UNIVERSITY „BABEŞ-BOLYAI”
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FACULTY OF ENVIRONMENTAL SCIENCE AND ENGINEERING

# STUDY OF PHARMACEUTICAL COMPOUND INVOLVED IN ENVIRONMENTAL POLLUTION

## PhD Thesis Summary

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**SUMMARY**

**Keywords:** pitofenone hydrochloride, the active substance, pesticides, adsorption, activated carbon, pharmaceutical compounds, surface, molecular sieves, zeolites, magnetic and optical spectroscopy, molecular modeling , differential scanning calorimetry .

## INTRODUCTION

The existence of multiple types of crystalline structures with different physical and chemical characteristics is called polymorphism and is a phenomenon of wide interest in the pharmaceutical industry. Worldwide, research regarding the obtaining of new solid forms involves the synthesis and physico-chemical and structural characterization of solids, in order to improve the quality of medicines and reduce adjacent effects. Identify as many solid forms has a major impact in the technology because different crystalline forms can generate a wide range of different physico-chemical properties that may affect the use of solid materials. Solid forms include polymorphs (the same compound with different crystalline structure), solvates (in the crystal structure of the compound is included the solvent) hydrate (crystal structure of the compound included water molecules), a salt or co-crystal that includes in the structure two different crystalline components. Parallel crystallization technique offers the possibility of applying a wide range of crystallization method that provides the diversity of experimental data required for the discovery of new solid forms of active pharmaceutical ingredients. To the parallel crystallization is using a crystallize platform which offers the possibility of up to 48 concurrent experiments using different solvents or mixtures of crystallization solvent which provides the ability to apply different thermal regimes (heating, controlled cooling) and also the possibility of stirring and filtering at various stages. The purposes of performed experiments which provide the achievement of thesis consist in applying the different crystallization conditions on the pitofenone hydrochloride compound and highlighting its various solid forms. In the paper are presented: the obtaining of single crystals, diffraction intensities collection and correction, determining the unit cell and space group, obtaining structural model, refining the obtained structural model. Since these methods are intertwined and largely resolved by the computer programs are determined by molecular modelling based on the theory of quantum chemistry, the geometric structure and molecular size.

In the present study it was proposed **structural characterization by X-ray diffraction and mass spectrometry of pharmaceutical compounds: pitofenone hydrochloride, efavinrez, inclusion compounds of pitofenone hydrochloride with β-cyclodextrin and also of same compounds from pesticides class. Also was studied the effect of the activated carbon on potable water in order to purify it. They developed methods on removing pharmaceutical compounds in water samples in the laboratories of the National Institute of Research - Development of Isotopic and Molecular Technologies. <u>The final objective</u>** of this paper consist in studying the pharmaceutical compounds of the pitofenone hydrochloride type, Ibuprofen, Naproxen, Indomethacin and Carbamazepine involved in the treatment of potable water based on the adsorption processes on activated carbon and molecular sieves and the preparation of novel inclusion compounds based on pitofenoe hydrochloride with β-cyclodextrin. Analytical techniques used are presented below.

*X ray diffraction* is an important tool used to obtain valuable structural information in crystalline materials. Since single crystals growth was possible by various crystallization methods, then single crystal X ray diffraction is the optimal technique used to determine the crystal structure.

*Mass spectrometry* is a physical method used in particular for the analysis of organic materials which consist essentially of ionization of the substance under investigation, followed by separation of produced ions (molecular ion plus fragment ion) according to the ratio between the mass and charge and registration of them. The mass spectrum represents the masses recording and the relative abundances of the ions produced, and are a characteristic of each compound. By mass spectrometry can be solve the following main aspects regarding an organic substance: finding the molecular mass, finding the molecular formula, elucidation of the molecular structure and determining the isotopic markings. Mass spectrometry is the standard method for analysing the experimental results of isotopic tracer, allowing to easily establishing the presence of isotopes and their position in the molecule.

By the *high performance liquid chromatography method* was recorded the calibration line of pitofenone that can be used later in order to determine its from water samples.

Pitofenone hydrochloride analysis was performed by *high performance gas chromatography method* with flame ionization detector, the detector being in this case a mass spectrometer. It has been experimentally determined the calibration line of the pitofenonă hydrochloride solution, and by the calibration line was determined the concentration of chromatographic peak in water samples taken from the river Somes. Considering the sample preparation of water results the limit of quantification and detection.

Spectrophotometric measurements UV-VIS aim the adsorption of pitofenone hydrochloride (pollutant in category of drugs) from waste water on porous molecular sieve and activated carbon. With the help of gas chromatography coupled with mass spectrometry, and UV-VIS spectrophotometry was determined the kinetics of adsorption on the porous material pitofenone hydrochloride. The studies may be maintained by the adsorption tests at different temperatures for different materials and with different adsorbing pollutants.

The paper is divided into seven chapters covering the topic of thesis.

**Chapter 1** presents the application of different crystallization conditions on pitofenone hydrochloride and highlighting its solid forms. Based on the solid samples results after the experiment, analyzed by the powder X-ray diffraction have identified three new forms of the pitofenone hydrochloride. Further treat structural characterization method that determines the crystal structure of single crystals.

It deduced the from the crystal structure of a solid by obtaining the crystallographic system in which the compound crystallized, the parameters of the unit cell, the space group and atomic positions in the cell. It is determined the crystal structure of (4S)-6-chloro-4- (2-ciclopropiletilin)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one, Efavirenz, more precisely the type of system, space group and how many molecules are found in the unit cell. Are defined as above thecrystal structures for compounds: 2- (4-Methy (phenylsulfanyl-amido-2-thiono-5,5-dioxaphosphorinane dimethyl 1,3,2 $C_{11}H_{16}O_4P_1S_2N_1$ and 2-Phenylsulfonyl-amido-2-thiono-5, 5-dimethyl1,3,2-dioxaphosphorinane $C_{12}H_{18}O_4P_1S_2N_1$ more precise crystallographic system, space group, unit cell parameters and position of atoms in the unit cell. And not least given the size of the molecule treated was calculated with Gaussian program.

In ***Chapter 2*** are determined the structure and molecular weight of the prepared compound using mass spectrometry. Thus this analyse may be carried out in some cases, succeeding the interpretation of fragments obtained from the molecular ion and the structural attribution may be made by comparison of spectral data with those existing in the literature. For the first part are determines pitofenone hydrochloride signals and internal standard using the temperature program at $90^0$C to $300^0$C and electron impact mass spectra. The deduction of the molecular weight can be determined directly if it is possible to accurately measure at least four decimal places. This precision requires high resolution spectrometer (greater than $10^4$). Pitofenone hydrochloride is analyzed using high performance liquid chromatography with ultraviolet-visible detection domain. By this method have been established retention time and wavelength of maximum specific pitofenone hydrochloride. ***Chapter 2*** continues pitofenone hydrochloride analysis using gas chromatography method coupled with mass spectrometer. The method presents the net advantage to gas chromatography with a flame ionization detector in vacuo. Coupled method gas chromatograph-mass spectrometer is very selective, meaning that they can be tracked ions originating from the interest compound eliminating in this mode interference. Further we analyze pitofenone hydrochloride achieved by high performance gas chromatography with flame ionization detector, the detector is in our case mass spectrometer and highlights the limit of detection and quantification limit.

In ***Chapter 3*** are determined the zeolite surface area and porosity from desorption isotherm using Dollimer Heal model. Described futher, absorption of pitofenone hydrochloride on porous materials, zeolite 13X and activated carbon, exactly the amount pitofenone hydrochloride deposited on porous material. ***Chapter 4*** discusses experimental results on determining the crystal structure and molecular weight for studied pharmaceutical compounds.

The goal of ***Chapter 5*** is to develop a sensible and sensitive method for determining polar pharmaceuticals in water samples. The method is used to study the extraction process of the pharmaceuticals in an aqueous medium in the solid phase. The concentration of same polar pharmaceuticals is determined in influent and effluent of wastewater treatment plant from Cluj-Napoca.

***Chapter 6*** discusses the study of the absorption of pharmaceutical compounds on the solid phase specifically on activated carbon and zeolite molecular sieves.

In ***Chapter 7*** the inclusion compounds of β cyclodextrin and pitofenone hydrochloride were prepared by methods coprecipitation method and lyophilization in order to increase the oral adsorption capacity by improving drug's taste and smell. For their physico-chemical characterized this inclusion compounds were analyzed by X-ray diffraction, Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, differential scanning calorimetry, scanning electron microscopy and molecular modeling techniques. Structural architecture of inclusion compound was proposed by molecular modeling techniques. The new product obtained can be used in the pharmaceutical industry because it provides improved odor and taste compared with the feedstock pitofenone hydrochloride.

The final part presents the conclusions of the paper. It highlights the main results and summarizes the author's contribution, by obtaining new solid forms by parallel crystallization experiments, by the crystal structure determination by X-ray diffraction. Is highlights the main achievements and summarizes the author's contribution so that the last chapter of the thesis general conclusions are drawn from all the results obtained and presented and remaining open issues representing new research directions.

## Chapter 1. Determination of molecular weight and crystalline structure for pharmaceutical compositions

**1.1 Pharmaceutical ingredients polymorphism**. The study of solid forms of pharmaceutical compounds (polymorphs, hydrates, solvates, salts, co-crystals) is an important aspect in the pharmaceutical industry, because a minor change in the structure of the

pharmaceutically active compound can lead to major changes of essential properties such as solubility and bioavailability thus the efficacy can be improved [Werner M, 2004].

**Chapter 2. Correlation of structure and molecular mass by mass spectrometry**

**2.1 General.** To confirm the structure and molecular weight of the prepared compound, it is necessary to analyze it by mass spectrometry. Mass spectrometry can elucidate the following key issues concerning organic substances: *(1) Molecular mass determination; (2) Determination of molecular formula; (3) Elucidation of the molecular structure (4) Establishing isotopic markings.*

**Chapter 3. Remove of pollutants from organic pharmaceutical class from aqueous medium by using porous materials**

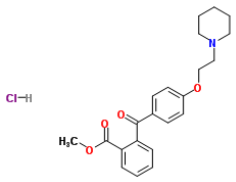
**3.1 General.** The presence of pharmaceuticals and personal hygiene in the aquatic environment has been recognized as an important issue in environmental chemistry. Residues of pharmaceutical products and personal hygiene are found in both sewage and surface waters. pitofenone hydrochloride is used in antispasmic contraction of smooth muscle in particular in tubular organs of the gastro-intestinal tract, resulting in the prevention of stomach, bladder and intestine cramps. The data about the concentration of pharmaceutical products in rivers and lakes, including sludge, are very rare. Detailed data related to the presence of transformations of these compounds into rivers and groundwater are limited to a few countries [Moldovan Z., 2006].

Pollutants variety of types and structures require advanced techniques and methods of water purification so that their concentrations drop to minimum levels allowed by law [Bhatnagar A., 2010]. Many researchers turn their attention to sorbents (ex. Biopolymers) and alternative methods to replace some conventional methods (precipitation, ion exchange, electrochemical methods) expensive or sorbents difficult to regenerate and expensive (ex. Activated carbon) [Monik A. 2013 Rahman N. 2013].
Literature shows that the investigations were conducted in the area combined adsorption-biological treatments to enhance biodegradation of dyes and to minimize the production of byproducts [Lilies G., 2006]. In recent decades zeolites have been investigated as drug transport systems and as adjunctive in therapy of cancer, dietary supplements or antimicrobial agents. Natural and synthetic zeolites are media that allowing adsorption of varying amounts of antibiotics and their gradual diffusion of these in the active forms so can be used as transport and controlled release systems [Bhatnagar A., 2010].

**Chapter 4 Experimental results for determining the crystal structure and molecular weight for pharmaceuticals**

**4.1 Experimental study for the preparation of new solid forms of the pharmaceutical compounds**

***4.1.1 The purpose of experiments***: applying various crystallization conditions on the pitofenone hydrochloride compound in order to obtain new solid forms which were subsequently highlighted [Popeneciu H., 2012]. pitofenone hydrochloride of (2- [4- (2-piperidin-1-yl-ethoxy) benzyl] benzoate) with molecular formula $C_{22}H_{25}NO_4$ HCl and molecular weight 403.9 g/mol is antispasmodic drug currently available in a product combined with fenpiverinium bromide and sodium metamisol.




*Pitofenone hydrochloride*

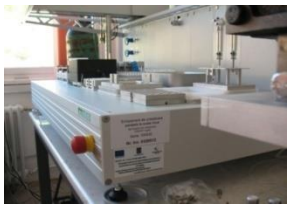


***Figure 4.1*** *Crystallization platform at small scale*

The first step in designing experimental design for the preparation of new solid forms of pharmaceutical compounds is to determine the solubility profile of the compound of interest in a wide variety of solvents. Depending on the solubility of the drug, is taking into account various preparation methods (Figure 4.2). Using the crystallization platform at parallel small-scale was determined the solubility profile of pitofenone hydrochloride in 24 solvents.

