ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 14, Issue 5, 2021

Online - 2455-3891
Print - 0974-2441

Research Article

DEVELOPMENT AND VALIDATION OF AN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF 17-B ESTRADIOL IN POLYMERIC NANOPARTICLES

ADRIANA YURIKO KOGA[1]*, BRUNA CARLETTO[1], LEANDRO CAVALCANTE LIPINSKI[2], TRAUDI KLEIN[3], PAULO VITOR FARAGO[1]

[1]Postgraduate Program in Pharmaceutical Sciences, Department of Pharmaceutical Sciences, State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil. [2]Department of Medicine, State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil. [3]Department of Pharmaceutical Sciences, State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil. Email: adri_yuriko@hotmail.com

Received: 12 February 2021, Revised and Accepted: 26 March 2021

ABSTRACT

Objective: A simple high-performace liquid chromatography method was developed and validated to determine 17-β estradiol in poly (ε-caprolactone) nanocapsules.

Methods: The chromatographic conditions were as follows: C18 GL column with a mobile phase of acetonitrile:water (92:8 v/v) at flow rate of 1.5 mL/min with detection at 280 nm. The evaluated parameters were specificity, linearity, limits of detection and quantification, precision, accuracy, and robustness.

Results: The method was specific and linear (r=0.9982). The limits of detection and quantification were 5.78 μg.mL^{-1} and 17.54 μg.mL^{-1}, respectively. Suitable accurancy and robustness were obtained. The stability assay showed that pH variation occured after 120 days of storage, and no changes were observed regarding the size and polydispersion parameters. The applicability of the method was evaluated by determining the encapsulation efficiency of the E2 nanocapsules after 120 days of storage. The results showed values >99%.

Conclusion: The results demonstrated the applicability of the developed and validated analytical method.

Keywords: High-performance liquid chromatography, Estradiol, Nanoparticles, Nanotechnology.

 DOI: http://dx.doi.org/10.22159/ajpcr.2021v14i5.41355. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Aging is directly related to structural and functional changes in tissues and organs such as the skin. Physiologically, skin aging is a slow process that is characterized by a decrease in elasticity, collagen synthesis, and an increase in age-related dermatoses such as dryness, wrinkles, and infections [1,2].

17-β estradiol (E2) (Fig. 1) is a steroid hormone that is mainly secreted by granulosa cells in the ovarian follicles. The biosynthesis of this hormone involves the conversion of cholesterol, and it is catalyzed by the aromatase of cytochrome P450 [3,4]. E2 has two types of nuclear receptors: Alpha (ERα) and beta (ERβ). The affinity of estrogenic receptors for E2 is similar; however, expression is prevalent in different tissues [5-7].

E2 is associated with the reproductive cycle of women; however, it is also known that this hormone has important functions in the male reproductive tract, has cardioprotective and wound healing effects, assists in the maintenance of bone density, and helps muscle regeneration [8-11].

A topical application of 17-β estradiol on cutaneous wound healing stimulates epithelization, extracelular matrix deposition, an increase in fibroblasts and keratinocytes, and modulates inflammatory response [12,13].

Advances in nanotechnology have focused on the development, characterization, and application of nanometric systems. Nanoparticles can be obtained by different processes and applied in many areas [14-16].

Polymeric nanoparticles are widely used for drug delivery. They can be divided into nanocapsules and nanospheres. Nanocapsules are colloidal systems that have a polymeric layer around an oil or aqueous nucleous. Nanospheres are composed of a matrix, where the drug is solubilized or dispersed homogeneously [17,18].

Poly (ε-caprolactone) (PCL) is a synthetic polymer widely used to obtain nanoparticles as a drug delivery system [19]. There are no studies in the literature regarding high-performance liquid chromatography (HPLC) methods to quantify 17-β estradiol in PCL nanoparticles. Msigala [20] described an optimized HPLC- ultraviolet (UV) method to detect estrogen in water. The column used was a C-18 with mobile phase of acetonitrile:water (50:50 v/v) and flow rate of 0.7 mL.min^{-1}.

