GIDA (2011) 36 (1): 1-7 *Research / Araştırma*

EXAMINATION OF CHANGES IN CERTAIN VITAMINS IN BABY FOODS BY CAPILLARY ELECTROPHORESIS

Ülkü Dilek Uysal[1]* , Muzaffer Tunçel[2], Necati Barış Tuncel[3]
[1]Anadolu University, Faculty of Science, Department of Chemistry, Eskişehir, Turkey
[2]Anadolu University, Faculty of Pharmacy, Department of Analytical Chemistry, Eskişehir, Turkey
[3]Çanakkale Onsekiz Mart University, Faculty of Engineering and Architecture,
Department of Food Engineering, Çanakkale, Turkey

Received / Geliş tarihi: 04.02.2010
Received in revised form / Düzeltilerek geliş tarihi: 24.06.2010
Accepted / Kabul tarihi: 30.06.2010

Abstract
This study describes the analysis of baby foods regarding time-dependent changes of d-pantothenate and niacin by a new capillary electrophoretic method at first and fifteenth days of opening the packages. D-pantothenate amounts in eight of the samples were found to be higher than those identified in the Turkish Standard and it was not detected in the nine samples. Niacin was not detected in the seven samples. Losses of mentioned vitamins were observed at the fifteenth-day for all samples

Keywords: Baby foods; Capillary electrophoresis; D-pantothenate, Niacine

BEBEK MAMALARINDA BAZI VİTAMİN DEĞİŞİMLERİNİN KAPİLER ELEKTROFOREZ İLE İNCELENMESİ

Özet
Bu çalışma, bebek mamalarının paketin açıldığı birinci ve onbeşinci günlerdeki d-pantotenat ve niasinin değişiminin yeni bir kapiler elektroforetik yöntemle zamana bağımlı olarak değişimini tanıtmaktadır. Sekiz numunede D-pantotenat miktarı Türk Standartlarının belirlediği değerlerden yüksek bulunmuş, dokuz numunede bulunmamıştır. Yedi numunede niasine rastlanmamıştır. On beşinci günde tüm numunelerde vitamin kayıbı gözlenmiştir.

Anahtar kelimeler: Bebek maması; Kapiler elektroforez; D-pantetonat, Niasin

* *Corresponding author* / Yazışmalardan sorumlu yazar ;
ulkudilek@yahoo.com, (+90) 222-335 0580/ 4790, (+90) 222-335 3616

INTRODUCTION

Baby foods are nutritionally inferior to breast milk, and superior to other substitutes such as animal milk. They have special functions in diets of infants because they are the major source of nutrients and a unique source of food during the first months of the infants' life (1-4). Nutritional content of infant formulas can be changeable depending on the producing country, but all formulas include protein, fat, linoleic acid, calcium, some elements and ions, phosphorus, iodine and certain vitamins in different amounts. As it is known, vitamins play an important role for healthy growth and development. They are a broad group of organic compounds with their small amounts, but essential constituents of food required for the normal growth, self-maintenance and functioning of human. Each one has different specific and vital functions in metabolism, and their lack or excess produces specific diseases. Human body can not produce vitamins and they should be taken by food or drugs. Moreover, they are not stored in the body, so it is needed to get a consistent amount on a regular basis. Vitamins have more importance for babies and children compared to the adults for healthy growth (5). Therefore, every step of their preparations should be inspected. For that purpose fast, easy and reliable methods are used for the determination of vitamins in baby foods.

The analytical methods used for the determination of vitamins especially for baby foods include chromatographic (6-14), high performance liquid chromatography-mass spectrometry (15) and biosensor based-assays (16). Among them, limited numbers of high performance liquid chromatographic (HPLC) reports on the water soluble vitamins have been published.

In the mentioned reports, different styles of analysis have been employed such as different elution modes, various mobile phases, detectors and ion-pair technique (6, 12).