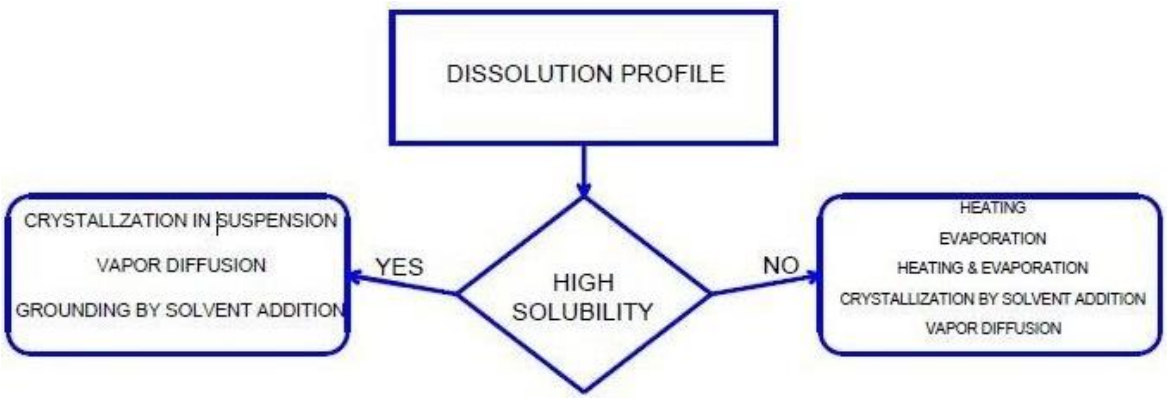


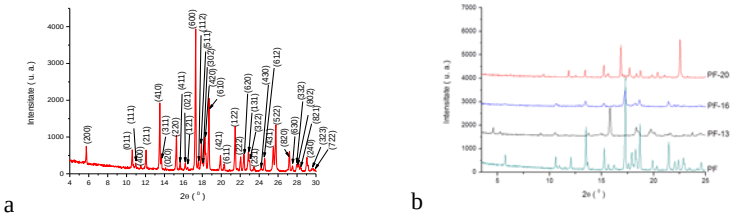

***Figure 4.2*** *Crystallization scheme based on compound solubility*

**4.1.2 Experimental method.** It was initially applied a method for estimating the solubility of the compound in a different solvent succedded by a crystallization method by cooling and evaporation. The methodology subsists in stages: ♦dispersing the solid starting material in bowls 4 mL; ♦ adding solvents using the Crissy equipment in 5 steps of 200 µl;♦ mixing the suspensions/solutions at 600 rpm for 10 minutes at each step; ♦ visual estimation of solubility at every step; ♦ heating the suspension/solution at $60^0$ C for 18 min with stirring at 600 rpm; ♦ visual estimation of solubility at $60^0$C;♦ cooling the solution/suspension withough stirring to $15^0$C in 3 hours; ♦ maintaining the solution/suspension at $15^0$C for 16 h without stirring; ♦ placing solutions / suspensions for 17 h at room temperature; ♦collecting the solid materials present and/or evaporation of the solvents.

**4.1.3 Experimental results.** The experimental results obtained regarding the solubility profile of the compound were used to index the new compound, i.e. to determine the crystallographic system unit cell parameters and to give each diffraction maxima corresponding Miller indices. To compound indexing method we used DICVOL. After indexing results that pitofenone hydrochloride belongs to orthorhombic crystallographic system and has the following network parameters: a = 30.836Å, b = 12.567Å, c = 11.225, α = β = γ = 90[°], Z = 8 and the volume V = 4349Å$^3$, respectively identified Miller indices from Figure. 4.3 a. Solid samples resulting from the experiment were analyzed by X-ray powder diffraction. Have been identified three new forms of pitofenone hydrochloride (Fig. 4.3 b).

**4.2 Methods of structural analysis.** After obtaining solid forms (polymorphs, solvates, hydrates, salts, co-crystals) one of the most important methods for their structural characterization is to determine the crystal structure of single crystal, becausethrough this way it is obtained the most complete structural information.

**4.2.1 Determination of crystalline structure from single crystals.** The determination of the crystal structure of solid forms involves the obtaining of crystallographic system in which the compound crystallized, the unit cell parameters, space group and the positions of atoms in the unit cell. From the positions of atoms in the unit cell is calculated the distances between atoms, bond angles and torsion angles. They allow complete description of the conformation of molecules from the structure and in of interactions between them. Basic steps applied in this thesis for determining the crystal structure of single crystals:♦ obtain single crystals; ♦ collected and correcting of diffraction intensities; ♦ determining the unit cell and space group; ♦ obtaining structural model; ♦ refining the structural model obtained. These methods are intertwined and mainly resolved by computer programs.




a b

***Figure 4.3. a.*** X-ray diffraction spectrum of the pitofenone hydrochloride.
***b****. The XRD patterns of three new forms (PF-13. given PF-16. given PF-20. given) as compared to the diffractogram of the base pitofenone hydrochloride (pitofenone hydrochloride given)*

**4.3 The size of the molecule**

**4.3.1 Functional Density Theory (DFT).** The total energy of a molecular system in Born-Oppenheimer approximation, the nonrelativistic and independent of time can be obtained by solving the equation:

$$\hat{H}\Psi(\mathbf{r_1},\mathbf{r_2},\mathrm{K},\mathbf{r_N}) = E\Psi(\mathbf{r_1},\mathbf{r_2},\mathrm{K},\mathbf{r_N}) \quad \textbf{(1.1)}$$

where the $\hat{H}$ is the Hamiltonian operator of the system, $\Psi(\mathbf{r_1},\mathbf{r_2},\mathrm{K},\mathbf{r_N})$ represents the wave function and E is energy. This system of equations defined by (1.1) is actually an equation of values and eigenvalues. The wave function $\Psi$ satisfies the fundamental properties of a fermion system that is an asymmetric function. Such a function can be constructed using a Slater determinant type (1.2)

By powder X-ray diffraction, was determined the crystal structure of starting form of pitofenone hydrochloride. The compound crystallizes in the monoclinic system from the space group $P2_1/c$ with two molecules in the asymmetric unit, having following network parameters: a = 12.547[Å], b = 11.205[Å], c = 30.786[Å], $\alpha = \gamma = 90[^o]$ and $\beta = 90.064[^o]$.

$$\Psi(\mathbf{r_1},\mathbf{r_2},\mathrm{K},\mathbf{r_N}) = \frac{1}{\sqrt{N!}}\begin{vmatrix} \varphi_1(r_1) & \varphi_1(r_2) & \Lambda & \varphi_1(r_N) \\ \varphi_2(r_1) & \varphi_2(r_2) & \Lambda & \varphi_2(r_N) \\ \mathrm{M} & \mathrm{M} & \mathrm{O} & \mathrm{M} \\ \varphi_N(r_1) & \varphi_N(r_2) & \Lambda & \varphi_N(r_N) \end{vmatrix} \quad \textbf{(1.2)}$$

where $\varphi_i(r_j)$ represents unielectronics wave function.

A projection of the unit cell is shown in Figure 4.4.

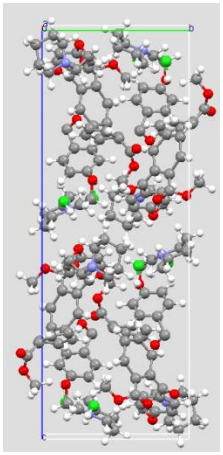


***Figure 4.4*** *The projection of pitofenone hydrochloride unit cell*

After determining the crystal structure was able to achieve a good concordance between experimental and calculated diffraction patterns (Figure 4.5).

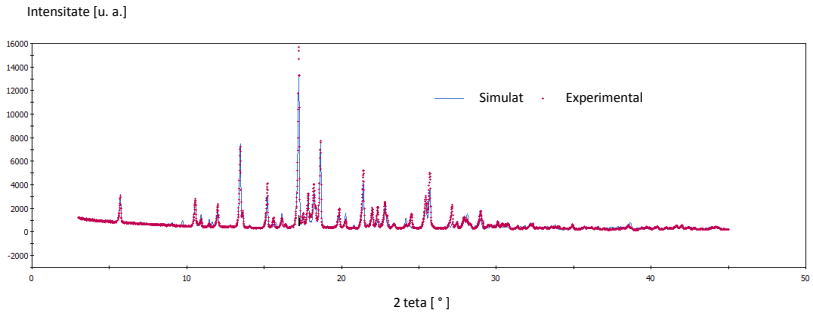



***Figure 4.5*** *Experimental and calculated diffraction pattern of pitofenone hydrochloride using the Pawley method with SuperNova X – ray diffractometer on monocrystals*

**4.3.2. The purpose of the experiment.** The determination of crystal structures was carried out for the compounds: **$C_{11}H_{16}O_4P_1S_2N_1$, $C_{12}H_{18}O_4P_1S_2N_1$** and **$C_{14}H_9C_lF_3NO_2$** using single crystal X-ray diffractometer.

**4.3.3 Research Methods**

***4.3.3.1 $C_{11}H_{16}O_4P_1S_2N_1$.***S compound has been determined that the compound crystallizes in the monoclinic system space group $P2_1/c$ which has 4 units in the asymmetric unit cell.

***Experimental results.***Next are presented the results obtained afterthe crystal structure determinationfor the compounds below. The molecular configuration of compound is shown in Figure 4.6 and in Figure 4.7 is presentet the packing mode of molecules in the unit cell. From the positions of atoms in the unit cell can determine the distances between the closest atoms. Alsoa from the positions of atoms in the unit cell can be determined and linking angles between neighboring atoms.

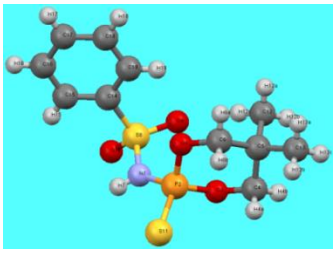


***Figure 4.6*** *Molecular onfiguration for $C_{11}H_{16}O_4P_1S_2N_1$*

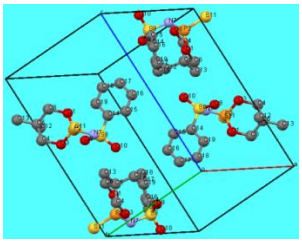


***Figure 4.7*** *Packing of molecules in the unit cell for $C_{11}H_{16}O_4P_1S_2N_1$*

***4.3.3.2 $C_{12}H_{18}O_4P_1S_2N_1$ compound***. Another compound from the pesticides class that we dealt with was $C_{12}H_{18}O_4P_1S_2N_1$. The compound crystallizes in the triclinic system, space group P-1 having two molecules in the unit cell. The molecular configuration of compound is shown in Figure 4.8 and in Figure 4.9 is presented the packing mode of molecules in the unit cell.

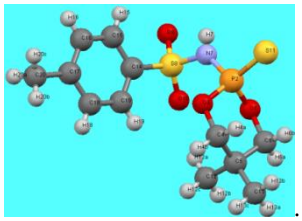


***Figure 4.8*** *Molecular configuration for $C_{12}H_{18}O_4P_1S_2N_1$*

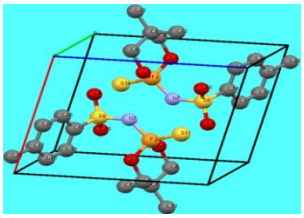


***Figure 4.9*** *Packing of molecules in the unit cell for $C_{12}H_{18}O_4P_1S_2N_1$*

From the positions of atoms in the unit cell results the distances between closest atoms and also can be calculated the linking angles to neighboring atoms. The new polymorph of efavirenz compound was obtained from a mixture of 1: 1 efavirenz and maleic acid dissolved in 1.5 mL methanol. The suspension was heated at $60^0$C for two hours and then cooled gradually to $15^0$C and keept for 24 hours. After 4 days were obtained white needle-shaped crystals [Popeneciu H., 2015].

**4.3.3.3 Compound $C_{14}H_9ClF_3NO_2$.** Computer programs used are: [Dolomanov O.V. 2009], [Macrae C.F., 2008], [CrysAlis PRO, 2014].

**Special details**

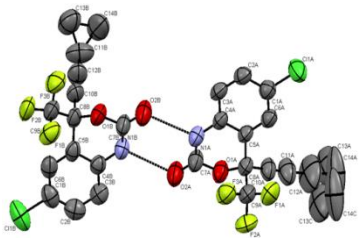
Geometry. All esds (except esd in the dihedral angle between two planes l. s) are estimated using the full covariance matrix. Esd cell sites are individual taken into account in the esds distances estimating, angles and torsion angles; esds

correlations between cell parameters are used only when are defined by the crystal symmetry. An approximate approach (isotropic) of the esds of cells is used to estimate esds involving planes l. s.

Refining.Refining $F^2$ against all reflections. R factor weighted wR and matching quality S is based on $F^2$, R conventional R factors are based on F, with F set to zero for negative $F^2$. The threshold of expression $F^2 > 2\ \sigma\ (F^2)$ is used only to calculate the R-factor (gt) etc. and is not relevant to choice of the reflections for refinement. R factors based on $F^2$ are statistical twice higher than those based on F and R factors based on all data will be even higher.

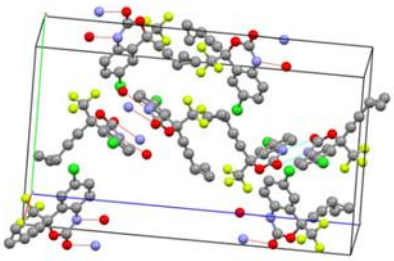
The new polymorph of the efavirenz compound was obtained from a mixture of 1: 1 efavirenz with maleic acid and dissolved in 1.5 mL methanol. The suspension was heated at $60^0$C for two hours and then cooled gradually to $15^0$C and held for 24 hours. After 4 days were obtained white needle-shaped crystals (Figure 4.10) [Popeneciu H., 2015].

***Figure 4.10****Disorder position of carbon atoms for* $C_{14}H_9ClF_3NO_2$



Efavirenz molecules in the asymmetric unit have 50% probability to replace elipsoizii. Hydrogen atoms are omitted for clarity. The dashed bonds represent hydrogen bonds (Figure 4.10).

***Figure 4.11*** *Bundling molecules in the unit cell for* $C_{14}H_9ClF_3NO_2$ *Efavirenz low similarity of crystals is represented with green and set room temperature are represented with red*



**4.3.4 The molecular size determination of pitofenone hydrochloride using density functional theory.** Spatial structure determination was performed using Density Functional Theory method on pitofenone hydrochloride and determining its size.

***4.3.4.1 Applied experimental methods.*** It was applied density functional theory using programs implemented in quantum chemistry package Gaussian 09 C.01.

***4.3.4.2. Interpretation of results.***Geometric structure was obtained by optimizing the total energy using the Density Functional Theory method (DFT) with functional M06-2X exchange-correlation [Garcia-Ac A., 2009] and the bases set of (such as triple zeta (TZV) [J Corcoran ., 2010] implemented in quantum chemistry software package Gaussian 09 C.01 [Daughton CG, 2010] (Figure 4.12).

