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Design of Experiments for the Establishment of the Dissolution Test Conditions of Rupatadine Fumarate 10 mg tablets

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ABSTRACT

Design of Experiments (DoE) is a statistical model that aims to determine if the factors under study affect the response, and if so, it determines the conditions under which this variable of interest can be optimized. In terms of pharmaceutical technology, independent variables are usually factors of the formulation, while dependent variables are properties of the product or parameters that indicate the performance of the process. Precisely, the dissolution test is a tool of interest for the developers of medicines since it allows them to evaluate the performance of a formulation designed in a solid pharmaceutical form, such as tablets. The present investigation used the design of experiments to establish and optimize the conditions of the dissolution test of a 10 mg Rupatadine fumarate tablets formulation, resulting in the use of HCl 0,1 N (pH 1) dissolution medium and a rotation speed of 100 rpm for the apparatus II USP, which allow the analysis of the product in a reproducible and reliable way.

Keywords: Analysis of variances, Design of experiments, Dissolution test, Factorial design, Rupatadine Fumarate, Tablets, Test conditions.

INTRODUCTION

Design of Experiments (DoE) is a concept used to organize, conduct and interpret the results of experiments in an efficient way, guaranteeing the collection of as much useful information as possible, through the execution of a small number of tests.[1]

In general, the DoE is considered a multivariate analysis technique, which requires the establishment or definition of levels (values) of the analyzed factors as well as prior knowledge about the problem under study, either by experience or by research.[2]

The main objective of an experimental study is to find the relationship between independent variables (factors) and dependent variables (results) that affect a certain process and its final product, according to a reference or workspace. An appropriate DoE can help to identify optimal conditions, critical material attributes (CMA), critical process parameters (CPP) and how these can affect critical quality attributes (CQA).[1]

The selection of an experimental design depends on the nature of the problem of interest, as well as the level of information that is wanted to obtain from it. In terms of pharmaceutical technology, independent variables are usually factors of formulation, while dependent variables are properties of the product or parameters that indicate the performance of the process. In the case of the development of pharmaceutical products, the DoE represents an excellent tool that allows to systematically manipulate factors according to a design prior to the establishment of specifications.[1, 3]

A test that allows evaluating the performance *in vitro* of a formulation is the dissolution test. Precisely, this test is a requirement for any solid dosage form of oral administration and is well used in all phases of the development of a drug product in order to study the release of the active pharmaceutical ingredient, as well as the product stability. [4, 5]

In the early stages of the development, the dissolution test allows to guide the optimization of the release profile shown by the formulations. Likewise, this test is used as a quality control procedure for medicines, which allows the Research and Development department to detect the influence of critical variables that may be affecting the quality of the designed formulation.[6]

The FDA guidance on dissolution testing for immediate release solid oral dosage forms, addresses the importance of using the Biopharmaceutical Classification System for the establishment of the dissolution test method to be performed, since this is based on the solubility and permeability of the active principle.[4, 7]

For the present study, the dissolution test will be used as a performance parameter of a 10 mg Rupatadine Fumarate tablet formulation prototype. The raw material is an active pharmaceutical ingredient that constitutes a potent second generation antihistamine selective H1 receptor, so it is widely used in the symptomatic treatment of allergic rhinitis and urticaria in adults and children over 12 years, with a daily dose of 10 mg.[8, 9]

The present research pretends to employ an optimization design in order to identify or find a combination of factors that correspond to an optimal response profile relative to the dissolution of the active pharmaceutical ingredient, that lets to establish the conditions of the test in such a way that the analysis can be done in a reproducible and reliable way.

MATERIALS AND METHODS

For the execution of this study it was decided to evaluate the performance of one formulation prototype shown in the table 1, regarding the dissolution test.

Table 1: Qualitative composition of the formulation prototype

Raw material
Rupatadine Fumarate
Monohydrate lactose
Microcrystalline cellulose
Sodium croscarmellose
Magnesium stearate

Equipment:

HPLC: Thermo Scientific, model Ultimate 3000.

