Journal of Chromatography, 200 (1980) 300–304
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,060

Note

Comparison of reversed-phase and adsorption modes of high-performance liquid chromatography for the assay of fat soluble vitamins in multivitamin tablets

D. THORBURN BURNS*

Department of Analytical Chemistry, The Queen's University of Belfast, Belfast BT9 5AG (Northern Ireland)

and

CHRISTEEN MACKAY

Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire LE11 3TU (Great Britain)

(Received June 19th, 1980)

Relatively simple mixtures of fat soluble vitamins prepared from dry concentrates may be separated by reversed-phase[1–3] and by adsorption modes[4–7] of high-performance liquid chromatography. Vitamin A acetate and its associated isomeric compounds[8] and also vitamin E acetate and its isomeric compounds[9,10] have been separated by adsorption chromatography. Vitamin D and its related thermal and photoisomers have been separated by reverse phase[11,12] and by adsorption modes[4–7] of chro·natography. The quantitative determination of the individual fat soluble vitamins in multivitamin tablets is a more complex analytical problem. Earlier work on the development of a method for the determination of vitamin D_2 and pre-vitamin D_2 in multivitamin tablets using adsorption chromatography[13,14] and recent reversed-phase studies of fat-soluble vitamins in admixture[15,16] suggested that a comparative study of the two modes of chromatography for the assay of all the fat-soluble vitamins in multivitamin tablets would be of interest. It was found that a combination of both reversed-phase and adsorption chromatography was necessary for the quantitative separation of the fat-soluble vitamins in a single multivitamin tablet.

EXPERIMENTAL

Reagents and stock solutions

The reagents used were of analytical-reagent grade unless otherwise stated: cyclohexane (laboratory grade); isopropanol; vitamin A acetate (pure, crystalline), vitamin E acetate (pure, N.F. XIII) and vitamin D_2 (pure, B.P., U.S.P.) (Koch-Light, Colnbrook, Great Britain); 4-hydroxybiphenyl (BDH, Poole, Great Britain).

Vitamin solutions

Vitamin A acetate + vitamin D_2. A 100-mg amount of vitamin A acetate and 10 mg vitamin D_2 were dissolved in 10 ml isopropanol and diluted to 100 ml with cyclohexane.

0021-9673/80/0000–0000/$02.25

Vitamin A acetate + vitamin E acetate. A 340-mg amount of vitamin A acetate and 500 mg vitamin E acetate were dissolved in 10 ml isopropanol and diluted to 100 ml with cyclohexane.

Isomerised vitamin D_2 solution. A 100-mg amount of vitamin D_2 was dissolved in 100 ml cyclohexane and heated under reflux (80°C) for 2 h. The isomerised solution contained vitamin D_2 and pre-vitamin D_2 in the ratio 78:22 (ref. 13). The isomerised solution was cooled to 4°C and 1 ml diluted to 10 ml with cyclohexane.

Vitamin A acetate + vitamin E acetate + vitamin D_2 + 4-hydroxybiphenyl. A 340-mg amount of vitamin A acetate, 500 mg vitamin E acetate, 10 mg vitamin D_2 and 4.5 mg 4-hydroxybiphenyl were dissolved in 10 ml isopropanol and diluted to 100 ml with cyclohexane.

Chromatography

The liquid chromatograph was equipped with a constant-flow pump (Waters Assoc., Milford, MA, U.S.A.; Model M6000), a dual-wavelength ultraviolet absorbance detector (Waters Assoc., Model 440) and a syringe-loop injection system (Waters Assoc., U6K). In the reversed phase systems the columns were: (a) Partisil 10 μm ODS (250 × 4.6 mm I.D.) (Whatman, Maidstone, Great Britain); (b) μBondapak C_{18} (300 × 4.6 mm I.D.) (Waters Assoc.). The chemical nature of the ODS and C_{18} phases are essentially the same. The mobile phase was methanol–water (90:10) at a flow-rate of 1 ml min^{-1}. In the adsorption system the column was packed with Partisil 10 microparticulate silica (250 × 4.6 mm I.D.) (Whatman). The mobile phase was cyclohexane containing 1.25% isopropanol[13] at a flow-rate of 0.8 ml min^{-1}.

