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DETECTION AND QUANTIFICATION OF ENVIRONMENTAL POLLUTION CAUSED BY STATIN AND ANTIFUNGAL DRUGS

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Abstract:
Lipophilic drugs, such as Simvastatin, Atorvastatin, Ketoconazole and Clotrimazole are present in the waste water in complex matrices. As a result, it is very difficult to detect them by conventional chromatographic methods since high flow rates are used in LC along with volatile buffers. The thermally labile nature of these compounds makes them difficult to detect. LC-UV and LC-MS are promising methods for detection of these drugs. In this review, the efficacy of these two techniques is discussed for the detection of these four lipophilic drugs.

Introduction:
Lipophilicity represents the affinity of a molecule for a lipophilic environment. Pharmaceutical wastes, especially those compounds with lipophilic structures, may persist in surface and ground water and accumulate in the sewage (Figure 1). Numerous lipophilic antibiotics have been detected worldwide in fresh water, industrial waste, sewage, manure, soil, plants, and living organisms[1,2]. The existence of antibiotic contamination in aquatic environments can reduce the diversity of microorganisms required for carbon processing[3].

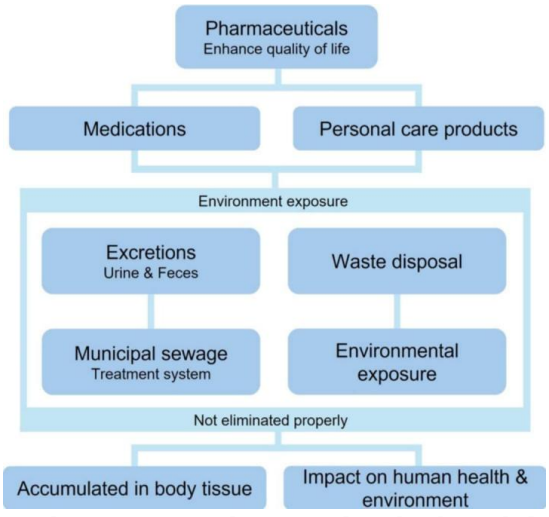

Figure 1: Schematic showing the various pathways taken by lipophilic drugs in the environment.

Lipophilic drugs, such as Statins and Antifungal drugs are typically used in hospitals. This leads to pollution in the environment such as in the waste water, surface water, groundwater and drinking water. Some researchers in Germany have reported that about half of the 2300 active ingredients used in Germany have potential of environmental toxicity[4].

Structure and Chemistry of Statins and Antifungal Drugs

Structurally, Statins resemble each other. They all have rigid, hydrophobic structures covalently linked to the HMG-like moiety. Both Simvastatin and Atorvastatin are derived from fungal metabolites and have elimination half-lives of 1-3 hours[5]. Simvastatin and Atorvastatin are both lipophilic compounds which means, they are more susceptible to metabolism by the Cytochrome P450 enzyme. The two Statin drugs, Atorvastatin and Simvastatin, use an acid to create a lactone-like structure[6]. Various researchers have reported that Simvastatin is more toxic than Atorvastatin. Statins are inhibitors for 3-Hydroxy-3-Methyl Glutaryl Coenzyme A Reductase (HMG-CoA Reductase) enzyme. They are widely used for the treatment of hypercholesterolemia. They function by binding to the active site of HMGR-CoA Reductase and inhibit the enzyme. The structural differences between Statins can account for differences in their ability to inhibit the enzyme, HMGR-CoA Reductase[7]. The Statins covered by this review, Atorvastatin and Simvastatin, can be described as:

(i) Simvastatin (Figure 2) is a derivative of a fungal product and has a hydronaphthalene ring[8].

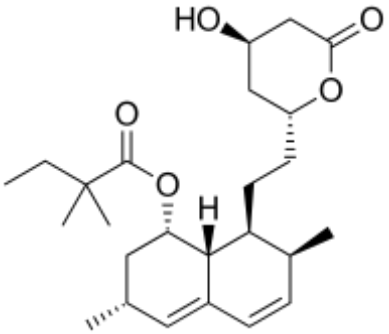

Figure 2: Chemical Structure of Simvastatin.