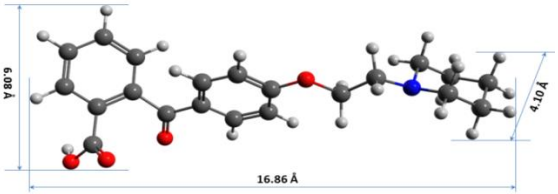



***Figure 4.12*** *The spatial structure of pitofenone hydrochloride*

**4.4 Conclusions of the chapter 4**

- It was determined by X-ray diffraction: ♦ complete crystalline structure for starting form of pitofenone hydrochloride drug, namely: crystallographic system; unit cell parameters; space group; atom positions in the elementary cell, which also gives: ♦ distances between atoms; ♦ bounding angles; ♦ bouding of hydrogen.
- The size of the molecule was calculated by quantum chemical program package Gaussian 09 C.01 and was obtained, 16,446Å.
- $C_{11}H_{16}O_4P_1S_2N_1$ compound crystallizes in the monoclinic system, having the space group $P2_1/c$ and the following parameters of the unit cell: a [Å]: 9.29217, b [A]: 11.87197, c [Å]: 14.26229, α [$^o$] 90, β [$^o$]: 107.7514, γ [$^o$] 90.
- $C_{12}H_{18}O_4P_1S_2N_1$ compound crystallizes in triclinic system, displaying the space group ***P-1***, and the following dimensions of the unit cell: a [Å] 7.4469 (4) b [Å] 10.9588, c [Å] 11.0534, α [$^o$]: 112.166 , β [$^o$]: 97.291, γ [$^o$] 102 556.
- After analyzing experimental data resulted the positions of atoms in the unit cell for both compounds, based which were calculated distances between neighboring atoms and bounding angles.$U_{iso}$ for H atoms were determined from the formula: $U_{iso}$ (H) = 1.2 $U_{eq}$ (CN). Disorder was resolved after cyclopropane ring of the Efavirenz molecule in which after positions washighlighted for $C_{13}$ and $C_{14}$ carbon atoms.

**Chapter 5 Experimental results regarding the correlation of structure and molecular mass in mass spectrometry. Determination of pollutants in the environment by chromatographic techniques**

**5.1 Experimental results regarding the correlation of structure and molecular mass in mass spectrometry**

***5.1.1 The purpose of the experiment***: Determining the structure and molecular weight for the pharmaceutical composition by high resolution mass spectrometry [Popeneciu H. 2014].

***5.1.2. Research Methods (i)*** the investigated substance: pitofenone hydrochloride (methyl-2- [4- (2-piperidin-1ilethoxi) benzoyl] benzoate); ***(ii)***The type of device used: *Mass spectrometer with ion trap;* Polaris Q manufacturer Thermo Electron model (Figure 2.15): ♦ Ionization system: electron ionization collision; ♦ Temperature: variable domain (25- 350)$^0$C; ♦ Field scaning: (25-450) atomic mass units (Daltons).

To the analysis of the pharmaceutical compound was used a solid sample in an amount of 0.5 mg. The sample was introduced by dedicated system for solids: input directly from the ion source. Experimental conditions: The sample temperature was programmed raised to 25$^0$C to 350$^0$C with a rate of 25$^0$C/min. It was used an electron impact ionization source: electron energy: 70 eV and emission current 100 μA.

Scaning mass range was between 25-400 u.a.m. During the climb range were recorded mass spectra with a frequency range from 10 sec. Mass spectra were recorded in normalized system to the base peak (base peak equal to 100% and the other signals are calculated as a percentage of it). It is shown in Figure 5.1 fully mass spectrum of the compound examined pitofenone hydrochloride.

In order to observe structural details, was recorded the normalized spectrum unto the mass ion, m/z = 55 which in the original spectrum is 10 %.

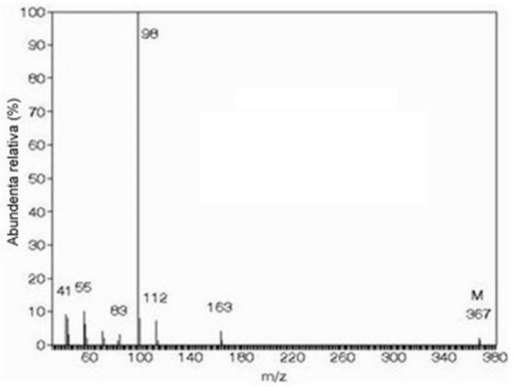



***Figure 5.1*** *Mass spectrum obtained by electron impact at energy of 70 eV with normalization at m / z = 98*

***5.1.3 Interpretation of results.***In the mass spectra are observed following significant features: ♦ It is observe the molecular ion peak (unfragmented whole molecule) M = 367 u.a.m. ♦ after the configuration of the isotope peaks, the ratio of the ion 368 to the ion 367, conclude that the molecule contains a 22 carbon atoms. ♦ Afterthe value of intensity of significant peaks are: 98, 112 and 163. The structure of these ions is presented in the analytical methods described below. They are produced by simple fragmentation of the molecular ion and have the atomic structures. *Ion m/z = 98* form the basic peak and is produced by a simple breaking of the C-C bond from the β to the N, being the effect of the location of the positive charge (+) on the N atom. The ion m/z = 112 is produced by tearing the simple bond C-O (ether bond) and location of the load on the the C atom to. The ion m/z = 163 is produced by breaking the C-C bond in the 2 position with respect to C = O.

**5.2 Determination of pitophenone hydrochloride from environment by high performance liquid chromatography**

**5.2.1.*The purpose of the experiment.***The analysis of pharmaceutical pitofenone hydrochloride was performed by high performance liquid chromatography coupled with UV-VIS spectroscopy.

***5.2.2 Applied investigative methods. Devices and chromatographic conditions:*** ♦ High performance liquid chromatograph Shimadzu 2010, consisting of a binary pump, degasser, autosampler thermostat set at $35^0$C, PDA detector (diode array detector). Column ♦ - LiChrosorb® RP-18 (250 mm x 4 mm i.d., 5 mm particles). ♦ The mobile phase - consisting of acetonitrile - water with formic acid (pH = 3) = 80:20, v/v. The mobile phase flow rate was 1 mL/min and the injected sample volume of 10 mL.

***5.2.3. Interpretation of results.*** After analysis it was determined the retention time 1.79 min of pitofenone hydrochloride and the maximum wavelength the absorbing (196 nm). For quantitative analysis of pitofenone hydrochloride from water samples is using the external standard method. Water samples taken from three different points on Somesriver were concentrated using rotovap. To establish the calibration curve were prepared pitofenone hydrochloride solution with a concentration in the range of 5-100 mg/mL, prepared by successive dilutions from a solution of 1 mg/mL. The solutions were prepared in methanol. The limit of detection was 3.345 mg/mL, while the limit of quantification was 6.532 mg/mL.

**5.3 Analysis of pitopenone hydrochloride by gas chromatography coupled with mass spectrometry**

***5.3.1 The purpose of the experiment.*** |Pitofenona hydrochloride analysis was performed by high performance gas chromatography and a mass spectrometer detector.

***5.3.2 Applied investigative methods.*** Devices and chromatographic conditions are: ♦ gas chromatograph of high performance Trade GC Ultra; ♦ column with low polarity: 30m length and 0.25 mm ♦ mobile phase - He used gas 1.5 mL / min; ♦ working in temperature program; ♦ stationary phase - column DB5-MS (+ 5% phenyl silicone metal silicon); ♦ Detector: mass spectrometer, Polaris Q. Has been prepared standard substances of pitofenona pure hydrochloride.

Pitofenone hydrochloride solution was prepared by weighing 1 mg of substance in 2 mL of acetonitrile and was obtained the compound with concentration of 500µg/ml corresponding to 500 ng/ml. By successive dilutions were prepared standard solutions D1-D7: D1 = 2000 ng/mL; D2 = 3000 ng/mL; D3 = 4000 ng/mL; D4 = 5000 ng/mL; D5 = 6000 ng/mL; D6 = 8000 ng/mL; D7 = 10000 ng/mL. Each sample was injected twice, and for the line of the calibration were used mean values.

***5.3.3. Interpretation of results.*** Chromatographic peaks area was determined by automatic integration. (Fig. 2.2).

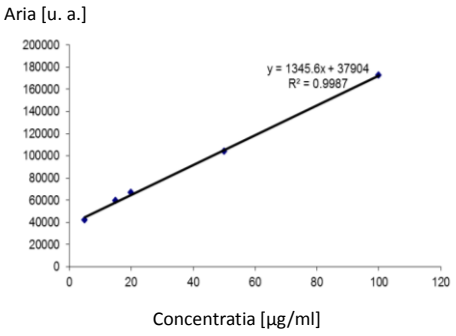



***Figure 5.2*** *The concentration of the chromatographic peak area based on the pitofenone hydrochloride concentration in solution*

**5.4 Conclusions Chapter 5**

- Was determined by mass spectrometry, based on registration and interpretation of mass spectra for the pitofenone hydrochloride: ♦ molecular weight; ♦ molecular formula; ♦ molecular structure.
- • Has developed the method of determining pitofenone hydrochloride from the water sample by gas chromatography. Was plotted the calibration line from which was determined: ♦ limit of detection; ♦ limit of quantification. Using the developed method and drowing the chromatogram was determined the difference in concentration of the support solution towards the zeolite solution.
- Were obtained signals of pitofenone hydrochloride and the internal standard and was determined the absorbed amount per gram of zeolite. The results show that we are dealing with a wide distribution of 13X zeolite pore size. The surface area is large and allows us to conclude that zeolite 13X can be used as absorbent because pores are in the mesopore domain (average size).

**CHAPTER 6. Experimental results regarding the use of adsorbents for the removal of pollutants in aqueous media**

**6.1 The organization of experiences.** The presence of pharmaceuticals and personal hygiene in the aquatic environment has been recognized as an important issue in environmental chemistry. Residues of pharmaceutical products and personal hygiene are both sewage and surface waters. Pitofenone hydrochloride is one of antispasmicele used in contraction of smooth muscle of tubular organs in particular in the gastro-intestinal tract, resulting in the prevention of stomach cramps, bladder and intestine. The data about the concentration and behavior of pharmaceutical products in rivers and lakes, including sludge, are very rare. Detailed data related to the presence and transform of these compounds into rivers and ground water are limited to a few countries [Moldovan Z., 2006]. Variety of types and structures of pollutants require advanced techniques and methods of water purification so that their concentrations drop to minimum levels allowed by law [Bhatnagar A., 2010]. Many researchers turn their attention to sorbents (ex. Biopolymers) and alternative methods to replace some conventional expensive methods (precipitation, ion exchange, electrochemical methods) or sorbents difficult to regenerate and expensive (ex. Activated carbon) [Monik A. 2013 Rahman N. 2013]. Literature shows that the investigations were conducted in the area of combined adsorption-biological treatments to enhance biodegradation of dyes and to minimize the production of byproducts [Lilies G., 2006]. In recent decades zeolites have been investigated as drug transport systems and as adjunctive in therapy of cancer, dietary supplements or antimicrobial agents. Natural and synthetic zeolites aremedia which permit the adsorption of varying amounts of antibiotics and gradual diffusion of them in the active forms, can be used as transport and controlled release systems [Bhatnagar A., 2010].

**6.2 Experiments to determine the total surface area and porosity of the adsorbent**

***The purpose of the experiment:*** ♦ zeolite surface area determination. ♦ determine the porosity of desorption isotherm using Dollimer Heal model [Cabrita I., 2010].

***The applied method:*** ♦ method of Brunauer, Emmett and Teller (BET) for interpretation of results from absorption-desorption isotherms of nitrogen at liquid nitrogen temperature (-186$^0$C).

***Methods of investigation:*** ♦ weigh 0.5-0.8 g of zeolite is put in degassed at 150$^0$C in vacuo for 3 hours; after degassing is measured isothermal nitrogen adsorption and desorption isotherm.

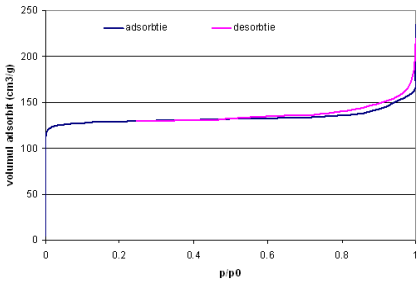



***Figure 6.1*** *Absorber isotherm is of type 1*

After ***results interpretation of the*** measurements was obtained (Fig. 6.1): ♦ absorption isothermal, ♦desorption isotherm, ♦ the amount of surface area♦ pore volume and the average radius of the pores. *Absorption isotherm*: is of type 1.

*Desorption isotherm*: shows moderate hysteresis at pressures. Total surface area: 401.6 $m^2$/g. Specific pore volume: 0.22 $cm^2$/g. *Porosity*. Pore radius is approximately between 20-200 Å.

***Conclusions:*** we have a broad pore size distribution. The surface area is large, and proposes its use as absorbent. The pores are mesopores area (average size). Has potential as absorbent material [Deegan A.M., 2011]. From pharmaceutically active compounds have been identified (♦pitofenona hydrochloride, ♦ Carbamazepine, ♦ Ibuprofen ♦ Naproxern, ♦ Indomethacin, ♦ Pentoxifylline), which was trying the adsorption onto porous materials (♦ charcoal, respectively ♦ molecular sieves).

**6.3 Experiments on activated carbon and molecular sieves.** The experiment starts with the dissolution of pharmaceutically active compounds: pitofenonahydrochloride, Bisoprolol Pentoxifylline, Naproxen, Carbamazepine, Indomethacin, Ibuprofen, Nifedipine, Nitrazepan to determine them solubility in solvents. Have ben carried out tests on several types of solvents: acetone, acetonitrile, chloroform and izoctan. After the preparation of solutions has been attempted adsorption of the compounds on activated carbon and molecular sieves.

