B173 An Investigation of the Correlation Between Human Age and Aspartic Acid and Asparagine Racemization and Isomerization of the Eye Lens Crystallins Proteins

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After attending this presentation, attendees will be able to recognize the use of proteomics in the age estimation of human eye lens proteins using high-resolution nano-liquid chromatography (nanoLC) in conjunction with high-resolution high-mass accuracy tandem mass spectrometry. Moreover, attendees will understand the mechanisms of the post-translational modifications (racemization and isomerization) of eye lens proteins and how these modifications can be used as potential biomarkers of aging.

This presentation will impact the forensic science community by demonstrating another method for determining the age of an individual at the time of death.

Isomerization of aspartic acids and asparagines results in the formation of several optical and structural isomers, such as the conversion of L-α-Asp to L-β-Asp, D-α-Asp, and D-β-Asp via succinimide intermediate. Identification of these complex isomers requires a high-resolution separation technique to differentiate between these optical and structural isomers of the tryptic peptides. Since these isomers all have the same *m/z*, their identification requires the use of synthetic peptides. Eye lens samples from 40 patients have been obtained and 28 of them have been analyzed by high-resolution nanoLC in conjunction with a high-resolution mass spectrometer. The samples include both male and female ranging in age from 45 years old to 87 years old. Eye lens proteins were extracted based on their solubility in water and 8M urea solution. The proteins were digested with trypsin and analyzed using a 50cm-long nanoLC column with 0.75µm ID.

To identify and quantify the tryptic isomers, three synthetic peptides (TVLDSGISEVR, IQTGLDATHAER, and DVTIQHPWFK) were prepared and analyzed. Baseline separation of the isomers for each of the peptides were achieved for both the synthetic peptides and for the tryptic digests of the lens samples. These isomers were identified by comparing their retention order with the synthetically prepared peptides containing the four isomers. Preliminary data demonstrated the ability to use high-resolution separation for the identification of aspartic acid and asparagine isomerization. Analysis of eye lens crystallins from individuals with varying ages indicated significant differences in relative intensities of the four isomers for these peptides. Work is underway to correlate the relative intensities of these isomers to the age of the individuals. This study will be expanded to include other tryptic peptides containing single aspartic acids or asparagine.

Human Age Estimation, Eye Lens Proteins Isomerization, High Resolution Proteomics