mixture of 0.1% trifluoroacetic acid : acetonitrile (80:20 v/v), delivered at 1.0 mL/min flow rate, at 30°C. 100 µL of each sample was injected into the chromatographic column.

Standard and plasma solutions

Ru-oflo stock solution (100 µg/mL) was prepared in a PEG 400 : water 1:1 (v/v) mixture. All solutions were kept protected from light, and filtered through 0.45-µm filter and degassed before use.

The standard stock solution of ofloxacin was prepared by dissolving 100 mg of USP reference standard substance in 100 mL ultrapure water.

Different solutions were prepared for the calibration curve samples and quality control.

Plasma solutions (calibration standards and quality control samples) were prepared by spiking various volumes of the Ru-oflo and ofloxacin stock solution into rat plasma. The calibration standards were prepared in the concentration range 0.05-20 µg/mL for Ru-oflo and 0.01-4 µg/mL for ofloxacin. Three standard solutions with high (4 µg/mL Ru-oflo and 1.6 µg/mL ofloxacin), medium (2 µg/mL Ru-oflo and 0.4 µg/mL ofloxacin) and low (0.4 µg/mL Ru-oflo and 0.08 µg/mL ofloxacin) containing were used as quality control samples.

Sample preparation method

Different extraction solvents were tested for optimizing analytes extraction from plasma (Figure 2). Difficulties resulted from the fact that ofloxacin is significantly more polar than Ru-oflo, and has poor extraction

yields in almost all tested solvents. The optimum extraction solvent for both Ru-oflo complex and ofloxacin was methylene chloride.

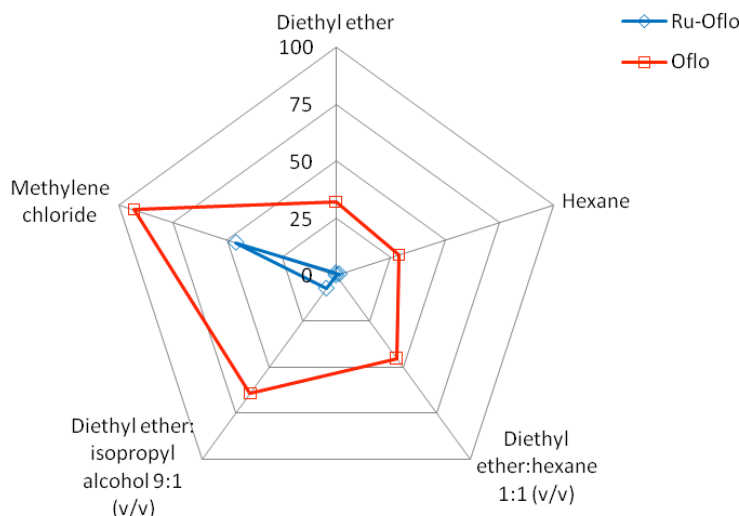


Figure 2.

Extraction solvents used for optimizing analytes extraction from plasma

Plasma samples (500 μL) were transferred to 10 mL disposable polypropylene tubes and 3 mL of methylene chloride were added. The tubes were shaken vigorously for 10 min by means of a horizontal Heidolph Promax 2020 shaker at a mixing speed corresponding to 400 rot/min. 2.5 mL of the organic layer were taken and dried under a stream of nitrogen at 50°C. The residue was dissolved in 300 μL of mobile phase solution, and a 100 μL aliquot of the mixture was injected into the HPLC system.

Pharmacokinetic analysis

PK analysis was carried out by using non-compartmental (NC) and compartmental (C) methods.

NC analysis was used for the estimation of half time ($t_{1/2}$), mean residence time (MRT), total clearance (Cl_T), volume of distribution (Vd), area under the time-concentration curve (AUC_{0-24} , AUC_{tot} , $\text{AUC}_{\text{extra}}$, $\%\text{AUC}_{\text{extra}}$).

Compartmental analysis was used for the estimation of the proper compartmental model followed by the complex and macro and micro constants associated to the identified model.

