Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 38 (2005) 910–917
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Hyphenation of capillary high-performance liquid chromatography to microcoil magnetic resonance spectroscopy—determination of various carotenoids in a small-sized spinach sample

Karsten Putzbach [a], Manfred Krucker [a], Marc David Grynbaum [a], Petra Hentschel [a], Andrew G. Webb [b], Klaus Albert [a,*]

[a] *Institute of Organic Chemistry, University of Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany*
[b] *4221 Beckman Institute, University of Illinois, 405N. Mathews Ave., Urbana, IL 61801, USA*

Received 24 September 2004; received in revised form 10 January 2005; accepted 22 January 2005
Available online 22 April 2005

Abstract

The development of miniaturized hyphenated systems such as capillary high-performance liquid chromatography–and nuclear magnetic resonance spectroscopy (HPLC–NMR) remains challenging in the field of structure elucidation. In combination with a highly specific sample preparation technique, matrix solid-phase dispersion (MSPD), and a highly selective C_{30} reverse phase HPLC–NMR enables the identification of small amounts of natural compounds. Here, the investigation of five carotenoids in a standard solution and two carotenoids from a spinach sample demonstrate the potential of this new development. The separation of the carotenoids is performed with self-packed fused-silica capillaries with a binary solvent gradient consisting of acetone and water. The miniaturized system allows the use of fully deuterated solvents for on-line HPLC–NMR coupling. The ^{1}H NMR spectra of the various carotenoids obtained in stopped-flow mode gave a high signal-to-noise ratio with a sample amount in the low nanogram range. All necessary parameters for structure elucidation such as multiplet structure, coupling constants and integration values can be detected unambiguously.

Keywords: Miniaturization; Capillary HPLC–NMR; Hyphenated techniques; Carotenoids

1. Introduction

The hyphenation of high-performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy (NMR) has matured to a routine method for structural elucidation of unknown compounds [1]. This method offers an effective tool to analyze complex mixtures of organic compounds in biological and pharmaceutical samples [2–7]. NMR spectroscopy has the capability to differentiate between structural and conformational isomers, which is an important advantage in comparison to HPLC–MS coupling. Disadvantages of classical HPLC–NMR coupling are the use of protonated solvents (due to the high cost of fully deuterated solvents) and therefore the necessity of solvent suppression, and the large amount of sample required for a high signal-to-noise ratio. Water can be replaced by D_2O, but use of other perdeuterated solvents incurs a very high economic cost. Over the past few years much effort has gone into overcoming these difficulties. One approach is the miniaturization of the separation technique. There are a number of benefits of capillary HPLC separation in comparison to classical HPLC. The amount of stationary phase and the solvent consumption are drastically reduced. A commonly used flow rate in capillary HPLC is 5 μl min^{-1} ($\sim$7 ml per day) which is around 200 times less than in classical HPLC and therefore allows the use of fully deuterated organic solvents [8,9]. Furthermore, mass-limited samples can be analyzed. Associated approaches are the miniaturization of the saddle coil [10]

* Corresponding author. Tel.: +49 7071 29 75335; fax: +49 7071 29 5875.
E-mail address: klaus.albert@uni-tuebingen.de (K. Albert).
URL: http://www.uni-tuebingen.de/uni/cok.

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.01.049

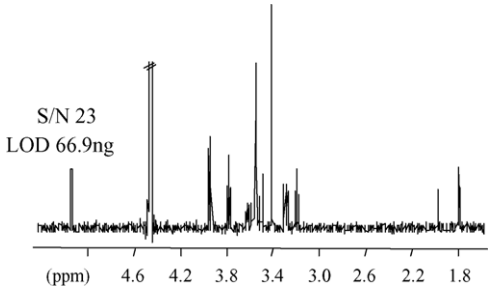

Fig. 1. Sensitivity test with a 1 mM sucrose solution (D_2O/ACN-d_3 = 90:10, v/v) measured with a single scan.

in analogy to conventional NMR flow probes, the development of solenoid microcoils, and the use of superconducting magnets with increased homogeneity and field strengths. The solenoidal probes are constructed by directly wrapping the rf coil around a capillary column [11]. The flow cell, which in this study has an active volume of 1.5 μl, is positioned perpendicular to the magnetic field B_0. The solenoidal-type design shows a roughly three-fold better sensitivity compared to the traditional saddle-shaped coils [12,13]. A sensitivity test with a 1 mM sucrose solution at 600 MHz shows that measurements in the low nanogram range are possible (Fig. 1). On-line NMR measurements can be carried out in two different modes, in continuous-flow as well as in stopped-flow mode. In continuous-flow mode the NMR instrument is used as a non-destructive real time detector. The required analyte amount is in the low microgram range (concentration approximately in gram per liter). The stopped-flow mode is used for analyte quantities in the nanogram range. Here, the separation is stopped when the peak of interest is in the microprobe detection volume. Two-dimensional NMR measurements can also be carried out in stopped-flow mode.

