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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF BUPROPION USING H-CLINOPTILOLITE AS A SORBENT FOR PLASMA AND URINE PURIFICATION

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Abstract. The coupling of solid-phase extraction (SPE) using H-clinoptilolite as an adsorbent with a high performance liquid chromatography (HPLC) method was developed for the high efficiency enrichment and rapid determination of bupropion in plasma and urine. The effect of the adsorbent particles size and pH on adsorption behavior of bupropion to H-clinoptilolite was evaluated. The experimental conditions of the sorbent preparation were developed, the effective solvents for the sorbent conditioning were chosen. The optimal eluent for bupropion extraction by H-clinoptilolite was chosen.

Keywords: H-clinoptilolite, bupropion, plasma, urine, HPLC.

1. Introduction

In the recent years solid-phase extraction (SPE) techniques have been of enhanced interest in the use and development of novel assays for toxic substances concentrating and complicated biological matrices purification in chemical-toxicological analysis [1-3]. Synthetic sorbents modified with specific reactive species or functional groups are commonly used for the selective adsorption of substances in accordance with their physical and chemical properties [4-6]. Porous HLB polymers, cyanopropyl (CN), phenylic (PH), and C1, C2, C8 types of SPE columns, as well as silicone covered with carboxymethyl cellulose as a filling material for SPE columns are commonly used for basic compounds extraction like amines from solutions and biological matrices [7-10]. However, in aggressive environment, particularly in strong acidic media, their sorption ability decreases significantly owing to the modifier reactant washing out. Thus the intensive studies of the efficient sorbents for purification, concentration and separation of complex multicomponent mixtures, like acidic extracts of corpses, blood, urine which are the common objects in forensic chemical analysis practice are the nowadays urgent challenges.

Natural sorbents, including zeolites, have been shown to exhibit the high rate of sorption equilibrium setting, resistance to aggressive environments, thermal stability, ability for regeneration, and low cost. Clinoptilolite is a zeolite of the heulandite group being the most abundant in nature. Large deposits of natural clinoptilolite occur in sedimentary deposits located in Transcarpathian region of Ukraine.

Clinoptilolite is a microporous crystalline hydrated aluminosilicate mineral characterized by cage-like structure and known composition, with high internal and external surface areas, and high cation exchange capacity. This sorbent has been particularly useful in concentrating and separating metals cationic species and adsorption of gases and catalyst support [11-15]. Natural clinoptilolite is suitable for use in industrial waste purification and filtration systems, in engineering, medicine, and agriculture [16-18]. However, there still have been no publications reporting its application in forensic chemical analysis for preparation and purification of probes obtained during toxic substances isolation from biological samples.

Our studies were focused on natural zeolites sorption ability evaluation and particularly on clinoptilolite originated from the Transcarpathian deposits. We demonstrated that natural zeolites, including clinoptilolite, were efficient absorbents for separation and purification of pharmaceutically active compounds. Zeolites have large cation exchange capabilities and are widely used as cation exchangers. Moreover, zeolite molecular sieves are in extensive use as selective adsorbents, which allow their using in analytical testing of biological fluids (blood, plasma, urine) as they are capable

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of concentrating and separating of organic compounds with basic and acidic properties in their mixtures with proteins, fats and other organic species contaminations, as well as electrolytes.

We used bupropion as a sample biologically active compound for evaluation and HPLC-UV assay of its detection development. Bupropion (1-(3-chlorophenyl)-2-[(1,1- dimetylethyl)-amino]-1-propanone) is the well-known antidepressant, overdose of which is accompanied with toxic effects, often fatal [19].

The objectives of the current study were to determine the sorption ability of natural zeolite – clinoptilolite originated from Transcarpatian region. The study was focused on the sorption of bupropion micro amounts introduced into plasma and urine to zeolite. Dependence of bupropion removal percentage from protein contaminations and trace endogenous substances in the biological fluids was also evaluated.