The compartmental model was estimated by using the Marquardt curve fitting algorithm. The Akaike information criterion and Schwarz criterion [13] were used for identifying the optimum model describing the complex and released ofloxacin kinetics.

Results and Discussion

Analytical method validation

The HPLC method was fully validated in accordance with international regulations [14-18].

The method proved to be selective towards separation of Ru-oflo and ofloxacin (Figure 3).

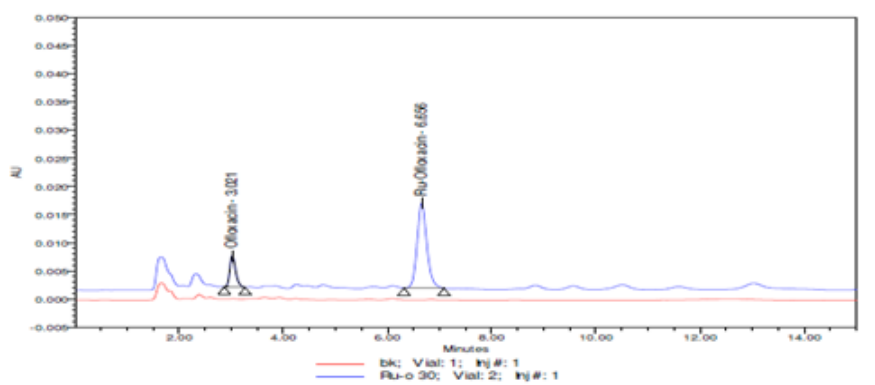


Figure 3.

Selectivity of the HPLC method. Overlay of a blank plasma sample (red line) and a standard plasma sample containing 10 µg/mL Ru-oflo and 2 µg/mL ofloxacin

The calibration curves (Figure 4) of the compounds in plasma were generated by using eight concentration levels, in the range 0.05-20 µg/mL for Ru-oflo and 0.01-4 µg/mL for ofloxacin. Three replicates for each concentration were used.

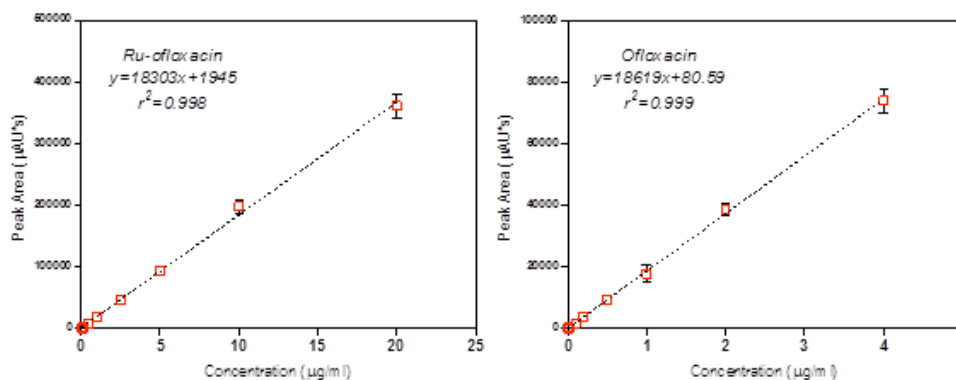


Figure 4.

Calibration curve for Ru-oflo and ofloxacin

The lower limit of detection (LLOD) and limit of quantification (LLOQ) were calculated by preparing diluted solutions of analytes in plasma to a final response equal to three times of the signal-to-noise ratio. The LLOQ was taken as ten times of signal-to-noise ratio. The specificity of the method was determined by using six different plasma samples.

For LLOQ concentration, the relative standard deviation for six replicates was 8.2%, and the deviation between the true and the measured value expressed in percent (as measure of accuracy) was -7.4%.

The accuracy and precision were determined by use of quality control sample prepared by spiking known amounts of analytes standard solution in plasma to result in three concentrations representing the entire range of standard curve; one towards the lower limit of quantitation (low quality control sample), one near the center (middle quality control) and one near the upper boundary of standard curve (high quality control). Measurements were made as five replicates at each concentration and mean and coefficient of variance (CV) were calculated.

