BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902017000300041

Article

Development and validation of a stability indicating HPLC method to determine diltiazem hydrochloride in tablets and compounded capsules

Mateus Araújo Castro e Souza[1]*, Carlos Eduardo de Oliveira Pereira[1], Fernando Henrique Andrade Nogueira[2], Gerson Antônio Pianetti[1]

[1]Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil, [2]Departamento de Farmácia, Centro de Ciências da Saúde, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil

A stability indicating HPLC method to determine diltiazem hydrochloride (DTZ) in tablets and compounded capsules was developed and validated according to Brazilian and the International Conference on Harmonization (ICH) guidelines. The separation was carried out on a Purospher Star® C18 (150 x 4.6 mm i.d., 5 µm particle size, Merck Millipore) analytical column. The mobile phase consisted of a 0.05% (v/v) trifluoroacetic acid aqueous solution and a 0.05% trifluoroacetic acid methanolic solution (44:56, v/v). The flow rate was 1.0 mL.min^{-1} with a run time of 14 minutes. The detection of DTZ and degradation products (DP) was performed at 240 nm, using a diode array detector. The method proved to be linear, precise, accurate, selective, and robust, and was adequate for stability studies and routine quality control analyses of DTZ in tablets and compounded capsules.

Keywords: Diltiazem hydrochloride/compounded capsules. Diltiazem hydrochloride/tablets. High performance liquid chromatography /method validation. Stability indicating method.

INTRODUCTION

Diltiazem hydrochloride (DTZ), chemically known as 1,5-benzothiazepin-4(5*H*)-one, 3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-, monohydrochloride (Figure 1), molecular mass of 450.98 g.mol^{-1}, is commonly prescribed to treat hypertension and angina pectoris, exerting its effects by promoting arterial smooth muscle relaxation, a reduction in coronary vascular resistance, and an increase in blood flow to the coronary arteries (Brunton, Lazo, Parker, 2006).

Diltiazem is almost completely absorbed by the gastrointestinal tract and undergoes extensive first-pass hepatic metabolism. The maximum plasma concentration is reached about three to four hours after the administration of diltiazem tablets (Sweetman, 2009).

DTZ is commercially available in tablet, extended-release capsule, extended-release tablet, suspension, oral solution, and intravenous injection dosage forms (AHFS, 2004; USP, 2014). Furthermore, diltiazem can also be compounded in specific pharmacies. These compounding pharmacies usually produce DTZ in immediate-release capsule dosage form. In addition, in Brazil, compounding pharmacies are allowed to produce capsules that contain the same strength of commercial products.

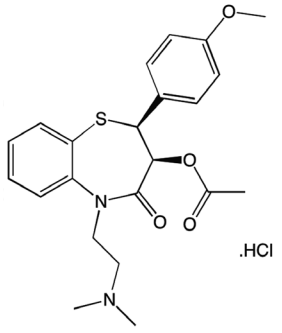

FIGURE 1 - Chemical structure of diltiazem hydrochloride (DTZ).

To date, there are no pharmacopeial monographs for the analysis of diltiazem in compounded capsules, only for

***Correspondence:** M. A. C. Souza. Departamento de Produtos Farmacêuticos. Faculdade de Farmácia. Universidade Federal de Minas Gerais. Av. Pres. Antônio Carlos 6627, 31270-901, Belo Horizonte, MG, Brazil. Tel: +55 31 34096983; fax: +55 31 34096976. E-mail: mateusarsouza@yahoo.com.br

raw material and tablet, extended-release tablet, extended-release capsule, suspension, and oral solution dosage forms (USP, 2014; British Pharmacopoeia, 2013). There are several HPLC methods reported to determine diltiazem in pharmaceutical dosage forms, but only one is a stability indicating method that is able to determine diltiazem in the presence of its degradation products (Chatpalliwar, Porwal, Upmanyu, 2012). However, the analytical method has a high running time and is incompatible for coupling with a mass detector. Furthermore, there are no stability indicating methods for diltiazem determination in compounded capsules.

