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Short Communication

Determination of retinyl palmitate and total vitamin A content in liver and liver-based ready-to-eat foods

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ABSTRACT

The determination of retinyl palmitate and total vitamin A in liver and liver-based ready-to-eat foods is described. The method is very simple as sample preparation is minimal, and the isocratic elution of the C_{18} column with pure methanol does not necessitate a sophisticated instrumental set-up. The method is accurate with high recoveries (100.6 ± 9.3%, mean ± S.D., n = 23), and precise with within-day and between-day coefficients of variation of less than 5.5% (n = 13) and less than 16% (n = 6), respectively.

INTRODUCTION

Present, widely used methods for the determination of vitamin A in liver and liver-based ready-to-eat foods have several disadvantages related to sample preparation, clean-up, precision and specificity [1–6]. A simpler, more precise and specific method is therefore needed. Most of those high-performance liquid chromatographic (HPLC) methods, which have been developed for the determination of vitamin A in foods and feed, require a sample saponification and/or evaporation of the sample extract [1–6]. These steps may result in losses of vitamin A during analysis. Furthermore, most of those methods do not correct for losses by means of internal standardization.

This paper describes a simple and rapid HPLC method for determination of retinyl esters in liver and liver-based ready-to-eat foods. The method does not necessitate saponification, and an internal standard is used to correct for losses of vitamin A during analysis.

EXPERIMENTAL

Apparatus

HPLC analysis was carried out with a Merck Hitachi (E. Merck, Darmstadt, Germany) liquid chromatograph equipped with a Model L-6200 intelligent pump, a Model AS-2000 autosampler and a Model D-2500 chromato-integrator. A variable-wavelength UV–VIS detector (Model L-4200) was used for detection at 295 nm and 0.05 a.u.f.s. A chromatographic system equipped with Perkin-Elmer (Norwalk, CT, USA) Model 250 binary LC pump, LC 235 diode-array detector and LCI 100 integrator were used, together with a Kontron (Basel, Switzerland) Model 460 autosampler for purity checking and identification of the chromatographic peaks.

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A ChromSep Nucleosil C_{18} (5 μm) glass cartridge column, 100 mm × 3 mm I.D. (Chrompack, Middelburg, Netherlands, Cat. No. 27665) with a guard column (10 mm × 2.1 mm I.D., Cat. No. 28141) was used.

The concentration of retinyl palmitate stock solution was determined with a Hitachi 150-20 (Tokyo, Japan) spectrophotometer. The centrifuge was a Heraus Christ Labofuge GL (Osterode, Germany).

Reagents

HPLC-grade methanol (Rathburn, UK) was used as the mobile phase. The flow-rate was 1.0 ml/min. Hexane (E. Merck) was used as the extraction solvent. Vitamin A standard (retinyl palmitate) was purchased from Sigma (St. Louis, MO, USA). The internal standard, *dl*-tocol, was a gift from Hoffmann La Roche (Basle, Switzerland). Ethanol (99%) was from Alko (Helsinki, Finland).

Standardization

A retinyl palmitate standard stock solution (25 mg per 50 ml) was prepared in methanol (retinyl palmitate was first diluted in a small amount of hexane). The working solution was prepared from the stock solution by diluting 1:100. The internal standard stock solution (100 mg per 50 ml) was prepared in ethanol, and the working solution was obtained from stock solution by diluting 1:200.

The concentration of the standard stock solution was determined daily by spectrophotometry. The specific absorbance ($A_{1\,cm}^{1\%}$) was 975 at 325 nm. The standard solution was stored at 4°C and was protected from light. The absorption spectrum of each standard lot was routinely checked.

