Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1007 (2003) 77–84
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Reversed-phase liquid chromatography on an amide stationary phase for the determination of the B group vitamins in baby foods☆

Pilar Viñas, Carmen López-Erroz, Nuria Balsalobre, Manuel Hernández-Córdoba*
Department of Analytical Chemistry, Faculty of Chemistry, University of Murcia, E-30071 Murcia, Spain

Received 7 March 2003; received in revised form 15 May 2003; accepted 27 May 2003

Abstract

A liquid chromatographic method for the separation and determination of several water-soluble vitamins of the B group is proposed. The procedure is based on the use of a new amide-based stationary phase, which avoids the need of using the ion-pair technique, leading to narrower peaks and a simpler mobile phase. Analyses were performed by gradient elution with acetonitrile–phosphate buffer as mobile phase and using a photodiode array detector. Specificity was demonstrated by the retention characteristics, UV spectra and by comparing the peak purity index with commercial standards. Linearity, precision, recovery and sensitivity were satisfactory. The vitamins are extracted from the baby food using a single digestion with hydrochloric acid followed by enzymatic digestion with taka-diastase. The method was successfully applied to the determination of nine vitamins: thiamine, riboflavin, nicotinamide, nicotinic acid, pyridoxine, pyridoxal, folic acid, cyanocobalamine and inosine in different baby foods as infant formulas, cereals and fruit products. Validation was performed using two certified reference materials.

Keywords: Food analysis; Infant formulas; Cereals; Fruits; Validation; Vitamins

1. Introduction

The water-soluble B group vitamins include many compounds of differing chemical structure and biological roles, which are essential for the health of children. Compared with the requirements of adults, there are additional needs for the normal growth of infants. Losses of vitamins are produced during food processing and storage and supplementation of baby food with vitamins compensates the loss of these nutrients during manufacture. Thus, vitamin fortification enables us to meet the nutritional requirements of the non-breastfed infant and infant formulas are submitted to rigorous quality control analysis. Other baby foods normally used are based on cereals and fruits.

Liquid chromatography (LC) is a separation technique that has important advantages in vitamin analysis and the reversed-phase technique with the addition of an ion-pair reagent has been commonly used for determining the B group [1–18]. Reversed-phase without ion-pairing formation has also been

☆Presented at the 2nd meeting of the Spanish Society of Chromatography and related techniques, Barcelona, 26–29th November 2002.

*Corresponding author. Fax: +34-968-364148.
E-mail address: hcordoba@um.es (M. Hernández-Córdoba).

0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00977-4

used [19–26] but problems of low reproducibility in the retention times of some vitamins appeared. The use of a stationary phase for basic compounds involving a ligand with amide groups (RP-AmideC_{16}) and the endcapping of trimethylsilyl permitted the residual silanol groups to react more strongly and gave good results for the determination of the vitamins without the use of the ion-pairing technique. This amide phase has been successfully applied to the separation of thiamine and its esters [27].

In the present study, the separation of nine vitamins of the B group: thiamine (vitamin B_1, T), riboflavin (vitamin B_2, RF), nicotinamide (vitamin B_3, N), nicotinic acid (NA), pyridoxine (vitamin B_6, PN), pyridoxal (PL), folic acid (FA), cyanocobalamine (vitamin B_{12}, CC) and inosine (IN) was optimized using a photo-diode array detector. The reversed-phase technique without ion-pair formation using a new amide-based stationary phase with endcapping of trimethylsilyl was employed. The method is based on a combination of acid digestion and enzymatic extraction to release protein-bound and phosphorylated vitamins followed by LC analysis. The procedure has been successfully applied to the determination of the B group vitamins in baby food products as infant formulas milk and soy-based and cereal and fruit based products. Separation using the amide-based column is advantageous with respect to other existing methods because the peaks are much narrower and column life is longer due to the simplicity of the mobile phase.

On the other hand, the US National Institute of Standards and Technology (NIST) has recognized the importance of food-matrix reference materials for organic nutrients since the 1990s [28]. The use of reference materials is invaluable for assessing method accuracy; however, only some recent papers have used these materials to prove the accuracy of the methods [18]. Thus, in the present paper the use of two certified reference materials allowed a good validation of the proposed method.