This study aimed to develop and validate a simple and selective HPLC method to quantify diltiazem in tablets and compounded capsules containing 30 mg DTZ in the presence of its hydrolytic and oxidative degradation products.

MATERIAL AND METHODS

Chemical, reagents, and material

The diltiazem hydrochloride reference standard (100.2% purity) was obtained from the Brazilian Pharmacopoeia (Brasília, DF, Brazil), DTZ capsules, and placebo capsules were obtained from a compounding pharmacy located in Belo Horizonte, MG, Brazil, while DTZ reference drug and generic tablets were obtained from a drugstore located in Belo Horizonte, MG, Brazil. Ultrapure water was obtained from a Millipore system (Bedford, MA, USA). Methanol (HPLC grade) and sodium hydroxide (analytical grade) were purchased from J. T. Baker (Phillipsburg, NJ, USA); trifluoroacetic acid (HPLC grade) was purchased from Tedia (Fairfield, OH, USA); glacial acetic acid and anhydrous formic acid (analytical grade) were purchased from Sigma-Aldrich (St Louis, MO, USA); and ammonium acetate, hydrogen peroxide, and hydrochloric acid (analytical grade) were purchased from Synth (Diadema, SP, Brazil). Quantitative filter paper was purchased from J. Prolab (São José dos Pinhais, PR, Brazil).

Instrumentation

The HPLC-DAD analyses were carried out in a Thermo Finnigan Surveyor System (San Jose, CA, USA), comprised of a quaternary pump, degasser, autosampler, column oven, and photodiode array detector. The chromatographic separation was performed on a Merck Millipore Purospher Star® C_{18} column (150 x 4.6 mm i.d.; 5 µm particle size) maintained at 30ºC. UV detection was performed at 240 nm. UV spectra from 200 to 400 nm were recorded online for peak identification. The injection volume was 20 µL. The optimized mobile phase was a mixture of a 0.05% (v/v) trifluoroacetic acid aqueous solution and a 0.05% (v/v) trifluoroacetic acid methanolic solution (44:56, v/v) at a flow rate of 1.0 $mL.min^{-1}$. To determine k (retention factor) and t_0 (column dead time) a 0.1% (w/v) uracil solution was injected onto the chromatograph.

The HPLC-ESI-MS/MS analyses were carried out in a Waters system (New Castle, DE, USA), comprised of a 1525 µ binary pump, a 2777 sample manager, a TCM/CHM column oven, and a Quattro LC triple quadrupole mass spectrometer, equipped with an electrospray ion source. Mass Lynx version 4.1 software was used for data acquisition and analysis. The separation was performed on the same column that was employed in the HPLC-DAD method development.

Preparation of solutions

Standard solutions

Approximately 15 mg of diltiazem hydrochloride were accurately weighed and transferred to a 100 mL volumetric flask, followed by the addition of 30 mL of methanol. The flask was sonicated for 10 minutes and filled to the mark with water. An aliquot of 10 mL of the solution was transferred to a 50 mL volumetric flask. The flask was filled to the mark with water. The final solution, with a concentration of 30 $\mu g.mL^{-1}$, was filtered using a PVDF syringe filter with a 0.45 µm pore size.

Capsule samples

The average content weight of 20 capsules containing 30 mg of DTZ was determined and the powder was further homogenized. An amount of powder equivalent to 30 mg of DTZ was transferred to a 100 mL volumetric flask, followed by the addition of 50 mL of methanol. The flask was sonicated for 10 minutes and was filled to the mark with water. The solution was filtered using filter paper. An aliquot of 5 mL of the solution was transferred to a 50 mL volumetric flask. The flask was then filled to the mark with water. The final solution, with a concentration of 30 $\mu g.mL^{-1}$, was filtered using a PVDF syringe filter with a 0.45 µm pore size.

