J.Sci.Soc. Thailand 12 (1986) 111 - 118

APPLICATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO DETERMINATION OF SEVEN WATER-SOLUBLE VITAMINS IN WHITE SAUCE

PRANEE NANDHASRI AND SOMPONG SUKSANGPLENG

Thailand Institute of Scientific and Technological Research (TISTR), Bangkok 10900, Thailand.

(Received 21 February 1986)

Abstract

High performance liquid chromatography (h.p.l.c) was successfully used to separate seven water-soluble vitamins: ascorbic acid (C), niacin, niacinamide(B_3), pyridoxine(B_6), thiamine(B_1), folic acid and riboflavin(B_2) by using reverse phase MCH-10 C18 column detected at the wavelength of 254 nm and at the specific wavelength of 290 nm for pyridoxine. This technique was applied to determine water-soluble vitamins in white sauce. In 1 g of white sauce, the quantity of vitamin C, niacin, B_6, B_1, and folic acid was found to be 7.96 µg, 0.50 µg, 0.20 µg, 0.17 µg and 4.58 µg respectively, and trace amounts of vitamin B_2 and niacinamide were also detected. The determination time was less than an hour in comparison with the chemical and microbiological methods which usually consume much logner time, usually more than five days. This method may be applied to determine the vitamin content in some sauces e.g. fish sauce, soy sauce and others.

Introduction

The determination of each vitamin is time-consuming and involves many sample preparation steps[1]. The h.p.l.c. method is considered simpler as it is less time-consuming and can determine several vitamins in the same experiment. Most h.p.l.c. techniques reported by various workers were reverse phase methods in which µ-Bondapak C18 was used as a column and a pairing ion reagent was added to the eluent[2-4]. Determination of ascorbic acid, niacin, niacinamide and pyridoxine in food products by using an MCH-10 column with tetramethylammonium chloride (TMA) pentanesulfonic acid (PSA) as the pairing ion reagents has been reported by Nandhasri[3]. But this failed in the separation of ascorbic acid and vitamin B_1 and in the detection of vitamin B_2. Efforts were then made by changing the solvent flow rate and compositions in order to successfully separate seven water soluble vitamins as described here. This method was used to analyse vitamin C, B_1, B_2, B_3, B_6, niacin and folic acid in white sauce within less than an hour.

Materials and Methods

Chemicals

All chemicals were of h.p.l.c. grade. Pentanesulfonic acid (PSA) and tetramethylammonium chloride (TMA) were obtained from Fluka AG and acetonitrile from Carlo Erba. Deionized water was double distilled in the TISTR laboratory.

Solvent A consisted of 1 % acetic acid in double distilled water containing 0.01 M PSA and 0.005 M TMA. Solvent B was a mixture of 0.005 M TMA and acetonitrile at the ratio of 10 : 90.

Standards

Ascorbic acid (C), niacin, niacinamide(B_3), pyridoxine(B_6), thiamine hydrochloride (B_1), folic acid and riboflavine (B_2) were all products of Roche obtained as a gift from Diethelm Pharmachem, Ltd., Bangkok, Thailand. Each vitamin was accurately weighed at 1-5 milligrams and dissolved in 10 ml of the solvent A in a dark brown volumetric flask.

Sample preparation

One millilitre (1.2205 g) of white sauce was diluted to 10 ml with solvent A in a 15 ml centrifuge tube. After mixing well and centrifuging, the supernatent was filtered through the Millipore pre-filters and 0.45 μm filters (13 mm) for aqueous solutions, using kits consisting of a 5 ml glass syringe, a stainless steel swinny adapter filter-holder, with stainless steel support screen and PTFE gasket. The sample solution was then kept in the dark brown bottle.

Instruments

Instruments used comprised a Varian 5500 high performance liquid chromatograph, equipped with Varian VISTA 402 data system and Varian 8055 autosampler. Reverse phase Micro-Pak MCH-10, end capped 4.6 mm i.d., 30-cm length was used as a column. The programmable UV-200 detector was programmed to read at 254 nm and 290 nm.

