

Dissolution Test for Mianserin Hydrochloride in Tablets

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A dissolution test for mianserin hydrochloride in coated tablets containing 30 mg was developed and validated using a fast ultraviolet spectrophotometric method. The appropriate conditions were determined after testing *sink* conditions, agitation speed and dissolution medium. The *sink* conditions tested showed that mianserin hydrochloride was soluble in 0.01 and 0.1 M hydrochloric acid (HCl), acetate buffer pH 4.1 and 5.0 and phosphate buffer pH 6.8. Then, dissolution tests were performed to investigate the drug release in each medium. Optimal conditions to carry out the dissolution test were 900 mL 0.1 M HCl and USP apparatus 2 (paddle) at 50 rpm stirring speed. The quantification method was also adapted and validated. The UV method showed specificity, linearity, precision and accuracy. The *in vitro* dissolution test can be used to evaluate the drug release profile and the data was used as an aid to establish a possible correlation with *in vivo* data.

Keywords: Mianserin hydrochloride; Dissolution test; UV method; Quality control; IVIVC

Introduction

Mianserin hydrochloride (Fig. 1) is a drug for the treatment of depressive illness and depression associated with anxiety. It is classified as an atypical antidepressant, based on their mechanism of action is not defined. Acts by blocking presynaptic alpha-2 receptors in the brain, resulting in higher levels of norepinephrine in the synaptic cleft and has antagonist activity at serotonin receptors [1,2].

In the literature is not described the biopharmaceutics classification of mianserin hydrochloride, but we believe that the drug is a class III based on data of solubility and bioavailability. In the case of high solubility/low permeability drugs (class III), permeability is the rate controlling step and a limited IVIVC may be possible, depending on the relative rates of dissolution and intestinal transit. IVIVC is defined as the correlation between *in vitro* dissolution and *in vivo* input rate and may be used as an alternative to minimize unnecessary tests with humans [3]. The biopharmaceutics classification system (BCS) suggests that for high solubility, high permeability (class I) drugs and in some instances for high solubility, low permeability (class III) drugs, 85% dissolution in 0.1 M HCl in 15 minutes can ensure that the bioavailability of the drug is not limited by dissolution. In these cases, the rate limiting step for drug absorption is gastric emptying [3].

There is real need to develop dissolution tests that can predict more effectively the *in vivo* behavior of dosage forms, should lead not only to reduce costs and work needed the development of a pharmaceutical form, but also the number and size of clinical studies required and more reliable quality control.

Dissolution testing should be carried out using basket at 50/100 or paddle at 50/75, at 15 min intervals to generate a profile. To evaluate the profile of drugs that dissolve quickly, sampling should occur at shorter intervals, from 5 to 10 min [3].

Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the product, the dissolution or solubilization of the drug

under physiological conditions and the permeability across the gastrointestinal tract. Then, *in vitro* dissolution may be relevant to the prediction of *in vivo* performance [3]. 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 [4].

Although the literature describe some analytical methods for quantification of mianserin hydrochloride in biological fluids [2,5,6] and pharmaceutical formulation [7], there is no dissolution test for this pharmaceutical dosage form reported. The aim of the present study was to develop dissolution test and validate an analytical method in order to evaluate the release profile of this drug.

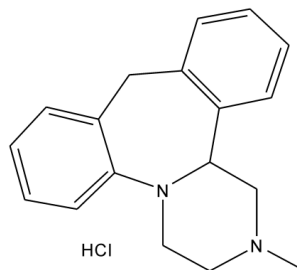


Figure 1. Chemical structure of mianserin hydrochloride.

