

# Effect of Clinoptilolite Acid Activation on Ceftriaxone Sorption from Wastewaters

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## Abstract

*Antibiotics residues are considered to be anthropogenic environmental pollutants and represent a serious danger to living organisms. Antibiotics residues in the environment, even in very small trace level amounts, cause resistance in bacterial populations, which inevitably reduce their therapeutic effectiveness against infectious diseases. Nowadays, antibiotics are found in soil, food, plants on the surface, in wastewaters and potable water. The presence of these substances is clearly associated with a hazardous risk to human health and requires research to prevent their appearance and spread in the environment.*

*The aim of the work was to study the adsorption properties of natural and modified clinoptilolite in relation to  $\beta$ -lactam broad-spectrum antibiotic namely ceftriaxone from the group of cephalosporins from aqueous solutions as a model of wastewaters as well as to develop an analytical method for estimation of ceftriaxone content in the above-mentioned solutions using high performance liquid chromatography (HPLC). Based on the results obtained, the possibility of using natural zeolites for adsorptive treatment of wastewater is considered. This will be allowed to solve the problem of the occurrence of antibiotic pharmaceuticals into the aquatic environment with lower costs.*

**Keywords:** Wastewater, Adsorption, Ceftriaxone, HPLC, Validation, Natural zeolite.

## Introduction

Antibiotics have been used in large quantities for several decades and the resistance of pathogens to antibiotics has been intensively studied for a very long time under clinical conditions from the environmental perspectives. Antibiotics and their metabolites are stable enough to obviate water treatment processes and enter the environment,<sup>12</sup> although they are diluted more than a million times in comparison with concentrations in the human body.<sup>10</sup> Antibiotics dispersed in the environment have important consequences for both human health and the ecosystem.<sup>4</sup>

A special problem is the presence of antibiotics and their metabolites in water as they have low biodegradability. It is also necessary to note the limited number of laboratory methods of drug analysis in environmental objects including

sewage.<sup>3,9</sup> In the field of environmental protection, the most important and perspective direction of use of natural zeolites is their use for conditioning of drinking water, treatment of domestic, industrial and agricultural wastes from ammonia nitrogen, oil products and toxic ions of non-ferrous and heavy metals.<sup>1,2,13,14</sup>

The researches have shown that zeolite-containing rocks possess a unique spectrum of physicochemical, adsorption and ion exchange properties due to which they find wide applications in practice of wastewaters treatment.<sup>2</sup> Natural zeolites are potentially industrial mineral raw materials for Georgia and their complex evaluation allows to fully use the mineral wealth and to increase their investment attractiveness.<sup>7,15</sup>

The aim of the present work was to study the possibility of using clinoptilolite (Handaki, Kaspi region, Georgia) as an adsorbent for one of the commonly used  $\beta$ -lactam antibiotics namely ceftriaxone (CEF –  $C_{18}H_{18}N_8O_7S_3$ ) from the group of cephalosporins and to develop an analytical method in model aqueous solution of wastewaters using HPLC.

## Material and Methods

The adsorbent, with a grain size of 0.5 - 1 mm was washed with deionized water and dried in a stream of nitrogen at 300°C for 3 hours. In order to obtain a modified H-form, clinoptilolite was treated by refluxing 2 N and 5 N HCl solution. The thermal stability of clinoptilolite-containing tuff was assessed by the thermogravimetric analytical method. It was found that there are two endothermic effects at 120 °C and 170 °C associated with a gradual loss of water. The total mass loss over a wide temperature range was 13.5%. The exothermic effect at 700 °C indicates the destruction of clinoptilolite structure. On this basis, it is assumed that with the loss of adsorption and constitutive water, the adsorption capacity of clinoptilolite tuff increases.<sup>8</sup>

The structure of zeolite – clinoptilolite was determined by infrared (IR) spectroscopy. IR analysis was performed using Agilent Cary 630 FTIR spectrometer (USA). The IR spectra of the zeolite were recorded in the region of vibrations of the aluminosilicate framework of the middle and far regions. The structures of both the initial and modified H-clinoptilolite were studied by X-ray diffractometry using diffractometer DRON-1.5 (Russia).

