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Optimization of dissolution test precision for a ketoprofen oral extended-release product

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Abstract

An example of application of experimental design methodologies to the set up of dissolution test conditions for a new ketoprofen oral extended-release dosage form is presented. The aim of the work was to find the best experimental conditions, using a USP apparatus 2 (paddle), for maximizing the method precision as degree of repeatability. The considered factors mainly influencing the dissolution test results were pH and volume of dissolution medium, and paddle stirring speed. Two distinct 4-run Plackett–Burman designs were carried out: one at gastric and the other at intestinal pH values. Each run was performed in triplicate in order to calculate the standard deviations of the drug dissolution efficiency at 60 and 120 min, selected as responses to be minimized. Optimum conditions to carry out the dissolution test were: 900 ml volume of dissolution medium and 70 rpm paddle stirring speed for both environments and pH 1 and 5.5, for the gastric and intestinal environment, respectively.

Keywords: Dissolution test; Paddle apparatus; Experimental design; Ketoprofen; Extended-release dosage form; Validation

1. Introduction

Dissolution test has emerged in the pharmaceutical field as a very important tool to characterize drug product performance. The significance of a dissolution test is based on the fact that for a drug to be adsorbed and available to the systemic circulation, it must previously be solubilized [1]. Therefore, dissolution tests are used not only for quality control of finished products, to assess batch-to-batch consistency of drug release from solid dosage forms, but they are also essential in several stages of formulation development, for screening and proper assessment of different formulations. In fact, the creative use of dissolution techniques can speed up the initial stages of formulation development, particularly in the case of extended-release products, enabling prompt identification of potential problems in drug release rate [2]. Basically, dissolution test makes it possible to assess the dissolution properties of the drug itself and thereby to select the most appropriate excipients and appropriate proportions among them for obtaining the desired drug release behavior. Moreover, when an 'in vitro–in vivo' corre-

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lation is available, it can also be used as a test to reflect the bioavailability of a product in humans and therefore to determine the actual bioequivalence of different products containing the same drug at the same dosage.

During a preformulation study, preliminary testing conditions are commonly elaborated taking into consideration the state of the art for dissolution testing. Different official apparatus are available and, for each, Compendia, e.g., USP 25, EP 4, report detailed specifications in both general chapters [3,4] and individual monographs on solid oral dosage forms.

Dissolution tests on conventional dosage forms have been successfully implemented, and formal Guidelines exist which provide useful recommendations for their evaluation [5].

Following the success obtained with dissolution testing of immediate release products, USP and the pharmaceutical industry attempted to standardize extended-release products in a similar manner. This would be important from the viewpoint of cost containment, standardization and international harmonization. On the other hands, extended oral release dosage forms, have very different physicochemical properties which often require specific considerations and need for case-by-case development, making it very difficult to establish proper generalized guidelines [6–8]. In fact, owing to the unique nature of the oral extended-release products, most of their monographs contain different dissolution test procedures, in order to cover multiple products with different release profiles in vivo [2].

In any case, it is important to point out that none of the purposes for which dissolution tests are used can be actually fulfilled by an in vitro test without sufficient reliability, where this is defined as a system being experimentally sound, yielding precise, accurate and repeatable results [7]. A recent international collaborative study indicated that drug dissolution testing is a highly variable technique [9]. As a consequence, in many cases the impact of formulation or manufacturing changes on drug release properties may not be detected, or, on the contrary, not true differences, but rather caused by test variability, could be evidenced. Thus, a careful control of experimental conditions is necessary in order to suitably reduce test-to-test variability and improve test reproducibility and reliability.

The validation of the dissolution test can be divided into two parts. The first regards equipment validation; equipment has to be calibrated taking into consideration the specifications for geometry and alignment of the dissolution apparatus [10]. The second concerns test validation and in this case, being the dissolution test an USP 25 category III assay, it requires the study of the performance parameter precision [11]. The evaluation of precision is very important in order to assess the reliability of the data obtained by the dissolution test. In fact, it is true that a more discriminative dissolution method is preferred, but it is also true that a precise assay is of utmost importance. A dissolution test with a good precision, for example, makes it possible to efficiently compare different alternative formulation candidates for selecting the dosage form with the most suitable and reproducible drug release profile.

