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Research paper

Dissolution test for citalopram in tablets and comparison of *in vitro* dissolution profiles

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Abstract

A dissolution test for tablets containing 20 mg of citalopram was developed and validated using a reverse-phase liquid chromatographic method and this dissolution test was applied to compare dissolution profiles. The sink conditions, filters, stability of the drug and specificity on different dissolution media were tested to choose a discriminatory dissolution method which uses USP apparatus 1 with baskets rotating at 50 rpm, 900 ml of deaerated 0.1 M hydrochloric acid (HCl) as the dissolution medium. The quantitation method was also adapted and validated. The parameters of difference factor, similar factor, according to current FDA guidelines, and dissolution efficiency were employed to compare dissolution profiles. The dissolution test developed and validated was adequate for its purposes and could be applied for quality control of citalopram tablets, since there is no monograph to citalopram in tablets, this work can be used to help pharmocopoeias.
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Keywords: Citalopram; Dissolution test; HPLC; Quality control; Dissolution profile comparisons

1. Introduction

In recent years, more emphasis has been placed on dissolution testing within the pharmaceutical industry and by regulatory authorities [1]. The dissolution tests for immediate release solid oral dosage forms, such as tablets, are used to assess lot-to-lot quality of a drug product; guide development of new formulations and ensure continuing product quality and performance after certain changes, such as changes in the formulation, and the manufacturing process [2]. From a quality assurance point of view, a more discriminating dissolution method is preferred because the test will indicate possible changes in the quality of the product before *in vivo* performance is affected [3].

The dissolution test is currently used as an *in vitro* bioequivalence (BE) test, generally for dissolution profile and profile comparison, establishing the similarity of pharmaceutical dosage forms [1,4]. For profile comparison for two model independent methods: the difference factor (f_1) and the similarity factor (f_2) [2] and dissolution efficiency (DE) [5,6] were used in this work.

Citalopram (Fig. 1), chemically 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-iso-benzofuranocarbonitrile [7], is one of the widely used antidepressants of the selective serotonin reuptake inhibitors (SSRI) for the treatment of various affective disorders such as major depression, anxiety, panic disorders, obsessive compulsive disorder [8].

Although there is a crescent number of works describing the determination of citalopram in biological fluids [9–16] and pharmaceutical formulation [17–22] by several methods, this drug as tablets is not listed in any pharmacopoeia and there is no dissolution test for this pharmaceutical dosage form reported in the literature.

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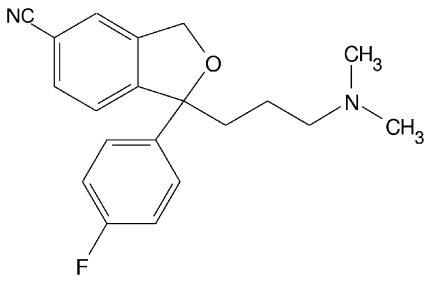

Fig. 1. The chemical structure of citalopram.

This paper describes the development and the validation of a dissolution test for citalopram tablets that contain 20 mg of the drug and a reverse-phase liquid chromatographic method for the quantitation of the drug from the dissolution test, as well as to evaluate the dissolution profiles for tablets.

2. Materials and methods

2.1. Chemicals

Citalopram hydrobromide reference substance (98.41%) was obtained from Cristália Produtos Químicos Farmacêuticos LTDA (São Paulo, Brazil), whereas the pharmaceutical formulations containing citalopram were obtained commercially.

Citalopram tablets used for development of the dissolution test and for comparison of profiles were:

Product A

(reference product, Cipramil®) – labeled to contain 20 mg of the drug and the following excipients: starch, lactose, microcrystalline cellulose, copolividone, 85% glycerol, croscarmellose sodium, magnesium stearate, hydroxypropyl methylcellulose, polyethylene glycol 400 and titanium dioxide.

Product B

– labeled to contain 20 mg of the drug and the following excipients: starch, lactose, microcrystalline cellulose, crospovidone, hydrogenated vegetable oil, croscarmellose sodium, magnesium stearate, hydroxypropyl methylcellulose, polyethylene glycol and titanium dioxide.

