ejpmr, 2020,7(7), 134-141

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ATORVASTATIN FATE STUDY IN WATER CONTAMINATED WITH QUALITY CONTROL REAGENTS IN THE PHARMACEUTICAL INDUSTRY. EFFECT OF UV LIGHT

Fateh Naitali* and Hafida Ghoualem

Laboratory of Electrochemistry-Corrosion, Metallurgy and Inorganic Chemistry. Faculty of Chemistry. University of Sciences and Technology. Houari Boumediene. Box N°32 El-Alia. Bab-Ezzouar. 16111. Algiers. Algeria.

***Corresponding Author: Dr. Fateh Naitali**
Laboratory of Electrochemistry-Corrosion, Metallurgy and Inorganic Chemistry. Faculty of Chemistry. University of Sciences and Technology. Houari Boumediene. Box N 32 El-Alia. Bab-Ezzouar. 16111. Algiers. Algeria.

Article Received on 03/05/2020 **Article Revised on 24/05/2020** **Article Accepted on 13/06/2020**

ABSTRACT

Releases from the pharmaceutical industry are increasingly being detected in aquatic matrices. Atorvastatin is an inhibitor of HMG Co-A reductase, an enzyme whose activity is early in the synthesis of cholesterol. The objective of this work is the study of the fate of atorvastatin in water polluted by chemical reagents during quality control in a laboratory of a pharmaceutical industry in the presence and in the absence of a UV light. The results of this study will be used later to predict the impact of the presence of atorvastatin in the receiving waters. The results of the HPLC analyzes show that there is a variety of interactions between the chemical reagents used and the atorvastatin.

KEYWORDS: Pollution, pharmaceutical industry, drug, reagents, HPLC, UV.

I. INTRODUCTION

The activities of the pharmaceutical industry and hospitals are the main sources of the presence of drugs in aquatic environments.

Several scientific studies[1-4] have shown the presence of various drugs in aquatic environments. The reactivity of these drugs in wastewater is poorly studied.

Statins were introduced in clinical practice in the 1980s. The active part of statins is the lactone ring. Statins are metabolised by the liver and intestine (except pravastatin), mainly by the major isoenzyme of hepatic and intestinal cytochromes P450, CYP 3A4.[5]

Atorvastatin is an inhibitor of HMG Co-A reductase, an enzyme whose activity is early in cholesterol synthesis. It is used as a cholesterol-lowering and hypotriglyceridemic drug.[5,6]

Statins have a short half-life (1 to 4 hours), with the exception of atorvastatin, which has a long elimination half-life of 14 hours for the parent compound and even more for active metabolites.

Statins are substances containing a pyrrole ring. The pyrrole ring is a 5-membered nitrogen heterocycle. It is one of the most studied simple heterocycles because of its presence in a large number of natural compounds. It thus represents the basic structural unit of the porphyrins of heme and chlorophyll, two pigments essential to life.

It is also found in vitamin B12 and many natural alkaloids, especially in compounds derived from marine organisms.

Statins also have anti-inflammatory and antimicrobial effects. According to literature, during the years 1999-2017, the research on atorvastatin with liquid chromatography has increased to 481 papers.[7]

Various chemicals are present in aquatic environments. Acids and bases are part of the majority of domestic and industrial effluents.

The effect of UV radiation on matter was discovered in 1801 by the German physicist Johann Wilhelm Ritter after their action on silver chloride.

These UV rays are able to degrade some chemical compounds and accelerate the kinetics of chemical reactions.

It is known that about 5% of the electromagnetic energy of the sun is emitted as ultraviolet radiation.

To better know the fate of drugs in wastewater, it is essential to study the reactivity of these drugs with organic and mineral materials that can constitute these waters.

The objective of this study is to evaluate the behavior of a drug under the guidance of various reagents of a quality control laboratory.

The fate of atorvastatin in water contaminated with chemical reagents of quality control (acid, base solvent) of the pharmaceutical industry in the presence and in the absence of UV radiation is monitored. Two wavelengths are studied for this purpose.