2. Experimental

2.1. Apparatus

The LC system consisted of a Shimadzu FCV-10ALvp (Shimadzu, Kyoto, Japan) liquid chromatograph operating at room temperature with a flow-rate of 1 ml min^{-1}. The solvents were degassed using a membrane system Shimadzu DGU-14A. The spectrophotometric detector was a photodiode array Shimadzu SPD-M10Avp operating at four wavelengths of 249 nm for IN; 266 nm for T, RF, N, NA and FA; 326 nm for PN and PL and 361 nm for CC. The software was Class-LC10 (Shimadzu) and the detector was connected to a SPD-MXA integrator. Aliquots of 100 μl were injected manually using a Model 7125-075 Rheodyne injection valve (Rheodyne, Berkeley, CA, USA). The analytical column used for the reversed-phase technique was packed with RP-AmideC_{16} with a particle size of 5 μm (Supelco). A guard column packed with the same stationary phase was also used. The ultrasonic processor UP 200 H (Dr Hielscher, Germany) was used for extraction of vitamins from the samples.

2.2. Reagents

Acetonitrile (ACN, Romil, Loughborough, UK) was of liquid chromatographic grade. Doubly distilled water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). The 10 m*M* potassium dihydrogenphospate solution of pH 6 was prepared from the commercial product (Panreac, Barcelona, Spain). Stock solutions (1000 μg ml^{-1}) of T, N, NA, PN, PL, CC and IN were prepared by dissolving 10 mg of the commercial products (Sigma, St. Louis, MO, USA), without previous purification, in 10 ml of water. Stock solution (1000 μg ml^{-1}) of RF was prepared by dissolving 10 mg of the commercial product (Sigma) in 3 ml of 1 *M* phosphoric acid and diluting up to 10 ml with water and sonicating for 2 min. A stock solution (1000 μg ml^{-1}) of FA was prepared by dissolving 10 mg of the commercial product (Sigma) in 10 ml of methanol. They were kept in dark bottles at 4 °C. Working standard solutions were prepared by dilution with the phosphate buffer just before use. Taka-diastase (Fluka, Buchs, Switzerland), trichloroacetic acid (Probus, Barcelona, Spain) and hydrochloric acid (Fluka) were also used.

2.3. Samples and certified reference materials

The samples were infant formulas (starting milk,

follow-on milk and soy-based) and other products based on cereals with honey, multifruits and rice cream commonly used as baby foods and were commercially obtained. The method was validated using two reference materials, milk powder CRM 421 and pig's liver CRM 487 were supplied by the Community Bureau of Reference, BCR (Belgium).

2.4. Procedures

All operations were performed in subdued light. Samples were submitted to successive hydrolysis with hydrochloric acid and enzyme hydrolysis using taka-diastase following a procedure described by the analytical methods committee [29]. Amounts of 5–10 g were weighed into polypropylene bottles and 25 ml of 0.1 *M* hydrochloric acid was added. The suspensions were homogenized by using an ultrasonic processor for 30 s and then heated in a water bath at 90 °C for 30 min. When the suspension was cold, the pH was adjusted to 4 using 1 *M* sodium acetate and 0.1 g of taka-diastase was added. The sample was maintained in a water bath with magnetic stirring at 50 °C for 2 h. Then, 1 ml of 50% (w/v) trichloroacetic acid was added and the bottle was again introduced into the water bath at 90 °C for 10 min. When the sample was cold, the pH was adjusted to 6 with 10 *M* potassium hydroxide and it was quantitatively transferred to a 50-ml calibrated flask using the mobile phase buffer (10 m*M* potassium dihydrogenphospate pH 6). Aliquots were centrifuged at 6000 rpm for 10 min, filtered through a 0.45-μm nylon Millipore chromatographic filter and injected into the chromatograph. Certified reference samples were analyzed in the same way.

3. Results and discussion

3.1. Optimizing the separation of B group vitamins using the amide-based column

Reversed phase chromatography using a common ODS column provided non-reproducible results for the separation of vitamins due to the interaction of the basic nature vitamins with the silanol groups of the silica-based columns. The RP-AmideC_{16} column is an ideal choice for basic compounds because it avoids the interaction of the vitamins with the silanol groups due to the endcapping of trimethylsilyl, which permitted the residual silanol groups to react more strongly. Thus, the RP-AmideC_{16} column with endcapping of trimethylsilyl was used to optimize the separation of the B group vitamins.