Tablet samples

The average weight of 20 tablets containing 30 mg of DTZ was determined. The tablets were crushed, and the powder was further homogenized. An amount of powder equivalent to 30 mg of DTZ was transferred to a 100 mL

volumetric flask, followed by the addition of 50 mL of methanol. The flask was sonicated for 10 minutes and was filled to the mark with water. The solution was filtered using filter paper. An aliquot of 5 mL of the solution was transferred to a 50 mL volumetric flask. The flask was filled to the mark with water. The final solution, with a concentration of 30 $\mu g.mL^{-1}$, was filtered using a PVDF syringe filter with a 0.45 µm pore size.

Method validation

The method was validated according to the Brazilian Guideline RE N° 899/2003 (ANVISA, 2003), International Conference on Harmonization (ICH) Guidance for industry Q1A R2 Stability testing of new drug substances and products (ICH, 2003) and ICH Guidance for Industry Q2 (R1) Validation of analytical procedures: text and methodology (ICH, 2005).

Selectivity

• *Intrinsic stability evaluation*

Stress tests were performed on DTZ to evaluate its stability in hydrolytic (acid, alkaline, and neutral) and oxidative conditions. The stress tests were conducted similarly to those described by Nogueira *et al*. (2011) for mefloquine hydrochloride and as recommended by the ICH (ICH, 2003).

A DTZ stock solution (0.2 $mg.mL^{-1}$) was prepared in methanol. Aliquots of 5 mL of this solution were transferred to four test tubes. Five mL of hydrochloric acid 0.2 M, 100 µL of sodium hydroxide 0.02 M (and 4.9 mL of water), 5 mL of water and 5 mL of hydrogen peroxide 6% (v/v) were added to each tube, separately. The total volume of the tubes was 10 mL, which was maintained in a water bath at 50°C for 21h. At 0, 1, 2, 3, 6, and 21h, aliquots of 1 mL of the solutions were drawn, and 20 µl were injected onto the chromatograph. The chromatographic conditions were adjusted in order to provide an adequate separation between DTZ and its degradants.

• *Selectivity in relation to the formulation excipients*

Capsules

A sample solution was prepared with the placebo capsules (containing microcrystalline cellulose and lactose monohydrate only), injected onto the chromatograph, and monitored for 30 minutes to evaluate interfering peaks in the diltiazem retention time and possible late eluting peaks. In addition, three solutions containing DTZ, capsule excipients, and diluent, as well as three solutions containing only DTZ and diluent, were prepared. The solutions were injected onto the chromatograph, the content of DTZ was determined and compared using a Student's t test. The statistical significance was set at 5%.

Tablets

Mixtures of excipients used in the manufacture of the tablets were prepared. The composition of the formulations was estimated based on information on the drugs labels and the percentages usually employed to produce tablets (Rowe, Sheskey, Quinn, 2009). A sample solution was prepared with the mixture of excipients and injected onto the chromatograph and monitored for 30 minutes to evaluate interfering peaks in the diltiazem retention time and possible late eluting peaks. In addition, three solutions containing DTZ, the mixture of excipients, and the diluent, as well as and three solutions containing only DTZ and diluent, were prepared. The solutions were injected onto the chromatograph, the DTZ content was determined and compared using a Student's t test. The statistical significance was set at 5%.

Linearity

The linearity of the method was evaluated at five concentration levels, between 50% and 150% of the working concentration (30 $\mu g.mL^{-1}$). A standard stock solution containing 150 $\mu g.mL^{-1}$ of DTZ in methanol was prepared. Aliquots of this solution were diluted in the mobile phase for five different concentrations, corresponding to 50%, 75%, 100%, 125%, and 150% of the working concentration. The solutions were randomly prepared and randomly injected onto the chromatograph.

The assumptions for the employment of the ordinary least squares method were evaluated according to Souza and Junqueira (2005): the verification of the outliers (the Jackknife residuals test), the normality (Ryan Joiner´s test), the homoscedasticity (modified Levene´s test), and the independence of the residuals (Durbin-Watson test). The regression significance and the linearity deviation were evaluated by analysis of variance (ANOVA). The statistical significance was set at 5%.

Accuracy, Intraday, and Interday Precision

Accuracy and precision were evaluated in a placebo contamination procedure. The DTZ working standard was added to the capsule matrix and to the mixture of excipients of the tablets to obtain three concentration levels: 50%, 100% and 150% of the working concentration. Twelve solutions were prepared for each concentration level, and the assay was performed on two separate days, separated by a one-week interval.