Starting from these considerations, it was considered important, in the context of a preformulation study aimed at the development of a new ketoprofen oral extended-release dosage form, the setting of the best dissolution test conditions for adequately assaying the prepared formulations. Thus, the work project was divided into two parts, the first concerning the dissolution testing set up, and the other the optimization of the pharmaceutical dosage form. In particular, the purpose of the present study was to evaluate, by means of statistical experimental design methodologies, the influence of critical dissolution test variables on ketoprofen release from an extended-release model dosage form, with the aim of optimizing the method precision as repeatability degree. The advantage of such methodology is in providing a rationale for evaluation of several variables simultaneously, without neglecting overall supervision.

This application of experimental design for optimizing the precision of dissolution test should reduce the amount of work needed for formulation development. In fact, the greater repeatability of results should make it possible to reduce of the number of single experiments and improve the method reliability in identifying possible effects of

process or formulation changes on drug release profile.

2. Experimental

2.1. Chemicals and solutions

Ketoprofen, (Sigma Chemical Co., St. Louis, USA), Carbopol 940 (Merck, Darmstadt, Germany), lactose and magnesium stearate (Carlo Erba, Milano, Italy) were used as received. All the other reagents were of analytical grade. Analytical grade water (Milli-Q system, Millipore) was used throughout the study.

2.2. Equipment and dissolution test conditions

Dissolution studies were performed with a USP paddle apparatus (Sotax AT7). The dissolution medium was constituted by 600–900 ml of pH 1–3 hydrochloric acid aqueous solution, with a constant ionic strength (by means of sodium chloride) of 0.11 M (for gastric juice simulation) or pH 5.5–7.5 phosphate buffer with a constant ionic strength (by means of sodium chloride) of 0.14 M (for intestinal juice simulation), thermostatted at 37 ± 0.5 °C and stirred at 70–140 rpm. The concentration of dissolved drug was spectrometrically monitored at 261 nm (UV/Vis spectrometer Lambda 2, Perkin Elmer). Dissolution efficiency (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 [12], according to the following equation:

$$\mathrm{DE} = \frac{\mathrm{AUC}_{t_i}}{c_{\mathrm{tot}} t_i} 100$$

where AUC_{t_i} is the area under the dissolution curve at the t_i time and c_{tot} is the concentration of 100% dissolved drug.

2.3. Tablet preparation

Model matrix tablets (75 mg ketoprofen, 120 mg Carbopol 940 as matrix forming material, 300 mg lactose as diluent and 5 mg magnesium stearate as lubricant) were prepared by direct compression of the components mixture using a hydraulic press.

2.4. Experimental design

Experimental design was generated and statistical analysis of experimental data was performed using NEMRODW software package [13]. A duplicate 4-run Plackett–Burman design was used. During the optimization, the dissolution experiments of ketoprofen, from the model matrix tablets, were carried out in randomized order.

3. Results and discussion

In developing a new drug solid dosage form, and particularly in the case of extended-release products, dissolution test is a very helpful tool for the assessment and adjustment of the drug release profile from candidate formulations, allowing easy and fast evaluation of the effects of formulation changes. However, this test is sensitive to many parameters such as temperature, stirring, solvent, pH, ionic strength and medium volume, shape of the vessel, etc. [14,15]. On the other hand, as for other analytical techniques, the precision of the method is essential for its reliability. Therefore, in a preliminary phase of a preformulation study for the development of ketoprofen extended-release tablets, a multivariate optimization strategy was carried out with the aim of finding the optimum conditions for testing the drug dissolution behavior from such tablets with the maximum precision as degree of repeatability.

DE is a model-independent parameter widely used as a significant index of drug dissolution performance. Thus, the standard deviations, calculated on three replicates, of DE values at 60 (SD–DE60) and at 120 min (SD–DE120) were selected as representative responses. The factors, among those previously cited, that, on the basis of preliminary experiments, were found to mainly

affect the drug dissolution behavior, were pH (U_1) and volume (U_2) of the medium, and the paddle rotation speed (U_3). These factors are important to simulate physiological conditions; only for such variables do the FDA guidelines for dissolution testing conditions of immediate release solid oral dosage forms report advisable values to use in carrying out dissolution tests [5]. In all the experiments temperature was set at 37 ± 0.5 °C, and ionic strength was maintained constant at 0.11 or 0.14 M for gastric and intestinal medium, respectively.