The flow of the mobile phase was 1 ml min^{-1} and potassium dihydrogenphosphate was introduced to avoid ionization of the vitamins. A detailed study was then performed on the influence of the phosphate concentration, the pH and the addition of an organic solvent, acetonitrile (ACN), to the aqueous mobile phase. The influence of pH was studied using a mobile phase 25 m*M* potassium dihydrogenphosphate without ACN. Several phosphate buffers at pH values ranging between 4 and 7 were used and Fig. 1A represented the variation of the retention factors for the different vitamins as a function of pH. As can be seen, retention increased for higher pH values, the vitamins FA, CC and RF were retained in the column in all the pH ranges studied and the best separation for the other analytes was obtained at pH 6, with only T and N being overlapped using this pH. When the influence of the KH_2PO_4 concentration at pH 6 was studied, retention slightly decreased when the phosphate concentration increased (Fig. 1B). A 10 m*M* concentration was chosen, which allowed separation of T and N, although FA, CC and RF were again retained. Finally, Fig. 1C shows the variation in vitamin retention when mixtures of 10 m*M* KH_2PO_4 (pH 6) with ACN at percentages between 0 and 20% were used. As appreciated, all the compounds were now eluted from the column and, as expected, retention decreased when the proportion of organic solvent was increased.

Isocratic elution of the vitamins was not possible since the optimal mobile phase, in the absence of ACN, for separating NA, PL, PN, T, N and IN did not elute the rest of the compounds. When a higher concentration of organic solvent was selected (15% ACN), FA, CC and RF were separated, while the rest of the vitamins eluted together at the void time. Consequently, a gradient elution technique was tried to achieve good peak resolution and to shorten the total analysis time. The gradient was started using an isocratic aqueous buffer, 10 m*M* KH_2PO_4 (pH 6) during 13 min, which allowed the separation of NA, PL, PN, T, N and IN. Then, several gradients with different ranges and profiles were tried and the best

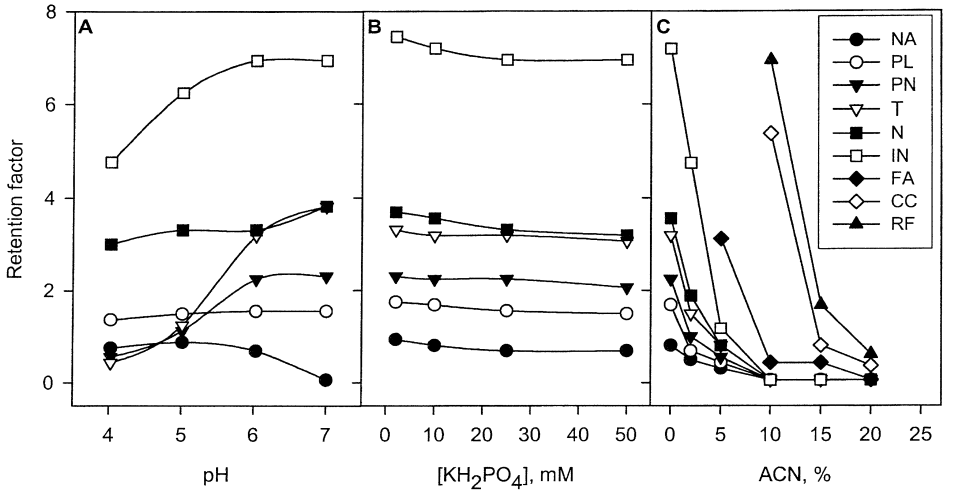

Fig. 1. Variation of the retention factors of the B group vitamins with (A) the pH at 25 m*M* KH_2PO_4; (B) the concentration of potassium dihydrogenphosphate at pH 6; and (C) the percentage of ACN at 25 m*M* KH_2PO_4 pH 6.

results were obtained using a first gradient with 6% v/v ACN to elute FA and a second gradient with 12% v/v ACN to elute CC and RF. The optimal gradient selected was the following. First, an initial isocratic step with 10 m*M* phosphate buffer at pH 6 for 13 min followed by a linear gradient to acetonitrile–buffer (6:94, v/v) mixture during 1 min, this mixture being held for 6 min. Then, a second linear gradient to acetonitrile–buffer (12:88, v/v) mixture in 1 min, this mixture being held for 10 min. Finally, the initial conditions were re-established in 1 min and held for 15 min. The flow-rate was 1 ml min^{-1}. Table 1 shows the elution order and the retention and separation characteristics for all the vitamins, expressed as the retention time (t_R), retention factor (k), separation factor (α) and resolution (R_s), using the gradient elution optimized.