The recovery results were analyzed for the presence of outliers, employing the Grubbs method, in a similar manner to that described by Gomes and Souza (2010). Precision was evaluated using the relative standard deviation of the sample assays.

Robustness and System Suitability

Method robustness was assessed by determining the DTZ content in six tablet and capsule sample solutions. Samples were assayed under nominal conditions and by the variation of the following analytical parameters: methanol concentration in the mobile phase (54-58%), flow rate (0.9-1.1 mL.min^{-1}), and oven temperature (25-35 ºC). Obtained data were submitted to statistical analyses (ANOVA test). The assays were performed in sextuplicate. The retention factor, tailing factor, and theoretical plate number were evaluated for five injections of the standard solution in each condition to set the system suitability parameters. The criteria established by the FDA (1994) for the system suitability parameters were used to judge the results.

Detection and quantitation limit

Detection and quantitation limits were estimated using the analytical curve for DTZ and later confirmed by injecting solutions at the estimated concentrations onto the chromatograph. The solutions were diluted and injected. The detection limit and quantitation limit were set at a signal/noise ratio of 3 and 10, respectively.

HPLC-ESI-MS/MS analyses

The LC separation was performed on a Purospher Star® C_{18} column (150 x 4.6 mm i.d.; 5 µm particle size). The oven temperature was maintained at 30°C. The mobile phase consisted of (A) 0.1% formic acid in methanol and (B) 0.1% formic acid in water, and delivered at a flow rate of 1 mL.min^{-1}.

Mass spectrometric detection was performed using an electrospray ion source in the positive ionization mode. Nitrogen was used as both the nebulizing and the desolvation gas. The ion source parameters were: capillary 2.75 kV, extractor 4 V, RF lens 0.4 V, source temperature of 100°C, desolvation temperature of 350 °C, and a DTZ cone of 35 V. The multiplier was set at 650 V.

The total ion current chromatograms (TIC) of diltiazem solutions subjected to degradation were acquired using a full-scan MS mode. To identify degradation products, mass spectra were obtained for each observed chromatographic peak, in the range of 0 to 500 Da.

RESULTS AND DISCUSSION

Intrinsic stability evaluation

Three degradation products were obtained in the experiments which employed acid, neutral, and alkaline reagents. These degradation products were initially named DP1, DP2, and DP3, respectively. Three degradation