Soranganba and Singh [21] developed an HPLC method for the simultaneous determination of testosterone, 17-β estradiol, and cortisol. They used a C18 column with a multiple-step gradient elution of water:acetonitrile, a flow rate of 1–1.5 mL/min, and a variation in UV detection of 203–242 nm. The validated method was applied to anayze plasma hormone levels in fish.

The development of polymeric nanocapsules containing 17-β estradiol to be applied in biomedicine could represent an efficient alternative for the treatment of various diseases. In this context, the present study describes the development and validation of a simple and low-cost HPLC method to quantify E2 in polymeric nanoparticles.

MATERIALS AND METHODS

Materials

17-β estradiol was purchased from a commercial pharmacy in the city of Ponta Grossa (Paraná, Brazil). HPLC- grade acetonitrile was

purchased from Vetec (Rio de Janeiro, RJ, Brazil). Water was purified in a Milli-Q Plus purification system (Milli-pore, Bedford, MA, USA). All the other reagents and solvents were of analytical grade.

Equipment and chromatographic conditions
The HPLC analyses were performed using a Merck-Hitachi Lachrom (Tokyo, Japan) L-7100 pump with D-7000 Interface, UV detector module, integral degasser, controller software (Chromquest), and manual injector (Rheodyne) equipped with 20 µl injector loop and 100 µl syringe (Hamilton, Microliter 710, Switzerland).

Chromatographic separation was performed using an Inertsil® C18 GL ODS3 (Torrance, CA, USA) reverse phase analytical column (150 mm×4.6 mm, 5 µm). The isocratic mobile phase consisted of acetonitrile and water (92:8, V/V) at a flow rate of 1.5 mL/min, and the detection was performed at 280 nm.

Preparation of standard and sample solutions
The 17-β estradiol standard stock solution was prepared in methanol solvent at 500 µg.mL^{-1}. Dilutions with the mobile phase were performed to obtain solutions with concentrations from 70 to 220 µg.mL^{-1}. These solutions were filtered through a polytetrafluoroethylene (PTFE) membrane filter (Cromafil® Xtra, 0.45 µm×25 mm, Macherey-Nagel GNBH & Co. KG, Duren, Germany) before injection into the HPLC system.

Preparation of polymeric nanoparticles
The 17-β estradiol nanoparticles were prepared using the preformed polymer method. To obtain the organic phase, 100 mg of PCL was dissolved in acetone and then 0.077 g of Span® 80, 50 mg of 17-β estradiol, and 0.33 of chain trygliceride medium were added. The aqueous phase was prepared with 0.077 g of Tween® 80 and 53 mL of distilled water. The organic phase was slowly added in aqueous solution under agitation at 40°C. The nanoemulsion was kept under agitation for 10 min. The organic solvent was subsequently removed by evaporation under reduced pressure at 40°C, resulting in a concentrated sample (10 ml).

Method validation
For the validation of this analytical method, the guidelines established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [22], and the Brazilian regulation RDC 166/2017 of the National Health Surveillance Agency [23] were followed. The following parameters were assessed: Selectivity, linearity, detection and quantification limits, precision, accuracy, and robustness.

Specificity
Specificity assesses the ability of a method to identify an analyte in the presence of impurities. This test was carried out using nanocapsule formulations, both with and without 17-β estradiol.

Linearity
The linearity was assessed using six concentration levels: 70, 100, 130, 160, 190, and 220 µg.mL^{-1}. The slope and other statistics in relation to the calibration curves were calculated by linear regression and analysis of variance (ANOVA). The calibration curve was fitted by linear regression and each point was performed in triplicate.

Limits of detection (LOD) and quantification (LOQ)
The LOD and LOQ were obtained using the standard deviation (SD) and the slope (S) of the calibration curve based on Equations 1 and 2, respectively.

$$LOD: \frac{3.3 \times SD}{S} \quad (1)$$

$$LOQ: \frac{10 \times SD}{S} \quad (2)$$

Precision
This parameter expresses the precision of results under the same conditions in a short period of time. Intermediate precision evaluates intraday and interday variation, and different analysts.

