SMGroup

Annals of Chromatography and Separation Techniques

Research Article

Development and Optimization of a High-Performance Liquid Chromatography Method for Resolution of Two Liposoluble Vitamins: Ergocalciferol (vitamin D2) and Cholecalciferol (vitamin D3) in Milk Samples

Jesús Alfredo Araujo-León[1]*, Jessica Torrez-Gámez[1], Angel Polanco-Rodríguez[2] and Durcy Verenice Ruiz-Ciau[1]

[1]*Facultad de Química, Universidad Autónoma de Yucatán, México*

[2]*Departamento de Medicina Social y Salud Pública, Centro de Investigaciones Regionales, Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán, México*

Article Information

Received date: Feb 27, 2017
Accepted date: Mar 28, 2017
Published date: Mar 30, 2017

***Corresponding author**

Araujo-León, Jesús Alfredo, Facultad de Química, Universidad Autónoma de Yucatán, México, Tel.: +52 (999) 922 57 11; Fax: +52 (999) 922 57 08; Email: jesus.araujo@correo.uady.mx

Distributed under Creative Commons CC-BY 4.0

Keywords Vitamin D; Draper-Lin optimization; HPLC

Abstract

A simple High-Performance Liquid Chromatography (HPLC) method was developed and optimized for quantification of the two fat-soluble (D2 and D3) vitamins in milk matrix. The optimization procedure was developed through a Plackett-Burman design (PBD) and Small Composite Design (SCD) (Draper-Lin). The significant (p<0.05) factors found in PBD were HPLC-column temperature, flow rate, composition of mobile phases and percentage of Triton X-100, and these were optimized by means of a Draper–Lin design. The study revealed that optimal operating conditions were found to be 15ºC HPLC-column temperature, 100% methanol (MeOH) as mobile phases, 0.8 mL/min as flow rate, and 7.2% Triton X-100. The final HPLC analytical procedure was validated according to Horwitz function and from the AOAC Peer Verified Methods (PVM) program on the analyte level, exhibit good linearity (>0.99), sensitivity (0.56-2.54 μg/mL), precision (<10%), and accuracy (>90%). The method was developed in this study is suitable for the quantification of vitamins D2 and D3 in milk samples and can be used for quality control and may replace the present Mexican Normative methods that employ a large amount of solvent and that are aggressive in the sample treatment, with low recovery, precision, and sensitivity.

Introduction

During the last several years, vitamins have been an object-of-study due to their nutritional properties and their effects on health that deficiencies of these cause. From a general panorama, vitamins are classified as water-soluble and fat-soluble [1]. The latter vitamins play an important role in the synthesis and degradation of nutrients, in promoting the development of the immunological system, for coping with diverse pathologies [2], and to the normal functioning in daily activities [3].

Among fat-soluble vitamins, one of the most important and one with global interest has been vitamin D [4], including vitamin D3 ($C_{27}H_{44}O$, PM = 384.62) and vitamin D2 ($C_{28}H_{44}O$, PM = 396.63). In recent years, both of these have been under discussion and study in terms of their potential health effects, specifically with regard to the deficiency or insufficiency of these vitamins [5].

Vitamin D plays an important role in human metabolism, especially in the synthesis of hormones, insulin, metabolism, and cognitive responses. Therefore, the deficiency of these vitamins has been related with metabolic syndrome diseases [5]. In Mexico, there are only a few studies on vitamin D deficiency in children and sick persons; therefore, the status of vitamin D in the Mexican population is not fully defined [4,6].

The U.S. Food and Drug Administration (FDA) have allowed vitamin D fortification in foods due to the scarcity of natural sources to obtain this vitamin. The main foods where this vitamin can be found are dairy products, mainly milk, which has been recommended to complement the dose of vitamin D for individuals who having adequate exposure to the sun [5].

In Mexico, the National Council for Evaluation of Social Development Policy (CONEVAL) has decided to fortify, with vitamin D, milk and infant formulas. These are the two most important foods of the basic food-consumption basket [7]; however, the identification and quantification of

Copyright © Araujo-León JA

vitamin D in dairy foods, according to the official Mexican Norm, is required preparative or semi-preparative HPLC methods and last quantified by the analytical HPLC system. Nonetheless, the extraction processes are very prolonged and aggressive (saponification), with significant analyte loss and the requirement of a large amount of organic solvents [8].

