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PHARMACOKINETIC STUDY OF DICLOFENAC SODIUM IN RAT PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The aim of this study is to determine pharmacokinetics of diclofenac sodium in the experiment on rats by the method of high-performance liquid chromatography. Identification and quantitative determination of diclofenac sodium in the substance of diclofenac sodium were carried out by the method of high-performance liquid chromatography. For study conduct, a total of 94 male rats with body weight of 250-300 g were used. Experimental animals were subdivided into 15 groups. Diclofenac sodium was diluted in purified water and administered per os at the doses of ED_{50} and $\frac{1}{2} ED_{50}$ equivalent to 8 mg/kg and 4 mg/kg of animal body weight, respectively. Animals were sacrificed in 15 min, 30 min, 60 min, 90 min, 120 min, 240 min, and 360 after drug administration. For extraction of diclofenac sodium from rat plasma samples, we used a method of solid-phase extraction, which had been modified due to micro-concentrations of the active substance. Dependence of diclofenac sodium concentration on time and dose was studied by the method of high-performance liquid chromatography. In the result of the study, quantitative content of diclofenac sodium substance and suitability of the doses studied (4 mg/kg and 8 mg/kg) were confirmed. The study conducted revealed no dynamics of time, during which diclofenac sodium was present in systemic blood flow, on the dose. The results, which had been proved by chromatograms of diclofenac sodium and control sample (plasma without diclofenac) for each time interval, were obtained. First concentrations of diclofenac sodium in rat plasma are registered in rat plasma in 15 min after dose administration at the doses of 4 and 8 mg/kg. That is why this time index is recommended for further preclinical and clinical studies.

Key words: pharmacokinetics; nonsteroidal anti-inflammatory drugs; diclofenac sodium; glucosamine; high-performance liquid chromatography

INTRODUCTION

Today, nonsteroidal anti-inflammatory drugs (NSAIDs) constitute one of the largest and clinically significant groups of drugs. In recent 30 years, its nomenclature has increased greatly and now it includes a great number of drugs that differ as for their peculiarities of action and use [3, 4, 6]. Diclofenac sodium (DNa) remains "the gold standard" in treatment of inflammatory diseases of connective tissue and is most widely used in modern medicine and pharmacy. So, studies of its pharmacological activity characteristics depending on dose and time are an important and urgent problem.

The aim of this work is to determine pharmacokinetics of DNa in experiments on rats by the method of high-performance liquid chromatography for further preclinical and clinical studies.

MATERIALS AND METHODS

Diclofenac sodium (DNa), sodium 2-[(2,6 - dichlorophenyl) amino] phenyl] acetate, has marked anti-in-

flammatory, analgetic, as well as moderate antipyretic activities [3, 6]. Substance of DNa manufactured by Borschahivskiy Chemical-Pharmaceutical Plant (BCPP), batch No. 20040609, was used for the study. Substance of DNa manufactured by "Amoli Organics Ltd" (batch No. 20061013) was used as a working reference standard (WRS).

Identification and quantitative determination of DNa in the substance of DNa were carried out by most sensitive method of high-performance liquid chromatography [2, 7, 8, 9, 10]. This method was used to determine concentrations of the test substance at all stages of the study.

The content of DNa in the substance (X), in per cent, was calculated according to the following formula:

$$X = \frac{S_x \cdot m_{WRS} \cdot P \cdot 100}{S_{WRS} \cdot m_x \cdot (100 - W)}$$

where: S_x is the mean peak area of diclofenac sodium calculated from chromatograms of the test solution; S_{WRS} is the mean peak area of diclofenac sodium calculated from chromatograms of the reference solution; m_{WRS} is the weight of diclofenac sodium WRS, g; P is the content

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Table 1

TACTICS OF PHARMACOKINETIC STUDY OF DICLOFENAC SODIUM ON RATS

Conditions of the experiment	Dose, mg/kg	Time of DNA entry into rat blood						
		360 min	240 min	120 min	90 min	60 min	30 min	15 min
Intact	-	Group No.						
		1						
Diclofenac sodium	4	2	3	4	5	6	7	8
Diclofenac sodium	8	9	10	11	12	13	14	15

of diclofenac sodium in the WRS, %; m_x is the weight of diclofenac sodium substance, g; W is the loss on drying of diclofenac sodium substance, %.

Experimental studies were carried out at experimental biological clinics of the State Enterprise "Sytenko Institute of Spine and Joint Pathology of the National Academy of Medical Sciences of Ukraine". Animals used in the experiment: nonlinear white rats of the population of experimental biological clinics of the State Enterprise "Sytenko Institute of Spine and Joint Pathology of the National Academy of Medical Sciences of Ukraine".

For study conduct, 94 male rats with body weight of 250-300 g were selected; they were kept according to sanitary norms on a standard diet [2]. Work with animals was carried out according to Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes.

Experimental animals were subdivided into 15 groups. An intact group (group 1) consisted of 10 rats, and groups 2-15 consisted of 6 rats each. Animals of group 1 received purified water in the volume of 0.5 mL per 100 g [2]. DNA was diluted in purified water and administered per os in the same volume at the doses of ED_{50} and $\frac{1}{2} ED_{50}$, equivalent to 8 mg/kg and 4 mg/kg of animal body weight, respectively [2, 3, 6].