The dynamic characteristics of the adsorption process were studied using the specially constructed dynamic type laboratory equipment. The equipment that was used to study

adsorption under dynamic conditions consists of three parts: 1) a glass adsorption column; 2) a cylindrical electric furnace and 3) a high-pressure pump. In dynamic condition through the prepared zeolite samples placed in a glass adsorption column the studied antibiotic test solution at a concentration of  $1.0 \text{ mg mL}^{-1}$  was passed. The experiment was carried out at  $20^\circ\text{C}$  with liquid flow rate of  $1.0 \text{ mL min}^{-1}$ .

The change in the concentration of ceftriaxone at the output of the adsorption column was determined by HPLC. Chromatographic analysis was performed using HPLC system - LC-20AD Prominence Shimadzu (Japan) and a column - Agilent SB-C18  $4.6 \times 250 \text{ mm}$ ,  $5 \mu\text{m}$ . To prepare standard (analytical standard EDQM of ceftriaxone diluted in deionized water) and model aqueous test solutions, water purification system - Aqua FX (China), analytical balance - LEX-210 (USA) and pH-meter - Hanna Instruments HI 2211 (USA) were used. All the measuring equipment was properly calibrated and certified.

Chromatographic system parameters were established for determination of ceftriaxone by HPLC analytical method. A mixture of different buffers and acetonitrile (ACN) was used as the mobile phase (MP). The value of pH of the MP was 5 and  $7 \pm 0.1$ . The analysis was performed in isocratic elution mode, the MP flow rate was  $1.0 \text{ mL min}^{-1}$ , the detector wavelength was  $270 \text{ nm}$  and the chromatographic column temperature was  $25^\circ\text{C}$ .

The concentration of CEF  $\text{mg mL}^{-1}$  in test solution is calculated by the eq. (1):

$$C = A_u \times W \times D \times P / A_s \times 100 \quad (1)$$

where  $A_u$  is peak area obtained with the test solution,  $W$  is weigh of standard of CEF (mg),  $D$  is dilution factor (mL),  $P$  is standard purity (%)  $A_s$  is peak area obtained with the standard solution.

The HPLC method has been validated with respect to the following validation parameters: standard solution stability, system suitability test (SST), specificity, linearity-range, accuracy, intra-day precision and sensitivity in accordance with the methodologies described in the reports.<sup>4,17,18</sup>

## Results and Discussion

**HPLC analysis and method validation:** In order to check the chromatographic system performance, the system suitability test was performed by using six replicate injections ( $n = 6$ ) of the CEF standard solution at the concentration -  $1.0 \text{ mg mL}^{-1}$ . The following parameters: the relative standard deviation (RSD) of peak areas -  $RSD_A$ , the RSD of the retention times -  $RSD_{RT}$ , the peak tailing factor (the USP coefficient of the peak symmetry), the column efficiency and the number of theoretical plates were measured. The specificity parameter was checked by injecting the standard solution, the negative control solution as a blank (solution without CEF) and the model test

solution. The CEF peak was pure. The purity factor (980) was greater than the purity threshold (950). Figure 1 shows the chromatogram obtained from the CEF standard solution.

For the linearity-range study, the standard working solutions were prepared from the initial CEF standard solution at six different concentrations from  $0.0001 \text{ mg mL}^{-1}$  to  $2.0 \text{ mg mL}^{-1}$ . Six replicate injections ( $n = 6$ ) were given for each concentration level. This parameter was checked by the square of the correlation coefficient  $R^2$  (acceptance criteria:  $> 0.999$ ), the  $RSD_A$  (acceptance criteria:  $< 2.0\%$  for concentrations  $> 1.0 \text{ mg mL}^{-1}$ ;  $< 5.0\%$  for concentration  $< 1.0 \text{ mg mL}^{-1}$  and for the last concentration estimated as the limit of quantification -  $LOQ < 10\%$ ) and the  $RSD_{RT}$  (acceptance criteria:  $< 1.0\%$ ).

The calibration curve (plot of linearity) was constructed by plotting the average peak area of CEF versus the corresponding concentration of the injected working standard solution. The calibration curve (linearity graph) is linear and the square value of correlation coefficient ( $R^2 = 0.99998$ ) indicates very good linearity in a wide range of concentrations of CEF at  $270 \text{ nm}$ . Figure 2 shows the linearity graph for CEF.

The LOQ was established for checking the sensitivity parameter of the method by injecting a series of stepwise-diluted working standard solutions. The LOQ was estimated by calculating the  $RSD_A$  for six replicate injections ( $n = 6$ ), which should be  $< 10\%$  and the ratio of signal to noise ( $s/N$ ) -  $> 10$  (acceptance criteria).