Methods

H.p.l.c. was performed by using a mobile phase consisting of solvent A and solvent B at a ratio of 90 : 10, at a flow rate of 2 ml/min. Detection was at 254 nm which is sufficiently sensitive to all water-soluble vitamins, except for pyridoxine which was determined at 290 nm. The retention time of each standard vitamin was determined by an automatic injection of 10 μl (0.7-3.0 μg) of standard solution into the h.p.l.c. system, followed by the injection of a mixture of seven standard vitamins as external standards (ES) programmed by entering "ES" in the analysis parameter. The resolution of the seven standard water-soluble vitamins was completed within 12 minutes. The result factors (amount standard, mg/ml, x 10^4/peak area) were computerized and kept in the data system.

The sample solution was injected in the same way as the standard mixture, and also detected at 254 nm and 290 nm. The analysis parameter was programmed for the calculation by entering "A" as the analysis parameter which resulted in the quantitation of vitamins in the sample solution.

Results and Discussion

The individual vitamins were injected into the h.p.l.c. to determine their retention

times as shown in Table 1. Pyridoxine proved to have less sensitivity of detection at the wavelength of 254 nm and had a resolution time which was rather close to that of niacinamide. However, at the wavelength of 290 nm, pyridoxine showed high detection sensitivity with no trace of niacinamide detectable (Figure 1). The mixture of 0.9681 mg/ml of the seven vitamins, as external standards, was injected into the h.p.l.c. and compared at the two wavelengths of 254 nm and 290 nm. The result factors were kept in the data system where the amount of standards were systematically programmed for further determination of amount of vitamins in the sample (Table 1).

TABLE 1. THE RETENTION TIMES, AMOUNTS, AND RESULT FACTORS OF EXTERNAL STANDARD VITAMINS AND THE VITAMIN CONTENT OF WHITE SAUCE

TIME min	NAME	AMOUNT mg/ml	RESULT FACTOR* 254 nm	290 nm	VITAMIN CONTENT IN WHITE SAUCE (μg/g)
1.37	Vitamin C	0.3000	0.003484	0.226432	7.96
1.65	Niacin	0.0789	0.002643	0.104172	0.50
2.03	Pyridoxine	0.0578	—	0.003108	0.20
2.07	Niacinamide	0.1052	0.004199	—	Trace
2.70	Thiamine	0.0842	0.003023	0.037484	0.17
6.70	Folic acid	0.2736	0.097187	0.035559	4.58
11.00	Vitamin B-2	0.0684	0.002113	0.008021	Trace

*Result factor of external standard (ES) $= \frac{\text{Amount (mg/ml)} \times 10^4}{\text{Peak area}}$

The white sauce sample solution at a concentration of 122.68 mg/ml was injected into the h.p.l.c. detected at 254 nm and this was repeated with detection at 290 nm (Figure 2). The vitamins in the white sauce were quantitated according to the amount of sample (mg/ml) with the result that the content of vitamins (μg/g) were 7.96, 0.50, 0.20, 0.17 and 4.58 for vitamin C, niacin, vitamin B_6, Vitamin B_1, and folic acid respectively, while a trace quantities of vitamin B_2 and niacinamide (B_3) were present.

Determination of the stability of the seven standard water-soluble vitamins was made by quantitation of vitamin solutions kept refrigerated in a brown bottle on the following day and on the fourth day. The results showed that the stability of vitamin C decreased at a rate of 5.10 μg/ml per hour while that of the other vitamins remained unchanged (Figure 3). Determination of vitamin C content in white sauce at 290 nm gave the value 7.96 μg/g,