It is known that HPLC is a time and material consuming method which needs preliminary steps to prepare the sample before injection. For that reason, capillary electrophoresis (CE) was preferred for the determination of vitamins in baby foods. It is mentioned that CE has been a relatively new separation technique used in food analysis recently (17-20). It has the advantages of high efficiency and resolution, automation, and rapid analysis duration. It is particularly suitable in the analysis of complex natural matrices, owing to its high resolution. Up to now, to the best of our knowledge, no capillary electrophoresis technique has been applied for the determination of water-soluble vitamins in baby food.

The objective of this research is to develop a new modified method for simultaneous determination of certain water-soluble vitamins in baby food by using CE and to examine the vitamin changes depending on the storage time. For this purpose, calcium D-pantothenate (D-PAN) and niacin (NIA) were selected randomly. There is a warning on the label of the packages of baby foods indicating that they should be consumed within two or three weeks after opening of the packages. Therefore, baby food samples were analyzed at first and fifteenth days.

MATERIALS AND METHODS

Apparatus, Reagents and Chemicals

A CE-L1 injection and power supply module separated the compounds and a Class Elegance Station (CE Resources Pte Ltd., Ayer Rajah Crescent, Singapore) provided the capillary electrophoresis (CE) signals. SPD-M10 a VP Diode Array Detector (DAD) (Shimadzu, Kyoto, Japan) detected the signals, and Class VP software (Shimadzu, Kyoto, Japan) processed the data. An uncoated fused silica capillary (Unimicro Technologies Inc., California, USA) having an ID of 75 μm was employed for resolution.

A Sonorex Ultrasonic Bath (Bandelin, Berlin, Germany) for degassing all of the solutions after centrifugation and a model of pH 301 pH/Ion meter with a Hanna HI 1131 glass electrode measured solution's pH (Hanna Instruments, Sarmeola di Rubano, Italy), a Daigger Vortex Genie 2 G 560-E (Scientific Industries Inc., Bohemia, USA) model vortex for dissolving and mixing baby food samples were used.

D-PAN and NIA were supplied from Fluka (Buchs, Switzerland) and methyl paraben (IS), borax, methanol, acetonitrile and sodium hydroxide were supplied from Merck (Darmstadt, Germany). All other chemicals were of analytical-reagent grade (Merck).

High quality water was obtained using a Model of Water Pro PS Labconco Corp. (Kansas City, USA). All standard solutions were daily prepared.

Baby food samples belonging to one brand were supplied from a local market. But, they had different contents such as without soy milk, soy protein, prebiotic fortified, special diet baby food, rice, different cereals or without cereals and etc. in the infant formula, up to 6 months, follow-on formula and from 6 months to 12 months categories.

Original packages of the samples of baby food were opened just before the analyses and they were analyzed capillary electrophoretically. The opened packages were stored for fifteen days at dry and cool home conditions by complying with the instructions on their labels. Then the mentioned analyses were performed once more at the fifteenth day.

Procedures
CE analysis
Preparation of CE solutions

Aqueous solution of 100 mM borate was prepared in a 100-mL flask. The stock solution was diluted to 20 mM with double distilled water. The pH of the solution was adjusted to 8.5 by the addition of 1 M HCl and this was used as a run buffer or solvent for the baby foods. It was always degassed in a sonicator for five minutes. 10 mM phosphate solution (10% MeOH, v/v) was used for extraction procedures. Vitamin standard solutions were prepared in 10 mM phosphate solution (10% MeOH, v/v) and their dilutions were made with the same solution. A $1.29x10^{-3}$ M concentration of methylparaben (internal standard, IS) was dissolved in water. All standard solutions were prepared daily by appropriately diluting their relevant stock solutions.

Although there were no reports of photo-sensitivity, all solutions were stored in the light-free and refrigerated conditions.

