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Use of capillary electrophoresis for the determination of vitamins of the B group in pharmaceutical preparations

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Abstract

The separation of four water-soluble vitamins, *i.e.*, thiamine, riboflavine, pyridoxine and nicotinamide, was investigated by capillary zone electrophoresis and micellar electrokinetic chromatography. The usefulness of the internal standard technique in order to improve the precision of peak area when either the migration time or the injection volume varied was demonstrated. Quantitative analyses of different pharmaceutical formulations were compared with the LC method of the US Pharmacopeia. A good correlation was obtained.

1. Introduction

Vitamins are structurally heterogenous substances which need elaborate chromatographic techniques for their separation and determination. Amin and Reusch [1] published a liquid chromatographic (LC) method for the simultaneous determination of vitamins B_1, B_2, B_6 and B_{12} in pharmaceutical preparations. Recently, the USP XXII [2] introduced a monograph for the LC determination of thiamine (B_1), riboflavine (B_2), pyridoxine (B_6) and nicotinamide (PP) in tablets and capsules. As these so-called water-soluble vitamin compounds are readily ionizable, except PP, capillary electrophoresis (CE) has been shown a valuable alternative technique for their separation. Micellar electrokinetic chromatography (MEKC) was proposed by Fujiwara *et al.* [3] and Nishi *et al.* [4] for the separation of water-soluble vitamins and Ong *et al.* [5] proposed the separation of water- and oil-soluble vitamins. However, only limited attention was paid to pharmaceutical analysis. In this work, a capillary zone electrophoresis (CZE) method and an MEKC method were developed for the determination of B_1, B_2, B_6 and PP in some commercial preparations. The results were compared with those obtained by the LC method given in the USP.

2. Experimental

2.1. Equipment

Electrophoresis was carried out on a Beckman (Palo Alto, USA) P/ACE 2100 system fitted with a UV detector. Separations were performed in a 570 mm × 0.075 mm I.D. fused-silica capillary tube (Beckman). Integration of the electropherograms was achieved by System Gold V.711 software (Beckman).

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The LC system consisted of an L-6000 LC pump, an L-400 variable-wavelength UV detector and a D-2000 integrator, all from Merck–Hitachi. A 250 mm × 4 mm I.D. LiChroCART column containing LiChrospher 100 RP-18 (5 μm) packing from Merck was used. Injections were made with a 20-μl loop valve.

2.2. Chemicals and reagents

Thiamine hydrochloride (B_1) and pyridoxine hydrochloride (B_6) were obtained from Merck (Darmstadt, Germany) and riboflavine (B_2) and nicotinamide (PP) from Bios (Belgium). All the reagents were of analytical-reagent grade. The solvent used in all CE experiments for dissolving and diluting the vitamin solutions consisted of 0.01 *M* hydrochloric acid containing 20% (v/v) acetonitrile. The addition of acetonitrile was necessary to ensure complete dissolution of B_2. Throughout all experiments paracetamol was used as an internal standard. The separation buffer of pH 9 was 0.02 *M* sodium tetraborate solution and the separation buffer of pH 7 was prepared by mixing an appropriate volume of 0.02 *M* borate solution with 0.02 *M* sodium dihydrogenphosphate solution. All solutions were prepared with water obtained from a Seralpur Pro $_{90}$CN purification system (Seral, Germany). For MEKC, sodium dodecyl sulphate (SDS) was added to the buffer solutions at a concentration of 0.1 *M*. All buffer solutions were filtered through a 0.2-μm membrane filter before use.

For the LC analyses according to the USP XXII [2], the solvent was dilute acetic acid containing 5% (v/v) acetonitrile; the vitamin solution was kept on a water-bath at 65–70°C for 10 min and cooled before injection.

2.3. Procedures

For CZE and MEKC, injections were made hydrodynamically by pressure for 3 s. The applied voltage was 20 kV, which provided a current of *ca.* 50 μA in CZE and 100 μA in MEKC. The temperature was kept constant at 25°C. Detection was performed at 214 nm. Before each injection, separating buffer was passed through the capillary for 4 min.

According to the LC method of the USP [2], separations were performed in the reversed-phase mode on a 5-μm C_{18} column. The mobile phase was water–methanol–glacial acetic acid (73:26:1) to which sodium hexanesulphonate was added. Detection was performed at 280 nm.