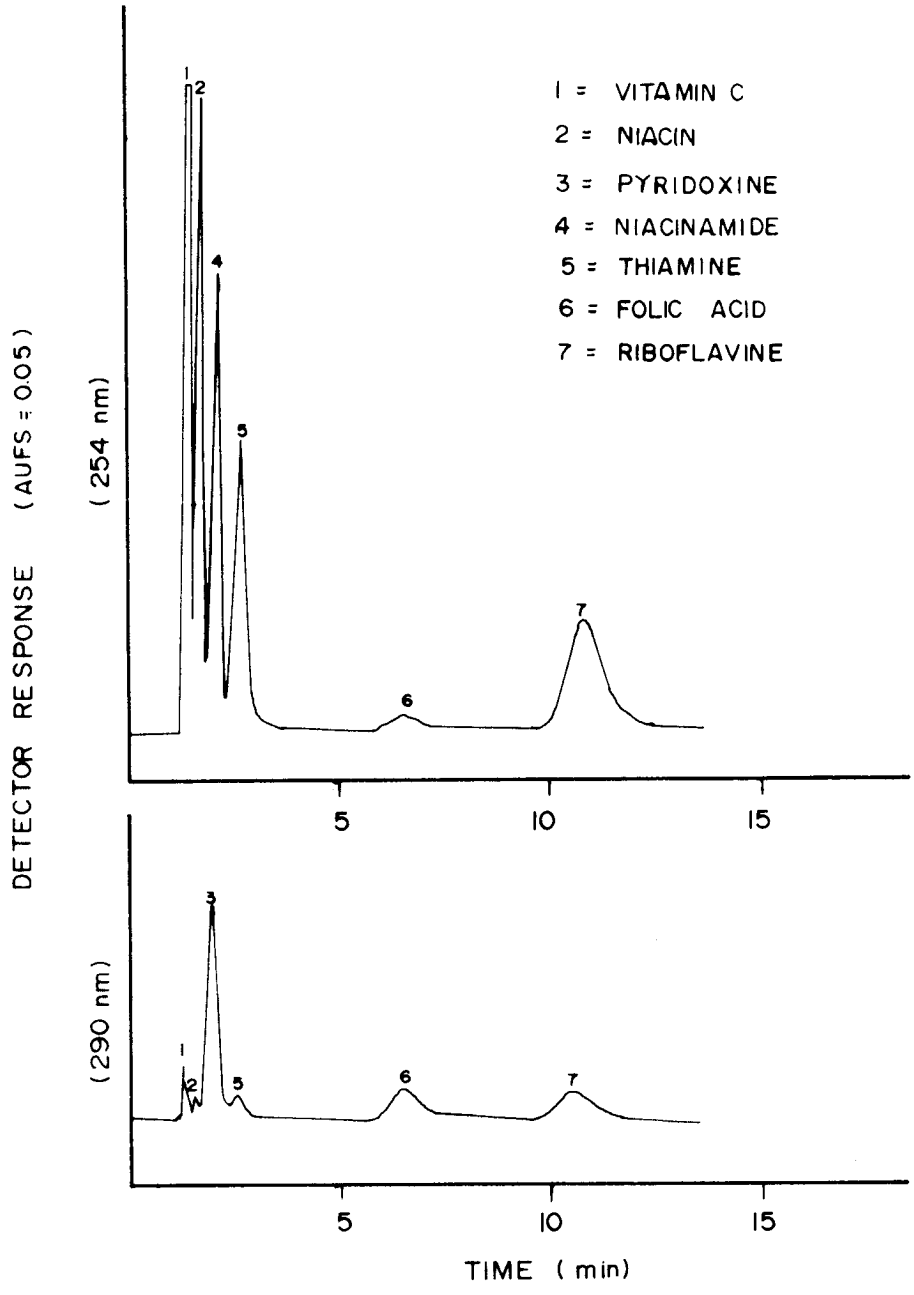

Figure 1. Chromatogram of seven standard water-soluble vitamins separated by h.p.l.c. using solvent A:solvent B in the ratio 90 : 10 and detected at 254 nm (upper) or 290 nm (lower).

