Simultaneous measurement of collagen cross-link markers and advanced glycation end-products by Q-TOF LC/MS

Mass Spectrometry

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Collagen is one of the major components of the extracellular matrix (ECM). Collagen biosynthesis involves both intracellular and extracellular steps including several posttranslational modifications, and results in covalent cross-links being formed between tropocollagen molecules, microfibrils and fibrils. Evidence has shown that cross-links play an important role in both physiological and pathological changes of collagen. Cross-links stabilize collagen structure and are essential for normal collagen physiological function. On the other hand, there is evidence that cross-links play a role in collagen changes during aging, resulting in loss of elasticity, decreased proteolytic susceptibility, and accumulation of yellow and fluorescent substances. Advanced glycation end-products (AGEs) are this type of senescent cross-link. High performance liquid chromatography (HPLC) with fluorescence detection has been commonly used to measure free fluorescent cross-links in biological samples. Reduction using sodium borohydride to stabilize immature divalent collagen cross-links that are acid labile allow for downstream detection using mass spectrometry. The purpose of this project is to develop a Q-TOF LC/MS method to measure collagen cross-links markers and AGEs simultaneously in various biological samples. Tissue samples were hydrolyzed in 6 N HCl at 105 °C overnight. Hydrolyzed samples were enriched using a cellulose column. Separation of hydrolyzed components was achieved using Cogent Diamond Hydride column using reverse phase-HPLC. MS analysis was performed on a Bruker maXis Q-TOF mass spectrometer. Preliminary results show that this method is suitable to quantify pentosidine, dihydroxylysinonorleucine (DHLNL), pyridinoline (Pyr), and carboxyethyl-lysine (CEL) in various tissue samples, including heart, kidney, and cartilage.