CE conditions

An uncoated fused silica capillary (total and effective lengths of 75 cm and 50 cm, respectively, and internal diameter of 75 μm) was used throughout the study. The capillary was conditioned and cleaned with solutions of 0.1 M sodium hydroxide (3 min.), water (3 min.) and a separation buffer (3 min.). Capillary column was flushed with the same solutions for same period of time after each injection. All experiments were conducted with an applied voltage of +18 kV (330 V/cm); the resulting current in the capillary was around 24 μA. A sample was injected through the capillary for 10 seconds at low hydrodynamic injection mode, and separated for 18 minutes in the separation running buffer. Signals were recorded at 200 nm.

CE extraction procedure and preparation of samples

Prior to CE analysis, fat of baby foods were removed by soxhlet extraction using diethyl ether. The dried fatless material was accurately weighed 1.5 g, it was placed into a test tube and 9 mL of pH 4 buffer solution (10 mM phosphate, 10% methanol, v/v) was added. A 5 mL aliquot of the solution was transferred to a test tube. 8 mL of acetonitrile was added and this mixture was centrifuged. A 4 mL aliquot of the solution was transferred into test tube, and then 1 mL of $1.29x10^{-4}$ M IS was withdrawn. The entire combination was vigorously shaken, and then it was injected to the CE for the determination of D-PAN and NIA.

Validation Studies

The method was validated according to ICH Guidelines for validation of analytical procedures (21). The precision of the method was determined as intra-day and it was evaluated by assaying the samples of the same concentration during the same day. Three sets (l=3), each one having fixed amount of IS ($2.58x10^{-4}$ M) and five dilutions (n=5) with increasing concentrations of D-PAN ($1.62x10^{-5}$ M-$1.35x10^{-4}$ M) and NIA ($1.86x10^{-5}$ M-$1.19x10^{-4}$ M) were prepared and they were injected to the CE at the optimum conditions. Quantification of peaks was accomplished by using the ratio of peak normalization of relevant vitamins and IS calculated (ratio of peak normalization is equal to the peak normalizations of D-PAN or NIA / peak normalization of IS) and the results were evaluated by linear regression analysis which was based on least square method.

The data were processed by Excel and GraphPad Prism statistical program (Version 3.02) throughout the study.

RESULTS AND DISCUSSION

The vitamin analyses of twenty-six baby foods were realized during fifteen-day period of screening (the samples were taken at first and fifteenth days). Capillary electrophoretic method was applied for the analysis on D-PAN and NIA because of its easiness and simplicity.

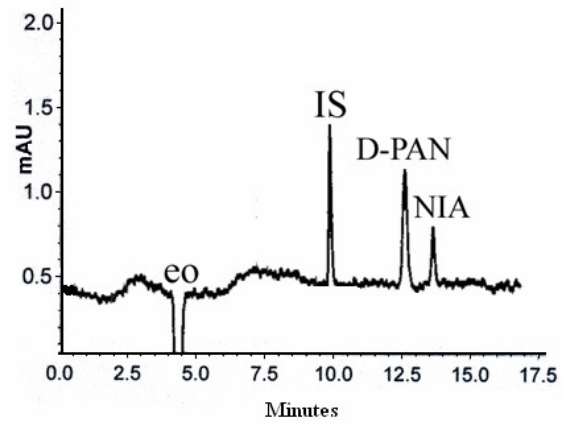
The analysis of vitamins by capillary electrophoresis

A run buffer consisting of 20 mM borate at pH 8.5 was used for the determination of D-PAN and NIA by using low hydrodynamic injection mode of 10 seconds and applying 18 kV potential, detecting at 200 nm employing CE.

There are many studies that were performed by CE for the determination of water soluble vitamins in different samples (17, 22-25). For this purpose, micellar electrokinetic chromatography has been mostly used, but we have tried to analyze D-PAN and NIA employing CE.

In the optimum conditions, the peaks for IS, D-PAN and NIA appeared, in turn, 10.0, 12.5 and 13.5 min, in the electropherogram. The signal of the electroosmosis was consistently observed at around 4.3 min. (Figure 1).