Material and methods

Instrumentation

The dissolution tests were performed in a Vankel® VK 7010 multi-bath (n=8), auto-sampling consisting of a bidirectional peristaltic pump, VK 750D digitally controlled heater, in accordance with United States Pharmacopoeia (USP) general methods [8]. Spectral and absorbance measurements were performed with an UV-Vis Shimadzu model UV 160A using quartz cells and detection at 278 nm. The Digimed potentiometer, model DM-20 (São Paulo, Brazil) was used to determine the pH of the solutions. The HPLC system (Agilent 1200 series, Santa Clara, USA) consisted of a G1311A

quaternary pump, G1322A vacuum degasser, G1316A thermostat column compartment, G1329A standard auto sampler and G1315B diode array detector set at 278 nm. Chromatographic analysis were performed in an Ace RP-18 octadecyl silane column (250mm×4.6mm i.d., particle size 5 µm) maintained at ambient temperature (25 °C). The mobile phase was composed of methanol, monobasic potassium phosphate buffer and triethylamine solution adjusted to pH 7.0 in isocratic mode at a flow rate of 1.0 mL/min and the sample injection volume was 20 µL.

Material and Reagents

Mianserin hydrochloride reference standard (99.90%) was kindly supplied by Pharmaceutical Industry Organon (Schering-Plough, São Paulo, Brazil) and the commercial tablets Tolvon® was obtained in the local market. The excipients of pharmaceutical formulation were starch, silicon dioxide, magnesium stearate, methylcellulose, calcium dibasic phosphate, hypromellose, macrogol and titanium dioxide. Water was purified using Millipore® system. All the other reagents were of analytical grade and buffer solutions were prepared according to USP 42 [8].

Dissolution tests conditions

The dissolution profiles should be performed in at least three different dissolution media, within the physiological pH range [3]. The selection of the dissolution medium is made by considering, in part, the solubility of the drug in order to ensure *sink* conditions [9]. The *sink* conditions were determined in different solvents: 0.01 and 0.1 M HCl, acetate buffer pH 4.1 and 5.0 and phosphate buffer pH 6.8. Water is not tested, since it is not an ideal dissolution medium. First, because the variability of the quality of the water from different sources, and second, the variability of the pH from day to day [3, 8]. For dissolution tests, 900 mL of each medium were deaerated, by stirring and heating, and maintained at 37 ± 0.5 °C. USP apparatus, paddle and basket, were tested. For paddle was tested stirring speeds of 50 and 75 rpm and for basket was tested 75 and 100 rpm. The test time was set on 90 min.

Validation

In order to demonstrate whether the method was adequate for dissolution test purposes, it was validated through the analysis of specificity, linearity, precision and accuracy [3,8,9].

Stability

Stability of mianserin hydrochloride in the dissolution medium selected was evaluated using standard and sample solutions. The solutions were kept at room temperature (25 °C) for 24 h. Aliquots of the solutions were analyzed at time 0, 1 and 24h. The obtained results were compared with a freshly solution.

Specificity

It is necessary to demonstrate that the results are not affected by placebo constituents [8]. Placebo sample of commercial product (Tolvon®) was prepared in its usual compositions. The equivalent of an average weight of the mixture of excipients was transferred to separate vessels ($n = 2$) with 900 mL of the dissolution medium at 37 ± 0.5 °C and stirred for 1 h at 150 rpm using paddle (USP apparatus 2). After 1 h, aliquots of these solutions were filtered and analyzed by the UV method at 278 nm. Placebo interference is available by the formula: $100C(AP/AS)(V/L)$, in which C is the concentration (mg/mL) of standard solution; AP and AS are the absorbances of placebo and standard; V is the volume of the medium and L is the label claim (mg). According to USP, the interference should not exceed 2.0%.

