Journal of Chromatography, 395 (1987) 609–612
Elsevier Science Publishers B.V , Amsterdam — Printed in The Netherlands

CHROMSYMP. 1187

SEPARATION OF SERUM CAROTENOIDS AND VITAMIN A ON CHROMSIL-AMINO AND -CYANO PHASES BY A BI-DIRECTIONAL GRADIENT ELUTION TECHNIQUE

R OHMACHT*, Gy. TÓTH and G. VOIGT
University Medical School, Szigeti út 12, H-7643 Pécs (Hungary)

SUMMARY

Vitamin A, β-carotene, lycopene, β-cryptoxanthin, lutein and zeaxanthin can be simultaneously separated in human serum extracts by normal-phase liquid chromatography. Two stationary phases (containing amino and cyano groups, respectively) were investigated. With a bi-directional (up-down) gradient-elution technique both packings are useful for the determination of serum levels of carotenoids and retinol in serum extracts. The small sample size, the simplicity of extraction, and a good reproducibility render these procedures ideal for clinical use.

INTRODUCTION

The knowledge of the vitamin A and carotenoid content in human serum is of importance from the clinical point of view[1–4]. The earlier photometric determination methods of the total serum carotenoid level do not meet the present requirements, because of the different physiological effects of the different carotenoids[5].

Several high-performance liquid chromatographic (HPLC) methods have been developed for the qualitative and quantitative determination of carotenoids and vitamin A in human serum[6–10]. The advantage of the presently proposed normal-phase chromatographic method is that the preparation of the serum samples is the same as in the above mentioned total carotenoid determination methods. Because of the presence of carotenoids with very different polarity in the serum, a gradient elution technique was used.

EXPERIMENTAL

Preparation of samples: 0.5 ml serum was extracted with *n*-hexane. The organic layer was removed after centrifugation at 3000 rpm for 2 min[11,12]. The solvent was evaporated under reduced pressure. The residue was dissolved in 200 μl of 5% benzene in *n*-hexane. Two types of stationary phases were investigated: Chromsil-CN and Chromsil-NH_2 (Labor, Esztergom, Hungary). The mobile phases and the gradient profiles are described in Figs. 1 and 2. The chromatographic system consisted

0021-9673/87/$03 50

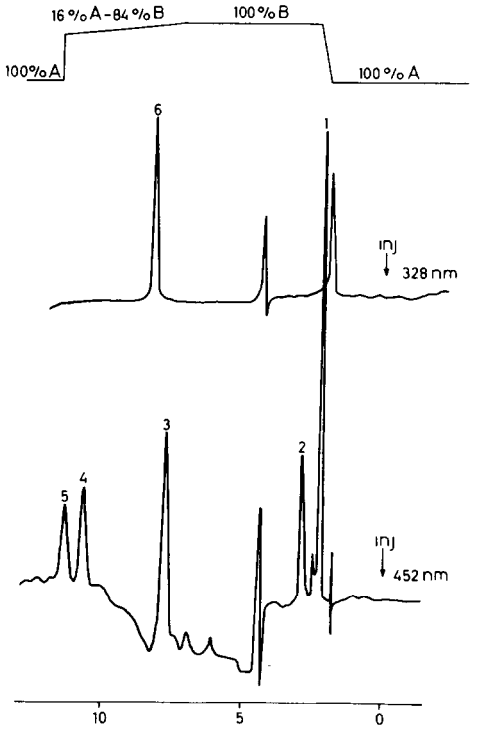

Fig. 1. Separation of carotenoids and retinol in serum on a Chromsil-NH_2 (6 μm) stationary phase. Columns, 250 × 4.6 mm; flow, 2.0 ml/min; mobile phase, A: benzene *n*-hexane (4:96), B: methanol–benzene (0.6:99.4). Upper chromatogram, separation of vitamin A; lower chromatogram, separation of carotenoids. Peaks: 1 = β-Carotene, 2 = lycopene, 3 = β-cryptoxanthin, 4 = lutein, 5 = zeaxanthin, 6 = vitamin A.

of a Model 250B Gradient-Former, and a Model 300B Precision Pump (Gynkotek, Munich, F.R.G.). A Model SV7 sample injection valve (Glenco, U.S.A.) with a 50-μl loop was used. A stainless-steel column (250 × 4.6 mm, Type 323 from Labor) and a home-built spectrophotometric detector (operated at 452 nm for carotenoids and at 328 nm for vitamin A) were employed.

RESULTS

The five most important serum carotenoids (as seen in Figs. 1 and 2), β-carotene (1), lycopene (2), β-cryptoxanthin (3), lutein (4) and zeaxanthin (5) were separated from each other and from vitamin A (6). The serum extracts were stable for 2 days when kept at temperatures below −25°C.