Figure 1



The electropherogram of standard vitamins and IS (methyl paraben), 20 mM borate buffer (pH 8.5), 10 s hydrodynamic injection, 18 kV, 200 nm, IS ($2.58x10^{-5}$ M), D-PAN ($4.43x10^{-5}$ M), NIA ($1.86x10^{-5}$ M).

Method Validation

After elucidation of the conditions presented above, the validity of the method was tested obeying the ICH suggestions (21).

Repeatability

Fixed concentration of D-PAN and NIA with IS was injected five times into the CE in the optimum conditions. Based on the results, the area of the peaks and peak normalizations (PN= peak area/peak retention time) and the rate of the peak normalizations ($R= PN_{vitamin}/PN_{IS}$) were evaluated. The experiments' precision increased in accordance with the rate of peak normalization. This can be attributed to the fact that the use of peak normalization and the processing of the internal standard become more repeatable by the use of the internal standard method. The results of repeatability tests, executed as intra-day precision, are demonstrated in Table 1.mostly used, but we have tried to analyze D-PAN and NIA employing CE.

In the optimum conditions, the peaks for IS, D-PAN and NIA appeared, in turn, 10.0, 12.5 and 13.5 min, in the electropherogram. The signal of the electroosmosis was consistently observed at around 4.3 min. (Figure 1).

Table 1. The repeatability results respect to the rate of peak normalization values ($PN_{vitamin}$ /PN_{IS}) for intra-day, CIS=$2.58x10^{-5}$ M, (n=5)

	Vitamins	
	D-PAN ($7.27x10^{-5}$ M)	NIA ($1.00x10^{-4}$ M)
x [a]	1.58	1.83
SD [b]	0.02	0.04
RSD % [c]	1.67	2.20
±CL(p=0.05) [d]	0.02	0.05

[a] x is mean.

[b] SD is standard deviation.

[c] RSD % is relative standard deviation.

[d] CL is confidence limits at (P=0.05).

The RSD values, which represent the repeatability, are 1.67 and 2.20 and these results indicate us that the method is very precise.

Calibration, limit of detection (LOD) and limit of quantification (LOQ)

Standard dilutions of D-PAN and NIA were prepared in the range of 1.62x10-5-$1.35x10^{-4}$ M for D-PAN and $1.86x10^{-5}$-$1.19x10^{-4}$ M for NIA as stated in the experimental section. They were injected through the capillary at the optimum conditions.

The calibration equations of D-PAN and NIA were separately computed so that it was based on least square method. Besides, correlation coefficients, LOD and LOQ values were also calculated.

The relevant results are presented in Table 2.

Table 2. The results of linearity and LOD, LOQ values for D-PAN and NIA.

Vitamin	Regression equation [b]	r [c]	Lineer range (M)	LOD (M)	LOQ (M)
D-PAN	Y=22914X-0.0839	0.9985	$1.62x10^{-5}$-$1.35x10^{-4}$	$2.62x10^{-6}$	$8.64x10^{-6}$
NIA	Y=17693X+0.0586	0.9999	$1.86x10^{-5}$-$1.19x10^{-4}$	$6.78x10^{-6}$	$2.24x10^{-5}$

[a] in the CE conditions as in Figure 1.

[b] In the regression equations, the X value is the concentration of analyte as molarity, the Y is the rate of the peak normalization value.

[c] correlation coefficient.

Highly repeatable and a good linearity was obtained for D-PAN and NIA and their LOD and LOQ values are low enough.

Baby food sample analysis by CE

The developed CE method was applied to the baby foods. D- PAN and NIA were analyzed in the twenty six of baby food samples employing the optimum CE conditions. A typical electropherogram of a sample is shown in Figure 2.