It is for this reason that this study aimed to develop and optimize an easy analytical methodology to identify and quantify vitamins D2 and D3 in a routine laboratory, under an optimized chromatographic system by Small Composite Design: Draper Lin, which allows orthogonality and rotability with a few experiments in comparison with other complex-model designs [9]. The method proposed in this paper significantly reduces analysis time, sample collection, as well as the excessive use of organic solvents.

Materials and Methods

Chemicals and reagents

Standards of the vitamins (D2 and D3) were supplied by Sigma-Aldrich (USA) and Supelco (USA), respectively. The solvents n-hexane (n-Hx; High Purity, México), Methyl Tert-Butyl Ether (MTBE; J. T. Baker, USA), Methanol (MeOH, J. T. Baker, USA), TetraHydroFuran (THF, EM Science, USA), DiChloroMethane (DCM; Fermont, México) used in the extraction method and chromatographic analysis were of chromatographic grade. Ammonium hydroxide (35%; Meyer, México) and ButylateHydroxyToluene (BHT; Sigma Aldrich, USA) were of analytical grade (ACS). Triton X-100 (Sigma-Aldrich, USA) was utilized for vitamin solubility.

Instrumentation

An Agilent Technologies 1200-series HPLC system (Agilent, San Jose, CA, USA) with a quaternary pump and a UV-DAD detector equipped with a ULTRASPHERE C18 column (150 mm × 4.6 mm i.d., 5 μm; Supelco, USA) was employed. The chromatography was performed under gradient conditions with MeOH:THF. The column was maintained at 15°C throughout analysis. The gradient system was programmed at 100% MeOH for 8 min, and was then increased at 15% of THF solvent between min 10 and 12 (85:15, MeOH:THF). Total time required for sample analysis was at25 min. Spectral data were collected at 254 nm for vitamins D2 and D3.

Milk sample characterization analysis

Lala[MR]-brand pasteurized milk samples were collected in regional supermarkets in the state of Yucatán, Mexico. The physicochemical characterization of the milk samples was tested according to NMX-F-026-1997 Mexican Guidelines for the following assays: acidity; casein percent; density; total fat analysis; refraction index; lactose; pH; total proteins, and non-fatty solids.

Experimental design to optimization of vitamins D2 and D3 in chromatographic resolution

Plackett-Burman Design (PBD) (2^8*3/24) was tested as the screening design-of-choice for significant factors. For statistical effects, the variables are coded according to the following:

$$x_i = \frac{X_i - X_0}{\Delta X} (1)$$

Table 1: Plackett-Burman factors and conditions in each analysis level.

Factors	Level			
	Symbol	-1	0	1
Aquoses phases (% de MeOH)	A	96	98	100
% Trixton X-100	B	0	2.5	5
Flow rate (mL)	C	0.4	0.6	0.8
Temperature (°C)	D	25	35	45
Injection volume	E	5	10	15
Acid type	F	CH_3COOH	H_2SO_4	H_3PO_4
Acid concentration (M)	G	0.5	0.75	1
Column length (mm)	H	100	150	250

Where Xi is the coded value of the variable; Xi is the actual value of variable, X0 is the actual value of Xi on the center point, and ΔX is the step change value. Each variable was tested at two levels: at -1 for the low level and at +1 for the high level (Table 1).

After the Plackett-Burman tested the significant factors design, a Draper-Lin SCD was constructed to improve D2 and D3 chromatographic resolution. Four factors (% Triton X-100, flow, temperature, and acid concentration) were tested and we used Statgraphics Centurion ver. XV software for data analysis and for equation modulation. The experiment was performed using the multiple linear regression models of nonlinear quadratic polynomials, which includes the following terms:

$$Y = b_0 + \Sigma_{i=1}^{k} b_i X_i + \Sigma_{i=1}^{k} b_{ii} X_i^2 + \Sigma_{i=1}^{k-1} \Sigma_{j=i+1}^{k} b_{ij} X_i X_j + \varepsilon (2)$$

Where Y is the chromatographic resolution, bo, bi, bii, and bij were the regression coefficients to intercept, linear model, quadratic, and interaction, respectively. Xi and Xj are the independent variables (Table 2).