To identify these compounds in surface water were used two types of grained charcoal with 60-80 mesh product by Pierce chemical company, Rokford, Illinois, S.U.A. charcoal grain 1-1,5-2 mm ( 18 to 10 mesh) purchased from Romcarbon factory in Buzau and 4 types of molecular sieves with their characteristics: molecular sieve 5A grained 60-80 mesh (0.250 to 0.177 mm) manufactured in Italy 18-10 mesh (1-2mm) grained molecular sieve of origin England, molecular site- 5A whith grained 70-18 mesh (0.2-1 mm) manufactured in Germany, and not least with grain 120-140 5A molecular sieve mesh (0.125 to 0.105 mm) purchased in England.

There have been many measurements as follows: first were weighed 10 mg of both coal (coal SK-4 and charcoal Buzău) then was weighed 1 mg of the five pharmaceutically active compounds. Were mixed the weighed quantities of pharmaceutical compositions with 3 mL of acetone, 100 mL of distilled water, and before being added to the stirred (300 rpm) to room temperature (18-20$^0$C) is sampled in both vials (blank or 0h sample) before being added the coal.

After it is added the porous material and start to stir for 4 days and take samples after each 24 h. A similar process is repeated for the molecular sieves, weigh out 10 mg of each sieve in part, is added 1 mg of pharmaceutical compositions,and was dissolved in 100 mL of distilled water. Before adding molecular sieves extract sample (blank). Meanwhile add sives and begin stirring for 4 days and after every 24 hours, is taken the samplefor analyzes.

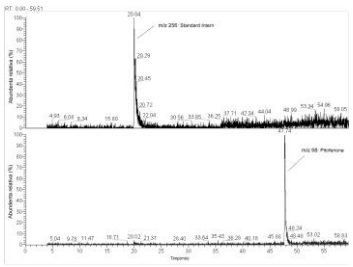
**6.4 The process of removing organic pollutants by using zeolite adsorbents**

***6.4.1 Experiments on pitofenone hydrochloride.*** Determination pitofenone hydrochloride signals and the internal standard where were determined from the adsorbed amount per a certain amount of zeolite.

***6.4.2 Experimental methods and interpretation of results.*** For the chromatography column was used temperature program in the range of 90$^0$C to 300$^0$C, and the mass spectra were obtained by electron impact at 70 eV (coupled system Gas chromatograph - Mass spectrometer Trade-GC Ultra-Polaris Q). Column type: DB5 - MS. We start with the preparation of 0.04 g of pitofenonahydrochloride in 100 mL of acetonitrile with concentration of $10^{-4}$ M. In the meantime 13X zeolite pores are conditioning in the oven by dry at 200$^0$C for 3 hours. We add 5 g zeolite 13X in solution of the pitofenone hydrochloride and acetonitrile and stirred for about 2-3 days at a temperature of 20$^0$C and 250 rpm speed. After stirring we centrifuge 10 minutes at a speed of 4000 rpm and determine with GS-MS whether or not the concentration changes.

There made measurements on the basis of internal standard [C. Y. Yin, 2007]. In each sample was placed aninternal standard sample. By comparing the signals of interest compound pitofenone hydrochloride with internal standard resulted in the original sample: 40 mg/100 mL = 0.40 mg/1 mL = 400 mg/1 mL. In the sample that came in contact with zeolite for 3 days, after comparing signal of standard sample with the signal pitofenone hydrochloride resulted the following concentrations: 32.71 mg/100 mL = 0.3271 mg/1 mL = 327.1 mg/1 mL. By the graphic representation:times vs. the relative abundance were obtained: pitofenone hydrochloride signals and the internal standard. The difference is 72.9 mg/1 ml = 7290 mg/100 mL = 7290 mg/100 mL. Resulting amount of absorbed zeolite/g is 1,458 mg. (1.458 mg/1 g zeolite).

***Figure 6.2*** *MS GC chromatogram of pitofenone hydrochloride: 1) The internal standard peak (m / z = 256), 2) pitofenone hydrochloride peak (m / z = 98)*



***Conclusion***: The pitofenone hydrochloride and internal standard signals was obtained, was determined the absorbed amount per gram of zeolite.

**6.5 Experimental results**

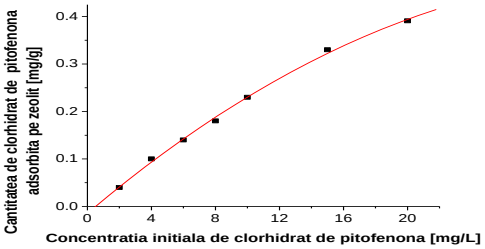
**6.5.1 Procedure.** From all that 6 standard solutions are taken 100 mL in flasks. In each flask is add 2.5g 13X zeolite. The samples were stirring at 400 rpm for 24 hours at temperature of $20^0$C. The obtained solutions were centrifuged with 4000 rpm for 20 minutes. pitofenone hydrochloride amount adsorbed was calculated using the formula.

$$q_{t\ PF} = (C_0 - C_t)\cdot\frac{V}{m}\ [\ g_{PF}/g_{zeolit}] \qquad \textbf{(3.2)}$$

unde: $q_{t\ PF}$ – capacitatea de adsorbţie de pitofenonă; $C_{0PF}$– concentraţia iniţială de pitofenonă;$C_{t\ PF}$ – concentraţia finală de pitofenonă; $V_{sol\ PF}$ – volumul soluţiei de pitofenonă; $m_{zeolit}$ –cantitatea de zeolit.

where: $q_{t\ PF}$- adsorption capacity of pitofenona; $C_{0PF}$- initial concentration of pitofenona; $C_{t\ PF}$- pitofenona final concentration; $V_{sol\ PF}$- volume of pitofenona solution; mzeolit zeolite quantity.

***Figure 6.3*** *Measurements by spectrophotometric method of pitofenone hydrochloride solutions and getting the calibration line (λ = 190 nm)*




**6.5.2.Interpretation of results** The data indicate that in every case, regardless of the initial concentration which is in the range 2-20 mg / L, the adsorption rate is constant (Figure 6.3). In studied case 2.5 g zeolite/sample the adsorption percentage was around 50 %. The load on the support was of 4 mg and 0.4 mg/g. Load balance is between 1/2500 and 1/25000 considering as reference the mass of adsorbent zeolite. The equation is shown below:

$$Y = A + B1\cdot X + B2\cdot X^2$$
$$Y = -0{,}0144 + 0{,}02853\cdot 10^{-4}\cdot X - 4.05244\cdot 10^{-4}\cdot X^2 \qquad \textbf{(3.3)}$$

where E correlation coefficient 0.99806; SD standard deviation 0.00675; N number of data points in experiment 7.

***Conclusion*** **the zeolite absorbs 0.391 mg of pitofenona/g zeolite.**

**6.6 Studies of elimination of pitofenone hydrochloride from aqueous solutions using active charcoal adorbents**

**6.6.1 Materials and equipment** ♦ pitofenone hydrochloride was purchased from Microsin Bucharest, Romania. ♦ Activated carbon is provenance: Alfa -Aesar GmbH & Co KG and is the size: -4 + 8 mesh. ♦ 13X Zeolite provenance: Linde,

USA, has size: 60-80 mesh. ♦ Analytical balance Radwag AS220/C /1 10-4g accurately; ♦ Magnetic Stirrer Heidolph MR Hei-Mix D; ♦ Hettich Protofix 32A centrifuge; ♦ UV-VIS Spectrophotometer UV Szimatzu 1800s.

**6.6.2 Working methods. Spectrophotometric method.**

*Procedure*. Weigh 20 mg of pitofenonahydrochloride using the analytical balance and brought to mark with distilled water in a flask of 1L. There was thus obtained the first standard of 20 mg/L concentration. The following standards are prepared by serial dilutions and have the following concentrations: 10 mg/L, 8 mg/L, 6 mg/L 4 mg/L, 2 mg/L. The standards were analyzed spectrophotometrically thus were obtained the two characteristic peaks of pitofenone hydrochloride at 192 nm, 289 nm, respectively. The absorbance values recorded with those standards were plotted vs their concentrations (Figure 6.2).

**The results analysis.** Thus was obtained the calibration line with ordinate at origin A = 0.05244 (+/- 0.02532) and slope B = 0.04589 (+/- 0.00249). The equation of calibration line is of the I order and have the form:

$$Y = 0{,}05244 + 0{,}04589 \cdot X \qquad \textbf{(3.1)}$$

where

Y absorbance; X pitofenone hydrochloride concentration; SD standard deviation = 0.03551; The number of points N = 6; A correlation coefficient (ideal is 1) = 0.99416.

**6.6.3 Experimental results**

**6.6.3.1 Procedure.** From all the 6 standard solutions are taken the 100 mL in flasks. In each flask are added 2.5 g charcoals. The samples were placed to sterredwith 400 rpm for 24 hours at $20^0$C. The obtained solutions were centrifuged with 4000 rpm for 20 minutes. The liquid phase was analyzed by the spectrometer in the range of 190-600 nm, and the absorbance values obtained were compared with those of the standards. The amount of absorbed pitofenone hydrochloride was calculated using the formula:

$$q_{t\,PF} = (C_0 - C_t)\cdot\frac{V}{m}\ [\,mg_{\,PF}/g_{cărbune\ activ}\,]\ \text{[Nguyen L.N., 2013]} \qquad \textbf{(3.4)}$$

where: $q_{t\,PF}$- adsorption capacity of pitofenone hydrochloride; $C_{0\,PF}$- initial concentration of pitofenone hydrochloride; $C_{t\,PF}$- final concentration of pitofenone hydrochloride; $V_{sol\ PF}$-volume of pitofenonahydrochloride solution; m cărb active quantity of charcoal.

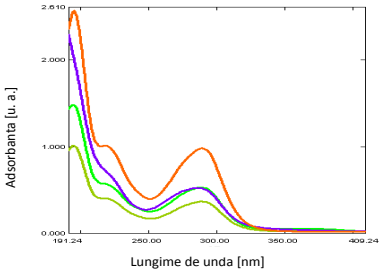



***Figure 6.4*** *absorption spectrum UV-VIS of pitofenone hydrochloride (25 mL of sample 20 mg of pitofenonă/L (red) absorbed by 10 mg of activated carbon (purple) and 8 mg/L (lime) and 6 mg/L ( green)*

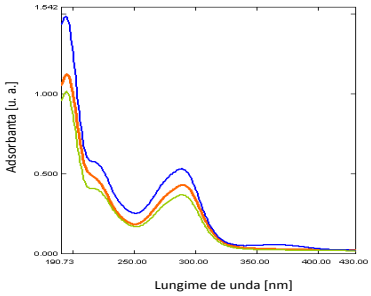
***6.6.3.2 Interpretation of results.*** The obtained data show that pitofenone hydrochloride was fully absorbed by the active carbon so we can not estimate the maximum amount of pitofenone hydrochloride that can be absorbed by the material (Figure 6.4). To determine the degree of saturation of pitofenone hydrochloride with charcoal were precede another series of measurements. The equation is described below:

$$Y = A + B{\cdot}X = \text{-5.55112}\cdot\text{E-17+ 0.04}\cdot X \qquad \textbf{(3.5)}$$

where R correlation coefficient 1; SD standard deviation of $6.2010^{-17}$; N number of experimental points 6.

Were prepared 4 samples of 25 mL with concentrations of 20 mg/L pitofenone hydrochloride where we added different amounts of the activated carbon 250 mg, 100 mg, 50 mg and 10 mg. Absorption time was 24 hours at a temperature of $20^0$C to 400 rpm. After centrifugation the samples were analyzed by spectrophotometer.

***Figure 6.5*** *UV-VIS absorption spectrum of the pitofenone hydrochloride (25 mL of 20 mg of sample pitofenona/L (red) absorbed onto 10 g of activated charcoal (blue) - overlapping with two calibration spectra, Figure 6.4*




From the results we can estimate the maximum amount of adsorbed pitofenonahydrochloride by activated carbon which is 27.5 mg/g (Figure 6.5).

**6.7 Adsorption study using Gas Chromatography coupled with Mass Spectrometry**

**6.7.1 Experimental conditions**. ♦ Compounds separation was done on a capillary column of 5DB-MS type with the following characteristics: L = 30 m, Ø 0.25 mm and stationary phase with a 0,25 µm layer thickness. Phase is of methyl polysiloxanes type plus phenyl polysiloxane (5 %); The temperature at the interface between the chromatograph and the spectrometer was $300^0$C; Sample introduction system was maintained at $250^0$C. ♦ Detection was performed by mass spectrometer programmed as follows: initial temperature of $90^0$C (1 minute) up to $120^0$C with $10^0$C/minute (0 minutes) goes up $3,5^0$C/min up to $190^0$C (0 minutes) and then goes up to $4^0$C / min up to $300^0$C where it is maintained 8 minutes. ♦ It was used He carrier gas at a flow rate of 1.5 mL/min. ♦ The amount of sample injected: 2 µL. ♦ Retention time was 46.91 minutes for pitofenone hydrochloride. ♦ For quantitative calculations hydrochloride pitofenonă peak area was compared to the area of internal standard, which elutes at 19.06 minutes. ♦ three experiments were performed: (1) Experiment with zeolite 13X 2.5 g/L; (2) Experiment with active charcoal 2.5 g/L; (3) Experiment with charcoal 0.005g/L. ***Note:*** 6 samples with zeolite 13X and 6 samples with charcoal were prepared in parallel.

***6.7.2 Interpretation of results***

***Experiment with zeolites 13X 2.5 g/L.*** They have been investigated a number of six samples with concentrations of pitofenonă of 2 mg/L 4 mg/L, 6 mg/L, 8 mg/L, 10 mg/L, 20 mg/L which have been put in contacted with zeolite with a concentration of 2.5 g/L. Samples were tested with coupled system gas chromatograph-mass spectrometer that has sensitivity 2 µg / L (detection limit). The amount of pitofenonă hydrochloride in each of the tested samples was below the detection limit. The explanation was that the mass of material, zeolite 2.5 g/L was too great and pitofenone hydrochloride was absorbed in 24 hours (it was produced a total adsorption).

***Experiment with charcoal 2.5 g/L.*** It was tested in another experiment pitofenone hydrochloride in water at the concentration of 0.2mg/L. It was added an amount of 6 mg/L of carbon and was provided thecontact between pitofenone hydrochloride and the activated charcoal by stirring for 24 hours and was found a 1.53 % concentration of pitofenona. Adsorption was therefore of 98.47 % (Figure 6.6).

