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Simultaneous separation and quantitation of thiamine, riboflavin, niacin and pyridoxine by high-pressure liquid chromatography

Tee E Siong and Khor Swan Choo

Division of Human Nutrition, Institute for Medical Research, 50588 Kuala Lumpur

Abstract

The AOAC methods have been widely used for the determination of B-vitamins in foods. However, these methods have been recognised as tedious and time-consuming, and each vitamin must be individually analysed. In recent years, there has been increasing interest in the use of liquid chromatographic methods for the analysis of these vitamins. Various methods are being developed, but few are suitable for the simultaneous determination of several B-vitamins, and for a wide variety of foods. This paper reports efforts to develop a HPLC method for simultaneous analysis of thiamine, riboflavin, niacin and pyridoxine. Mixtures of these water-soluble vitamins were studied systematically to derive at optimal chromatographic and detection procedures. Studies on UV-absorption characteristics of the vitamins showed that the vitamins can be simultaneously detected at 260 and 290 nm in a dual-wavelength detector. Separation was effected on a C18 μBondapak column, and different proportions of methanol and water in the mobile phase were studied. The effect of the addition of varying amounts of glacial acetic acid into the mobile phase was also studied. To further improve the separation of the vitamins, the effect of the addition of different amounts and types of ion-pairing reagents was examined. The various studies carried out have shown that a mobile phase comprising of a mixture of methanol, glacial acetic acid and water in the ratio of 34.5:0.5:65 and containing hexane sulphonic acid at a concentration of 5 mM as an ion-pairing reagent gave satisfactory separation of the four vitamins. Suitability of the method developed for the analysis of food samples will be tested in the next phase of the study.

Introduction

The AOAC methods have been widely used for the analysis of the B-vitamins, thiamine, riboflavin, niacin and pyridoxine. However, these methods, based on fluorescence and colour reactions have been recognised as tedious and time-consuming, and each vitamin must be individually analysed. The need for hazardous chemicals for the analysis of niacin poses additional difficulties.

In recent years, there has been increasing interest in the use of liquid chromatographic methods for the analysis of these vitamins. Various methods are being developed, but few are suitable for the simultaneous determination of

several B-vitamins, and for a wide variety of foods. This paper reports efforts to develop a HPLC method for simultaneous analysis of thiamine, riboflavin, niacin and pyridoxine.

Materials and Method

Apparatus

The liquid chromatography system used consisted of a Gilson 305 piston pump connected to a Gilson 805S manometric module, and a model 7161 Rheodyne injector with a variable 2 ml sample loop. The detector used was a Gilson 116 UV detector set at 0.01 absorbance unit full scale. The pump and detector was controlled by the Gilson 714 system controller software operating in a micro-computer which also permited storage, reviewing and re-integration of all chromatographic data. Chromatograms and results analysed were then recorded on a Panasonic KX-P1081 printer.

A stainless steel, 30 cm by 3.9 mm I.D., 10 µBondapak C18 column was used for the chromatographic separation. This was preceded by a Supelco guard column holder containing a 2 cm disposable guard column insert which was packed with the same material as that in the analytical column.

A Perkin-Elmer model 550S UV/VIS Spectrophotometer with a model 561 chart recorder was used to determine absorption spectra of the vitamins.

Chemicals and Reagents

Methanol used was of HPLC grade obtained from BDH, and analytical grade glacial acetic acid was obtained from Merck. Water for preparation of mobile phase and standards was purified with Elgastat UHQ II. All mobile phase solutions were filtered with a 47 mm 0.45 µm Schleicher & Schuell RC 55 membrane filter paper utilising a millipore filtration set and subsequently degassed for 15 minutes with a Branson 3200 sonic bath. Before use, the HPLC column was washed with at least 100 to 150 ml of mobile phase and then equilibrated at a flow rate of 1 ml per minute for about 30 minutes.

The 4 vitamin B standards studied, i.e. thiamine hydrochloride (B1), riboflavin (B2), nicotinic acid (niacin) and pyridoxine hydrochloride (B6) were obtained from Sigma chemicals. Solutions of 5 mg per ml thiamine, niacin and pyridoxine were prepared in 0.1N hydrochloric (Convol, BDH). Riboflavin standard solution of 0.1 mg per ml was also prepared in 0.1N hydrochloric acid; there was, however, a slight turbidity in the preparation. These standard solutions were kept refrigerated and were stable for several months. Fresh standard solutions of mixtures containing 10 µg/ml of thiamine, 2.5 µg/ml of riboflavin, 5 µg/ml of niacin and 50 µg/ml pyridoxine were prepared in the appropriate mobile phase and filtered with a 13 mm 0.45 µm Schleicher & Schuell RC 55 membrane filter paper using a Water's sample filteration kit. These standard mixtures were kept in 25 ml amber bottles to prevent degradation of riboflavin as the vitamin is sensitive to light.

Ion pairing reagents were obtained from Waters Division of Millipore and they were packed in vials of 25 ml each. They were 1-pentane sulphonic acid (PIC B-5), 1-hexane sulphonic acid (PIC B-6), 1-heptane sulphonic acid (PIC B-

7) and 1-octane sulphonic acid (PIC B-8).

Results and Discussion

Absorption spectra and HPLC chromatograms of the four vitamins

The characteristic absorption spectra of thiamine, riboflavin, niacin and pyridoxine in 0.1N hydrochloric acid are shown in Figures 1a, 1b, 1c and 1d respectively. The main absorption maxima was found to be 246 nm for thiamine, 266 nm for riboflavin, 260 nm for niacin and 290 nm for pyridoxine. The wavelengths 260 nm and 290 nm were therefore selected for detection of the vitamins during chromatography. The detection procedure made use of the capability of the detector to permit simultaneous monitoring at 2 wavelengths, namely 260 nm at channel 1 for thiamine, riboflavin and niacin, and 290 nm at channel 2 for pyridoxine.