This paper deals with an application of a miniaturized hyphenated system for determination of various carotenoids in a standard solution and in plant material (e.g. spinach leaves). The miniaturized chromatographic separation is coupled on-line to solenoidal microcoil ^{1}H NMR detection in stopped-flow mode.

Carotenoids are naturally occurring tetraterpenes found in various plants, fruits and vegetables. An important structural feature of these compounds is the existence of a highly conjugated system of double bonds resulting in *cis*- and *trans*-isomers. Carotenoids play an important role as antioxidants due to their ability to quench highly damaging singlet oxygen, and scavenge free radicals in vivo. Additionally, they decrease the risk of degenerative diseases, such as certain kinds of cancer and cardiovascular disease [14–16]. Because carotenoids are light- and oxygen-sensitive, an analytical method, such as a closed-loop hyphenated system, for the artifact-free determination of carotenoids has to be employed. The extraction method also has to be optimized for the analysis of carotenoids. A mild and rapid extraction method has to be used to extract the carotenoids from the plant material without degradation. Matrix solid-phase dispersion (MSPD) combines both requirements, and allows the direct extraction of natural compounds from solid or viscous samples [17–19]. The separation of the carotenoids is performed on a self-packed C_{30} capillary column with an inner diameter of 250 μm. C_{30} silica phases offers high shape selectivity and high loading capacity [20,21]. These properties are necessary for successful separation of stereoisomers of the carotenoids and their structure determination with the on-line capillary HPLC–NMR system.

2. Experimental

2.1. Reagents and materials

Acetone (LiChrosolv, gradient grade), acetone-d_6 (Uvasol), deuterium oxide (Uvasol), and carbon tetrachloride (LiChrosolv) were obtained from Merck (Darmstadt, Germany). HPLC-grade water was obtained from a Milli-Q water purification system (Millipore AS, Bedford, MA, USA). The solid-phase material used for MSPD was silica based octadecyl (C_{18} end capped, IST Ltd, Hengoed Mid Glam, UK). (All-E) β-carotene was purchased from Fluka (Taufkirchen, Germany), (all-E) lutein, (all-E) zeaxanthin, (all-E) canthaxanthin, and (all-E) lycopene were gifts from BASF (Ludwigshafen, Germany). The spinach sample was home-grown, harvested after 8 weeks, and stored below −30 °C.

2.2. System set-up

For capillary HPLC–NMR coupling a ternary Waters CapLC system (Waters, Milford, MA, USA) equipped with a micro injection valve kit (Upchurch Scientific, Oak Harbor, WA, USA) with a 100 nl and 500 nl fused silica injection loop, a CapLC Selector Valve Modul (Waters, Milford, MA, USA) for stopped-flow NMR measurements, and a Bischoff Lambda 1010 UV detector (Bischoff Chromatography, Leonberg, Germany) with on-column (150 μm i.d.) UV detection at 450 nm was used. All NMR spectra were recorded on a Bruker AMX 600 spectrometer equipped with an ^{1}H-^{13}C inverse capillary NMR probe (Protasis/MRM, Savoy, IL, USA) with an active volume of 1.5 μl. A 3 m fused silica transfer capillary (50 μm i.d./360 μm o.d.) was used to connect the capillary HPLC with the NMR probe.

2.3. Chromatography

All separations were performed on a self-packed C_{30} capillary (15 cm × 250 μm i.d.) with Bischoff ProntoSil (Bischoff Chromatography, Leonberg, Germany), with a particle size of 3 μm and a pore width of 200 Å. The slurry technique introduced by Boughtflower et al. [22] was used for packing. 20 mg of the stationary phase were suspended in 300 μl carbon tetrachloride and placed in an ultrasonic bath for 5–10 min. The resulting slurry was transferred into a slurry chamber and forced downward into the empty fused-silica

capillary with a pneumatic HPLC pump (Knauer, GmbH, Berlin-Zehlendorf, Germany). Starting at 400 bar the pressure was continuously increased up to 650 bar over a period of 5 min. This final pressure was maintained for 30 min. The capillary end fittings consisted of zero dead volume unions, steel screens, and graphite ferrules (Vici AG Valco Int., Schenkon, Switzerland).

The solvent gradient for the capillary HPLC-UV method (5 μl min^{-1}, 295 K) consisted of a binary mixture of acetone and water. The separation starts isocratically with acetone: water = 80:20 (v/v) for 15 min, followed by a 5-min linear gradient to 99:1 (v/v) which was maintained for 25 min. The amount of sample injected was 100 nl for capillary HPLC-UV. For the capillary HPLC–NMR method fully deuterated solvents with the same gradient were used. The injection volume was increased from 100 to 500 nl for the NMR investigation.