For the repeatability, three concentrations (100.0, 160.0, and 220.0 µg.mL^{-1}) were each evaluated in triplicate. The intermediate precision was evaluated by the SD and relative SD (RSD) of six injections at 100 µg.mL^{-1}.

Accuracy
The determination of accuracy by the recovery assay was performed by adding an exact amount of 17-β estradiol to a known sample solution (5 µg.mL^{-1}), resulting in concentrations of 90, 120, and 180 µg.mL^{-1}. The analyses were performed in triplicate and the results were expressed as the percentage of recovery.

Robustness
The robustness analysis was performed with the samples at 90, 160, and 220 µg.mL-1 by variations in flow (1495 and 1505 mL.min-1) and concentrations of the mobile phase to 93:7 (V/V) and 91:9 (V/V). To assess the influence of the variations, the analysis of variance (ANOVA) followed by Tukey's test was performed. The results were analyzed using the RSD to compare the values of the standard conditions.

Method applicability
Determination of encapsulation efficiency (EE) in 17- β *estradiol nanoparticles*
The quantification of the encapsulated 17-β estradiol was performed by the indirect method, which determines the concentration of a non-encapsulated drug. A 500 µg aliquot of the nanocapsule suspension was subjected to ultrafiltration/centrifugation using Amicon® 10,000 Mw (Milipore) equipment at 14,000 rpm for 10 min. The amount of 17-β estradiol was determined (in triplicate) in the ultrafiltrate using a previously validated method.

The EE (%) was calculated by the difference between the total and free drug concentration (Equation 3).

$$EE: \frac{\text{Theoretical 17}\beta\text{estradiol} - \text{Free 17}\beta\text{estradiol}}{\left(\text{Theoretical 17}\beta\text{estradiol}\right) \times 100} \quad (3)$$

The theoretical 17-β estradiol corresponded to the amount initially added to the formulation and the free 17-β estradiol that was not incorporated in the nanocapsules. Quantification was expressed by the mean±SD.

Stability of the nanocapsules
The stability of the nanocapsules was evaluated at 0, 30, 60, 90, and 120 days. Analyses of pH, particle size, zeta potential, and polydispersion were performed. After 120 days of storage, the encapsulation of E2 was evaluated using the HPLC validated method.

Statistical analysis
The data of validation were evaluated by linear regression, analysis of variance (ANOVA), Tukey's test, and the other validation parameters (mean, SD, and relative SD) using GraphPad Prism 6.0 software.

RESULTS AND DISCUSSION

Method validation
After running in an exploratory gradient, the proportion of the mobile phase acetonitrile:water (92:8) (V/V) and flow of 1.5 mL.min-1 were defined as the standard conditions to perform the other analyses. The running time was determined as 6 min, which was adequate for the laboratory analysis and provided a symmetric peak for the 17-β estradiol with a lower retention time.

The short analysis time (6.0 min) and retention time (2.5 min) were very suitable for routine analysis.

Specificity
The specificity was demonstrated by the comparison between the chomatogram of the unloaded nanoparticles and the 17-β estradiol (Fig. 2). The results showed that there was no interference in the area under the curve of the E2 from the other components used in the nanoparticle formulation. This data showed that the proposed method was specific.

Linearity
The linearity was evaluated using six concentration levels: 70, 100, 130, 160, 190, and 220 μg.mL-1. A linear relationship was observed between the peak area and the 17-β estradiol concentration, which produced a linear equation y = 4623.8403x – 20193.

According to the Analytical Methods Committee, adjustment must be applied or tested (lack of it) if the value of the correlation coefficient closes to 1 does not indicate the result of a linear relationship [24]. The negative value of b (−2019.3) was in the confidence interval of the calibration curve by the ANOVA test. The results of the linearity parameters are summarized in Table 1.

