

The aim of this Master's thesis was the development of an oxysterols fingerprinting as tool to understand the CYP450 metabolic pathway modification during embryos development by means of UHPLC-MS/MS. The main issues were efficient chromatographic separation and robust sample preparation. The selective extraction of the target compounds was developed with suitable enrichment factor, maintaining an acceptable matrix effect during all embryos growth stages. The investigation on oxysterols profile in a suitable *in vivo* system could provide useful information on the amount, roles and biological functions of these molecules that are enzymatically generated by cytochrome P450 (CYP450) family, or via autooxidation, or even via both pathways in synergy. Zebrafish is an animal model used in toxicology to estimate the effects of xenobiotics and their teratogenic consequences; this animal model presents several advantageous features as high fecundity, rapid embryonic development (24 h) and external fertilization. The lipidomic analyses in zebrafish early-life stages could represent a reliable tool for studying the global lipid profile during embryos development, but the knowledge about the sterols derivatives in the context of embryogenesis of these vertebrates remains limited. The knowledge of the oxysterols profile in zebrafish, during early embryonic stages, provides important information on the role and biological function of these molecules. Moreover, different oxysterols have been investigated in zebrafish as liver X receptor (LXR) activators of the liver and regulators in the metabolism of carbohydrates and lipids and the cytotoxic effects of 25-hydroxycholesterol on nervous system cells in zebrafish larvae was demonstrated. From analytical point of view, oxysterols present many challenging aspects: first of all, deriving from the same precursor molecule (cholesterol), these compounds are an isomeric group, so that tandem mass spectrometry is not able to obtain an univocal identification, and complete chromatographic separation is mandatory. Anyway, the separation of oxysterols it is a challenging task as these compound differs each other only by hydroxylic moiety position (i.e. 27 hydroxycholesterol and 25.hydroxycholesterol). Moreover, the changing in composition and matrix characteristic of embryos during their growth need a robust analytical method with high versatility. Finally, the poor ionization efficiency of oxysterols, due to the high lipophilicity, is another challenging aspect to take into account.