2.4. Sample preparation

For tablets, ten tablets were ground to a homogeneous powder and an aliquot was suspended in 50.0 ml of solvent, sonicated and centrifuged. Appropriate volumes were taken, internal standard solution was added and the volumes were adjusted with the same solvent so as to obtain concentrations of each vitamin up to 500 μg/ml.

For soft gelatin capsules, ten capsules were immediately dissolved in 500.0 ml of solvent, sonicated and further treated as for tablets.

For syrups, an aliquot volume was weighed, the internal standard solution was added and the volume was adjusted with the solvent.

3. Results and discussion

Under the conditions used in the CZE mode, a mixture of the four vitamins was completely resolved within 6 min. The migration sequence of the vitamins observed in borate buffer (pH 9) was the same as that found by Nishi *et al.* [4], *i.e.*, B_1, PP, B_2 and B_6, and paracetamol eluted between PP and B_2. Thiamine elutes first owing to its positive charge, followed by PP, which is neutral. Paracetamol, B_2 and B_6 are negatively charged at pH 9 and their migration velocity depends largely upon their degree of ionization and molecular size. The fact that B_2 elutes after paracetamol is the result of the complexation of the ribose moiety of riboflavine with borate ions. By this reaction B_2 becomes more negatively charged than expected from its pK_a value of 10.2, corresponding to only 6% ionization, in contrast with paracetamol ($pK_a = 9.5$), which provides about 25% ionization.

In MEKC, a concentration of 100 m*M* SDS in the borate buffer (pH 9) was found to provide the separation of PP from acetonitrile, both neutral compounds eluting together with the

electroosmotic flow (EOF) in the CZE mode. The addition of acetonitrile to this buffer improved the peak efficiency, especially for PP. On the other hand, it can be seen from Fig. 1A that, as a function of the acetonitrile concentration of the buffer, the migration times are prolonged except for B_1, resulting from the decrease in the EOF as indicated by the EOF marker formamide.

In contrast to CZE, in MEKC B_1 elutes with the slowest velocity owing to the electrostatic interaction of the positively charged compound with the negatively charged micelles. However, when the separating buffer contained larger amounts of acetonitrile, a second peak of B_1 appeared between the peaks of B_2 and B_6. This peak was found to originate from the degradation of B_1 in alkaline medium, which might be enhanced by the addition of acetonitrile. This degradation was not observed in the aqueous buffer of pH 9 in CZE.

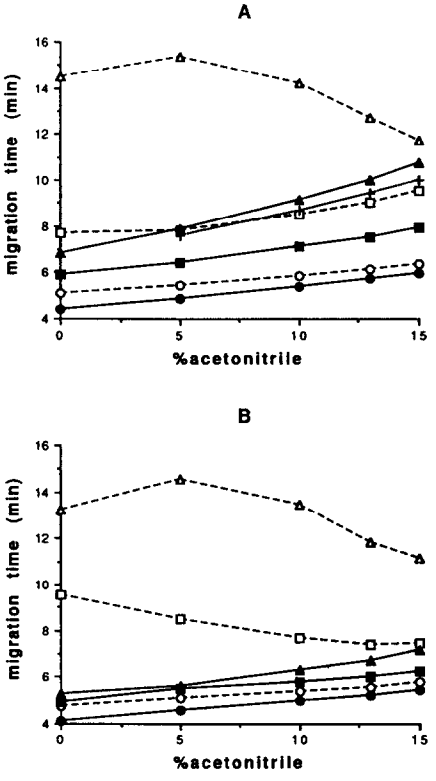

Fig. 1. Effect of acetonitrile on the migration time in MEKC. Buffer, 0.1 *M* SDS in 0.02 *M* sodium borate, (A) pH 9 and (B) pH 7; capillary, fused silica, 0.075 mm I.D., length to detector 500 mm, total length 570 mm; injection time, 3 s; voltage, 20 kV; temperature, 25°C. ● = Formamide; ○ = PP; ■ = paracetamol; □ = B_2; ▲ = B_6; △ = B_1; + = B_1 (peak 2).

On lowering the pH of the running buffer from 9 to 7, the degradation peak disappeared in the mixed aqueous–acetonitrile solution (see Fig. 1B). At this pH thiamine is completely ionized whereas the other vitamins are no longer ionized. It can also be noted from Fig. 1B that at pH 7 the migration of thiamine and, to a lesser extent, riboflavine becomes progressively faster in buffers with a higher acetonitrile content. However, the overall longer migration times of these two compounds is due to their stronger interaction with the micelles: ionic interaction for thiamine and hydrophobic interaction for riboflavine. In the presence of acetonitrile these interactions become weaker and result in smaller capacity factors.