Linearity

Linearity is established by preparing solutions, ranging in concentration from below the lowest expected concentration to above the highest concentration during release. Aliquots of a 100.0 µg/mL solution of mianserin hydrochloride reference standard, prepared in 0.1 M HCl, were transferred to 20 mL volumetric flasks to obtain the final concentration of 5.0, 10.0, 15.0, 20.0, 25.0, 30.0 and 40.0 µg/mL. To evaluate the linearity of the analytical method, three standard curves were constructed in three different days. The linearity was evaluated by linear regression analysis, which was calculated by the least squares 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 [4] at two different days and the intermediate precision through the RSD inter-day. The recovery data were performed, in triplicate, by adding an amount of powder equivalent to 30.0 mg (100% of the nominal assay) of mianserin hydrochloride reference standard to a placebo sample. The dissolution test was done for 30 min using 900 mL of dissolution medium 0.1 M HCl at 37 ± 0.5 °C, apparatus 2 (paddle) rotating at 50 rpm. Aliquots of 10.0 mL were filtered with 0.45 µm nylon filter and analyzed by UV method at 278 nm.

Accuracy

This parameter was determined by the recovery test, which consists in adding known amounts of mianserin hydrochloride reference standard to placebo solution at 10, 100 and 150% (3.0, 30.0 and 45.0 mg) of the nominal assay of mianserin. These concentrations were determine based on other papers published in the literature. The dissolution test was done for 30 min using 900 mL of dissolution medium 0.1 M HCl at 37 ± 0.5 °C, USP apparatus 2 rotating at 50 rpm. Aliquots of 10.0 mL were filtered with 0.45 µm nylon filter and analyzed by UV method at 278 nm. Each concentration was prepared in duplicate.

Robustness

It was evaluated by the influence of dissolution medium deaeration affects the liberation profile of mianserin hydrochloride from the tablets. Medium deaeration was used to eliminate air bubbles before the dissolution test.

Dissolution profiles

The dissolution profiles were obtained after the determination of the best dissolution conditions tests. Aliquots of 10.0 mL were withdrawn from each vessel without replacement of dissolution medium. Later, calculations were performed for volume correction. The times selected were: 5; 10; 15; 30; 45; 60 and 90 min. The samples were first filtered in a 10.0 μm filter and then in 0.45 μm nylon filter. The samples were analyzed by UV method at 278 nm.

In vitro-in vivo correlation

The data was used as an aid to establish a possible correlation with *in vivo* data. The average plasma concentration versus time curve was fitted with a non-linear software (Micromath Scientist®, v.2.01) using a one-compartment model. An IVIVC for mianserin hydrochloride was evaluated by plotting the mean percentage of drug absorbed (FA) versus the mean percentage of drug dissolved (FD).

Results and discussion

The *sink* conditions tested showed that mianserin hydrochloride was soluble in 0.01 and 0.1 M HCl, acetate buffer pH 4.1 and 5.0 and phosphate buffer pH 6.8. Then, dissolution tests were performed to investigate the drug release in each medium. Initially was used a validated stability-indicating HPLC method [10] to analyze the dissolution profile of the samples (Fig. 2).

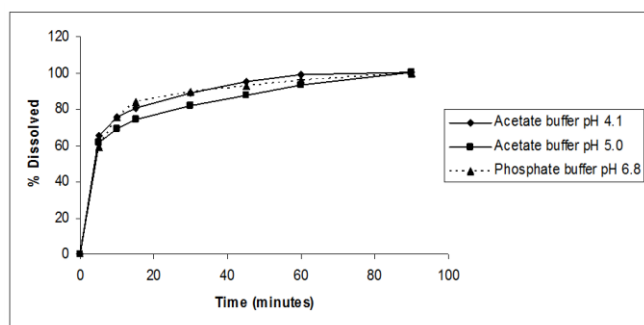


Figure 2. Dissolution profiles of mianserin hydrochloride tablets using acetate buffer pH 4.1, acetate buffer pH 5.0 and phosphate buffer pH 6.8 and apparatus 2 rotating at 75 rpm.

However, optimal conditions to carry out the dissolution test were 900 mL 0.1 M HCl at 37 ± 0.5 °C, USP apparatus 2 (paddle) at 50 rpm stirring speed using UV method at 278 nm (Fig. 3). The UV method requires no expensive or complex equipment and needs no toxic solvents [11]. The preferred method of analysis is spectrophotometric determination because results can be obtained faster, the analysis is simpler and fewer solvents are used, making this valuable in routine analysis [4,12]. The choice of 0.1 M HCl as medium is due to its good solubility, accessibility, low cost and the fact that is a typical dissolution medium [9].