The F value for lack of fit was less than the tabulated F value for the 95% confidence level and therefore the linear regression showed no lack of fit (Table 2). The DPR of the inclination was 0.86%, which was less than that proposed by the ICH [22] and ANVISA [23], namely, 5%.

Limit of detection (LD) and limit of quantification (LQ)
The LOD and quantification are the lowest and highest concentrations that a method can detect and quantify, respectively. The LD was 5.78 $\mu g.mL^{-1}$ and the LQ was 17.54 $\mu g.mL^{-1}$.

Precision
The precision of a method describes the proximity of the results when a test is applied repeatedly. The results of the intermediate precision and repeatability (Table 3) showed RDS values lower than 5.0%, which was established by the RDC 166/2017, confirming the precision of the method.

Accuracy
The accuracy of a method determines the proximity of agreement between the value obtained in a test and the theoretical value of an analyte in a sample [22]. The mean±SD (RSD) values obtained were 89.79±2.65 (2.95); 119.59±2.99 (2.52); and 178.40±1.44 (0.81) for 90, 120, and 180 $\mu g.mL^{-1}$, respectively. The results showed that the method was accurate.

Table 1: Analytical method parameters for quantification of 17-β estradiol in Poly (ε-caprolactone) nanocapsules

Linearity	
Linear range ($\mu g.mL^{-1}$)	70–220
Detection limit ($\mu g.mL^{-1}$)	5.78
Quantification limit ($\mu g.mL^{-1}$)	17.54
Regression data	
n	3
Slope (a)	4.6238
Standard deviation of slope	39.82
Relative standard deviation of slope (%)	0.86
Intercept (b)	−20193
Correlation coeficient (r)	0.9982

Table 2: ANOVA results for linearity of the method

	SS	DF	MS	F	Ftab
Model	8.146896E+06	1	8.146896E+06	0.036	3.048
Residual	3.601018E+09	16	225063624	Linear	
Lack of fit	416385716	4	104096429	0.3922	2.48
Pure error	3.184632E+09	12	265386023	No lack of fit	

SS: Sums of squares, DF: Degrees of freedom, MS: Mean squares, F: F value of the test, Ftab: Fixed F value

Robustness
The robustness of a method indicates its capacity to remain unaffected under small variations in parameters and demonstrates its reliability during a test. The method was considered robust because no difference was detected ($p<0.05$) when the mobile phase and flow were altered. The results are presented in Table 4.

EE
The evaluation of the drug content and EE of the 17-β estradiol in the nanocapsules were carried out using the previously validated HPLC method. The formulations presented excellent EE with values higher than 99%. The theoretical concentration was about 5000 $\mu g.mL^{-1}$ and the experimental result was 4989.11±0.1672. This value correponded to 99.8%. The relative SD was 0.03%.

Yilmaz and Kadioglu [25] described the validation of an HPLC method to quantify E2 in samples for hormone replacement. The mobile phase used was methanol:water (70:30) in a C18 reverse phase column (Merck, Darmstadt, Germany) with 5 μm particles and 20 μl volume injection. Fluorescence detector was used and it was possible to quantify the drug content in the samples.

Other authors have described the validation of differents methods to quantify 17-β estradiol in waste water and rivers [26,27]. However, there are no validated methods in the literature regarding quantifying 17-β estradiol in polymeric nanoparticles.

