



ELSEVIER

Journal of Chromatography A, 771 (1997) 127–134

JOURNAL OF
CHROMATOGRAPHY A

Determination of cyanocobalamin in foods by high-performance liquid chromatography with visible detection after solid-phase extraction and membrane filtration for the precolumn separation of lipophilic species¹

Hiroshi Iwase*, Ichiro Ono

Ajinomoto Co., Inc., Central Research Laboratories, 1-1 Suzuki-cho Kawasaki-ku, Kawasaki 210, Japan

Received 30 October 1996; revised 27 January 1997; accepted 12 February 1997

Abstract

Determination of trace amounts of cyanocobalamin (18 ng/g) in fatty foods was performed by solid-phase extraction and high-performance liquid chromatography (HPLC) with visible detection at 550 nm using a membrane filter for the precolumn separation of particulate material. It was found that a membrane filter (HLC-Disk 25, 0.45 μm) was most suitable for the separation of oily particulates. A sample solution was applied to a solid-phase extraction cartridge and then cyanocobalamin was eluted using a 50% aqueous acetonitrile solution followed by HPLC. This method was suitable for the determination of trace amounts of cyanocobalamin in nutrient samples. The proposed method was simple, rapid (extraction time: ca. 12 min, analysis time: ca. 12 min), sensitive (detection limit: ca. 0.15 ng at a signal-to-noise ratio of 3:1), highly selective and reproducible (relative standard deviation: 2.67%) for cyanocobalamin. The calibration graph for cyanocobalamin was linear in the range of 0.1 to 30 ng. Recovery of cyanocobalamin was over 90% by the standard addition method.

Keywords: Food analysis; Sample handling; Cyanocobalamin

1. Introduction

A simple, rapid and highly selective analytical method is required for the determination of trace amounts of cyanocobalamin in the presence of many kinds of compounds in foods and biological samples.

Numerous methods have been developed for the analysis of cyanocobalamin, including spectropho-

tometry [1,2], microbiological methods [3] and high-performance liquid chromatography (HPLC) [4–15].

Spectrophotometry is not suitable for a complex sample matrix. Microbiological methods generally have been used for the routine analysis of vitamins. However, this method is tedious and time-consuming, because it requires that the tissue is cultured and the strain is preserved. Furthermore, it takes a long time to prepare the sample and to incubate it.

HPLC is a useful tool for the separation and determination of trace amounts of various compounds.

Numerous techniques have been developed to

*Corresponding author.

¹ Presented at the 20th International Symposium on High-Performance Liquid Phase Separations and Related Techniques, San Francisco, CA, 16–21 June 1996.

extend the application of HPLC, including pre- and postcolumn labelling, column switching and solid-phase extraction (SPE). SPE is a simple and rapid technique and has been applied to complex sample matrices for the extraction of nutrients from foods and drugs and their metabolites from biological fluids [16].

It is necessary to remove the oily particles from samples with a high fat content to optimize the retention of trace amounts of water-soluble compounds before the SPE, because large amounts of lipophilic species, which are strongly retained on reversed-phase SPE cartridges such as Bond-Elut C₁₈, reduce the capacity of the cartridge for these water-soluble compounds. The use of SPE is necessary, however, because the analytes, present in trace amounts, must be concentrated to permit detection, and oily components in these samples must be removed.

Usually, a hexane extraction method is used for the separation of oily particles in a sample, after the addition of inorganic salt, such as sodium chloride, to the sample in a separating funnel. However, this method takes a long time, because the sample often forms an emulsion, which may take as long as 1 h to break up. In addition, this procedure is tedious and exposes the analyst to hexane, which is not good for our health or for the environment.

The trace amounts of cyanocobalamin in samples have been determined by HPLC with visible detection at 550 nm after SPE [10,15]. However, SPE conditions required to optimize the retention and elution of trace amounts of cyanocobalamin in sample solution were not examined in detail.