II. MATERIALS AND METHODS

The drug making the goal of this study is atorvastatin. The structure of the molecule is shown in Figure1.

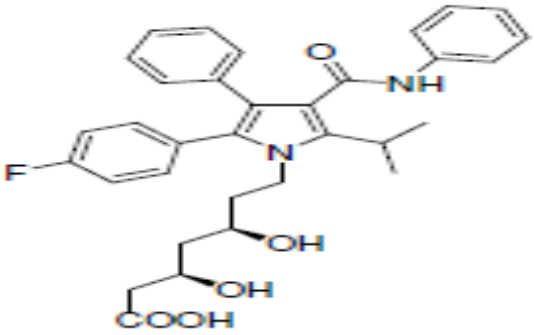

Figure 1: Chemical structure of atorvastatin.

The chemical structure of the various statins can be broken down into three parts: an analogue of the substrate of the target enzyme, HMG-CoA; a hydrophobic complex side chain covalently bound to the analogous substrate and involved in the binding of the statin to the enzyme reductase; chemical groups grafted onto the chain conditioning the solubility of the molecule and thus several of its pharmacokinetic properties.

Atorvastatin such as fluvastatin, lovastatin and simvastatin are relatively lipophilic compounds, whereas pravastatin and rosuvastatin are more hydrophilic.[8]

Atorvastatin molecular weight is 558.2530 g / mol, pKa is 4.5 and log KOW is 6.36.[9]

All solutions are prepared by dissolving organic and / or inorganic compounds (Table 1) in ultra-pure water with pH ranging from 6 to 7.2 and conductivity less than 3 µS/cm and obtained at using a Millipore system.

The chemical reagents used in this study (Table 1.) are prepared according to the methods described by the European Pharmacopoeia.

Table 1: Chemicals used.

Reagent	Chemical formula	M (g/mol)
H_2SO_4 (98%)	H_2SO_4	98
Ammonia	NH_4OH	35
Diméthylsulfoxyde_(DMSO)	C_2H_6OS	78

Atorvastatin is determined by high performance liquid chromatography (HPLC) according to the procedure of the European Pharmacopoeia (EP.9.5) using a Waters analysis chain.

Chromatographic analysis conditions (composition of the mobile phase, retention time, wavelength, etc.) for atorvastatin are given by the European Pharmacopoeia.

The solutions to be analyzed are prepared as follows

To six solutions atorvastatin at a concentration of 1.175 mg / mL, the reagents (Table 1) are added with a volume percentage of (2 µL of each reagent per 5 mL of each atorvastatin solution).

Three sets of solutions are prepared: the first is exposed to UV light (254 nm) for 5 min; the second is exposed to UV light (366 nm) for 5 minutes and the third is analyzed as it is.

III. RESULTS AND DISCUSSION

III.1 Chromatogram of control atorvastatin

The chromatogram obtained for the reference solution containing atorvastatin is represented in the following figure:

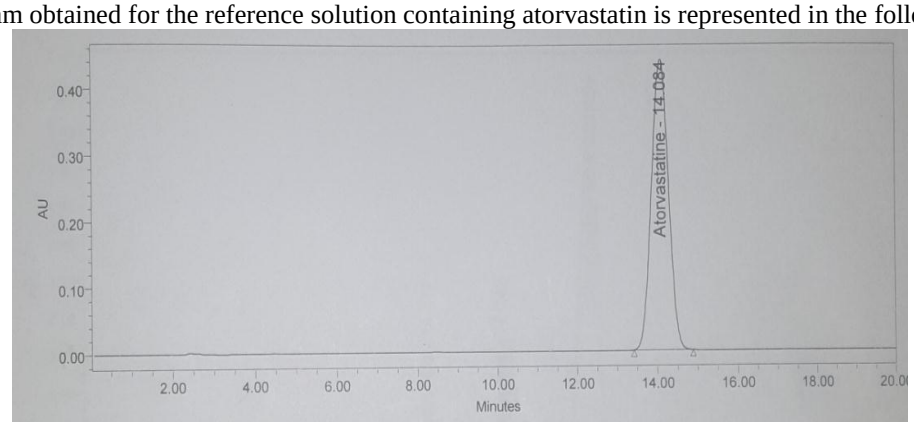

Figure 2: Chromatogram of atorvastatin.

