Chulalongkorn University
จุฬาลงกรณ์มหาวิทยาลัย

CU Pharmacy Centennial

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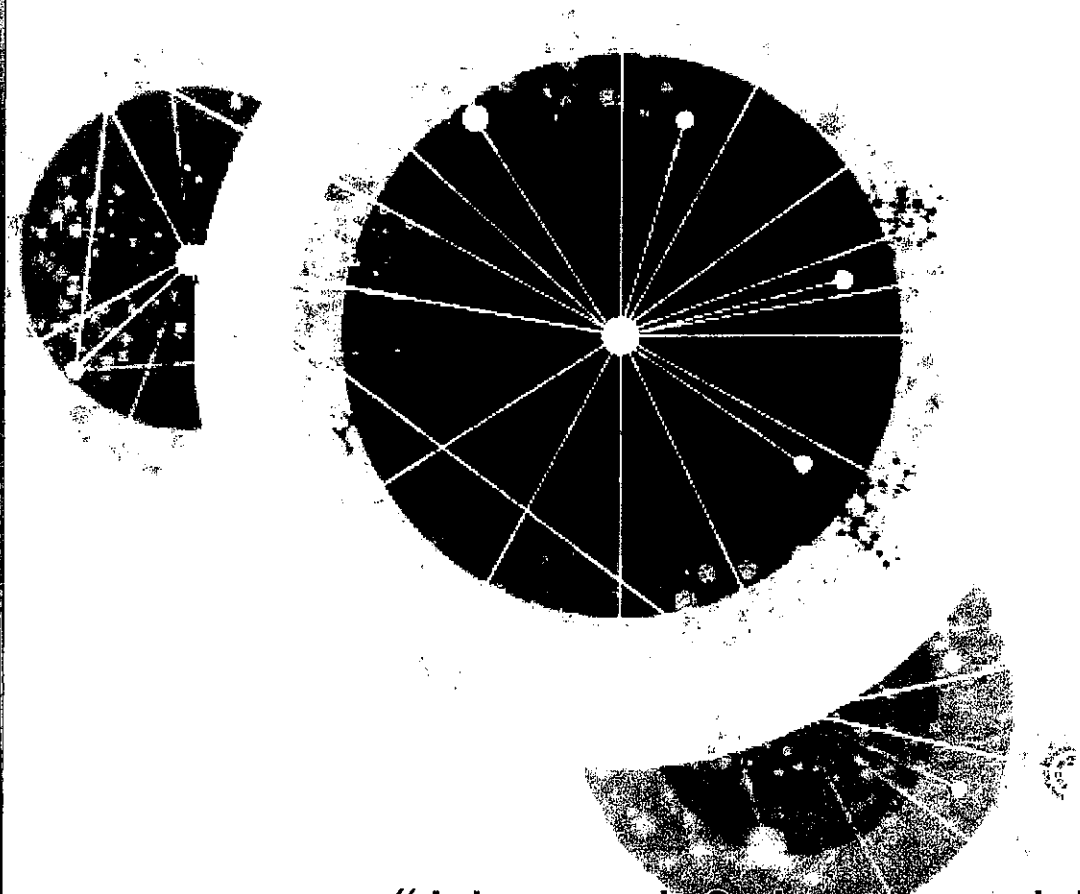
ChulaGlobal Network

วช. NRCT

The Celebrations on the Auspicious Occasion of Her Royal Highness Princess Maha Chakri Sirindhorn's 5th Cycle (60th) Birthday Anniversary

The JSPS-NRCT Follow-Up Seminar 2015 and 31st International Annual Meeting in Pharmaceutical Sciences (JSPS-NRCT 2015 and 31st IAMPS)



"Advanced Science and Technology in Pharmaceutical Research"

Faculty of Pharmaceutical Sciences,
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DEVELOPMENT AND VALIDATION OF SIMULTANEOUS DETERMINATION OF ATORVASTATIN AND ITS METABOLITES IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY AND ELECTROSPRAY TANDEM MASS SPECTROMETRY

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The development and validation of a sensitive liquid chromatography and electrospray tandem mass spectrometry (LC-MS/MS) for simultaneously quantitative analysis of atorvastatin, *o*-hydroxylated atorvastatin and *p*-hydroxylated atorvastatin were performed. Liquid-liquid extraction was done with diethyl ether after the addition of atorvastatin-d5, *o*-hydroxylated atorvastatin-d5 and *p*-hydroxylated atorvastatin-d5 as internal standards. The chromatographic separation of atorvastatin, *o*-hydroxylated atorvastatin and *p*-hydroxylated atorvastatin was achieved with a reversed phase C18 column (150mm x 4.6mm) under an isocratic condition consisting of a mobile phase of 0.1% formic acid and acetonitrile (30:70). Quantification by MS/MS was performed in positive polarity mode using multiple reaction monitoring with mass transition (m/z) of 559.260 → 440.250 for atorvastatin, 575.258 → 466.200 for *o*-hydroxylated atorvastatin, 575.260 → 440.250 for *p*-hydroxylated atorvastatin, 564.280 → 445.270 for atorvastatin-d5, 580.285 → 471.200 for *o*-hydroxylated atorvastatin-d5 and 580.280 → 445.270 for *p*-hydroxylated atorvastatin-d5. The acceptable linearity ranges were determined to be 0.102-81.568 ng/mL for atorvastatin, 0.250-40.670 ng/mL for *o*-hydroxylated atorvastatin and 0.100-10.036 ng/mL for *p*-hydroxylated atorvastatin. Within batch precision and accuracy (LLOQ included) ranged from 0.9% to 5.7% and from 101.9% to 118.7% for atorvastatin, from 0.9% to 9.7% and 88.3% to 105.4% for *o*-hydroxylated atorvastatin and from 0.7% to 11.6% and 93.0% to 111.8% for *p*-hydroxylated atorvastatin. Between batch precision and accuracy (LLOQ included) ranged from 2.1% to 7.8% and from 104.0% to 109.6% for atorvastatin, from 2.8% to 10.5% and 93.1% to 97.5% for *o*-hydroxylated atorvastatin and from 2.7% to 11.6% and 95.5% to 103.4% for *p*-hydroxylated atorvastatin. Satisfactory selectivity, linearity, precision, accuracy, robustness and ruggedness of this method were obtained and met the acceptance criteria as per US Food and Drug Administration and European Medicines Agency guidelines. Recoveries of atorvastatin, *o*-hydroxylated atorvastatin and *p*-hydroxylated atorvastatin from plasma were 75%, 67% and 67%, respectively. Atorvastatin, *o*-hydroxylated atorvastatin and *p*-hydroxylated atorvastatin were stable in human plasma after four freeze-thaw cycles, bench top stability for 13.0 hours and wet extract stability (within 2-8 °C) for 177.0 hours. This method fulfils all the regulatory requirements and could be applied to a clinical pharmacokinetic study.