This paper demonstrates a simple, rapid and highly selective analytical method for the trace detection of cyanocobalamin (18 ng/g) in nutrients with high fat content (ca. 2%) by SPE and HPLC with visible detection at 550 nm, which is specific for cyanocobalamin, and which uses a commercially available membrane filter for the precolumn separation of oily particles. The concentration of oily particles is at least 1 000 000 times higher than that of cyanocobalamin. The nutrient samples used were in powder form and had to be dissolved prior to use. They are complex sample types, containing, for example, amino acids, vitamins, organic acids, plant oil, dextrin and minerals at concentrations at least

50–1 000 000 times higher than that of cyanocobalamin. Evaluation of the separation of oily particles was made by measuring the transmittance (*T*%) value at 650 nm.

This paper deals with the choice of membrane filter for the separation of oily particles, effects of sodium sulfate concentration and of sample concentration on the separation of fatty components using a membrane filter. In addition, a comparison of *T* (%) values for samples and filtrates in many kinds of foods was made using a membrane filter and an aqueous layer that was extracted with hexane. Our efforts were also focused on optimizing the recovery of cyanocobalamin using a Bond-Elut C₁₈ cartridge. We investigated the effect of pH value in aqueous solution, eluent composition and elution volume to order to optimize the retention and elution of cyanocobalamin. We demonstrate the validity of the determination of cyanocobalamin in nutrient samples with high fat contents, by HPLC with visible detection at 550 nm after SPE and membrane filtration for the precolumn separation of oily particles.

2. Experimental

2.1. Reagents and materials

the cyanocobalamin used in this study was of Japanese Pharmacopeia Standard. Other reagents were all of analytical or HPLC grade. Nutrient and food samples were commercially available. Light-resistant brown volumetric flasks and glassware [1,2,17] were used in this study. The membrane filters (HLC-DISK 25, 0.45 μm, polyvinylidene fluoride) were purchased from Kanto Kagaku (Tokyo, Japan). Bond-Elut C₁₈ cartridges (500 mg, 3 ml) were purchased from Varian (Harbor City, CA, USA). The Bond-Elut C₁₈ cartridges were washed with 5 ml of methanol and then with 10 ml of deionized water prior to use.

2.2. Apparatus and conditions

A Model 655 A-11 high-performance liquid chromatograph (Hitachi, Tokyo, Japan) equipped with a Model UV-DEC 100-IV detector (JASCO, Tokyo) set at 550 nm was used. The samples were

applied using a Rheodyne Model 7125 sample loop injector with an effective volume of 200 μl . Separation took place on a 15 \times 0.46 cm I.D. column of Inertsil ODS-2 (5 μm) (GL Sciences, Tokyo, Japan) using 50 mM KH_2PO_4 (pH 2.1, adjusted with phosphoric acid)–acetonitrile (90:10, v/v) as the mobile phase. The flow-rate was 1 ml/min at 40°C. A Shimadzu UV variable-wavelength recording spectrophotometer UV-2100 (Shimadzu, Kyoto, Japan) was used to obtain the transmittance ($T\%$) value at 650 nm.

2.3. Sample preparation

After the nutrient (50 g) was dissolved in a brown volumetric flask (250 ml) with a 15% aqueous solution of sodium sulfate containing 1 mM ethylenediaminetetraacetic acid disodium dihydrate ($\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$), the solution was filtered using a membrane filter (0.45 μm) to separate the oily particles and then this filtrate (10 ml) was applied to a Bond-Elut C_{18} cartridge for the determination of trace amounts of cyanocobalamin. The cartridge was washed with deionized water (5 ml) and then was eluted with 50% acetonitrile in deionized water (10 ml). The eluate was concentrated to dryness in a water bath at 50°C in vacuo. Concentrates were dissolved in exactly 0.5 ml of deionized water. An

aliquot (200 μl) was injected into the chromatograph.