Table 3: Repeatability and intermediate precision data for 17-β estradiol analysis

Precision		Theoretical concentration ($\mu g.mL^{-1}$)	Experimental concentration ($\mu g.mL^{-1}$) – Mean±SD*	RSD (%)**
Repeatability		100	100.8±0.70	0.70
160		155.8±2.83	1.83	
220		217.3±3.92	1.81	
Intermediate precision	Intraday (n=3)	100	101.96±1.67	1.64
	Interday (n=3)	100	102.72±0.70	0.69
	Different analysts (n=3)	100	103.0±0.27	0.27
	Mean±SD*	100	101.98±0.71	0.70

SD*: Standard deviation, RSD**: Relative standard deviation

Table 4: Robustness parameters for 17-β estradiol analysis

Parameters	Theoretical concentration ($\mu g.mL^{-1}$)	Experimental concentration ($\mu g.mL^{-1}$) – Mean±SD*	DPR** (%)
Flow 1.495 $mL.min^{-1}$	100	103.55±2.21	2.16
	160	161.03±2.38	1.48
	220	221.92±2.14	0.96
Flow 1.505 $mL.min^{-1}$	100	103.02±2.51	2.43
	160	161.29±2.30	1.43
	220	220.88±2.35	1.07
Mobile phase 93:7 (V/V)	100	103.93±2.54	2.44
	160	161.87±2.11	1.31
	220	222.98±5.01	2.25
Mobile phase 91:9 (V/V)	100	101.60±0.75	0.73
	160	163.13±3.44	2.11
	220	256.64±41.4	16.16

Stability analysis

All the formulations had milky and opaque aspects with a blue reflection due to the Brownian motion of the nanoparticles.

pH determination

The pH evaluations of the nanoparticle solutions made on 0, 30, 60, 90, and 120 days are set out in Fig. 3. All the results differed statistically from 0 to 120 days, and the E2 samples stored at 10°C showed little variation after 30 days of storage.

There was a greater pH variation in the samples of nanoparticles without the drug. This result can be explained due to the exposure of a greater number of terminal carboxylic acid groups resulting from the hydrolysis of the ester bond present in the PCL structure [28].

Some factors, such as pH, ionic concentration, temperature, and storage time, can influence the stability of particles. The pH of the solution can influence the nanoparticle surface load, which can lead to variations in the polydispersity and homogeneity of the sample [29]. The results showed that the samples stored at 10°C presented the best pH stability.

There was no statistical difference in relation to the nanoparticle size during the 120 days. This suggests a high level of stability of the samples at various temperatures. This is an important parameter because size is essential for the anticipated drug action.

The zeta potential varied from −31 to −36 mV, and from −29 to −37 mV for E2 and PCL at 25°C, respectively. At 10°C, there was a variation from −28 to −19 mV for E2 and from −29 to −23 mV for PCL. At 37°C, the range was from −33 to −21 for E2 and −36 to −19 mV for PCL. There was no significant difference in the evaluation of the zeta potential, confirming adequate stability for the nanoparticles.

The high magnitude of the zeta potential corresponded to greater electrostatic repulsion and, consequently, adequate stability. The agglomeration of nanoparticles can occur due to factors such as high concentrations of particles in solutions, thermodynamic conditions, and variations in pH [30].

The analysis of polydispersion showed values lower than 0.14 for all the samples evaluated at 10°C, 25°C, and 37°C. The results showed that the nanoparticles remained homogeneous during the 120 days of storage. The variation in the polydispersion values indicates the aggregation or breaking of particles, resulting in size variations [31].

The results were in accordance with results shown by Alex *et al.* [32], who described the stability of PCL carboplatin-loaded nanoparticles. After 3 months of storage at room temperature, there was no difference in zeta potential, size, or polydispersion of the particles.

Melo *et al.* [33] described the stability of PCL nanoparticles containing articain; they evaluated pH, size, and polydispersion for a period of 120 days. The results showed a decrease in pH values and and an increase in polydipersion, suggesting polymer hydrolysis, and aggregation of particles. There was no change in particle size.

The evaluation of the E2 EE after 120 days of storage showed values higher than 99% for all the analyzed temperatures. The results are shown in Table 5 and indicate appropriate stability.