Pharmacokinetic study of DNA was carried out according to the tactics under conditions indicated in Tab. 1. Animals were sacrificed in 15 min, 30 min, 60 min, 90 min, 120 min, 240 min, and 360 after drug administration.

Blood was sampled in the quantity of 7-10 ml into labeled test-tubes that were heparinized. Blood samples were centrifuged (3.000 rpm, 15 min), and plasma was obtained. The interval between blood sampling and its processing did not exceed 5 min. Before analysis, plasma samples were kept at -80°C .

For extraction of DNA from rat plasma samples, a method of solid-phase extraction was used [10]. The method had been modified due to micro-concentrations of the active substance via sample concentration [1].

After determination of DNA concentration depending on the time of its administration, the following pharmacokinetic parameters were calculated by the noncompartmental method of statistical moments with the use of application software program M-ind on IBM PC Pentium III: C_{\max} (maximal concentration); T_{\max} (time to maximum drug concentration); $T_{1/2}$ (half-life); Cl_T (total clearance); $AUC^{0 \rightarrow t}$ (area under the concentration-time curve within

the limit of drug concentration observation); $AUC^{0 \rightarrow \infty}$ (total area under the concentration-time curve within the limit of 0 to ∞); $C_{\max}/AUC^{0 \rightarrow \infty}$ (coefficient that characterizes drug absorption rate).

Statistic processing of experimental data obtained was carried out with software STATISTICA (StatSoft Inc., the USA). Reliability of the results obtained was assessed at the level of significance not less than 95 % ($p \leq 0.05$) [5].

RESULTS AND DISCUSSION

Quantitative determination of diclofenac sodium in rat plasma

Comparative characteristic of diclofenac sodium substance

Results of quantitative determination of DNA in substance samples are indicated in Tab. 2.

Chromatograms of DNA substance samples obtained under the same conditions are presented in Fig. 1, where the following is indicated: 1. – chromatogram of DNA manufactured by Amoli Organics Ltd; 2. – chromatogram of DNA used in the experiment as WRS manufactured by Borshchahivskiy Chemical-Pharmaceutical Plant (BCPP).

Quantitative content of DNA in DNA substance (BCPP) used in the experiment is 98.5 %.

Retention time of DNA in the chromatogram of the test sample of the solution (5.673 min) corresponds to that of DNA in the chromatogram of DNA WRS (5.673 min), so the sample provided is DNA substance. In the result of the studies conducted, quantitative content of DNA and

Table 2

RESULTS OF QUANTITATIVE DETERMINATION OF DICLOFENAC SODIUM IN SUBSTANCE SAMPLES

Mean peak area of diclofenac sodium manufactured by Amoli Organics Ltd, India (S_{WRS})	15570622
Exact weight of diclofenac sodium (Amoli Organics Ltd) (m_{WRS}), g	0,0120
Content of diclofenac sodium (Amoli Organics Ltd) in the substance (P), %	99,4
Loss on drying (Amoli Organics Ltd) (W_{WRS}), %	0,36
Mean peak area of diclofenac sodium (BCPP) (S_x)	14993617
Exact weight of diclofenac sodium (BCPP) (m_x), g	0,0117
Content of diclofenac sodium (BCPP) in the substance (X), %	98,5

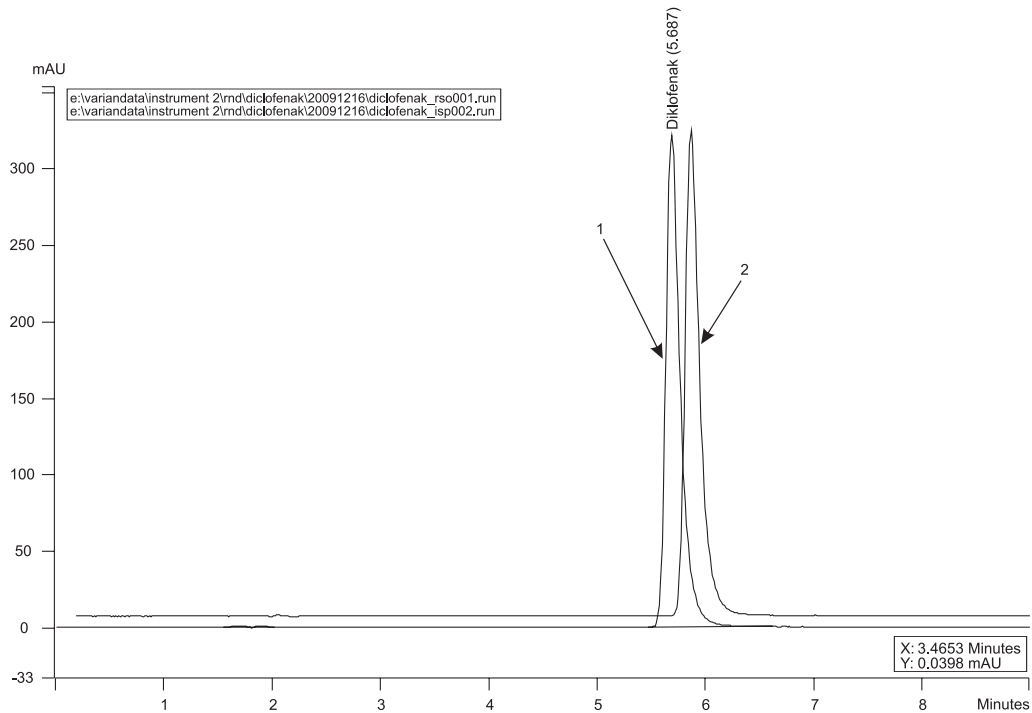


Fig. 1. Chromatograms of samples of diclofenac sodium substance.