Product C

– labeled to contain 20 mg of the drug and the following excipients: starch, lactose, microcrystalline cellulose, polividone, croscarmellose sodium, coloidal silicium dioxide, magnesium stearate, polyethylene glycol, dimethylaminoethyl methacrylate and a yellow colorant.

All of the excipients were obtained from different local distributors. Water was purified using Millipore® system. HPLC grade acetonitrile and triethylamine, orthophosphoric acid (reagent grade) were purchased from Merck® (Darmstadt, Germany). Hydrochloric acid (HCl), sodium hydroxide and sodium acetate were purchased from Quimex® (Merck, Brazil). Monobasic potassium phosphate was obtained from Synth® (São Paulo, Brazil). Glacial acetic acid was obtained from Nuclear® (Brazil). The 0.01 and 0.1 M HCl, and sodium acetate USP buffer (pH 4.1), monobasic potassium phosphate USP buffer (pH 6.8) were prepared according to the directions in USP 29 [23]. These media were deaerated prior to use in the ultrasonic bath for 20 min.

2.2. Instrumentation

The dissolution test was performed in a Sotax AT7 multi-bath ($n = 7$) dissolution test system (Basel, Switzerland), in accordance with United States Pharmacopoeia (USP) general methods [23].

A liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a model LC-10ADvp binary pump, SIL-10ADvp autosampler, CTO-10ACvp column oven, SPD-M10Avp PDA detector, SCL-10Avp system controller and a Class – VP software was used to quantify the samples. A UV–VIS Recording Spectrophotometer UV-160A (Shimadzu) at 239 nm, using 1.0 cm quartz cells and SPECTRA MANAGER software was used for all absorbance measurements.

The Digimed potentiometer, model DM – 20 (São Paulo, Brazil), was used to determine the pH of all solutions.

The ultrasonic bath used for deaeration was the model USC 5000 (Unique, São Paulo, Brazil). The filter used for mobile-phase filtration was the MFS®0.45 μm, 47 mm, nylon membrane. Sample filtration was carried out using as centrifuge the model excelsa 2, Fanem®. The three filters evaluated for sample filtration were:

- Millipore® 0.45 μm, 13 mm, nylon membrane;
- Framex®, quantitative filter, 10 mm;
- Frama®, qualitative filter, 3.0 μm.

2.3. Chromatographic conditions

An HPLC method with UV detection, developed in our laboratory, was selected because of its ability to separate citalopram from the tablet excipients and because it did not use any buffer in the mobile phase contributing to increase the column's lifetime. The reverse-phase HPLC procedure utilized an ACE® RP-18 (250 × 4.6 mm I.D., 5 μm particle size, Aberdeen, Scotland) and UV detection at 239 nm. The column temperature was maintained at 25 °C. The mobile phase was prepared daily by mixing triethylamine solution 0.3%–acetonitrile (55:45, v/v), pH 6.6 (adjusted with 10% orthophosphoric acid). The injection volume was 20 μl and the run time

was 7 min. The mobile phase was filtered using a 0.45 μm membrane filter (Millipore, Milford, MA) and degassed with helium. The mobile-phase flow rate was 1.0 ml min^{-1}.

2.4. Dissolution test conditions

Citalopram sink conditions were determined in different solvents. The solubility of the drug was tested using an amount of citalopram equivalent to three times of the dose in the pharmaceutical formulation in 900 ml of 0.01 and 0.1 M HCl, acetate buffer, pH 4.1, and phosphate buffer, pH 6.8. To FDA (1997) the *sink* conditions are desirable, but not mandatory. The solubility in water was not tested, since it is not an ideal dissolution medium [2].