Conditions:

• Column: C18; 4.6 x 250 mm and pore size of 5 μm.

• Mobile phase: 20% acetates buffer (pH 6) and 80% methanol.

• Temperature: 35 ° C.

• Injection volume: 20 μL.

• Wavelength: 244 nm.

Disolutor: Agilent Technologies, model 708-DS.

Conditions: According to the sections "Preliminary study" and "Design of Experiments".

Methods

Preliminary study:

- Dissolution test: Six units of the formulation prototype, apparatus II USP (pallets), 75 rpm rotation speed, evaluating its performance in two dissolution mediums: One consisting of 900 mL of water and another by 900 mL of a phosphate buffer at pH 5, both at 37 ± 0.5ºC, for 30 minutes.

- Chemical analysis: Samples were analyzed by the HPLC under the conditions established in the "Equipment" section.

- Statistical analysis: An ANOVA was done to find out significant differences between the dissolution medium according the following:

✓ Null hypothesis: The means are equal.

✓ Alternative hypothesis: The means are different.

✓ Level of significance: $\alpha = 0.05$

Design of Experiments:

The DoE was done according the following:

- Controlled variables: HPLC equipment and method of analysis, dissolutor, apparatus II USP (pallets), analyst, duration of the test (30 minutes), temperature of the dissolution medium (37.5 ± 0.5 °C), formulation to analyze, number of units per test (6 tablets), weight of the tablets (118-122 mg) and their hardness (5 - 6.5 kp).

- No controlled or noise variables: pH of the distilled water for the preparation of the mediums, environmental conditions, days of the tests.

- Input variables or study factors and their respective levels: pH of the medium (1 and 5) and agitation speed (75 and 100 rpm).

- Output variable or response: Average quantity of dissolved active pharmaceutical ingredient at 30 minutes.

- Type of design: Factorial design 2^2.

- Repetitions: Six for each treatment.

- Planning and organization of the experimental work: The dissolution tests were carried out according the design proposed in this section and the chemical analysis was done as established in the conditions of the HPLC equipment.

- Interpretation of the results: The statistical analysis of the results was carried out with the Minitab 17® program through a variance analysis (ANOVA) for the means of the factors studied (Ho: The means are the same, Ha: At least one of the means is significantly different), whose level of significance was 95% (α = 0.05). We analyzed the factors and effects that significantly influenced the response variable (P value <0.05), as well as the interactions that were presented. The verification of the normality compliance, constant variance and independence of the residuals was carried out by using graphical methods.

Table 2: Treatments for the proposed DoE.

Treatment	Medium pH	Apparatus Speed (rpm)
1	1	75
2	1	100
3	5	75
4	5	100

RESULTS

Preliminary study

The results obtained in the preliminary study allow to observe significant differences in the performance of the formulation in the dissolution test, when it was evaluated in a medium constituted by water and another by a buffer of phosphates, where the second one showed the highest percentages of dissolved amount at 30 minutes.

Table 3. Results of the dissolution tests for the preliminary study.

Medium	Dissolved amount at 30 minutes / %					
	1	2	3	4	5	6
Water	82,0	81,2	81,0	80,7	86,2	87,2
Buffer	95,1	95,9	102,5	90,6	99,0	99,5

Tabla 4: ANOVA one way for the exploratory study of the dissolution medium.