The accuracy was assessed by comparing the analyte amount determined versus the known amount spiked at three different concentration levels (80%, 100% and 120% of the model test solution  $1.0 \text{ mg mL}^{-1}$  CEF) with three replicate injections ( $n = 3$ ). The average percentage recovery should be within 95.0-105.0% and the RSD of percentage recovery rates for three individual determinations should be not more than 5.0% for each concentration level of spiked test solution (acceptance criteria).

The intra-day precision of the analytical method was estimated by measuring repeatability on six individual determinations of CEF concentration in model test solution at the same concentrations (100% of the model test solution). The parameter was checked during the accuracy study. The precision was checked by the  $RSD_C$  for six individual determinations of CEF concentration (mg/mL) which should not be more than 3.0% (acceptance criteria).

Standard solution stability study indicated that CEF solution was stable within 24 hours at ambient temperature. The results obtained from SST, precision and accuracy studies and the value of LOQ are given in table 1. Hence, the analytical HPLC method was precise, accurate, specific and linear to determine quantitatively CEF in test solution during sorption experiment.

**Sorption properties of the initial and modified clinoptilolite:** Based on IR spectra of clinoptilolite, the zeolite structure is characterized by Si–O–Si–O–Al intertetrahedral deformation and valence oscillations in the region of  $593.6\text{ cm}^{-1}$  and  $520.9\text{ cm}^{-1}$  (Figure 3a). After treatment of the sample with 2 N acid solution (HCl), various

impurities are removed and the zeolite structure improvement takes place. This is indicated by the disappearance of bands in the spectrum of initial clinoptilolite in the areas of  $1430.4\text{ cm}^{-1}$  and  $875.0\text{ cm}^{-1}$ . These bands are typical for  $\text{CO}_2$  and  $\text{SO}_3$  (Figure 3b).

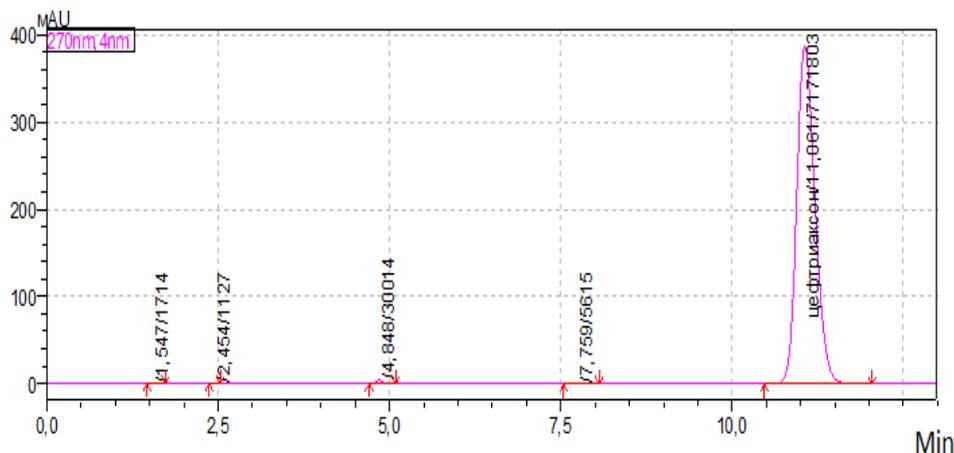


Figure 1: The chromatogram of the CEF standard solution (peaks with RT=1.547, 2.454, 4.848, 7.759 belong to solvent; principal peak with RT=11.061 belongs to CEF)