Concerning the experimental domain of the considered independent variables, information about the repeatability of the dissolution test in both gastric and intestinal conditions was needed. Towards this aim, and according to the values reported in the FDA guidelines [5], for the pH factor, an experimental domain ranging between 1 and 3 for the gastric medium, and between 5.5 and 7.5 for the intestinal one, was considered. As for this last experimental domain, actually, pH 6.8 is the maximum advisable value for intestinal pH suggested by the FDA guidelines, for testing immediate release dosage forms. However, when an extended-release dosage form is considered, its dissolution characteristics should be determined over the entire range of physiological pH, which is generally described as being from approximately pH 1 to 7.5 [2]. For the other two factors, the experimental domain was the same in the two designs and ranged from 600 to 900 ml and from 70 to 140 rpm, for dissolution medium volume and paddle rotation speed, respectively. Actually, the FDA recommends mild agitation conditions and, in particular, for the paddle apparatus, a stirring from 50 to 75 rpm is suggested [5]. The effect on the considered responses of high rotation speed (e.g. 140 rpm) was chosen for study due to the fact that most dissolution apparatus allow such stirring rates to be used. Therefore, it is useful to obtain information about the precision of dissolution test also at high values of stirring speed. On the other hand, the 50 rpm value was not included in the studied experimental domain since, in preliminary experiments, it did not allow a good homogeneity of solution to be obtained.

A Plackett–Burman design was used to obtain information about the effect of the change in the factor levels on the considered responses. According to this design, each factor is present at two levels and the number of experiments is a multiple of 4. The levels may be simply represented as '−' and '+'. A Plackett–Burman design is very easily constructed: the first line is given and the remaining lines are obtained by permutation, except for the last line, which consists entirely of minus signs [16]. Table 1 reports the experimental matrix, where the three columns correspond to the three factors, and the eight rows, to four experiments, each replicated. Substituting the real values for the codified values, the two experimental plans, for gastric and intestinal pH, respectively, were obtained.

Usually, in order to have an estimation of the experimental variance for quantitative factors (such as those studied in this case) it is sufficient that central points are added and replicated [16]. However in our case, since the drug release process from tablets was subject itself to variations, the whole design was replicated. In this way, the run-to-run variability was a combination of the variation between drug products (dissolution behavior) and that due to other experimental variations [16]. In this context, randomization was essential in order to ensure genuine run replicates, where the variation between runs made at the same experimental conditions is a reflection of the total variability afflicting runs made at different experimental conditions [17].

Table 1
Duplicate 4-run Plackett–Burman design

Exp. no.	x_1	x_2	x_3
1	+	+	−
2	+	+	−
3	−	+	+
4	−	+	+
5	+	−	+
6	+	−	+
7	−	−	−
8	−	−	−

In summary, there were 24 experiments carried out for gastric pH and 24 for intestinal pH. By means of the statistical treatment of the responses and by means of experimental design tools, we obtained the desired information on the influence of the factor level change on the responses. Tables 2 and 3 report the experimental plans with the obtained responses at gastric and intestinal pH, respectively.

As experimental design tool, graphic analysis of the effects was used. This analysis allowed the important factors for the considered responses to be pointed out and the better factor level to be selected. Construction of a bar graph is required in which the bars that exceed the two reference lines, calculated according to the experimental variance, correspond to the factors that are active on the response. In particular the active factors are those where a level change determines a response variation which is statistically different from the variation due to the experimental error. Fig. 1a reports the graphic analysis for the response SD–DE60 at gastric pH. For this response, the factors pH and paddle rotation speed were significant, whereas the

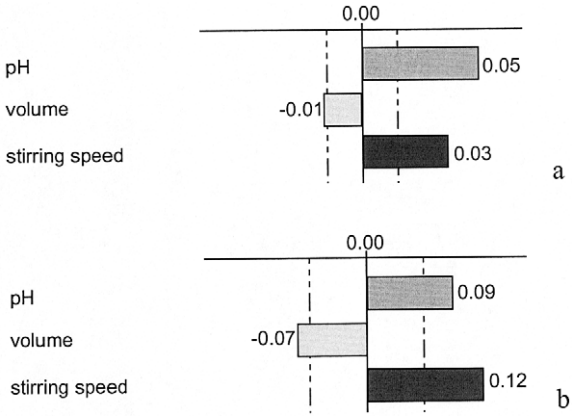

Fig. 1. Graphic analysis of effects at gastric pH. (a) Response SD–DE60 min; (b) response SD–DE120 min.