Figure 2

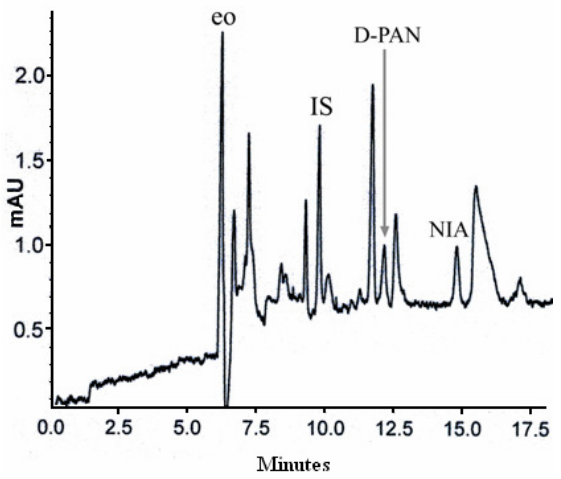


Figure 2. The electopherogram of one type baby food and IS (C_{IS}=$2.58x10^{-5}$ M), 20 mM borate buffer (pH 8.5), injecting 10 s, applying 18kV (direct polarity), recording at 200 nm.

Since there are many peaks appeared, the peaks were detected by spiking D-PAN and NIA before quantification. The vitamin contents of various baby foods are demonstrated in Table 3.

It was found that D-PAN amounts in the Samples 9, 15, 16, 21, 22, 24, 25 and 26 were higher than those identified in the Turkish Standard (26). D-PAN has not been detected in the samples 2, 3, 5, 6, 7, 11, 12, 19 and 20. It can be attributed to the matrix effect. Generally, loss for D-PAN was usually observed at fifteenth day after opening their packages in all samples. It is thought that one of the reasons of the loss of the vitamins in baby foods can be sourced from the storage as in the literature (12). Our data are in agreement with the mentioned study.

NIA has not been detected in the Samples 2, 3, 5, 11, 12, 19 and 20. It was determined in the interval of Turkish Standard limits for all the other samples (26-29). The loss for NIA for all samples was observed at fifteenth day.

Conclusions

The possible changes of certain vitamins (D-PAN - NIA) in baby foods were examined for fifteen days in this study. A new capillary electrophoresis method was developed for the mentioned vitamins by using a simple extraction procedure. Good results were obtained with a 15-minute CE analysis. The limits of detection and quantification for D-PAN and NIA were calculated to be $2.62x10^{-6}$M, $8.64x10^{-6}$ M and $6.78x10^{-6}$ M and, $2.24x10^{-5}$ M, respectively. According to the results of above mentioned CE analysis, D-PAN amounts in the Sample 9, 15, 16, 21, 22, 24, 25 and 26 were higher than those identified in the Turkish Standard (26). D-PAN was not detected in the samples 2, 3, 5, 6, 7, 11, 12, 19 and 20. NIA was not detected in the Samples 2, 3, 5, 11, 12, 19 and 20. Amounts of the D-PAN and NIA were determined in the interval of Turkish Standard limits for all the other samples (26-29). It was determined that there were some losses in the amount of D-PAN and NIA within fifteen days analysis.

Acknowledgements

The authors are grateful to the Anadolu University Scientific Research Projects (Project No 041021) for financial support.

Table 3. Vitamin contents (D-PAN and NIA) of baby food samples by CE method (n=3)