Both intra-assay accuracy and precision were within the accepted limits. CV was lower than 10% and the bias did not exceed 8% at all concentration levels tested (Table I).

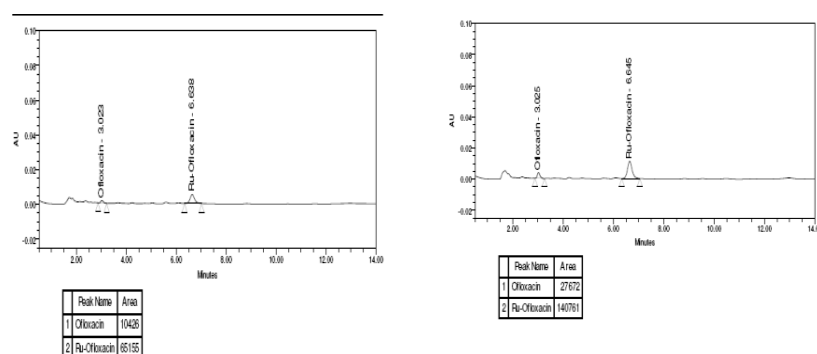
Table I
Accuracy and precision of the analytical method (mean of 5 determinations)

Test	Ru-oflo				Oflo			
	Concentration ($\mu\text{g/mL}$)		Accuracy (Bias%)	RSD (%)	Concentration ($\mu\text{g/mL}$)		Accuracy (Bias%)	RSD (%)
	Added	Measured			Added	Measured		
Intra-assay accuracy and precision	4	3.985	-0.38	2.63	0.8	0.789	-1.38	3.32
	2	2.124	6.20	3.64	0.4	0.421	5.30	4.26
	0.4	0.429	7.25	9.16	0.08	0.073	-9.25	6.92
Inter-assay accuracy and precision	4	4.050	1.25	6.25	0.8	0.821	2.62	5.68
	2	2.156	7.80	8.26	0.4	0.436	9.07	11.49
	0.4	0.412	3.07	10.12	0.08	0.076	-5.49	7.16

Samples analysis

The HPLC method was applied in an experimental PK study for the estimation of Ru-oflo and ofloxacin PK parameters, following a single dose administration.

Representative chromatograms of Ru-oflo plasma samples are presented in Figure 5.

**Figure 5.**

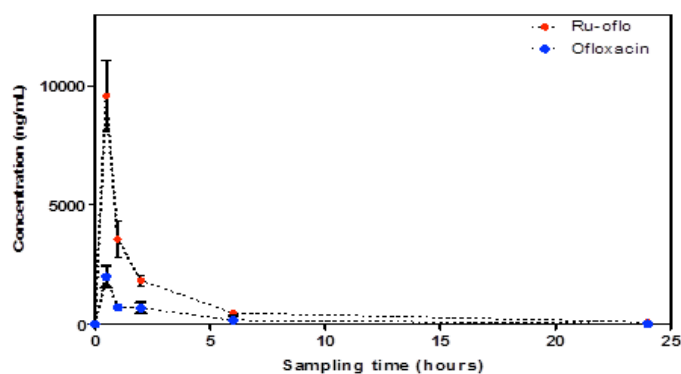
Representative chromatograms of plasma sample containing Ru-oflo and ofloxacin obtained at 0.5 hours after ip administration of 100 mg Ru-oflo complex/ kg b.w. in rats

The mean plasma concentrations of Ru-oflo complex and ofloxacin according to the sampling moments, are shown in Table II and graphically represented in Figure 6.

Table II

Mean plasma concentration of Ru-oflo complex and ofloxacin

Sampling time (h)	Ru-oflo (ng/mL)	Ofloxacin (ng/mL)
0	0	0
0.5	9580 ± 1480	1999 ± 453
1	3565 ± 736	704 ± 147
2	1839 ± 222	688 ± 229
6	471 ± 44	169 ± 193
24	79 ± 21	14 ± 24

**Figure 6.**

Comparative plasma concentration time profile for Ru-oflo complex and ofloxacin

*Pharmacokinetic analysis**Non-Compartmental (NC) analysis*

The pharmacokinetic parameters estimated by the NC methods are presented in Table III.