Validation

The method was validated according to International Conference Harmonization (ICH) guidelines; Sections Q2A and B. linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy were evaluated.

Table 2: Draper-Lin conditions and factors in each analysis level.

Factors		Level			
	-1. 68179	-1	0	1	1.68179
% Triton X-100	3.3	5	7.5	10	11.7
Flow rate (mL)	0.1	0.4	0.8	1.2	1.5
Temperature (°C)	11.6	15	20	25	28.4
Acid concentration (M)	0.1	0.3	0.5	0.8	0.9

Linearity: Linearity was evaluated by linear regression analysis by the least-square regression method and graphic examination within the range of 5 and 100 mgmL^{-1}. With these results, we calculated the following statistical parameter: slope (b); intercept (a); correlation coefficient (r); residual standard deviation ($s_{(y/x)}$); slope standard deviation (s_b) and intercept standard deviation (s_a). Calibration curves were prepared using fortified milk matrix.

Limited of detection and limited of quantification: LOD and LOQ were calculated as 3 and 10 times the residual standard deviation

Copyright © Araujo-León JA

($s_{(y/x)}$) from the linearity test, respectively, divided by the respective slopes of the calibration curve analysis.

Precision and accuracy: Precision and accuracy were tested with intra- and interday assays utilizing three concentration levels: 5; 10, and 50 mgmL^{-1}; all samples were run in triplicate each day (n = 9) on 3 different days. With the results, we calculated Relative Standard Deviation (RSD). The blank matrix samples were fortified with each concentration level, respectively, and were considered as an RSD with ≤10% 10% for precision, and % Bias between -15 and 10%. One-way ANalysis of VAriance (ANOVA) was run and significant differences were found between the in intra- and interday assay.

Vitamins D2 and D3 sample extraction

The extraction step consisted of 5 mL of the milk sample added into a 50-mL screw-cap tube with a conical bottom; 0.625 mL of NH_4OH, 10 mL of MeOH, and 3 mg of BHT were subsequently added, and the tube was shaken by vortex for 1 min. Following that, 25 mL of MTBE:n-Hexane (1:1, v/v) was added and shaken for 1 min in vortex again. Subsequently, this was centrifuged at 4,000 rpm for 10 min at 4°C.

Then, 5 mL of upper phase (organic phase) was transferred into a vial; the extraction step was repeated three times. Organic phase was concentrated to dryness in a rotary evaporator and reconstituted with 1 mL of MeOH:DCM (1:1, v/v). Finally, the samples were filtered with a 0.45-μm nylon membrane filter for HPLC analysis.

Results and Discussion

Significant Factors Employing the Plackett-Burman Design (PBD) Criterion

The Plackett-Burman Design (PBD) criterion was applied to identify the significant factor for chromatographic resolution in D2 and D3 vitamins (Figure 1). The equation model and the main factors with significant effects ($p<0.05$) were selected by ANOVA test. Afterward, the significant variables that resulted from PBD were optimized using a Small Composite Design (SCD): Draper-Lin.

According to the Pareto Distribution Diagram Chart (Figure 1), column length comprised the most important factor with statistical significance. However, in chromatographic terms, in terms of the capacity factor (k'), vitamins D2 and D3 were shown with k' value >15 utilizing a 250-mm HPLC-column with a retention time of >20 min. Under the conditions of these analyses, retention times were reported in the literature of 16 min [10] and 20 min [11,12]. However, the analytes underwent a significant retention-time increase in the optimization process; therefore, this factor was discarded, and the 150-mm HPLC column was used.