2. Experimental

The clinoptilolite samples were obtained from the Sokyrnytsia deposits in Transcarpathian region (Ukraine). Chemical composition (mas %) of the clinoptilolite samples was determined as: SiO_2 – 71.5; Al_2O_3 – 13.1; Fe_2O_3 – 0.9; TiO_2 – 0.5; CaO – 3.44; MgO – 0.68; P_2O_5 – 0.014 and K_2O-Na_2O – 3.03; SiO_2/Al_2O_3 mass ratio was 5.5. Sorption capacity of natural zeolite samples varied with its batch. To avoid zeolite batch origin influence on its sorption capacity clinoptilolite was modified to H^+-form with the ion-exchange process when its exchange sites were saturated with H^+ ions. It was found that sorption ability of H-clinoptilolite type was enhanced. Analytical purity HCl was used to treat clinoptilolite and to modify it with H^+ ions.

In the present studies natural clinoptilolite samples were crushed in a rotary mill and sieved into five fractions with the particles size ranges of 0.10–0.12; 0.20–0.22; 0.30–0.32; 0.50–0.70 and 0.80–1.00 mm. The sorbent was washed with distilled water to remove dust and then dried on air. The clinoptilolite chemical transformation in H-form was achieved by the samples treatment with 1 M HCl solution (20 ml of solution per 1 g of the adsorbent) and tumbling for 6 h. The samples were then decanted and washed with distilled water until chloride ions were not identified in the supernatant. The clinoptilolite samples were dried at 383 K and stored in dessicator.

Adsorption properties of H-clinoptilolite were determined in dynamic conditions using SPE technique. H-clinoptololite was applied for SPE cartridges construction: medical syringes of 12 mm in diameter and 2 ml by volume were filled with 0.6 g of solid and dried clinoptilolite fractions of each size. Processes of adsorption and desorption were studied at 293 ± 1 K on blank samples of bupropione as its aqueous solutions with concentration 400 μg/ml.

Before testing the cartridges were firstly rinsed with 2 ml of 0.1 M HCl solution in methanol and 2 ml of distilled water. Afterwards 4 ml of bupropione aqueous solution were passed through the cartridges. Bupropion desorption from the sorbent also was carried out in dynamic conditions. Cartridges were firstly rinsed with 4 ml of water and 3 ml of water-methanol solution (1:1). Sorbent was dried in a stream of nitrogen and bupropion was eluted with 3 ml of 0.1 M HCl solution in methanol. Flow rate of solvents, bupropion blank solutions, plasma and urine through cartridges was 0.5 ml/min, which was ensured with Automated Solid Phase Extraction System "Manifold" application.

Biological systems such as plasma and urine are characterized by different pH values, so the effect of pH on the bupropion adsorption capacity to H-clinoptilolite packed cartridges from its aqueous solutions was studied. For the pH effect testing diluted hydrochloric acid or NaOH were added to bupropion aqueous solutions (400 mg/ml) for adjusting pH in the range of 2.5–10.0 and the entire samples were injected through the prepared sorbent.

The samples of plasma and urine were subjected to the same experimental procedure developed for the high efficiency bupropion extraction. Various amounts of bupropion solution were added to 4 ml of plasma. Bupropione solutions concentrations were set in the range of its therapeutic dose (0.025–0.1 mg/l), toxic dose (0.17–0.20 mg/l), and fatal dose – 0.45 mg/l. All plasma volume was injected through zeolite packed cartridges at a flow rate of 0.5 ml/min. The sorbent was preliminary rinsed with 2 ml of 0.1 M HCl solution in methanol and 2 ml of distilled water. After the plasma sample injection the sorbent was rinsed with 4 ml of water and 3 ml of water-methanol solution (1:1). Sorbent cartridges were dried in a stream of nitrogen and bupropion was then eluted with 3 ml of 0.1 M HCl in methanol. Methanol elutes were dried in the stream of nitrogen, quantitatively dissolved in 200 μl of methanol and were then subjected to HPLC analyzing.

The urine samples volume was 10 ml. Bupropion was added to these samples in amounts ensuring its concentration being in the range of 0.025–0.45 mg/l. Cartridges with the sorbent preparation procedure, rinsing and elution protocols were the same as for plasma testing. Acidic methanolic elutes were dried in a stream of nitrogen and then were dissolved in 200 μl of methanol. The bupropion solutions concentrations were analyzed using HPLC method.