Table 5: Encapsulation efficiency of 17-β estradiol nanoparticles after 120 days of storage at different temperatures

Temperature (°C)	Theoretical concentration (μg.mL^{-1}±SD*)	Experimental concentration (μg.mL^{-1}±SD*)	Encapsulation efficiency ± SD
25	5000	4993.27±0.27	99.8±0.01
10	5000	4994.33±0.51	99.7±0.04
37	5000	4986.21±1.96	99.8±0.01

The validated method was successfully applied to determine E2 in PCL nanocapsules. This method fulfilled all the parameters regarding reliability and could be applied for others assay involving E2 quantification.

CONCLUSION

A simple and effective HPLC method was validated to quantify E2 in PCL nanocapsules. This method was linear, specific, accurate, precise, and robust according to the RDC 166/2017 [23] and ICH guidelines [22]. The samples showed pH variation that may have been related to the degradation of the polymer; there were no changes in the size or polydispersion parameters. The method was effective to determine the EE of E2 in nanocapsules stored for 120 days.

ACKNOWLEDGMENTS

The authors are grateful to CLABMU-UEPG and LAPMED for technical support.

AUTHOR CONTRIBUTIONS

Adriana Yuriko Koga, Traudi Klein, and Paulo Vitor Farago designed and developed the study. Bruna Carletto and Leandro Cavalcante Lipinski contributed to the statistical analysis and revised the manuscript. Paulo Vitor Farago supervised the study. All the authors approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' FUNDING

This research received no external funding.

REFERENCES

1. Blume-Peytavi U, Kottner J, Sterry W, Hodin MW, Griffths TW, Watson RE, *et al*. Age-associated skin conditions and diseases: Current perspectives and future options. Gerontologist 2016;56:230-42.
2. Tobin DJ. Introduction to skin aging. J Tissue Viability 2017;26:37-46.
3. Gao WL, Wu LS, Zi JH, Wu B, Li YZ, Song YC, *et al*. Measurement of serum estrogen and estrogen metabolites in pre- and postmenopausal women with osteoarthritis using high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. Braz J Med Biol Res 2015;48:146-53.
4. Fuentes N, Silveyra P. Estrogen receptor signaling mechanisms. Adv Protein Chem Struct Biol 2019;116:135-70.
5. Seleit I, Bakry OA, Repey HS, Ali R. Intrinsic versus extrinsic aging: A histopathological, morphometric and immunohistochemical study of estrogen receptor β and androgen receptor. Skin Pharmacolol Physiol 2016;29:178-89.
6. Yasar P, Ayaz G, User SD, Gupur G, Muyan M. Molecular mechanism of estrogen-estrogen receptor signaling. Reprod Med Biol 2017;16:4-20.
7. Rzepecki AK, Murase JE, Juran R, Fabi SG, McLellan BN. Estrogen-deficient skin: The role of topical therapy. Int J Womens Dermatol 2019;5:85-90.
8. Cooke PS, Nanjappa MK, Ko C, Prins GS, Hess RA. Estrogens in male physiology. Physiol Rev 2017;97:995-1043.
9. Mondockova V, Adamkovicova M, Lukacova M, Grosskopf B, Babosova R, Galbavy D, *et al*. The estrogen receptor 1 gene affects bone mineral density and osteoporosis treatment efficiency in Slovak postmenopausal women. Med Genet 2018;19:2-13.
10. Mahmoodzadeh S, Dworatzek E. The role of 17β-estradiol and estrogen receptors in regulation of Ca2 + channels and mitochondrial function in cardiomyocytes. Front Endocrinol 2019;10:1-15.
11. Liao ZH, Huang T, Xiao JW, Gu RC, Ouyang J, Wu G, *et al*. Estrogen signaling effects on muscle-specific immune responses through controlling the recruitment and function of macrophages and T cells. Skelet Muscle 2019;9:20.
12. Lobo RA. Menopause and aging. In: Yen and Jaffe's Reproductive Endocrinology. Amsterdam: Elsevier; 2009. p. 325-55.
13. Brufani M, Rizzi N, Meda C, Filocamo L, Ceccaci F, Bartolli VG, *et al*. Novel locally active estrogens accelerate cutaneous wound healing-part