A concentration of 13% of acetonitrile in the separating buffer at pH 7 containing 0.1 *M* SDS was chosen to perform the MEKC analyses. Under these conditions the separations was completed within 13 min. The order of elution was PP, paracetamol, B_6, B_2 and B_1.

For quantification purposes, some workers report the necessity to correct the raw peak area by dividing by the corresponding migration time, [6,7] whereas others [8–11] stress the use of the internal standard technique to obtain precise results.

Before starting quantitative analyses of the vitamins we investigated the problem of peak area precision under two different experimental conditions, first such that the migration times were not altered, and second when the migration times exhibited large differences. A 0.01 *M* HCl solution containing 600 μg/ml of B_1, 100 μg/ml of paracetamol and 150 μg/ml of B_6 was analysed in the CZE mode at 10, 15 and 20 kV, performing six replicate injections at each voltage. B_1 and B_6 were considered as test substances: B_1 eluted before and B_6 behind the internal standard paracetamol. The results are given in Table 1.

It can be seen that at any voltage, correcting the peak area by the migration time does not improve the precision (R.S.D. 7–10%) which in fact is expected from the low R.S.D. values of

Table 1
Influence of using corrected area and/or internal standard technique on the precision of peak area

Voltage (kV)	Parameter	t_m (min)			A			$A/A_{I.S.}$		A_c			$A_c/A_{c\,I.S.}$	
		B_1	I.S.	B_6	B_1	I.S.	B_6	B_1	B_6	B_1	I.S.	B_6	B_1	B_6
20	$\bar{x}_{20}$	2.98	4.49	5.94	0.628	1.140	0.982	0.551	0.862	0.211	0.254	0.165	0.831	0.651
	R.S.D. (%)	0.18	0.44	0.33	7.30	7.64	7.981	1.14	0.72	7.34	7.55	7.94	0.95	0.68
15	$\bar{x}_{15}$	4.02	6.04	8.04	0.882	1.529	1.409	0.577	0.922	0.219	0.253	0.175	0.867	0.692
	R.S.D. (%)	0.16	0.20	0.15	6.80	6.99	6.82	0.52	0.42	6.94	6.93	6.86	0.48	0.39
10	$\bar{x}_{10}$	6.15	9.31	12.44	1.103	2.049	1.839	0.538	0.898	0.179	0.220	0.148	0.815	0.672
	R.S.D. (%)	0.15	0.14	0.10	9.76	10.20	9.59	0.76	1.18	9.87	10.18	9.68	0.59	1.03
	$\bar{x}_{10}/\bar{x}_{20}$	2.06	2.07	2.08	1.79	1.87	2.09	0.97	1.04	0.85	0.86	0.89	0.98	1.03
	$\bar{x}_{15}/\bar{x}_{20}$	1.34	1.34	1.35	1.40	1.34	1.43	1.04	1.06	1.03	0.99	1.05	1.04	1.06
	$\bar{x}_{10}/\bar{x}_{15}$	1.52	1.54	1.54	1.25	1.34	1.30	0.93	0.97	0.81	0.87	0.84	0.94	0.97

Capillary, fused silica, 0.075 mm I.D., length to detector 500 mm, total length 570 mm; sample, B_1 600 μg/ml, paracetamol (I.S.) 100 μg/ml and B_6 150 μg/ml in 0.01 *M* HCl; injection time, 3 s; running buffer, 0.02 *M* sodium borate (pH 9); voltage, 20, 15 and 10 kV; temperature, 25°C. t_m = migration time; A = peak area; $A_c = A/t_m$ = corrected area; $\bar{x}$ = mean of six replicate analyses.

the migration times. The precision is greatly improved, however, by the use of an internal standard (R.S.D. 1%).

Moreover, in situations where a variation in migration times was induced by injecting the same solution at different voltages, it can be seen from Table 1 that the ratios of the mean areas calculated from the results obtained at 10, 15 and 20 kV nearly attain unity when the internal standard is taken into account.