The filter evaluation is necessary to determine if it could be used in the dissolution test without adsorption of the drug and that it removes insoluble excipients that may otherwise cause high background or turbidity [3]. A standard and a sample solutions were prepared in different dissolution media proposed (0.01 and 0.1 M HCl and phosphate buffer, pH 6.8) with a final concentration of 11.11 μg/ml. The sample solutions were prepared using a placebo added an amount of reference standard equivalent to 20 mg of citalopram in a beaker with 900 ml dissolution medium maintained at 37.0 ± 0.5 °C and stirred with a magnetic stirrer for 1 h. Aliquots of 10.0 ml were withdrawn at the same point and each one was centrifuged, filtered with a quantitative filter, a 0.45-μm nylon filter and a 3.0-μm filter. The standard solutions were prepared in volumetric flasks and the final solution was analyzed without filtration and filtered with the same filters listed above. All the filtrates were analyzed by UV method. For a filter to be acceptable for use, the results of the filtered portions are to approach (98–102%) the original concentrations of the unfiltered standard solution and the centrifuged sample solution [3,24].

Dissolution testing was performed in compliance with USP 29 [23] testing apparatus 1 and 2 rotating at 50 rpm and 900 ml of the different dissolution media. The medium, which was deaerated in the temperature of 48 °C using an ultrasonic bath for 20 min, was maintained at 37 ± 0.5 °C. The 900 ml glass dissolution vessels were covered to minimize evaporation. Manual sampling aliquots of 15.0 ml were withdrawn at 5, 10, 15, 20, 30, 45 and 60 min, and after dissolution optimization, aliquots of 10.0 ml were withdrawn at 5, 9, 12, 15, 20, 25 and 30 min. The replacement of the same volume of the medium at 37 ± 0.5 °C was done for constant maintenance of the volume.

The standard solution, used in all dissolution tests, was prepared using an amount of powder equivalent to 11.11 mg of citalopram that was transferred to a 50 ml volumetric flask with the dissolution medium (222.2 μg ml^{-1}). Aliquot of 5.0 ml of this standard solution was transferred to a 50 ml volumetric flask and diluted with the same diluent obtaining the final concentration of 22.22 μg ml^{-1}. The solution was filtered in a 0.45 μm membrane filter before injection in the column.

2.5. Validation

In order to demonstrate whether the method was adequate for dissolution test purposes, it was validated through the analysis of stability, specificity, linearity, precision, accuracy and robustness parameters [2,3,24].

Stability

The standard solution stability was evaluated for 24 h stored at 4 °C and with the sample solution, they were evaluated for 24 h at room temperature and kept at 37 °C for 2 h in 0.01 and 0.1 M HCl and phosphate buffer, pH 6.8, verifying the chromatograms obtained by the HPLC method (peak area and degradation product formation).

Specificity

It was evaluated by preparing a placebo sample of the reference commercial formulation of tablets in their usual concentration. The placebo sample was transferred to vessels with 900 ml of three different dissolution media deaerated (0.01 and 0.1 M HCl and phosphate buffer, pH 6.8) and stirred at 37 °C for 1 h at 150 rpm using paddle (USP apparatus 2). Aliquots of this solution were filtered with quantitative filter and 0.45 μm nylon filter and analyzed by HPLC and UV methods.

Linearity

Aliquots of a 100.0-μg ml^{-1} solution of citalopram reference standard, prepared with 0.1 M HCl, were transferred to volumetric flasks to obtain the final concentrations of 1, 5, 15, 20, 25, 30 and 40 μg ml^{-1}. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method and analysis of variance (ANOVA).

Precision

Repeatability and intermediate precision were used to assess the precision of the method. Repeatability was evaluated through relative standard deviation (RSD) from the recovery data at 100% level [3] at two different days and the intermediate precision through the RSD inter-day and using another HPLC equipment (Agilent Technologies, model 1200, Agilent ChemStation software). The recovery data were performed, in triplicate, by adding an amount of powder equivalent to 20.0 mg (100% of the nominal assay) of citalopram reference substance to a placebo sample. The dissolution test was done for 30 min using 900 ml of dissolution medium 0.1 M HCl, apparatus 1 rotating at 50 rpm. Aliquots of 10.0 ml were filtered with quantitative filter and then with 0.45 μm nylon filter and analyzed by HPLC method.

