

DEVELOPMENT AND VALIDATION OF A DISSOLUTION TEST FOR DILTIAZEM HYDROCHLORIDE IN IMMEDIATE RELEASE CAPSULES

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This work describes the development and validation of a dissolution test for 60 mg of diltiazem hydrochloride in immediate release capsules. The best dissolution *in vitro* profile was achieved using potassium phosphate buffer at pH 6.8 as the dissolution medium and paddle as the apparatus at 50 rpm. The drug concentrations in the dissolution media were determined by UV spectrophotometry and HPLC and a statistical analysis revealed that there were significant differences between HPLC and spectrophotometry. This study illustrates the importance of an official method for the dissolution test, since there is no official monograph for diltiazem hydrochloride in capsules.

Keywords: dissolution; high performance liquid chromatography; spectrophotometry.

INTRODUCTION

In recent years, progressively more emphasis has been placed on dissolution testing within the pharmaceutical industry and by regulatory authorities.¹ The quality assurance of pharmaceutical products is a wide-ranging concept covering all matters that individually or collectively influence the quality of a product. In this sense, the importance of *in vitro* dissolution tests for immediate release solid oral dosage forms, such as tablets and capsules, must be highlighted, since they are essential to evaluate the lot-to-lot quality of a drug product, to guide the development of new formulations and to ensure continuing product quality and performance after certain changes, such as changes in the formulation, the manufacturing process, the site of manufacture, and scale-up of the manufacturing process.²

In light of this, there is clearly a real need to develop dissolution tests for pharmaceutical products to assure the quality and to attend to the regulatory demands.

Diltiazem hydrochloride (Figure 1), the 1,5-Benzothiazepin-4(5H)-one, 3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-, monohydrochloride, (2*S*-*cis*),³ is a benzothiazepine calcium channel antagonist, mainly used in the treatment of angina, hypertension and supraventricular arrhythmias and is commercially available in tablets, extended release tablets, immediate release capsules, extended release capsules or injectable dosage forms.⁴

Diltiazem hydrochloride has a molecular weight of 451. It is a white, odorless, crystalline powder that is freely soluble in water, dichloromethane, methyl alcohol, chloroform, and formic acid. It is slightly soluble in dehydrated alcohol and insoluble in ether. The pH of a 1% solution in water is 4.3 to 5.3.^{4,5}

Many high-performance liquid chromatography (HPLC) methods have been reported for the analysis of diltiazem hydrochloride and its

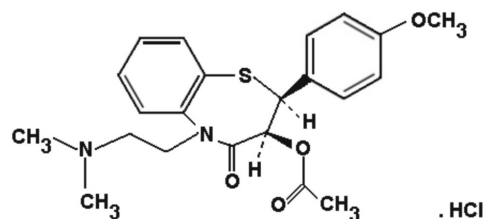


Figure 1. Chemical structure of diltiazem hydrochloride

metabolites in human plasma.⁶⁻²⁴ Liquid chromatography-mass spectrometry (LC-MS)²⁵ and capillary electrophoresis (CE) methods have also been reported.²⁶ For applications in pharmaceutical products, there are methods that make use of spectrophotometry,^{27,28} high-performance thin-layer chromatography (HPTLC),^{29,30} HPLC,³¹⁻³³ micellar electrokinetic chromatography (MEKC)³⁴ and supercritical fluid chromatography (SFC).³⁵ However, to the best of our knowledge, there are no dissolution tests described in the literature for diltiazem hydrochloride in pharmaceutical immediate release capsules and no pharmacopoeia describes an official method for this pharmaceutical product.

This study aims to establish the dissolution conditions for diltiazem hydrochloride assessment in pharmaceutical immediate release capsules, to validate the analytical methods used to quantify the drug, to evaluate three different commercial products and to compare HPLC and spectrophotometric methods.

EXPERIMENTAL

Chemical and reagents

All reagents were of analytical grade. Potassium dihydrogenphosphate (KH₂PO₄), phosphoric acid, sodium hydroxide, HPLC grade

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acetonitrile, triethylamine and hydrochloric acid were purchased from Vetec® (Rio de Janeiro, Brazil). Diltiazem hydrochloride salt (99.10% purity) was supplied by Galena® (São Paulo, Brazil). Diltiazem hydrochloride capsules (60 mg) were supplied by three magistral pharmacies from three different Brazilian manufactures, codified as A, B and C. The placebo mixtures used in accuracy and selectivity studies were obtained from the magistral pharmacies (A, B and C) and were described as containing the following inactive excipients: magnesium stearate, aerosil®, sodium lauryl sulfate, talc and starch (Product A); microcrystalline cellulose, starch and aerosil® (Products B and C).