The peak of the atorvastatin control appears at t equal to14.084 min
The symmetry factor (k) for atorvastatin is equal to 1.13.
The peak area is equal to 13077688.
The method of dosing followed is therefore suitable for the determination of atorvastatin.

III.2 Effect of sulfuric acid

The chromatogram obtained after the addition of sulfuric acid to the solution of atorvastatin is shown in figure 3.

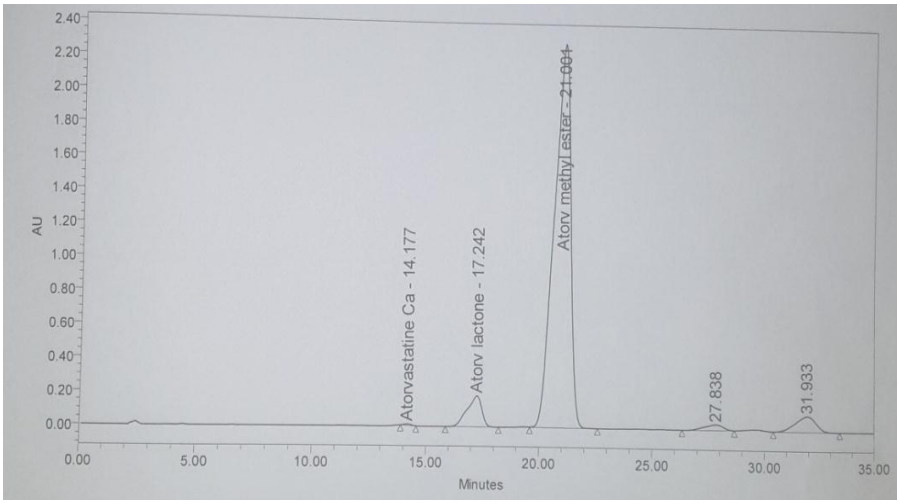

Figure 3: Chromatogram of atorvastatin after the addition of sulfuric acid.

These results show the absence of a principal peak of atorvastatin.

Thus, five other peaks appear at t equal to 14.177, 17.242, 21.001, 27.838 and 31.933 min successively. The first three peaks are identified such as atorvastatin calcium (0.13%), atorvastatin lactone (6.21%) and Atorvastatin methyl ester (87.61%). The other two peaks have a percentage of 1.5 and 4.55% successively.

These results are explained by an interaction between atorvastatin and sulfuric acid.

The sulfonation of the pyrrole and furan heterocycles requires the use of sulfur trioxide in pyridine to avoid polymerization reactions that result from the use of H2SO4.

The presence of a hetero element in heterocycles such as pyridine, pyrrole or thiophene orients the attack of electrophilic reagents.

For pyrrole and thiophene, the 2-position is most often attacked because of its higher electron density which results from the effect of the hetero element.

Thus, the presence of reactive species[10-11], the presence of catalysts such as NaCl, Fe^{3+}[12-13], the presence of humic acids and algae[14-15], the presence of Nitric acid (HNO_3) and its salts (KNO_3) are also parameters that can influence reactivity with organic matter.

III.3 Effect of sulfuric acid under UV light (254 nm)

The chromatogram obtained after the addition of sulfuric acid to the solution of atorvastatin under UV light (254 nm) is shown in figure 4.