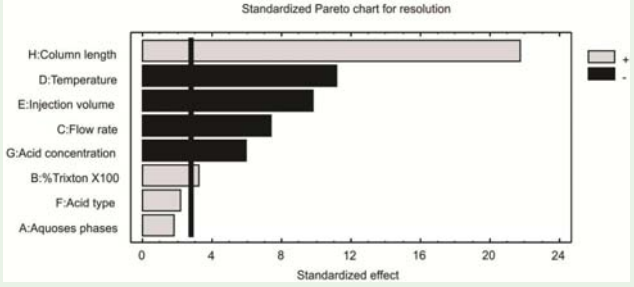

Figure 1: Pareto Chart showing significant variable obtained in PBD.

The result for volume injection (E) demonstrated a small amount of increase in volume in the resolution; however, for operational reasons, the automatic injector does not permit a volume of <5 μL. Also, factors F and A did not exhibit statistical significance ($p \geq 0.05$); thus, these three factors were discarded for the optimization process.

The second factor with greatest statistical relevance was thermostat temperature (D); chromatographic resolution of the vitamins increased with a decrease in thermostat temperature. The results showed that at 45°C, resolution was 0.26, and 25°C, this increased to 1.03. In RP-HPLC, temperature plays an important role with respect to chromatographic selectivity and capacity factor; for the latter, it is possible get obtain the chromatographic resolution, when the whole optimized the temperature with mobile phase (% organic phase) in isocratic or gradient system elution [13]; these results were shown in the PBD experiment when the temperature decreased and there was a 1.26-resolution of the mobile phase

Another important factor was the Triton X-100 (B, nonionic surfactant) concentration; this increased proportionally the chromatographic resolution with high concentration; the main Triton X-100/Vitamin interaction concerns the micellar analyte/surfactant, which significantly affects the solubility and absorption of D vitamins. These interactions have been determined, showing micelle formation among nonpolar compounds and modifying adsorption interactions [14,15], which in this system are reflected in the chromatographic resolution.

The final factor was the mobile-phase flow rate, demonstrating a inversely proportional trend. The ratio of this parameter has been described in terms of capacity factor (k'), column efficiency (theoretical plates), and selectivity (α), and has a relatively low bandwidth in chromatographic-process effects. However, but flow rate was described as useful for fine resolution. However, it is important to consider the flow rate %/aqueous-phase ratio, because k' may be increased and because the analytical process do not possess an application [16-18].

Optimization of the chromatographic Process by Draper–Lin Design

The significant factors (D, C, G, and B) of PBD were selected for Draper–Lin optimization. The main process for optimizing these factors involves maintaining the use of classic response-surface designs, such as Central Composite Designs (CCD), this requiring a total of 30 experiments, or incomplete designs such as the Box–Behnken response surface design, with 27 experiments. The Draper-Lin Design (DLD), a Small Composite Design (Small Composite Design [SCD]), was employed to reduce the number of experiments and to increase efficiency. This allowed, in the first eight experiments, a middle fraction of 24 design, and in the second set (eight experiments), the star points; for this case, we utilized as star points α = 1.68, which was completed with seven central points to maintain the orthogonal design conditions [19,20].

In the ANOVA test for DLD, we tested the model's lack-of-fit; this test showed the adequacy of the model. In this case, there was a p value of 0.062; the model did not exhibit statistical differences

(p>0.05). The DLD model can predict data behavior in 96.32% (R^2), which demonstrates a high degree-of-fit to the regression equation, to (R^2_{ajs}) = 0.90 with a high degree of correlation between with predicted and observed values.

According to multiple regression analyses regarding experimental information, the mathematical model for chromatographic resolution between D2 and D3 as an independent variable function, was expressed in the following equation:

$$Y_{resolucion} = 2.275 - 0.084x_1 + 0.005x_2 - 0.015x_3 - 0.084x_4 - 0.155x_1^2 - 0.833x_1x_2 - 0.011x_1x_3 - 0.021x_1x_4 - 0.118x_2^2 + 0.011x_2x_3 - 0.056x_2x_4 - 0.135x_3^2 - 0.008x_3x_4 - 0.819x_4^2 (3)$$

