Simultaneous Profile and Determination of Statin Composition in Various Media and Biological Matrices by Accurate Mass and High Resolution LC-QTOF-MS

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Introduction

The class of lipid-lowering statins such as atorvastatin, fluvastatin, pravastatin, rosuvastatin and simvastatin can undergo interconversion between a lactone and hydroxyl acid form depending on physiological conditions, this could potentially induce myotoxicity. Interconversion causes problems in developing and validating an effective bioanalytical method for the quantitation of statins. Regulated bioanalysis requires investigating back-conversion and interconversion for compounds like acidic metabolites to ester, unstable N-oxides or glucuronide metabolites, and lactone-ring structures to ensure the validity of the method and results derived from it. It is essential to know the content and relative ratio between lactone and its hydroxyl acidic forms for various experimental conditions to develop and validate robust bioanalytical methods for the analysis of statins.

Methods

Atorvastatin, fluvastatin, simvastatin, pravastatin and rosuvastatin were obtained from Sigma-Aldrich. Biological matrices such as human plasma, human urine, cell culture media, solvent and buffer at different pH were prepared for this evaluation. Samples were analyzed by direct sample infusion into MS or LCMS using an Elute UHPLC system with a reversed phase LC column (1.8 µ, 2.0 x 50 mm) and ultra-high resolution LC-ESI-MS (QTOF maXis II, Bruker, Figure 1).

Figure 1. Elute UHPLC and maXis II QTOF MS

Results and Discussion

Detection of statins in acid and lactone forms

Several statins and their lactone forms were used to evaluate hydroxyl acid-lactone inter conversions (Figure 2). The nomenclature of simvastatin is different from other statins and its hydroxyl acidic form is named simvastatin acid and its lactone form is called simvastatin. Statins lactone samples could not be detected in ESI negative mode but both statins and lactone forms could be detected under ESI positive mode (Figure 3). For example, simvastatin acid was detected by negative ESI

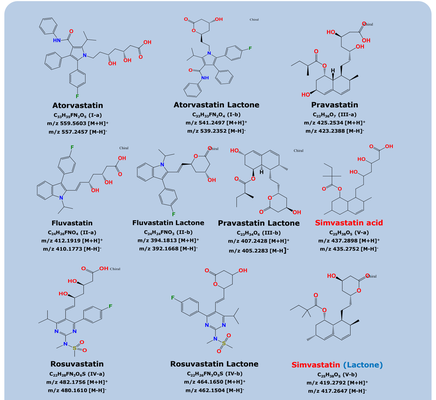

Figure 2. Chemical Structure of Statins

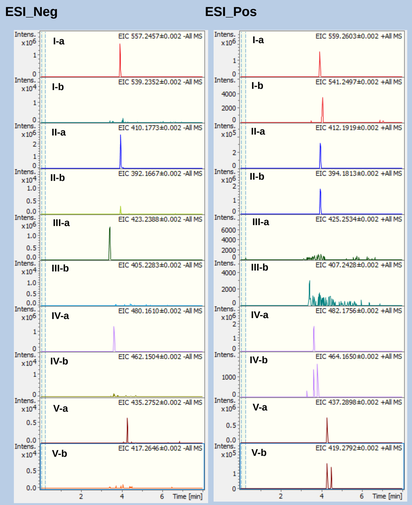

Figure 3. Statins LC_MS Chromatograms

LC gradient elution with MP-A = 0.1% formic acid in H_2O; MP-B = 0.1% formic acid in acetonitrile, injection 2 µL and total run time 8 min, 1 µg/mL. I-V (a, b) were listed in Figure 2.

but simvastatin (lactone form) was not, even at high concentrations. Therefore, ESI positive ionization mode was used to investigate statins inter-conversion to lactones.

Initial tests in a 0.1% formic acid solution showed simvastatin was mostly converted to simvastatin acid; fluvastatin mainly exists as fluvastatin lactone; atorvastatin and rosuvastatin acidic forms are predominant; sensitivity for pravastatin was poor under ESI positive mode; and in source fragmentation of rosuvastatin and simvastatin acid was observed (Figure 3 VI-b, V-b, ESI_Pos)

Statins in different pH solutions

Three different mobile phases at pH ~2.7 containing 0.1% formic acid; pH ~ 7 containing no acidic or basic additive and pH ~10 containing 0.1% ammonium hydroxide (pH ~10) were applied to evaluate the pH dependency of the interconversion process. Increase in acidic forms at higher pH indicated a reversible equilibrium of statins shifting to its parent compound with the hydrolysis of the cyclic carboxylic esters of the lactone group (Figure 4).

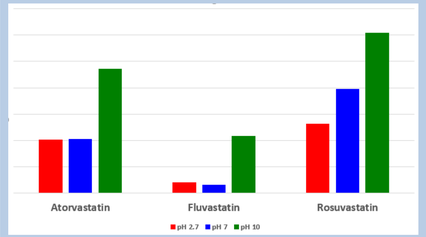

Figure 4. Statins at different pH

Statins in organic solvent

The influence of organic solvent concentration on statin solutions was evaluated. The response decreased for lower organic solvent amounts (Figure 5) and no signal was observed for atorvastatin and fluvastatin in 40% methanol and rosuvastatin at 20%. This is expected due to the lipophilic nature of these molecules.

Statins in biological matrices

Similar results were achieved when spiking and extracting statins from biological matrices of human plasma, human serum, human urine, and in different cell culture media of NCTC-109, HAM F-10/F-12 and MCCoy 5A as long as the final reconstitution solution is selected to promote statins hydroxyl acidic form.

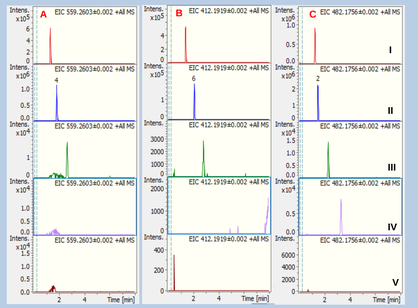

Figure 5. Statins at different organic solvent level

A – atorvastatin; B – fluvastatin; c – rosuvastatin
MP-A = water, MP-B = MeOH (I – 100%, II – 80%, III – 60%, IV – 40%, V – 20%); isocratic elution

Conclusions

- Ultra-high resolution accurate-mass LC-MS QTOF analysis provided reproducible qualitative and quantitative information for statins composition
- A reliable workflow was established to simultaneously monitor the dynamic balance of statin's hydroxyl acidic form and its lactone
- Statin lactone should not be ignored during statins sample preparation and analysis as it might be significantly higher than its hydroxyl acidic form
- pH and organic solvent level are the keys to control statins inter-conversion

Statins Conversion