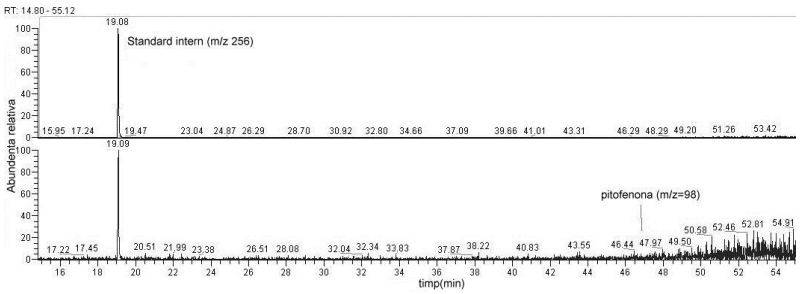



***Figure 6.6*** *CG-MS chromatogram after adsorption*

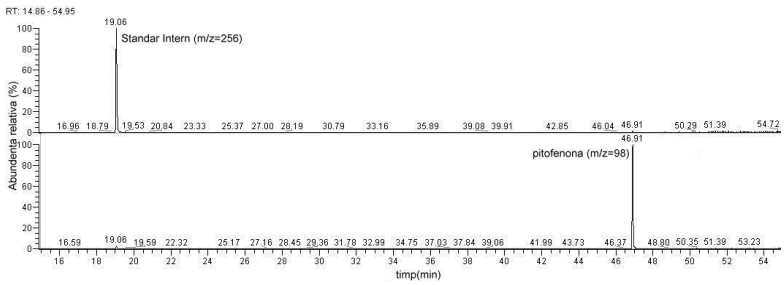



***Figure 6.7*** *GC-MS chromatogram for initial sample*

***The experiment with active charcoal 0.005 g/L.*** They have been investigated a number of 4 samples with concentrations of pitofenone hydrochlorideof 20 mg/L. Samples were tested with Gas Chromatography - Mass Spectrometercoupled system. This sample has the same amount of pitofenona hydrochloride as the original sample, 20 mg/L but was added 50 mg/L (0.05 g/L) activated charcoal and was stirred for 24 h and from analysis results a smaller amount ofpitofenona hydrochloride than the control sample (original sample). It was calculated the percentage of adsorption of pitofenone hydrochloride/charcoal in 24 hours. The obtained results are in consistent with literature data. Even at the end our data can be used in purification process of purification stations. pitofenone hydrochloride is adsorbed on the activated charcoal and is related to the amount of with activated charcoal it comes into contact. In 24 h occurs an adsorption of 83.30 % (Figure 6.7).

**6.8 Study of the adsorption process at low concentrations of pitofenone hydrochloride on active carbon**

**6.8.1 Procedure**. It has been investigated a total of 6 samples resulting from the concentration of pitofenone hydrochloride each of 0.002 mg/mL in water. At 10 mL pitofenone hydrochloride solution in water (2 mg/L) was added 2mg/L activated charcoal and stirred for 6 h. They took samples of 1 mL every hour. Samples were tested with coupled system gas chromatograph - mass spectrometry and were determine the amount of pitofenone hydrochloride adsorbed onto activated carbon over time.

**6.8.2 Interpretation of results.** The mass of pitofenone hydrochloride in the solution is given by:

$$q_{t\,PF} = (C_0 - C_t)\cdot\frac{V}{m}\ [\ mg_{\ PF}/g_{active\ charcoal}]\ \text{[Hohenberg P., 1956]} \qquad \textbf{(3.6)}$$

where: $q_{tPF}$ - adsorption capacity of pitofenone hydrochloride; $C_{0PF}$ - initial concentration of pitofenone hydrochloride; $C_{t\ PF}$ - pitofenone hydrochloride final concentration; $V_{sol\ PF}$ - solution volume of the pitofenone hydrochloride; $m_{active\ charcoal}$ - the amount of active charcoal.

The obtained absorption curve is described by the equation of the second degree of the form:

$$Y = AX^2 + B1\cdot X + B_2 \qquad \textbf{(3.7)}$$

where: Y is the final concentration of pitofenone hydrochloride (in %) A, B1, B2 adsorption curve parameters and have the values: A = 0.0008, $B_1$ = 0.5215, $B_2$ = 105.09; X = the concentration of pitofenone hydrochloride after adsorption; $R^2$ = 0.9558 correlation coefficient; N = 5 the total number of data points.

**6.9 Experimental data for the adsorption of pitofenone hydrochloride to zeolite13X**

**6.9.1 Procedure.** The samples which were tested with zeolite were extracted from water by liquid-liquid (L-L) method. The extraction was done in the following way: it was took 0.5 mL of sample and was transferred to a 5 mL tube. It was added the internal standard (IS), and then was added 0.5 mL ($N_{c6}$) hexane. It has been vigorous stirring to the mechanical stirrer for 3 minutes, after has been collected hexane from the top of the sample and was introduced into GC-MS system.

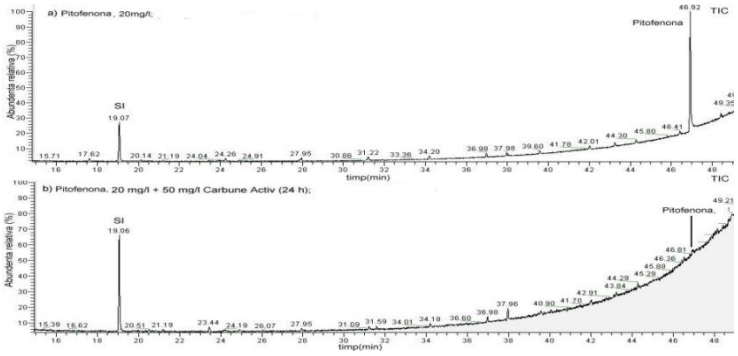
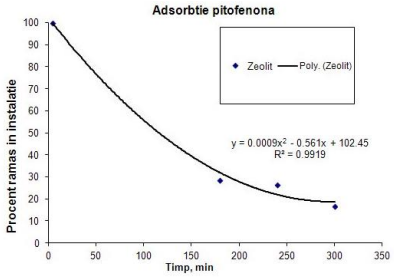



*Figure 6.8 pitofenone hydrochloride adsorption on activated* charcoal*: a) the chromatogram of the total ion at original sample 20 mg/L pitofenone hydrochloride; b) cthe hromatogram of the total ion at pitofenone hydrochloride sample 20 mg/L + 50 mg activated* charcoal *after 24 hours adsorption*

**6.9.2 Interpretation of results.** Samples taken after 1h, 2h respectively were diluted by accident and were not considered in graph (Figure 6.8).

***Figure 6.9****Absorption curve which represents the concentration of pitofenone hydrochloride absorbed on activated charcoalfor 6 h*




Was obtained absorption curve (Figure 6.9):

$$Y = AX^2 + B_1 \cdot X + B_2 \qquad \textbf{(3.8)}$$

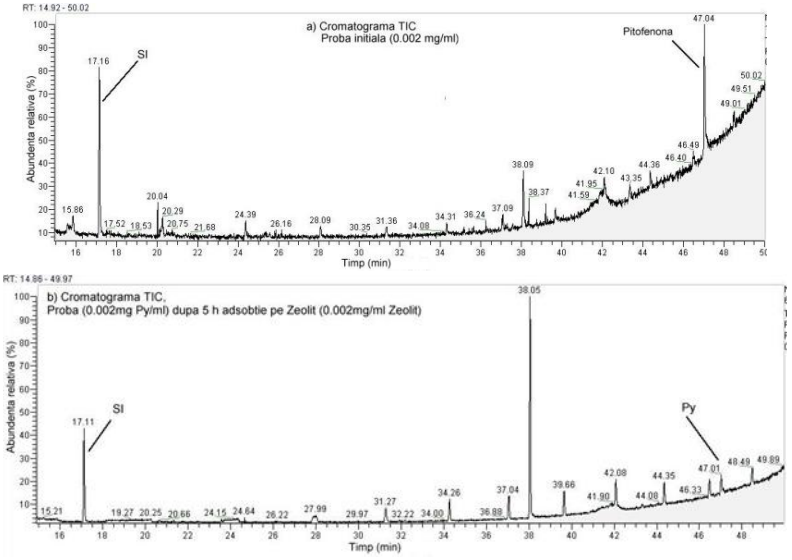



**Figure 6.10** *a) GC MS in the original sample; b) GC-MS after 5 hours of adsorption on zeolite*.

Referring to the two studies of the adsorption process, the data leads to the conclusion that the adsorption process is governedin both cases the zeolite and activated charcoalby the processes which are described by a function of the second degree

(Figure 6.10). The parallelism processes can be seen in Figure 6.11, where are shown both adsorption functions (both for 13X zeolite and for activated charcoal). It was tested in water a solution of pitofenona by concentration of 0.002 mg/mL. It was determined the adsorption on two types of substances: ♦ Activated charcoal with specific surface area: 1230$m^2$/g; ♦ 13X zeolite with specific surface: 401,6 $m^2$/g;

The amount of adsorbent (activated charcoal) is 0.004 mg/ml experiments were done at room temperature. In order to study the adsorption process were collected every hour, 1 mL of samples. In order to analyze the quantity of pitofenonă remaining in solution after adsorption was passed to the analysis of amount of pitofenona from aqueous phase. For this, first was made the extraction of pitofenone hydrochloride from solid phase extraction process. Final elution solid phase was done with acetonitrile solvent. There were done two tests: ♦ elution with 0.5 mL of acetonitrile; ♦ Elution with 2 mL of acetonitrile.

Satisfactory results were obtained by elution with 2 mL of acetonitril. As a result all extraction was used for the final elution 2 mL of acetonitrile.

$$Q_{t\ PF} = (C_0 - C_t)\cdot\frac{V}{m}\ [\ mg_{\ PF}/g_{\ active\ \ charcoal}] \qquad \textbf{(3.9)}$$

where:

-$q_{t\ PF}$ - adsorption capacity of pitofenone hydrochloride; $C_{0\text{-}PF}$ - initial concentration of pitofenone hydrochloride; $C_{t\ PF}$ - final concentration of pitofenone hydrochloride; $V_{sol\ PF}$ - solution volume of pitofenone hydrochloride; $m_{active\ charcoal}$ - the amount of active charcoal.

Was obtained the adsorption curves (regression polynomial equations) of the form:

$$Y = A\cdot X^2 + B_1\cdot X + B_2 \qquad \textbf{(3.10)}$$

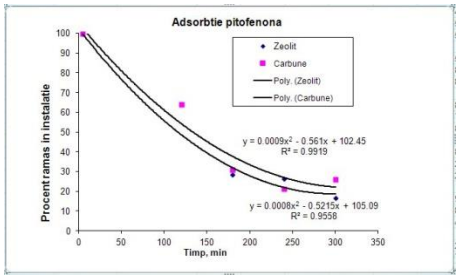
where:

A, $B_1$, $B_2$ zeolite adsorption curve parameters, A = 0.0008, $B_1$ = 0.5215, $B_2$ = 105.09; $R^2$ - 0.9919 correlation coefficient

A, $B_1$, $B_2$ curve adsorption parameters on activated charcoal, A = 0.0009, $B_1$ = 0.561, $B_2$ = 102.45; $R^2$ - 0.9558 correlation coefficient; Y – pitofenone hydrochloride amount remained in the solution (%); X - time (minutes).

Adsorption process is very similar in both cases (Figure 3.11). In 6 hours pitofenone hydrochloride solution reached 16.8 % from the initialwhen using at adsorbent zeolite or 26.27 % when using the activated charcoal as adsorbent.

***Figure 6.11*** *Adsorption curves resulting from representing the remaining precent of pitofenone hydrochloride in the system depending on the time of interaction with the adsorbent on activated charcoaland zeolite respectively*




**6.10 Separation and identification of pharmaceutical compounds by Gaseous Coupling Chromatography coupled with Mass Spectrometry**

**6.10.1 The purpose of the experiment.**Were used4 pharmaceutical compounds, by the adsorption on molecular sieves and activated charcoal process whereof only: Carbamazepine, Ibuprofen, Naproxen, Indomethacin was adsorbed to 90 % by gas chromatography coupled with mass spectrometry

***Spectrometric conditions.*** Detection was performed by mass spectrometer POLARIS Q type of Ion Trap type under the following conditions: ♦ ionization mode: Electron impact (EI); ♦ Electron energy: 70 eV; ♦ Bale mass range: 50-650 Daltons; ♦ data acquisition method; ♦ The amount of sample injected: 2µL.

**6.10.2 Sample preparation mode**. The concentration of each pharmaceutical was calculated: eg 2.1 mg + 100 mL distilled water + 3 mL acetone = 104.02 concentration = 1.02 mg naproxen 100 / 104.02 = 0.98 %

***Description of adsorbent.*** Granulation: active charcoal SK-4 0250-0177 mm (60-80 mesh), wood charcoal: 1-1,5-2 mm (18-10 mesh) molecular sieve 5A: 0.125 to 0.177 mm (60-80 mesh) molecular sieve 5A: 0.2-1 mm (70-18 mesh), molecular sieve 5A: 0.125 to 0.105 mm (120-140 mesh).

***Derivatisation of samples.*** In the gas chromatography for analysis with precision of compounds with active hydrogen is required a derivatization. In this category are includes compounds from class: alcohols, acids, phenols, amines, steroids. By derivatization reactions the compounds are thermally stable and interaction with metal surfaces is much less. Reaction conditions were as follows: ♦ the sample is taken to dryness; ♦ it is added the silylating reagent; ♦ sample with the reagent is maintained at $80^0$C for 30 minutes; ♦ sample ispassed in iso-octane and is injected into the GC-MS system.

**6.10.3 Experimental results.** Compounds concentrations are determined from the chromatographic areas taken on characteristics ion respectively, 160, 185, 193 and 139 for Ibuprofen, Naproxen, Indomethacin and Carbamazepine. The chromatographic separation is given in Figure 6.12. The amounts of the compound expressed in the remaining concentration percentage after the interaction with the adsorbent as a function of time of interaction are shown in Figures 6.13-6.18 for 6 types of adsorbents (two types of activated charcoal and four species of zeolites).