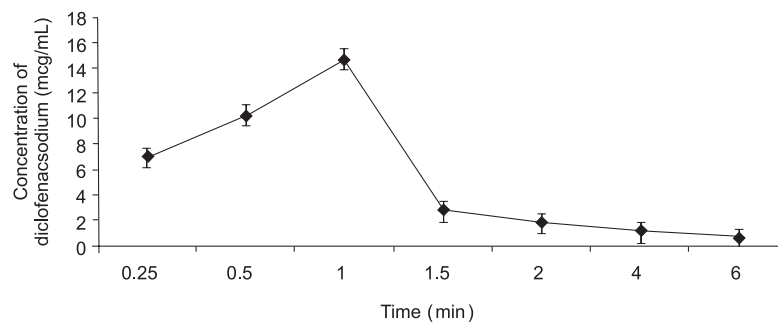


Fig. 2. Pharmacokinetic curve of diclofenac sodium (dose of 4 mg/kg).

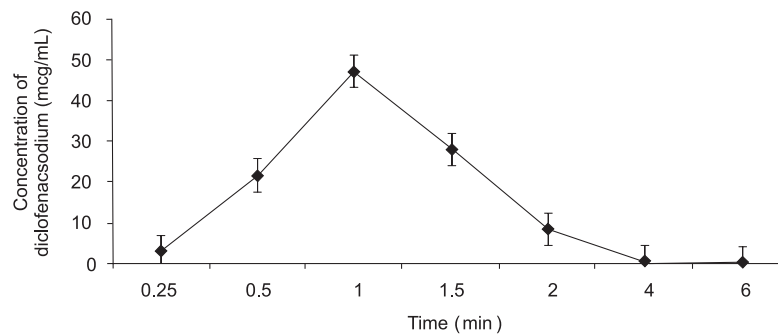


Fig. 3. Pharmacokinetic curve of diclofenac sodium (dose of 8 mg/kg).

suitability of the doses studied. Actual doses of DNA during study conduct are 4 mg/kg and 8 mg/kg, respectively.

Assessment of pharmacokinetic parameters of diclofenac sodium

Profiles of averaged pharmacokinetic curves of dynamics of DNA rat plasma concentrations after single oral administra-

tion of 4 mg/kg and 8 mg/kg and their comparative characteristics are presented in Fig. 2-4. DNA is registered in plasma already in 15 min after substance administration of both doses studies. At the same time, DNA concentration at this time is 4.88 ± 0.82 mcg/mL at administration of 4 mg/kg, and 17.45 ± 1.38 mcg/mL at administration of 8 mg/kg.

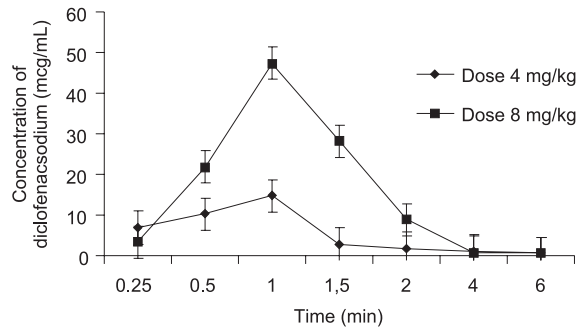


Fig. 4. Comparison of pharmacokinetic curves of diclofenac sodium (doses of 4 mg/kg and 8 mg/kg).