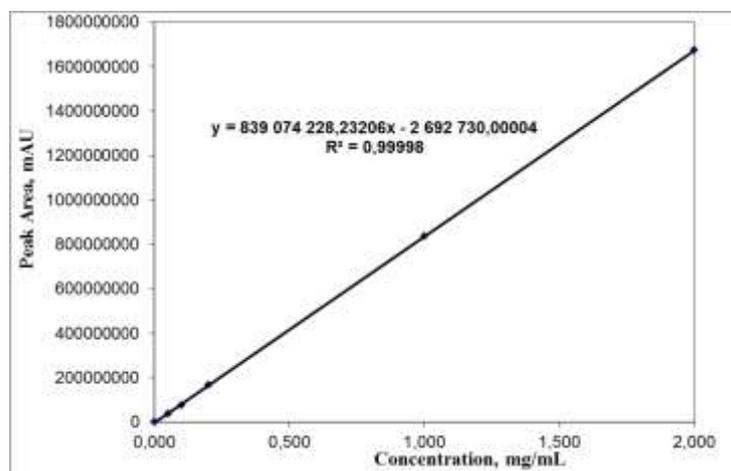


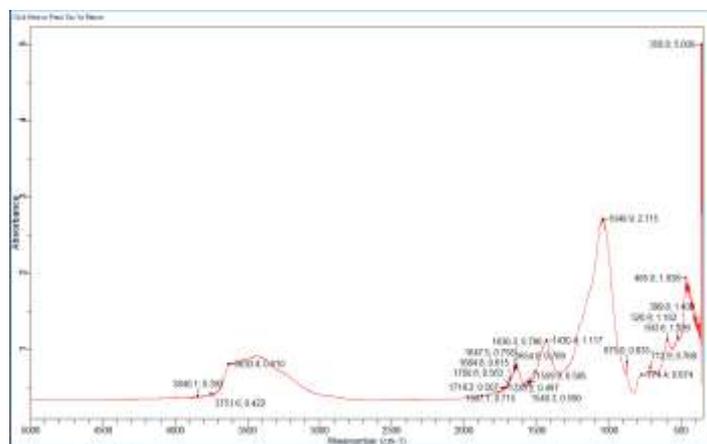
Figure 2: The calibration curve at the concentration range of  $0.0001\text{--}2.0\text{ mg mL}^{-1}$  of CEF

Table 1  
The method validation data

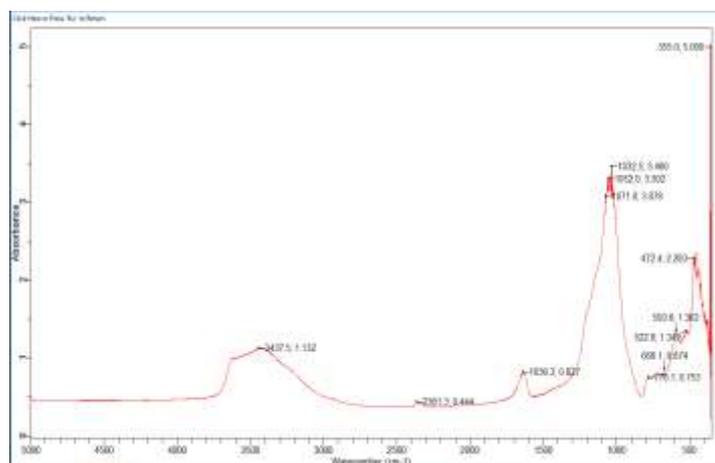
Validation parameter	Characteristic	Result	Acceptance criteria
SST	Column efficiency	>3600	> 2000
	Symmetry	0.95-1.2	< 2.0
	RSD <sub>A</sub> (n = 6), %	0.130	≤ 2.0
	RSD <sub>RT</sub> (n = 6), %	0.261	≤ 1.0
Precision	RSD <sub>C</sub> (n = 6), %	2.114	≤ 3.0
Accuracy	Recovery, % at 80%	98.5	95.0 - 105.0
	Recovery, % at 100%	99.7	
	Recovery, % at 120%	101.1	
	Main recovery, %	99.8	
LOQ	Concentration, mg mL <sup>-1</sup>	0.0001	-
	s/N	> 23	> 10

In addition, dealumination of zeolite begins. This is indicated by the change of frequencies in the spectrum of aluminosilicate carcass of intra-tetrahedral valence vibrations Si–O–Si from 1040.9 cm<sup>-1</sup> to 1052.0 cm<sup>-1</sup>. Worth mentioning, that in the characteristic spectrum of clinoptilolite structure, the intensity of Si–O–Si inter-tetrahedral bands of oscillations has changed insignificantly.