Table 2
Experimental plan at gastric pH and obtained responses

Exp. no.	Ran order	pH	Volume (ml)	Stirring speed (rpm)	SD–DE60[a] ($n = 3$)	SD–DE120[a] ($n = 3$)
1	11	3	900	70	0.071	0.099
2	1	3	900	70		
3	4	3	900	70		
4	19	3	900	70	0.092	0.049
5	9	3	900	70		
6	16	3	900	70		
7	2	1	900	140	0.072	0.205
8	5	1	900	140		
9	12	1	900	140		
10	13	1	900	140	0.042	0.071
11	22	1	900	140		
12	3	1	900	140		
13	20	3	500	140	0.174	0.514
14	24	3	500	140		
15	10	3	500	140		
16	23	3	500	140	0.184	0.415
17	21	3	500	140		
18	7	3	500	140		
19	18	1	500	70	0.024	0.033
20	6	1	500	70		
21	15	1	500	70		
22	14	1	500	70	0.014	0.038
23	17	1	500	70		
24	8	1	500	70		

[a] DE, area under the dissolution curve at 60 (DE60) or 120 (DE120) min, expressed as percent of the area of the rectangle described by 100% dissolution in the same time.

Table 3
Experimental plan at intestinal pH and obtained responses

Exp. no.	Ran order	pH	Volume (ml)	Stirring speed (rpm)	SD–DE60[a] ($n = 3$); unità	SD–DE120[a] ($n = 3$)
1	8	7.5	900	70	0.163	0.304
2	21	7.5	900	70		
3	14	7.5	900	70		
4	11	7.5	900	70	0.064	0.219
5	4	7.5	900	70		
6	20	7.5	900	70		
7	2	5.5	900	140	0.141	0.318
8	1	5.5	900	140		
9	13	5.5	900	140		
10	7	5.5	900	140	0.134	0.346
11	22	5.5	900	140		
12	3	5.5	900	140		
13	18	7.5	500	140	0.424	0.603
14	24	7.5	500	140		
15	5	7.5	500	140		
16	23	7.5	500	140	0.420	0.594
17	17	7.5	500	140		
18	16	7.5	500	140		
19	10	5.5	500	70	0.042	0.090
20	6	5.5	500	70		
21	15	5.5	500	70		
22	12	5.5	500	70	0.330	0.316
23	9	5.5	500	70		
24	19	5.5	500	70		

[a] DE, area under the dissolution curve at 60 (DE60) or 120 (DE120) min, expressed as percent of the area of the rectangle described by 100% dissolution in the same time.

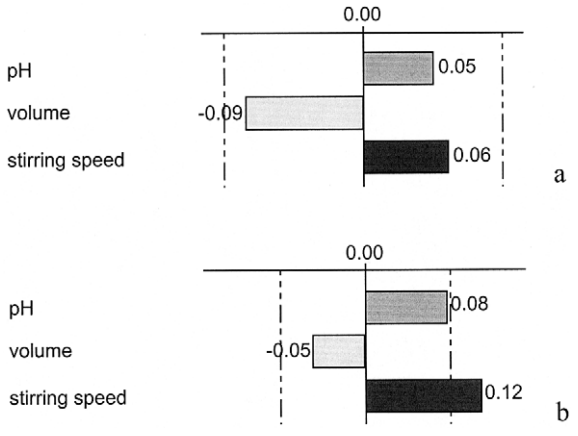

Fig. 2. Graphic analysis of effects at intestinal pH. (a) Response SD–DE60 min; (b) response SD–DE120 min.

medium volume was not important to minimize the response.

As for the level corresponding to the best results, the graph gives the following information. On the right are reported the bars corresponding to factors for which we have the maximization of the response when moving from the low to the high level, and on the left the bars of the factors for which, on the contrary, we have the maximization of the response when moving from the high to the low level. Thus, in this case, since the response has to be minimized, we have to use the low level of both pH and paddle rotation speed, while we can choose indifferently both levels for the factor volume. Fig. 1b reports the graphic analysis of the effects for the SD–DE120 response at gastric pH; in this case, in order to minimize the response we have to choose the high level of the factor volume,

while the level change of the other two factors was not important.