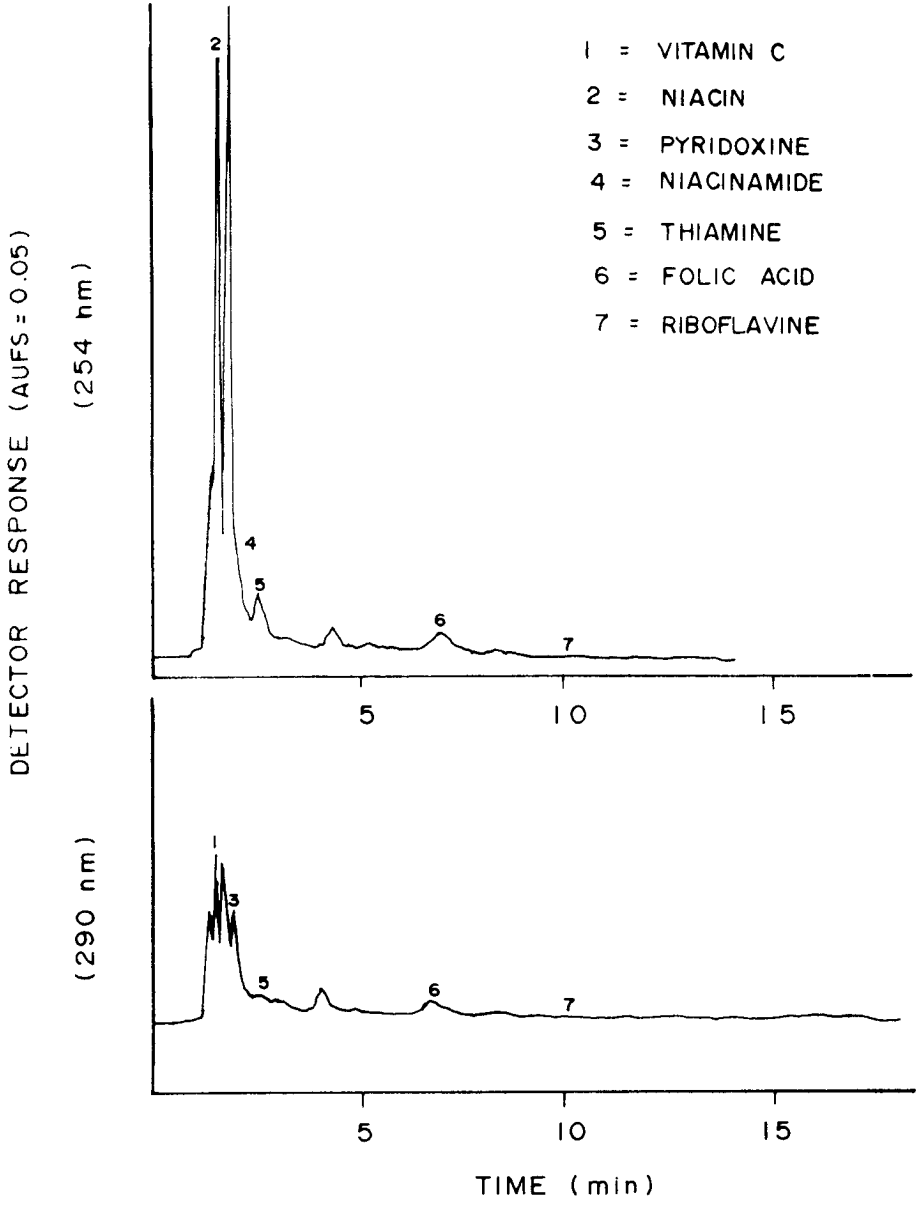

Figure 2 Chromatogram of water-soluble vitamins in white sauce separated by h.l.p.c. using solvent A:solvent B in the ratio 90 : 10 and detected at 254 nm (upper) or 290 nm (lower).