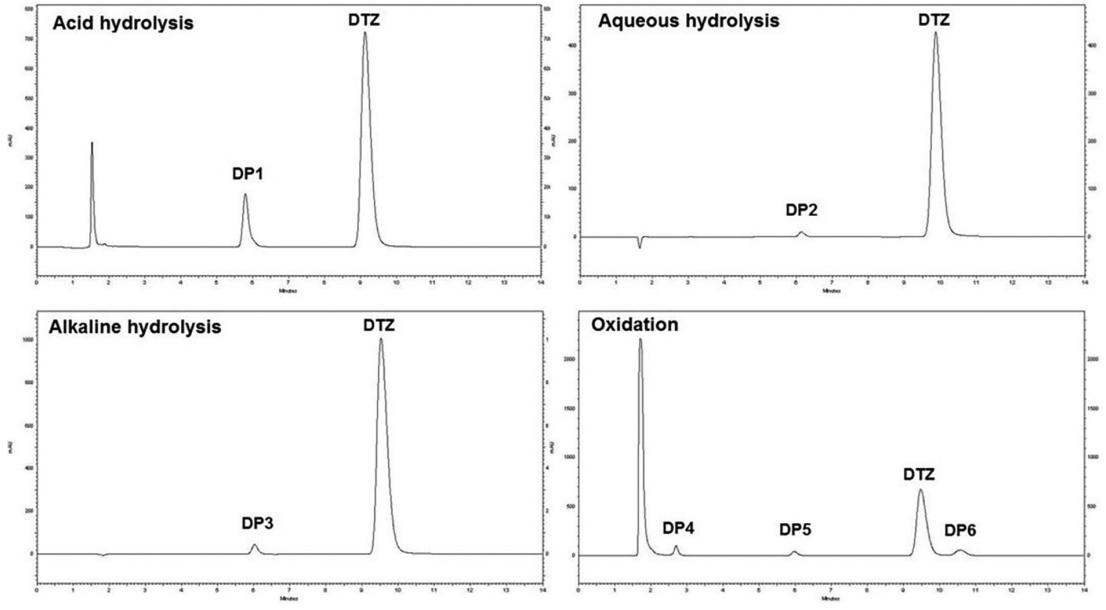

FIGURE 2 - Chromatograms of the sample solution obtained from HPLC-DAD experiments after degradation by acid hydrolysis, aqueous hydrolysis, alkaline hydrolysis and oxidation, where one can see the DTZ peaks (k=4.46) and the degradation products 1 (k=2.47), 2 (k=2.93), 3 (k=2.60), 4 (k=0.61), 5 (k=2.71), and 6 (k=5.75).

products, named DP4, DP5, and DP6, were obtained in the oxidative experiment. The chromatograms of the degradation experiments are shown in Figure 2.

The degradation products DP1, DP2, DP3, and DP5 presented similar retention factors and UV spectra. Moreover, the UV spectra of the degradation products were similar to the diltiazem spectrum. HPLC-ESI-MS/MS was employed to identify the degradation products.

The HPLC-ESI-MS/MS analyses

The mass spectrum of a standard DTZ solution (500 ng.mL^{-1}) was recorded using direct injection onto the mass spectrometer. The protonated molecule $[M+H]^+$ presented an m/z of 415. The solutions subjected to forced degradation were analyzed using HPLC-ESI-MS/MS and the mass spectra of the degradation products were recorded. The degradation products DP1, DP2, DP3, and DP5 presented a protonated molecule $[M+H]^+$ of 373, which suggests that they are the same compound, since their retention factors were also similar.

It can be observed, in diltiazem hydrochloride chemical structure, that there is a group which can suffer hydrolysis – the ester carbonyl. Moreover, the loss of this group and the addition of one hydrogen atom results in a molecule with a molecular mass of 372 g.mol^{-1}, which agrees with the m/z obtained for DP1, DP2, DP3, and DP5. British Pharmacopoeia describes a DTZ impurity (Impurity F) as having a molecular mass of 372 g.mol^{-1} and one less carboxyl group than diltiazem (British Pharmacopoeia, 2013). These findings made us believe that the main degradation product of diltiazem is the impurity F. The degradation scheme, as well as the possible chemical structure of the degradation products DP1, DP2, DP3, and DP5, are shown in Figure 3.

The other degradation products obtained in the oxidation experiment (DP4 and DP6) were not detected in the HPLC-ESI-MS/MS analysis, probably due to their low concentration.

Among the many analytical methods available in the literature, only one is a stability indicating method, in other words, it is capable of assaying DTZ in the presence of its degradation products (Chatpalliwar, Porwal, Upmanyu, 2012). However, this method presents a run time of 80 minutes. The analytical method developed in our work employed real samples and was capable of separating DTZ from its degradation products within a significantly lower run time (14 minutes). Furthermore, its mobile phase is compatible with tandem mass detection, which can be useful in further studies conducted with DTZ.

Analytical method validation

Selectivity

No interfering peaks were observed in the DTZ retention time and no late eluting peaks were observed in the runs performed with placebo solutions. In addition, no statistical difference ($p = 0.21$ for compounded capsules, $p=0.86$ for reference drug, and $p = 0.66$ for generic drug, being the last two tablet formulations) was found between the sample solutions with and without added excipients, proving that the latter does not interfere in the quantitative analysis of diltiazem.

The intrinsic stability evaluation demonstrated that the analytical method is capable of determining diltiazem in the presence of its degradation products, proving to be selective to determine diltiazem in compounded capsules and tablets.