The general equation must be derived from partial derivatives, which resulted in four new equations:

$$\frac{\delta(Y_{resolucion})}{\delta(x_1)} = -0.084 - 0.310x_1 - 0.833x_2 - 0.011x_3 - 0.021x_4 (4)$$

$$\frac{\delta(Y_{resolucion})}{\delta(x_2)} = -0.005 - 0.833x_1 - 0.236x_2 - 0.011x_3 - 0.056x_4 (5)$$

$$\frac{\delta(Y_{resolucion})}{\delta(x_3)} = -0.015 - 0.011x_1 + 0.011x_2 - 0.270x_3 - 0.008x_4 (6)$$

$$\frac{\delta(Y_{resolucion})}{\delta(x_4)} = -0.084 - 0.021x_1 + 0.056x_2 - 0.008x_3 - 1.638x_4 (7)$$

The four system equations were resolved by the substitution method. For this method, we found the critical points of each factor in the equation system by response surface, including the following terms: temperature = 20.21°C; %triton X-100: 7.26%; acid concentration: 0.48 M, and Flow: 0.78 mL/min. Under these conditions, chromatographic resolution was 1.57, with a total analysis time of 14 min. However, in an ANOVA test evaluation, temperature and aqueous phases (acid concentration) did not show statistical significance (p<0.05) for the model. Furthermore, these factors have a negative effect on chromatographic resolution, decrease temperature and aqueous phases, and increase resolution. Therefore, we decided to develop the method only with MeOH (thus eliminating the aqueous phases) and employed the lower temperature (15°C) and controlled this. Under these conditions, it was possible to reduce the retention time of 11.5 min to 6.8 min and to increase the chromatographic resolution of 1.57 to 2.14 (Figure 2).

Method validation

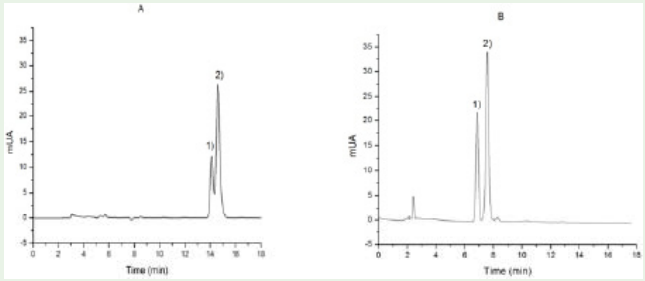

Figure 2: Chromatograms of (A) non-optimized method, and (B) optimized method at a detection wavelength of 294 nm.

Table 3: Results of milk samples to physicochemical parameters test.

Physicochemical properties	Limit allowed	Assay test	Milk sample
Acidity (lactic acid) g/L	Min. 1.3 Max. 1.7	NOM- 155- SCFI- 2012	1.65 g/L
Casein (g/L)	Min. 24	NOM- 155- SCFI- 2012	30.59 g/L
Density at 15°C (g/mL)	Min. 1.029	NOM- 155- SCFI- 2012	1.03 g/mL
Total fat g/L	Min. 30	NMX- F- 311- 1977	36.88 g/L
Refraction index	Min. 1.35	NMX- F- 148- S- 1982	1.35
Lactose (g/L)	Min. 43 Max. 60	NOM- 155- SCFI- 2012	47.28 g/L
pH	Min. 6.5 Max. 6.8	NMX- F- 317- S- 1978	6.56
Total protein (g/L)	Min. 30	NOM- 155- SCFI- 2012	30.96 g/L
Non-fatty solids (g/L)	Min. 83 Max. 89	NMX- F- 026- 1997	85.90 g/L

For the validation method, it was necessary to test the physicochemical parameters from the milk matrix; these parameters were tested according in accordance with the Mexican Normative Method (Table 3). For this Norm, the milk matrix was found at among the established ranges, which was used for recovery assays by fortification, thus performing the validation tests.

The efficiency of the method was considered in terms of linearity, accuracy (% recovery), and precision (% RSD); the experiments were tested in triplicate for intra- and interday evaluation.

Linearity was evaluated by linear regression analysis, starting with analysis of the peak areas, calculated by the least-square regression method (Table 4). Visual inspection revealed a proportional-increase value for the tested concentration, and good linearity between vitamin D concentration and peak areas was found with R2 values, of >0.99. According to the Confidence Interval (CI) for the Student t test, we found that the slope was different from zero and that the intercept was not statistically different from zero.