Instrumentation

Dissolution tests were performed in an Electrolab® TDT-08 L multi bath (n=8) dissolution test system (Mumbai, India), in accordance with the United States Pharmacopoeia (USP) general method.³

The UV visible spectrophotometer used was a Shimadzu® model 1601 (Kyoto, Japan), connected to a computer loaded with Shimadzu UVPC version 3.9 software.

The HPLC equipment used was a Shimadzu® series LC-10 A (Kyoto, Japan), consisting of LC AVP pump, CLASS-VP 5.02 integration system, DGU-14 A degasser, 7725i manual injector with a 20 µL loop, SPD-10 AVP integrated UV detector, FCV-10 ALVP valve, CTO-10 AVP column oven and SCL-10 AVP controller. A Hypersil BDS RP-18 analytical column, with a 3-µm particle size, and 10.0 cm × 4.0 mm i.d., was used in this study.

The following equipment was also used: digital pHmeter PA 200 (Marconi® S.A., Piracicaba, Brazil), ultrasonic Bath model USC 2800 A (Unique®, São Paulo, Brazil), analytical balance model 410 (Kern®, Kern, Germany), reverse osmosis water purifier OS 10 LTH (Gehaka®, São Paulo, Brazil) and vacuum filtration system (Millipore®, Bedford, United States).

Solutions

All dissolution media used in this study (water, 0.1 mol L⁻¹ hydrochloric acid and phosphate buffer, pH 6.8) were degassed at 41 °C in an ultrasonic bath for 30 min prior to use.

Potassium phosphate buffer, pH 6.8, was prepared by adding 13.609 g of potassium dihydrogenphosphate and 22.38 mL of 2 mol L⁻¹ sodium hydroxide in 2.0 L of water.

A 0.1 mol L⁻¹ hydrochloric acid (HCl) solution was prepared by adding 8.5 mL of hydrochloric acid in 1.0 L of water.

Potassium phosphate buffer, pH 6.2, was prepared by adding 13.609 g of potassium dihydrogenphosphate and 8.10 mL of 2 mol L⁻¹ sodium hydroxide in 2.0 L of water.

The stock solutions of diltiazem hydrochloride were prepared in a 25 mL volumetric flask by dissolving an accurately weighed amount (16.7 mg) of diltiazem hydrochloride standard (99.10% purity) in potassium phosphate buffer, hydrochloric acid or purified water according to the dissolution medium used. These solutions were filtered in a quantitative Vetec® filter paper. Working standard solutions were prepared immediately before use by appropriate dilutions of the corresponding stock solutions of diltiazem hydrochloride with potassium phosphate buffer, hydrochloric acid or purified water.

Placebo solutions were prepared by dissolving an accurately weighed amount of placebo mixture (containing the same amount of the products A, B and C) in potassium phosphate buffer.

Sample solutions were prepared by putting one capsule in each vessel containing the dissolution medium (900 mL) at a temperature of 37 ± 0.5 °C. Samples were collected at the end of the specified time and filtered in quantitative Vetec® filter paper. For the HPLC analysis,

samples were directly injected into the HPLC system. In the spectrophotometric analysis, 2.5 mL of the samples were transferred into a 25 mL volumetric flask, later completed with dissolution medium.

To avoid interference from the capsule shell in the spectrophotometric quantitation, blank solutions were prepared by dissolving capsule shells of each brand (A, B and C) into the same medium. The dissolution test was performed in the same manner as the samples. Any absorbance obtained from the blank solutions was subtracted from the absorbance of the sample solutions.

Spectrophotometric measurements

Spectra of diltiazem hydrochloride standard were obtained in the range from 200 to 400 nm using 1 cm quartz cuvettes in the fast scan speed (about 3200 nm min⁻¹), 2.0 nm data interval and 2 nm bandwidth. The drug release percent (DR%) was assayed at the wavelength of 237 nm.