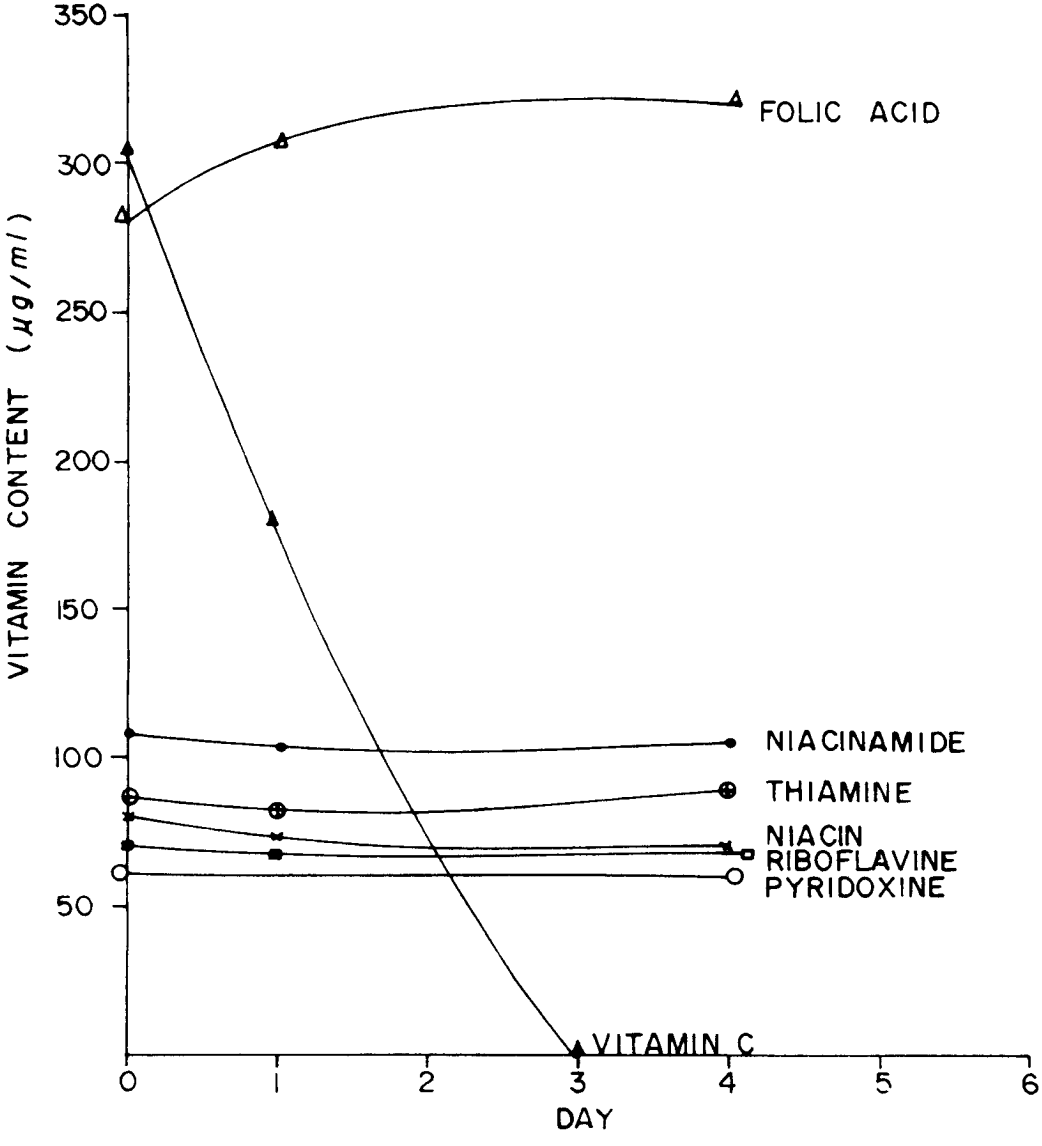

Figure 3 The stability of the seven standard water-soluble vitamins as determined by h.p.l.c using solvent A:solvent B in the ratio 90 : 10.