Table III

Pharmacokinetic parameters calculated by the NC methods

Parameter	Ru-oflo	Ofloxacin
	Value	
$t_{1/2}$ (h)	5.5	4
MRT (h)	4.5	4
Cl_T (L/h)	0.72	2.50
Vd (L)	5.59	14.96
AUC ₀₋₂₄ (ng/mL*h)	18016.4	5234.18
AUC _{extra} (ng/mL*h)	564.774	75.59
AUC _{tot} (ng/mL*h)	18581.2	5309.77
% AUC _{extra}	3.04	1.42

The extrapolated AUC percent for both Ru-oflo complex and ofloxacin are under the commonly accepted limit of 20% (3.04 for the complex and respectively 1.42 for ofloxacin); therefore the applied PK study design is proper for the further estimation of the PK parameters both for the complex and released ofloxacin.

Ru-oflo C analysis

Data fitting was performed for the monocompartmental, bicompartamental and tricompartamental models with immediate or delayed absorption. In the Figures 7-12 there are presented the fitting results of the experimental data among each model statistics.

Statistic methods Akaike's information criterion (AIC) and Schwartz criterion (SC) (with minimum values) pointed out the bicompartamental model with delayed absorption for Ru-oflo complex as an optimum PK model. The fitting results for the tricompartamental model appear to be inconsistent most probably due to the reduced amount of data.

Taking in consideration physiological particularities of the route of administration (rapid absorption due to rich vascularisation of the peritoneal cavity), the use of DMSO as an enhanced absorption agent [19] and the enhanced absorption of the complex administered as DMSO solution (K_a estimated by bicompartamental model with delayed absorption was found 31.29 h^{-1} , corresponding to a half time of absorption calculated as $t_{1/2\text{abs}} = \ln 2 / K_a$ less than 2 minutes) it was possible to simplify the PK model, from a model with extravascular absorption to an apparent model with intravascular

administration. The simplification of the model is sustained by the intravascular fitting results presented in Figure 13.

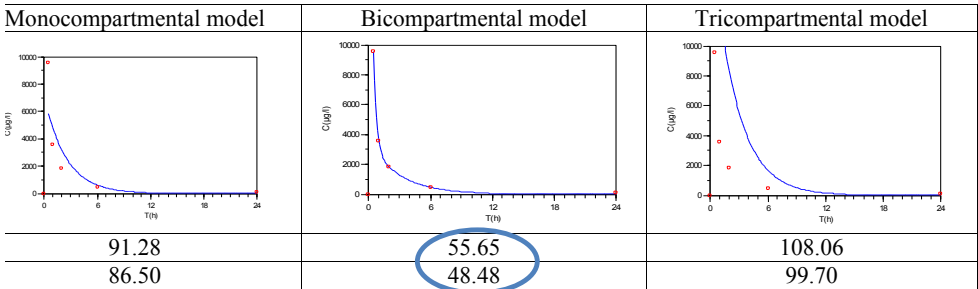


Fig. 7. Fitting curve the monocompartmental model with delayed absorption for Ru-oflo complex

Fig. 8. Fitting curve the bicompartmental model with delayed absorption for Ru-oflo complex

Fig. 9. Fitting curve the tricompartmental model with delayed absorption for Ru-oflo complex

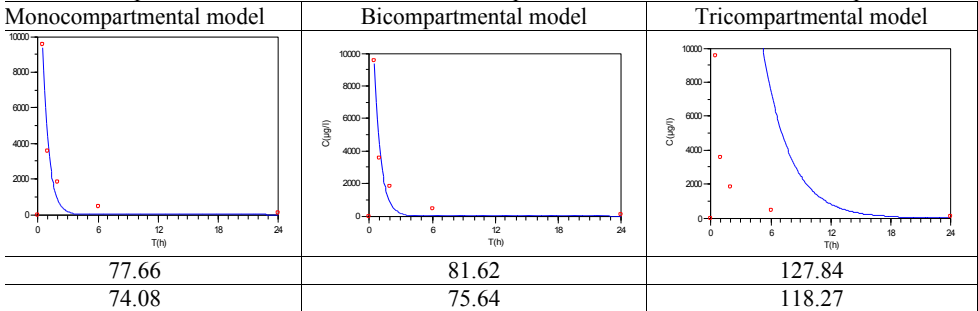


Fig. 10. Fitting curve the monocompartmental model with immediate absorption for Ru-oflo complex