3.1. Calibration

For CZE and MEKC, calibration lines were constructed with concentrations up to 600 μg/ml for B_1, 1200 μg/ml for PP and 120 μg/ml for B_2 and B_6. Linear regression lines of the ratios of the peak areas to that of the internal standard as a function of the concentration were calculated using the least-squares method. Good linearity was obtained for all components in the range studied.

3.2. Precision and accuracy

The accuracy and the within-day precision (repeatability) were evaluated by determining the four vitamins in an artificially prepared

Table 2
Recovery ($\bar{x}$), repeatability (R.S.D.) and accuracy [95% confidence interval (C.I.)] for spiked powder samples ($n = 6$)

Method	Parameter	PP	B_6	B_2	B_1
CZE	$\bar{x}$	143.6	100.5	99.7	101.2
	R.S.D. (%)	1.7	1.3	2.2	2.2
	C.I.	141.3–145.8	99.3–101.7	97.6–101.7	99.1–103.2
MEKC	$\bar{x}$	100.4	99.8	99.7	100.9
	R.S.D. (%)	0.8	1.2	1.8	2.8
	C.I.	99.1–101.7	98.7–101.0	97.8–101.6	98.4–103.6

Sample, B_1 and B_2 120 μg/ml, B_6 85 μg/ml and PP 400 μg/ml in 20% acetonitrile in 0.01 *M* HCl; running buffer, CZE 0.02 *M* sodium borate (pH 9), MEKC 13% acetonitrile in 0.02 *M* borate–phosphate (pH 7) containing 0.1 *M* SDS; voltage, 20 kV; other conditions as in Table 1.

mixture composed of 3.3% B_1, 11.0% PP, 3.3% B_2 and 2.2% B_6, added to a mixture of tablet excipients composed of lactose, starch, polyvinylpyrrolidone (PVP), talc and magnesium stearate. Samples were treated as described in the procedure for sample preparation for tablets and the final concentrations of vitamins were 120 μg/ml of B_1, 400 μg/ml of PP, 120 μg/ml of B_2 and 85 μg/ml of B_6, using 2.0 ml of 50 μg/ml paracetamol solution as the internal standard. Six replicate samples were analysed by comparison with an external reference solution of the vitamins. Mean recoveries are given in Table 2, together with the relative standard deviations and 95% confidence intervals (C.I.) of the mean. Acceptable repeatability (R.S.D. 3%) and good accuracy were obtained for all vitamins. However, a high recovery was observed in the CZE analysis of PP, which is attributed to PVP, which was present in a high concentration (6% of the total mass) in the excipient mixture. PVP is an uncharged compound and interferes with nicotinamide, both migrating at the velocity of the EOF. Such interferences could be resolved in the MEKC mode.

3.3. *Analyses of commercial vitamin preparations*

Three commercial tablet formulations, one syrup and one soft gelatin capsule preparation were analysed by CZE and MEKC. For comparison, the LC method of the USP XXII [2] for the analysis of vitamin tablets was also applied to the same samples. Fig. 2 shows typical CZE and MEKC results and the LC results for tablet 1. For most of the preparations investigated, each vitamin could be determined without interference by both CE and LC methods. It can be noted from Fig. 2 that calcium pantothenate could also be determined with with the CE systems used. In tablets that contained PVP, the result for PP by CZE was not different from those given by LC and MEKC; probably the amount of PVP present in the tablets was lower than the detectable concentration. However, with soft gelatin capsules LC could not be used owing to the presence of oily droplets in the sample solution. In CZE, these droplets were

