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DEVELOPMENT OF AN LC-MS METHOD FOR THE IDENTIFICATION OF POST-TRANSLATIONAL MODIFICATIONS IN HUMAN LENSES.

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Around 90% of protein present in the human lens is represented by crystallins. These proteins are grouped into three main classes, α , β and γ , based on their aggregation behaviour and sequence homology. As there is little or no turnover of crystallins during the lifetime of an organism, these proteins are susceptible to the accumulation of post-translational modifications. These modifications include, but are not limited to; N- and C-terminal truncations, deamidation, phosphorylation, acetylation, disulfide bonding, methylation and oxidation of methionine residues. Most, if not all, of these modifications are found in aged normal lenses in addition to cataractous lenses. It is therefore important to be able to qualitatively, and ultimately quantitatively, determine the level of modification in both aged normal and cataractous lenses.

A hallmark of age related nuclear cataract, the main cause of blindness worldwide, is massive protein oxidation manifested in the form of disulfide bonded crystallins and oxidised methionine residues. We are developing an on-line LC-MS method for the identification of disulfide bonded cysteine and oxidised methionine residues. The nuclear portion of cataractous lenses is taken, dissolved in a strong chaotrope (7M guanidine hydrochloride). The identification of cysteine residues is performed by parent ion scanning for a 106.1m/z ion after alkylating with 4-vinylpyridine. Free and bonded cysteine residues are differentiated by either reducing and alkylating (bonded), or alkylation only (free). Neutral loss scanning is used to identify methionine sulphoxide. Upon CID, methionine sulphoxide produces a neutral loss ion of 64m/z. Prior to MS analysis, the lens proteins are digested with trypsin and analysed by LC-MS (ESI-QqQ).

MS parameters were optimised using Met(O)-ACTH (4-9), where the neutral loss was found to be very sensitive to the charge state and collision energy used. Parent ion scanning was optimised with Somatostatin I that had been alkylated with 4-vinylpyridine.