Extraction and Normal-Phase Separation of Brain Carotenoids and Tocols

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Outline

- Background
- Previous analysis of brain
  - C18 RP-LC
  - C30 RP-LC
- Extraction
- NP-LC Separation

Background

Alzheimer's Disease (AD) is a severe neurodegenerative disease that afflicts >4,500,000 US elderly. Tocopherols and carotenoids have been implicated in reduced risk of Alzheimer's Disease and improved cognition. The results of the EVA study suggest that low carotenoid levels could play a role in cognitive impairment.[1] Our lab was the first to measure individual carotenoids in human brain.[2] The process involved direct saponification, extraction and C18 reversed-phase HPLC separation. This approach may result in alteration of the vitamins and carotenoids originally present in the brain. To minimize the potential for chemical artifacts, a direct extraction and normal-phase HPLC system was developed to measure tocopherols, tocotrienols, and carotenoids in human brain for the Rush Memory and Aging Project.

Background

Brain tissue from several donors of different Alzheimers grading were composited and blended with water (50/50 wt/wt) containing 50mmol EDTA and 0.05% ascorbic acid. One gram samples of brain homogenate were extracted with several solvent combinations: including SDS, MTBE, hexane, and THF. Two treatments were saponified to compare results with unsaponified samples. The extracts were separated using a Diol column with a gradient of hexane and dioxane and included two internal standards (Tocol and b-apo 8' carotenoate). Carotenoids were measured at 450nm and tocols by fluorescence (296nm Ex/340nm Em).

Additionally saponified extracts were measured by reversed phase using a C18 separation (historic method) and a C30 separation.

Brain homogenate was separated by HPLC and the zeaxanthin fractions were collected and concentrated. Brain zeaxanthin enantiomers were separated using a Chiralpak AD column.

What's the interest?

- Specific carotenoids (Lutein and Zeaxanthin) are located in the eye.
- The eye is considered to be neuronal tissue and similar to the brain.
- Cognitive function
- Individual carotenoids in human brain were first measured by our lab.
- Dietary manipulation of brain carotenoids
- Could carotenoids be integral to brain function?

- Adrenic acid (22: 4 n-6) was three to four times higher in the grey matter but lower in the white matter in each of the three regions in the Alzheimer brains than in the control group. These alterations were compensated by reciprocal changes in 18: 0 in the grey matter and 16: 1 fatty acids in the white matter.

Differences in the fatty acid composition of the grey and white matter of different regions of the brains of patients with Alzheimer's disease and control subjects

E. R. Skinner, C. Watt, J. A. O. Besson and P. V. Best

- Tocopherols and tocotrienols plasma levels are associated with cognitive impairment. Neurobiology of Aging

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