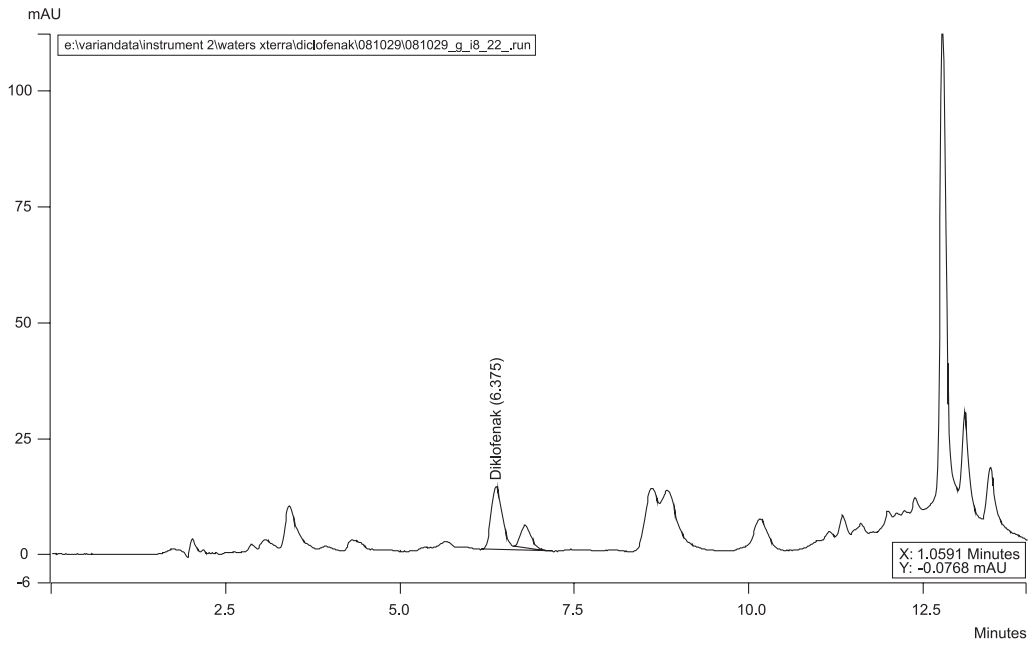


Fig. 5. Chromatogram of diclofenac sodium (in 15 min after drug administration).

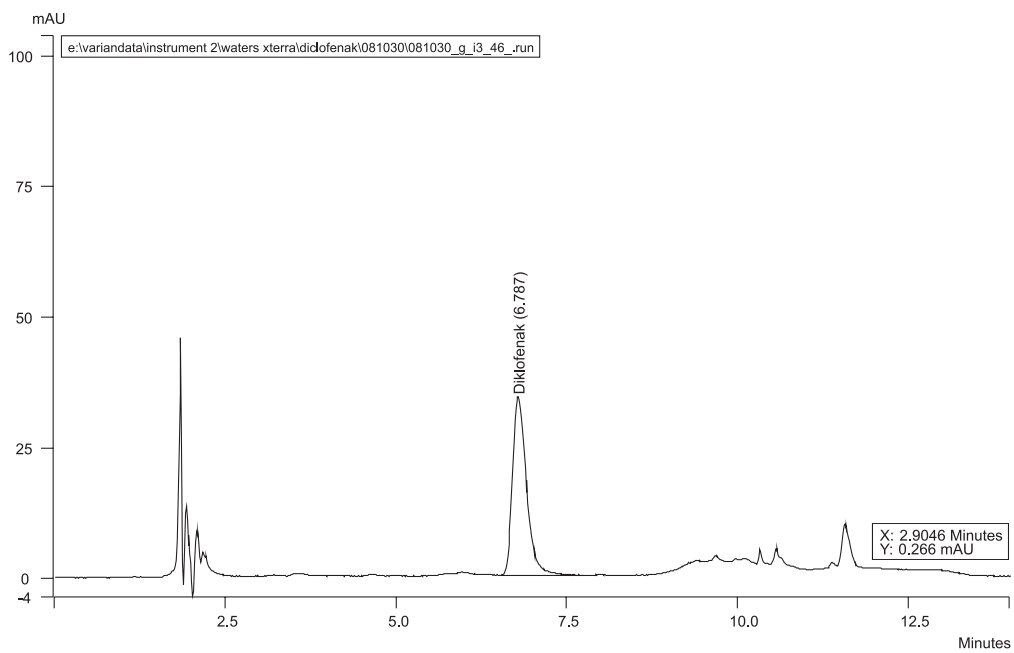


Fig. 6. Chromatogram of diclofenac sodium (in 30 min after drug administration).