Table 1
Chromatographic parameters using the RP-AmideC_{16} column

Vitamin	Parameter		Vitamins	Parameter	
	t_R (min)	k		α	R_s
NA	3.2	1.0	NA/PL	1.7	2.1
PL	4.3	1.7	PL/PN	1.3	1.2
PN	5.2	2.3	PN/T	1.4	2.1
T	6.7	3.2	T/N	1.4	1.0
N	7.3	3.6	N/IN	2.0	7.5
IN	13.1	7.2	IN/FA	1.5	9.3
FA	19.2	11.0	FA/CC	1.4	11.8
CC	26.7	15.7	CC/RF	1.1	2.1
RF	28.4	16.7			

The chromatographic profile obtained using this gradient elution program is shown in Fig. 2. The elution order was: 1, nicotinic acid; 2, pyridoxal; 3, pyridoxine; 4, thiamine; 5, nicotinamide; 6, inosine; 7, folic acid; 8, cyanocobalamine; and 9, riboflavin. As can be seen, very good results, defined by total elution of the mixture components and the appearance of narrow and non-tailed peaks, were obtained using the AmideC_{16} column. In addition, this experimental procedure is simpler than that using the ion-pair technique because column life is increased and smaller quantities of reagents are necessary.

3.2. Calibration, detection limits and repeatability

Calibration graphs were performed by the external standard technique following linear regression analysis by plotting concentration (μg ml^{-1}) against peak area. Table 2 shows the equations obtained for the calibration graphs and the regression coefficients at the optimal wavelength for each compound. The precision of the method was demonstrated by repetitive analyses, calculating the average relative standard deviation (RSD) for 10 replicate injections of the same sample at the 0.5 μg ml^{-1} level. The detection limits were calculated on the basis of 3σ and the quantitation limits on the basis of 10σ, using

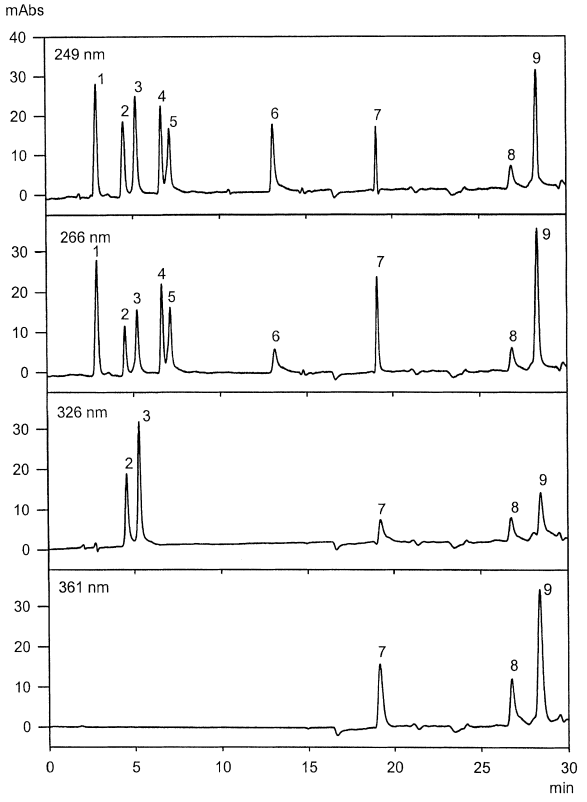

Fig. 2. Chromatographic profile using the amide-based column and gradient elution. Flow-rate, 1 ml min^{-1}; injected sample (containing 0.5 μg ml^{-1} of each vitamin), 100 μl. The peaks correspond to: 1, NA; 2, PL; 3, PN; 4, T; 5, N; 6, IN; 7, FA; 8, CC; and 9, RF.

the regression lines for the standards according to Miller and Miller [30]. Values are also given in Table 2.

Table 3
Recovery percentages for two spiked infant formula samples

Vitamin	Recovery[a] (%)	Recovery[b] (%)
PL	94.8	95.6
PN	99.1	102.8
T	66.5	99.4
N	103.4	98.6
IN	92.1	90.3
FA	98.3	98.4
CC	96.7	97.2
RF	70.3	99.0

[a] Using acid hydrolysis.
[b] Using both acid and enzymatic hydrolysis.

