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Statistical method for evaluation of dissolution stability in the formulation development of solid dosage forms: tablets of amonafide

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A statistical method for the evaluation of the dissolution stability results and for selecting the most stable formulation within a solid dosage form development is discussed. Three types of tablets of an antineoplastic drug, amonafide, stored at a relative humidities (RH), 45% and 75%, were used. The drug release from tablets was tested before and after storage. The experimental data were statistically fitted to empirical model equations. Furthermore, the best mathematical fit was the statistical comparison of the residuals. From the selected model equation, time-dependent dissolution (Q_{45} and DE^{45}) and dissolved quantity-dependent parameters (t_{70}, t_{100} and MDT) were calculated. An useful parameter to present and evaluate the results obtained in comparative stability studies was defined: the Modification Factor (MF). It allowed the selection of the most stable formulation in the easiest and fastest way: the most stable formulation should present the smallest modification of the studied characteristics, in other words, the smallest MF value. In this way, tablets II (manufactured by wet granulation and with Emcompress as main excipient) showed the greater dissolution stability of the three types of tablets studied. Amonafide tablets must be packaged in impermeable containers, since the environmental relative humidity strongly modifies their dissolution characteristics.

1. Introduction

The objective of the present work is to evaluate the dissolution stability of different formulations of tablets of a new drug with the aim of selecting the optimum formulation. Different ways to express the drug dissolution characteristics should be analyzed in order to determine the most useful, to investigate the modifications in the drug dissolution during storage.

The objective of this work to quantify these modifications to compare promptly the dissolution stability of different formulations in order to select the most stable one. A comparative study of the dissolution stability of an antineoplastic drug, amonafide, from tablets was designed to evaluate the procedure proposed in this work. The dissolution characteristics of the active substance formulated in several types of immediate-release tablets (manufactured by different techniques and with different constituents) were determined and their modifications during the storage quantified.

In this work, the environmental humidity is of special importance as amonafide · 2 HCl is a hygroscopic substance (Torres and Camacho 1991).

Amonafide is a 1.8 naphtalimide with antineoplastic activity (Malviya et al. 1994). Amonafide · 2 HCl presents pKa values of 2.63 and 7.31. These values suggest that the drug will be absorbed in the first segments of the duodenum. The aqueous solubility of the drug is high, with values of the solubility coefficient from 11.57% w/v at pH = 6 to 25.19% w/v at pH = 1.2. These data indicate that, as drug substance, the dissolution of amonafide · 2 HCl does not limit its oral absorption rate (Torres and Camacho 1992).

2. Investigations, results and discussion

The Figures 1A, 1B and 1C show the amonafide · 2 HCl dissolution profile for the tablets I, II and III, respectively, at initial time (t = 0) and after the storage under the different conditions.

In the Tables 1, 2 and 3, the mathematical models that describe the dissolution profiles Q (%) versus t (min), as well as the goodness of fit (WSSQ value for χ^2-test for residuals) are presented.

To define the dissolution characteristics of the tablets before and after storage, the dissolution parameters Q_{45}, DE^{45}, t_{70}, t_{100} and MDT were calculated from the mathematical equations and gathered in the tables 4, 5 and 6.

The values of the dissolution parameters before and after the storage were transformed into the parameters Storage Initial Ratio (SIR) (Fig. 2) and Modification Factor (MF) (Fig. 3).

2.1. Dissolution characteristics of each formulation

All the fitted equations were exponential (Tables 1–3), because the polynomial equations were too flexible and followed outlines, leading to oscillating curves, rather than data smoothing that was really required.