3. Results and discussion

3.1. Removal of oil components using a membrane filter

Our first effort was focused on the effect of membrane filtration on the separation of oily particles in nutrient samples. Various commercially available membrane filters were tested for the separation of oily particles. Evaluation was made by measuring the transmittance ($T\%$) value at 650 nm.

The T (%) values for filtrates, obtained using various membrane filters, the relative standard deviations (R.S.D.) and their ability to remove oily particles from samples diluted in 10% aqueous solutions of sodium sulfate were determined and the results are shown in Table 1.

The membrane filter materials examined in this study were cellulose acetate, cellulose nitrate, polypropylene and acrylic copolymer, which were used for aqueous samples, and polytetrafluoroethylene and polyvinylidene fluoride, which were used for both aqueous and non-aqueous samples. The porosity of the membrane filters was either 0.2 or 0.45 μm .

Table 1

T (%) values (at 650 nm) of filtrate obtained using various membrane filters and the suitability of filters for the removal of fatty components in nutrient samples diluted with 10% Na_2SO_4

| Filter composition | Use for | T (%) | R.S.D. (%; $n=3$) | Suitability |
|--|---------|---------|--------------------|-------------|
| Sample | | 2.9 | 3.4 | |
| Filtrate | | | | |
| Cellulose acetate (0.45 μm) | A | 4.7 | 2.1 | No |
| Cellulose nitrate (0.45 μm) | A | 9.4 | 1.1 | No |
| Cellulose acetate+nitrate (0.45 μm) | A | 68.8 | 0.1 | No |
| Polypropylene (0.45 μm) | A | 3.3 | 3.0 | No |
| Polypropylene (Chromatodisc, 0.2 μm) | A | 96.4 | 0.1 | Yes |
| Acrylic copolymer (0.2 μm) | A | 78.5 | 0.1 | No |
| Acrylic copolymer (0.45 μm) | A | 5.5 | 1.8 | No |
| Polytetrafluoroethylene (0.45 μm) | B | 0.9 | 6.2 | No |
| Polytetrafluoroethylene (0.2 μm) | B | 28.9 | 0.3 | No |
| Polyvinylidene fluoride (HLC-DISK 25, 0.45 μm) | B | 98.6 | 0.1 | Yes |

Sample: 10 g/100 ml.

A: Can be used for aqueous solutions.

B: Can be used for both aqueous and non-aqueous solutions.

3.1.1. Aqueous samples

The data in Table 1 show that cellulose acetate, cellulose nitrate and polypropylene (0.45 μm) were not suitable for the samples diluted in 10% sodium sulfate. However, polypropylene (0.2 μm) was effective for the separation of oily particles, whereas acrylic copolymer (both 0.2 and 0.45 μm) was not suitable.

3.1.2. Non-aqueous samples and aqueous samples

The data in Table 1 show that polytetrafluoroethylene (with porosities of both 0.2 and 0.45 μm) was not suitable for the removal of oily particles. Polyvinylidene fluoride (0.45 μm) was the most suitable for the separation of oily particles. The R.S.D. was 0.1% ($n=3$) and a typical filtration time was within 1 min.

A comparison of particle sizes showed that 0.2 μm filters gave better results than the 0.45 μm filters in all of the membrane filters examined. A comparison of membrane materials was also made. It was found that cellulose was not suitable, polypropylene (0.2 μm), used for aqueous samples, was suitable and polyvinylidene fluoride (0.45 μm) was the most effective for removing oily particles.

It was concluded that membrane materials and the particle size of membrane filters were important considerations for the separation of oily components. The ability to separate oily particles simply and rapidly using commercially available membrane filters was a great advantage, because it removes the need to use an extraction solvent such as hexane.

3.2. Effect of sodium sulfate concentration on the T (%) value of filtrate

A secondary purpose of this work was to de-

termine the effect of sodium sulfate concentration on the T (%) values of filtrate using HLC-DISK 25 (0.45 μm) filters.