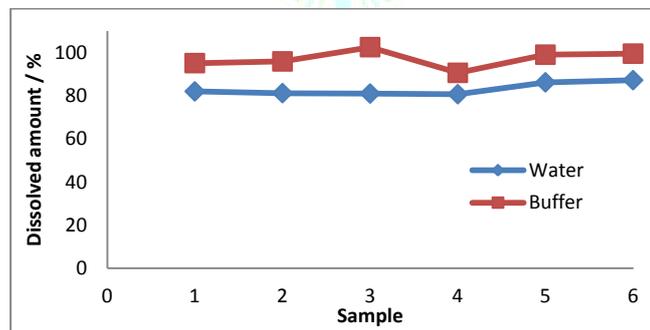| Source	DF	Adj SS	Adj MS	F value	P value
Medium	1	592,2	592,21	46,41	0,000
Error	10	127,6	12,76		
Total	11	719,8			

Figure 1: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage, in the preliminary dissolution study.
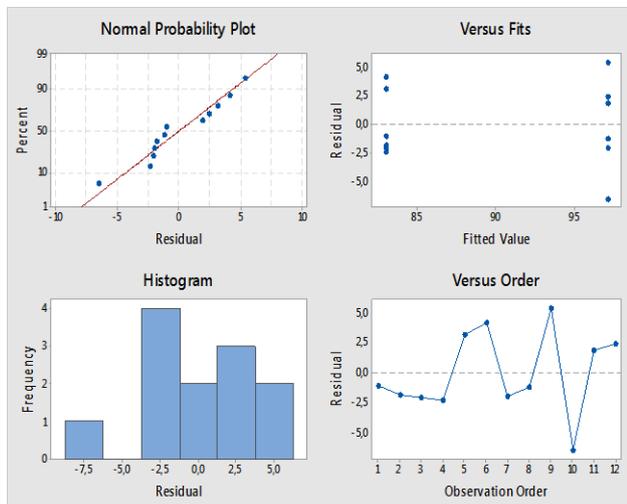

Figure 2: Residual plots for disolved percentage in the preliminary study.

Design of Experiments

Regarding the results obtained through the DoE, it was observed that the dissolution medium is a critical variable to be considered in the test, where the use of a HCl 0.1 N (pH 1) allows to obtain the highest dissolution percentages. On the other hand, the speed of the dissolution apparatus does not constitute a factor that shows significant differences between the two speeds studied. However, the interaction between the two factors is of great interest since certain combinations result in higher dissolution percentages.

Table 5: DoE execution.

Std Order	Run Order	Rotation speed (rpm)	pH	Dissolved % at 30 minutes
11	1	75	5,0	95,1
3	2	75	5,0	95,9
4	3	100	5,0	95,3
21	4	75	1,0	102,4
24	5	100	5,0	100,5
19	6	75	5,0	102,5
2	7	100	1,0	101,3
8	8	100	5,0	94,3
13	9	75	1,0	101,8
22	10	100	1,0	108,6
17	11	75	1,0	99,0
10	12	100	1,0	105,9
7	13	75	5,0	90,6
15	14	75	5,0	99,0
18	15	100	1,0	104,3
12	16	100	5,0	96,2
9	17	75	1,0	98,0
5	18	75	1,0	96,5
6	19	100	1,0	101,0
20	20	100	5,0	95,8
1	21	75	1,0	98,8
14	22	100	1,0	99,8
16	23	100	5,0	96,0
23	24	75	5,0	99,5

Table 6: One Way ANOVA for the DoE.

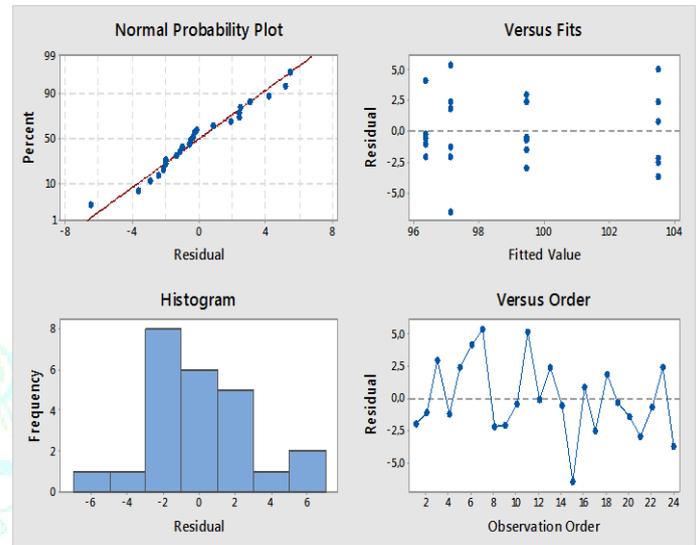| Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	3	185,25	61,752	6,43	0,003
Linear	2	150,45	75,227	7,83	0,003
Rotation speed	1	16,50	16,500	1,72	0,205
Medium pH	1	133,95	133,954	13,95	0,001
2-Way interaction (Medium pH * Rotation speed)	1	34,80	34,800	3,62	0,071
Error	20	192,03	9,602		
Total	23	377,29			