3.3. Extraction procedure and recovery study

Extraction of vitamins from food samples normally requires the use of successive acid or enzymatic hydrolysis steps. To test the need of such treatments, two infant formula samples were submitted to a recovery study. Absolute recoveries were evaluated by adding a fixed amount (0.5 μg ml^{-1}) of all the vitamins to the infant formulas. These were submitted to an extraction procedure based on an acid hydrolysis without enzymatic treatment. The second extraction procedure consisted on both acid and enzymatic hydrolysis using taka-diastase. After extraction, the chromatographic procedure was carried out and the concentrations were obtained using the calibration graphs. Table 3 shows the results obtained. As can be seen, the enzymatic hydrolysis was essential to achieve total extraction of thiamine and riboflavin, probably because these vitamins are present as esters in the baby foods, while for the other

Table 2
Calibration characteristics of B group vitamins

Vitamin	λ (nm)	Intercept	Slope (ml μg^{-1})	Correlation coefficient	Linearity (μg ml^{-1})	Detection limit (μg ml^{-1})	Quantitation limit (μg ml^{-1})	RSD (%)
NA	266	−5048±802	124 313±941	0.9999	0.07–1.5	0.019	0.064	2.0
PL	326	−6320±1160	184 022±2252	0.9998	0.07–1.0	0.019	0.063	2.5
PN	326	−7421±1262	184 811±803	0.9999	0.10–1.5	0.020	0.068	2.7
T	266	−4011±402	126 225±794	0.9997	0.05–1.0	0.010	0.032	4.1
N	266	−7621±1216	94 812±997	0.9994	0.20–1.5	0.038	0.128	2.6
IN	249	−4227±663	227 584±1799	0.9998	0.07–1.0	0.009	0.029	1.4
FA	266	−4224±326	212 989±797	0.9998	0.02–1.0	0.005	0.015	1.4
CC	361	−3210±413	127 938±778	0.9999	0.05–1.0	0.010	0.032	2.7
RF	266	−2973±414	424 629±1206	0.9999	0.02–1.0	0.003	0.010	1.6

vitamins the recovery was similar using both procedures. The recovery of the vitamins added to the infant formula prior to the extraction was not influenced by the vitamin forms present in the infant formula before the vitamin addition. Consequently, both the acid and the enzymatic hydrolysis were necessary for the treatment of the samples.

To confirm the efficiency of the extraction method, a recovery study was carried out by the standard addition technique, by spiking six samples of different baby foods (starting milk, follow-on milk, soy-based formula, cereals with honey, multifruits and rice cream) with the vitamin standards prior to hydrolysis at a level of 5 μg g^{-1}. Data of average recovery±SD (n=6) for the different vitamins in the baby food samples were: PL, 98.1±1.5; PN, 99.1±2.5; T, 98.7±1.1; N, 99.7±4.0; IN, 99.0±4.4; FA, 98.3±1.1; CC, 96.7±3.9 and RF, 99.7±0.5. These results demonstrated that recoveries of all vitamins were quantitative.

3.4. Analysis of baby food samples

The chromatographic procedure was applied to the determination of the nine B group vitamins in the different baby food samples. The chromatograms were obtained by injecting the samples of the different products and the peaks were identified by: (1) comparing the retention data obtained for the sample, the standards and the sample spiked with the standards under identical conditions; and (2) using the photo-diode array detector to continuously measure the UV-visible spectrum while the solute passed through the flow-cell and measuring the absorbance ratio at two wavelengths.

When the absorption spectra of the different peaks obtained for the standards, the baby food samples and the spiked baby food samples were compared, a good agreement was found. The following criteria were used to confirm the purity of the peaks: (a) peak purity curve, which is based on the similarity between the spectrum at the top of the target peak and the spectra at each point on that peak. If there is any part where this curve deflects toward the negative side, impurities are considered to be included at that part. (b) The ratio between chromatograms obtained using two wavelengths, the purity of the target peak being displayed as a chromatogram ratio. The peak purity curves and chromatogram ratios obtained for the peaks corresponding to the vitamins present in the baby foods showed that no impurities coeluted with the analytical peaks. The averaged values (mean±SD) for the purity index were: pyridoxal, 0.9974±0.0033 (n=5); pyridoxine, 0.9488±0.0513 (n=8); thiamine, 0.9951±0.0050 (n=8); nicotinamide, 0.9754±0.0192 (n=8); folic acid, 0.9855±0.0070 (n=8); cyanocobalamine, 0.9962 (n=1) and riboflavin, 0.9681±0.0328 (n=6). These values indicate that the purity of the peaks was satisfactory.

