The Enantioselective Separation and Quantitation of the Hydroxy-Metabolites of Arachidonic Acid by Liquid Chromatography – Tandem Mass Spectrometry

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Arachidonic acid (AA) is a polyunsaturated fatty acid with a structure of 20:4(ω-6). Cytochrome P450s (CYPs) metabolize AA to several regioisomers and enantiomers of hydroxyeicosatetraenoic acids (HETEs). The hydroxy-metabolites (HETEs) exist as enantiomers in the biological system. The chiral assays developed for HETEs are so far limited to a few assays reported for midchain HETEs. The developed method is capable of quantitative analysis for midchain, subterminal HETE enantiomers, and terminal HETEs in microsomes. The peak area or height ratios were linear over concentrations ranging (0.01 -0.6 µg/ml) with r2 > 0.99.The intra-run percent error and coefficient of variation (CV) were ≤ ±12%.The inter-run percent error and CV were ≤ ±13%, and ≤15%, respectively. The matrix effect for the assay was also within the acceptable limit (≤ ±15%,). The recovery of HETE metabolites ranged from 70-115 %. The method showed a reliable and robust performance for chiral analysis of cytochrome P450-mediated HETE metabolites.

Keywords: Arachidonic acid, enantiomer, hydroxyeicosatetraenoic acid, LC-MS/MS, assay, microsome matrix