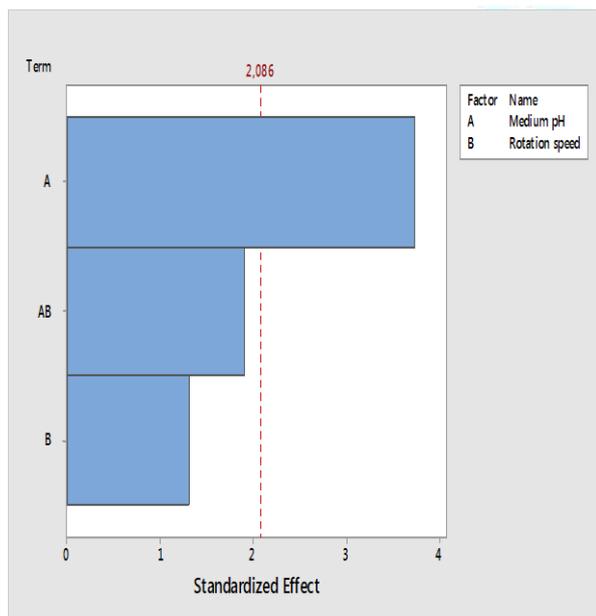**Figure 3:** Residual plots for the DoE.

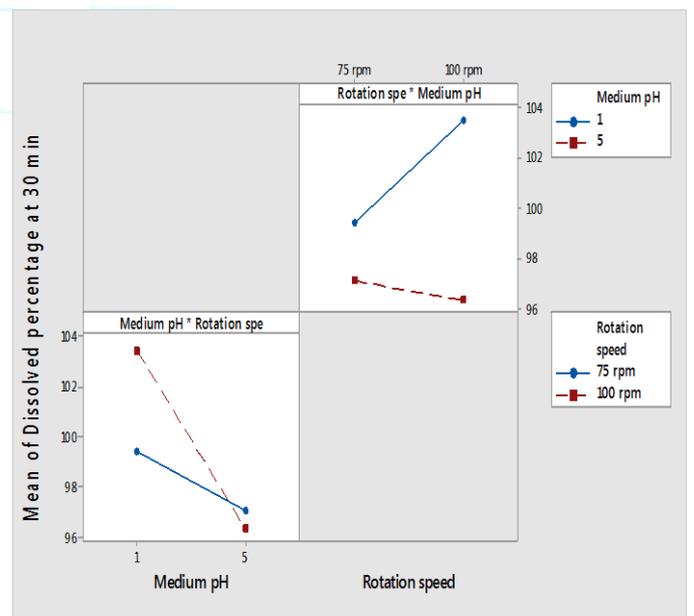**Figure 4:** Pareto diagram for the analysis of standardized effects.