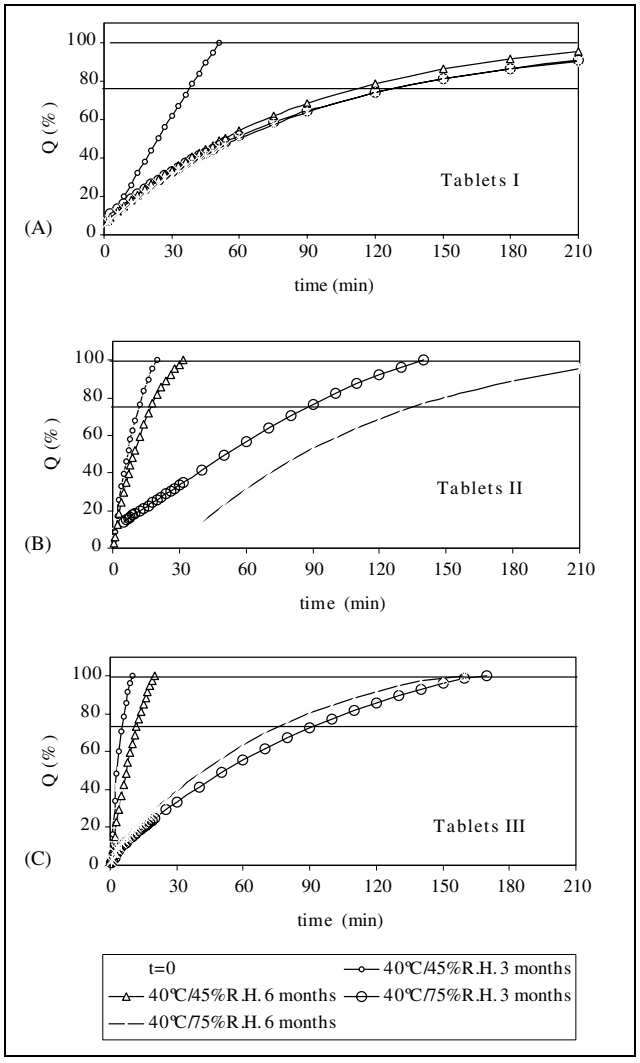

Fig. 1: Amonafide · 2 HCl dissolution profile from the tablets I (A), II (B) and III (C), at initial time (t = 0) and after the storage in the different conditions

The experimental data have been fitted to mathematical equations and dissolution theoretical models have not been searched. In fact, most authors have proposed different theoretical models that try to explain the dissolution characteristics of immediate-release tablets. The most common ones are the Noyes-Whitney equation and the cubic root or Hixon-Crowell equation. Higuchi and Hiestand, Brooke and Carstensen, and Masa, among others authors (Banakar 1991), have undertaken other theoretical models

Table 1: Dissolution profile of amonafide · 2 HCl from tablets I under the storage conditions studied: mathematical equation, goodness of fit and parameters of the equation

	Goodness of fit: WSSQ	$Q(\%) = B'(1 - e^{-k't}) + B''(1 - e^{-k''t}) + C$				
		B′ σ (%)	k′ σ (min^{-1})	B″ σ (%)	k″ σ (min^{-1})	C σ (%)
Initial	65.90*	116.30 1.67	0.047 0.003	–	–	1.32** 1.89
40 °C 45% RH	52.50*	551.10 83.82	0.0039 0.0009	–	–	−2.33** 3.14
40 °C 75% RH	99.0*	92.78 0.59	0.0103 0.0002	–	–	8.23 0.47

* $P > 0.05$ (χ^2-test). **$P < 0.05$ (t-test for parameter redundancy). WSSQ = weighted sum of squares value, σ = standard deviation, RH = Relative Humidity

Table 2: Dissolution profile of amonafide · 2 HCl form tablets II under the storage conditions studied: mathematical equation, goodness of fit and parameters of the equation

	Goodness of fit: WSSQ	$Q(\%) = B'(1 - e^{-k't}) + B''(1 - e^{-k''t}) + C$				
		B′ σ (%)	k′ σ (min^{-1})	B″ σ (%)	k″ σ (min^{-1})	C σ (%)
Initial	84.7*	136.20 5.53	0.070 0.006	–	–	0.44** 0.69
40 °C 45% RH	73.8*	136.20 5.49	0.070 0.006	–	–	5.17** 6.58
40 °C 75% RH	64.6*	−285.00 41.33	0.021 0.003	399.20 75.45	0.016 0.003	12.42 2.12

* $P > 0.05$ (χ^2-test). **$P < 0.05$ (t-test for parameter redundancy)

Table 3: Dissolution profile of amonafide · 2 HCl form tablets III under the storage conditions studied: mathematical equation, goodness of fit and parameters of the equation