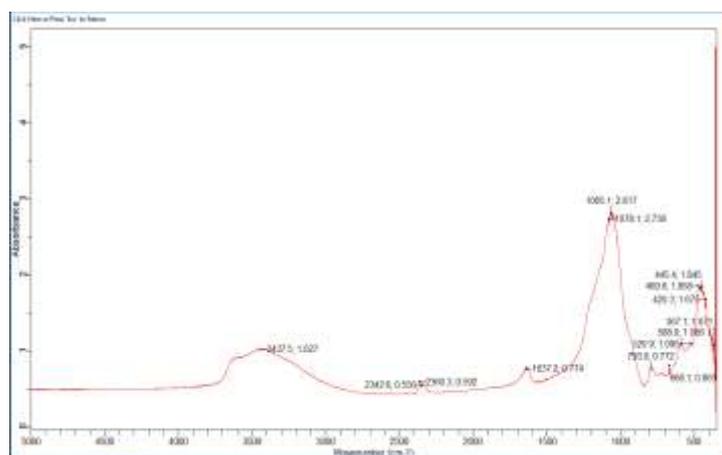
Treatment of zeolite with 5 N acid solution (HCl) has significantly reduced these vibration bends and accordingly, the intensity of intra-tetrahedral valence Si–O–Si vibrations has increased to 1065.1 cm<sup>-1</sup>. This indicates an increase of module of SiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub> in zeolite (Figure 3c). Thus, acid treatment of zeolite does not destroy the structure of zeolite. X-ray diffractometric analysis of the structures of both the initial and modified clinoptilolite indicates that the initial zeolite is preserved after modification (Figure 4).



(a)



(b)



(c)

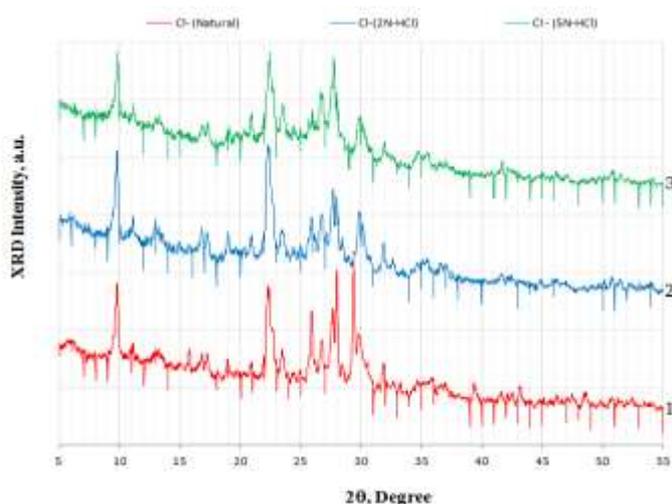
Figure 3: IR Spectra of (a) initial clinoptilolite, (b) modified clinoptilolite (with 2 N HCl solution) and (c) modified clinoptilolite (with 5 N HCl solution)

Clinoptilolite-containing rocks belong to the zeolite group and are characterized by a two-dimensional system of channels and have a more open structure. Depending on the configuration and the size of pollutant molecules, their adsorption on zeolite can occur both on the principle of competing adsorption and on the principle of molecular-sieve effect. The study involved studying the equilibrium of the liquid-zeolite system and determining the effect of the nature of the antibiotic and the nature of the sorbent on the equilibrium data. The dynamics of sorption processes considers the spatiotemporal distribution of components between the phases of the system (one of which is solid) that occurs when these phases move relative to each other.

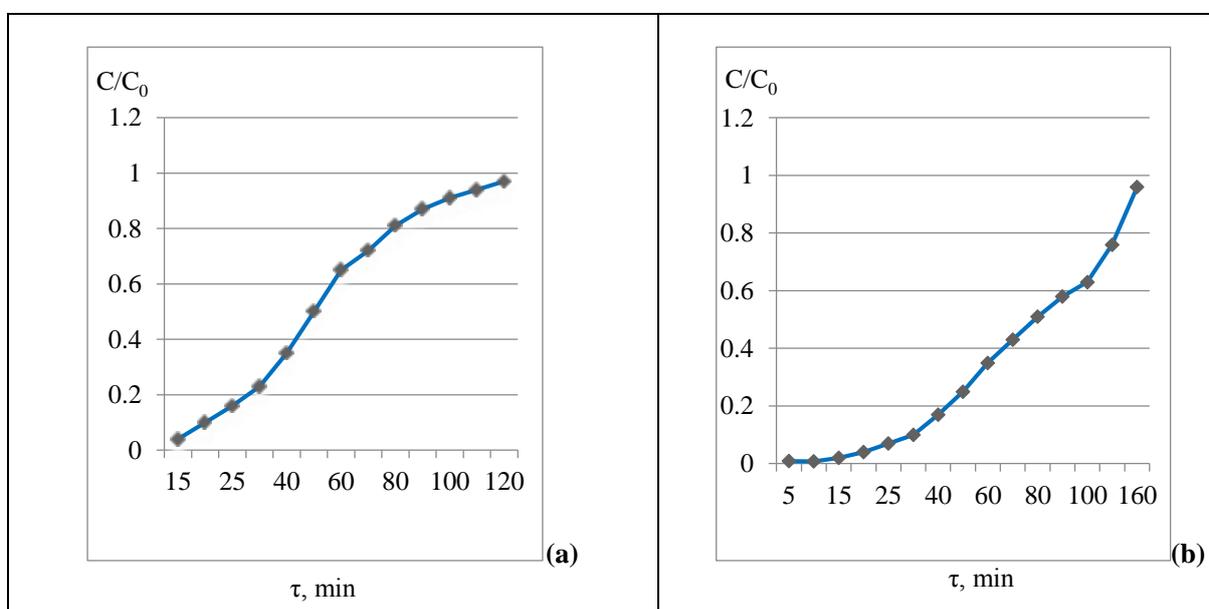
Previously, the authors have investigated adsorption properties of natural zeolite – original and modified clinoptilolite in relation to some fluoroquinolone antibiotics (moxifloxacin and norfloxacin).<sup>16,19</sup> Experimental study of

