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Microwave Assisted Synthesis for A2E and Development of LC-ESI-MS Method for Quantification of Ocular Bisretinoids in Human Retina

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Highlights

• Microwave Synthesis of A2E and Quantification of Retinal Bisretinoids

Abstract :

Purpose: To develop a microwave assisted method for the rapid synthesis of A2E and also to develop a method to quantify N-retinylidene-N-retinylethanolamine(A2E), all-trans retinal dimer (ATRD), A2-glycerophospho ethanolamine (A2GPE), dihydropyridine phosphatidyl ethanolamine (A2DHPE) and monofuran A2E (MFA2E) in age matched retina.

Methods: The development of microwave assisted synthesis of A2E, its purification and Characterization for its utility in quantification in human retina. The semi-quantitative method development using LC-ESI-MS, LC-ESI-MS/MS and LC-APCI-MS/MS from pooled macula and peripheral retina for the bisretinoid analysis has been done.

Results: Maximum A2E conversion using microwave assisted process took place at 80 °C for 45 min with a yield of 55.01%. Highly sensitive and specific mass spectrometric method was developed using reverse phase C-18 separation with positive electrospray ionization and positive atmospheric phase chemical ionization of tandom mass spectrometry. A gradient mobile phase separation was achieved using water and methanol with 0.1% TFA. Multiple reaction monitoring acquisition for ESI and APCI was performed at ATRD m/z 551.2/522.2, A2GPE m/z 746.4/ 729.5, A2DHPEm/z 594.4/576.5, MFA2E m/z 608.2/591.2, A2E m/z 592.4/ 418.2. Method was validated using LC-ESI-SIM mode to determine selectivity, linearity, sensitivity, precision and accuracy.

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