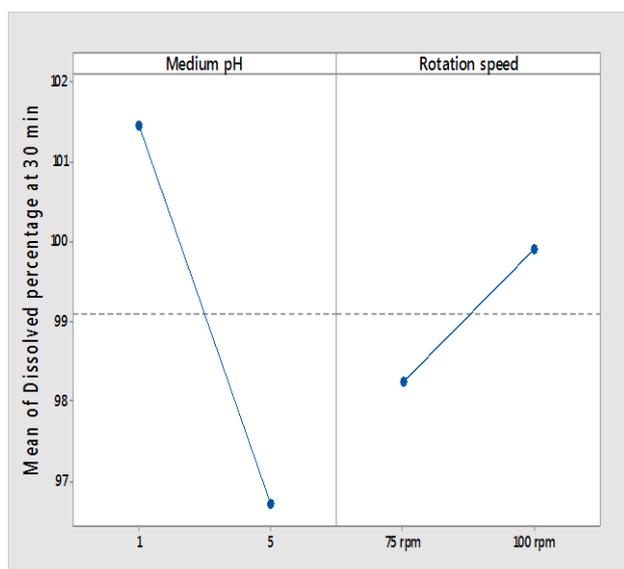**Figure 5:** Diagram of interactions between the study factors.
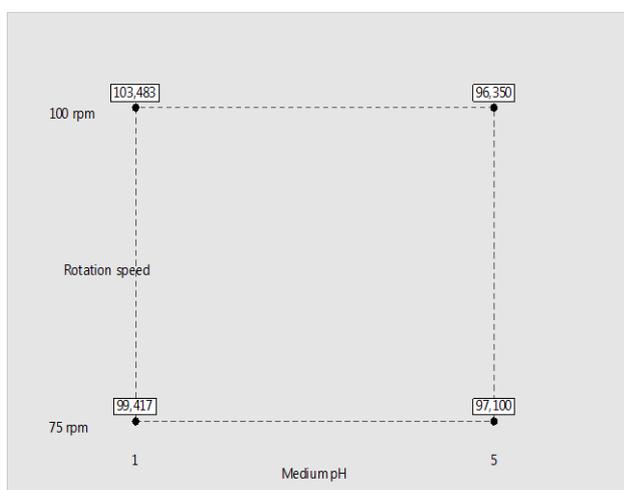
Figure 6: Main effects diagram.

Figure 7: Geometric representation of the factorial design 2^2.

DISCUSSION

When carrying out the dissolution test initially in water, it was possible to observe that the data did not provide a reliability of the method of analysis, since there were anomalous situations in terms of the dissolved quantity of the active pharmaceutical ingredient as a function of time.

In a study consulted about the dissolution of Rupatadine Fumarate tablets, the test was executed for 30 minutes in a medium consisting of 900 mL of water and another by 900 mL of an acetate buffer (pH 4.5), at 37.0 ± 0.5 ° C, using the apparatus II at 75 rpm. This study revealed large differences in the performance of the formulation in both media, since in water it showed dissolution percentages around 80% and in the buffer the values were under 70%.[10]

Although the matrix of the formulation presented in that study differs with the proposed in the present investigation, it was observed that the performance of our formulation in water was even poorer than in the reference study, so it was decided to compare such parameter in this medium, with the one of another constituted by a phosphate buffer at pH 5 as an exploratory study.

It was then necessary to determine between both mediums, which one was suitable to be taken into consideration for the execution of the DoE. Figure 1 shows the performance of the tablets of the proposed formulation in the two mediums. In

addition, it should be noted that distilled water as a medium represents a factor that can´t be controlled at all, due to the variability of its pH.

When performing the analysis of variances between these two mediums, the p-value turned out to be lower than the level of significance (0.01 <0.05), so the alternative hypothesis is accepted, which implies that there are significant differences between the water and buffer as a dissolution medium. Also, as can be seen in Figure 2, compliance of the normality, equality of variances and independence of the respective residues is checked for the ANOVA in question.

From the results of the preliminary study, and unlike the reference one, it was determined that the phosphate buffer does constitute a medium in which it is possible to obtain a good performance of the proposed formulation in the dissolution test.