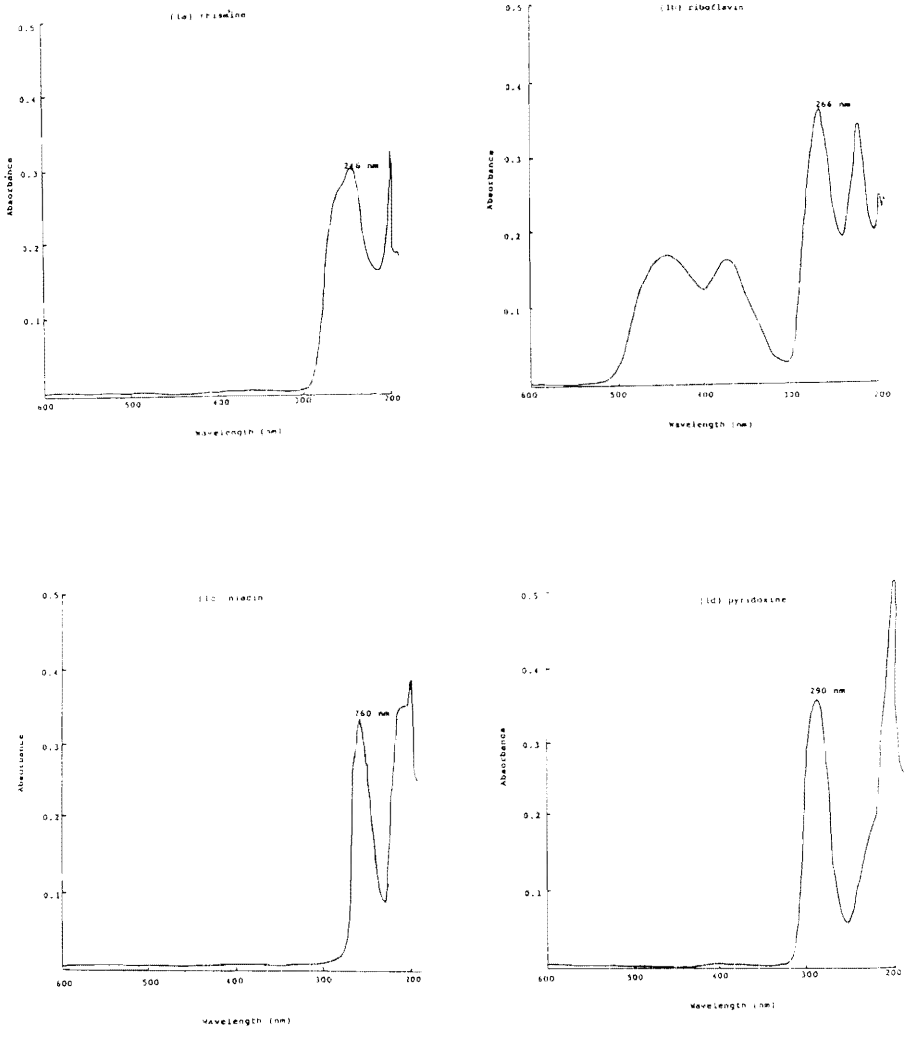

Figure 1, Absorption spectra of the four B-vitamins

(a) thiamine, 10 μg/ml
(b) riboflavin, 5 μg/ml
(c) niacin, 10 μg/ml
(d) pyridoxine, 10 μg/ml

HPLC chromatograms of the four vitamins were obtained using a mobile phase comprising of a mixture of methanol, glacial acetic acid and water (39:1:60, v/v) with 5 mM Water's PIC B-6. All four vitamins gave single peaks at the retention times of 6.56 for thiamine, 5.27 for riboflavin, 3.63 for niacin and 4.60 for pyridoxine.

Evaluation of reported systems

Trials were first carried out on reported chromatrographic systems for the separation of these vitamins. Wimalasiri and Wills (1985) reported the use of a mixture of methanol and water (40:60, v/v) with 5 mM PIC B-6 for the separation of thiamine and riboflavin on a μBondpak C18 (10 μm) Radial-Pak column. However when this mobile phase was used in this study, resolution for riboflavin and pyridoxine was only 0.5 (Figure 2a).

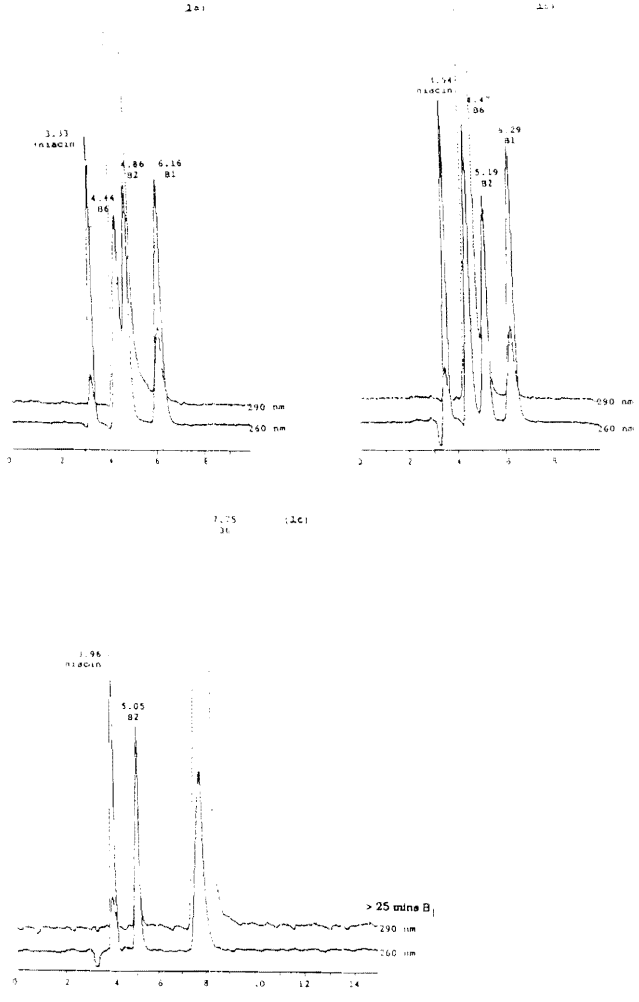

Figure 2. HPLC chromatograms of the four B-vitamins using a mobile phase of methanol-water with and without glacial acetic acid

(a) m.p. = methanol-water, 40:60 (v/v), with 5mM PIC B-6
(b) m.p. = methanol-glacial acetic acid-water, 39: 1:60 (v/v), with 5 mM PIC B-6
(c) m.p. = methanol-glacial acetic acid-water, 39: 1:60 (v/v), with 5 mM PIC B-8

Dong et al. (1988) described the use of glacial acetic acid as an additive to acidify the mobile phase to facilitate the ion-pairing of basic solutes with the alkylsulphonates. They suggested that satisfactory effect is obtained with the addition of 1% acetic acid. The mobile phase was therefore modified to a mixture of methanol, glacial acetic acid and water at a ratio 39:1:60 (v/v) with 5mM PIC B-6. The separation between riboflavin and pyridoxine improved markedly (Figure 2b) and the four vitamins were well separated within 7 minutes. In another trial, the same mobile phase as above was used, but the ion-pairing reagent PIC B-6 was replaced with B-8. There was good separation of riboflavin, niacin and pyridoxine, but thiamine was not eluted even after 25 minutes (Figure 2c).