After equilibration with solvent to obtain a steady base line (15–30 min) 5-μl aliquots of samples were injected and chromatograms recorded.

RESULTS AND DISCUSSION

Chromatograms shown in Figs. 1–5 are typical and illustrate the main features.

The chromatography of vitamin A acetate and vitamin E acetate is complicated by the presence of products of side chain oxidation and by degradation to the parent alcohols, that of vitamin D_2 is complicated by the presence of thermal and photoisomers. Reverse-phase chromatograms, Figs. 1 and 2, show that vitamin A and E acetates and vitamin D_2 can be separated from one another and that the degradation products of vitamin A acetate and vitamin E acetate can be separated from the parent vitamins. The reversed-phase chromatograms in Fig. 3 show that vitamin D_2 could be separated from pre-vitamin D_2 on the ODS column but not on the C_{18} column although the phases are chemically similar. Reversed-phase separation of vitamin D from related isomeric compounds has been reported previously[11,12]. In the present work these separations were not consistent and it is the view that these separations are obtained by an adsorption mechanism based on residual uncoated silica which varies from column to column and with usage.

Reversed-phase chromatography is thus considered suitable for the determination of vitamin A acetate and vitamin E acetate and adsorption mode chromatography for the determination of both vitamin D_2 and pre-vitamin D_2 as required for the determination of the potency of a product[13]. Adsorption chromatography on

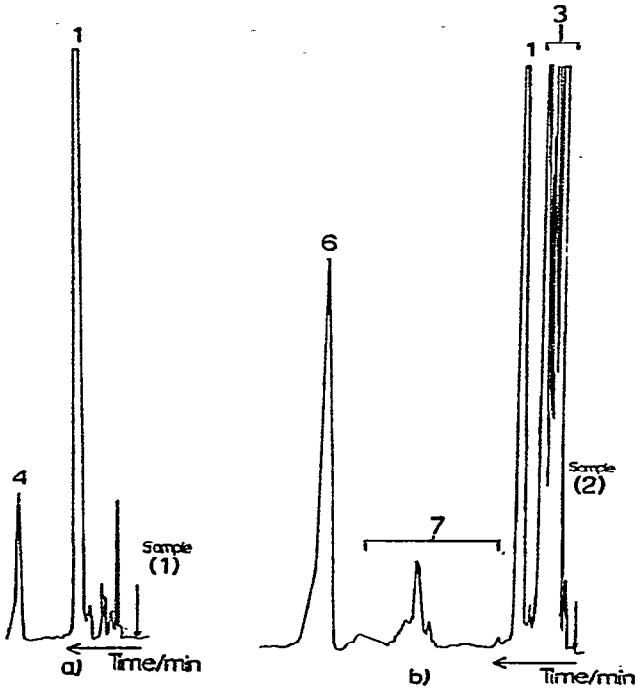

Fig. 1. Reversed-phase (μBondapak C_{18}) chromatograms of vitamin A acetate (1), vitamin D_2 (4) and vitamin E acetate (6) with isocratic elution using methanol–water (9:1). Other peaks: 3 = degradation products of vitamin A; 7 = degradation products of vitamin E.