(ii) Atorvastatin (Figure 3) is a synthetic Statin that contains a penta-substituted Pyrrole ring.

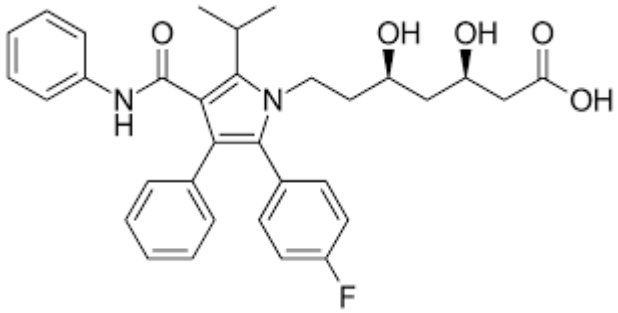

Figure 3: Chemical Structure of Atorvastatin[9].

Antifungal drugs interact with the liver P-450 enzyme system[10]. They are metabolized by cytochrome P-450 3A (CYP 3A) and may inhibit the elimination of other drugs metabolized by this enzyme such as Anti-arrhythmics, Cortisol, Cyclosporins, Estradiol and Tacrolimus. The two antifungal drugs, covered by this review are Ketoconazole and Clotrimazole:

(i) Ketoconazole(Figure 4) is used for the treatment of fungal infections such as those caused by *Tinea capitis*[11].

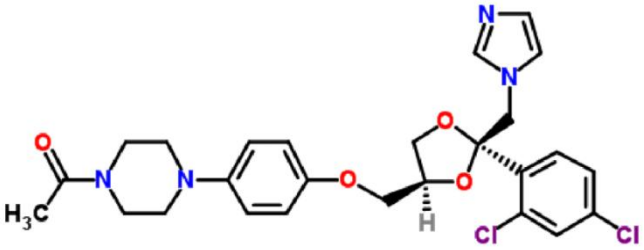

Figure 4: Chemical Structure of Ketoconazole.

(ii) Clotrimazole (Figure 5) is being used to treat *Tinea versicolor* infections, different types of ringworm infections including athlete's foot and jock itch as well as vaginal yeast infections, oral thrush and diaper rash[12].

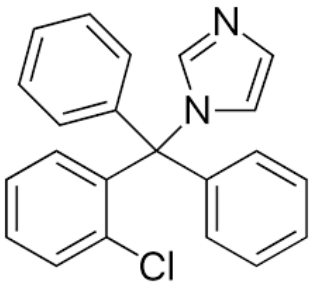

Figure 5: Chemical Structure of Clotrimazole.

Environmental and Health Damage Caused by Lipophilic Drugs

Lipophilic Antifungals and Statins[13] pose a risk for both the environment and the human health. Antifungals, such as Azoles, have been detected in surface and ground waters. Another group of drugs that pose a risk to the environment and human health are Statins[14] that protect against hypercholesterolemia. Exposure to lipophilic drugs has been linked to various diseases in humans such as: cancers, neurological disorders, diabetes mellitus, etc. Cardiac toxicity is one of the side effects of anti-fungal therapy[15]. Some studies have shown that Miconzole and Ketoconazole inhibit oxidative phosphorylation in the mitochondria[16,17].

Analytical Methods and Instrumentation being used for Analyses

Two types of chromatographic methods are currently being used for quantification and detection of Lipophilic Drugs are gas chromatography (GC) and liquid chromatography (LC). LC-MS is the more commonly used technique for analyses of pharmaceuticals, since sensitivity of LC-MS allows detection of a wider range of pharmaceuticals at sub-ppb levels[18].

Fast Protein Liquid Chromatography (FPLC) is a medium-pressure system. It employs an operating pressure of up to 3,500 psi (24 MPa). Medium-pressure systems are compatible with a wide range of pre-packed columns and resins and can be used for complex analytical purposes. High-pressure liquid chromatography (HPLC) uses very high pressures of up to 5,000 psi (34 MPa)[19]. After the sample has been extracted and injected in the instrument through the injector, it passes through the chromatography column. There are various columns that are being used depending upon the analyses. HPLC systems use small particle-size resins in the chromatographic columns which increases the resolution[20].