Toma and Tabekhia (1979) also employed a mobile phase of a mixture of methanol, glacial acetic acid and water (39:1:60, v/v) for the separation of thiamine, riboflavin and niacin on a 30 cm x 4 mm mBondapak C18 column. However, the ion-pairing reagents used was a mixture of 5 mM PIC B-5 and PIC B-7. The procedure was found to give poor separation for riboflavin and pyridoxine (Figure 3a). When the same mobile phase was used with only PIC B-7 as the ion-pairing reagent, there was better separation of these two vitamins (Figure 3b) but the retention time for thiamine was increased significantly. However, when PIC B-5 alone was used, there was poor separation for thiamine, niacin and pyridoxine (Figure 3c) although retention times were reduced.

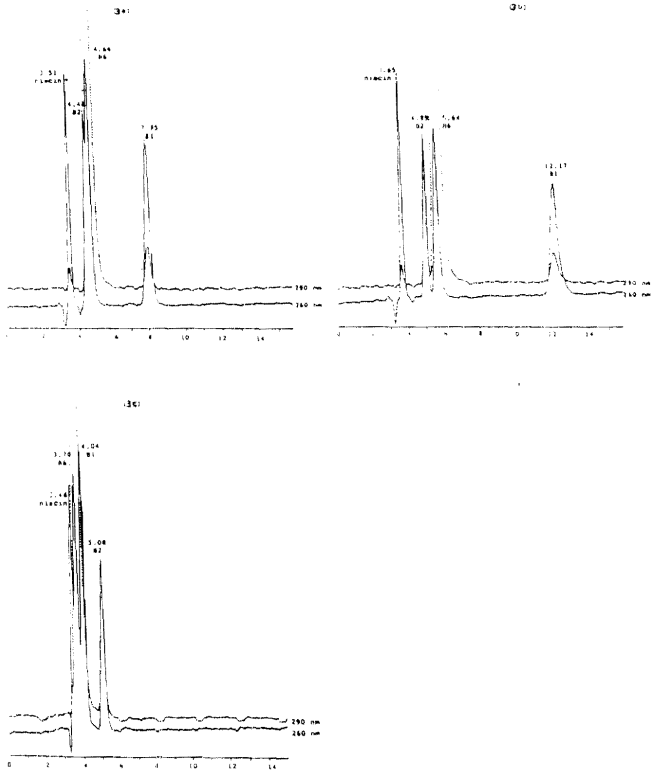

Figure 3. HPLC chromatograms of the four B-vitamins using a mobile phase of methanol-glacial acetic acid-water (39:1:60, v/v) with 5 mM of different ion-pairing reagents
(a) PIC B-5 & PIC B-7
(b) PIC B-7
(c) PIC B-5

Since the two reported methods did not give satisfactory results, several studies were then carried out systematically to determine the most suitable mobile phase and ion-pairing reagents to be used for optimal separation of the vitamins, including type and strength of ion-pairing reagents, different proportions of methanol and glacial acetic acid.

Separation without ion-pairing reagents

Several trials were first carried out using mobile phase of varying proportions of methanol and water with an addition of 1% glacial acetic acid but without the addition of any ion-pairing reagent. The proportion of methanol was gradually increased from 15% to 45%, with a concomitant decrease in the proportion of water (Table 1). Using 15% methanol, there was no separation for niacin and pyridoxine, and riboflavin did not appear even after 50 minutes of chromatography (Figure 4a). Increasing the proportion of methanol improved the resolution between niacin and pyridoxine, and decreased the retention time of riboflavin considerably (Figures 4b, 4c, 4d and 4e). Nonetheless, separation of the four vitamins cannot be said to be satisfactory. It would appear that ion-pairing reagents are necessary for achieving the desired separation.

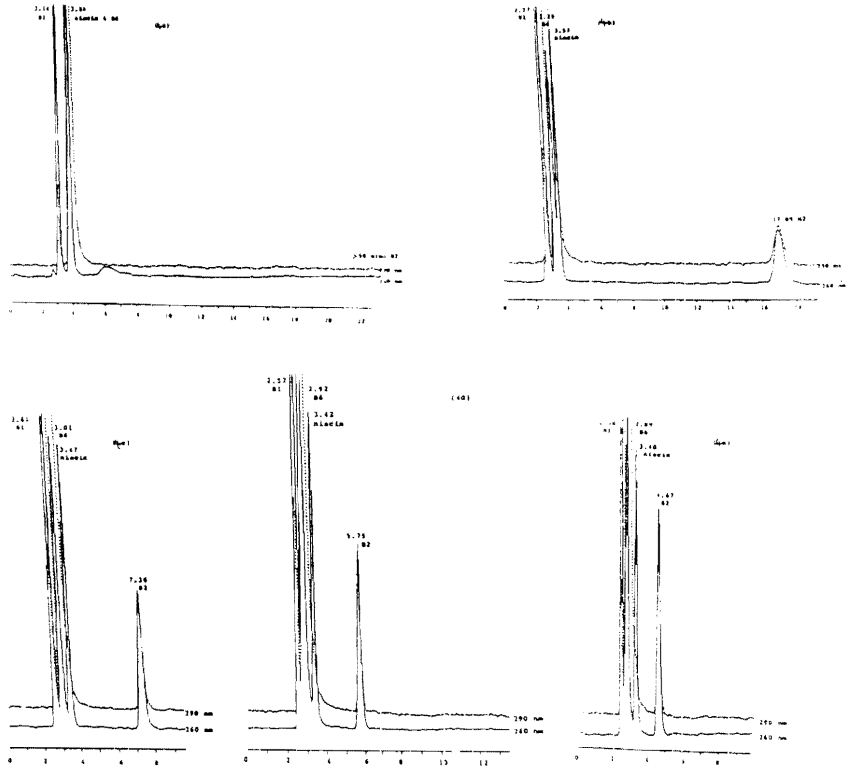

Figure 4. Chromatograms showing the effect of different proportions of methanol on the separation of the four B-vitamins without the addition of any ion-pairing reagent
(a) m.p. = methanol-glacial acetic acid-water, 15:1:84 (v/v)
(b) m.p. = methanol-glacial acetic acid-water, 25:1:74 (v/v)
(c) m.p. = methanol-glacial acetic acid-water, 35:1:64 (v/v)
(d) m.p. = methanol-glacial acetic acid-water, 39:1:60 (v/v)
(e) m.p. = methanol-glacial acetic acid-water, 45:1:54 (v/v)