2.4. Sample preparation

The carotenoid standards (0.5 mg) were dissolved separately in acetone-d_6 (1 ml). The spinach leaves (0.5 g) were deep frozen with liquid nitrogen and ground with 1.5 g of C_{18} (EC) MSPD-material to a homogeneous and dry powder. Then the mixture was loaded into an empty SPE column and pressed to a compact column bed between two frits. After this the column was washed with 10 ml water to elute polar impurities. Elution of the carotenoid fraction was performed with 4 ml acetone. After evaporation of acetone under a nitrogen stream, the residue was redissolved in 100 μl acetone-d_6.

2.5. Stopped-flow NMR experiments

The stopped-flow experiments were recorded with a pulse program to suppress the residual signals from deuterated acetone-d_6 and deuterium oxide, using rectangular shaped pulses for low-power presaturation (rectangular pulses, length 100 ms). ^{1}H NMR spectra from the standards and the spinach sample were recorded with between 2 and 16 k transients, a spectral width of 6024 Hz and 32 k time domain points. The relaxation delay was set to 1 s and the pulse angle to 30°. For all spectra, before Fourier transformation, a squared sine bell function was applied to the FID. Baseline correction and phasing were performed manually. The chemical shift axis was reference with respect to acetone-d_6, δ = 2.04 ppm, for all ^{1}H NMR spectra.

3. Results and discussions

Fig. 2 depicts the structures of the investigated carotenoids: (all-E) lutein, (all-E) zeaxanthin, (all-E) canthaxanthin, (all-E) β-carotene and (all-E) lycopene. They are all centrosymmetric except for (all-E) lutein.

The first step of the analysis was to translate the analytical HPLC separation of the carotenoids [23] to capillary-scale. The separation of a carotenoid standard solution (each 100 μg ml^{-1}) using a self-packed capillary C_{30} column is shown in Fig. 3A, monitored by UV absorbance at 450 nm. The injection volume was 100 nl, corresponding to an absolute amount of 10 ng of each carotenoid. The separation was performed with a solvent gradient of acetone: water = 80:20 (v/v) to 99:1 (v/v) and a flow rate of 5 μl min^{-1}. The carotenoids eluted between 8.7 min for (all-E) lutein and 36.7 min for (all-E) lycopene, corresponding to their decreasing polarity. The more polar xanthophylls (all-E) lutein, (all-E) zeaxanthin and (all-E) canthaxanthin elute in the first part of the chromatogram, after these the less polar (all-E) β-carotene and finally the non-polar (all-E) lycopene. The chromatogram obtained shows narrow and symmetrical peaks, which reflects the high quality of the self-packed capillary column. Column tests with a standard solution result in a similar number, 100,000, of theoretical plates per meter (TP/m) as for commercially available classical C_{30} columns. The peak

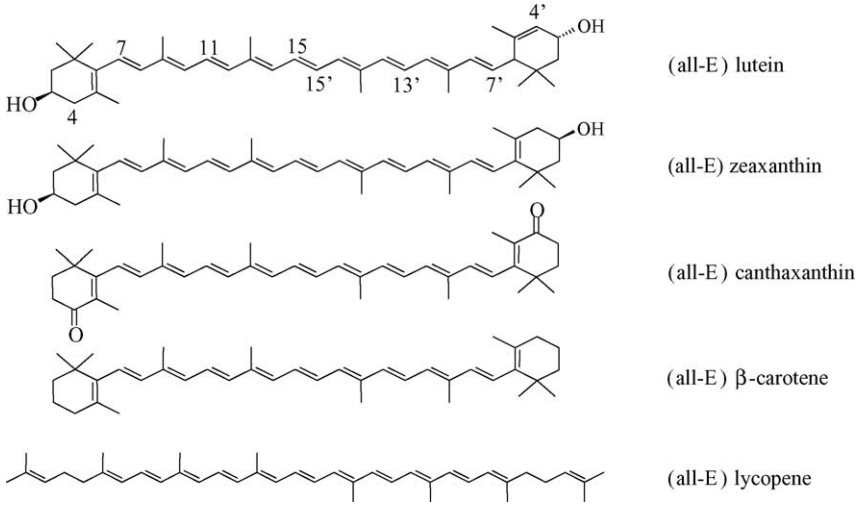

Fig. 2. Structures of: (all-E) lutein; (all-E) zeaxanthin; (all-E) canthaxanthin; (all-E) β-carotene; and (all-E) lycopene.

