ABSTRACT

Atorvastatin is a synthetic member of the drug class known as statins, which are used primarily as cholesterole-lowering agents. Therapy is usually long term therefore, it is one of the key parameters in addition to the effectiveness of a drug that they contains as less impurities as possible. This could be obtained by controlling the formation of impurities during the synthesis and by formulating and packing the drug appropriately hence preventing the forming of degradation impurities. It is crucial to prepare reference standard materials for all the impurities monitored during synthesis and on API. Reference standard materials could be synthesised or isolated. For isolation various chromatographic methods are used, in most cases preparative HPLC.

In PhD thesis it is shown that the isolation of related substances of Atorvastatin API (Active Pharmaceutical Ingredient - API) with supercritical fluid chromatography (SFC) is a good alternative to other isolation techniques, such as column, TLC or preparative HPLC chromatography. In certain cases I have shown also some advantages of isolation with SFC over the mentioned techniques. Atorvastatin is a complex organic molecule with a significant number of potencial related degradation impurities.

The isolation of impurities was carried out from degradation samples, which preparation was based on stress tests study. I have prepared degradation samples based according to the experimental plan, combining the influence of reaction conditions such as temperature, pH, effect of solvents, light and oxidation. The purpose was to isolate as much as possible of numerous degradation products and to provide impurities in sufficient quantities for isolation utilizing the potentials of semi preparative SFC.

Further on, the methods for the isolation of impurities with the SFC were developed and optimized. A variety of columns, solvents and modifiers were tested, also gradient programme, the temperature of the column, back pressure, etc. This way the optimal or sufficiently good conditions for collecting fractions and isolation of impurities with targeted chromatographic purity over 97 area % were achieved. Furthermore in case of some impurities the collection of the fractions into the flask had to be optimised under specific conditions (chilled solvent, another solvent). With that operation I have stabilized impurities, because they were unstable in the mobile phase. With some of impurities I had to repeat the additional chromatography step under different conditions to get enough pure material (impuritiy).

In the isolation of a solid impurities, the volatility of mobile phases proved beneficial, and that is one of the main advantages of supercritical fluid chromatography. The fractions were simply evaporated with a rotary evaporator, while in the case of preparative HPLC chromatography desolvation, extraction or lyophilization would be required, which are time consuming and not suitable for treatment of less stable degradation impurities.

The isolated impurities were structurally characterized and compared with the available standards or the structure of impurities was determined by LC-MS and / or NMR. Some of the impurities are unstable at normal conditions and / or they are difficult to obtain as a standard.

The impurities E, L, Z and AG were isolated for the first time. All four impurities are degradation impurities of atorvastatin. The isolation with supercritical fluid chromatography and the following characterization with LC-MS and NMR, confirmed the formation of impurity E under acidic conditions and simultaneous irradiation with light in water or combination of acetonitrile and water. Mainly atorvastatin decomposes into impurity E: 4-(7-(4-fluorophenyl)-1,1-dymethyl-8-phenyl-9-(phenylcarbamoyl)-4,5-dyhidro-1*H*,3*H*-pyrrolo[2,1-*c*][1,4]oxazepin-3-yl)-3-hydroxyibutanoic acid. In the same time in acetonitrile also arises impurity L, which is very unstable in oil state. With help of LC-MS I predicted the structure with chemical name (7-(4-fluorophenyl)-1,1-dimethyl-3-((4-oxooxetan-2-yl)methyl)-*N*,8-diphenyl-4,5-dihydro-1*H*,3*H*-pyrol[2,1-*c*][1,4]oxazepin-9-carboxamido).

Impurity Z ((E)-7-(-2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl)-5-hydroxyhept-3-enoic acid) and AG ((3*E*, 5*E*)-7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl)hepta-2,4-dienoic acid) were isolated from degradation sample of atorvastatin solid, which was heated on 180 °C. Impurities Z in AG are kinetic impurities of thermal degradation. Under the same conditions the main two thermodynamic impurities P and R are formed.

With supercritical fluid chromatography 21 impurities were isolated and characterized, of which according to the the available literature, until now four are unknown. According to the obtained results, the degradation path of active ingredient atorvastatin was reviewed and explained in details.

Keywords: Supercritical fluid chromatography, atorvastatin, degradation impurities, isolation