However, taking into consideration the above, the same reference study also carried out dissolution tests in a medium constituted by 900 mL of 0.1 N HCl, which obtained the best results. That behave is logical due to the fact that the active pharmaceutical ingredient is an acid salt or weak base salt.[8, 10, 11]

Thus, since our formulation didn´t show a good dissolution in water, it was then decided to execute the DoE using mediums constituted by phosphate buffer pH 5 and by HCl 0.1 N of pH 1, to evaluate the performance of the proposed formulation in the dissolution test.

The mentioned above is due to the fact that both mediums provide suitable sink conditions for the execution of the test, in such a way that they allow to reliably evaluate the performance of the formulation of interest.[11]

The general objective of the design of experiments performed is not far from the universal, which lies in improving the response, being in this case the average of the dissolved amount of drug at 30 minutes. [12]

All those variables that had to be controlled during the tests were defined, as well as the identification of the factors in which that was not possible or commonly referred to as noise variables; where in addition to the pH of the distilled water, stand out the environmental conditions.

However, noise variables were minimized thanks to the randomization of the analysis of the samples, as can be seen in Table 5, where there were a standard order and an order of analysis. The above allowed distributing the error attributable to those factors, among all the results.[1, 12] Therefore, the only two variables that remained to be analyzed were the already defined pH of the medium (1 and 5) and, in addition, the speed of the dissolution apparatus (75 and 100 rpm). Working with a 2^2 factorial design made the treatment of the data more manageable, analyzing only four treatments with six repetitions each as established in the dissolution test, for a total of 24 samples.

With the implementation of the proposed experimental design, its ANOVA indicates that there are no significant differences between the speeds used in the test, since the p-value is greater than the level of significance (0.205> 0.05).

However, for the pH of the medium it is established that there are differences between HCl 0.1 N (pH 1) and the phosphate buffer (pH 5), because the p value corresponds to 0.003 which is lower than the established level of significance.

Likewise, it is important to mention that the interaction between the two factors could be significant as long as a level of significance of 90% (α = 0.1) or close to 92% (α = 0.08) is established.

The Pareto diagram that can be observed in figure 4, allows to determine the magnitude and importance of the standardized effects, which are the changes observed in the response variable due to a change of level in the factors.

In this case only the respective bar for the pH of the medium manages to overcome the reference line. Therefore only that factor is statistically significant at the level of α = 0.05 according to the terms of the established model.[13]

Figure 6 is the graphic representation of the main effects, where it is possible to observe the levels of the factors on the horizontal axis and in the vertical one there is located the average of the response observed according to each level. In this case, the main effect of the pH of the medium is greater than the one of the speed, because the difference between the means of its two levels is greater.[12]

In figure 5, it can be seen that when the speed increases from 75 rpm to 100 rpm, the average of the dissolved amount decreases slightly at pH 5. Whereas, if there is such an increase in speed when the test is carried out in a medium at pH 1, the mean of the dissolved amount increases markedly.

This implies that if it is desired to maximize, minimize or bring the response to a target value, the speed level can´t be changed without taking into account the level of the pH of the medium. Based on this interpretation, it can be assumed that there is an interaction between the factors. However, as mentioned above, such interaction is not significant for the level of significance initially established.

Precisely, one of the main uses of an interaction chart lies in the contribution made in the selection of the optimal condition for the operation of a particular process and the consequent improvement in its performance.

In addition to the above, it is also possible to make this determination through a geometric representation. In the 2^2 factorial design, each vertex of a square is considered as a design or treatment point. The area bounded by this square is known as the experimental region and, in principle, the conclusions obtained from the experiment only have validity in this region.[12, 13]

If Figure 7 is analyzed, it is possible to determine that the treatment which combines a medium with pH 1 at a speed of 100 rpm gives the higher percentages of drug dissolved (103.48 %), while the worst of the treatments integrates a medium with pH 5 and a speed of 100 rpm (96,35 %).

Finally, as a requirement for any analysis of variance, it is necessary to do a verification of the assumptions of normality, equality of variances and independence of residuals.