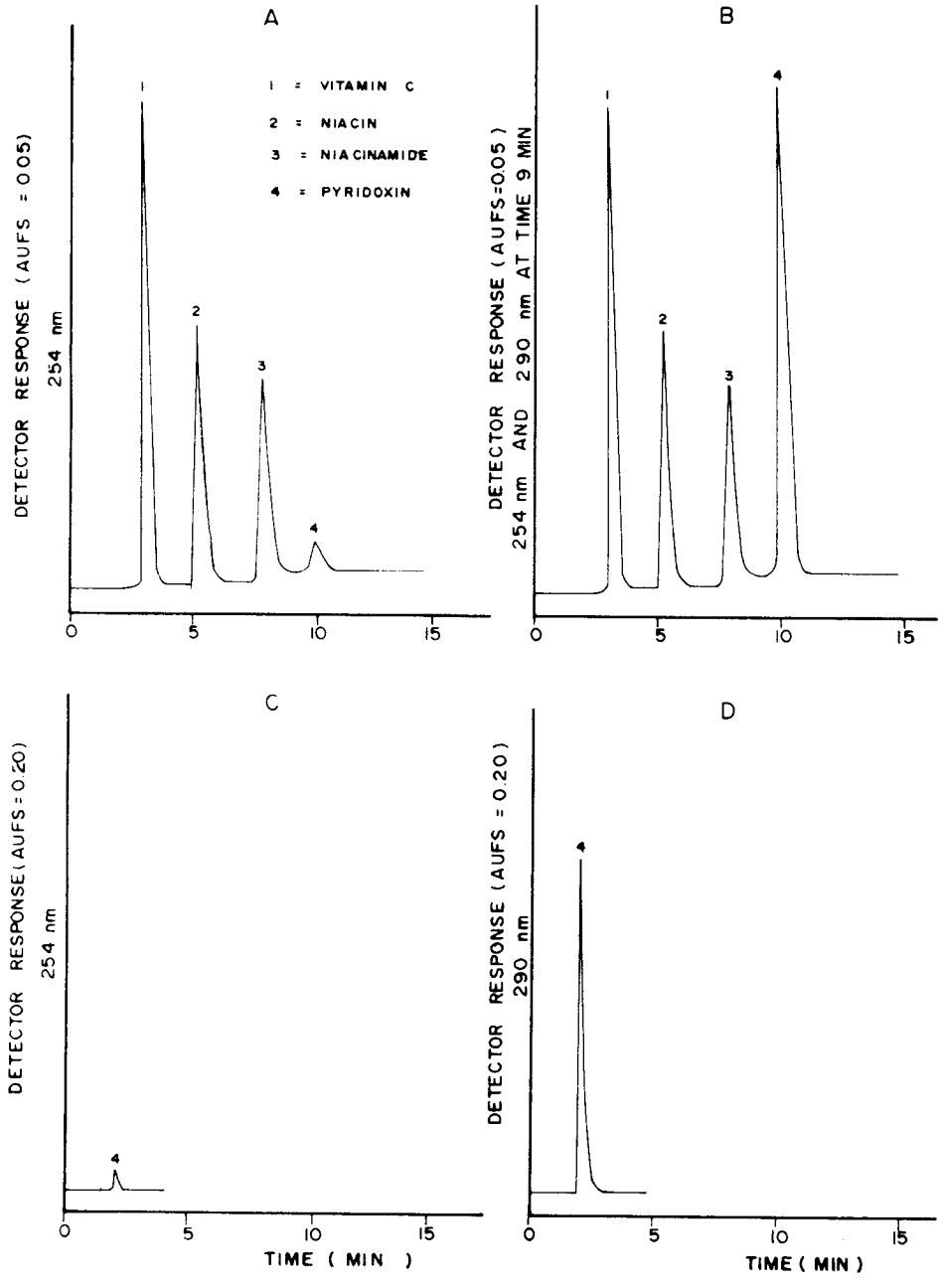

Figure 4 Chromatograms of water-soluble vitamins determined by h.p.l.c. *A* and *B:* vitamin C, niacin, niacinamide and pyridoxine separated using a gradient of 0-15% solvent B in solvent A, flow rate 1 ml/min, detected at 254 nm throughout *(A),* or detected at 254 nm for 0-9 min and at 290 nm from 9 min *(B). C* and *D:* pyridoxine chromatographed in solvent A:solvent B mixture in the ratio 90:10, flow rate 2 ml/min, detected at 254 nm *(C)* or at 290 nm *(D).*