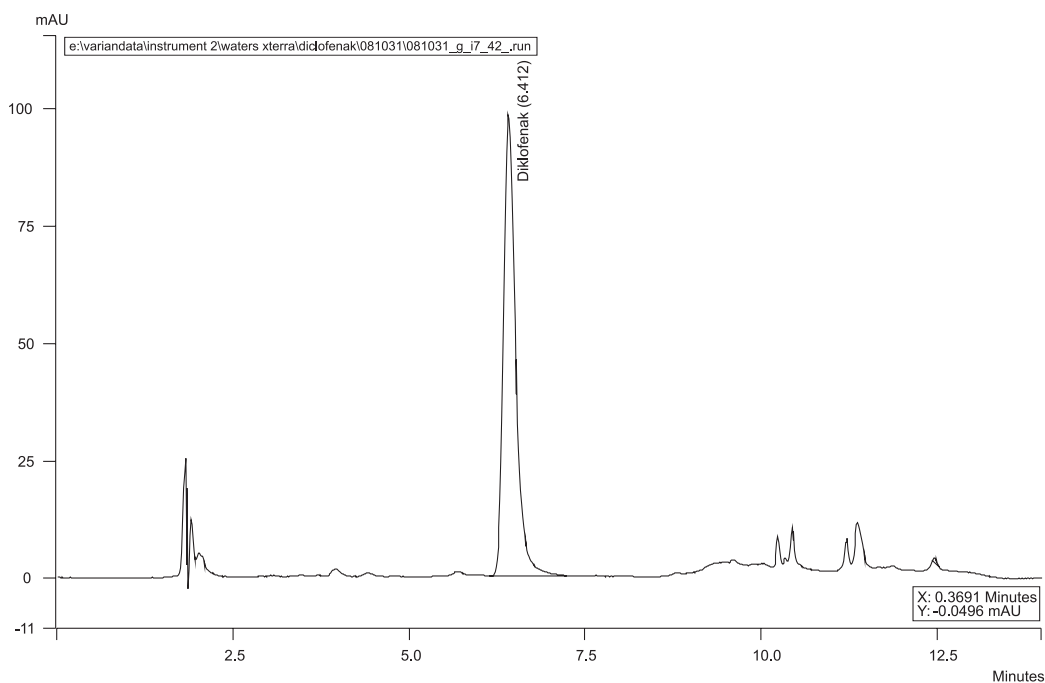


Fig. 7. Chromatogram of diclofenac sodium (in 60 min after drug administration).

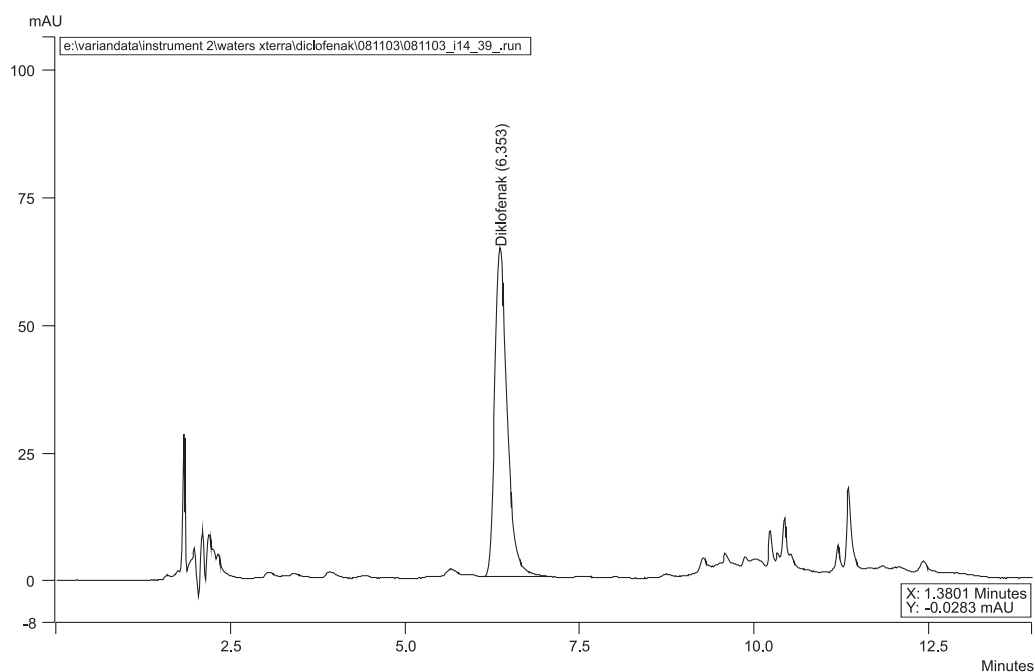


Fig. 8. Chromatogram of diclofenac sodium (in 90 min after drug administration).

After that, DNA plasma content increases rapidly and reaches its maximum within 60 min. At the same time, its concentration 14.72 ± 0.07 mcg/mL at administration of 4 mg/kg, and 47.41 ± 0.44 mcg/mL at administration of 8 mg/kg.

Starting with 90 min, there is noted a decrease in DNA plasma concentration, which is decreased 5.37-folds at administration of 4 mg/kg, and 1.68-folds at administration of 8 mg/kg when compared to initial product concentration. This indicated to the fact that DNA at the dose

of $\frac{1}{2} ED_{50}$ is excreted from rat blood flow much more rapidly.

Starting with 120 min, there is noted too low level of DNA plasma concentration (be close to the limit of analytical method).

Pharmacokinetic parameters calculated are as follows: at administration of DNA 4 mg/kg, C_{max} is 15.16 ± 0.24 mcg/mL, T_{max} is 0.8 ± 0.06 h, $T_{1/2}$ is 0.36 ± 0.02 h, Cl_T is 0.04 ± 0.00 L/h, MRT is 1.63 ± 0.04 h, AUC_{0-t} is 20.38 ± 0.54 mcg×h/mL, $AUC^{0-\infty}$ is 20.93 ± 0.51 mcg×h/mL,