Accuracy

A recovery study was collected by adding known amounts of citalopram reference substance to placebo solution at 10, 100 and 150% of the nominal assay of citalopram. The dissolution test was done for 30 min using 900 ml of dissolution medium 0.1 M HCl, apparatus 1 rotating at 50 rpm. Aliquots of 10.0 ml were filtered with quantitative filter and then with 0.45 μm nylon filter and analyzed by HPLC method. Each concentration was prepared in duplicate and each one was injected in triplicate.

Robustness

It was evaluated during development by making small, but deliberate, changes to the method's parameters. An experimental design was used to determine how the influence of dissolution medium deaeration affects the liberation profile of citalopram from the tablets.

2.6. Dissolution profiles

The dissolution profiles were obtained after determination of the best dissolution test conditions. Aliquots of 10.0 ml were withdrawn from each vessel and the same volume of the dissolution medium was replaced to maintain a constant total volume. The times selected were 5, 9, 12, 15, 20, 25 and 30 min. Twelve samples were assayed for each dissolution profile. The withdrawn samples were first filtered in a quantitative filter and then in 0.45 μm nylon filter. The samples were analyzed directly by HPLC.

The content uniformity of the three products tested was assessed, individually, using 10 U of each product. The percentage found was used to calculate the percentage drug release on each time of the dissolution profile. The standard preparation for content uniformity was done using an amount of powder equivalent to 10 mg of citalopram that was weighted and transferred to a 50 ml volumetric flask. The volume was completed with water. An aliquot of 5.0 ml of this solution was transferred to a 25 ml volumetric flask and diluted with water obtaining the final concentration of 40.0 μg ml^{-1}. For the sample preparation, one tablet was transferred to a 200 ml volumetric flask containing 180 ml of water, pH 1.85 (adjusted with 10% orthophosphoric acid). They were kept in the ultrasonic bath for 10 min and the volume was completed with the same diluent. Aliquots of 10.0 ml were transferred to 25 ml volumetric flasks and diluted with water obtaining the final concentration of 40.0 μg ml^{-1}. The standard and the sample solutions were filtered in a 0.45 μm nylon filter before the analysis by HPLC.

The dissolution profiles were compared through two model independent methods: the difference factor (f_1) and the similarity factor (f_2) and dissolution efficiency (DE). The DE was calculated from the area under the dissolution curve at time t_i (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

The f_1 factor measures the percent error between two curves over all time points. The percent error is zero when the test and drug reference profiles are identical and increases proportionally with the dissimilarity between the two dissolution profiles.

The f_2 factor is a logarithmic transformation of the sum-squared error of differences between the test and the reference products over all time points. This factor is 100 when the test and reference profiles are identical and tends to 0 as the dissimilarity increases. Two dissolution profiles are declared similar if f_1 is between 0 and 15 and if f_2 is between 50 and 100 [2,6].

3. Results and discussion

The discriminatory power of the dissolution method depends on the method's ability to detect changes in the drug product. Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium [3]. The *sink* conditions tested showed that citalopram bulk was soluble in 0.01 and 0.1 M HCl, acetate buffer pH 4.1 and phosphate buffer, pH 6.8. Then, dissolution tests for citalopram tablets (product A) were performed using this dissolution medium at the stirring speed of 50 rpm, to investigate the drug release in each media.

The initial parameters for filtration and solution stability must be established prior to the completion of any dissolution samples [25]. The evaluation of the filters demonstrated that the quantitative and 0.45 μm nylon filters were within 98–102% of the initial values and could be used in the dissolution tests in the different dissolution medium.