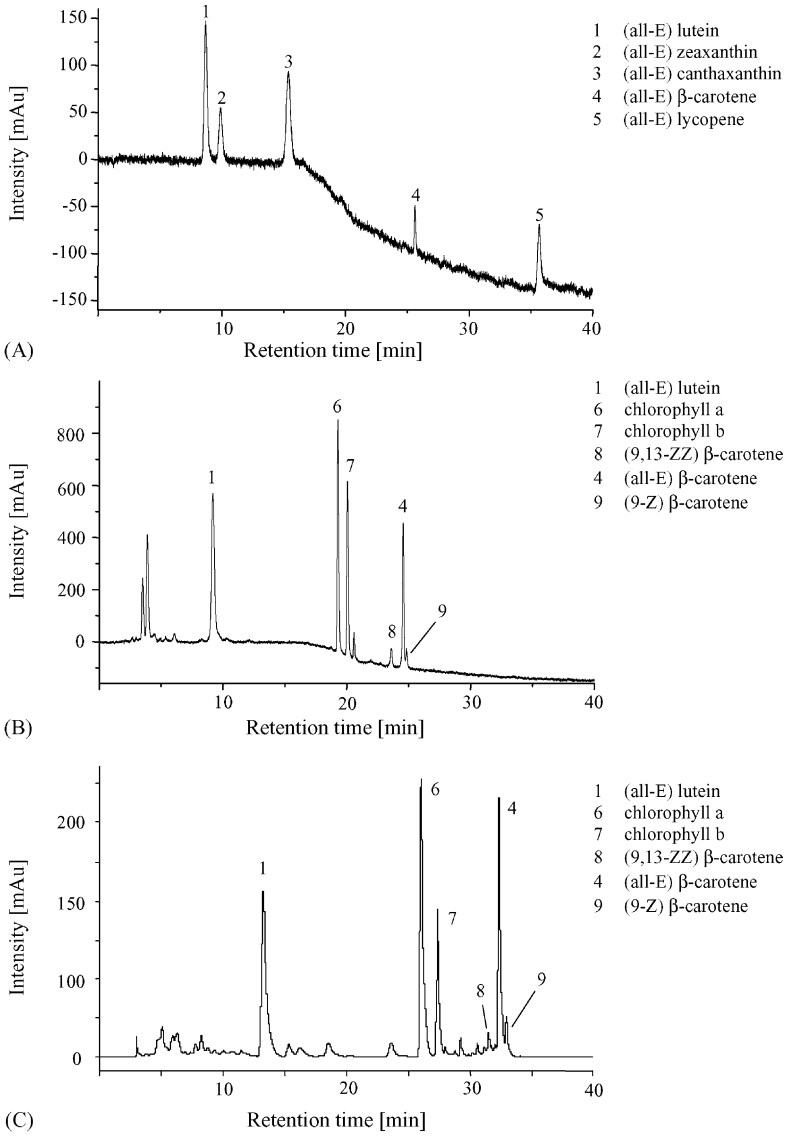

Fig. 3. (A) Capillary HPLC-UV chromatogram of a standard mixture (lutein, zeaxanthin, canthaxanthin, β-carotene, lycopene); (B) capillary HPLC-UV chromatogram of a spinach extract; and (C) HPLC–UV chromatogram of a spinach extract.

shape additionally, shows good system performance with less dead volume.

The next step was the necessary experimental adjustments for the hyphenated capillary HPLC–NMR experiments. For ^{1}H NMR measurements of the carotenoid standards fully deuterated solvents were used and the injection loop size was enlarged to 500 nl. Despite the high injection volume of 500 nl, which is necessary to get a sufficient analyte concentration in the NMR detection cell, no overloading effects are visible (chromatogram not shown). In spite of the high injection volume of the sample, the concentration of the carotenoids in the detection cell is still too small for continuous-flow NMR measurements. Therefore, stopped-flow measurements were performed to obtain ^{1}H NMR spectra of the carotenoids. The chromatographic run was stopped when the peak maximum of the analyte of interest reached the NMR probe detection volume. When the NMR measurements were finished, the separation was resumed, and subsequently other analytes of interest were trapped in the NMR probe. The NMR spectra obtained are shown in Fig. 4, in the order of their elution in the chromatographic separation. Only the olefinic region from 5 to 7 ppm is displayed. The chemical shifts of the olefinic protons are summarized in Table 1. In all five spectra a clear assignment of the olefinic protons is possible. In the spectra of (all-E) canthaxanthin, (all-E) β-carotene and (all-E) lycopene there is a small signal from dichloromethane-d_2, which was added for improved solubility of these carotenoids. A detailed analysis of the NMR spectra shows the following: the spectrum of the noncentrosymmetric (all-E) lutein shows at ∼6.60 ppm a multiplet for the protons 11/11′ (6.62 ppm) and 15/15′ (6.59 ppm) with an integration value of four. The protons 12/12′ (6.30 ppm) and