Baby food sample	D-PAN [a] (mg/ 100 kcal)			NIA [b] (mg/ 100 kcal)		
	First day	15th day		First day	15th day	
	Found	Found (changes,%, compare to first day)	Certified	Found	Found (changes,%, compare to first day)	Certified
1	1.718	1.573(-8)	0.580	0.437	0.355(-19)	1.234
2	-	-	0.431	-	-	0.389
3	-	-	0.455	-	-	0.576
4	1.247	1.050(-16)	0.405	0.271	0.262(-3)	0.581
5	-	-	0.443	-	-	1.057
6	-	-	0403	3.555	0.606(-83)	0.545
7	-	-	0.697	1.200	0.832(31)	0.697
8	1.220	1.023(-16)	0.448	0.553	0.430(-22)	0.627
9	2.454	2.220(-10)	0.519	0.650	0.341(-48)	1.195
10	1.504	1.207(-20)	0.379	0.364	0.348(-4)	0.576
11	-	-	0.449	-	-	1.067
12	-	-	0.420	-	-	0.398
13	1.273	-	0.429	0.708	-	0.600
14	1.105	0.920(-17)	0.482	0.279	0.261(-6)	0.562
15	1.889	-	0.573	0.400	-	0.987
16	1.551	1.247(-20)	0.519	0.572	0.530(-7)	1.195
17	1.232	1.133(-8)	0.403	0.263	-	0.625
18	0.818	-	0.448	0.670	0.647(-3)	0.612
19	-	-	0.509	-	-	0.916
20	-	-	-	-	-	-
21	2.371	1.952(-18)	0.369	1.332	1.029(-23)	2.192
22	2.248	-	0.525	0.427	0.235(-45)	1.188
23	1.431	-	-	1.386	1.228(-11)	0.161
24	3.305	2.684(-19)	0.278	1.524	1.270(-17)	0.278
25	3.664	1.483(-60)	0.365	1.882	1.693(-10)	2.214
26	2.288	-	0.382	1.389	1.324(-5)	2.029

[a] Limits: (min.-max.)/100 kcal: For D-PAN: 0.300 mg-unknown (Turkish Standard, TS 11983, 1996)

[b] Limits: (min.-max.)/100 kcal: for NIA: 0.250 mg-unknown (Turkish Standard, TS 11983, 1996)

(-): not detected

REFERENCES

1. Anon 1997. US Food and Drug administration (FDA). Overview of infant formulas. USA: Center for Food Safety and Applied Nutrition, Office of Special Nutritionals.

2. Bermejo P, Pena E, Dominguez R, Bermejo A, Fraga JM, Cocho JA. 2000. Speciation of iron in breast milk and infant formulas whey by size exclusion chromatography-high performance liquid chromatography and electrothermal atomic absorption spectrometry. Talanta, 50: 1211-1222.

3. Rodriguez Rodriguez EM, Sanz Alaejos M, Diaz Romero C. 2000. Concentrations of iron, copper and zinc in human milk and powdered infant formula. Int J Food Sci Nutr, 51: 373-380.

4. Ikem A, Nwankwoala A, Odueyungbo S, Nyavor K, Egiebor N. 2002. Levels of 26 elements in infant formula from USA, UK, and Nigeria by microwave digestion and ICP–OES. Food Chem, 77: 439-447.

5. Combs Jr GF. 1992. Discovery of the vitamins. In: The vitamins: fundamental aspects in nutrition and health, Academic Press, San Diego, USA.

6. Albalá-Hurtado S, Novella-Rodríguez S, Veciana-Nogués MT, Mariné-Font A. 1997. Determination of vitamins A and E in infant milk formulae by high-performance liquid chromatography. J Chromatogr A, 778: 243-246.

7. Albalá-Hurtado S, Veciana-Nogués MT, Izquierdo-Pulido M, Mariné-Font A. 1997. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography. J Chromatogr A, 778: 247-253.

8. Gonzalez-Corbella MJ, Tortras-Biosca M, Castellote-Bargallo AI, Lopez-Sabater MC. 1999. Retinol and α-tocopherol in infant formulas produced in the EEC. Food Chem, 66 (2): 221-226.

9. Majchrzak D, Elmadfa I. 2001. Phylloquinone (vitamin K1) content of commercially-available baby food products. Food Chem, 74: 275-280.

10. Rodrigo N, Alegría A, Barberá R, Farré R. 2002. High-performance liquid chromatographic determination of tocopherols in infant formulas. J Chromatogr A, 947: 97-102.

11. Mendoza BR, Morera Pons S, Castellote Bargalló AI, López-Sabater MC. 2003. Rapid determination by reversed-phase high-performance liquid chromatography of vitamins A and E in infant formulas. J Chromatogr A, 1018: 197-202.