According to the results for LOD and LOQ, we found that the method was significantly more sensitive for vitamin D2 than for vitamin D3; furthermore, a highest absorption of λ=294 nm under UltraViolet (UV) conditions was found for both vitamins. Vitamin

Table 4: Linearity, LOD, LOQ, precision and accuracy of vitamin D2 and D3.

Parameter	D_2				D_3			
t_R	6.855				7.292			
$b \pm t_{(n-2)}S_b$	6.789±0.002				10.091±0.003			
$a \pm t_{(n-2)}S_a$	0.193±0.258				-0.275±0.495			
$S_{y/x}$	2.993				3.285			
R	0.9998				0.9997			
LOD (μg/ mL)	0.764				2.548			
LOQ (μg/ mL	0.564				1.881			
Accuracy/ Precision	**Intra-day**		**Inter- day**		**Intra- day**		**Inter- day**	
μg/ mL	%R	R.S.D	%R	R.S.D	%R	R.S.D	%R	R.S.D
50	82.8	12.1	84.6	9.1	94.7	5.1	81	2.1
100	93.7	5.25	92.5	6.6	99	0.86	96.2	3.1
500	93.2	0.96	93.4	1.1	90.5	0.14	90.5	0.2

Citation: Araujo-León JA, Torrez-Gámez J, Polanco-Rodríguez A and Ruiz-Ciau DV. Development and Optimization of a High-Performance Liquid Chromatography Method for Resolution of Two Liposoluble Vitamins: Ergocalciferol (vitamin D2) and Cholecalciferol (vitamin D3) in Milk Samples. Ann Chromatogr Sep Tech. 2017; 3(1): 1029.

D2 had one instauration more than vitamin D3, which allows better radiation absorption. Similar results for LOD and LOQ have been reported in the literature for High-Performance Liquid Chromatography-UltraViolet Diode Array detector (HPLC-UV-DAD) [21]. However, currently, new techniques, such as HPLC-Mass Spectrometry (MS), have demonstrated better results for LOD. With the working range reported in the present paper, it is possible to determine and quantify vitamins D2 and D3 reliably according to the fortification standards present in the Mexican Normative method.

The intraday average recoveries were 90.03 and 92.45%, with an RSD of 5.85 and 3.81% for D2 and D3, respectively. In the interday evaluation, average recoveries were 91.98 and 89.23%, with an RSD of 1.91 and 1.80%, respectively, for vitamins D2 and D3. The data obtained were compared with those reported by the AOAC, and Horwitz function provisions [22] for recovery was observed, and accurately determined that the values found were within the acceptance range of the concentrations evaluated. With the data obtained by the Student t test, we determined whether there were statistically significant differences (p 0.05), and we also determined that there was no significant statistical evidence to reject the null hypothesis (H_0:μ_1=μ_2) in both vitamins. There are some reports in the literature on the recovery of fat-soluble vitamins [11,23,24], in which the authors have reported similar results; however, their evaluations were carried out by different extraction methods, mainly involving saponification as first step to remove fatty acids.

Conclusion

The method developed in this work is appropriate as an alternative in routine analysis to ensure quality control for traditional methods in the Mexican normative method with regard to in the quantification of vitamins D2 and D3 in smaller amounts (5 mL) of milk samples and can replace the aggressive (saponification) extraction techniques and significantly reduce the amount of extraction organic-solvent extraction. Validation results demonstrated that the method has good linearity, sensitivity, and recovery percentages, with >90%. Thus, the method developed complies with international guides for the determination of D2 and D3 vitamins.

Acknowledgement

This work was funded by “Desarrollo de cuerpos académicos” support PIFI 2013.