After identification of the vitamins and confirmation of the absence of a matrix effect, the compounds were quantified in the different baby foods. Table 4 shows the results obtained by the proposed procedure

Table 4
Determination of B group vitamins in baby food samples

Vitamin	Found[a] (labeled) (μg g^{-1})					
	Infant formula (starting milk)	Infant formula (follow-on milk)	Infant formula (soy-based)	Cereals with honey	Multifruits	Rice cream
NA	ND	ND	ND	ND	ND	ND
PL	2.0±0.12 (9.6[b])	1.1±0.03 (3.8[b])	0.75±0.03 (3.2[b])	ND	ND	ND
PN	6.2±0.1	3.2±0.09	2.6±0.03	3.5±0.22 (3.6[b])	1.8±0.03 (2.6[b])	3.3±0.06 (3.6[b])
T	8.0±0.09 (7.2)	3.6±0.2 (3.6)	3.1±0.05 (3.2)	6.8±0.16 (6.0)	4.0±0.06 (5.5)	5.5±0.17 (6.0)
N	139±3 (130)	51±2 (51)	73±3 (71)	44±2 (50)	69±2 (70)	56±2 (50)
IN	ND	ND	ND	ND	ND	ND
FA	1.1±0.07 (1.4)	0.5±0.01 (0.46)	0.91±0.03 (0.8)	0.6±0.01 (0.5)	0.56±0.01 (0.4)	0.61±0.02 (0.5)
CC	ND	ND	ND	ND	ND	ND
RF	11.4±0.5 (11.0)	8.5±0.12 (7.6)	8.8±0.1 (7.9)	ND	5.7±0.1 (4.79)	ND

[a] Mean±standard deviation (n=6).
[b] Labeled the total pyridoxal+pyridoxine as pyridoxine.

Table 5
Determination of B group vitamins in certified reference materials

Vitamin	Milk powder (CRM 421)		Pig's liver (CRM 487)	
	Found[a] ($\mu g\ g^{-1}$)	Certified ($\mu g\ g^{-1}$)	Found[a] ($\mu g\ g^{-1}$)	Certified ($\mu g\ g^{-1}$)
PN total	6.40±0.24	6.66±0.85	17.7±1.1	19.3±2.9
T	6.08±0.09	6.51±0.48	9.1±0.4	8.6±1.1
N	70.3±0.9	68.0±2.0	–	No certified
FA	1.24±0.05	1.42±0.14	11.6±0.5	13.3±1.3
CC	No detected	0.034±0.005	1.17±0.06	1.12±0.09
RF	14.4±0.1	14.5±0.6	105.2±3.3	106.8±5.6

[a] Mean±standard deviation, $n=6$.

and the contents labeled by the manufacturers. No nicotinic acid, inosine or cyanocobalamine were identified in any of the baby food samples above the detection limits. Higher levels of vitamins were generally found in the infant formula than in the cereal products. A statistical study was carried out to compare the contents found and the labeled values. The paired *t*-test for PN, T and N and the Wilcoxon signed rank test for FA and RF were used. The values obtained for the statistics and the associated *P* values at 95% confidence interval were: PN, $t=-1.164$ ($P=0.297$); T, $t=-0.236$ ($P=0.823$); N, $t=0.768$ ($P=0.477$); FA, $W=-9.000$, P(exact)=0.438 and RF, $W=-10.000$; P(exact)=0.125. These values indicated that there was no significant difference between the results obtained and the labeled levels.

3.5. Validation using certified reference materials

The use of reference materials is invaluable for assessing method accuracy. Thus, the reliability of the method was further established by using two certified reference materials, milk powder (CRM 421) and pig's liver (CRM 487). The statistical study using the paired *t*-test showed the following values obtained for the *t* statistic and the associated *P* value (95% confidence interval): PN, $t=-1.388$ ($P=0.397$); T, $t=0.0753$ ($P=0.952$); FA, $t=-1.237$ ($P=0.433$) and RF, $t=-1.133$ ($P=0.460$). These values indicated that there was no significant difference between the results obtained and the certified values (see Table 5). Such data also confirm the efficacy of the extraction procedure in recovering both free supplemented and endogenous vitamins in the baby foods.