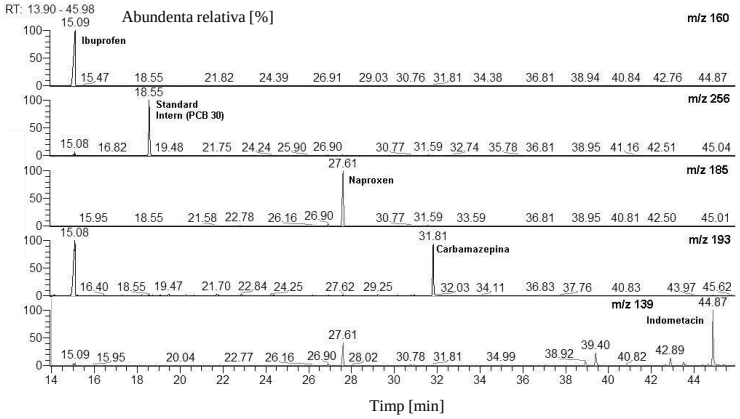



***Figure 6.12*** *GC-MS separation of compounds ibuprofen, naproxen, indomethacin and Carbamazepine in the form of silylated compounds (1TMS)*

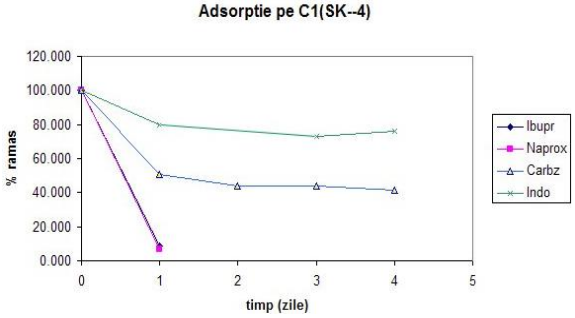



***Figure 6.13*** *Absorption variation of pharmaceutical compounds in time*

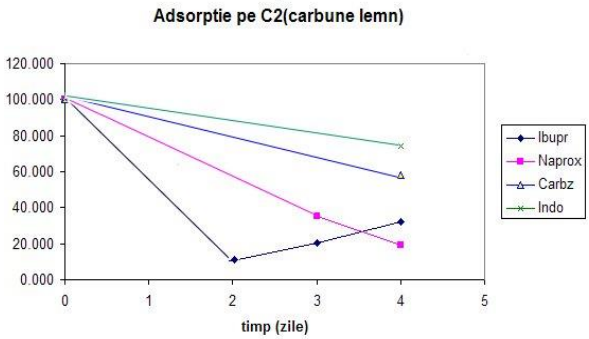



***Figura 6.14*** *Absorption variation of pharmaceutical compounds in time*

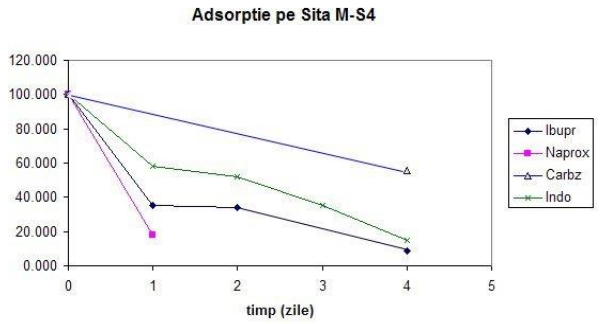



***Figura 6.15*** *Absorption variation of pharmaceutical compounds in time*

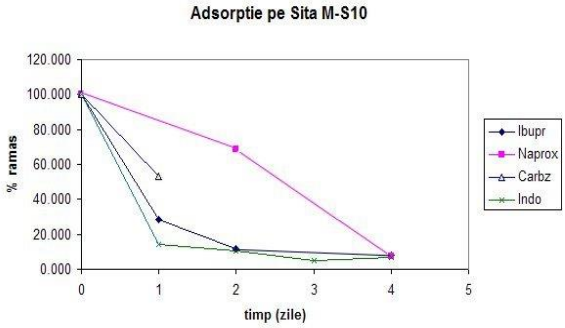



***Figura 6.16*** *Absorption variation of pharmaceutical compounds in time*

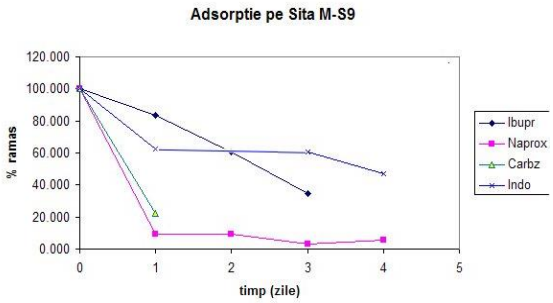



***Figura 6.17*** *Absorption variation of pharmaceutical compounds in time*

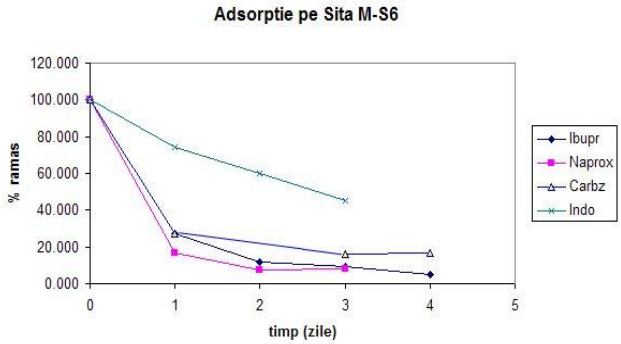



***Figura 6.18*** *Absorption variation of pharmaceutical compounds in time*

**6.11 Experiments for prevention of pharmaceutical residues from aqueous medium.**

Occurrence of pharmaceuticals in the aquatic environment has been recognized as one of the problems in environmental chemistry. After admission, pharmaceuticals are excreted in urine and fecals, either as active substances or metabolites. Widespread presence of pharmaceuticals in the aquatic environment due to their incomplete removal in wastewater treatment plants (WWTP). The objective of this paper is developing of methods for removal of pharmaceuticals from water samples based on adsorption processes involving charcoal and molecular sieves. It is presented the results from analyzes of several

pharmaceutical acid removal efficiency on the solids and results on determining the rate of removal of pharmaceuticals by municipal treatment plants with wastewater under normal conditions. The purpose of this paper is to develop a sensible and sensitive method based on coupled system Gas Chromatograph - Mass Spectrometer to determine polar pharmaceuticals in water samples. The method is used to: a) Study of the pharmaceuticals extraction process in an aqueous medium in the solid phase. b) Determination of concentration of polar pharmaceuticals (commonly prescribed) in the influent and effluent of wastewater treatment plant (WWTP) in Cluj-Napoca [Popeneciu H., 2016].

**6.11.1 Sampling and sample preparation.** The samples used for adsorption study were prepared in the laboratory. In order to investigate the efficacy of the treatment plant samples were taken samples from Treatment Station Someseni of Somes Water Company S.A. at selected points. The samples were concentrated by liquid-liquid procedure (L-L) [www.chromacademy.com 2014, Juhascik M. P., 2009] using n-hexane as organic phase. There have been used a volume of 250 mL aqueous samples (250 ng PCBs were added as an internal standard), and 5 mL of n-hexane. After 30 minutes of stirring, the organic phase was collected and concentrated to 250 µl. The sample was silylated and 2 pl was injected into the gas chromatograph coupled with a mass spectrometer. In order to achieve the required precision by gas chromatography and mass spectrometry analysis the compounds were derived by silylating [Moldovan, Z., 2002]. Derivatized compounds are thermally stable and interactions with metal surfaces are much lower. By silylating process the active hydrogen is substituted with trimethylsilyl group -Si $(CH_3)_3$. The reaction conditions were as follows: a.) The sample is taken to dryness; b) the silylating reagent is added; c) the sample with the reagent is maintained at $70^0$C for 60 minutes; d) the sample is extracted with iso-octane and 2 µl is injected into the gas chromatograph coupled with a mass spectrometer. For removal of selected organic compounds obtained after water-absorption processes on the solid materials were studied two types of activated charcoal and four types of molecular sieves.

**6.11.2 Analysis by gas chromatography method coupled with mass spectrometer.** Analyses were performed on a gas chromatograph system coupled with mass spectrometer (Thermo Electron Polaris Q). The mass spectrometer was operated in the method Electron Impact (EI) at 70 eV. The source temperature was $250^0$C and emission current of 300 mA. The gas chromatograph was equipped with an HP-5MS (30mm x 0.25mm) capillary column, with a film thickness of 0.25 µm. The temperature was programmed from $90^0$ C (1 min) to $120^0$C to $100^0$C/min and then to $200^0$ C at $3.5^0$C/min after to $315^0$C to $5^0$C/min (maintaining this temperature for 11 min). The injector temperature was $250^0$C, and 2 µl of sample was injected through the undivided technique with dividing flow 50 mL/min and 1 min time undividing. Mass spectra were obtained in the full scan mode in the range of 50 to 650 Daltons.

**6.12 Interpretation of experimental results**

**6.12.1 The adsorption of compounds on solid**. The pharmaceutical studied in this paper were Ibuprofen, Indomethacin, Naproxen and Carbamazepine. For removal of selected organic compounds from water have been studied the adsorption processes on some solid material including two kinds of activated charcoal and four types of molecular sieves. In our studies we selected activated charcoal having grain with range of 60-80 mesh (Charcoal - TL Service LLC, Milan, Italy) and a charcoal with a grain size of 10 -18 mesh (Romcarbon Buzau, Romania). Also were reported experiments of removal on molecular sieves (zeolites) [Li J., 2001, Damjanović L., 2010, Yousef RI, 2011, Senturk HB 2009, UF Alkar, 2009, Khalid M., 2004 Anderson MA, 2000 Kamble SP, 2008]. Were used four types of molecular sieves with granular 60-80 mesh (Machery Nagel, Duren, Germany, 10-18 mesh (Carlo Erba Strumentazione Tuscany, Italy), and 18-70 mesh (Service TL SRL, Milan, Italy) and 120-140 mesh (TL S.RL. service, Milan, Italy). The experiments were carried out as follows.

For each studied adsorbent were prepared original solution (sample) into a Berzelius flask of 250 ml to which were added 100 mL of distilled water and 1 mg of each pharmaceutical. Alsso was prepared the control sample containing mixture of pharmaceutical in the same amount, but not the adsorbent. Both the sample and the control sample were shaken (300 rpm) at room temperature for four days. Every 24 hours, 5 ml of sample were collected and pharmaceuticals were extracted by liquid-liquid processes using 2 mL of n-hexane. The final extract was brought to 0.25 ml, and after derivatization (silylation) 2 µl was introduced into the gas chromatograph coupled with a mass spectrometer. The GC-MS sealing is shown in Figure 6.12. For the quantification of each compound were used the maximum base area. The results were expressed as a percentage from the initial concentration, on the six kinds of adsorbents (two types of activated charcoal and four species of molecular sieve) obtained within four days are presented in Table 6.1.

***6.12.2 The rate of removal of pharmaceuticals in wastewater treatment plant effluent wastewater***. Prior to removal from the medium, usually in rivers, the used water must be purified. Pollutant removal rate is a fundamental objective to characterize the efficiency of wastewater treatment [Moldovan Z., 2007]. In the paper was determined the rate of removal of

common pharmaceuticals such as ibuprofen, naproxen, indomethacin carbamazepine in the biological stage treatment plant in Cluj-Napoca. Samples were collected from the influent and effluent treatment plant in Epuration Station Someşeni. The compound concentration was determined by a liquid-liquid process (as solvent extraction using n-hexane) described in Experimental.

Samples were collected in two different precipitation seasons: one dry, and one wet respectively. Good result was achieved in the dry period. During wet,in effluent samples the compounds concentration it was below the detection limit. After concentration the samples were silylated and analyzed using gas chromatography coupled with mass spectrometer. Quantification was achieved using chromatogram compounds peak area on based maximum and compared with the internal standard (30-2,4,6-triclorobifenil PCB).

The obtained data are presented in Table 6.1 on the three samples average. Removal rate for ibuprofen obtained for the biological unit of 95.15 %. The amount is in concordance with recently published works on wastewater treated in pilot station of hospital [Langenhoff A., 2013] or in experiments for study the extraction efficiency by biological treatment using activated sludge at laboratory scale [M. Zupanc 2013]. For galaxolides, the removal rate was 9.01 % and for tonalide the elimination was 30.54 %. The galaxolide and tonalide compounds are freely detected in waste water, and they are used in detergent formulations.