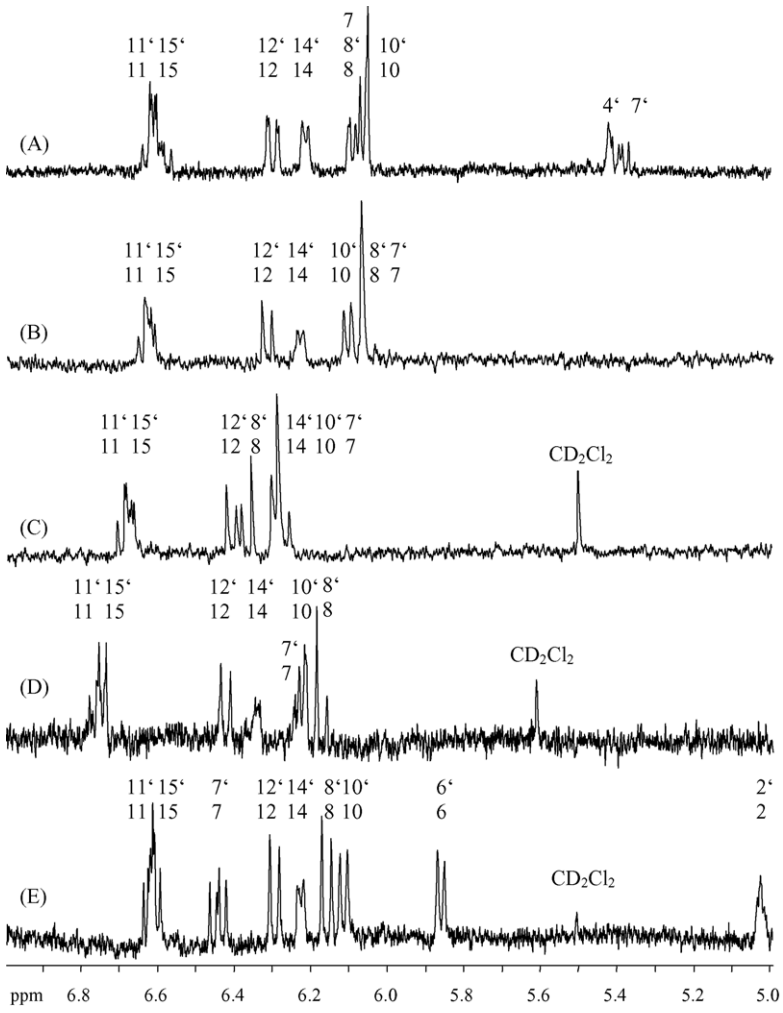

Fig. 4. Stopped-flow 1D ^{1}H NMR spectra (600 MHz, olefinic region) of: (A) (all-E) lutein; (B) (all-E) zeaxanthin; (C) (all-E) canthaxanthin; (D) (all-E) β-carotene; and (E) (all-E) lycopene.

Table 1
^{1}H NMR data of the olefinic protons for the carotenoid standard in ppm (reference was set to acetone-d_6 $\delta = 2.04$ ppm)

	(all-E) Lutein	(all-E) Zeaxanthin	(all-E) Canthaxanthin	(all-E) β-Carotene	(all-E) Lycopene
H 2	–	–	–	–	5.00
H 2′	–	–	–	–	5.00
H 4′	5.42	–	–	–	–
H 6	–	–	–	–	5.85
H 6′	–	–	–	–	5.85
H 7	6.06	6.07	6.25	6.24	6.44
H 7′	5.39	6.07	6.25	6.24	6.44
H 8	6.09	6.08	6.36	6.18	6.15
H 8′	6.07	6.08	6.36	6.18	6.15
H 10	6.07	6.11	6.27	6.23	6.11
H 10′	6.09	6.11	6.27	6.23	6.11
H 11	6.62	6.65	6.68	6.76	6.63
H 11′	6.62	6.65	6.68	6.76	6.63
H 12	6.30	6.32	6.40	6.43	6.29
H 12′	6.30	6.32	6.40	6.43	6.29
H 14	6.22	6.23	6.29	6.34	6.22
H 14′	6.22	6.23	6.29	6.34	6.22
H 15	6.59	6.62	6.65	6.74	6.60
H 15′	6.59	6.62	6.65	6.74	6.60

14/14′ (6.22 ppm) are each doublets with integration values of two. The next multiplet structure in the spectrum arise from the protons 8/8′ (6.09/6.07 ppm), 10/10′ (6.07/6.09 ppm) and 7 (6.06 ppm) and has a integration value of five. Due to the shift of a double bond in one ionone ring system, the proton 7′ (5.39 ppm) is shifted to higher field because of its altered relationship to the aliphatic C-6′. In comparison to the olefinic proton 7 this is a difference of 0.67 ppm. Another consequence is the olefinic proton 4 (5.42 ppm). In contrast to (all-E) lutein the centrosymmetric (all-E) zeaxanthin and the other carotenoids shows only proton pairs. The chemical shifts of the olefinic protons from (all-E) zeaxanthin are similar to the olefinic protons from (all-E) lutein except for proton 7′. The proton pairs 11/11′ (6.65 ppm) and 15/15′ (6.62 ppm) lead to a multiplet with an integration value of four. The chemical shifts of the pairs 12/12′ (6.32 ppm) and 14/14′ (6.23 ppm) are slightly different to the values of (all-E) lutein. The integration values are two for both. The main difference in the spectrum is located in the signal group for the protons 10/10′ (6.11 ppm), 8/8′ (6.08 ppm) and 7/7′ (6.07 ppm). Due to the centrosymmetric structure of the (all-E) zeaxanthin molecule the proton 7 and 7′ appear as one signal and all protons from one pair show the same chemical shift. The integration value is six, in accordance with the number of protons.