To evaluate the citalopram stability three dissolution media were used, which were over the physiologic pH range of 1.2 to 6.8. The chromatograms for each medium were obtained. According to the literature, the acceptable range for solution stability is 98–102% of the initial value

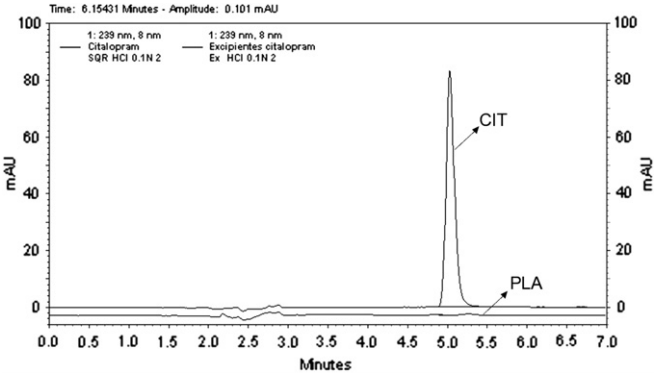

Fig. 2. Chromatogram of citalopram reference standard (CIT) and placebo (PLA) before dissolution with 0.1 M HCl at 37.0 ± 0.5 °C and apparatus 2 rotating at 150 rpm.

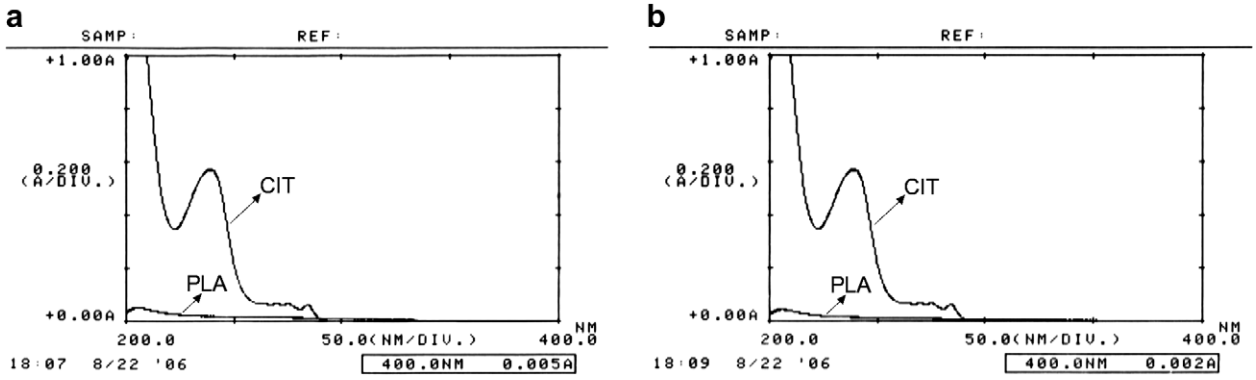

Fig. 3. UV spectrum of citalopram reference standard (CIT) and placebo (PLA) before filtration with a quantitative filter (a) and a 0.45 μm nylon membrane (b) to the dissolution test with 0.1 M HCl at 37.0 ± 0.5 °C and apparatus 2 rotating at 150 rpm.

[24]. The solutions remained stable in all dissolution media tested for the time period specified and no degradation products were observed in any chromatogram. So, it was possible to guarantee the integrity of the drug during all the analysis time. The standard solution is therefore considered stable for at least 24 h at 4 °C.

The specificity analysis revealed the HPLC method did not suffer interference by the formulation excipients, since there was not another peak in the retention time of citalopram (about 5.0 min) (Fig. 2). The chromatographic peak purity too, applied for citalopram peak, demonstrated that there were no impurities on the peak. The same analysis was done using the UV method. The results obtained suggested that the UV method could not be used for citalopram tablets quantitation in dissolution tests, once the formulation excipients had significative absorbance (interference exceeds 2% of the reference absorbance) at 239 nm (Fig. 3). Thus, the HPLC method is useful to quantify citalopram in pharmaceutical formulation from the dissolution tests.