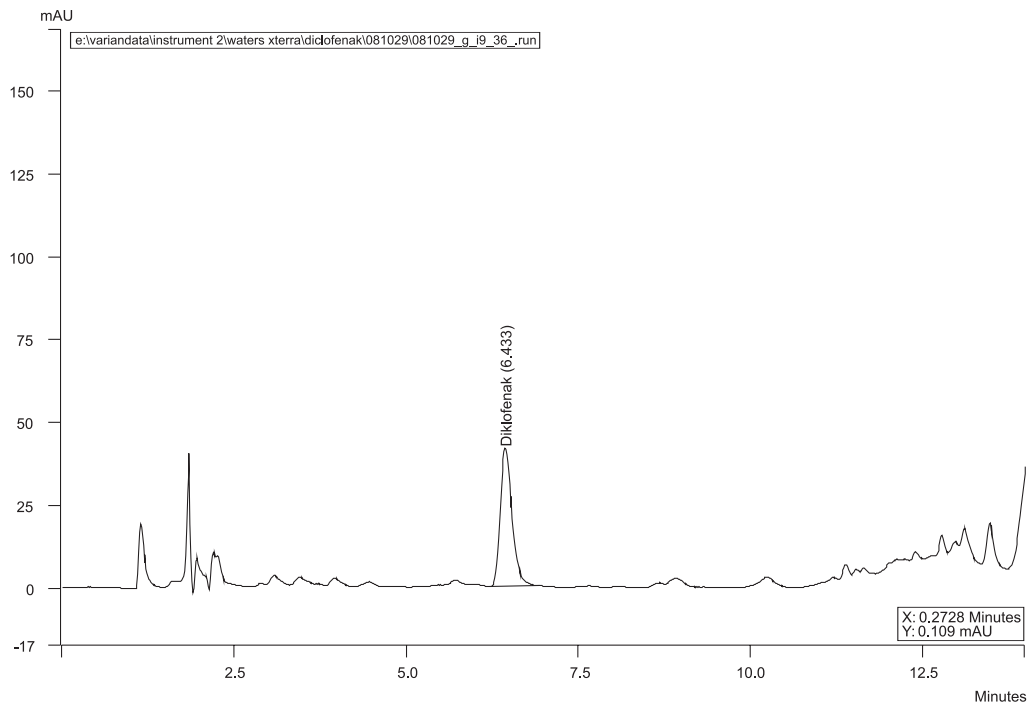


Fig. 9. Chromatogram of diclofenac sodium (in 120 min after drug administration).

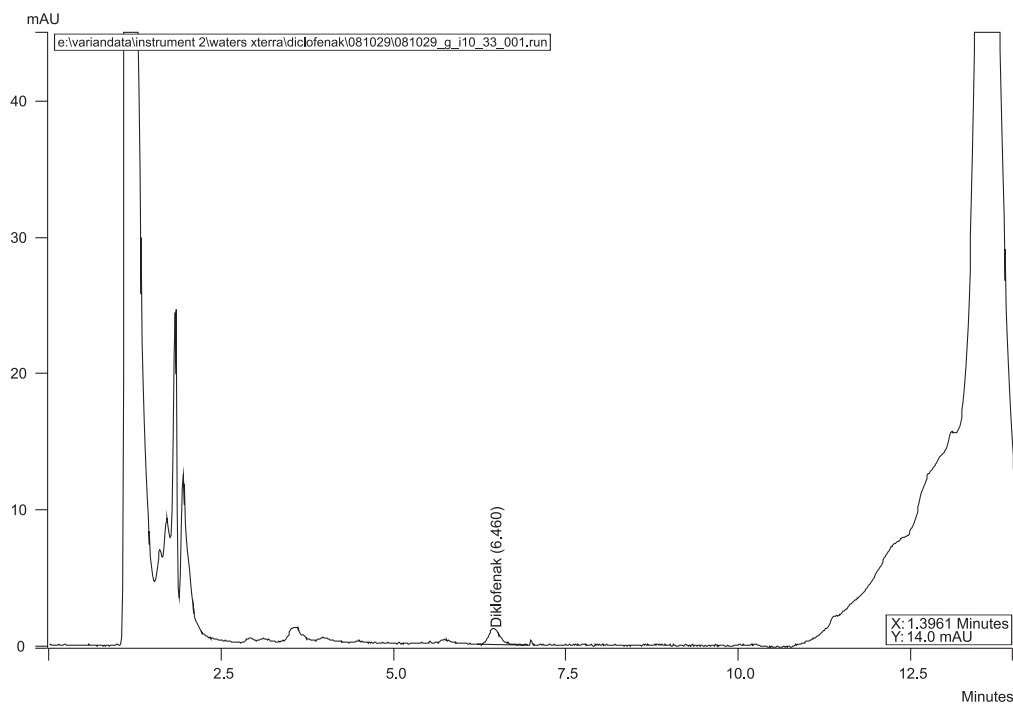


Fig. 10. Chromatogram of diclofenac sodium (in 240 min after drug administration).

$C_{max}/AUC^{0 \rightarrow \infty}$ is 0.73 ± 0.01 mcg/h. At administration of DNA 8 mg/kg, C_{max} is 47.14 ± 0.44 mcg/mL, T_{max} is 1.00 ± 0.00 h, $T_{1/2}$ is 0.09 ± 0.00 h, Cl_T is 0.03 ± 0.00 L/h, MRT is 1.3 ± 0.00 h, AUC_{0-t} is 63.52 ± 0.18 mcgAUC^{0 \rightarrow t} is 63.61 ± 0.18 mcgC_{max}/AUC^{0 \rightarrow \infty} is 0.74 ± 0.01 1/h. The value of $C_{max}/AUC^{0 \rightarrow \infty}$ index administration of 4 mg/kg practically completely corresponds to that of 8 mg/kg.