HPLC analytical procedure

The volume of injection was 20 mL. All solutions were filtered through a 0.45 µm millex-LCR filter before injection into the column. The flow rate was set at 1.0 mL min⁻¹ with a mobile phase of acetonitrile-potassium phosphate buffer (pH = 6.2; 50 mM)-triethylamine (45:55:0.2, v/v/v). The mobile phase was filtered under vacuum through a 0.45 µm modified hydrophilic PTFE membrane and degassed ultrasonically for 30 min prior to use. The column temperature was maintained at 30 °C. Peak areas (in volts) were used as the measured analytical response, with detection at 240 nm.

Sink conditions

In order to establish sink conditions, the solubility of the drug was tested using 6 mg of diltiazem hydrochloride in 30 mL of 0.1 mol L⁻¹ HCl, 0.01 mol L⁻¹ HCl, phosphate buffer at pH 6.8 and water.

Dissolution test optimization

The dissolution experimental conditions were established by submitting products A, B and C, respectively, under the following conditions: water, 0.1 mol L⁻¹ HCl and potassium phosphate buffer (pH 6.8) as the dissolution media, a paddle and basket as the apparatus and a stirring speed of 50, 75 and 100 rpm. A volume of 900 mL was used in cubes and the temperature stabilization in the test was 37.0 ± 0.5 °C. Samples (10 mL) were withdrawn from the dissolution medium at 5, 10, 15, 30 and 60 min, followed by immediate replacement, analyzed by both HPLC and UV methods and employed to the acquisition of dissolution profiles. Six samples were assayed for each product (A, B and C).

Method validation

After dissolution test optimization, the method was validated according to ICH guidelines.³⁶ In order to demonstrate whether the method was adequate for dissolution test purposes, it was validated through the analysis of stability, selectivity, linearity, precision, accuracy, detection limit and quantitation limit parameters.

Stability

The standard and sample solutions of each product (A, B and C) in phosphate buffer (pH 6.8) at the concentrations of 66.8 and 6.68 mg L⁻¹ of diltiazem hydrochloride were prepared, maintained at room temperature for 24 h and evaluated by HPLC and spectrophotometry, respectively.

In order to evaluate the diltiazem hydrochloride stability on dissolution test conditions, an amount of standard and a sample of each product (A, B and C) containing 60 mg of diltiazem hydrochloride were transferred to separate vessels containing 900 mL of phosphate buffer (pH 6.8) at 37 ± 0.5 °C and stirred for 2 h at 50 rpm using a paddle as the apparatus. Aliquots were collected and evaluated by HPLC and spectrophotometry.

Selectivity

For selectivity determination, the placebo samples and empty capsules of each product (A, B and C, respectively) were transferred to separate vessels (n=6) containing 900 mL of the dissolution medium phosphate buffer (pH 6.8) at 37 ± 0.5 °C and stirred for 60 min at 150 rpm using a paddle as the apparatus. Aliquots were collected and the interference of the empty capsules and placebo mixture of each formulation was evaluated by HPLC and spectrophotometry.³⁷

Linearity

Linearity was assessed by the analysis of standard solutions at concentrations of 36.0, 48.0, 60.0, 72.0 and 84.0 mg L⁻¹ of diltiazem hydrochloride in phosphate buffer at pH 6.8 using the HPLC method. A calibration curve using spectrophotometry was obtained at the concentrations of 3.6, 4.8, 6.0, 7.2 and 8.4 mg L⁻¹ of diltiazem hydrochloride in phosphate buffer at pH 6.8. Linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The acceptance criteria were a correlation coefficient of 0.999 or greater and a relative standard deviation (R.S.D) of each point (n=5) smaller than 2%.³⁶

Repeatability (intra-assay) and intermediate precision (inter-assay) of the analytical methods

The repeatability of the analytical methods was validated by analysis of 6 standard solutions of diltiazem hydrochloride dissolved in phosphate buffer at pH = 6.8 at the concentrations of 66.8 and 6.68 mg L⁻¹ using HPLC and spectrophotometry, respectively. The intermediate precisions of the methods were determined similarly at different days by different analysts. The R.S.D. values for the determinations were calculated.³⁶ The acceptance criterion was a R.S.D. of 2.0% or less.