As regards the results for the intestinal pH, no factor level change was important for the response at 60 min (Fig. 2a). On the contrary, for the response at 120 min (Fig. 2b), the factor rotation speed, was active and, in order to minimize the response, we have to move towards the low level. The results regarding the use of low stirring rate are, in particular, in agreement with that reported in the FDA guidelines [5] where conditions of mild agitation are required.

Summarizing, the optimum conditions to carry out the dissolution test resulted to be: 900 ml volume of dissolution medium and 70 rpm paddle stirring speed, for both environments, and pH 1.0 and 5.5, for the gastric and intestinal environment, respectively. Applying these optimum conditions the highest SD value found was 0.083 for the DE at 120 min and at intestinal pH.

4. Conclusions

The possibility to obtain with a dissolution test reliable results on the pharmaceutical to be tested, is essential to ensure the quality, safety and efficacy of the developed drug product.

In this work, the potential of experimental design to assist in the search for the optimum conditions for dissolution experiments is illustrated. In particular, the precision, as repeatability degree, of a paddle dissolution method for assaying ketoprofen extended-release tablets has been optimized. The chosen strategy, a Plackett–Burman design, was suitable to point out the significant factors affecting standard deviation of drug DE in the considered experimental domain and to determine the level of each, leading to optimal repeatability of the dissolution experiments.

The obtained results allowed the pH and volume of the dissolution medium and the paddle rotation stirring to be properly set according to the optimized conditions for both gastric and intestinal dissolution experiments.

This same approach may be easily applied for other dissolution procedures, as well as for other solid dosage forms.

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References

[1] B. Hoener, L.Z. Benet, in: G.S. Banker, C.T. Rhodes (Eds.), Modern Pharmaceutics, Marcel Dekker, New York, 1990, p. 143.
[2] E.D. Jorgensen, D. Bhagwat, Pharm. Sci. Technol. Today 1 (1998) 128–135.
[3] The United States Pharmacopeia 25, The United States Pharmacopeial Convention, Rockville, MD, USA, 2002, pp. 2011–2012.
[4] The European Pharmacopoeia, fourth ed., Council of Europe, Strasbourg, 2002, pp. 194–197.
[5] Guidance for Industry, Dissolution testing of Immediate Release Solid Oral Dosage Forms, US Department of Health and Human Services, FDA (CDER), August 1997.
[6] M.Z.I. Khan, Int. J. Pharm. 140 (1996) 131–143.
[7] International Pharmaceutical Federation (FIP) guidelines for dissolution testing of solid oral products, Drug Inf. J. 30 (1996) 1071–1084.
[8] Guidance for Industry, Extended Release Oral Dosage Forms, Development, evaluation and application of in vitro/in vivo correlations, US Department of Health and Human Services, FDA (CDER), September 1997.
[9] S.A. Qureshi, I.J. McGilveray, Eur. J. Pharm. Sci. 7 (1999) 249–258.
[10] A. Frick, H. Moller, E. Wirbitzki, Eur. J. Pharm. Biopharm. 46 (1998) 305–311.
[11] The United States Pharmacopeia 25, The United States Pharmacopeial Convention, Rockville, MD, USA, 2002, pp. 2258–2259.
[12] K.A. Khan, J. Pharm. Pharmacol. 27 (1975) 48–49.
[13] D. Mathieu, J. Nony, R. Phan-Tan-Luu, NEMRODW, LPRAI sarl, Marseille, 2000.
[14] K. Wingstrand, B. Abrahamsson, B. Edgar, Int. J. Pharm. 60 (1990) 151–156.
[15] P.O. Quist, G. Östling, J. Pharm. Biomed. Anal. 28 (2002) 1081–1089.
[16] G.A. Lewis, D. Mathieu, R. Phan-Tan-Luu, Pharmaceutical Experimental Design, Marcel Dekker, New York, 1999.
[17] G.E.P. Box, W.G. Hunter, J.S. Hunter, Statistics for Experimenters, Wiley, New York, 1978.