The cyano packing is a moderately polar stationary phase. In order to achieve sufficient separation in the case of β-carotene and lycopene, dry hexane was used as eluent at the beginning of the analysis. Because this eluent has limited solubility for certain carotenoids, the procedure can only be used in the case of samples containing several 100 ng of carotenoids or less. Since the amount of carotenoids in serum samples varies between 5 and 50 ng in 50 μl, solubility difficulties do not affect the determi-

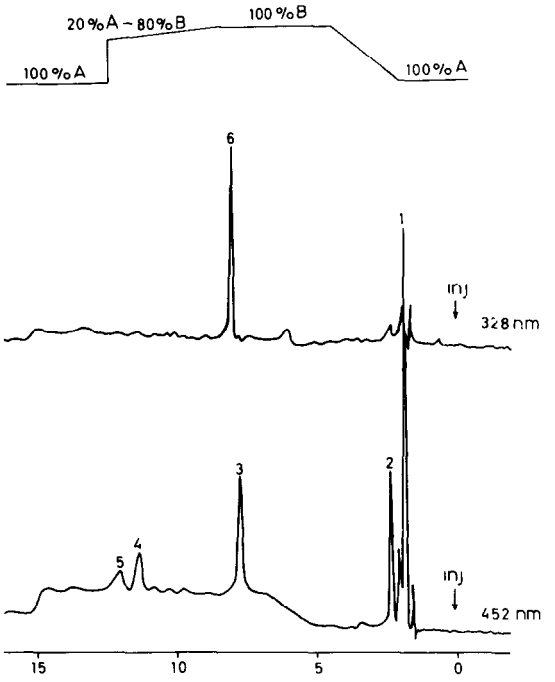

Fig. 2. Separation of carotenoids and retinol in serum on a Chromsil-CN (6 μm) stationary phase. Mobile phase, A: *n*-hexane (dry), B: dichloromethane–*n*-hexane (30:70). For other conditions see Fig. 1.

nation. Duplicate measurements of each sample were made, with resulting variations in peak height of less than 3%. A linear calibration range was found between the concentration in the samples and the peak height from several nanograms to the 100-ng level. (The minimum detectable amount of β-carotene and lycopene is less than 1 ng.)

Although the separation of β-cryptoxanthin and vitamin A is not complete, the quantitative evaluation is not hampered because the detection of the two components is carried out at different wavelengths. The polarity of lutein and zeaxanthin is similar, and for this reason they can be readily separated using a negative gradient. The amino packing is also suitable for the separation of carotenoids using a bi-directional gradient.

In both systems, but especially on the amino phase unevenness of the base line is observed during separation. This is caused by differences in the refractive indices of the solvent systems (*e.g.* in the case of the amino phase the first eluent is rich in hexane, and the second is rich in benzene). Although UV detection is not so sensitive to the refractive index, large refractive index differences cause discernible changes in the base line. These circumstances do not influence the accuracy of the determination. The amino phase is suitable for the separation of carotenoids, but it should be mentioned that in the case of oxocarotenoids (*e.g.* capsanthin), the formation of Schiff-bases cannot be excluded.

CONCLUSION

It was established that the above suggested stationary phases, in particular the amino-phase, are suitable for the qualitative and quantitative determination of serum carotenoids.

REFERENCES

1 *Brit. Med. J.* (Editorial), 281 (1980) 957.
2 M. M. Mathews-Roth, *Proc. 6th Int. Symp. Carotenoids*, 1981, p. 297.
3 *Nutr. Rev.* (Editorial), 40 (1982) 265.
4 M. M. Mathews-Roth, *Pure Appl. Chem.*, 57 (1985) 717.
5 T. Jávor, M. Bata, M. Lovász, L. Nagy, I. Patty, J. Szabolcs, F. Tárnok, Gy. Tóth and Gy. Mózsik, *Int. J. Tiss. Reac.*, V (1983) 289.
6 R. Ohmacht and Gy. Tóth, in H. Kalász and L. S. Ettre (Editors), *Chromatography, the State of the Art*, Akadémiai Kiadó, Budapest, 1985, p. 119.
7 K. W. Miller, N. A. Lorr and Cl. S. Yang, *Anal. Biochem.*, 138 (1984) 340.
8 J. G. Bieri, E. D. Brown and J. C. Smith, Jr., *J. Liq. Chromatogr.*, 8 (1985) 473.
9 D. B. Milne and J. Botnen, *Clin. Chem.*, 32 (1986) 874.
10 R. Ohmacht, Gy. Tóth and G. Voigt, *Chromatographia*, 22 (1986) 189.
11 H. K. Biesalski, W. Ehrenthal, G. Haffner and O. Hartl, *GIT Fachz. Suppl. Chromatogr.*, (1981) 5–8.
12 K. W. Miller, S. Yang and S. Chung, *Anal. Biochem.*, 145 (1985) 21.