Repeatability (intra-assay) and intermediate precision (inter-assay) of the dissolution procedure

Dissolution procedure repeatability was accomplished according to the literature,³⁸ by submitting 6 samples of each product (A, B and C) to the optimized dissolution test. Aliquots were collected and evaluated by HPLC and spectrophotometry at 237 nm. The intermediate precisions were determined similarly at different days by different analysts. The R.S.D. values for the determinations were calculated. The acceptance criterion was a R.S.D. of 5.0% or less.³⁹

Accuracy of the analytical methods

The accuracy of the analytical methods was verified by the addition of diltiazem hydrochloride stock standard solution to placebo mixtures A, B and C in 25-mL volumetric flasks, to obtain three final concentration levels, corresponding to 80, 100 and 120% of the target concentration of each quantitation method (HPLC and spectrophotometry). Each solution was prepared in triplicate. The percent recoveries were calculated according to Equation 1.

$$R(\%) = \frac{C_{\text{measured}}}{C_{\text{added}}} \times 100 \quad (1)$$

where C_{measured} is the sample concentration obtained from each quantitation method (HPLC and spectrophotometry) at each level

concentration and C_{added} is the theoretical concentration at each level concentration. The acceptance criterion was recovery between 98.0 and 102.0%.³⁶

Accuracy of the dissolution procedure

The accuracy of dissolution tests was determined according to the literature,³⁸ by the addition of 48, 60 and 72 mg of diltiazem hydrochloride (80, 100 and 120% of the target concentration) to placebo mixtures A, B and C, respectively, in vessels containing 900 mL of the dissolution medium (phosphate buffer, pH = 6.8). The samples were submitted to an optimized dissolution test. Aliquots were collected and analyzed by HPLC and spectrophotometry at 237 nm. These studies were performed in triplicate. The percent recoveries were calculated according to Equation 1. The acceptance criterion was recovery between 95.0 and 105.0%.³⁹

Detection limit and quantitation limit

The detection limit (DL) and quantitation limit (QL) of the methods were obtained from Equations 2 and 3:

$$DL = 3(S.D/a) \quad (2)$$

$$QL = 10(S.D/a) \quad (3)$$

where S.D. is the intersection standard deviation and a is the slope of the calibration curves obtained in the linearity study.³⁶

Assay of pharmaceutical products

After determination of the best dissolution conditions as well as analytical validation, three different commercial products (A, B and C) were evaluated. The dissolution experimental conditions were potassium phosphate buffer (pH 6.8) as the dissolution media and a paddle as the apparatus stirring at a rate of 50 rpm. Ten milliliters of dissolution medium (controlled at 37.0 ± 0.5 °C) were sampled after 5, 10, 15, 30 and 60 min, followed by immediate replacement and then analyzed by spectrophotometry and HPLC methods. Six samples were assayed for each product. Additionally, a comparison between the dissolution profiles of the commercial products (A x B, A x C and B x C) using the HPLC method was carried out. The similarity of the dissolution profiles was determined by a difference factor (f_1) and a similarity factor (f_2), calculated from Equations 4 and 5 as follows:

$$F1 = \left\{ \frac{\sum_{t=1}^n |R_t - T_t|}{\sum_{t=1}^n R_t} \right\} \times 100 \quad (4)$$

$$F2 = 50 \times \log \left\{ \left[\left(1 + \frac{1}{n} \right) \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \right\} \times 100 \quad (5)$$

For curves to be considered similar, f_1 values should be close to 0, and f_2 values should be close to 100. Generally, f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100) ensures equivalence of the two curves.⁴⁰

Comparison between HPLC and UV methods

In order to compare the analytical methods (UV spectrophotometry x HPLC), a statistical method based on the analysis of variance (ANOVA) at a significance level of 0.05 was applied. The drug release percent (DR%) of products A, B and C at each collection time was used considering the analytical methods as treatments and the products as blocks.

RESULTS AND DISCUSSION

Spectrophotometric conditions

The ultraviolet spectrum for diltiazem hydrochloride, in water, hydrochloric acid and potassium phosphate buffer showed a maximum drug absorption wavelength at 237 nm (results not shown). Therefore, this wavelength was used for the diltiazem hydrochloride spectrophotometric quantitation throughout the study.

Optimization of HPLC conditions

The chromatographic conditions were optimized to yield an adequate analytical performance in a low run time. After several preliminary experiments, the following parameters were considered adequate for our purpose: acetonitrile-potassium phosphate buffer (pH = 6.2; 50 mM)-triethylamine (45:55:0.2, v/v/v) as the mobile phase, a flow rate of 1.0 mL min⁻¹ with a column temperature of 30 °C, a Hypersil BDS RP-18 analytical column with a 3 µm particle size and an internal diameter of 10.0 cm × 4.0 mm, a UV detection wavelength at 240 nm and an injection volume of 20 µL.