Figure 3 is representative of a sample of residues that have a normal distribution (p value 0.701 > 0.05). Also, this figure allows to check the independence of the residuals, since the represented points do not follow a specific pattern and also allows satisfying the constant variance assumption, because the points are distributed in a random way on the horizontal band.[1, 13]

CONCLUSIONS

The preliminary study allowed discarding water as a medium of dissolution since the performance of the tablets of the formulation under study did not prove to be adequate. On the other hand, the phosphate buffer turned out to be a medium in which the tablets showed an acceptable performance.

However, the experimental design carried out determined that the medium of dissolution constituted a factor that significantly affected the response variable, which is, the average percentage of active pharmaceutical ingredient dissolved at 30 minutes, whereas the apparatus rotation speed does not represent a significant impact on the response studied. Thus, it was shown that better results were obtained at higher speeds in the ideal medium.

Therefore, the present study allowed to optimize the performance of the formulation in the dissolution test, by providing the appropriate tools for the establishment of the ideal analysis method, which consists of a medium constituted by 900 mL of HCl 0.1 N, at 37.5 ± 0.5 ° C, apparatus II and at a speed of 100 rpm.

REFERENCES

1. Djuris J, Ibric S, Djuric Z. Experimental design application and interpretation in pharmaceutical technology. Computer-aided applications in pharamceutical technology. 1st ed. Belgrado: Woodhead Publishing Limited; 2013. p. 31-56.

2. Djuris J, Ibric S, Djuric Z. Quality by Design in pharmaceutical developments. Computer-aided applications in pharamceutical technology. 1st ed. Belgrado: Woodhead Publishing Limited; 2013. p. 1-16.

3. Yu L, Amidon G, Khan M, Hoag S, Poli J, Woodcock J. Understanding Pharmaceutical Quality by Design. AAPS Journal. 2014; 16(4):771-783.

4. U.S. FDA/CDER, Guidance for Industry, "Dissolution testing of immediate release solid oral dosage forms", 1997.

5. Farmacopea de los Estados Unidos (USP). Disolución (711). USP 39 - NF 34. Maryland: Convención de la Farmacopea de los Estados Unidos; 2016. p. 579-590.

6. Zhang H, Yu L, "Dissolution Testing for Solid Oral Drug Products: Theoretical Considerations", American Pharmaceutical Review, September/October 200;, 7(5):26-31.

7. Wang Q, Fotaki N, Mao Y, "Biorelevant Dissolution: Methodology and Application in Drug Development", Dissolution Technologies, August 2009, 6-12.

8. Uriach, J. & Compañía S.A. 8-cloro-11-(1-((5-metil-3-piridil)metil)-4-piperidiliden)6,11-dihidro-5H-benzo-(5,6)cicloheptal(1,2-b)piridina, fumarato. España; ES 2 087 818, 2017.

9. Zhuhai Jin Hong Pharmaceutical. One kind of fumaric rupatadine compounds, their synthesis and pharmaceutical compositions thereof. China; CN103804357 B, 2016.

10. Shangguan Qing. Rupatadine fumarate compound as well as synthesis method and pharmaceutical composition thereof. China; CN103804357 A, 2014

11. Dalmora S, Nogueira D, Zanini G. Development and Validation of a Dissolution Test with Reversed-Phase Liquid Chromatography Analysis for Rupatadine in Tablet Dosage Forms. Quim Nova. 2010; 33(5):1150-1154.

12. Azeem S, Sharma S. Design & Evaluation of Immediate Release Tablet of Rupatadine Fumarate. International Journal Of Pharma Professional's Research. 2012; 3(4):660-668.

13. Gutiérrez Pulido H, Vara Salazar R. Análisis y diseño de experimentos. México: McGraw-Hill; 2008.

14. Pukelsheim F. Optimal design of experiments. 2nd ed. Philadelphia: Society for Industrial and Applied Mathematics; 2006.