Thus, in the result of studies conducted, no differences in the dynamics of time during which DNA is present in systemic blood flow on the dose are determined. The results obtained are proved by chromatograms of DNA and control sample (plasma without DNA) for each time interval; they are presented in Fig. 5-12.

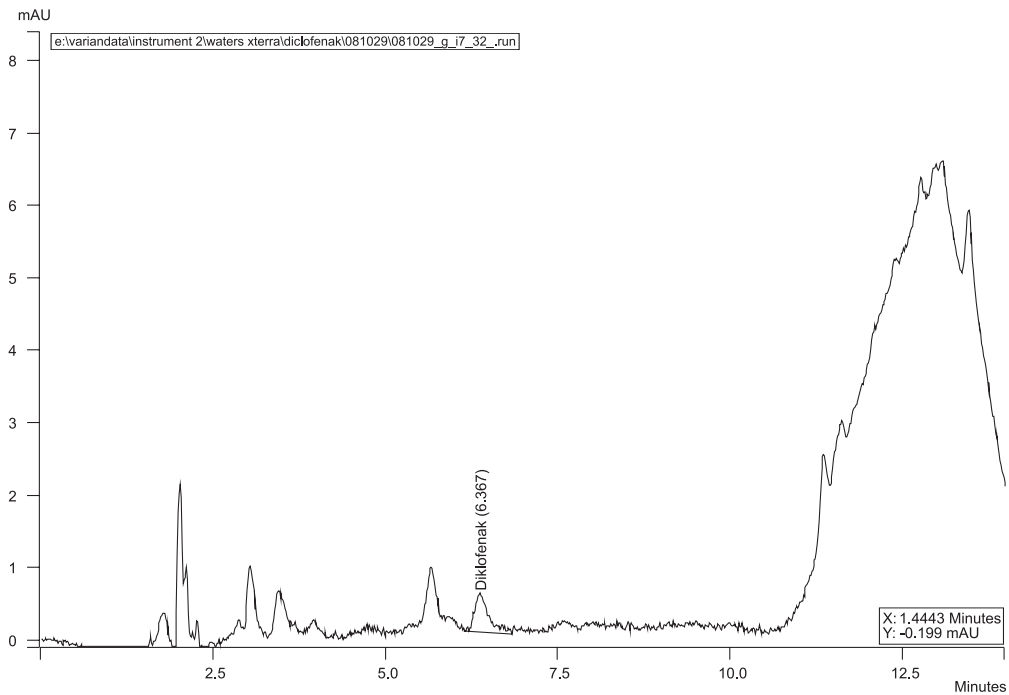


Fig. 11. Chromatogram of diclofenac sodium (in 360 min after drug administration).

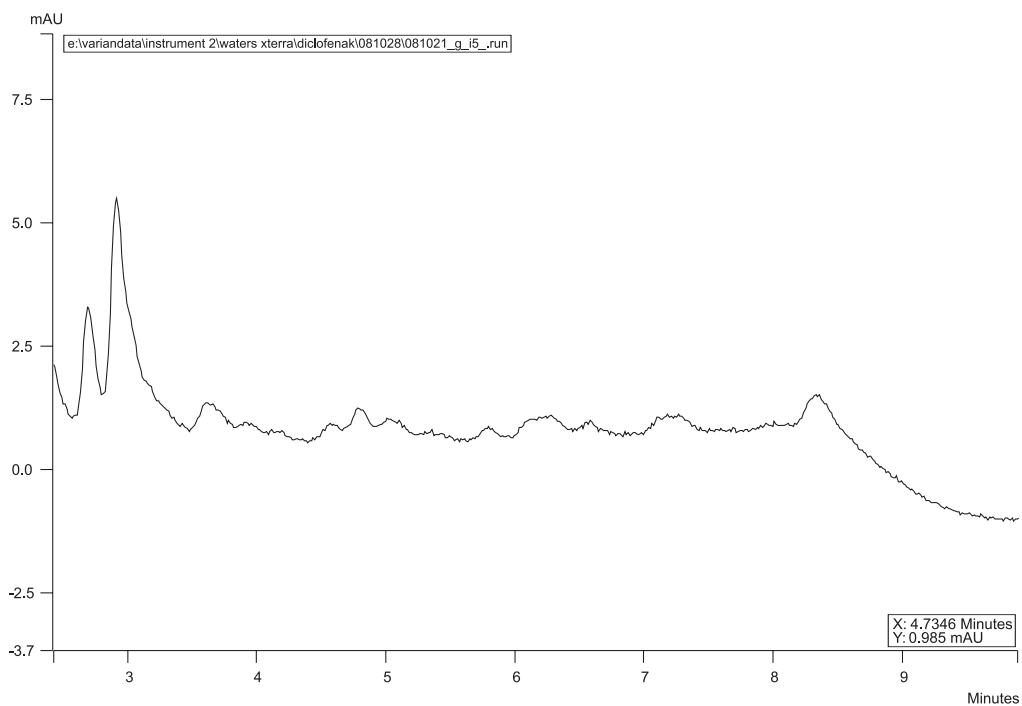


Fig. 12. Chromatogram of the control solution (plasma without diclofenac).