In order to confirm if the chromatographic parameters were in accordance with the literature, a system suitability test was carried out by injecting six replicates of a working standard solution of diltiazem hydrochloride containing 66.8 mg L⁻¹. The following results were found: retention time of 3.89 min, theoretical plates higher than 6000, a capacity factor of 2.57 and an asymmetry of 1.39. The relative standard deviation of the peak area was 1.28%. Thus, all parameters were in agreement with the United States Pharmacopeia recommendations.³

Dissolution test optimization

The dissolution test was optimized in terms of the dissolution medium, the paddle (type II)/basket (type I) apparatus and the rotation speed. Regarding the dissolution medium, pure water was not considered as an "ideal" dissolution medium because it was experimentally observed that diltiazem capsules showed a low and constant dissolution without reaching a maximum up to 60 min. Besides, the use of water as a dissolution medium is discouraged because test conditions such as pH and surface tension can vary depending on the water source and may change during the dissolution test itself. Additionally, water is not considered a physiologically relevant medium as it is not representative of the gastrointestinal environment.

Two alternative media were employed in our studies: 0.1 mol L⁻¹ HCl, to simulate gastric fluid conditions, and phosphate buffer at pH 6.8 to simulate intestinal conditions in terms of pH. When HCl was used as the dissolution medium, diltiazem hydrochloride capsules showed a fast dissolution rate, which is not desired because this condition cannot reflect in vivo performance and an adequate method's capacity to differentiate between the tested pharmaceutical formulations cannot be observed. The dissolution profiles obtained in phosphate buffer (pH 6.8) were slower and a better method's capacity to differentiate between the formulations A, B and C should be obtained. Therefore, the phosphate buffer (pH 6.8) was chosen as the optimal dissolution medium. Using phosphate buffer as the dissolution medium, experiments were carried out to compare the performance of basket versus paddle agitation. The experimental results showed that the dissolution rate was similar when both apparatus were used. Paddles were selected for further studies because paddle apparatus is generally recommended by United States Pharmacopeia 31 for dissolution test of tablets.³

The effect of the rotation speed of the paddles on the dissolution

profile of diltiazem hydrochloride immediate release capsules was examined at 50, 75 and 100 rpm and the experimental results showed a fast dissolution rate at 75 and 100 rpm. Therefore, a rotation speed of 50 rpm was selected for further experiments in order to produce a maximum method's capacity to differentiate between the products A, B and C.

Thus, the established dissolution conditions for the assessment of diltiazem hydrochloride immediate release capsules were 900 mL of phosphate buffer (pH 6.8) at 37 °C as the dissolution medium and a paddle as the apparatus at a stirring speed of 50 rpm.

Method validation

Stability

The standard and sample solutions remained stable at room temperature for 24 h and for 2 h after the dissolution test, since the relative standard deviations (between initial moment and after described procedure) did not exceed those obtained in precision tests.

Selectivity

Placebo formulations for products A, B and C did not present absorbance at 237 nm. However, A, B and C capsule shells show UV absorbance at this wavelength (Figure 2). For this reason, one capsule shell of each product was analyzed in a separate vessel under the same optimized conditions and the absorbance value was subtracted from the sample solution absorbance to eliminate this interference in the spectrophotometric method.

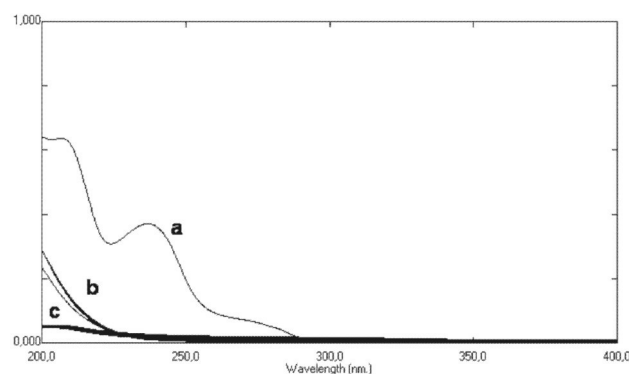


Figure 2. UV spectrum of diltiazem hydrochloride (a), empty capsules A, B and C (b) and placebo samples A, B and C (c), after the dissolution test using a paddle at 150 rpm for 60 min. The aliquots were diluted 10 fold

In the HPLC method, no additional chromatographic peaks from the placebo formulations and empty capsules were observed with the same retention time for diltiazem hydrochloride, which demonstrates the selectivity of this method (Figure 3).