Table 1: Separation of B-vitamins without ion-pairing reagents

Methanol (%)	Glacial acetic acid (%)	Water (%)	Ion-pairing reagent	Chromatogram
15	1.0	84	nil	Figure 4a
25	1.0	74	nil	Figure 4b
35	1.0	64	nil	Figure 4c
39	1.0	60	nil	Figure 4d
45	1.0	54	nil	Figure 4e

Addition of hexane sulphonic acid

In order to determine if the separation of the four vitamins with 5 mM of PIC B-6 in a mobile phase of methanol, glacial acetic acid and water (39:1:60, v/v) (Figure 2b) can be further improved, various concentrations of the reagent were studied (Table 2A). Figure 5 shows the changes in the separation of the four vitamins using 1 mM, 3 mM, 5 mM and 7 mM of PIC B-6. The best separation was achieved using 5 mM of the ion-pairing reagent.

Table 2: Effect of different concentrations of hexane sulphonic acid and varying proportions of methanol and glacial acetic acid

Methanol (%)	Glacial acetic acid (%)	Water (%)	Ion-pairing reagent	Chromatogram
A. Varying concentrations of PIC B-6				
39	1.0	60	*1 mM PIC B-6*	Figure 5
39	1.0	60	*3 mM PIC B-6*	Figure 5
39	1.0	60	*5 mM PIC B-6*	Figure 5
39	1.0	60	*7 mM PIC B-6*	Figure 5
B. Varying proportions of methanol				
19	1.0	80	5 mM PIC B-6	Figure 6
24	1.0	75	5 mM PIC B-6	Figure 6
27	1.0	72	5 mM PIC B-6	Figure 6
29	1.0	70	5 mM PIC B-6	Figure 6
34	1.0	65	5 mM PIC B-6	Figure 6
39	1.0	60	5 mM PIC B-6	Figure 6
C. Varying proportions of glacial acetic acid				
35.0	0	65	5 mM PIC B-6	Figure 7
34.5	0.5	65	5 mM PIC B-6	Figure 7
34.0	1.0	65	5 mM PIC B-6	Figure 7
33.5	1.5	65	5 mM PIC B-6	Figure 7
33.0	2.0	65	5 mM PIC B-6	Figure 7

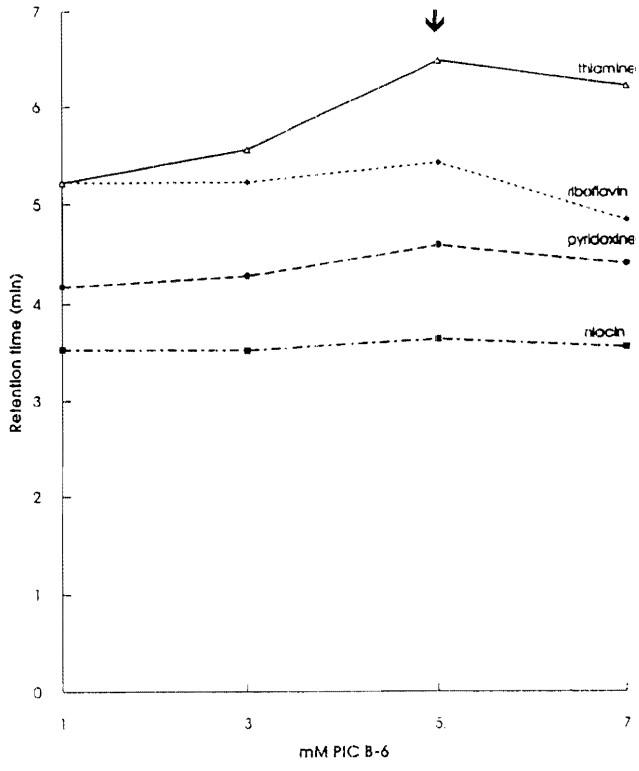

Figure 5. Effect of different concentrations of PIC B-6 on the separation of B-vitamins in a mobile phase of methanol-glacial acetic acid-water, 39:1:60 (v/v)

To determine the optimal proportion of methanol in the mobile phase, the percentage of methanol was changed gradually from 19% to 39% with glacial acetic acid fixed at 1 % and the addition of 5 mM PIC B-6 (Table 2B). Optimum separation at resonable retention times was obtained using 34% methanol in the mobile phase (Figure 6).

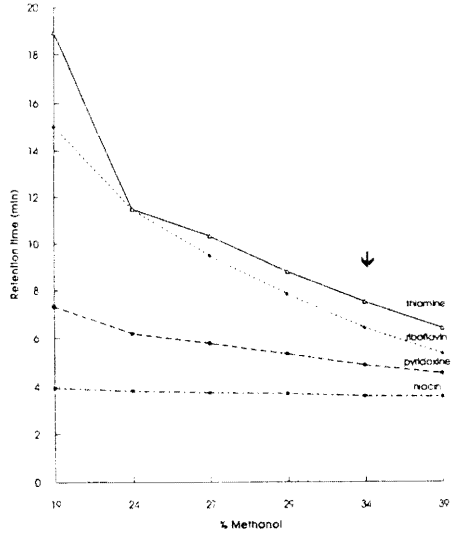

Figure 6. Effect of different proportions of methanol on the separation of B-vitamins in a mobile phase of methanol - 1% glacial acetic acid-water with 5 mM PIC B-6.

The effect of changing the proportion of glacial acetic acid on the separation of the vitamins was next studied (Table 2C). It was found that decreasing the proportion of glacial acetic acid from 2.0-0% increased the resolution between the vitamins (Figure 7). However, when glacial acetic acid was omitted totally, there was tailing of pyridoxine peak. Therefore 0.5 % glacial acetic acid is thought to be the most appropriate.