The dissolution test conditions were selected based on a screening study with USP apparatus 1 (50 rpm baskets) and USP apparatus 2 (50 rpm paddles). The tablets were tested in 900 ml of 0.01 and 0.1 M HCl, acetate buffer, pH 4.1, and phosphate buffer, pH 6.8 (Figs. 4 and 5). The data for citalopram are given in Table 1. As expected for a highly soluble compound, the dissolution of citalopram was rapid and essentially complete within 30 min under all of these test conditions.

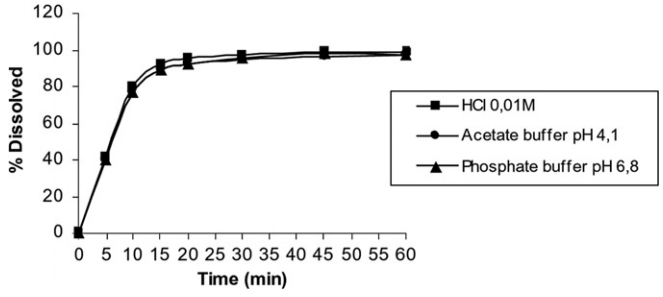

Fig. 4. Dissolution profiles of citalopram tablets using 0.01 M HCl, acetate buffer, pH 4.1, and phosphate buffer, pH 6.8, and apparatus 2 rotating at 50 rpm.

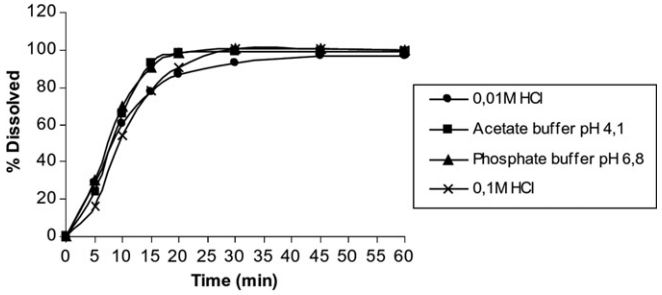

Fig. 5. Dissolution profiles of citalopram tablets using 0.01 and 0.1 M HCl, acetate buffer, pH 4.1, and phosphate buffer, pH 6.8, and apparatus 1 rotating at 50 rpm.

Table 1
Screening study for citalopram (% dissolved at 30 min)[a]

Medium	USP apparatus 1 50 rpm	USP apparatus 2 50 rpm
0.01 M HCl	100.81 (100.24–101.37)	97.20 (91.81–99.73)
0.1 M HCl	92.67 (84.11–99.21)	–[b]
Acetate buffer (pH 4.1)	99.17 (98.24–100.10)	94.57 (87.05–99.95)
Phosphate buffer (pH 6.8)	100.80 (98.66–100.18)	95.28 (84.27–98.90)

[a] The average result is reported followed by the range in parentheses.
[b] Data not collected.

USP apparatus 1 with baskets rotating at 50 rpm was selected as the dissolution apparatus and 900 ml of 0.1 M HCl was chosen as the dissolution medium. This dissolution test condition was selected because in general, mild conditions should be maintained during dissolution testing to allow maximum discriminating power and the drug release profile obtained in the developed dissolution test was considered satisfactory and discriminative (Fig. 6). Using USP apparatus 2, it was observed that the discriminatory power of the dissolution profile was obtained at high pH, therefore, this apparatus was not tested at 50 rpm with 900 mL of 0.1 M HCl.

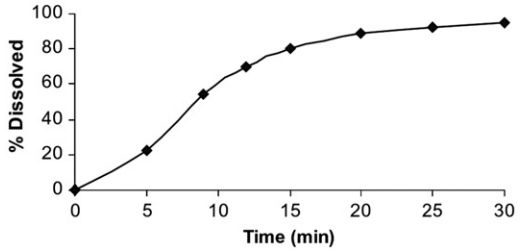

Fig. 6. Dissolution profile of citalopram tablets using 0.1 M HCl and apparatus 1 rotating at 50 rpm.