LC-UV Technique

The combination of HPLC and a UV detector is called LC-UV. UV detection typically works well with organic compounds containing unsaturated bonds, aromatic groups, or functional groups containing heteroatoms. The analyte concentration can be determined using the Beer–Lambert Law (equation 1)[21]:

$$A = \varepsilon cl \quad \text{(equation 1)}$$

Where 'ε' is the molar absorptivity coefficient (L $mol^{-1}cm^{-1}$), c is the concentration ($molL^{-1}$), 'A' is the absorbance, and 'l' is the path-length of the light through the flow cell (cm).

LC-MS Technique

LC-MS combines the physical separation capability of liquid chromatography (HPLC) with the mass analysis capability of MS (Mass Spectrometer). LC-MS detects the mass-to-charge ratios of molecular ions created by electrospray, electron-impact, or other ionization methods. The two important parts of these instruments are the ion source, which generates the ions, and the mass analyzer, which sorts the ions[22]. LC-MS is useful for environmental sampling, drug discovery, and proteomics.

Examples of Methodologies being used for Detection

Several (LC-MS) and liquid chromatography–ultraviolet (LC-UV) techniques have been described in literature for the quantification of target drugs in various biological and environmental samples as shown in (Table 1).

Table 1: Summary of some of the techniques currently being used to detect Statins and Azoles.

Lipophilic Drug/Metabolite	Technique Used for Detection	Reference
Simvastatin and Simvastatin hydroxy acid	RP-HPLC	[23]
Simvastatin and Simvastatin hydroxy acid	HPLC–MS/MS	[24]
Simvastatin, Atorvastatin	LC–MS/MS	[25]
Atorvastatin	LC–MS/MS	[26]
Atorvastatin and its metabolites	HPLC–ESI-MS/MS	[27]
Atorvastatin, Simvastatin	HPLC	[28]
Simvastatin	UV spectroscopic method	[29]
Atorvastatin and its two active metabolites	LC–MS/MS	[30]
Atorvastatin and its two active metabolites	HPLC/MS	[31]
Atorvastatin and its acid and lactone metabolites	LC–MS/MS	[32]
Ketoconazole	HPLC-UV	[33]
Ketoconazole	LC–MS/MS	[22]
Ketoconazole	LC–MS–MS	[34]
Clotrimazole	LC-UV-ESI-MS	[35]

Various LC–MS/MS methods for the determination of Simvastatin and Simvastatin acid in human plasma have been reported using different extraction procedures such as solid-phase extraction[36] and liquid–liquid extraction[22]. A method using LC–MS/MS to detect Simvastatin and Simvastatin acid has also been developed[37]. This method does not involve sample preparation except for adding an internal standard to the samples[38,39]. Recently, Zhou et al.[27] have developed a simple, rapid and sensitive method for the determination of Atorvastatin and its metabolites (in combination with other drugs) in biological fluids.

LC-UV is being used for the detection and quantification of both the antifungals, Ketoconazole and Clotrimazole. It uses reverse phase HPLC column and a mobile phase comprising Acetonitrile, Trishydroxymeihyl and Aminomethene[40,41].

Future Directions

At present, among the various methodologies that are being employed, LC-MS can handle a large number of lipophilic drugs. However, much work remains to be done on improving the interface between the LC and the MS. There are no limitations for the mass range of the samples that can be analyzed by LC but there are limitations for the mass analyzer of the MS. LC uses high pressure to separate a liquid phase and produces a high gas load whereas MS requires a vacuum and a limited gas load. Furthermore, LC operates at near ambient temperature whereas MS needs elevated temperatures. Finally, LC uses inorganic buffers whereas MS requires volatile buffers.

For the coming future, it would be important to develop a comprehensive and multi-faceted approach to handle the issue of pharmaceutical pollution. Public and healthcare professional education campaigns can promote responsible antibiotic use and adherence to prescribed treatments.

Conclusion

Currently, no methodology exists for the simultaneous determination of all four drugs of interest: Simvastatin, Atorvastatin, Ketoconazole and Clotrimazole, in one step. Both LC-UV and LC-MS are specific and accurate.However, LC-UV is more specific than LC-MS. The advantage of LC-MS over LC-UV is that very small amount of sample in the range of nanograms to micrograms can be analyzed.

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