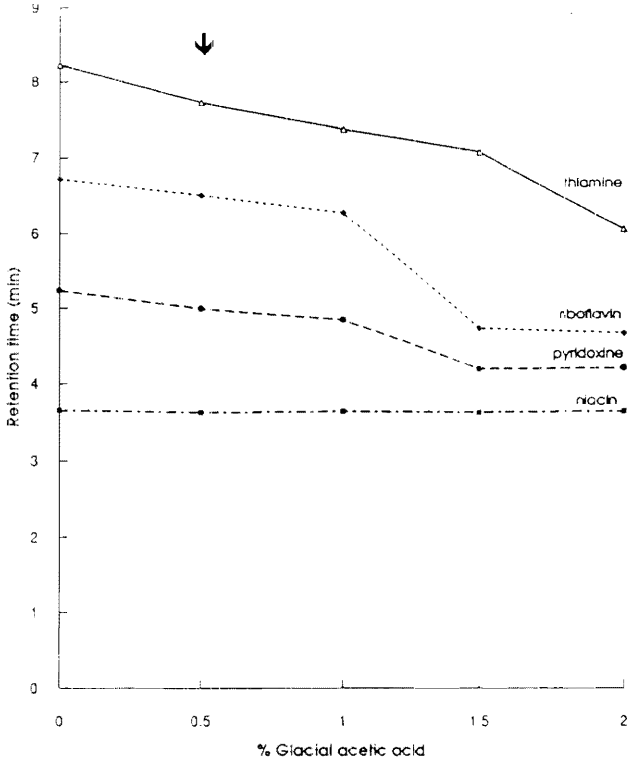

Figure 7. Effect of different proportions of glacial acetic acid on the separation of B-vitamins in a mobile phase of methanol-glacial acetic acid-65% water with 5 mM PIC B-6

Addition of other ion-pairing reagents

A series of studies were carried out to determine the effect of the addition of other ion-pairing reagents, namely PIC B-5, PIC B-7, PIC B5 plus PIC B7 and PIC B-8, to the mobile phase.

With the concentration of ion-pairing reagent fixed at 5 mM and glacial acetic acid at 0.5%, the proportion of methanol in the mobile phase was gradually increased from 19.5 to 49.5%. The studies were repeated for each of the reagent or combination of reagents mentioned above (Table 3) (Figures 8a, 8b, 8c, and 8d). Decreasing the proportion of methanol increased the resolution between niacin and pyridoxine. There was, however, a concomitant increase in the retention time of thiamine, the increase being greater with increase in alkyl chain length of the ion-pairing reagent. The retention time of niacin remained unchanged with all types of ion-pairing reagents. For the different ion-pairing

reagent, the optimum proportion of methanol varied. The best separation appeared to be achieved with 24.5% methanol for PIC B-5, 39.5% for PIC B-7, 24.5% for the combination of PIC B-5 and B7 and 44.5% for PIC B-8.

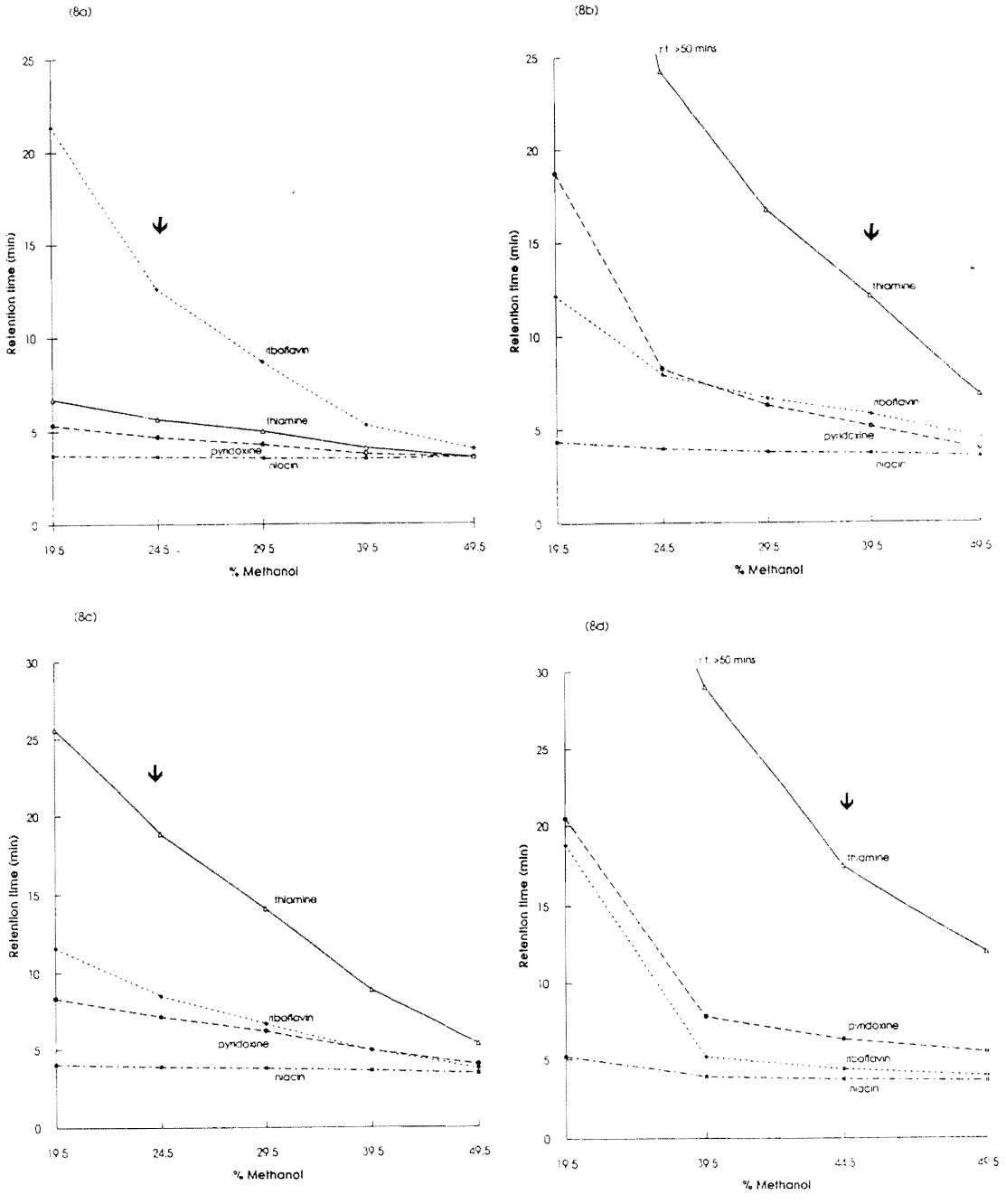

Figure 8. Effect of different proportions of methanol on the separation of the four B-vitamins in a mobile phase of methanol-0.5% glacial acetic acid-water with 5 mM of different ion-pairing reagent
(a) PIC B-5
(b) PIC B-7
(c) PIC B-5 & B-7
(d) PIC B-8