12. Vinas P, Lopez-Erroz C, Balsalobre N, Hernandez-Cordoba M. 2003. Reversed-phase liquid chromatography on an amide stationary phase for the determination of the B group vitamins in baby foods. J Chromatogr A, 1007: 77-84.

13. Perales S, Delgado MM, Alegría A, Barberá R, Farré R. 2005. Liquid chromatographic determination of Vitamin D3 in infant formulas and fortified milk. Anal Chim Acta, 543: 58-63.

14. Chávez-Servín JL, Castellote A, López-Sabater MC. 2006. Simultaneous analysis of Vitamins A and E in infant milk-based formulae by normal-phase high-performance liquid chromatography–diode array detection using a short narrow-bore column. J Chromatogr A 1122: 138-14315. Mittermayr R, Kalman A, Trisconi MJ, Heudi O. 2004. Determination of vitamin B5 in a range of fortified food products by reversed-phase liquid chromatography–mass spectrometry with electrospray ionisation. J Chromatogr, 1032: 1-6.

16. Gao Y, Guo F, Gokavi S, Chow A, Sheng Q, Guo M. 2008. Quantification of water-soluble vitamins in milk-based infant formulae using biosensor-based assays. Food Chem, 110: 769-776.

17. Jorgenson JW, Lukacs KD. 1983: Capillary zone electrophoresis. Science, 222: 266-272.

18. Dong Y. 1999. Capillary electrohoresis in food analysis. Trends Food Sci Tech, 10: 87-93.

19. Trenerry VC. 2001. The application of capillary electrophoresis to the analysis of vitamins in food and beverages. Electrophoresis, 22: 1468-1478.

20. Fraizer RA. 2005. Capillary electrophoresis-food chemist applications. Encyclopedia of Analytical Science, 374-381.

21. ICH Topic Q2A, 1996. Validation of analytical procedures: Methodology, CPMP/ICH/281/95.

22. Terabe S, Otsuka K, Ichikawa K, Tsuchiya A, Ando T. 1984. Electrokinetic separations with micellar solutions and open-tubular capillaries. Anal Chem, 56: 111-113.

23. Heiger DN. 1992. High performance capillary electrophoresis-An introduction. Hewlett-Packard Primer. Publication number 12-5091-6199E.

24. Hu Q, Zhou T, Zhang L, Li H, Fang Y. 2001. Separation and determination of three water-soluble vitamins in pharmaceutical preparations and food by micellar electrokinetic chromatography with amperometric electrochemical detection. Anal Chim Acta, 437: 123-129.

25. Klejdus B, Petrlová J, Potěšil D, Adam V, Mikelová R, Vacek J, Kizek R, Kubáň V. 2004. Simultaneous determination of water- and fat-soluble vitamins in pharmaceutical preparations by high-performance liquid chromatography coupled with diode array detection. Anal Chim Acta, 520: 57-67.

26. Anon 1996. Turkish Standard, Baby Food I-Infant Formula, TS 11983.

27. Anon 2007. Turkish Food Codex, Baby and infant supplementary food, 01.11.2007-26687, No: 2007-50.

28. Anon 2008. Turkish Food Codex, Baby Formula, 04.09.2008-26987, No: 2008/52.

29. Anon 2008. Turkish Food Codex, Follow-on formula, 04.09.2008-26987, No: 2008/53.

GIDA

Yazım Kuralları

GIDA (2009) 34 (1): 55-58

www.gidadernegi.org / Gıda Dergisi / Yayın kuralları

Makale Gönderimi ve Telif Hakkı Devir Formu

GIDA (2009) 34 (1): 65

www.gidadernegi.org / Gıda Dergisi / Makale Gönderimi ve Telif Hakkı Devir Formu

Son Kontrol Listesi

GIDA (2009) 34 (1): 66

www.gidadernegi.org / Gıda Dergisi / Son Kontrol Listesi

adreslerinden erişilebilir. Yazarlar, makale göndermeden önce yazım kurallarını tam olarak okumalı ve makalelerini burada verilen kurallara göre hazırlamalıdırlar.