To evaluate mianserin hydrochloride stability, the standard and sample solutions dissolved in 0.1 M HCl, were kept at room temperature for a period of 24 h. According to the literature, the acceptable range for solution stability is 98-102% of the initial value [9]. The solutions remained stable for the time period specified.

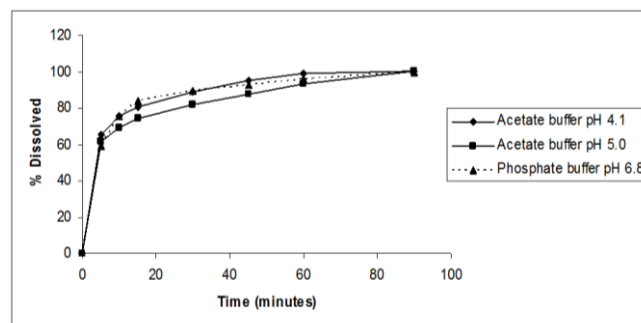


Figure 3. Tablets dissolution profile using HCl 0.1 M and paddle at 50 rpm.

The specificity analysis of placebo solution revealed that the UV method suffered no interference from the formulation excipients when the solution is filtered by 0.45 μm nylon filter. However, when the placebo solution was only filtered by 10.0 μm filter, the interference from pharmaceutical excipients is above the allowed (Fig. 4). According to the calculation of specificity recommended by USP, the interference of excipients was less than 2.0%. Thus, the UV method is useful to quantify mianserin hydrochloride in pharmaceutical formulation from the dissolution tests.

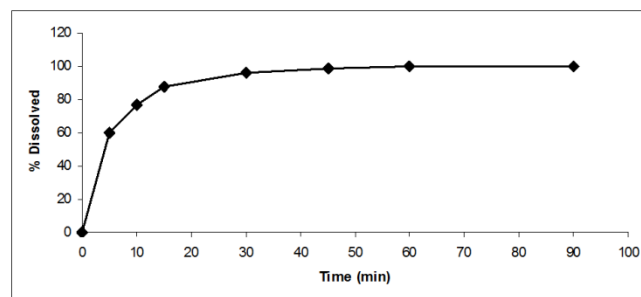


Figure 4. UV spectrum of mianserin hydrochloride reference standard (—) and placebo (...) before (A) and after (B) filtration with 0.45 μm nylon filter to the dissolution test in 0.1 M HCl at 37 ± 0.5 °C and paddle rotating at 150 rpm for 1h.

To assess the linearity, three standard curves for mianserin hydrochloride were constructed, plotting concentrations ($\mu\text{g}/\text{mL}$) versus absorbance and showed good linearity on the 5.0 – 40.0 $\mu\text{g}/\text{mL}$ range. The representative linear equation was $y = 0.0073x + 0.0111$ and the correlation coefficient was $r = 0.9998$. The data were validated by means of the analysis of variance (ANOVA), which demonstrated significant linear regression and no significant linearity deviation ($p < 0.05$) ($F_{\text{cal}} = 1.15$).

The precision of the dissolution tests was determined by repeatability (intra-day) and intermediate precision (inter-day) and it was expressed as RSD (%). The low values of relative standard deviations for the repeatability (1.32% and 1.05%) and intermediate precision (1.77%) demonstrated adequate precision of the analytical method (Table 1).

Table 1. Precision of the dissolution method for mianserin.