**Table 6.1** The results obtained from water samples collected before and after biological stage treatment plant Cluj-Napoca. Note: (*) is the molecular weight after silylation; (**); The detection limit was 10 ng / l.

| Compound | Elution time (min) | Molecular weight (M) (*) | Ion used for quantification (m/z) | The influence of concentration [ng/L] | The effluent concentration [ng/L] | Remuval percentage [%] |
|---|---|---|---|---|---|---|
| Ibuprofen | 15.09 | 278 | 160 | 245,92±13.2 | 11,88±1.05 | 95,17 |
| Internal standard | 18.55 | 256 | 256 | 1000.00 | 1000.00 | - |
| Naproxen | 27.61 | 302 | 185 | <LOQ (**) | <LOQ | n.d. |
| Carbamazepină | 31.81 | 308 | 193 | <LOQ | <LOQ | n.d. |
| Indometacin | 44.87 | 429 | 139 | <LOQ | <LOQ | n.d. |

**6.13 Conclusions of Chapter 6**

- Both activated charcoal and zeolite can be successfully used for adsorption of pitofenone hydrochloride in municipal treatment plants. pitofenone hydrochloride can be adsorbed from wastewater with porous materials of molecular sieve and activated charcoal type. Due to the small size of the pores, 13X zeolite adsorbs a small amount of pitofenone hydrochloride than the activated charcoal. Studies can proceed with tests of adsorption at different temperatures also the adsorption rate of pitofenone hydrochloride on most effective adsorbent can be evaluated, and activated charcoal respectively with experiments which monitor pitofenone hydrochloride concentration during adsorption on this material.
- For a treatment plant with a flow rate of 2 $m^3$/s results in the following quantities: flow rate = 2 $m^3$/s equivalent to 2·3600 = 7.200 $m^3$/h equivalent to 7200·24 = 172 800 $m^3$/day. If the amount of the pollutant is 1 mg/$m^3$ 518 400 results a necessary amount = 518.400 mg of activated charcoal = 518 g/day = 5.18 kg/10 days = 15 kg/month = 93 kg/6 months.
- For economic reasons it is preferable zeolite, cost prices are much lower.
- Adsorption processes for Ibuprofen, Naproxen, Indomethacin and Carbamazepine have been studied on six kinds of adsorbents: 2 kinds of activated charcoal and 6 kinds of zeolite, for a period of 4 days (96 hours). In all cases, Ibuprofen and Naproxen have been adsorbed on the activated charcoal (C2) which ibuprofen is adsorbed in an amount of 80 % and naproxen of about 70%. Ibuprofen has been adsorbed at a rate of only 60 % on the zeolite SM-S9.
- Carbamazepine and Idometacine showed a ratio of adsorption of about 54%. An exception is observed for carbamazepine on the SM-S6 zeolite where the adsorption is about 80 % after 4 days, and adsorption for Indomethacin is about 90 % on SM -S10 adsorbent. The most effective adsorbent seems to be SM-S10 in which case the three compounds (Ibuprofen, Naproxen, Indomethacin) are absorbed in 4 days of over 90 %, and Carbamazepine is absorbed in an amount of about 50 %. These data are in concordance with the reported results in a recently published paper [A. M. Anderson, 2000], which concluded that the retention rate is increasing for higher ratio Si/Al.
- Obtained concentration for ibuprofen in the influent sewage plants in Cluj-Napoca was 245.92 ng/L and 11.88 ng/L in the effluent. Elimination rate for ibuprofen by sewage plants in Cluj-Napoca was 95.17 %, remaining concentration in the effluent was 4.83 % compared to the influent. By dilution in the receptor river (Small Somes River with a flow of 12 $m^3$/s) concentration of ibuprofen is reduced to 1.98 ng / L. For compounds naproxen, carbamazepine and indomethacin the elimination rate was not calculated because test concentrations were below the limit of quantification (LOQ = 10 ng/l).

**Chapter 7. Associated studies on pharmaceutical compounds of pitofenone chlorhydrate type included in β-cyclodextrin**

**7.1 Introduction**

Spasmalgon product with pitofenone hydrochloride as active substance is a combination of drugs used to relieve pain and muscle spasms. The purpouse was to prepare inclusion compounds of pitofenone hydrochloride with β-cyclodextrin by co-precipitation and lyophilization and characterize them using X-ray powder diffraction, Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, differential scanning calorimetry, scanning electron microscopy and the density functional theory- molecular modelling [Popeneciu H., 2016].

**7.2 Experimental conditions**

**7.2.1. Powder X-ray diffraction.** Powder diffractogrammes were collected in the angular range of 2θ = 3.5-40 with Bruker D8 Advance diffractometer using Cu $K\alpha_1$ (λ = 1.5406 Å) (40 Kv; 40 Ma) radiation. In order to enhance the resolution, the Ge (111) monocromator was used to eliminate CuKα2 radiations. Data collection was conducted with the package program DIFFRAC plus XRD Commander, at room temperature. The scanning mode was performed with a step $0.01^0$ at a rate of 1 step/s. The samples were gently milled in a mortar of agate to control the size of the crystals and to minimize the effects of preferred orientation.

**7.2.1.1 Interpretation of X-ray diffraction results.** The XRD patterns of pitofenone hydrochloride, β- cyclodextrin and of the inclusion compounds obtained by co-precipitation and lyophilization are shown in Figura.7.1. It can be seen that diffraction X-ray diffractograms of the products obtained by co-precipitation and lyophilization are different compared with diffractograms of starting compounds, so the inclusion compounds are obtained. The most important reflections of hydrochloride pitofenona - β- cyclodextrin not belong to β-CD, nor for pitofenone hydrochloride. These reflections occur at the following angles (2θº): 4.70º, 4.94º, 5.87º, 7.31º, 9.44º, 9.88º and 14.94º. The crystallite size was measured using the Scherrer relationship [Klug, H. P., 1974]. I obtained the crystallite size D = 300 Å for hydrochloride pitofenonă - β-cyclodextrin while the crystallites size of β-cyclodextrin was D = 1200 Å and D = 1300 Å for pitofenonă hydrochloride. It is confirmed the amorphous nature of the lyophilized product and also the partial amorphous nature of the co-precipitated product.

The most important reflections that appear in hydrochloride pitofenona - β- cyclodextrin do not belong to β-CD, nor pitofenone hydrochloride. These reflections occur at the following angles ($2\theta^0$): $4.70^0$, $4.94^0$, $5.87^0$, $7.31^0$, $9.44^0$, $9.88^0$ and $14.94^0$. The crystallite size was measured using the Scherrer relationship [Klug, H. P., 1974]. I obtained the following crystallite size: D = 300 Å for pitofenone hydrochloride - β-cyclodextrin, D = 1200 Å for β-cyclodextrin crystallites and D = 1300 Å for pitofenone hydrochloride. It is confirmed the amorphous nature of the lyophilized product and also partial amorphous nature of the co-precipitated product.

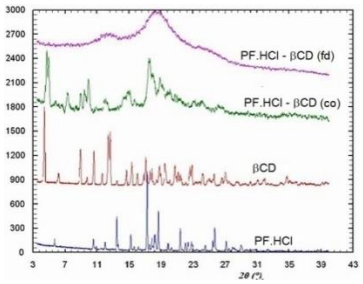



***Figure 7.1*** *Diffraction patterns of : pitofenone hydrochloride powder, β-cyclodextrin inclusion compounds produced by co-precipitation (co) and lyophilization (fd)*

**7.2.2 Analysis by differential scanning calorimetry**. The DSC thermograms were recorded on a Shimadzu differential scanning DSC 60 calorimeter by heating 1.5 mg of the sample from room temperature to $400^0$C in a box of aluminum crimp under a stream flow of nitrogen, the heating rate being $100^0$C/min. For data collection was used WS60 and Shimadzu TA-60 TA 2.1 software.

**7.2.2.1 Results and conclusions in Differential Scanning Calorimetry**. DSC thermograms of pure pitofenone hydrochloride, β-cyclodextrin and of the inclusion compounds formed between pitofenonă hydrochloride-β-cyclodextrin are shown in Fig. 7.2. The DSC curve of β-cyclodextrin shows a broad endothermic signal between 74-$118^0$C, with ΔH = 190 kJ/mol, which corresponds to a loss of water molecules contained in the cavity [J.A.Castro-Hermida, 2004, M.K. Rotich, 2003] and of them existing as residual moisture. The melting occurs at $290^0$C followed by decomposition of the compound [J. Szejtlij, 1988, F. Trotta, 2000]. Hydrochloride pitofenonă DSC curve has an endothermic sharp peak at $171.6^0$C, with ΔH = 117.7 kJ/mol,

corresponding to the melting of the product, the compound exhibits thermal stability up to $230^0$C when the degradation begins (Figure 7.2). The DSC curve of the compounds obtained by co-precipitation or lyophilization of pitofenone hydrochloride and β-cyclodextrin shows no endothermic signal specific to β-cyclodextrin dehydration or the one corresponding to the melting pitofenone hydrochloride. These facts sustaine the formation of hydrochloride inclusion complex between β-cyclodextrin and pitofenone [HP Klug 1974].

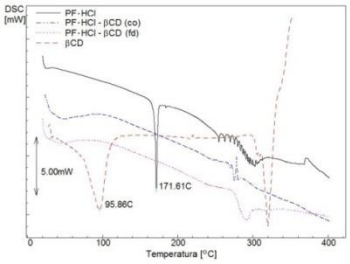



***Figure 7.2*** *The DSC curves of pitofenone hydrochloride, β-cyclodextrin and of the inclusion compounds obtained by co precipitation and lyophilisation*

**7.2.3 Solid stade NMR spectroscopy**. The $^{13}$C NMR spectra were obtained on a Bruker Advance III 500 MHz spectrometer large bore-NMR operating at room temperature, using a 4 mm probe head CP-MAS. The RAMP standard spectrum CP-MAS was acquired by the spinning frequency of 14 kHz, 2ms contact time CP, high power (100 kHz) proton decoupling under TPPM, delay recirculation 5S average in 1200 transitional pitofenone hydrochloride and β-cyclodextrin, and 15,500 transitional pitofenone hydrochloride-β-cyclodextrin. The recorded spectra are calibrated with respect to the line $CH_3$ in TMS (tetramethylsilane) through an indirect procedure using L-glycine α as the external standard (C - O Glycine at 176.5 ppm).

**7.2.3.1 Interpretation of NMR results.** The magnetic resonance spectroscopy spectra of $^{13}$ C CP-MAS in solid pitofenone hydrochloride, β-cyclodextrin and the two compounds of pitofenone hydrochloride-β-cyclodextrin, prepared by co-precipitation techniques (co) and lyophilization (fd) are shown in Figure 7.3. The pitofenone hydrochloride-β-cyclodextrin compound obtained by co-precipitation (Figura.7.3.c) is a mixture of amorphous and crystalline phase while the one obtained by lyophilisation is amorphous (Figure 7.3.d). Despite the amorphousness of the spectrum, the most obvious difference observed between free state and complexed state of pitofenone hydrochloride is the displacemet of line located at 66.1 ppm in pitofenone hydrochloride spectrum, marked with black lines in the NMR spectra (Figure 7.3b, 7.3c , 7.3d) thus indicating a different chemical environment for C $^{16}$ atom corresponding to pitofenone and the possibility of forming inclusion compounds.

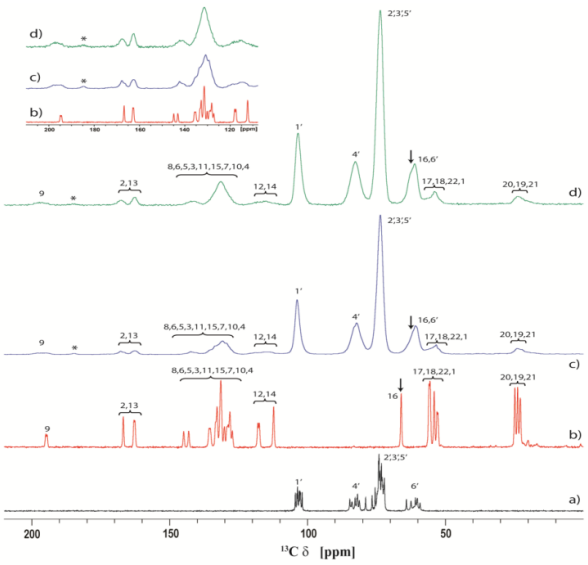



Figure 7. *$^{13}$C CP-MAS NMR spectrum of: a) β-cyclodextrin, b) hydrochloride pitofenona, c) Ethyl pitofenona-β-cyclodextrin (co-precipitation) d) Ethyl pitofenona-β-cyclodextrin (lyophilization). In figure inset are the appropriate lines of pitofenone hydrochloride in the 110-200 ppm range. Side bands are marked by asterisks*

**7.2.4 FTIR spectroscopy.** FTIR spectra were recorded in the spectral range 4000 - 400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ on 6100 JASCO FT-IR using Br pellets technique; the spectra were processed by spectral analysis software.

**7.2.4.1 Fourier Transform Infrared Spectroscopy results interpretation.** In Figure 7.4 A and B are shown the FTIR spectra of pitofenone hydrochloride, β-cyclodextrin and inclusion compounds obtained by co-precipitation and lyophilisation.

It can be concluded (see Figure 7.4b.) that the vibration frequency ν of (C = O) located at 1721 $cm^{-1}$ is not moved by complexation, while the vibrational mode ν (COO-) at situated at 1679 $cm^{-1}$ is shifted at 1658 $cm^{-1}$ by complexation with β-cyclodextrin. This is an indication of the formation of the inclusion compound, both by co-precipitation and lyophilisation processes.

***Figure 7.4A*** *FTIR spectra of pitofenone hydrochloride, β-cyclodextrin, inclusion compounds β-cyclodextrin pitofenone hydrochloride obtained by co precipitation and lyophilization, in 4000-400 $cm^{-1}$ spectral range*

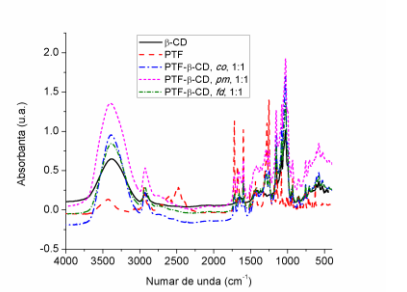
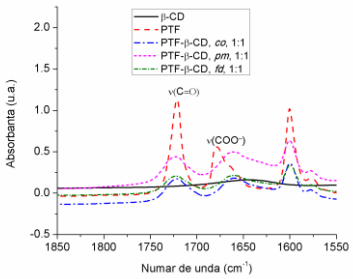
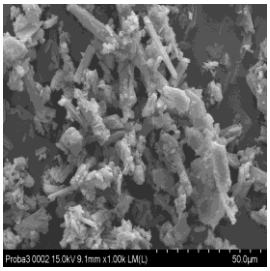



***Figure 7.4B*** *FTIR spectra of pitofenone hydrochloride, β-cyclodextrin, inclusion compounds β-cyclodextrin pitofenone hydrochloride obtained by coprecipitation and by lyophilization, in 1850-1550 $cm^{-1}$ spectral range*

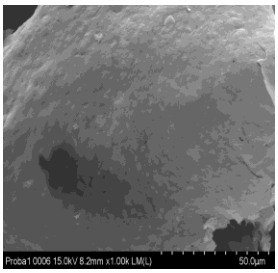



**7.2.5 Scanning electron microscopy**. SEM was performed on a HITACHI 8230 UHR-SEM. The samples were pipetted and evenly distributed on the silicon wafer. The samples were analyzed in their natural state, without a covering with a conducting layer. The SEM analysis was conducted in vacuum. Advantages of EDX (EDS) X-ray spectroscopy: $x_{max}$ were conducted with 80 $mm^2$ SDD Oxford instrument type (silicon detector movement). It was used a voltage of 15 kV, hight vacuum $\geq 10^{-4}$, secondary electron imaging was used.