The next ^{1}H NMR spectrum shown is assigned to (all-E) canthaxanthin. The proton pairs 11/11′ (6.68 ppm) and 15/15′ (6.65 ppm) appear as multiplets with the greatest shift to low-field, as in all other investigated carotenoid spectra. The doublets are from protons 12/12′ (6.40 ppm) and 8/8′ (6.36 ppm). This low-field shift of the proton pair 8/8′ and of the multiplet from the proton pairs 14/14′ (6.29 ppm), 10/10′ (6.27 ppm) and 7/7′ (6.25 ppm) arise from the oxygen molecule in the C-4 position in the ring system. The spectra of (all-E) β-carotene and (all-E) lycopene show a lower signal-to-noise ratio than the other three spectra. The reason is a poorer solubility of these two relatively nonpolar carotenoids. Because of the higher solubility and diffusion band broadening characteristics in dichloromethane, a small amount of dichloromethane-d_2 was added to the standard solution (in acetone-d_6) in order to increase the solubility of the carotenoids and thereby the concentration of the standard solution. Nevertheless, the resolution and the signal intensity is still sufficient for an unambiguous peak assignment. The multiplet signal at ∼6.75 ppm arises from the proton pairs 11/11′ (6.76 ppm) and 15/15′ (6.74 ppm) and has an integration value of four. The next two doublets are from protons 12/12′ (6.43 ppm) and 14/14′ (6.34 ppm). This order of the first four signals in the spectra of (all-E) β-carotene is equivalent to the order in the spectrum of (all-E) lutein and (all-E) zeaxanthin. The proton pairs 7/7′ (6.24 ppm) and 10/10′ (6.23 ppm) represent a multiplet with an integration value of four. Finally, the proton pair 8/8′ (6.18 ppm) shows a doublet with an integration value of two. The structure of (all-E) lycopene deviates from the structure of the other four carotenoids. (All-E) lycopene has no ring system in the molecule and has two additional double bonds at the end of the chain. Therefore, two additional olefinic signals appear in the spectra and the order of the other signals is different. Specifically, the multiplet signal at ∼6.60 ppm is from the proton pairs 11/11′ (6.63 ppm) and 15/15′ (6.60 ppm) and has an integration value of four. The next signals show the proton pairs 7/7′ (6.44 ppm), 12/12′ (6.29 ppm), 14/14′ (6.22 ppm), 8/8′ (6.15 ppm) and 10/10′ (6.10 ppm). The integration value is two in each case. The two additional proton pairs are 6/6′ (5.85 ppm) and 2/2′ (5.00 ppm) and show a high-field shift due to their position in the chain. The protons 6 and 6′ are at the end position (smaller high-field shift) and the protons 2 and 2′ are completely isolated from the conjugated double bond system (larger high-field shift). Furthermore, the signal structure for the protons 2 and 2′ is different from the other olefinic protons, because it shows coupling to the nearby methylene groups instead of couplings to olefinic protons. This leads to a different signal pattern and coupling constants. The signal pattern of an olefinic proton with another olefinic proton is a doublet (e.g. proton 6 in (all-E) lycopene) and with two other olefinic protons a doublet of doublets (e.g. proton 7 in (all-E) lycopene). In some cases only a general multiplet can be detected due to overlapping with other signals. In all spectra the coupling constants are between 11 and 12 Hz for the *trans* conformation of the olefinic protons, except for the proton pair 2/2′ in (all-E) lycopene. These coupling constant show that all carotenoids are in the all-E configuration. Minor differences between these chemical shifts and values in the literature are due to a different solvent composition [24–27].

The next step after successful measurements with standard solutions was the investigation of plant material. For this experiment we used spinach, because it contains mainly lutein and β-carotene in the all-E configuration, although some small peaks in the chromatogram show the presence of low levels of various Z stereoisomers. Due to the instability of carotenoids in the presence of UV light, and to avoid artifacts or isomerisation during the extraction step, a mild and rapid extraction method had to be used. Therefore, MSPD was chosen for the extraction of the carotenoids from the spinach leaves. After harvest, the spinach leaves were deep frozen with liquid nitrogen and reduced to small pieces. Afterwards, 0.5 g of the chopped spinach leaves were ground with 1.5 g C_{18} sorbent material. The obtained mixture was loaded into an empty SPE cartridge, and pressed in a stable column bed. After a wash step with water, the carotenoids were extracted with acetone and after drying under a nitrogen stream redissolved in 100 μl of acetone-d_6.