but in the subsequent experiment at 254 nm only 0.21 μg/g of vitamin C remained. This was due to the instability of vitamin C, probably, after deproteinization of white sauce by solvent A during sample preparation. It is, therefore, recommended that EDTA (ethylenediaminetetra-acetic acid) be used as a sequestrant to prevent the oxidation of vitamin C.

The interference between pyridoxine and nicotinamide standards was studied. The results indicate that only traces of pyridoxine can be detected at 254 nm (Fig. 4 A and C) but detection of pyridoxine at 290 nm showed very high sensitivity (Fig 4 B and D). On the other hand, no trace of nicotinamide could be measured at 290 nm (Figs. 1 and 2).

Further studies are needed not only to improve peak shape of folic acid and vitamin B_2 but also to determine the identity of other unknown peaks in the sample.[2]

References

1. *Official Methods of Analysis* (1984), 14th Ed., AOAC, Arlington, VA, Sections 43.024 - 43.082.
2. Callmer, K. and Davies, L. (1974). Separation and determination of vitamins B_1, B_2 B_6, and nicotinamide in commercial vitamin preparation using High Performance Cation-Exchange Chromatography. *Chromatographia* **7**, 644.
3. Nandhasri, P. (1985). Determination of ascorbic acid, niacin, niacinamide and pyridoxine in food products by using High Performance Liquid Chromatography (HPLC). The *ASEAN Workshop on Food Technology Research and Development,* Bangkok, Thailand.
4. Wills, R.B.H., Shaw, C.G. and Day, W.R. (1977), Analysis of water soluble vitamins by high pressure liquid chromatography. *J. Chromatog. Sci.* **15** : 262-266.

บทคัดย่อ

การแยกวิตามิน ซึ่งละลายได้ในน้ำ 7 ชนิด คือ กรดแอสคอร์บิค (วิตามิน ซี), ไนอะซิน, ไนอะซินนาไมด์ (วิตามิน บี-3), ไพริดอกซิน (วิตามิน บี-6), ไทอะมีน (วิตามิน บี-1), กรดโฟลิค และ ไรโบเฟลวิน (วิตามิน บี-2) ด้วยวิธีลิควิดโครมาโตกราฟี (HPLC) โดยใช้รีเวอร์สเฟสคอลัมน์ (Reverse phase MCH-10 C-18 column) และตรวจวัดค่าแอบซอร์แบนซ์ที่ความยาวคลื่น 254 นาโนเมตร และที่ความยาวคลื่นเฉพาะไพริดอกซิน ที่ 290 นาโมเมตร ใช้เทคนิคนี้ตรวจสอบปริมาณวิตามินต่าง ๆ ในซีอิ้วขาวจำนวน 1 กรัม พบว่ามีปริมาณของวิตามิน ซี 7.96 ไมโครกรัม, ไนอะซิน 0.50 ไมโครกรัม, ไพริดอกซิน 0.20 ไมโครกรัม, ไทอะมิน 0.17 ไมโครกรัม, กรดโฟลิค 4.58 ไมโครกรัม และปริมาณเล็กน้อยที่วัดไม่ได้ของ วิตามิน บี-2 และไนอะซินนาไมด์ วิธี HPLC นี้ใช้ตรวจสอบวิตามินต่าง ๆ ในน้ำปลา, ซีอิ้วชนิดต่าง ๆ โดยใช้เวลาตรวจสอบน้อยกว่า 1 ชั่วโมง เปรียบเทียบกับวิธีเคมีและจุลชีวะที่ใช้เวลาในการตรวจสอบวิตามินเหล่านี้นานมากกว่า 5 วัน