Fig. 11. Fitting curve the bicompartmental model with immediate absorption for Ru-oflo complex

Fig. 12. Fitting curve the tricompartmental model with immediate absorption for Ru-oflo complex

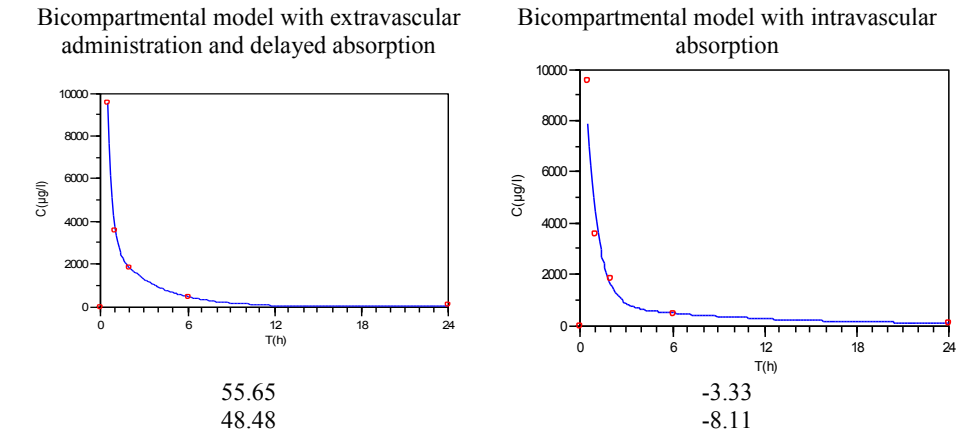


Figure 13.
Comparative fitting curves for Ru-oflo complex extravascular model vs. intravascular model

The proposed PK model for Ru-oflo complex is summarized in Figure 14.

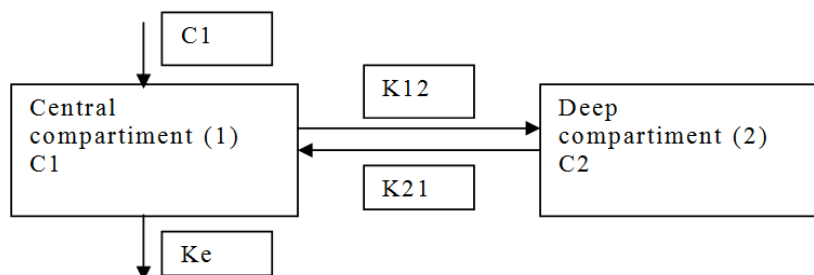


Figure 14.
Simplified PK model of Ru-oflo complex

In this model we can consider that the central compartment is represented by blood and the deep compartment by low vascularised tissues (bone, fat).

By simplifying the model it is possible to assimilate concentration values at the absorption site with plasma concentration from the central compartment. In this case the PK equations associated to the model became:

$$dC_1/dt = dC_2/dt = -(k_e + k_{23}) C_2 + k_{32} C_3 \quad (5)$$

$$dC_3/dt = k_{23} C_2 + k_{32} C_3$$

By solving the equation we find a possible solution:

$$C_1(t) = C_2(t) = B \times e^{-\beta t} + C \times e^{-\gamma t} \quad (6)$$

$$C_2(t) = B' \times e^{-\beta t} + C' \times e^{-\gamma t} \quad (7)$$

where the coefficients B, C, B', C' are complex related constants and β , γ are constants associated to the transfer between compartments, distribution and elimination processes.

The estimated constants of the proposed model are presented in Table IV.

