

Development of an LC-MS/MS method for determination of atorvastatin and its acid and lactone metabolites in human plasma

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Purpose: To develop and validate a LC-MS/MS method for quantification of atorvastatin acid (atv, Lipitor®, Pfizer Pharmaceuticals) and lactone forms and its para- and ortho-hydroxy metabolites (a total of 6 compounds).

Methods: A turboion spray LC-MS/MS was used (PE Sciex API 2000) at positive ion mode. Deuterium labeled analogs (d5) of each compound was used as internal standards (IS). Solid phase extraction was carried out from a 0.5mL plasma sample spiked with the 20 µL internal standard mixture on a pre-conditioned 3mL C18 (200mg) cartridges. The analytes were eluted using 750µL of acetonitrile-ammonium acetate (0.1M, 95:5 % v/v) and evaporated to dryness, reconstituted in acetonitrile:1mM formic acid (80:20 % v/v) and 5 µL was injected onto the analytical column. Chromatographic separation was achieved on a YMC Basic (2 x 100mm, 5µm) column, maintained at 40°C. The mobile phase composition was 55% of solution A and 45% of solution B at a flow rate of 0.3mL. [Solution A: 950mL water + 50mL methanol + 43µL formic acid (88%) and Solution B: 950mL acetonitrile + 50mL methanol + 43µL formic acid (88%)].

Results: The m/z transitions for atv, p-OH-atv and o-OH-atv in acid forms were 559.2→440.3, 575.4→440.1, 575.4→440.2 and their lactone forms were 541.3→448.0, 557.4→448.1, 557.4→448.1, respectively. These transitions in the MRM mode were obtained using the following instrument parameter: curtain gas: 10 psi, collision gas:4 psi, ion spray voltage: 4500 V, capillary temperature: 400°C, ion source gas1 and 2: 20 and 60 psi, respectively. The lower limit of quantification was 0.5 ng/mL and limit of detection was 0.2 ng/mL. No matrix interference was observed at the retention times of the analytes indicating the specificity of the method. Acceptable accuracy ($\pm 10\%$) and precision ($\pm 10\%$ CV) was observed for the linear range of 0.5 – 200 ng/mL ($r^2 = 0.9993 \pm 0.0007$) for all the analytes.

Conclusion: A robust, sensitive, specific, accurate, and reliable method was developed. The method will be used for pharmacokinetic and drug interaction studies.