The T (%) values of sample and filtrate are shown in Table 2. The results in Table 2 show that higher T (%) values of both sample and filtrate at 650 nm were attained on increasing the sodium sulfate concentration. The sample was not transparent at 650 nm at any sodium sulfate concentration. Increasing the sodium sulfate concentration above 10% gave higher T (%) values (over 90%) of filtrate, at 650 nm. The R.S.D. was 0 to 0.1% ($n=3$).

From the above, it was found that the sodium sulfate concentration affects the separation of the sample's fatty components.

3.3. Effect of sample concentration on the T (%) value of filtrate in each fraction

We also looked at the effect of sample concentration (10 to 20 g per 100 ml) on the T (%) value of filtrate in the fractions (5 ml) (Table 3). The results in Table 3 show that the T (%) values of each filtrate decrease as the sample volume loaded increases.

Fraction 3 in the sample solution (20 g per 100 ml) could not be obtained, because the solution was not filtered with a membrane filter. The R.S.D. was 0 to 0.2% ($n=3$).

3.4. Comparison of the T (%) values of samples and filtrates (5 ml) in foods, obtained using membrane filtration and extraction of the aqueous layer with hexane

We next compared the T (%) values of samples

Table 2
Effect of Na_2SO_4 concentration on the T (%) value of sample and filtrate at 650 nm

| Na_2SO_4 (%) | Sample | | Filtrate | |
|------------------------------|---------|--------------------|----------|--------------------|
| | T (%) | R.S.D. (%; $n=3$) | T (%) | R.S.D. (%; $n=3$) |
| 0 | 0.2 | 0.3 | 65.6 | 0.2 |
| 2.5 | 0.3 | 0.2 | 73.5 | 0.1 |
| 5 | 0.5 | 0.1 | 76.5 | 0.1 |
| 10 | 2.9 | 1.9 | 98.6 | 0.1 |
| 15 | 4.5 | 1.2 | 100 | 0 |
| 20 | 6.5 | 1.5 | 99.6 | 0.1 |

Sample: 10 g/100 ml.

Table 3
Effect of the nutrient concentration on the *T* (%) value of filtrate at 650 nm in fractions

| Sample | 10 g/100 ml | | 15 g/100 l | | 20 g/dl | |
|-------------------|--------------|-------------------------|--------------|-------------------------|--------------|-------------------------|
| | <i>T</i> (%) | R.S.D. (%; <i>n</i> =3) | <i>T</i> (%) | R.S.D. (%; <i>n</i> =3) | <i>T</i> (%) | R.S.D. (%; <i>n</i> =3) |
| Sample | 6.5 | 1.5 | 0.1 | 0 | 0.3 | 0 |
| Filtrate | | | | | | |
| Fraction 1 (5 ml) | 100 | 0 | 99.6 | 0.1 | 99.6 | 0.1 |
| Fraction 2 (5 ml) | 100 | 0 | 99.0 | 0.1 | 99.8 | 0.1 |
| Fraction 3 (5 ml) | 95.1 | 0.1 | 89.8 | 0.1 | Not filtrate | |

Concentration of Na₂SO₄: 15 g/100 ml

and filtrates (5 ml) in various foods obtained by using membrane filtration or extraction of aqueous sample with hexane (Table 4).

Nutrient (10%), health food (10%), mayonnaise (5%), plant oil (50%), cow's milk (1%) and milk powder (1%) were tested in this study. Both cow's milk and milk powder could not be filtered using a membrane filter in higher concentration. It seems that these sample solutions were difficult to pass through a membrane filter, because the sample solutions contain proteins such as casein.

The results in Table 4 show that the *T* (%) values of all sample solutions at 650 nm were lower. A comparison of *T* (%) values of filtrates obtained using membrane filtration or extraction with hexane showed that the former gave higher *T* (%) values than the latter. This is a great advantage because it shows that it is possible to separate oily components in many kinds of foods using commercially available

membrane filters, in a simple and rapid procedure compared to that of hexane extraction.