Separation and detection of bupropion in the aqueous phases were achieved using HLPC chromatograph

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Waters 2690 Separation Module, UV diode array detector Waters 996, ACE 5 C18 column (250 x 4.6 mm), thermostatic column compartment ensured the temperature of 298 K. The mobile phase consisted of acetonitrile (solution A) – 0.05 % aqueous solution of trifluoroacetic acid (TFA, solution B). Acetonitrile and TFA had HPLC quality (Merck), water was double-distilled and demineralized. The mobile phase flowed in the mode of sudden step changes in the relative concentrations of A and B solutions. The solutions A and B volumes ratio was 95:5 for the first 2 min, 45:55 during next 20 min, 10:90 between 23 and 24 min, and 95:5 during 3 final min. The volume of the injected sample was 10 μl, the mobile phase flow rate was 1 ml/min. Bupropion in the flow from the HPLC was identified by UV spectrum at 248 nm and according to its retention time. The amount of the adsorbed bupropion was determined with calibration curve.

For the calibration curve plotting bupropion solutions in methanol were prepared in the concentration range of 0.1–40 μg/ml. The solutions were prepared with bupropion hydrochloride standard (Sigma, USA). The calibration curve trend line equation was calculated as the relationship between the surface area of bupropion peak and its solution concentration using Empover Pro device software.

3. Results and Discussion

The adsorption behavior of bupropion to H-clinoptilolite was preliminary investigated by varying the adsorbent particles size used to pack the column. The experimental studies indicated that bupropion showed increased sorption capacity with H-clinoptilolite particles size decreasing in dynamic conditions (Table 1). Thus H-clinoptilolite presented high extraction efficiency due to its specific surface area increasing. Clinoptilolite in H-form with the particles size ranges of 0.10–0.12 and 0.20–0.22 mm almost completely absorbed bupropion from its aqueous solutions. Therefore the sorbent fraction with particles size 0.20–0.22 mm was used in our further studies.

To summarize the effect of pH on bupropion removal, its percentage extraction was found to be pH-dependent. In the current study bupropion showed the highest sorption capacity up to 98 % at pH range of 5.5–7.5 (Fig. 1). It was demonstrated also that bupropion percentage extraction was significantly lower from its acidic solutions and from solutions with pH ≥ 8.

The correlation between pH and absorption efficiency of clinoptilolite was attributed to both zeolite surface chemical characteristics changing and different forms of bupropion in its aqueous solution at varying pH levels. The limited sorption capacity of bupropion to clinoptilolite at low pH may be explained by the fact that surface available OH-groups dissociation was almost completely suppressed. It was also assessed that bupropion adsorption increased with increasing pH, which may indicate the clinoptilolite surface OH-groups dissociation increasing. Bupropion was characterized by two different moieties of the molecule that could undergo protonation-deprotonation depending on the pH of the aqueous solution in which it was dissolved (Fig. 2). Therefore, absorption efficiency of clinoptilolite was found to be affected with both electrostatic attraction of bupropion to –O– groups of the adsorbent and their donor-acceptor interactions.

Table 1

Effect of H^+-modified clinoptilolite particles size on the percentage removal of bupropion from its aqueous solutions (dynamic conditions, *n* = 5)

Particles size ranges, mm	Percentage removal
0.10–0.12	97.2–98.5
0.20–0.22	97.8–99.4
0.30–0.32	92.4–93.9
0.50–0.70	84.1–86.0
0.80–1.00	75.1–77.4

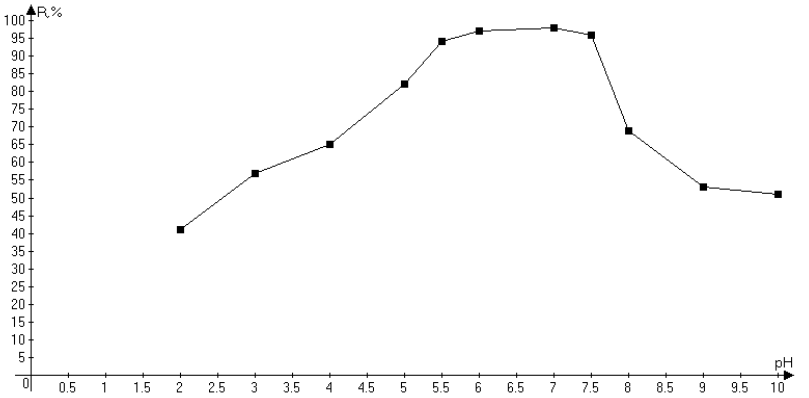

Fig. 1. Effect of pH on the bupropion percentage extraction (*R*) in dynamic conditions