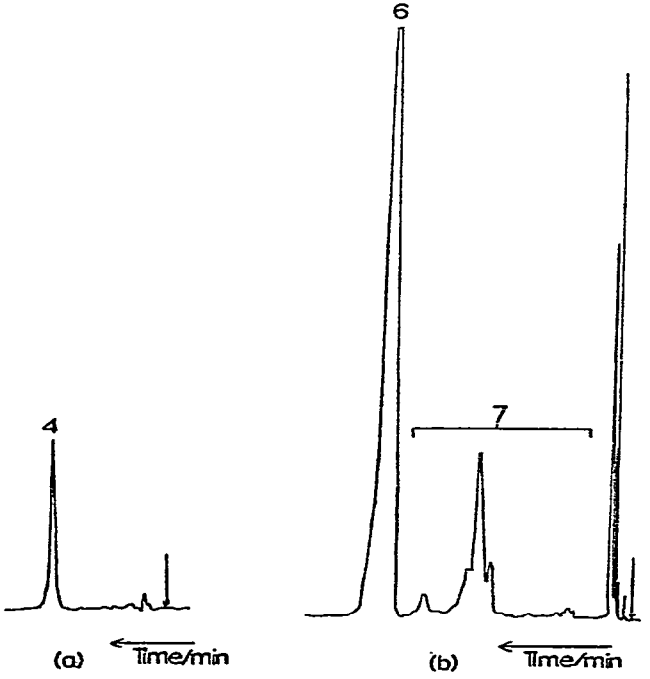

Fig. 2. Reversed-phase (μBondapak C_{18}) chromatograms of (a) vitamin D_2 and of (b) vitamin E acetate. Details as in Fig. 1.

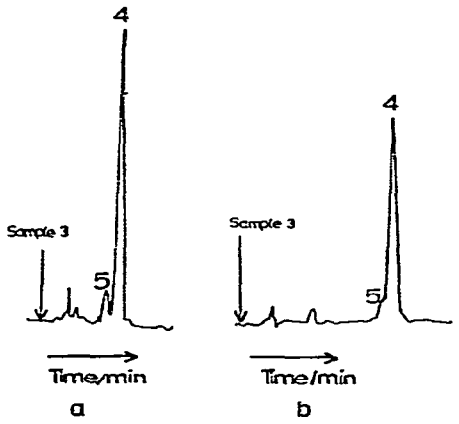

Fig. 3. Reversed-phase chromatograms of isomerised vitamin D_2 on (a) Partisil 10 μm ODS, (b) μBondapak C_{18}. Details as in Fig. 1. Peak 5 = pre-vitamin D_2.