3.5. SPE

3.5.1. Effect of acetonitrile concentration on the elution of cyanocobalamin

Next, we focused on the recovery of cyanocobalamin using a Bond-Elut C₁₈ cartridge.

First, the optimal elution and retention characteristics of a cyanocobalamin standard were determined. Cyanocobalamin in an acetonitrile–water eluent was examined for the complete elution of cyanocobalamin on a Bond-Elut C₁₈ cartridge.

Optimal elution of cyanocobalamin was achieved using 50% acetonitrile in water as the eluent (Fig. 1). To determine if all of the cyanocobalamin was retained on the cartridge, the aqueous (unbound)

Table 4
T (%) value at 650 nm of sample and filtrate (5 ml) in foods obtained by using various membrane filters and aqueous layer extraction with hexane

| Nutrient type | Sample | | Filtrate | | Aqueous layer ^a | |
|-------------------|--------------|-------------------------|-------------------|-------------------------|----------------------------|-------------------------|
| | <i>T</i> (%) | R.S.D. (%; <i>n</i> =3) | <i>T</i> (%) | R.S.D. (%; <i>n</i> =3) | <i>T</i> (%) | R.S.D. (%; <i>n</i> =3) |
| A (10%) | 7.5 | 1.3 | 99.9 | 0.1 | 91.5 | 0.1 |
| B (10%) | 6.5 | 1.5 | 98.3 | 0.1 | 97.1 | 0.1 |
| C (10%) | 7.2 | 1.4 | 99.1 | 0.1 | 93.2 | 0.1 |
| D (10%) | 6.5 | 1.5 | 100 | 0 | 69.3 | 0.2 |
| Health food (10%) | 32.9 | 0.4 | 100 | 0 | 88.1 | 0.1 |
| Mayonnaise (5%) | 0.02 | 0 | 98.7 | 0.1 | 0.9 | 11.1 |
| Plant oil (50%) | 3.9 | 2.6 | 95.3 | 0.1 | — ^c | |
| Cow's milk (1%) | 9.5 | 1.1 | 100 ^b | 0 | 9.9 | 1.1 |
| Milk powder (1%) | 0.4 | 0 | 98.7 ^b | 0.1 | — ^c | |

Concentration of Na₂SO₄: 15 g/100 ml.

^a Aqueous layer after extraction with hexane.

^b 3 ml.

^c Not examined.

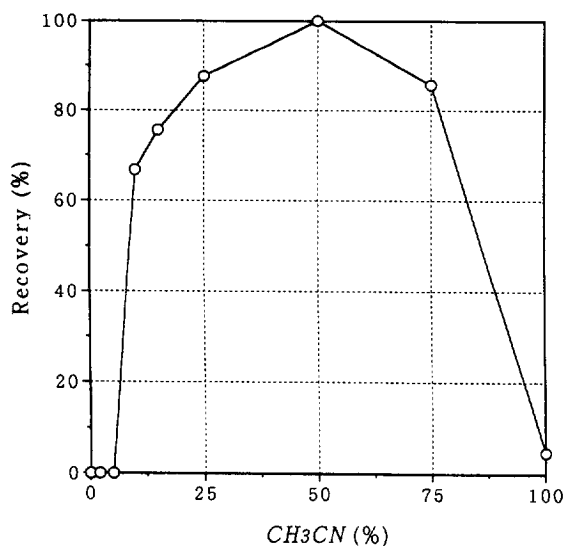


Fig. 1. Effect of acetonitrile concentration on the elution of cyanocobalamin. Cyanocobalamin (5 μ g) samples were injected into the cartridge and were eluted with 10 ml of each of the acetonitrile solutions, separately.

fraction was examined and was found not to contain cyanocobalamin.

Therefore, cyanocobalamin in aqueous solution was completely retained on the cartridge and could be eluted with a 50% acetonitrile solution.