Linearity

The calibration equations obtained from HPLC and spectrophotometry were $y = 33569x + 99340$ and $y = 0.0533x - 0.002$, respectively. The least squares regression showed satisfactory correlation coefficients: $r = 0.9999$ and 1.0000 for HPLC and spectrophotometric methods, respectively. The relative standard deviation of each point ($n=5$) was smaller than 2%. These results meet the acceptance criteria.³⁶

Repeatability (intra-assay) and intermediate precision (inter-assay) of the analytical methods

The results of the repeatability (intra-assay) and intermediate precision (inter-assay) of the methods are shown in Table 1. The relative standard deviations did not exceed 2.0% for both methods, demonstrating suitable precision.³⁹

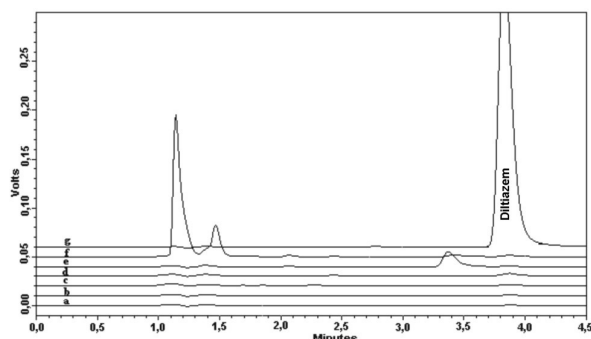


Figure 3. Chromatogram of a placebo sample of product A (a), a placebo sample of product C (b), a placebo sample of product B (c), empty capsule A (d), empty capsule C (e), empty capsule B (f) and a work standard solution of 66.8 mg L⁻¹ diltiazem hydrochloride in phosphate buffer at pH 6.8 (g)

Table 1. Precision of the HPLC and spectrophotometric methods for the analysis of diltiazem hydrochloride in capsules

| Method | Level | Concentration (mg L ⁻¹) | R.S.D. (%) |
|-------------------|------------------------|-------------------------------------|-------------|
| HPLC | Repeatability | 66.8 | 0.80 (n= 6) |
| | Intermediate precision | 66.8 | 0.90 (n=12) |
| Spectrophotometry | Repeatability | 6.68 | 0.25 (n=6) |
| | Intermediate precision | 6.68 | 0.50 (n=12) |

Repeatability (intra-assay) and intermediate precision (inter-assay) of the dissolution procedure

The results of the repeatability (intra-assay) and intermediate precision (inter-assay) of the dissolution procedure using HPLC and spectrophotometry are shown in Table 2. The relative standard deviations did not exceed 5.0%, demonstrating suitable precision for the dissolution test.³⁹

Table 2. Precision of the dissolution procedure using HPLC and spectrophotometry for diltiazem hydrochloride in capsules

| Method | Level | Product | Concentration (mg L ⁻¹) | R.S.D. (%) |
|-------------------|------------------------|---------|-------------------------------------|-------------|
| HPLC | Repeatability | A | 66.8 | 3.17 (n= 6) |
| | | B | 66.8 | 3.45 (n= 6) |
| | | C | 66.8 | 1.39 (n= 6) |
| | Intermediate precision | A | 66.8 | 3.71 (n=12) |
| | | B | 66.8 | 2.64 (n=12) |
| | | C | 66.8 | 2.68 (n=12) |
| Spectrophotometry | Repeatability | A | 6.68 | 3.65 (n= 6) |
| | | B | 6.68 | 3.76 (n= 6) |
| | | C | 6.68 | 1.45 (n= 6) |
| | Intermediate precision | A | 6.68 | 3.61 (n=12) |
| | | B | 6.68 | 2.70 (n=12) |
| | | C | 6.68 | 2.34 (n=12) |

Accuracy of the analytical methods

The values of analytical methods accuracy for diltiazem hydrochloride in all drug products are summarized in Table 3. The obtained mean recoveries indicated the suitability of the developed method in quantifying the concentration of diltiazem hydrochloride in pharmaceutical capsules.

