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HPLC Analysis of Vitamin A and Carotenoids

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Introduction

Retinol and carotenoids are extracted from plasma with ethanol containing butyl-hydroxytoluene (BHT). Retinol is determined by HPLC with spectrofiuorimetric detection (λex 340 nm; λcm 460 nm). Carotenoids are also determined by HPLC with UV detection (A453 nm) or with an electrochemical detector (ECD, 900mV). Figure 1 shows a typical chromatogram from human blood plasma carotenoids [1,2].

Protocol

1. Add BHT in ethanol (0.015%, 1 ml) to plasma (0.2 ml) and shake.
2. Add n-hexane (5 ml) and shake.
3. Centrifuge for 10 min at 3000 rpm.
4. Evaporate upper layer (4 ml) under N_2 gas.
5. Add ethanol (100 ml) and analyse by HPLC.

HPLC was performed with an lrica Instruments (Kyoto, Japan) Σ-871 chromatograph equipped with a 250 mm × 4 mm i.d. lrica RP-18 column and fluorescence detection (λex 340 nm; λem 460 nm). The mobile phase was 95:5 (v/v) ethanoi-H_2O at a flow rate of 0.7 ml/min. The external standard was retinal prepared from retinyl palmitate

Discussion

ECD is highly sensitive and is used when small amounts of biological sample are available. The more the applied voltage increased, the lesser the selectivity. However, the sensitivity increased with increased applied voltage. ECD is in general 10 to 100 times more sensitive than UV at 220 nm. The sensitivity of fluorescence detection was almost identical to ECD.

In figure 1 separation of the interest compounds from the admixtures must be further improved. For that purpose, change of constituent of the mobile phase, selection of column, gradient elution and solid phase extraction must be considered. These studies may result in appropriate separation.

Reference

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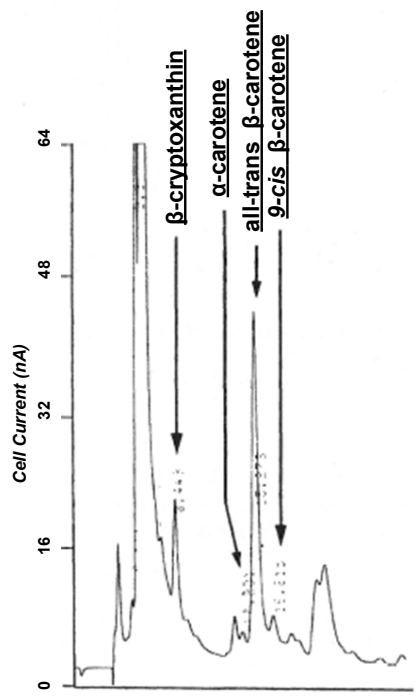

Figure 1: Typical HPLC chromatogram of carotenoids from blood plasma Detection was by ECD at 900 mV.

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