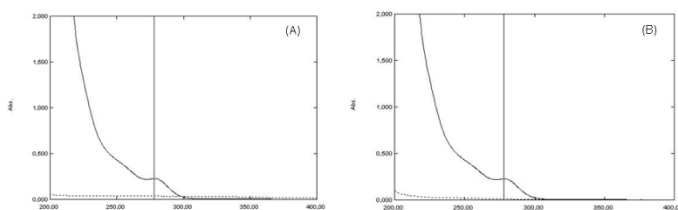
Samples	Assay (%)	
	Day 1	Day 2
1	102.73	99.40
2	104.77	101.80
3	101.47	99.78
Mean	102.99	100.33
RSD (%)	1.32	1.05

Mean/RSD Interday: 101.66 / 1.77%

The accuracy was demonstrated by the recovery of known amounts of mianserin hydrochloride in the dissolution vessels. Percentage recoveries from 95.0% to 105.0% are recommended [4,8]. In the accuracy test, three concentrations were evaluated (3.33, 33.33 and 50.0 µg/mL) and mean recoveries were 99.08 ± 1.95 , 102.17 ± 1.88 and $100.27 \pm 0.81\%$, respectively.

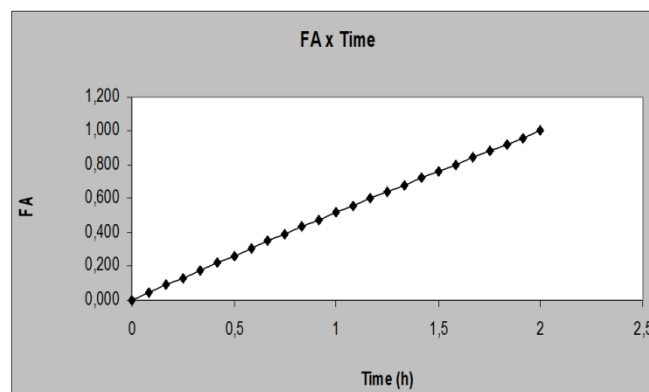
In the evaluation of the robustness of the method [13], the presence of possible air bubbles in the dissolution medium no interference in the dissolution profile of the mianserin hydrochloride in tablets.

To establish a possible correlation with *in vivo* data, was used the pharmacokinetic data available from the literature [2]. In this paper referenced, the values of C_{max} (peak plasma concentration) and t_{max} (time to reach C_{max}) were calculated and the others parameters were calculated using one compartmental model by Drug and Statistics Software. Based on these results, the plasma concentration versus time profile curve was transformed into percentage of drug absorbed (FA) versus time, using Wagner-Nelson method (Fig. 5).

**Figure 5.** Percentage of drug absorbed (FA) versus time curve for mianserin hydrochloride tablets using Wagner-Nelson method.

The level-A correlation is the highest level of correlation that can be obtained. Represents a point to point relationship between *in vitro* dissolution data and *in vivo* absorption data of the drug from the dosage form [14].

The analysis of percentage of drug absorbed (FA) versus the percentage of drug dissolved (FD) cannot be evaluated by linear regression, because there was not obtained a straight (fig. 6). It was not possible to establish a level-A *in vitro-in vivo* correlation for mianserin hydrochloride and one factor that may have hindered the correlation was the rapid drug release in the initial points of dissolution test. For BCS class III drugs - high solubility, low permeability - there is a limited crossing of the GI wall. *In vitro* dissolution will not predict *in vivo* absorption. It was not expect a level A correlation, but a limited correlation was possible.

**Figure 6.** Plot of mean percentage of drug absorbed (FA) versus mean percentage of drug dissolved (FD) for Tolvon®.

Conclusions

The dissolution test developed and validated for mianserin hydrochloride was considered satisfactory. The method has the advantages of being very easy, fast and simple. The better conditions for dissolution was: 900 mL of medium containing 0.1 M HCl, USP apparatus 2 (paddle) at 50 rpm and 37 ± 0.5 °C. The UV method showed specificity, linearity, precision and accuracy. The *in vitro* dissolution test can be used to evaluate the drug release profile, but it was not possible to establish a correlation with *in vivo* data.

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Conflict of interest

None to declare.

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