Accuracy of the dissolution procedure

The values of dissolution procedure accuracy for diltiazem hydrochloride in all drug products shown in Table 4 are between 95.0-105.0%, showing acceptable recovery according to the literature.³⁹

Table 3. Accuracy of the HPLC and spectrophotometric methods for the analysis of diltiazem hydrochloride in capsules

| | Product | Concentration (mg L ⁻¹) | | R.S.D. (%) n=3 | Mean recovery (%) |
|-------------------|---------|-------------------------------------|-------|----------------|-------------------|
| | | Added | Found | | |
| HPLC | A | 53.44 | 53.02 | 0.21 | 101.19 |
| | | 66.80 | 65.20 | 1.01 | |
| | | 80.16 | 78.71 | 0.77 | |
| | B | 53.44 | 50.87 | 0.99 | 100.77 |
| | | 66.80 | 66.11 | 0.96 | |
| | | 80.16 | 79.82 | 0.83 | |
| | C | 53.44 | 51.81 | 1.27 | 101.11 |
| | | 66.80 | 65.38 | 0.04 | |
| | | 80.16 | 78.46 | 0.44 | |
| Spectrophotometry | A | 5.34 | 5.50 | 0.34 | 101.52 |
| | | 6.68 | 6.83 | 0.27 | |
| | | 8.02 | 8.17 | 0.23 | |
| | B | 5.34 | 5.19 | 0.36 | 100.13 |
| | | 6.68 | 6.46 | 0.29 | |
| | | 8.02 | 7.75 | 0.14 | |
| | C | 5.34 | 5.27 | 0.35 | 101.75 |
| | | 6.68 | 6.49 | 0.17 | |
| | | 8.02 | 7.89 | 0.24 | |

Table 4. Accuracy of the dissolution procedure using HPLC and spectrophotometry for the analysis of diltiazem hydrochloride in capsules

| | Product | Concentration (mg L ⁻¹) | | R.S.D. (%) n=3 | Mean recovery (%) |
|-------------------|---------|-------------------------------------|-------|----------------|-------------------|
| | | Added | Found | | |
| HPLC | A | 53.33 | 50.70 | 0.89 | 96.76 |
| | | 66.67 | 64.04 | 0.49 | |
| | | 80.00 | 79.33 | 0.45 | |
| | B | 53.33 | 52.48 | 0.71 | 97.08 |
| | | 66.67 | 63.26 | 0.67 | |
| | | 80.00 | 78.34 | 0.44 | |
| | C | 53.33 | 51.85 | 0.36 | 99.13 |
| | | 66.67 | 66.09 | 0.59 | |
| | | 80.00 | 80.82 | 0.79 | |
| Spectrophotometry | A | 5.33 | 5.08 | 0.20 | 97.21 |
| | | 6.67 | 6.55 | 0.27 | |
| | | 8.00 | 7.85 | 0.23 | |
| | B | 5.33 | 5.30 | 0.39 | 97.98 |
| | | 6.67 | 6.52 | 0.69 | |
| | | 8.00 | 7.74 | 0.35 | |
| | C | 5.33 | 5.17 | 0.53 | 98.66 |
| | | 6.67 | 6.61 | 0.16 | |
| | | 8.00 | 7.99 | 0.47 | |

Detection limit and quantitation limit

The detection limits of the HPLC and spectrophotometric methods were found to be 2.28 and 0.15 mg L⁻¹, respectively. The quantitation limits of the HPLC and spectrophotometry were found to be 7.62 and 0.49 mg L⁻¹, respectively. These results demonstrated that the analyses were being performed in a region above the quantitation limit value.

Assay of pharmaceutical products

The validated methods were used in the analysis of diltiazem hydrochloride capsules supplied from three different manufacturers (products A, B and C). Dissolution profiles of each product using HPLC and spectrophotometric detection are presented in Figures 4 and 5, respectively. The results are expressed as drug release percentage versus time (min). All three products tested showed >85% dissolution in 30 min using the HPLC method. These results are in accordance with the FDA,² which recommended that for highly soluble and rapidly dissolving drug products (BCS classes 1 and 3), a single-point dissolution test specification of 85% in 60 min or less is sufficient as a routine quality control test for batch-to-batch uniformity. Therefore, 30 min can be considered a satisfactory duration for a routine quality control test for diltiazem hydrochloride in capsules. Therefore, the acceptance criterion of 85% in 30 min was established. Moreover, a comparison of three commercial products (A x B, A x C and B x C) using a difference factor (f_1) and a similarity factor (f_2) are shown in Table 5. A difference factor between 0 and 15 and a similarity factor between 50 and 100 suggests that the two dissolution profiles are similar.⁴⁰ As observed in Table 5, only products B and C showed similar profiles. This effect was attributed to same composition of the product B excipients as that of the product C excipients. A comparison between products A and B and between products A and C did not reflect the similarity of these products, which was observed probability due to the difference in the composition of these product excipients. Therefore, these results showed the method's capacity to differentiate between the tested pharmaceutical formulations.