2. Sci Rep 2017;7:1-36.
14. Ealias AM, Saravanakumar MP. A review on the classification, characterisation, synthesis of nanoparticles and their application. Mater Sci Eng 2017;263:2-14.
15. Radwan F. Nanotechnology and medicine. Mater Sci Nanotechnol 2018;2:10-1.
16. Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: History, sources, toxicity and regulations. Beilstein J Nanotechnol 2018;9:1050-74.
17. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B Biointerfaces 2010;75:1-18.
18. Jarai BM, Kolewe EL, Stillman ZS, Raman N, Fromen CA. Polymeric nanoparticles. In: Nanoparticles for Biomedical Applications. ; 2020. p. 304-23.
19. Azimi B, Nourpanah P, Rabiee M, Arbab S. Poly (ε-caprolactone) fiber: An overview. J Eng Fiber Fabr 2014;9:74-90.
20. Msigala SC, Mabiki FP, Styrishave B, Mdegela RH. Optimized HPLC-UV method for separation, detection and quantification of endocrine disrupting estrogens in low quality water. Int J Chem 2017;9:19-27.
21. Soranganba N, Singh IJ. Simultaneous determination of fish steroidal hormones using RP-HPLC with UV detection by multi-step gradient elution technique. Int J Curr Microbiol App Sci 2018;7:1397-404.
22. ICH. International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use guidelines, Validation of analytical procedures: Text and Methodology, Q2(R1). Vol. 1. Geneva: ICH; 2005.
23. Agencia Nacional de Vigilância Sanitária. ANVISA, Resolução No. 166, de 24 de Julho de 2017. Diário Oficial [da] República Federativa do Brasil, Brasília, DF; 2017.
24. Rawski RI, Sanecki PT, Kijowska KM, Skital PM, Saletnik DE. Regression analysis in analytical chemistry. Determination and validation of linear and quadratic regression dependencies. S Afr J Chem 2016;69:166-73.
25. Yilmaz B, Kadiouglu Y. Determination of 17 b-estradiol in pharmaceutical preparation by UV spectrophotometry and high performance liquid chromatography methods. Arab J Chem 2017;10:1422-8.
26. Liz MV, Amaral B, Stets S, Nagata N, Peralta-Zamora P. Sensitive estrogens determination in wastewater samples by HPLC and fluorescence detection. J Braz Chem Soc 2017;28:1453-60.
27. Moraes FC, Rossi B, Donatoni MC, Oliveira KT, Pereira EC. Sensitive determination of 17 b-estradiol in river water using a graphene based electrochemical sensor. Anal Chim Acta 2015;881:37-43.
28. Loh XJ. The Effect of pH on the hydrolytic degradation of poly (e-caprolactone)-block-poly (ethylene glycol) copolymers. J Appl Polym Sci 2012;127:1-11.
29. Xu L, Liang H, Yang Y, Yu S. Stability and reactivity: Positive and negative aspects for nanoparticle processing. Chem Rev 2018;118:3209-50.
30. Selvamani V. Stability studies on nanomaterials used in drugs. Characterization and Biology of Nanomaterials for Drug Delivery. Amsterdam: Elsevier; 2019. p. 425-44.
31. Souza PM, Lobo FA, Rosa AH, Fraceto LF. Desenvolvimento de nanocápsulas de poli-e-caprolactona contendo o herbicida atrazina. Quim Nova 2012;35:132-7.
32. Alex AT, Joseph A, Shavi G, Rao JV, Udupa N. Development and evaluation of carboplatin-loaded PCL nanoparticles for intranasal delivery. Drug Deliv 2014;23:2144-53.
33. Melo NF, Campos EV, Franz-Montan M, Paula E, Silva CM, Muruyama CR, *et al*. Characterization of articaine-loaded poly (ε-caprolactone) nanocapsules and solid lipid nanoparticles in hydrogels for topical formulations. J Nanosci Nanotechnol 2018;18:4428-38.