	Goodness of fit: WSSQ	$Q(\%) = B'(1-e^{-k't}) + B''(1-e^{-k''t}) + C$				
		B′ σ (%)	k′ σ (min^{-1})	B″ σ (%)	k″ σ (min^{-1})	C σ (%)
Initial	77.8*	229.50 37.20	0.041 0.009	–	–	−021** 0.45
40 °C 45% RH	66.4*	139.40 5.69	0.158 0.015	–	–	−0.49** 0.43
40 °C 75% RH	101.2*	121.90 13.45	0.008 0.001	–	–	5.63 0.65

* $P > 0.05$ (χ^2-test). **$P < 0.05$ (t-test for parameter redundancy)

Table 4: Dissolution parameters obtained in the study of the tablets I. X = mean, σ = standard deviation, t-Student value with respect to initial time (t = 0)

Conditions	Statistics (n = 6)	Parameter				
		t_{70} (min)	t_{100} (min)	Q_{45} (%)	DE^{45} (%)	MDT (min)
Initial	X	18.59	37.08	100	69.43	13.76
	σ	2.73	5.17	0	3.19	1.43
40 °C	X	33.86	48.38	93.13	46.76	23.96
45% RH	σ	1.56	1.87	3.57	2.74	1.23
	t-Student	11.90*	5.03*	4.30*	13.20*	13.24*
40 °C	X	106.11	425.95	42.73	26.76	142.56
75% RH	σ	5.29	83.65	1.82	1.80	24.99
	t-Student	32.86*	11.36*	70.36*	28.54*	12.60*

*$P < 0.05$

Table 5: Dissolution parameters obtained in the study of tablets II

Conditions	Statistics (n = 6)	Parameter				
		t_{70} (min)	t_{100} (min)	Q_{45} (%)	DE^{45} (%)	MDT (min)
Initial	X	10.47	18.57	100	82.99	7.66
	σ	1.48	2.27	0	1.88	0.84
40 °C	X	10.49	19.44	100	82.83	7.73
45% RH	σ	0.75	0.62	0	1.28	0.58
	t-Student	0.032	0.91	–	0.16	–
40 °C	X	88.53	129.20	36.46	26.46	60.39
75% RH	σ	5.55	10.43	1.88	0.94	4.01
	t-Student	33.30	25.39	75.57*	65.98*	31.52*

*$P < 0.05$

Table 6: Dissolution parameters obtained in the study of tablets III

Conditions	Statistics (n = 6)	Parameter				
		t_{70} (min)	t_{100} (min)	Q_{45} (%)	DE^{45} (%)	MDT (min)
Initial	X	8.81	13.69	100	86.39	6.12
	σ	0.97	0.88	0	1.29	0.55
40 °C	X	4.75	8.43	100	91.97	3.61
45% RH	σ	0.55	1.01	0	0.67	0.30
	t-Student	8.91*	9.57*	–	9.76*	9.81*
40 °C	X	96.96	149.35	38.47	25.19	66.24
75% RH	σ	8.68	15.64	3.20	3.65	5.94
	t-Student	24.71*	21.21*	42.99*	38.95*	24.69*

*$P < 0.05$

of drug dissolution from tablets, which disintegrate into granules or particles with homogeneous or heterogeneous particle-size distribution.

For dissolution studies, to find the mathematical equation that experimental data are better fitted to (from this equation, the dissolution parameters are calculated) is more important than an only fair fit to a theoretical model of dissolution.

At initial time, the experimental data of the amonafide · 2 HCl release from the three types of tablets studied (I, II and III) were fitted to a monoexponential equation. No significant y-intercept was detected. Then, the mathematical equation that describes the drug dissolution process from the beginning to the end, for all the tablets studied, was:

$$Q(\%) = B'(1 - e^{-k't}) \quad (1)$$

After the storage of tablets I and III under both relative humidities, and tablets II under 45% R.H., the drug dissolution kinetics was also fitted to a monoexponential equation. However, for tablets II stored at 75% R.H., the best fit was achieved with biexponential equations:

