AN IMPROVED REVERSED PHASE HPLC METHOD FOR THE SEPARATION OF CAROTENOIDS, THEIR ISOMERS, THE XANTHOPHYLL CYCLE PIGMENTS AND α- AND ß-CAROTENE FROM PHEOPHYTIN *a*

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HPLC is a wide-spread method for the analysis of photosynthetic pigments. However, peak resolution is sometimes not good enough to allow accurate quantifications. This is often the case for the *cis*- and *trans*-carotenoid isomers, α-carotene (Car), β-Car and pheophytin *a* (Pheo), and for the pigments involved in the xanthophyll cycle. In the later case this difficulty mainly originates from the fact that zeaxanthin (Z) elutes between the lutein (L) isomers. Another problem is the coelution of nonpolar pigments (α-Car, β-Car and Pheo).
In this communication we present an improved reversed-phase HPLC method for the determination of photosynthetic pigments present in higher plant green leaves.
The experiments were performed on fully developed green leaves of horseweed (*Erigeron canadensis* syn. *Conyza canadensis* (L.)). The plants were grown under natural daylight and then exposed to high-light irradiation to induce Z formation. The original elution programme (Schoefs *et al.*, 1995) was modified by setting the proportion of methanol from 30 to 17 % at the begining (0-10 min) and by setting the proportion of methylene chloride (MC) at either 28 or 45 % at the end (25-30 min) of the elution programme.
After these modifications a better separation of carotenoids and their isomers was reached. This was especially clear for the separation of *trans*-L,Z,*cis*-L.
In the original method, the separation of α-Car, β-Car and Pheo is not satisfactory since β-Car and Pheo are coeluting. To improve this, different proportions of MC, between 20 and 50 % were tested. No perfect separation of the 3 pigments was achieved with any MC proportion. Therefore, we searched for conditions allowing a perfect coelution of Pheo with either β-Car or α-Car. In this condition the contribution of Pheo, which can be determined at 666 nm, can be easily substracted from the elution peak recorded at either 450 nm (α-Car) or 457 nm (β-Car). Perfect coelution of Pheo with either β-Car or α-Car was obtained at 28% or 45% of MC.
Under these conditions, equations should be used to calculate the Car contents:

$$S_{\alpha\text{-Car, 450 nm}} = S_{\text{measured, 450 nm}} - R_1 S_{\text{Pheo. 666 nm}} \quad \text{(eq.1)}$$

$$S_{\beta\text{-Car,457 nm}} = S_{\text{measured, 457 nm}} - R_2 S_{\text{Pheo, 666 nm}} \quad \text{(eq.2)}$$

where $R_1 = 0.040$ and $R_2 = 0.047$ are the proportions of Pheo absorbances at 666 nm and 450 (eq.1) or 457 (eq.2), and S is the surface of the elution peak corresponding to α-Car, β-Car and Pheo measured at the indicated waveglength.

Schoefs, B., Bertrand, M. and Lemoine, M. (1995) *J. Chromatogr.* **692A**, 239-245.