To assess the linearity, three standard curves for citalopram were constructed, plotting concentration ($\mu g\,ml^{-1}$) versus absolute area (mV s) and showed good linearity in the range of 1.0–40.0 $\mu g\,ml^{-1}$ range, with a correlation coefficient of 0.9999. The slope obtained was 54,162 and the intercept was −317.49. The analysis of variance (ANOVA) showed significative linear regression and no-significative linearity deviation ($P < 0.05$) [26]. These data indicate that the method is linear for citalopram.

The precision of the dissolution tests was evaluated through repeatability and intermediate precision. The repeatability demonstrated small RSD for each day analyzed (1.59% in the first day and 0.80% in the second day). The RSD for intermediate precision was 0.45% inter-day and 0.52% to the citalopram quantification in another HPLC. These results can demonstrate the good precision of the method for dissolution test.

The accuracy expresses the agreement between the accepted value and the value found. According to Marques (2002), the recovery must to be in the range of 95–105% of the reference standard weight [24]. The recovery found was in the range of 99.69–101.30% for citalopram. The accuracy of the method was considered acceptable based on its intended use.

In the evaluation of the robustness of the method, the presence of possible air bubbles in the dissolution medium no interference in the dissolution profile of the citalopram in tablets. These results demonstrate that the method is robust.

The mean values found for the uniformity of content in product A, product B and product C tablets were 98.69% (RSD = 1.40), 101.24% (RSD = 2.36) and 110.71% (RSD = 1.11), respectively. These results were in accordance with the specifications [26].

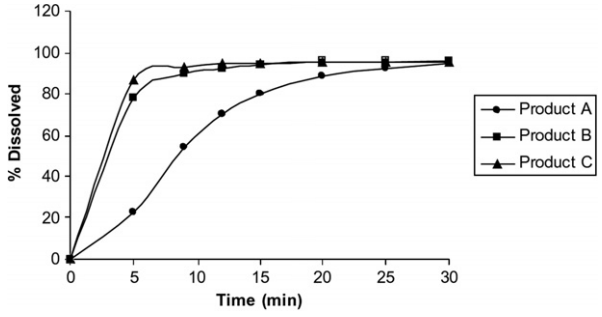

Fig. 7. Comparison of citalopram tablet's dissolution profiles using 0.1 M HCl and apparatus 1 rotating at 50 rpm.

Table 2
Comparison of tablet's dissolution profiles through the dissolution efficiency (DE), difference factor (f_1) and the similarity factor (f_2)

Parameter	Product A	Product B	Product C
DE%	65.69	83.72	85.69
f_1	–	14.81	16.14
f_2	–	33.24	31.45

The comparison of the dissolution profiles for different products cited in Section 2.1 was realized (Fig. 7). The results of dissolution efficiency (DE), difference factor (f_1) and the similarity factor (f_2) are presented in Table 2. Since the product A is the reference brand, the factors f_1 and f_2 were calculated between product A and B/C. Two dissolution profiles are declared similar if f_1 is between 0 and 15, and if f_2 is between 50 and 100. The results of f_1 and f_2 for the comparison of products A and B and for products A and C, showed that the profiles are not similar. The dissolution efficiency was calculated for all products. The analysis of variance of the DE values shows that the profiles are not similar for the tablets ($F_{calc} = 93.40 > F\text{-}_{crit} = 3.28$). *The Tukey test showed that products B and C are significantly different from product A, but product B is not significantly different from product C.*

The differences between the products evaluated could change their bioavailability. Amidon et al. (1995) suggested that products with high solubility drugs, as citalopram, can be considered bioequivalent if they cause 85% of the drug release in 15 min [27]. The products B and C were in agreement with this specification. It is necessary to carry out *in vivo* studies to guarantee the bioequivalence between the products. The comparison of the dissolution profiles for these products allowed to observe the differences between the formulations.

Typical acceptance criteria for the amount of drug dissolved are in the range of 70–80% dissolved. These criteria including test times are usually established on the basis of an evaluation of the dissolution profile data [3,24]. In this article, it was observed that for all products a dissolution of 80% in 30 min takes place. So, this acceptance criterion was utilized.