CONCLUSIONS

Dependence of diclofenac sodium concentration of dose and time has been determined in rat experiments by the method of high-performance liquid chromatography. According to study results, first concentrations of diclofenac sodium in rat plasma are registered in rat plasma in 15 min after dose administration at the doses of 4

and 8 mg/kg. That is why this time index is recommended for further preclinical and clinical studies.

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УДК 615.454.2:543.544.5:001.891**Л. В. Брунь, В. І. Маколінець, С. М. Губарь****ДОСЛІДЖЕННЯ ФАРМАКОКІНЕТИКИ ДИКЛОФЕНАКУ НАТРІЮ У ПЛАЗМІ КРОВІ ЩУРІВ МЕТОДОМ ВИСОКОЕФЕКТИВНОЇ РІДИННОЇ ХРОМАТОГРАФІЇ**

Метою даного дослідження є встановлення фармакокінетики диклофенаку натрію в експерименті на щурах методом високоефективної рідинної хроматографії (ВЕРХ). Ідентифікацію та кількісне визначення диклофенаку натрію в субстанції диклофенаку натрію проводили методом ВЕРХ. Для проведення експерименту було використано 94 щурів-самців масою 250-300 г. Тварини в експерименті були розділені на 15 груп. Диклофенак натрію розчиняли у воді очищеній та вводили перорально в дозах ED_{50} та $\frac{1}{2} ED_{50}$, що складає відповідно 8 мг/кг та 4 мг/кг маси тіла тварин. Тварин виводили з досліду через 15 хв, 30 хв, 60 хв, 90 хв, 120 хв, 240 хв та 360 хв після введення препарату. Для екстрагування диклофенаку натрію із зразків плазми крові щурів був використаний метод твердофазової екстракції, який був модифікований нами з урахуванням мікроконцентрації діючої речовини. Залежність концентрації диклофенаку натрію від часу та дози дослідили за допомогою методу ВЕРХ. При проведенні досліджень підтверджено кількісний вміст субстанції диклофенаку натрію та відповідність доз, які вивчаються (4 мг/кг і 8 мг/кг). У результаті проведених досліджень не виявлено відмінностей у динаміці часу знаходження диклофенаку натрію у системному кровообігу від дози. Отримані результати підтверджені хроматограмами диклофенаку натрію та контрольного зразка (плазма крові без диклофенаку) на кожний інтервал часу. Перші концентрації диклофенаку натрію реєструються в плазмі крові щурів через 15 хв після введення субстанції у дозах 4 та 8 мг/кг. Тому цей часовий показник рекомендується для подальших доклінічних та клінічних досліджень.

Ключові слова: фармакокінетика; нестероїдні протизапальні препарати; диклофенак натрію; глюкозамін; високоефективна рідинна хроматографія

УДК 615.454.2:543.544.5:001.891**Л. В. Брунь, В. І. Маколінець, С. Н. Губарь****ИССЛЕДОВАНИЕ ФАРМАКОКІНЕТИКИ ДИКЛОФЕНАКА НАТРИЯ В ПЛАЗМЕ КРОВИ КРЫС МЕТОДОМ ВИСОКОЕФЕКТИВНОЇ ЖИДКОСТНОЇ ХРОМАТОГРАФІЇ**

Целью данного исследования является установление фармакокинетики диклофенака натрия в эксперименте на крысах методом высокоэффективной жидкостной хроматографии (ВЭЖХ). Идентификацию и количественное изучение диклофенака натрия в субстанции диклофенака натрия проводили методом ВЭЖХ. Для проведения эксперимента были использованы 94 крысы-самцы массой 250-300 г. Животные в эксперименте были разделены на 15 групп. Диклофенак натрия растворяли в воде очищенной и вводили перорально в дозах ED_{50} та $\frac{1}{2} ED_{50}$, что составляет 8 мг/кг и 4 мг/кг массы тела животных. Животных выводили из эксперимента через 15 мин, 30 мин, 60 мин, 90 мин, 120 мин, 240 мин та 360 мин после введения препарата. Для экстрагирования диклофенака натрия из образцов плазмы крови крыс был использован метод твердофазовой экстракции, который был модифицирован нами с учетом микроконцентрации действующего вещества. Зависимость концентрации диклофенака натрия от времени и дозы исследовали с помощью метода ВЭЖХ. При проведении исследований подтверждено количественное содержание субстанции диклофенака натрия и соответствие изучаемых доз (4 мг/кг и 8 мг/кг). В результате проведенных исследований не определены различия в динамике времени нахождения диклофенака натрия в системном кровотоке от дозы. Полученные результаты подтверждены хроматограммами диклофенака натрия и контрольного образца (плазма крови без диклофенака) на каждый интервал времени. Первые концентрации диклофенака натрия регистрируются в плазме крови крыс через 15 мин после введения субстанции в дозах 4 та 8 мг/кг. Поэтому этот показатель времени рекомендуется для дальнейших доклінічних и клинических исследований.

Ключевые слова: фармакокінетика; нестероїдні протизапальні препарати; диклофенак натрію; глюкозамін; високоефективна жидкостная хроматографія

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