Table 3: Effect of varying proportions of methanol with several ion-pairing reagents

Methanol (%)	Glacial acetic acid (%)	Water	Ion-pairing reagent (%)	Graphs
19.5	0.5	80	5 mM PIC B-5/B-7/B-5 + B-7/B-8	Fig. 8a-8d
24.5	0.5	75	5 mM PIC B-5/B-7/B-5 + B-7/B-8	Fig. 8a-8d
29.5	0.5	70	5 mM PIC B-5/B-7/B-5 + B-7/B-8	Fig. 8a-8d
39.5	0.5	60	5 mM PIC B-5/B-7/B-5 + B-7/B-8	Fig. 8a-8d
49.5	0.5	50	5 mM PIC B-5/B-7/B-5 + B-7/B-8	Fig. 8a-8d

The effect of different concentrations of each of the above ion-pairing reagents was studied, with glacial acetic acid fixed at 0.5% and the most appropriate proportion of methanol for each PIC as stated above (Table 4). Figure 9a shows that with PIC B-5, concentrations of 5 mM to 9 mM could be used for the separation of the four vitamins. With PIC B-7, 7 mM gave the best separation (Figure 9b). As for the combination of PIC B-5 and PIC B-7, 3 mM of the reagent gave the best separation (Figure 9c), but the chromatography time took more than 20 minutes. Increasing the concentration of PIC B-8 showed a significant increase in retention time of thiamine with not much difference in the separation of the three other vitamins. Figure 9d shows that the best separation in using PIC B-8 was a concentration of 2 mM.

Table 4: Effect of different concentrations of several ion-pairing reagents

Methanol (%)	Glacial acetic acid (%)	Water (%)	Ion-pairing reagent	Chromatogram
24.5	0.5	75	*1 mM PIC B-5*	Fig. 9a
24.5	0.5	75	*3 mM PIC B-5*	Fig. 9a
24.5	0.5	75	*5 mM PIC B-5*	Fig. 9a
24.5	0.5	75	*6 mM PIC B-5*	Fig. 9a
24.5	0.5	75	*7 mM PIC B-5*	Fig. 9a
24.5	0.5	75	*9 mM PIC B-5*	Fig. 9a
39.5	0.5	60	*1 mM PIC B-7*	Fig. 9b
39.5	0.5	60	*2 mM PIC B-7*	Fig. 9b
39.5	0.5	60	*3 mM PIC B-7*	Fig. 9b
39.5	0.5	60	*4 mM PIC B-7*	Fig. 9b
39.5	0.5	60	*5 mM PIC B-7*	Fig. 9b
39.5	0.5	60	*6 mM PIC B-7*	Fig. 9b
39.5	0.5	60	*7 mM PIC B-7*	Fig. 9b
24.5	0.5	75	*1 mM PIC B-5 + B-7*	Fig. 9c
24.5	0.5	75	*3 mM PIC B-5 + B-7*	Fig. 9c
24.5	0.5	75	*4 mM PIC B-5 + B-7*	Fig. 9c
24.5	0.5	75	*5 mM PIC B-5 + B-7*	Fig. 9c
44.5	0.5	55	*1 mM PIC B-8*	Fig. 9d
44.5	0.5	55	*2 mM PIC B-8*	Fig. 9d
44.5	0.5	55	*3 mM PIC B-8*	Fig. 9d
44.5	0.5	55	*4 mM PIC B-8*	Fig. 9d
44.5	0.5	55	*5 mM PIC B-8*	Fig. 9d