Linearity

After having performed the statistical tests, it was

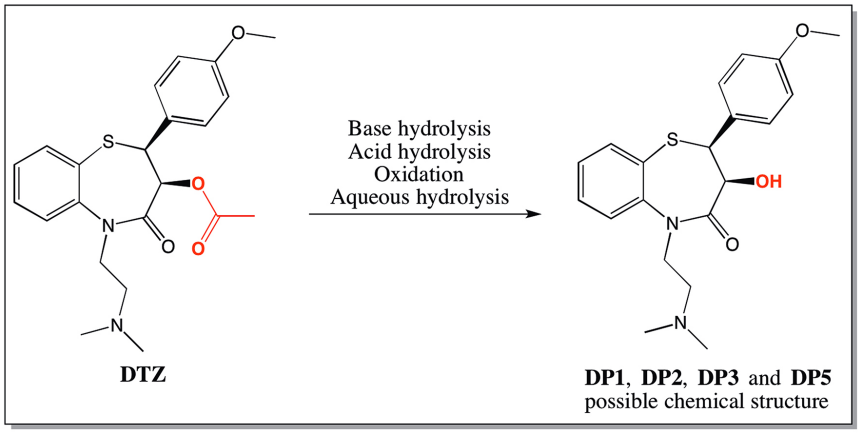

FIGURE 3 - Degradation scheme of DTZ and the possible chemical structure of the degradation products DP1, DP2, DP3, and DP5.

observed that the data is in agreement with all of the least squares method assumptions. Three outliers were excluded, and this number is in accordance with the rule of excluding a maximum of 2/9 of the data (Souza, Junqueira, 2005).

The residues presented random distribution, the regression was statistically significant ($p<0.01$), and no lack of adjustment to the linear model was observed ($p = 0.32$). The correlation coefficient was higher than 0.99, as required (ANVISA, 2003; FDA, 1994). The regression analysis data are shown in Table I.

TABLE I - Overview of the linearity data for diltiazem

Regression Parameter	Result
r^2	0.9995
Slope ± SE	307.46 ± 2.12
Intercept ± SE	-473.47 ± 68.29
Concentration range (μg.mL^{-1})	15.0 - 45.0
Number of points	5

Accuracy, Intraday, and Interday Precision

The mean recovery for the concentration levels of 50%, 100%, and 150% after the removal of outliers are shown in Table II. Mean recoveries are within the 98% to 102% range, which is in agreement with the literature (Green, 1996).

With respect to the method precision, the data followed normal distribution and were homoscedastic. The relative standard deviation for the intraday and interday precision are also described in Table II.

The relative standard deviation values were lower than 5%, as established by Brazilian law (ANVISA, 2003). It is suggested in the literature that the RSD values for intraday precision should be below 2% (Green, 1996). The RSD values obtained in the interday precision were also close to 2%.

Robustness and system suitability

Statistical analysis showed no significant difference ($p = 0.28$) between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of some parameters were introduced. Thus, the method proved to be robust for changes within the mobile phase flow rate from 0.9 to 1.1 mL.min^{-1}, methanol proportion from 54% to 58%, and column temperature from 25 ºC to 35 ºC.

The parameters of system suitability, such as the retention factor, the number of theoretical plates, the asymmetry factor, and repeatability, were considered adequate at all variations in chromatographic parameters, as shown in Table III, confirming that the analytical method is suitable to determine DTZ within the conditions for which it was considered robust.

Detection and quantitation limit

Employing the signal/noise ratio method, the detection and quantitation limits were lower than 0.15 μg.mL^{-1} and 0.75 μg.mL^{-1}, respectively, for an injection volume of 20 μL.