References

1. Goudarzi N, Farsimadan S, Chamjangali MA, Bagherian GA. Development of coupled ultrasound-assisted and reversed-phase dispersive liquid–liquid microextraction before high-performance liquid chromatography for the sensitive determination of vitamin A and vitamin E in oil samples. J Sep Sci. 2015; 38: 3254- 3261.
2. Karaźniewicz- Lada, M Glówka A. A review of chromatographic methods for the determination of water- and fat-soluble vitamins in biological fluids. J Sep Sci. 2016; 39: 132-148.
3. Yamada H, Kitagawa S, Ohtani H. Simultaneous separation of water- and fat-soluble vitamins in isocratic pressure-assisted capillary electrochromatography using a methacrylate-based monolithic column. J Sep Sci. 2013; 36: 1980-1985.
4. Schoor vNM, Lips P. Worldwide vitamin D status. Best Pract Res Clin Endocrinol Metab. 2011; 25: 671-680.
5. Byrdwell WC. Extract–filter–shoot liquid chromatography with mass spectrometry for the analysis of vitamin D_2 in a powdered supplement capsule and standard reference material 3280. J Sep Sci. 2014; 37: 2095-2110.
6. Calderon-Garciduenas L, Franco-Lira M, D'Angiulli A, Rodriguez-Diaz J, Blaurock-Busch E, Busch Y, et al. Mexico City normal weight children exposed to high concentrations of ambient PM 2.5 show high blood leptin and endothelin-1, vitamin D deficiency, and food reward hormone dysregulation versus low pollu- tion controls. Relevance for obesity and Alzheimer disease. ENVIRON RES. 2015; 140: 579-592.
7. http://www.coneval.gob.mx/Medicion/MP/Paginas/Lineas-de-bienestar-y-canasta-basica.aspx
8. http://www.dof.gob.mx/normasOficiales/4692/seeco/seeco.htm
9. Noguerol-Cal R, López-Vilariño JM, González-Rodríguez MV, Barral-Losada LF. Development of an ultraperformance liquid chromatography method for improved determination of additives in polymeric materials. J Sep Sci. 2007; 30: 2452-2459.
10. Bilodeau L, Dufresne G, Deeks J, Clément G, Bertrand J, Turcotte S, et al. Determination of vitamin D3 and 25-hydroxyvitamin D3 in foodstuffs by HPLC UV-DAD and LC–MS/MS. J Food Compos Anal. 2011; 24: 441-448.
11. Jakobse J, Saxholt E. Vitamin D metabolites in bovine milk and butter. J Food Compos Anal. 2009; 22: 472-478.
12. Hymoller L, Jensen SK. Vitamin D analysis in plasma by high performance liquid chromatography (HPLC) with C30 reversed phase column and UV detection – Easy and acetonitrile-free. J Chromatogr A. 2011; 1218: 1835-1841.
13. Dolan JW. Temperature selectivity in reversed-phase high performance liquid chromatography. J Chromatogr A. 2002; 965: 195-205.
14. Roy D, Kongara S, Valsaraj KT. Application of surfactant solutions and colloidal gas aphron suspensions in flushing napthalene from a contaminated soil matrix. J Hazard Mater. 1995; 42: 247-263.
15. Takeuchi E, Matsuoka K, Ishii S, Ishikawa S, Honda C, Endo K. Solubilization of polycyclic aromatic hydrocarbons in C16E7nonionicsurfactant solutions. Colloids and Surfaces A: Physicochem. Eng. Aspects. 2014; 441: 133-139.
16. Synder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development, John Wiley and Sons: New York. 1997.
17. Kortrikla A, Lekkas TD, J Liq. Chromatogr. RT. 2001; 24: 543-553.
18. Guillaume YC, Peyrin E. Talanta. 2000; 51: 579-586.
19. Draper NR. Technometrics. 1985; 27: 173-180.
20. Draper NR, Lin DKJ. Technometrics. 1990; 32: 187-194.
21. Dabre R, Azad N, Schwämmle, Lämmerhofer M, Linder W. J. Sep. Sci. 2011; 34: 761-772.
22. González AG, Herrador MA. Trends in Analytical Chemistry. 2007; 26: 227-238.
23. Kwak BM, Jeong IS, Lee, MS, Ahn JH, Park JS. Food Chemistry. 2014; 165: 569-574.
24. Kucevorá B, Krcmová L, Solichová D, Plísek J, Solich P. J. Sep. Sci. 2013; 36: 2223-2230.