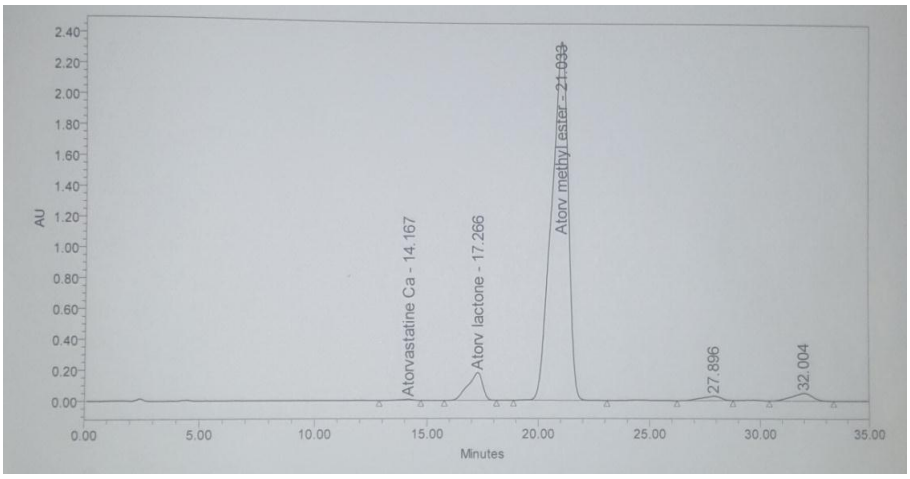

Figure 4: Chromatogram of atorvastatin after addition of sulfuric acid under UV light (254 nm).

These results show the absence of a principal peak of atorvastatin.

Thus, five other peaks appear at t equal to 14.167; 17.266; 21.033; 27.896 and 32.004 min successively.

We can say that this result is the same as that of the previous experiment. Therefore UV light (254 nm) has no effect on atorvastatin in the presence of sulfuric acid.

The first three peaks are identified such as atorvastatin calcium (0.21%), atorvastatin lactone (6.07%) and atorvastatin methyl ester (89.96%).

The other two peaks have a percentage of 1.31 and 2.44% respectively.

III.4 Effect of sulfuric acid under UV light (366 nm)

The chromatogram obtained after the addition of sulfuric acid to the solution of atorvastatin under UV light (366 nm) is shown in figure 5.

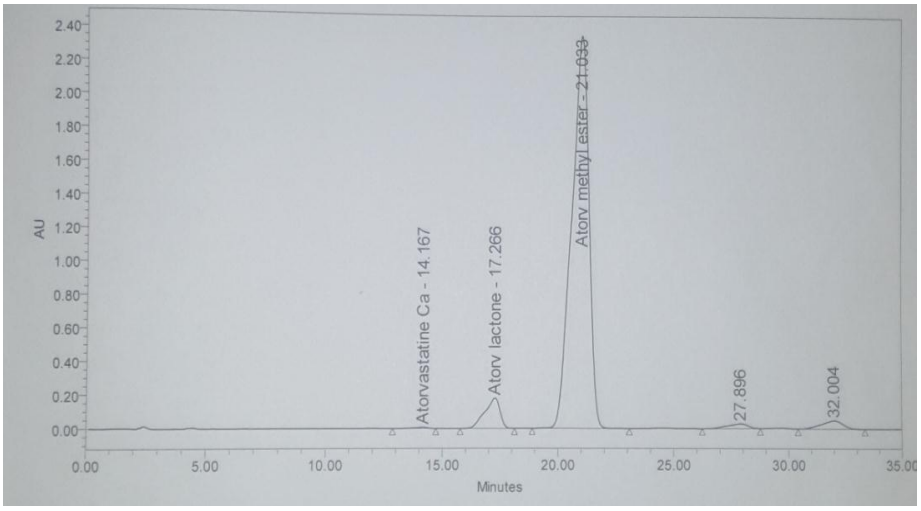

Figure 5: Chromatogram of atorvastatin after addition of sulfuric acid under UV light (366 nm).