adsorption dynamics usually has two goals: checking the adequacy of the model to the experiment and obtaining information from the output adsorption curves necessary to select the adsorbent. As a result, the basic information was obtained on the operation of the layer - sorbent-output curves for various process parameters.

The experiments were carried out until the zeolite layer was fully worked out (until the appearance behind the sorbent layer pollutant concentration equals to its concentration in the initial solution -  $C_0$ ). Output (breakthrough) curves are plotted in coordinates  $C/C_0$  and  $\tau$  where  $C$  is concentration of pollutant component – CEF ( $\text{mg mL}^{-1}$ ),  $\tau$  is time (min). The following interrelated parameters were determined from graphical data: the dynamic ( $a_d$ ) and equilibrium ( $a_e$ ) adsorption activity of the nozzle; the time of sorbent layer protective action ( $\tau$ ); the length of mass transfer zone ( $L_0$ ) and the degree of sorbent layer utilization ( $\eta$ ).



**Figure 4: X-ray diffractograms of (1) initial clinoptilolite, (2) clinoptilolite modified (with 2N HCl solution) and (3) clinoptilolite modified (with 5N HCl solution)**



**Figure 5: Output adsorption curves of CEF on clinoptilolite activated with (a) 2 N HCl solution and (b) on clinoptilolite activated with 5 N HCl solution**

Dynamic adsorption activity of the sorbent layer is characterized by the amount of substance adsorbed before the breakthrough and is calculated by the eq. (2):

$$a_d = a_e \times (1 - L_0/L) \quad (2)$$

where  $a_e$  is equilibrium adsorption activity of the sorbent layer ( $\text{mg g}^{-1}$ ),  $L$  is length of the sorbent layer (cm) and  $L_0$  is length of the mass exchange zone (cm).<sup>6,11</sup>

When the sorbent is treated with acid solution, an equivalent exchange of extra-frame cations for hydrogen ion and dealumination occurs accompanied by the formation of silanol groups. The change in the chemical nature of the active sites and the porosity of clinoptilolite as a result of its treatment with acid leads to an increase in the sorption activity of clinoptilolite, which is associated with an increase in pore size due to the unlocking of the channels of the aluminosilicate skeleton of the sorbent during dealumination and this contributes to a greater accessibility of sorbent molecules to active centers. Figure 5 shows the adsorption curves of ceftriaxone on acid-activated clinoptilolite.

Analysis of the data obtained from the output adsorption curves showed that the degree of use of the clinoptilolite layer activity in the case of modification with 5 N HCl solution is higher than in the case of the sorbent modified with 2 N HCl solution. The indices of dynamic ( $a_d$ ) and equilibrium activity ( $a_e$ ) of the sorbent layer are improved. Adsorption isotherms in both the first and second cases are elongated relative to the axis of the equilibrium concentration  $C_{eq}$  based on the monomolecular adsorption of ceftriaxone on the surface of the zeolite.

## Conclusion

Thus, natural zeolite – clinoptilolite of the Georgian deposit (Khandaki), as a selective, effective, eco-friendly and inexpensive adsorbent can be successfully used in process of treatment of industrial wastewaters from pharmaceutical pollutants of different nature.

## Acknowledgement

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