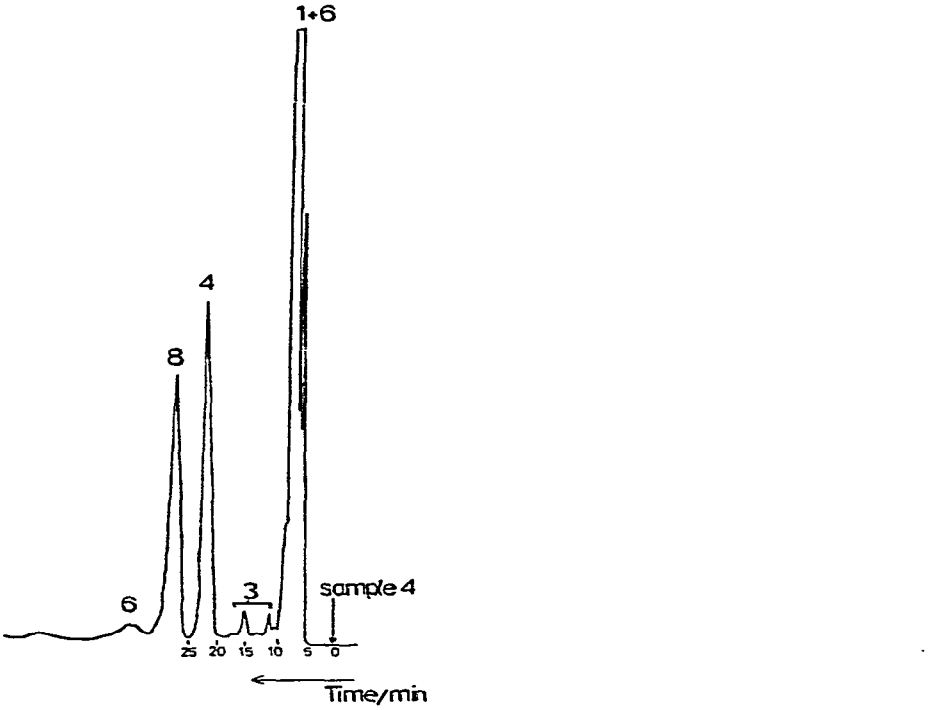

Fig. 4. Adsorption mode chromatogram of vitamins A and E acetates, D_2 and internal standard (4-hydroxybiphenyl). Partisil 10 column, isocratic elution with 1.25% isopropanol in cyclohexane. Peaks as in Fig. 1, except 8 = 4-hydroxybiphenyl.

silica does not separate vitamin A acetate and vitamin E acetate under the conditions used by us (Figs. 4 and 5).

Single multivitamin tablets may be assayed using the previously described sample pretreatment procedure[13], which was designed to avoid thermal degradation

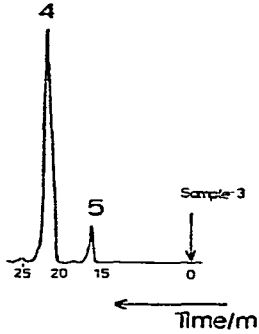

Fig. 5. Adsorption mode chromatogram of isomerised vitamin D_2. Details is in Fig. 4, except peak 5 = pre-vitamin D_2.

of vitamin D, making up extracts to 0.5 ml and injecting 10-μl aliquots for the determination of vitamin D_2 and 5-μl aliquots for the determination of vitamin E acetate ($\lambda_{max.}$ 287 nm; ε 2500). Due to the high molar absorptivity and amounts of vitamin A acetate ($\lambda_{max.}$ 325; ε 5200) it is necessary to dilute the extract 100:1 and made a second injection for the best quantitation. The chromatographic procedures developed are suitable for product impurity screening and for stability studies.

REFERENCES

1 R. C. Williams, J. A. Schmit and R. A. Henry, *J. Chromatogr. Sci.*, 10 (1972) 494.
2 M. Osadca and M. Araujo, *J. Ass. Offic. Anal. Chem.*, 60 (1977) 993.
3 M. Eriksson, T. Eriksson and B. Sörensen, *Acta Pharm. Suecica*, 15 (1978) 274.
4 D. F. Tompkins and R. J. Tscherne, *Anal. Chem.*, 46 (1974) 1602.
5 H. Steuerle, *J. Chromatogr.*, 115 (1975) 447.
6 K. A. Tartivita, J. P. Sciarello and B. C. Rudy, *J. Pharm. Sci.*, 65 (1976) 1024.
7 H. Hofsass, A. Grant, N. J. Alicino and S. B. Greenbaum, *J. Ass. Offic. Anal. Chem.*, 59 (1976) 251.
8 M. Vecchi, J. Vesely and G. Oesterhelt, *J. Chromatogr.*, 83 (1973) 447.
9 P. J. van Niekerk, *Anal. Biochem.*, 53 (1973) 533.
10 J. F. Cavins and G. E. Inglett, *Cereal Chem.*, 51 (1974) 605.
11 R. A. Wiggins, *Chem. Ind. (London)*, (1977) 841.
12 E. J. de Vries, J. Zeeman, R. J. E. Esser, B. Borsje and F. J. Mulder, *J. Ass. Offic. Anal. Chem.*, 62 (1979) 129.
13 C. Mackay, J. Tillman and D. Thorburn Burns, *Analyst (London)*, 104 (1979) 626.
14 D. Thorburn Burns, C. Mackay and J. Tillman, *J. Chromatogr.*, 190 (1980) 141.
15 J. W. Dolan, J. R. Gant, N. Tanaka, R. W. Giese and B. L. Karger, *J. Chromatogr. Sci.*, 16 (1978) 616.
16 S. A. Barnett and L. W. Frick, *Anal. Chem.*, 51 (1979) 641.