4. Conclusions

The dissolution test developed and validated for citalopram tablets was considered satisfactory. It was carefully studied in order to guarantee the drug stability during all the analysis time. The conditions that allowed the dissolution profile determination were 900 ml of 0.1 M HCl medium at 37 °C, baskets (USP apparatus 1), 50 rpm stirring speed and filtration with quantitative filter and 0.45 μm nylon membrane. The comparison of

the obtained dissolution profiles was realized by DE and the factors, f_1 and f_2, and showed that the profiles were not similar for tablets of products A, B and C. However, for all products the drug delivery was satisfactory since at least 80% was dissolved in 30 min. This method demonstrated to be adequate for quality control of citalopram dosage form, since there is no official monograph, collaborating to the official codes.

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References

[1] T. O'Hara, A. Dunne, J. Butler, J. Devane, Pharm. Sci. Technol. Today 5 (1998) 214–223.
[2] FDA, Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, Food and Drug Administration, Rockville, 1997.
[3] The United States Pharmacopoeia, Pharmacopeial Forum, 30 (2004) pp. 351–363.
[4] V.P. Shah, Dissol. Tech. (2001) 1–2.
[5] N.H. Anderson, M. Bauer, N. Boussac, R. Khan-Malek, P. Munden, M. Sardaro, J. Pharm. Biomed. Anal. 17 (1998) 811–822.
[6] P. Costa, J.M.S. Lobo, Eur. J. Pharm. Sci. 13 (2001) 123–133.
[7] The Merck Index, an Encyclopedia of Chemicals, Drugs, and Biologicals. Merck & Co., Inc. Whitehouse Station, NJ, 2001.
[8] I.M. Anderson, Curr. Anaesthesia Critic. Care 10 (1999) 32–39.
[9] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 909 (2001) 87–93.
[10] H. Juan, Z. Zhiling, L. Huande, J. Chromatogr. B 820 (2005) 33–39.
[11] C. Pistos, I. Panderi, J.A. Politou, J. Chromatogr. B 810 (2004) 235–244.
[12] Q.H. Meng, D. Gauthier, Clin. Biochem. 38 (2005) 282–285.
[13] J. Macek, P. Ptácek, J. Klima, J. Chromatogr. B 755 (2001) 279–285.
[14] C. Frahnet, M.L. Rao, K. Grasmader, J. Chromatogr. B 794 (2003) 35–47.
[15] P. Molander, A. Thomassen, L. Kristoffersen, T. Greibrokk, E. Lundanes, J. Chromatogr. B 766 (2001) 77–87.
[16] C.B. Eap, P. Baumann, J. Chromatogr. B 686 (1996) 51–63.
[17] J.J. Berzas-Nevado, A.M. Contendo-Salcedo, Chromatographia 55 (2002) 369–373.
[18] R. Flores, J. Berzas-Nevado, J.J. Contento-Salcedo, M.P. Cabello-Dias, J. Sep. Sci. 27 (2004) 33–40.
[19] V. Pucci, S. Fanali, C. Sabbioni, M.A. Raggi, J. Sep. Sci. 25 (2002) 1096–1100.
[20] J.J. Berzas, C. Guiberteau, A.M. Contento, V. Rodríguez, Chromatographia 56 (2002) 545–551.
[21] R. Skinbisnki, G. Mistzal, Chem. Anal. 47 (2002) 531–538.
[22] A. Raza, Chem. Pharm. Bull. 54 (2006) 432–434.
[23] The United States Pharmacopoeia, 29th ed., United States Pharmacopoeial Convention, Rockville, 2006.
[24] M.R.C. Marques, W. Brown, Analytica 1 (2002) 48–51.
[25] D. Fortunato, Dissol. Tech. (2005) 12–14.
[26] Farmacopéia Brasilera, fourth ed., Atheneu, Rio de Janeiro, 1988.
[27] G.L. Amidon, H. Lennernas, J.MR. Crison, Pharma. Res. 12 (1995) 413–420.