4. Conclusion

The use of an Amide-C_{16} column appeared a good choice for the determination of the water-soluble B group vitamins using reversed-phase liquid chromatography with diode array detection. A procedure for analyzing nine vitamins in a single chromatogram using gradient elution is proposed. Peak purity evaluation was carried out using the peak purity curve and the ratio of the chromatogram at two wavelengths. The procedure was applied to the determination of the vitamins in baby foods as infant formulas and cereal and fruits products with very good results. The reliability of the method was verified using two certified reference materials.

Acknowledgements

The authors are grateful to the Spanish INIA (project CAL01-025) and to Comunidad Autónoma de la Región de Murcia (CARM, Fundación Séneca, Project 01474/CV/02) for financial support.

References

[1] R.B. Wills, C.G. Shaw, W.R. Day, J. Chromatogr. Sci. 15 (1977) 262.
[2] R.L. Kirchmeier, R. P Upton, J. Pharm. Sci. 67 (1978) 1444.
[3] R.P. Kwok, W.P. Rose, R. Tabor, T.S. Pattison, J. Pharm. Sci. 70 (1981) 1014.
[4] M. Amin, J. Reusch, Analyst 112 (1987) 989.
[5] Y. Maeda, M. Yamamoto, K. Owada, S. Sato, T. Masui, H. Nakazawa, J. Assoc. Off. Anal. Chem. 72 (1989) 244.
[6] K. Johansen, P.O. Edlund, J. Chromatogr. 506 (1990) 471.
[7] M.C. Gennaro, J. Chromatogr. Sci. 29 (1991) 410.

[8] A. Rizzolo, C. Baldo, A. Polesello, J. Chromatogr. 553 (1991) 187.
[9] G.W. Chase Jr., W.O. Landen Jr., R.R. Eitenmiller, A.G.M. Soliman, J. Assoc. Off. Anal. Chem. Int. 75 (1992) 561.
[10] D. Blanco, L.A. Sanchez, M.D. Gutierrez, J. Liq. Chromatogr. 17 (1994) 1525.
[11] S. Albalá-Hurtado, M.T. Veciana-Nogués, M. Izquierdo-Pulido, A. Mariné-Font, J. Chromatogr. A 778 (1997) 247.
[12] T.S. Agostini, H.T. Godoy, J. High Resolut. Chromatogr. 20 (1997) 245.
[13] P.K. Sarkar, E. Morrison, U. Tinggi, S.M. Somerset, G.S. Craven, J. Sci. Food Agric. 78 (1998) 498.
[14] D. Ivanovic, A. Popovic, D. Radulovic, M. Medenica, J. Pharm. Biomed. Anal. 18 (1999) 999.
[15] S. Albalá-Hurtado, M.T. Veciana-Nogués, M.C. Vidal-Carou, A. Mariné-Font, J. Food Sci. 65 (2000) 1052.
[16] S. Albalá-Hurtado, M.T. Veciana-Nogués, M.C. Vidal-Carou, A. Mariné-Font, J. Food Sci. 66 (2001) 1191.
[17] I. Almagro, M.P. San Andrés, S. Vera, Chromatographia 55 (2002) 185.
[18] D.C. Woollard, H.E. Indyk, J. Assoc. Off. Anal. Chem. Int. 85 (2002) 945.
[19] R.M. Kothari, M.W. Taylor, J. Chromatogr. 247 (1982) 187.
[20] M. Amin, J. Reusch, J. Chromatogr. 390 (1987) 448.
[21] S.M. Elgizawy, A.N. Ahmed, N.A. Elrabbat, Anal. Lett. 24 (1991) 1173.
[22] I.N. Papadoyannis, G.K. Tsioni, V.F. Samanidou, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 3203.
[23] C.M. Cho, J.H. Ko, W.J. Cheong, Talanta 51 (2000) 799.
[24] P. Moreno, V. Salvadó, J. Chromatogr. A 870 (2000) 207.
[25] H.B. Li, F. Chen, Chromatographia 54 (2001) 270.
[26] H.B. Li, F. Chen, J. Sep. Sci. 24 (2001) 271.
[27] P. Viñas, C. López-Erroz, N. Balsalobre, M. Hernández-Córdoba, J. Chromatogr. B 757 (2001) 301.
[28] K.E. Sharpless, S. Margolis, J.B. Thomas, J. Chromatogr. A 881 (2000) 171.
[29] Analytical Methods Committee, Analyst 125 (2000) 353.
[30] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 3rd Edition, Ellis Horwood, Chichester, 1993.