Sample preparation

The samples were thoroughly homogenized in a blender. Four standard samples were prepared by adding 25 μl and/or 50 μl of retinyl palmitate stock solution to a known amount of sample (*ca.* 50 mg). The samples were then diluted with 200 μl of freshly distilled water. A 200-μl volume of *dl*-tocol working solution was then added to each tube and vortex-mixed for 5 s. Retinoids were extracted with 1 ml of hexane and vortex-mixed for 60 s. Samples were then centrifuged at 4933 *g* (5000 rpm) for 10 min. The hexane-containing upper layer was removed and evaporated to dryness under a nitrogen stream, and the residue was dissolved in 250–1000 μl of methanol, depending on the injector used. A 20-μl aliquot of the solution was injected into the chromatograph. The sample analysis was carried out in the same way without the standard addition.

All samples were prepared under subdued light.

Quantitation

Quantitation was based on an internal standard method. The linearity was tested by adding known amounts of retinyl palmitate stock solution (10–50 μl) to identical liver sausage samples. Analyses for the linearity study were carried out in five replicates, with five samples spiked with five different aliquots of stock solution and one in triplicate with no standard addition.

The total vitamin A content is a mixture of two or three retinyl esters (the free retinol concentration is not included). Some liver extracts were also analysed by an essentially similar chromatographic system, but equipped with a diode-array detector, for purity checking and identification of the chromatographic peaks.

The results are expressed as retinol equivalents (RE), where the activity of retinol is 1.83 times the activity of retinyl palmitate.

RESULTS AND DISCUSSION

Retinyl palmitate was identified in samples by comparing its retention time and its absorption spectrum with those of an authentic standard (Fig. 1).

The HPLC conditions used to separate vitamin A derivatives resulted in good separations with sharp peaks. A time of *ca.* 10 min was required to separate all vitamin A derivatives. Retinyl acetate was also found to be a suitabe candidate as an internal standard, but required the addition of some water to the mobile phase for better resolu-

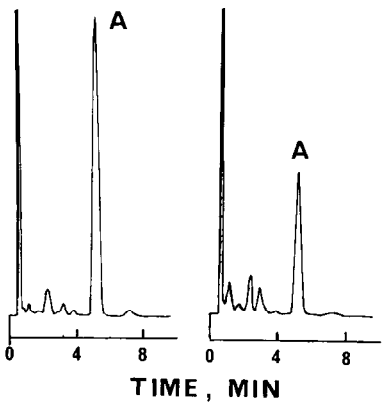

Fig. 1. Chromatogram of the retinyl palmitate standard. A diode-array detector was used. Detection at 325 nm (left panel) and 295 nm (right panel); 0.05 a.u.f.s. Peak A = retinyl palmitate standard at 5.30 min (A_{max} 326 nm).

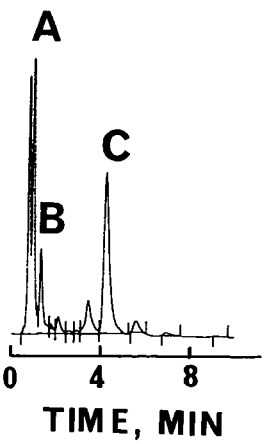

Fig. 3. Typical chromatogram of liver-based ready-to-eat food (liver pastry). Peaks: A = retinol at 0.95 min; B = *dl*-tocol at 1.31 min; C = retinyl palmitate at 4.29 min.

tion. The method is accurate with high recoveries (100.6 ± 9.3% mean ± S.D., n = 23), and precise with within-day coefficient of variation (C.V.) of less than 5.5% (n = 13) and between-day C.V. of less than 16% (n = 6). The method was found to be linear over the studied range of concentrations (retinyl palmitate added 0–0.4 μg/mg).

The samples analysed in this study were liver sausage and liver pastry from different factories. Liver samples were obtained from one slaughterhouse.

Typical chromatograms of the different samples are shown in Figs. 2–4. Peaks of vitamin A derivatives obtained by the HPLC method were well separated in all chromatograms from liver and liver-based ready-to-eat foods, and no interference from other sample compounds was observed. This indicates that this method is specific

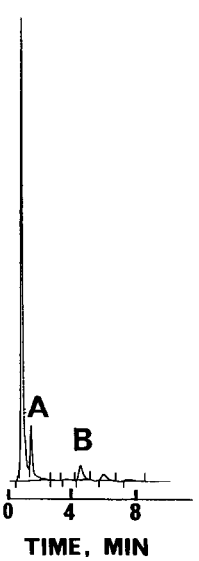

Fig. 2. Typical chromatogram of liver-based ready-to-eat food (liver sausage). Peaks: A = *dl*-tocol at 1.34 min; B = retinyl pamitate at 4.45 min.