Thus, four other peaks appear at t equal to 14.274; 17.400; 21.194 and 32.263 min respectively. Compared to the two previous results, the fourth peak does not exist.

Therefore, UV light (254 nm) has no effect on atorvastatin in the presence of sulfuric acid. Also, the peak area regarding atorvastatin calcium to decrease while the last peak area to increase.

The first three peaks are identified such as atorvastatin calcium (0.15%), atorvastatin lactone (6.43%) and atorvastatin methyl ester (89.39%). The fourth peak at a percentage of 4.03%.

III.5 Effect of DMSO

The chromatogram obtained after the addition of DMSO to the solution of atorvastatin is shown in figure 6.

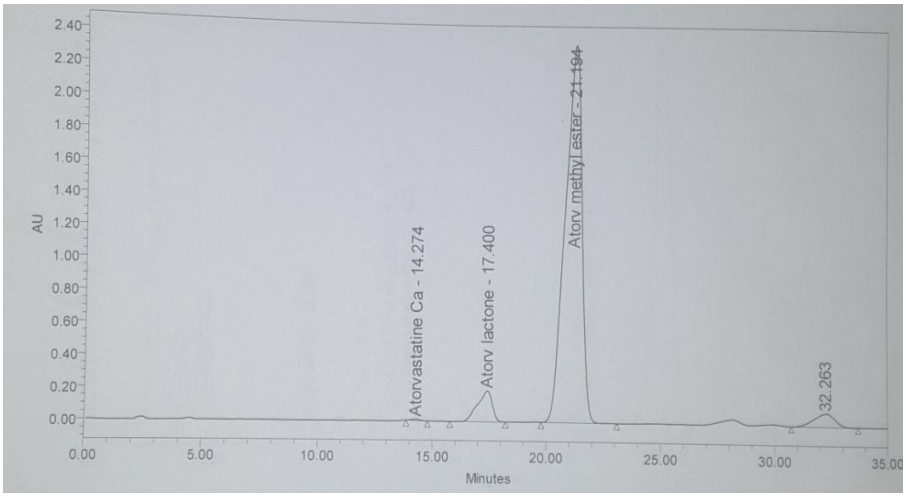

Figure 6: Chromatogram of atorvastatin after addition of DMSO.

These results show the absence of a principal peak of atorvastatin. Thus, three other peaks appear at t equal to 13.058; 14.165 and 21.150 min respectively.. The three peaks are identified as: Desfloroatorvastatin (0.06%),

atorvastatin calcium (99.82%) and atorvastatin methyl ester (0.11%). Many studies[16-18] have studied the influence of different types of cations and anions (Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, $HClO^{-}$, NO_3^{-}, SO_4^{2-}, $HCOO^{-}$, etc.) on the different chromatographic parameters (retention, enantioselectivity, resolution, etc.).

The results obtained by[17-18] showed that the separation between chemical molecules are obtained with a solution of $CuSO_4$.

Also, researchers[19] mention the case of the transformation of paracetamol by chlorination of the effluents which can lead to the formation of quinone forms,

III.6 Effect of DMSO and UV light (254 nm)

The chromatogram obtained after the addition of DMSO to the solution of atorvastatin under UV light (254 nm) is shown in figure 7.