Table IV

Calculated micro- and macro- constants for the proposed PK model

Constant	h^{-1}
K_{12}	0.53
K_{21}	0.17
K_e	0.79

In the proposed model, the initial concentration of complex is 0. After the administration, the distribution of complex in the body compartments is described by the microconstants k_{12} and k_{21} . The distribution process indicates a cumulative trend for the complex. The

global transfer constant between the compartments K (where $K=k_{12}/k_{21}$) has a high value (>3). The elimination from the central compartment is the main process that contributes to the decrease of plasma concentration probably with the dissociation of the complex.

We can assume that Ru-oflo plasma concentrations represent the equilibrium between the absorption, distribution and elimination processes. Elimination appears to be the main process that contributes to the concentration decrease of complex in the organism. In this context Ru-oflo concentration from the central compartment decreases by 2 exponential processes, represented in Figure 15. The first one may be correlated with the distribution in the deep compartment in the first 2 hours after the administration. The second process may be correlated to elimination from the central compartment.

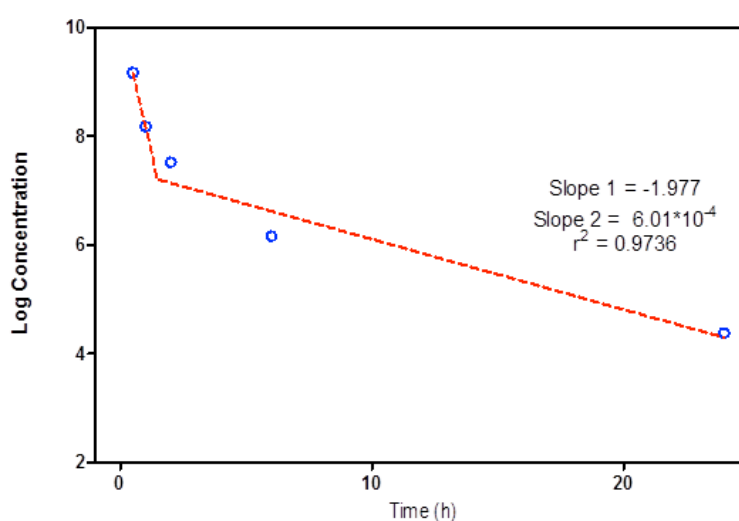


Figure 15.
Biphasic decrease of Ru-oflo complex plasma

Ofloxacin C analysis:

Data fitting was performed for the monocompartmental, biocompartmental and tricompartmental models with immediate or with lag time. In the Figures 16-21 there are presented the fitting results of the experimental data along with each model statistics.

Model statistics (AIC, SC with minimum values) pointed out the biocompartmental model with lag time for ofloxacin as an optimum PK model. In this case also, the fitting results for the tricompartmental model appear to be inconsistent most probably due to the reduced amount of data.

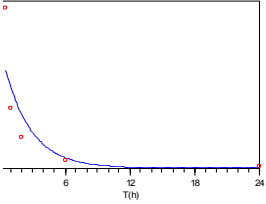
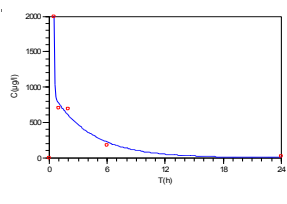
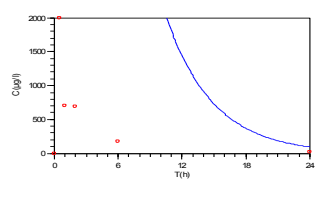
Monocompartmental model	Bicompartmental model	Tricompartmental model
		
91.28 86.50	60.08 52.91	118.41 108.85

Fig. 16. Fitting curve the monocompartmental model with lag time for oflo

Fig. 17. Fitting curve the bicompartmental model with lag time for oflo

Fig. 18. Fitting curve the tricompartmental model with lag time for oflo

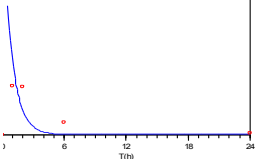
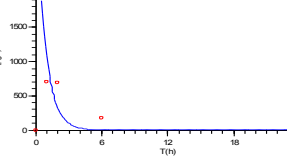
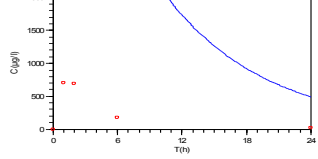
Monocompartmental model	Bicompartmental model	Tricompartmental model
		