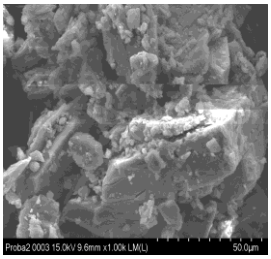
**7.2.5.1 Results and conclusions of scanning electron microscopy SEM characterization**. The pitofenone hydrochloride, β-cyclodextrin and the inclusion compounds pitofenone hydrochloride-β-cyclodextrin images obtained by co-precipitation and lyophilisation are shown in Figure 7.5 A-D. On scanning electron microscopy images can be seen that the crystallite sizes are different in the case of pure substances (crystalline substance) and inclusion compounds.



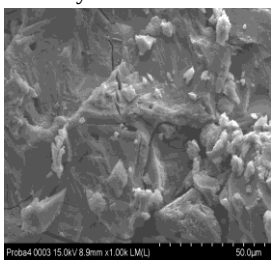
A β-cyclodextrin



B pitofenone hydrochloride



C 1:1 inclusion compounds (co-precipitation)



D 1:1 inclusion compounds (lyophilization)

***Figure 7.5*** *A-D* Scanning electron microphotographs: A -β-CD, B - pitofenone HCl, C- the inclusion complex 1:1 co precipitation, and D - 1:1 inclusion complex (lyophilization).

**7.2.6 Molecular modeling**. **Density Functional Theory.** The geometry optimisation and frequency calculations for β-cyclodextrin, pitofenone hydrochloride molecules and their host-guest compound were carried out in the gas phase density of functional theory level, taking into account the M11 [R Peverati ., 2011] correlation with exchange def2-SVP [Weigend F., 2005] have been implemented in the software package Gaussian mode 09 [Gaussian 09, 2009]. No negative wave number has been obtained for all three cases, showing that the true minimum of potential energy surface has been found in optimization.

**7.2.6.1 Conclusions in molecular modelling.** The equilibrium geometry of pitofenone hydrochloride, β-cyclodextrin, supramolecular host-guest complex was obtained using the theory of density functional M11 [Peverati R., 2011] exchange functional correlation on the basic of set-TZVP def2 [Weigend F., 2005] implemented in Gaussian 09 program package quantum chemistry [Frisch M.J., 2009]. Geometry configuration of the inclusion compound β-cyclodextrin - pitofenone hydrochloride can be seen from two points of view: side and bottom view, see Figure 7.6 A and B respectively.

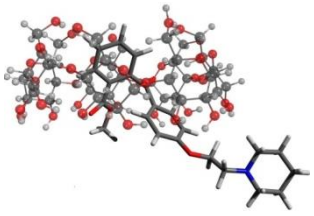


*Figure7.6A Side view of the inclusion compound pitofenone hydrochloride-β-cyclodextrin*

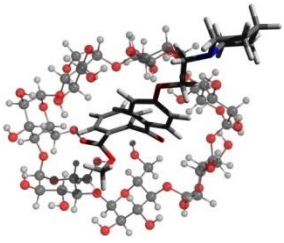


*Figure 7.6B Bottom view of the inclusion compound pitofenone hydrochloride-β-cyclodextrin*

To estimate the thermodynamic effects on inclusion process was carried out further analysis to calculate normal mode Gibbs free energy by using smaller size def2-SVPP [Weigend F., 2005] in the base set. Therefore, the intermolecular interaction energy calculation was carried out using both sets of base, small and large. Intermolecular energy interaction resulting from the M11/def2-SVPP theory is -61.41 kcal/mol, while the value obtained with the same method M11/def2-TZVP is -41.87 kcal/mol. As we can see, by increasing the size of basis set, the intermolecular interaction energy fall sharply. To take into account of the thermodynamic effects, we calculated also the Gibbs free energy given the M11/def2-SVPP theory. For this value -6.36 kcal/mol was obtained showing a complexing spontaneous process, but should be accepted with reservations due to the effects of base size. On the other hand, it is important to know the effects that may prevent complexation. In this regard, we calculated the strain energy, which is the energy difference between the energy values of the constituents of molecular geometry obtained at equilibrium configuration in isolated and complex cases. Strain energy of β-CD is 29.28 kcal/mol for M11 case/def2-SVPP and 19.67 respectively kcal/mol for M11/def2-TZVP. Similar values for pitofenone hydrochloride are 6.04 kcal/mol for the case M11/def2-SVPP respectively 2.81 kcal/mol for M11/def2-TZVP. It can be seen that the inclusion process also induces a strong deformation of the β-cyclodextrin cavity, while in the case of pitofenone hydrochloride is relatively low. Another interesting feature is that the complexation of pitofenone hydrochloride molecule bonds only two hydrogen with OH groups of β-cyclodextrin, most of the interaction is given by the contribution of long-term dispersion forces. At the same time the movement of the nuclear magnetic resonance line in $C^{16}$ carbon atom spectra can be induced by one of the hydrogen bonds realized by $O^{13}$ with a lower OH group of the β-cyclodextrin.

**7.3 Conclusions of Chapter 7**. The inclusion compounds of pitofenone hydrochloride with β-cyclodextrin were prepared by co-precipitation and lyophilisation. We have characterized the solid phase by X-ray diffraction, differential scanning calorimetry, Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, scanning electron microscopy and molecular modeling. X-ray diffraction indicates that the compound obtained by lyophilisation was in amorphous state. By co-precipitation was obtained a compound in partly amorphous phase but with an important crystalline phase. Differential scanning calorimetry curves indicate the formation of compounds which are different from the started compounds. The infrared spectroscopy indicates the involvement of $COO^-$ group in the complexation process. Nuclear magnetic resonance spectroscopy indicated the formation of an amorphous compound (obtained by lyophilization), and a partially crystalline compound (obtained by co-precipitation) with the possibility of forming inclusion compounds (hydrochloride pitofenona line shifted in $^{13}C$ CP-MAS spectra of the new compounds compared with pitofenone hydrochloride spectrum).

## CONCLUSIONS

- Three new solid forms were obtained by parallel crystallisation experiments.
- It was obtained by X-ray diffraction, the complete crystalline structure of the hydrochloride pitofenona drug start form, such as: crystallographic system, unit cell parameters, the space group, the positions of the atoms in the elementary cell which also gives: the distance between atoms, link angles, hydrogen connections.
- The compound $C_{11}H_{16}O_4P_1S_2N_1$ crystallizes in monoclinic system, having $P2_1/c$ space group and the following unit cell parameters: a [Å]: 9.29217, b [Å]: 11.87197, c [Å]: 14.26229, α [$^o$] 90, β [$^o$]: 107.7514, γ [$^o$] 90.
- The compound $C_{12}H_{18}O_4P_1S_2N_1$ crystallizes in triclinic system, having the P-1 space group and the following unit cell parameters: a [Å]: 7.4469 (4), b [Å]: 10.9588, c [Å]: 11.0534, α [$^o$] 112 166, β [$^o$]: 97.291, γ [$^o$] 102 556.
- It was determined by mass spectrometry, based on the recording and interpretation of the mass spectra, for the pitofenone hydrochloride: the molecular weight, the molecular formula and molecular structure.
- It was attempted to determine the concentration of pitofenone hydrochloride in Somes river water.
- In analyzed water samples, the pitofenone hydrochloride wasn't found.
- For pollutants such as pharmaceutical compounds have been developed methods of remediation.
- The pitofenone hydrochloride can be adsorbed from wastewater by porous materials like molecular sieve and activated carbon. Due to the small size of the pores, 13X zeolite adsorbs a smaller amount of pitofenone hydrochloride compared to the activated carbon.
- The studies may continue with the adsorption test at various temperatures and also with the evaluation of the pitofenone hydrochloride adsorption speed on the more effectively adsorbent, namely on active coal by experiments which follow the pitofenone hydrochloride concentration during the adsorption on this material.
- In all cases the ibuprofen and naproxen have been adsorbed in a proportion of 90 %. An exception was observed in the case of adsorption on activated carbon, the ibuprofen adsorbtion ratio was 80 % and for naproxen about 70 %. The ibuprofen was absorbed at a rate of only 60 % on Zeolite MS-S9.

- Both the activated carbon and zeolite can be successfully used for pitofenone hydrochloride adsorption in municipal treatment plants.
- For a treatment plant with a flow of $2m^3/s$ results the following quantities: flow = $2m^3/s$ equivalent to $2 \cdot 3600 = 7.200m^3/h$ equivalent to $7200 \cdot 24 = 172.800m^3/day$.
- If the amount of pollutant is 1 $mg/m^3$ results an amount of 518.400mg active coal = 518 g/day, 5.18 kg / 10 days = 15kg/month = 93kg/6 months.
- For economic reasons, the zeolite is preferable, the prices being much lower.
- The adsorption of Ibuprofen, Naproxen, Indomethacin and carbamazepine has been studied on six kinds of adsorbents, two types of activated carbon and six types of zeolite for a period of 4 days (96 hours).
- In all cases the ibuprofen and naproxen have been adsorbed to an extent of more than 90 %. The exception is the case of ibuprofen adsorption on activated carbon where a ratio of 80% was obtained and naproxen adsorption ratio of about 70 %. The ibuprofen was absorbed at a rate of only 60% on Zeolite SM-S9.
- Carbamazepine and indomethacin showed a ratio of adsorption of less than 54%. An exception in carbamazepine case was observed for SM-S6 zeolite where the adsorption is about 80 % after 4 days, and in the case of indomethacin, the adsorption is about 90 % in the SM-S10 adsorbent.
- The most effective adsorbent seems to be zeolite SM-S10, case in which the three compounds (Ibuprofen, Naproxen, Indomethacin) are absorbed 90 % within 4 days and whereas carbamazepine is adsorbed in a ratio of about 50% .
- These data are consistent with the reported results of a recently published paper [M. A. ANDERSON, 2000], which concluded that the retention rate is increasing for high Si/Al ratio.
- The quantity obtained for ibuprofen in influent waste water of treatment plants in Cluj-Napoca was 245.92 ng/L and 11.88 ng/L in the effluent. The Ibuprofen rate of disposal by sewage plants in Cluj-Napoca was 95.17 %. Therefore, the compound concentration in the effluent was 4.83 % compared to the influent. By dilution in the receiver river (River Somes Mic at a rate of 12 $m^3/s$) the Ibuprofen concentration is reduced to 1.98 ng/L. For Naproxen, Indomethacin and Carbamazepine compounds the elimination rate was not calculated because the concentrations were below the limit of quantification (LOQ = 10 ng/l).
- The inclusion compounds of β-cyclodextrin pitofenone hydrochloride were prepared by coprecipitation and lyophilisation. We have characterized the obtained solid phase by X-ray diffraction, differential scanning calorimetry, Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, scanning electron microscopy and molecular modeling. X-ray diffraction indicates that the compound obtained by lyophilization was in amorphous state. The compound obtained by coprecipitation was partially crystalline with an important amorphous phase. Differential scanning calorimetry curves indicate the formation of compounds which are different from the starting reactants.
- The infrared spectroscopy indicates the involvement of $COO^-$ group in the complexation process.
- The resonance spectroscopy indicates the formation of an amorphous compound (obtained by lyophilization), and a partially crystalline compound (obtained by co-precipitation) with the possibility of forming inclusion compounds (the hydrochloride line is shifted in $^{13}C$ CP-MAS spectra of the new compounds compared to pitofenone hydrochloride). Based on the X-ray diffraction results, and magnetic resonance spectroscopy, the compounds thus obtained (co-precipitation and lyophilization) are amorphous and partially amorphous. There are indications that the X-ray diffractograms of inclusion compounds obtained by co-precipitation are different from the start. The density functional theory modeling technique gives the spatial molecular architecture of inclusion compounds.

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**LIST OF SCIENTIFIC PUBLICATIONS PRODUCED TO DATE PHD THESIS THEME AND INCLUDED**

**Articles published rated ICI**

1. **Horea Popeneciu**, Ioan Bratu,Gheorghe Borodi, Attila Bende, Lucian Barbu – Tudoran,Dumitru Ristoiu, Inclusion compounds of β-cyclodextrin-pitofenone hydrochloride.Invetigations of solid forms, Studia Univeristatea Babeş-Bolyai Seria Chemia, Cluj Napoca Edition 2 Vol. 61 2016 (p.61), http://chem.ubbcluj.ro/~studiachemia/, impact factor ISI: 0.81
2. **Horea Popeneciu**, Zaharia Moldovan, Dumitru Ristoiu,Removing residues of pharmaceuticals in aqueous medium by adsorption on solid phase,Revista de chimie, Bucharest Vol. 67 Nr. 10, 2016, http://www.revistadechimie.ro, impact factor ISI:0.677.
3. **Horea Popeneciu**, Zaharia Moldovan, Dumitru Ristoiu, 2013 *Characterization of pitofenone by EI mass spectrometry and GC/MS system*, **A** IX-aInternational Conference on Processes in Isotopes and Molecules, Cluj-Napoca Romania, AIP Conference Proceeding 1565, 67 25-27 September, http://www.itim-cj.ro/contact rated ISI,without impact factor.
4. **Horea Popeneciu**, Adrian Calborean, Cristian Tudoran, Luiza Buimaga-Iarinca 2013*DFT investigation on the electronic structure of Faujasite***,** **A** IX-aInternational Conference on Processes in Isotopes and Molecules, Cluj-

NapocaRomania, AIP Conference Proceeding 1565, 71 25-27, Septembrie, http://www.itim-cj.ro/contact, rated ISI without impact factor.

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**Work-communication**

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