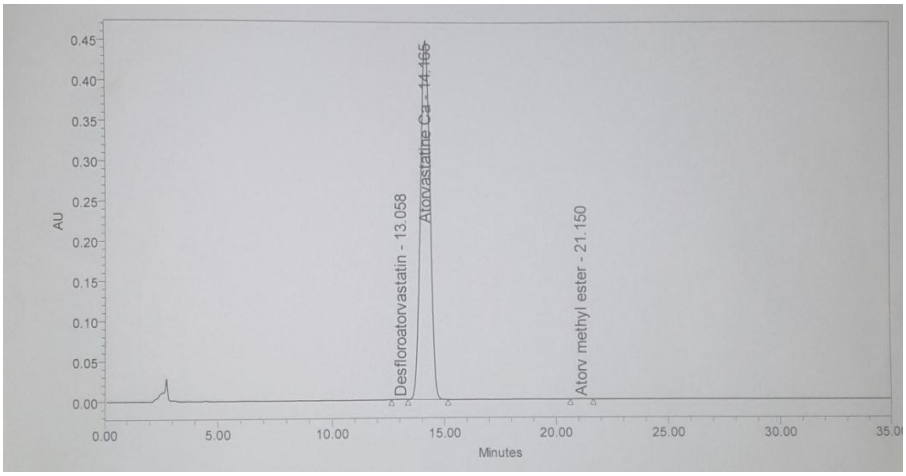

Figure 7: Chromatogram of atorvastatin after addition of DMSO under UV light (254 nm).

The same results as the previous ones (Effect of DMSO) are obtained.

Therefore, UV light (254 nm) has no effect on atorvastatin in the presence of DMSO.

III.7 Effect of DMSO and UV light (366 nm)

The chromatogram obtained after the addition of DMSO to the solution of atorvastatin under UV light (366 nm) is shown in figure 8.

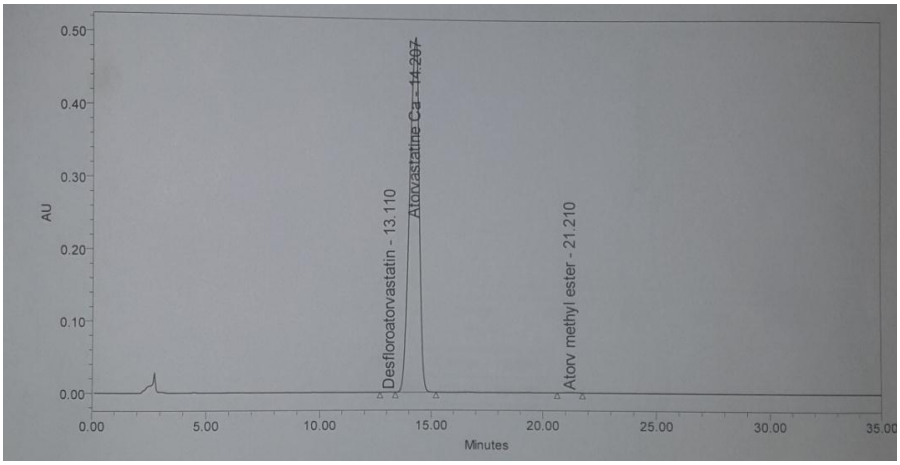

Figure 8: Chromatogram of atorvastatin after addition of DMSO under UV light (366nm).

The same results as the previous ones (Effect of DMSO) are obtained.

Therefore, UV light (366 nm) has no effect on atorvastatin in the presence of DMSO.

III.8 Effect of ammonia

The chromatogram obtained after the addition of ammonia to the solution of atorvastatin is shown in figure. 9.

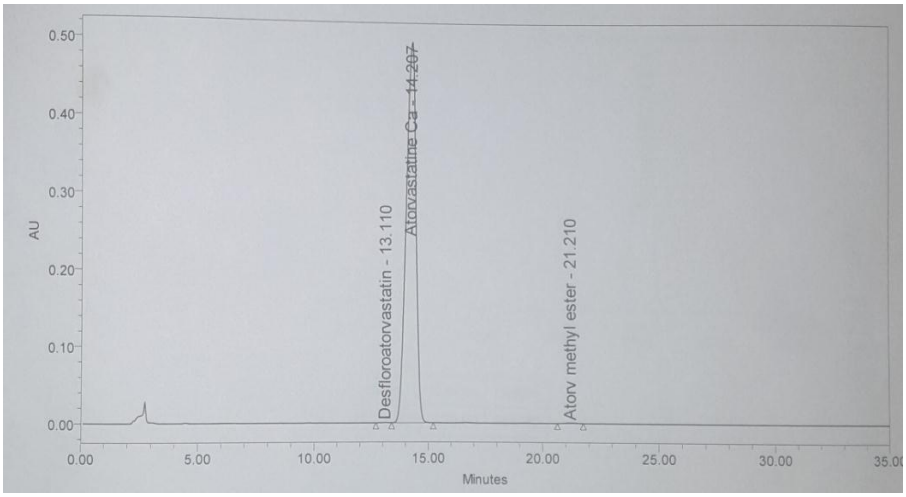

Figure 9: Chromatogram of atorvastatin after addition of ammonia.