68.92 65.33	72.92 66.94	103.96 95.59

Fig. 19. Fitting curve the monocompartmental model with immediate absorption for oflo

Fig. 20. Fitting curve the bicompartmental model with immediate absorption for oflo

Fig. 21. Fitting curve the tricompartmental model with immediate absorption for oflo

The estimated constants of the proposed model are presented in Table V.

Table V
Calculated micro- and macro- constants for the proposed PK model

Constant	h^{-1}
K_{12}	14.88
K_{21}	1.41
K_e	3.40

In the proposed model, prior to administration of complex, the ofloxacin concentration is 0. After the administration, the ofloxacin appears in the circulation with an estimated delayed time of 0.34 h. Ofloxacin was not observed in the plasma standards of Ru-oflo complex used, in this case the ofloxacin identified in the rat plasma appears to be a degradation product of the complex, under physiological conditions. The apparent absorption constant estimated ($k_a=42.22 \text{ h}^{-1}$) by the model can be assimilated to an appartion constant that describes the complex degradation with the release of ofloxacin most likely at the absorption site and in the blood stream.

After the occurrence in the blood stream, ofloxacin is distributed in the profound compartment, the distribution process has a cumulative trend ($K > 10$). The elimination of ofloxacin is made from the central compartment. Ofloxacin plasma concentration decrease is described by two decay periods that may be correlated with the distribution in the first 6 h after the administration of complex and the terminal elimination (Figure 22).

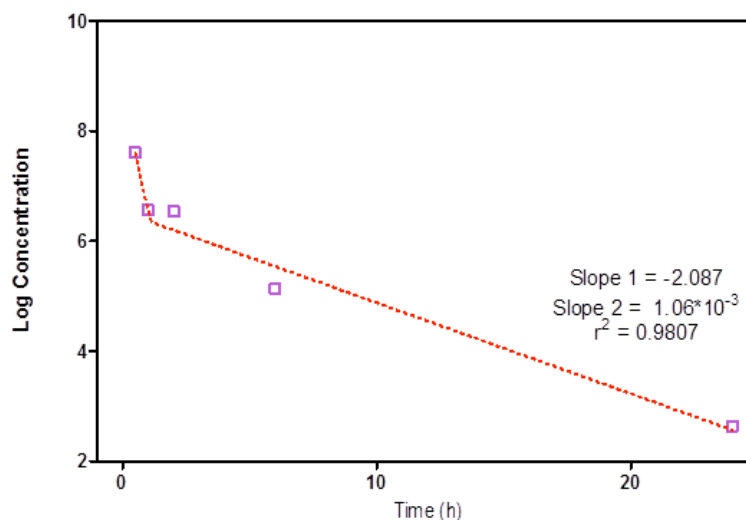


Figure 22.

Biphasic decrease of Ofloxacin plasma concentration (log scale)

Conclusions

A HPLC method for the simultaneous quantification of ruthenium (III)-ofloxacin complex and ofloxacin from rat plasma samples was developed, validated and applied in a single dose PK study.

The study design was adequate and by the PK analyses it was possible to estimate Ru-oflo complex and the resulted ofloxacin PK parameters. Both compounds kinetics, following the intraperitoneal administration, are described by a bi-compartmental model and the plasma concentration decrease is described by two phases that might be correlated to the distribution and elimination processes. The PK model of the complex administered as DMSO solution could be approximated to a bi-compartmental model with intravascular administration.

The distribution profiles of both compounds indicate a cumulative effect described by the global transfer constant between the central and the deep compartment > 1 . The apparent elimination constant estimated by the PK models indicates a faster elimination for ofloxacin than the complex.

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