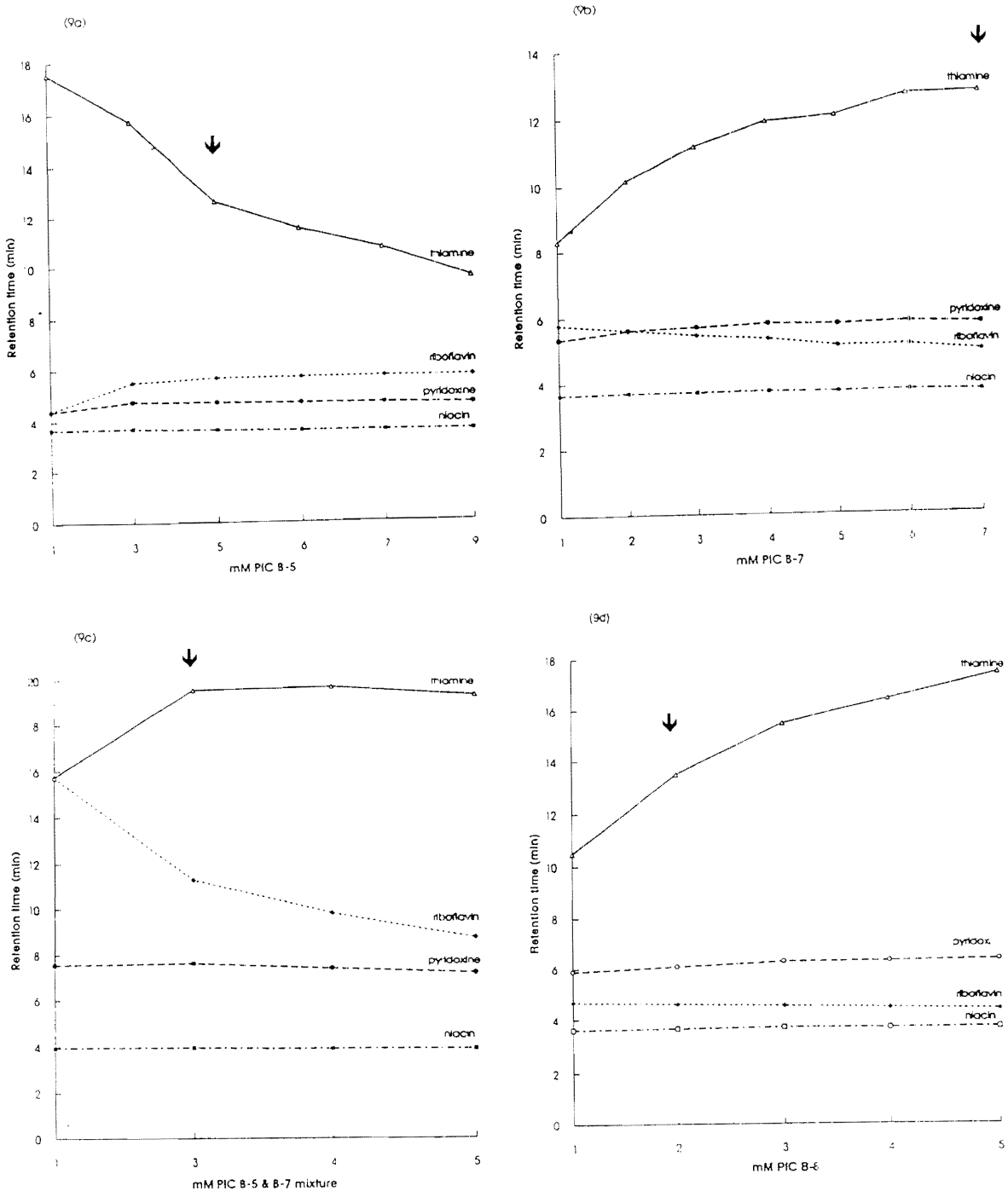

Figure 9. Effect of different concentrations of several ion-pairing reagents in the following mobile phases

(a) m.p. = methanol-glacial acetic acid-water, 24.5:0.5:75 (v/v)
(b) m.p. = methanol-glacial acetic acid-water, 39.5:0.5:60 (v/v)
(c) m.p. = methanol-glacial acetic acid-water, 24.5:0.5:75 (v/v)
(d) m.p. = methanol-glacial acetic acid-water, 44.5:0.5:55 (v/v)

To determine if changing the proportions of glacial acetic acid could further improve the separation of the four vitamins with different ion-pairing reagent, the proportion of the acid was gradually increased from 0.5% to 2%. The proportion of water and ion-pairing reagent were fixed as follows: 75% water and 5 mM PIC B-5; 60% water and 7 mM PIC B-7; 75% water and 3 mM PIC B-5 plus PIC B-7; and 55% water and 2 mM PIC B-8 (Table 5). As can be seen in Figures 10a, 10b, 10c and 10d, all four combinations showed only minor differences in the retention times of the four vitamins with different amounts of glacial acetic acid. However the absence of glacial acetic acid appeared to lead to peak tailing of pyridoxine. Therefore in most cases, 0.5% appeared to be the best percentage to use except for the combination of PIC B-5 plus B-7 where 2% acid was even better since it shortened the chromatography time to less than 18 minutes.

Table 5: Effect of varying proportions of glacial acetic acid with several ion-pairing reagents

Methanol (%)	Glacial acetic acid (%)	Water (%)	Ion-pairing reagent	Chromatogram
25.0	*0*	75	5 mM PIC B-5	Fig. 10a
24.5	*0.5*	75	5 mM PIC B-5	Fig. 10a
24.0	*1.0*	75	5 mM PIC B-5	Fig. 10a
23.0	*2.0*	75	5 mM PIC B-5	Fig. 10a
40.0	*0*	60	7 mM PIC B-7	Fig. 10b
39.5	*0.5*	60	7 mM PIC B-7	Fig. 10b
39.0	*1.0*	60	7 mM PIC B-7	Fig. 10b
38.0	*2.0*	60	7 mM PIC B-7	Fig. 10b
25.0	*0*	75	3 mM PIC B-5 + B-7	Fig. 10c
24.5	*0.5*	75	3 mM PIC B-5 + B-7	Fig. 10c
23.0	*1.0*	75	3 mM PIC B-5 + B-7	Fig. 10c
22.0	*2.0*	75	3 mM PIC B-5 + B-7	Fig. 10c
45.0	*0*	55	2 mM PIC B-8	Fig. 10d
44.5	*0.5*	55	2 mM PIC B-8	Fig. 10d
44.0	*1.0*	55	2 mM PIC B-8	Fig. 10d
43.0	*2.0*	55	2 mM PIC B-8	Fig. 10d