The chromatographic separation of the spinach extract is depicted in Fig. 3B. The obtained chromatogram shows four major peaks, at 9.18 min (all-E) lutein, around 20 min two peaks for chlorophyll a and b, and at 24.56 min (all-E) β-carotene. In addition, two Z stereoisomers of β-carotene, (9,13-ZZ) β-carotene and (9-Z) β-carotene, can be detected. Minor differences in the retention times in comparison with the standard separation are due to matrix effects. A comparison to a classical separation of a spinach sample (Fig. 3C) shows that the retention time is reduced from 32.30 to

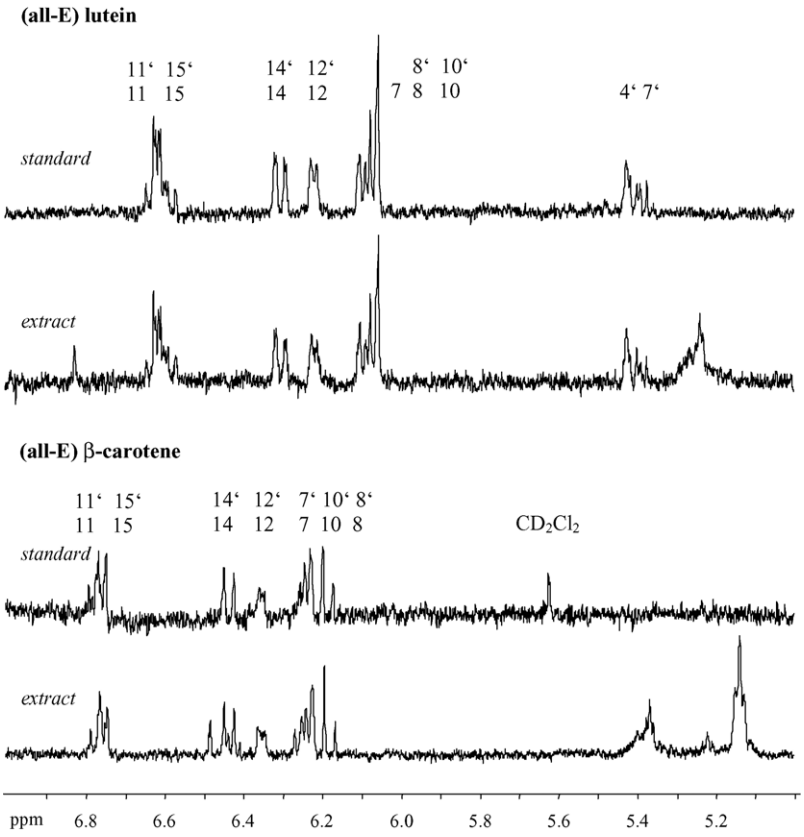

Fig. 5. Comparison of the 1D ^{1}H NMR spectra (600 MHz, olefinic region) of (all-E) lutein and (all-E) β-carotene from the standard solution and MSPD-extract.

24.56 min for the last eluting carotenoid β-carotene with the capillary column. Despite the reduction of the retention factor k', the separation factors α for the stereoisomers are almost equal for both column sizes. This fact shows the good quality of the self-packed capillaries.

Stopped-flow ^{1}H NMR measurements of the MSPD-extract were performed with deuterated solvents and a 500 nl loop, as with the standard solution. The olefinic part (5–7 ppm) of the obtained ^{1}H NMR spectra of (all-E) lutein and (all-E) β-carotene is depicted in Fig. 5. Both spectra of the extract show the same good spectral resolution as the standard spectra and all olefinic protons can be clearly assigned. Also, the integration values, multiplet structure and coupling constants can be determined unambiguously. In spite of the chromatographic separation some additional signals in the olefinic region can be seen. In the (all-E) lutein spectrum there is a small and sharp signal at 6.83 ppm and also a broad signal at 5.30 ppm. In the spectrum of (all-E) β-carotene there are two broad signals at 5.15 and 5.40 ppm, and a small and sharp signal next to the proton pair 14/14′. However, these additional signals do not overlap with the olefinic proton signals from the carotenoids nor complicate the assignment.