These results show the absence of a principal peak of atorvastatin.

A series of peaks appears:

Before the 5 min, four peaks appear. These peaks have not been identified.

After 5 minutes, several peaks appear. Four peaks are identified:

Atorvastatin amide (0.06%), desfloroatorvastatin (3.13%), atorvastatin calcium (3.01%), and atorvastatin methyl ester (6.28%).

The percentage of other peaks varies between 7.52 and 80%.

Lactones, cyclic esters, are most often converted into lactams (cyclic amides) by the action of ammonia or primary amines.

Ammonia reacts on lactones forming alanine or its alkylated derivatives

Lactones with rings greater than 6 members lead to unsaturated acids with ring opening. Nonhydroxylated heterocycles such as pyrrole, indole, quinoline, thiazole provide formyl derivatives. Side reactions are often concomitant.

The extrusion reactions are specific, for the most part, heterocycles. The elimination of a molecule of nitrogen, carbon monoxide, carbon dioxide, sulfur dioxide, or a sulfur atom, among others, leads to a contraction of cycles, or to a double, even a triple bond, in the case of three-membered rings (Ramberg-Bäcklund reaction).

Also, studies have shown that atorvastatin interacts with other molecules in the body.

Kahri[20] described in 2005 a case of rhabdomyolysis of fatal evolution, after association of atorvastatin and fluconazole.

In a randomized, double-blind study, 10 healthy volunteers received itraconazole 200 mg or placebo once a day for 4 days. On the fourth day, 40 mg of atorvastatin was ingested and on the fifth day a final dose of 200 mg of itraconazole was absorbed.

Itraconazole increases atorvastatin AUC by 3.2-fold compared with placebo.

The increase in AUC ranged from 2.4 to 4.3 times, depending on interindividual variations. ASCo-n of the active inhibitors of HMG-CoA reductase and total was multiplied by 1.6 and 1.7 respectively.[21] Atorvastatin has been described as increasing estrogen levels in the blood when taken with oral contraceptives containing estrogens.[22]

Food intake may decrease the bioavailability of atorvastatin from 13% to 20%.[23]

It can also be said that the small quantity detected of certain drugs is due to the interactions of these molecules with the substances present in the environment.

The effluents of the pharmaceutical industry can interact with other organic and mineral materials in the industry where they are manufactured but also with the contact of other effluents.

IV. CONCLUSION

During this study, the interaction between various chemical reagents used in the pharmaceutical industry and a drug of the statin family (atorvastatin) was evaluated by HPLC analyzes. The chromatograms obtained during the experiments vary according to the reagent used. This study shows that it is necessary to introduce and consider environmental factors in the decision-making process and procedures when

researching and developing drug manufacturing and treatment processes.

The interactions between pharmaceutical substances with mineral and organic wastewater appear to be paramount for the understanding of the fate of these molecules in aquatic environments and their impact on the environment.

The bioconcentration factor in the presence of dissolved organic matter is proportional to the fraction of free pollutant in the medium.[24-26]

Since the drug studied reacts with the materials present in the waste water, the mutagenic, teratogenic or carcinogenic effects appear after degradation of the initial molecule by oxidation byproducts.[1-27-28]

This study must be supplemented by other qualitative and quantitative analyzes to determine the reaction mechanisms, the nature and the concentrations of the molecules obtained after the addition of each reagent.

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