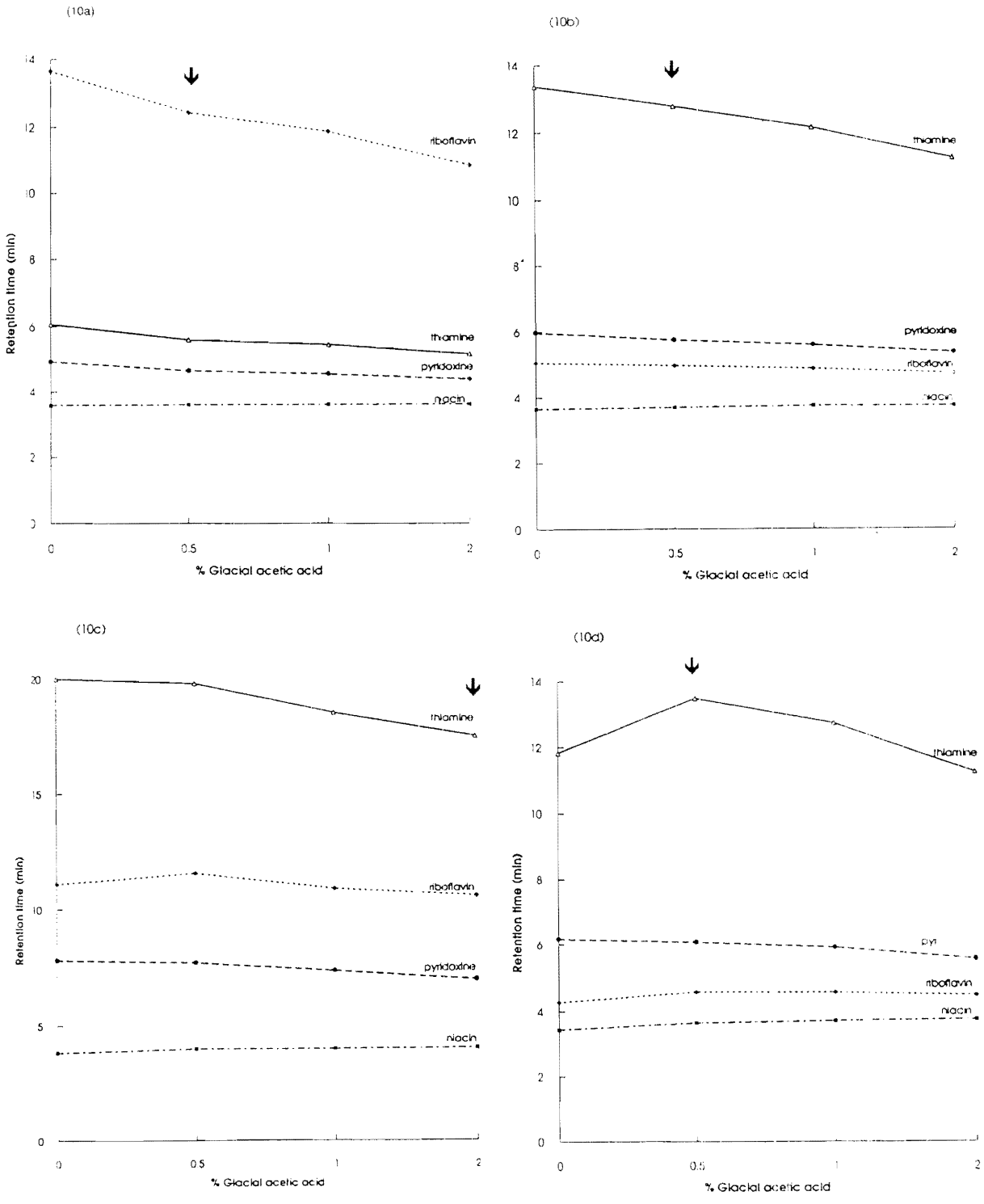

Figure 10. Effect of different proportions of glacial acetic acid on the separation of the four B-vitamins in the following mobile phases

(a) m.p. = methanol-glacial acetic acid-75% water with 5 mM PIC B-5
(b) m.p. = methanol-glacial acetic acid-60% water with 7 mM PIC B-7
(c) m.p. = methanol-glacial acetic acid-75% water with 3 mM PIC B-5 & B-7
(d) m.p. = methanol-glacial acetic acid-55% water with 2 mM PIC B-8

Summary of mobile phase systems

The series of experiments carried out have shown that several combinations of methanol, glacial acetic acid and water may be used for satisfactory separation of thiamine, riboflavin, niacin and pyridoxine. Each of these combination of solvents, however, has to be used in conjunction with the appropriate type and concentration of ion-pairing reagent. Based on the findings from this study, the best chromatography systems are shown in Table 6 and Figure 11.

Table 6: Mobile phase systems suitable for separation of thiamine, riboflavin, niacin and pyridoxine

Methanol (%)	Glacial acetic acid (%)	Water (%)	Ion-pairing reagent	Chromatogram
24.5	0.5	75	5 mM PIC B-5	Figure 11a
34.5	0.5	65	5 mM PIC B-6	Figure 11b
39.5	0.5	60	7 mM PIC B-7	Figure 11c
22.0	2.0	76	3 mM PIC B-5 + B-7	Figure 11d
44.5	0.5	55	2 mM PIC B-8	Figure 11e

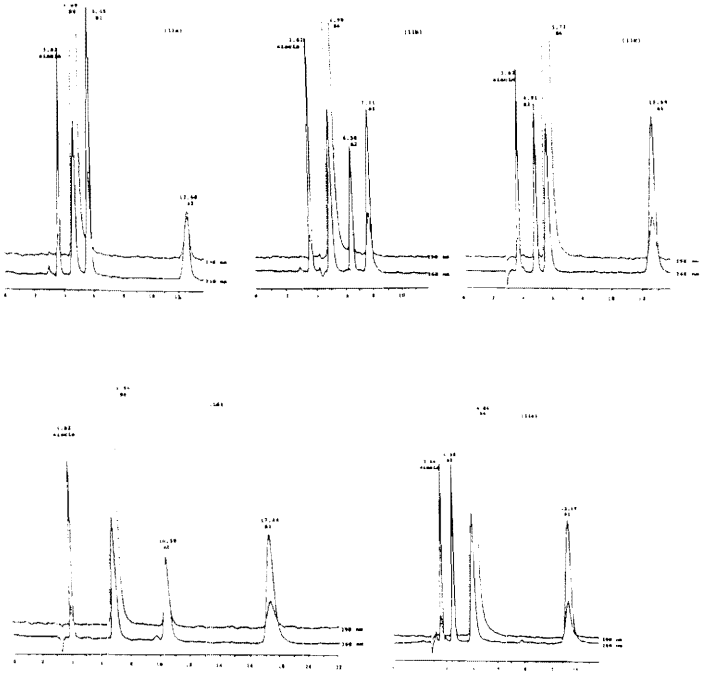

Figure 11. Chromatographs showing suitable mobile phase systems for the separation of the four B-vitamins.
(a) methanol-glacial acetic acid-water, 24.5:0.5:75 (v/v), with 5 mM PIC B-5
(b) methanol-glacial acetic acid water, 34.5:0.5:65 (v/v), with 5 mM PIC B-6
(c) methanol-glacial acetic acid water, 39.5:0.5:60 (v/v), with 7 mM PIC B-7
(d) methanol-glacial acetic acid-water, 24.5:0.6:75 (v/v), with 3 mM PIC B-5 & PIC B-7
(e) methanol-glacial acetic acid-water, 44.5:0.5:55 (v/v), with 2 mM PIC B-8

The best separation appeared to be the mobile phase with 34.5% methanol, 0.5% glacial acetic acid and 65% water with 5 mM of PIC B-6, and the chromatography time was less than 8 minutes (Figure 11b).

Conclusions

Simultaneous separation of thiamine, riboflavin, niacin and pyridoxine can be effected satisfactorily on a 30 cm by 3.9 mm I.D. 10 µm µBondapak C18 column using a mobile phase comprising of a mixture of 34.5% methanol, 0.5% glacial acetic acid and 65% water with 5 mM of PIC B-6. Quantitation of the vitamins can be carried out using an integrator, or a system controller computer programme that enable quantitation and storage of chromatography data. The developed method will next be used for the analysis of these B-vitamins in various pharmaceutical preparations and food samples. In the analysis of these samples, particularly for the latter, considerable amount of sample cleaning procedures have to be carried out prior to separation by HPLC. A series of studies will be carried out to determine appropriate procedures for these analyses.

References

1. Dong MW, Lepore J, Tarumoto T. Factors affecting the ion-pairing chromatography of water-soluble vitamins. J Chromatogr 1988; 442: 81-95.
2. Toma RB, Tabekhia MM. High performance liquid chromatographic analysis of B-vitamins in rice and rice products. J Food Sci 1979; 44(1): 263-265.
3. Wimalasiri P, Wills RBH. Simultaneous analysis of thiamin and riboflavin in foods by high-performance liquid chromatography. J Chromatogr 1985; 318:412-416.

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EDITORS

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