3.5.2. Effect of the volume of 50% aqueous acetonitrile used to elute cyanocobalamin from the cartridge

The content (%) of cyanocobalamin in a fraction (2 ml) was tested to determine if cyanocobalamin was eluted completely from the cartridge with the eluent (Fig. 3). Standard cyanocobalamin (5 μ g) was injected into the cartridge and was eluted with a 50% aqueous acetonitrile solution. The cyanocobalamin present in fractions 1-5 (each 2 ml) was analyzed (Fig. 2).

Recoveries (%) of cyanocobalamin in fractions 1-3 were over 90%. It was found that complete elution of cyanocobalamin from the cartridge was obtained in the fractions 1 to 5 (10 ml of eluent).

3.5.3. Effect of the pH value of the sample solution on the retention of cyanocobalamin

The effect of the pH of the sample solution on the

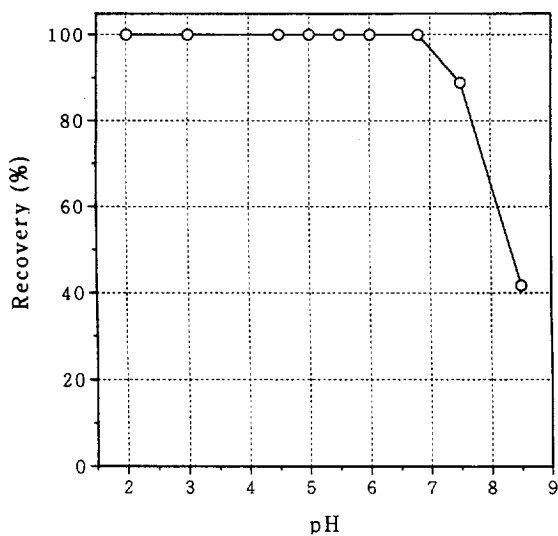


Fig. 2. Effect of the volume of the 50% acetonitrile solution on the elution of cyanocobalamin from the cartridge. Standard cyanocobalamin (5 μ g) was injected into the cartridge and was eluted using a 2-ml volume of 50% aqueous acetonitrile.

retention of cyanocobalamin on a Bond-Elut C₁₈ cartridge was tested (Fig. 3). Standard cyanocobalamin (5 μ g), diluted in 2 ml of 10 mM potassium

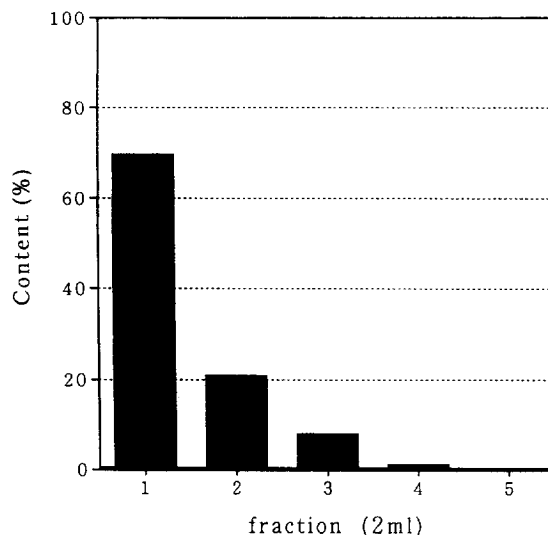


Fig. 3. Effect of the pH of the sample on the retention of cyanocobalamin. Standard cyanocobalamin (5 μ g), diluted in 2 ml of 10 mM potassium phosphate buffer (pH 2 to 8.5), was applied to the cartridge and was eluted with 10 ml of 50% aqueous acetonitrile.

phosphate buffer (pH 2–8.5) was applied to the cartridge and was eluted using 50% aqueous acetonitrile solution.

The cyanocobalamin present in each eluate (10 ml each) was analyzed.

As shown in Fig. 3, the optimal pH for the retention of cyanocobalamin on a Bond-Elut C₁₈ cartridge was in the range of 2 to 7.5.