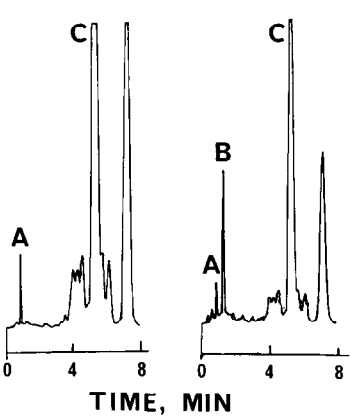

Fig. 4. Typical chromatogram of beef liver sample. A diode-array detector was used. Detection at 325 nm (left panel) and 295 nm (right panel); 0.05 a.u.f.s. Peaks: A = retinol at 0.95 min (A_{max} 326 nm); B = *dl*-tocol at 1.31 min (A_{max} 299 nm); C = retinyl palmitate at 5.37 min (A_{max} 326 nm).

TABLE I

HPLC ANALYSIS OF VITAMIN A IN SOME LIVER-BASED READY-TO-EAT FOODS

Liver sausage samples from different factories.

Sample	Retinyl palmitate (mg RE/100 g)	C.V. (%)	Total vitamin A (mg RE/100 g)	C.V. (%)
I[a]	2.08 ± 0.08	3.85	2.08 ± 0.08	3.85
II[b]	6.48 ± 0.32	4.94	7.68 ± 0.40	5.21
III[b]	2.54 ± 0.14	5.51	2.54 ± 0.14	5.51
IV[b]	1.11 ± 0.05	4.50	1.11 ± 0.05	4.50
V[a]	5.82 ± 0.12	2.06	6.80 ± 0.12	1.76
VI[a]	4.90 ± 0.09	1.84	5.53 ± 0.04	0.72

[a] Mean of duplicate determinations ± S.D.
[b] Mean of triplicate determinations ± S.D.

TABLE II

HPLC ANALYSIS OF VITAMIN A IN BEEF AND PORK LIVER

Values are mean of triplicate determinations ± S.D.

Sample	Retinyl palmitate (mg RE/100 g)	C.V. (%)	Total vitamin A (mg RE/100 g)	C.V. (%)
Beef				
I	26.05 ± 1.42	5.45	39.49 ± 2.17	5.50
II	28.13 ± 1.47	5.23	41.10 ± 2.23	5.43
III	7.04 ± 0.25	3.55	10.34 ± 0.34	3.29
IV	26.62 ± 0.73	2.74	41.26 ± 1.17	2.84
Pork				
I	9.50 ± 0.07	0.74	11.65 ± 0.07	0.60
II	10.71 ± 0.40	3.73	13.41 ± 0.50	3.74
III	11.46 ± 0.61	5.32	14.31 ± 0.77	5.38

for vitamin A derivatives under investigation. No attempt was made to identify other minor isomers of retinol, because of a lack of corresponding standards. However, we concluded that they were retinyl stearate and retinyl oleate [3,7].

Results obtained from HPLC of liver and liver-based ready-to-eat foods are shown in Tables I and II.

Some attempts were made to analyse retinyl palmitate and total vitamin A content from non-homogenous material (liver rice casserole), but the within-day C.V. was found to be too high. It would, therefore, be necessary to increase the sample amount and/or freeze-dry and grind the samples [7] when analysing non-homogenous material. However, when analysing homogenous material, the relatively small sample size results in more rapid analysis and savings in the amounts of solvents and reagents needed. Because there is no saponification step, the reliability of the method is better than that of other widely used methods.

Because of the advantages mentioned above, in addition to simplicity and convenience, the present method should be useful, especially in routine use.

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