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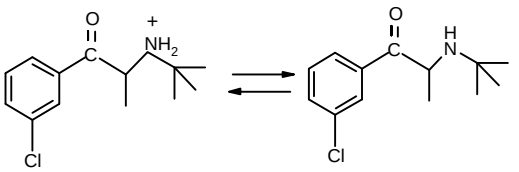
It was also found that the optimum eluent for bupropion desorption from clinoptilolite was 0.1 M HCl solution in methanol. Organic solvents such as methanol, ethanol, and ethyl acetate can elute less then 30 % of bupropion from the sorbent.



pH < 7 pH > 7

Fig 2. Ionic and molecular forms of bupropion in increasing pH

Bupropion was detected and identified in the flow from HPLC with UV spectrum (Fig. 3) and according to the retention time of 12.277 ± 0.051 min. In blank samples, which served as backgrounds during the analysis obtained by passing water, plasma and urine through the clinoptilolite no peaks with similar to bupropion retention parameters were found. Protein contaminations and trace components originating from urine did not affect the bupropion elution profiles (Figs. 4a and 4b).

Calibration curve for bupropion extraction was linear in the concentration range of 0.1–40 μg/ml. The regression analysis allowed to determine the trend line equation of the relationship between the surface area of bupropion peak (Y) and its concentration, μg/ml (X) as $Y = 2.51 \cdot 10^4 \cdot X - 1.78 \cdot 10^3$, with the correlation coefficient $r = 0.9998$. The relative error of bupropion quantitative HPLC-UV analytical determination in its solutions was 1.27 %.