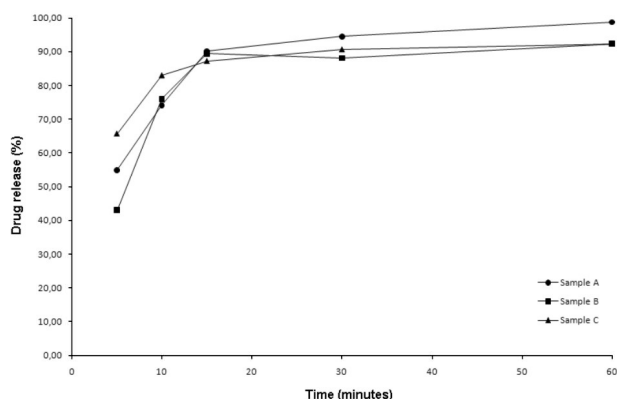


Figure 4. Dissolution profile of 60 mg capsules of diltiazem hydrochloride products codified as A, B and C under optimal dissolution conditions using the HPLC method

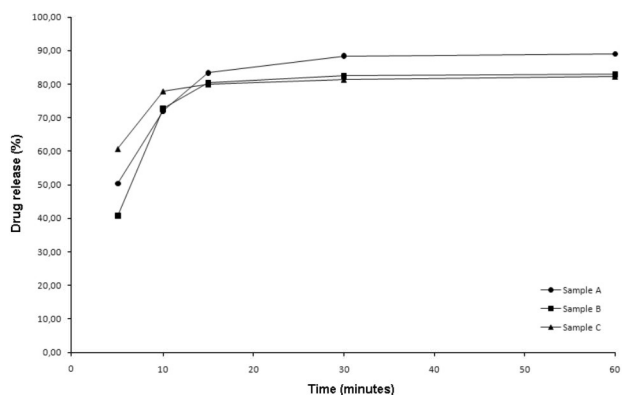


Figure 5. Dissolution profile of 60 mg capsules of diltiazem hydrochloride products codified as A, B and C under optimal dissolution conditions using the spectrophotometric method

Table 5. Difference factor and similarity factors between products A, B and C

| Products | Difference factor (f_1) | Similarity factor (f_2) |
|----------|-----------------------------|-----------------------------|
| A x B | 10.18 | 38.09 |
| A x C | 12.52 | 33.61 |
| B x C* | 2.13 | 69.36 |

* Two dissolution profiles are declared similar capsules

Comparison between HPLC and UV methods

The ANOVA revealed that there were significant differences between HPLC and spectrophotometric analytical methods at the 5% significance level. The calculated F values were found to be greater than the critical F values at 10, 15, 30 and 60 collection times and the mean drug release percent (DR%) of products A, B and C were found to be lower when spectrophotometry was used. These results suggest that the spectrophotometric method may underestimate the drug release percent. Considering that HPLC provides the following features in comparison with spectrophotometry: wide dynamic linear range, improved specificity via separation and increased sensitivity,⁴¹ the HPLC detection was considered more reliable in evaluating the release-time of diltiazem hydrochloride from capsules.

CONCLUSION

In this work, a dissolution test for diltiazem hydrochloride in capsules was developed. The established dissolution conditions were 900 mL of phosphate buffer (pH 6.8) at 37 ± 0.5 °C as the dissolution medium and a paddle as the apparatus at a stirring speed of 50 rpm. The validation results demonstrated that all the data meet the acceptance criteria when both HPLC and spectrophotometry are used as analytical methods. An assessment of commercial pharmaceutical products shows that 30 min can be considered a satisfactory duration for a routine quality control test for diltiazem hydrochloride in capsules. Moreover, a comparison of the three commercial products suggested the method's capacity to differentiate between the tested pharmaceutical formulations. Statistical analysis revealed that there were significant differences between HPLC and spectrophotometry, and that the HPLC method was considered more reliable in evaluating the release-time of diltiazem hydrochloride from capsules. This study illustrates the importance of an official method for the dissolution test, since there is no official monograph for diltiazem hydrochloride in capsules.

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