4. Conclusion

The results obtained demonstrate that the combined use of an optimized extraction MSPD technique, miniaturized HPLC chromatographic separation and solenoidal- microprobe ^{1}H NMR detection is a powerful tool for the structural determination of natural compounds in mass-limited biological samples. In this study, the identification of UV light and air-sensitive carotenoids in the low nanogram range was accomplished with ^{1}H NMR spectroscopy. The excellent performance of the self-packed separation capillaries in comparison to the classical HPLC columns was shown by a reduction in retention times without a loss of selectivity. The ^{1}H NMR spectra obtained enable the determination of all the important parameters such as multiplet structure, coupling constants and integration values. The next step will be the detection of carotenoid stereoisomers as well as other bioactive compounds in biological samples, and further developments in tailor-made stationary phases with a higher loadability and similar, or even better, shape selectivity.

Acknowledgements

This work was supported by the European Union project HPRI-CT-1999-50018 and the Deutsche Forschungsgemeinschaft (AL 298/10-1). A.G.W. acknowledges the support of the Alexander von Humboldt Foundation, Wolfgang Paul Preis.

References

[1] K. Albert (Ed.), On-line LC–NMR and Related Techniques, Wiley, Chichester, UK, 2002.
[2] J.-L. Wolfender, K. Ndjoko, K. Hostettmann, Phytochem. Anal. 12 (2001) 2–22.
[3] K. Albert, M. Dachtler, T. Glaser, H. Händel, T. Lacker, G. Schlotterbeck, S. Strohschein, L.-H. Tseng, U. Braumann, J. High Resol. Chromatogr. 22 (1999) 135–145.
[4] J.C. Lindon, J.K. Nicholson, I.D. Wilson, J. Chromatogr. B 748 (2000) 233–258.
[5] K. Levsen, A. Preiss, M. Godejohann, TRAC 19 (2000) 27–48.
[6] U.G. Sidelmann, I. Bjornsdottir, J.P. Shockcor, S.H. Hansen, J.C. Lindon, J.K. Nicholson, J. Pharm. Biomed. Anal. 24 (2001) 569–579.
[7] T. Glaser, A. Lienau, D. Zeeb, M. Krucker, M. Dachtler, K. Albert, Chromatographia 57 (2003) 19–25.
[8] M.E. Lacey, Z.J. Tan, A.G. Webb, J.V. Sweedler, J. Chromatogr. A. 922 (2001) 139–149.
[9] M. Krucker, A. Lienau, K. Putzbach, M.D. Grynbaum, P. Schuler, K. Albert, Anal. Chem. 76 (2004) 2623–2628.
[10] B. Behnke, G. Schlotterbeck, U. Tallarek, S. Strohschein, L.-H. Tseng, T. Keller, K. Albert, E. Bayer, Anal. Chem. 68 (1996) 1110–1115.
[11] N. Wu, T.L. Peck, A.G. Webb, R.L. Magin, J.V. Sweedler, Anal. Chem. 66 (1994) 3849–3857.
[12] D.I. Hoult, R.E. Richards, J. Magn. Res. 24 (1976) 71–85.
[13] K. Albert, Habil, University Tübingen, Germany, 1988.
[14] H.N. Basu, A.J. Del Vecchio, F. Flider, F.T. Orthoefer, JAOCS 78 (2001) 665–675.
[15] G. Tang, X.-D. Wang, R.M. Russell, N.I. Krinsky, Biochemistry 30 (1991) 9829–9834.
[16] G. Tang, N.I. Krinsky, Methods Enzymol. 214 (1993) 69–74.
[17] S.A. Barker, A.R. Long, C.R. Short, J. Chromatogr. 475 (1989) 353–361.
[18] S.A. Barker, J. Chromatogr. A 880 (2000) 63–68.
[19] M. Dachtler, T. Glaser, K. Kohler, K. Albert, Anal. Chem. 73 (2001) 667–674.
[20] C. Emenhiser, L.C. Sander, S.J. Schwartz, J. Chromatogr. A 707 (1995) 205–216.
[21] M. Dachtler, T. Glaser, H. Händel, T. Lacker, L.-H. Tseng, K. Albert, Encyclopedia of Separation Science, Level II, Academic Press, London, 2000, pp. 747–760.
[22] R.J. Boughtflower, T. Underwood, C.J. Paterson, Chromatographia 40 (1995) 329–335.
[23] C. Rentel, S. Strohschein, K. Albert, E. Bayer, Anal. Chem. 70 (1998) 4394–4400.
[24] T. Glaser, K. Albert (Eds.), On-line LC–NMR and Related Techniques, Wiley, Chichester, UK, 2002, pp. 129–140.
[25] G. Englert, Carotenoids, vol. 1B, Birkenhäuser-Verlag, Basel, 1995, 147–260.
[26] S. Strohschein, M. Pursch, H. Händel, K. Albert, Fresenius J. Anal. Chem. 357 (1997) 498–502.
[27] F. Khachik, G. Englert, C.E. Daitch, G.R. Beecher, W.R. Lusby, L.H. Tonucci, J. Chromatogr. 582 (1992) 153–166.