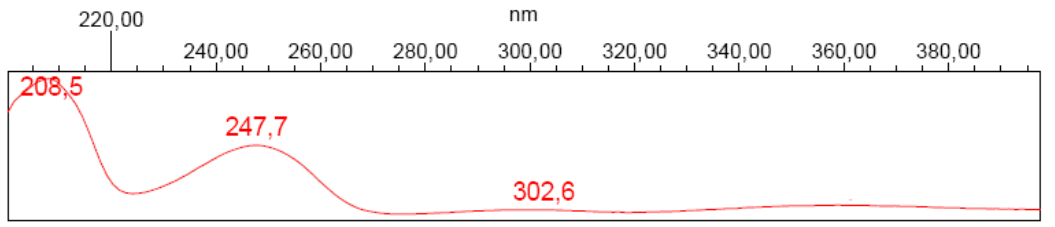


Fig.3. UV spectrum of bupropion

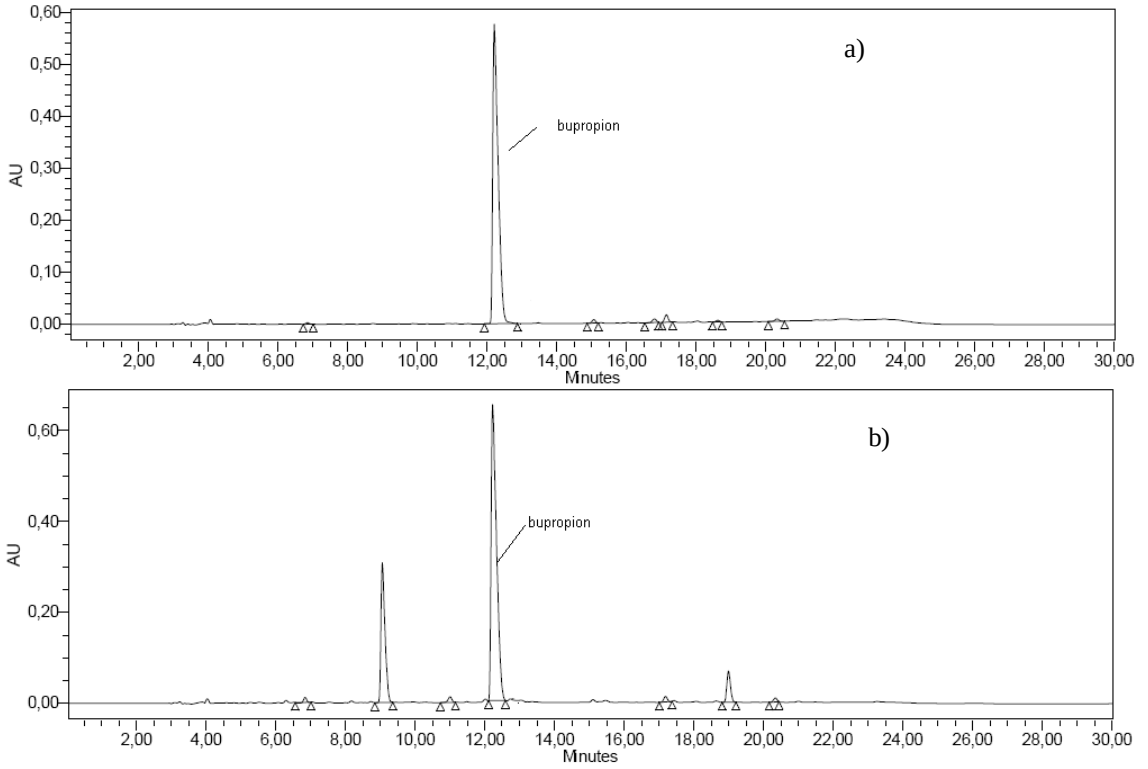


Fig. 4. Chromatograms obtained for bupropion separated from plasma (a) and urine (b) on H-clinoptilolite packed columns with HPLC system

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Table 2

Efficiency of bupropion removal from plasma and urine on H-clinoptilolite packed columns (*n* = 5 for each series)

Biological fluid	Bupropion injected, ng	Eluted fraction of bupropion		RSD, %
		ng ± S.D.	%	
Plasma, 4 ml	100	74.14 ± 1.83	74.14	2.47
	400	306.80 ± 3.96	76.70	1.29
	680	524.80 ± 5.31	77.17	1.01
	800	635.20 ± 4.92	79.37	0.77
	1800	1458.8 ± 6.83	81.04	0.47
Urine, 10 ml	250	230.2 ± 3.3	92.08	1.43
	1000	930.6 ± 7.5	93.06	3.25
	1700	1602.2 ± 8.5	94.24	0.53
	2000	1892.4 ± 9.9	94.97	0.52
	4500	4328.6 ± 14.4	96.19	0.33

The experimental data concerning bupropion HPLC removal from plasma and urine with H-clinoptilolite packed columns extraction are summarized in Table 2. All main experiments were conducted five times. The percentage of bupropion adsorbed to the clinoptilolite is presented by the averages, and standard deviation was performed on the repetitions.

The obtained results proved bupropion percentage removal on H-clinoptilolite packed column from plasma at the level of 74–49 % while the relative standard deviation (RSD) did not exceed 2.47 %. The bupropion extraction percentage from urine was assayed as 92–96 % with high precision (RSD did not exceed 3.25 %).

4. Conclusions

H-clinoptilolite was assayed as the efficient sorbent for solid phase extraction of bupropion from biological fluids (plasma and urine) and its HPLC-UV detection in chemical-toxicological analysis routine. The capacities of bupropion extraction from plasma and urine were 74–79 % and 92–96 %, respectively, with the RSD under 2.47 % and 3.25 % for each series.

The optimal conditions for bupropion separation using ACE 5 C18 column and its HPLC-UV analytical detection at l = 248 nm were developed. The relative error of bupropion quantitative determination in its solutions was 1.27 %.

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ВИКОРИСТАННЯ Н-КЛИНОПТИЛОЛІТУ ЯК СОРБЕНТУ ДЛЯ ОЧИЩЕННЯ ПЛАЗМИ ТА СЕЧІ ПРИ ВИЗНАЧЕННІ БУПРОПІОНУ МЕТОДОМ ВИСОКОЕФЕКТИВНОЇ РІДИННОЇ ХРОМАТОГРАФІЇ

Анотація. *Вивчена ефективність застосування Н-клиноптилоліту як адсорбенту для твердофазної екстракції (ТФЕ) бупропіону з плазми та сечі з використанням високоефективної рідинної хроматографії (ВЕРХ). Досліджено залежність сорбції бупропіону Н-клиноптилолітом від розміру зерен фракції та pH середовища. Розроблено умови підготовки сорбенту, підібрано розчинники для кондиціонування сорбенту, а також підібрано оптимальний елюент для екстракції бупропіону з Н-клиноптилоліту.*

Ключові слова: *Н-клиноптилоліт, бупропіон, плазма, сеча, ВЕРХ.*

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