It can be seen in Figs. 1–3 that the three parameters have an effect on the complete retention of cyanocobalamin on the cartridge and elution of cyanocobalamin from the cartridge. Figs. 1–3 show that the optimum pH for retention of cyanocobalamin was 2–7.5, the optimum eluent was 50% acetonitrile in water, and the optimum elution volume was 10 ml.

The pH of nutrient dissolved in 15% sodium sulfate containing 1 mM Na₂EDTA was ca. 6. Therefore, this sample could be applied to the SPE cartridge without adjusting the pH value.

3.6. Chromatography

Cyanocobalamin has ultraviolet absorbance maxima at 260 and 360 nm and visible absorbance at 550 nm. The absorbance at 550 nm is specific to cyanocobalamin.

The assay of cyanocobalamin in pharmaceutical preparations using an internal standard [Co- α -(5-hydroxybenzimidazolyl) - Co - β - cyanocobamide], which is not commercially available, was reported [10]. If it was commercially available, it would be a better internal standard for the analysis. The trace amounts of cyanocobalamin present in a sample treated with a Sep-Pak C₁₈ cartridge after removing the oil with hexane extraction by monitoring at 550 nm was observed with sufficient intensity without an internal standard [15], despite the large injection volume (100 ml) without an interference by other compounds.

By monitoring the absorbance at 550 nm, we obtained a reproducible peak [R.S.D.=2.7% ($n=5$)] of cyanocobalamin in the sample treated with a Bond-Elut C₁₈ cartridge after removal of the oily components using a membrane filter. This peak had sufficient intensity (Fig. 4) for quantitation purposes and the analysis time was about 12 min. The limit of

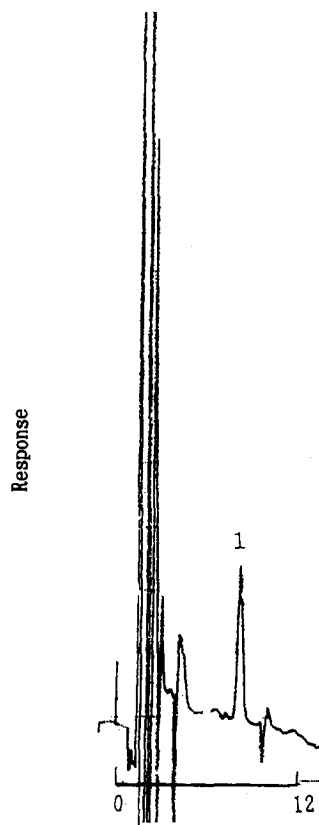


Fig. 4. Chromatograms of cyanocobalamin in nutrient analyzed by HPLC with visible detection at 550 nm after SPE using a membrane filter for the precolumn removal of oily particles. HPLC was carried out on a 15×0.46 cm I.D. column of Inertsil ODS-2 (5 μ m) using 50 mM KH₂PO₄ (pH 2.1, adjusted with phosphoric acid)–acetonitrile (90:10, v/v) as the mobile phase, at a flow-rate of 1 ml/min at 40°C. Peak 1=cyanocobalamin.

detection was ca. 0.1 ng per injection (200 μ l) at a signal-to-noise ratio 3:1 (see Fig. 4).

From the above results, it seems that the trace amounts of cyanocobalamin were completely retained and concentrated on the Bond-Elut C₁₈ cartridge, without interference by other compounds.

3.7. Determination of cyanocobalamin

The calibration graph for cyanocobalamin was constructed by plotting the peak height of cyanocobalamin against the amount of cyanocobalamin. Satisfactory linearity was obtained in the range of 0

Table 5
Recoveries of cyanocobalamin added to nutrient^a

| Added (μg per 100 g) | Found | Recovery (%) |
|-------------------------------------|-------|-----------------|
| 0 | 1.65 | – |
| 0.45 | 2.05 | 88.8 |
| 0.9 | 2.47 | 91.1 |
| 1.8 | 3.31 | 92.2 |

^a R.S.D.: 2.7% ($n=5$) when no cyanocobalamin has been added.

to 30 ng on column ($y=1.5004x+5.5778$, y =peak height, x =amount of cyanocobalamin, in ng).