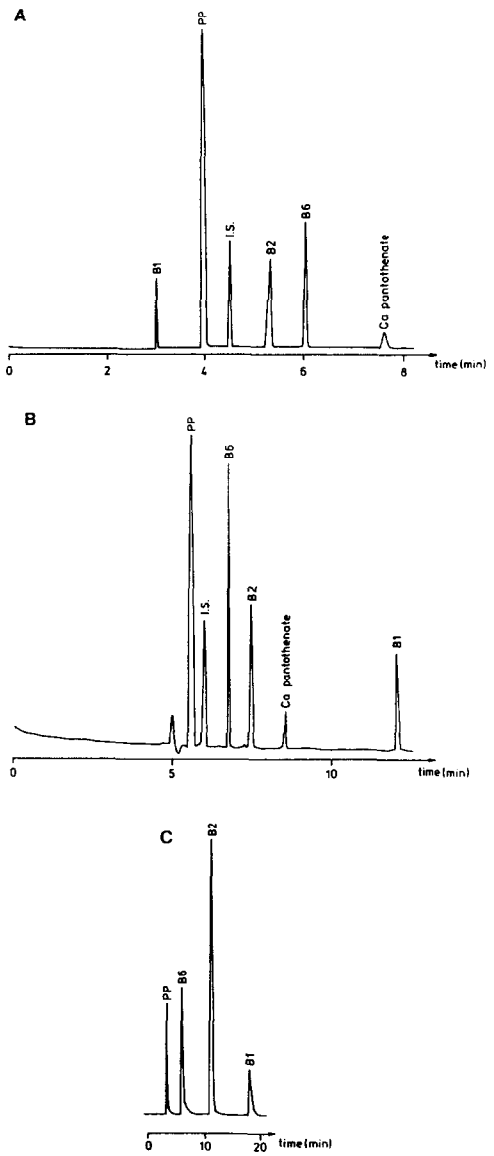

Fig. 2. Comparison of CE separations and LC separation of tablet 1. (A) CZE separation, 0.02 *M* borate buffer (pH 9), 214 nm, 20 kV; (B) MEKC separation, 13% acetonitrile in 0.02 *M* borate–phosphate buffer (pH 7) containing 0.1 *M* SDS, 214 nm, 20 kV; (C) LC separation, USP method [2].

probably adsorbed on the capillary surface and caused broadening of the zones and increased migration times resulting from a decrease in the EOF [12]. This was not a problem in MEKC as the surfactant SDS probably dissolves the oily droplets. The results of the analyses of the commercial preparation are given in Table 3.

Table 3
Determination of vitamins B_1, B_2, B_6 and PP in commercial preparations

Sample	Analyte	$\bar{x}$ ± S.D. (%)		
		CZE	MEKC	LC
Tablet 1	B_1 (15 mg)	118.7 ± 1.7	123.6 ± 2.6	123.8 ± 3.6
	PP (50 mg)	110.8 ± 3.1	108.0 ± 1.2	108.7 ± 2.1
	B_2 (15 mg)	99.0 ± 2.2	99.4 ± 2.1	103.9 ± 0.7
	B_6 (10 mg)	110.9 ± 3.3	113.7 ± 1.7	112.4 ± 3.2
Tablet 2	B_1 (10 mg)	130.3 ± 1.3	132.3 ± 2.1	129.0 ± 4.6
	PP (25 mg)	107.7 ± 0.7	105.2 ± 2.1	109.0 ± 1.8
	B_2 (5 mg)	106.9 ± 3.5	102.1 ± 2.3	101.1 ± 4.4
	B_6 (5 mg)	108.4 ± 1.6	108.2 ± 5.3	102.7 ± 3.1
Tablet 3	B_1 (50 mg)	104.4 ± 2.0	103.7 ± 1.3	106.4 ± 3.7
	PP (100 mg)	99.8 ± 0.7	98.2 ± 1.3	100.2 ± 1.4
	B_2 (10 mg)	104.1 ± 1.6	109.6 ± 3.1	108.0 ± 3.0
	B_6 (10 mg)	96.3 ± 1.5	95.0 ± 1.6	95.7 ± 3.0
Syrup (5 ml)	B_1 (10 mg)	117.2 ± 4.0	112.4 ± 1.7	111.6 ± 1.6
	PP (20 mg)	111.2 ± 1.4	109.4 ± 0.9	111.5 ± 3.4
	B_2 (1 mg)	115.4 ± 1.5	119.3 ± 2.9	117.2 ± 2.2
	B_6 (5 mg)	109.9 ± 1.4	106.2 ± 3.1	113.2 ± 3.9
Soft capsule	B_1 (10 mg)	122.0 ± 2.2	126.6 ± 1.7	
	PP (30 mg)	111.3 ± 1.8	108.6 ± 1.7	
	B_2 (7 mg)	112.1 ± 3.1	114.9 ± 1.6	
	B_6 (5 mg)	108.6 ± 1.8	108.2 ± 1.6	

Results from five determinations are given as mean percentages of the labelled amount (values in parentheses) ±S.D.

4. Conclusions

The analysis of water-soluble vitamins by capillary electrophoresis (both CZE and MEKC) can be considered as a valuable alternative to LC. Short analysis times and low running costs are the dominant features in CE. It was found that the use of the internal standard technique is necessary in order to obtain good precision with either constant or varying migration times.

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