CONCLUSIONS

The developed method proved to be selective, linear, precise, accurate, and robust. DTZ was separated from its degradation products with an adequate run time of 14 minutes, and the method is compatible for coupling with a mass detector. The method proved to be a simple

TABLE II - Mean recovery to determine the accuracy and relative standard deviation (RSD) for intraday and interday precision of the analytical method (n=12 for each concentration level)

Concentration level	Mean recovery (%)	Intraday precision RSD (%)	Interday precision RSD (%)
Capsules			
50%	98.3	1.5	2.4
100%	99.6	0.8	1.0
150%	100.8	0.8	1.0
Tablets			
50%	101.9	2.1	2.3
100%	100.7	1.5	1.6
150%	98.7	2.5	2.7

TABLE III - The values of system suitability parameters of the developed method compared to that recommended by the US Food and Drug Administration (FDA, 1994)

Parameter	Results	Recommended by the FDA
Repetitivity (n=5)	RSD=0.22%	RSD of ≤ 1% for n ≥ 5
Retention factor (*k*) (n=5)	4.789 RSD=0.34%	Generally >2
Number of theoretical plates (N) (n=5)	5848 RSD=0.56%	Generally >2000
Resolution	>2 for all peaks	Generally >2
Asymmetry factor (n=5)	1.26 RSD=0.30%	≤2 *

* The FDA recommends values for USP tailing factor only.

and appropriate technique that could be employed in the quality control and stability testing of diltiazem tablets and compounded capsules.

ACKNOWLEDGEMENTS

The authors would like to thank CAPES and the Brazilian Pharmacopeia for their financial support.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding this manuscript.

REFERENCES

Agência Nacional de Vigilância Sanitária. ANVISA. Resolução RE n.899, de 29 de Maio de 2003. Guia para validação de métodos analíticos e bioanalíticos. [cited 2016 Ago 10]. Available from: http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2003/res0899_29_05_2003.html.

AHFS Drug Information 2004. Bethesda: American Society of Healthy-System Pharmacists; 2004. p.10223-270.

British Pharmacopoeia 2014. London: Her majesty´s Stationary Office; 2013.

Brunton LL, Lazo JS, Parker KL, editores. As bases farmacológicas da terapêutica. 11ªed. Rio de Janeiro: McGraw Hill; 2006. 1821 p.

Chatpalliwar VA, Porwal PK, Upmanyu N. Validated gradient stability indicating HPLC method for determining diltiazem hydrochloride and related substances in bulk drug and novel tablet formulation. J Pharm Anal. 2012;2(3):226-37.

Food and Drug Administration. FDA. Department of Health and Human Services. Center for Drug Evaluation and Research (CDER). Reviewer Guidance: validation of chromatographic methods. Rockville: FDA, 1994. [cited 2016 Ago 10]. Available from: http://www.fda.gov/downloads/drugs/guidances/ucm134409.pdf.

Gomes MLPC, Souza SVC. Validação de método para determinação de resíduos de amoxicilina aplicado à validação de limpeza em indústria farmacêutica de penicilânicos. Quim Nova. 2010;33(4):972-77.

Green JM. A practical guide to analytical method validation. Anal Chem. 1996;68(9):305A-06A.

International Conference on Harmonization. ICH. Q1A R2 Stability testing of new drug substances and products. Geneva: International Conference on Harmonization; 2003. [cited 2016 Ago 10] Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2__Guideline.pdf.

International Conference on Harmonization. ICH. Validation of analytical procedures: text and methodology Q2 R1. Geneva: International Conference on Harmonization; 2005. [cited 2016 Ago 10] Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.

Nogueira FHA, Goulart LPL, César IC, Campos LMM, Pianetti GA. Development and validation of an HPLC method for mefloquine hydrochloride determination in tablet dosage form. J AOAC Int. 2011;94(4):1089-93.

Rowe RC, Sheskey PJ, Quinn M, editores. Handbook of pharmaceutical excipients. 6th ed. London: Pharmaceutical Press and American Pharmacists Association; 2009. 888 p.

Souza SVC, Junqueira RG. A procedure to assess linearity by ordinary least squares method. Anal Chim Acta. 2005;552(1-2):25-35.

Sweetman SC, editor. Martindale: the complete drug reference. 36th ed. London: Pharmaceutical Press; 2009. p. 1265-1268.

The United States Pharmacopeia and National Formulary. USP. USP 38 - NF 33 2015. Rockville, MD: The United States Pharmacopeial Convention; 2014.

Received for publication on 19th September 2016
Accepted for publication on 22nd December 2016