A known amount of cyanocobalamin was added to nutrient and overall recoveries were estimated by the standard addition method. The results in Table 5 show the analytical data for cyanocobalamin in nutrient. As listed in Table 5, over 90% of the cyanocobalamin was recovered using the standard addition method. The R.S.D. was 2.7% ($n=5$) when no cyanocobalamin was added.

This method was suitable for the determination of trace amounts of cyanocobalamin in nutrient samples with high fat contents, because it was simple, rapid, highly selective, reproducible and recoveries were over 90%.

4. Conclusion

We have demonstrated that it is possible to separate the oily components in foods using membrane filters (HLD-DISK 25, 0.45 μm , polyvinylidene fluoride). The membrane filter's pore size, membrane type, sodium sulfate concentration and sample concentration effect the separation of fatty components. A comparison of T (%) values of filtrates obtained using either membrane filters or hexane-extraction of the aqueous layer showed that the former gave higher T (%) values than the latter.

The effects of elution solvent, pH of the sample solution and eluate volume on the retention and elution of cyanocobalamin on a Bond-Elut C_{18} cartridge were also examined. This method is applicable to the analysis of cyanocobalamin in nutrient samples, because of its simplicity, speed (analysis time of cyanocobalamin: ca. 12 min), reproducibility (R.S.D.=2.7%) and selectivity, and gives recoveries of over 90% by the standard addition method. The use of the proposed method for the determination of cyanocobalamin in other foods and biological fluids is being studied.

References

- [1] The United States Pharmacopeia, 23rd Revision, 1995, p. 363.
- [2] The Pharmacopeia of Japan, 11th ed., 1988, pp. 6–847.
- [3] T. Ujiue, M. Mori, T. Ito, Y. Iida, M. Mabuchi, K. Okamura, *Vitamins* 63 (1989) 87.
- [4] E.P. Frenkel, R.L. Kitchen, R. Prough, *J. Chromatogr.* 174 (1979) 393.
- [5] T. Hattori, N. Asakawa, M. Ueyama, A. Shinoda, Y. Miyake, *Yakugaku Zasshi* 100 (1980) 386.
- [6] D.W. Jacobsen, R. Green, E.V. Quadros, Y.D. Montejano, *Anal. Biochem.* 120 (1982) 394.
- [7] M. Binder, J.F. Kolhouse, K.C. Van Horne, R.H. Allen, *Anal. Biochem.* 125 (1982) 253.
- [8] W.B. Whitman, R.S. Wolfe, *Anal. Biochem.* 137 (1984) 261.
- [9] S.H. Ford, J. Gallery, A. Nichols, M. Shambee, *J. Chromatogr.* 537 (1991) 2356.
- [10] C.C. Jansen, J.P. de Kleijn, *J. Chromatogr. Sci.* 28 (1990) 42.
- [11] R.M. Kothari, M.W. Taylor, *J. Chromatogr.* 247 (1982) 187.
- [12] P. Wimalasiri, R.B.H. Wills, *J. Chromatogr.* 318 (1985) 412.
- [13] M. Yoshida, K. Iriyama, *J. Liq. Chromatogr.* 9 (1985) 177.
- [14] K. Hayakawa, J. Oizumi, *J. Chromatogr.* 413 (1987) 247.
- [15] H. Iwase, *J. Chromatogr.* 590 (1992) 187.
- [16] P.A. Harris, *Sorbent Extraction Technology*, 1985.
- [17] A.P. De Leenheer, W.E. Lambert and H.J. Nelis, *Modern Chromatographic Analysis of Vitamins*, Marcel Dekker, New York, 2nd ed., 1992, p. 469.