$$Q(\%) = B'(1 - e^{-k't}) + B''(1 - e^{-k''t}) + C \quad (2)$$

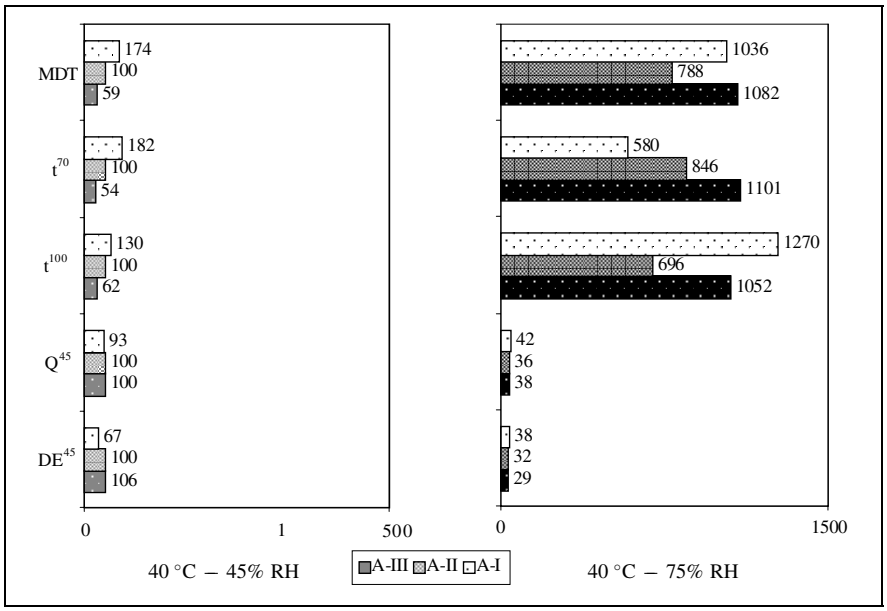

Fig. 2:
SIR values for the three types of tablets

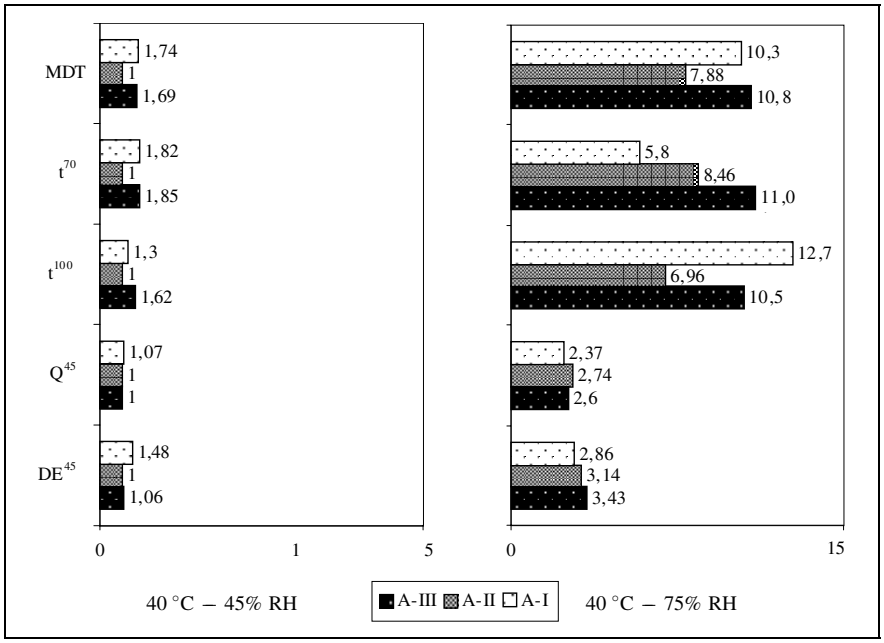

Fig. 3:
MF valvues for the three types of tablets

It is not strange that, after storage, not only the dissolution rate, but also the kinetic model were modified.

When dissolution models are fitted to experimental data, it is common to characterise the dissolution process through the rate constant in a general way. However, this constant is not a useful parameter for dissolution stability studies. When the dissolution kinetic model changes after storage, as in tablets II, the constants cannot be compared.

Thus, it is not useful for comparing the dissolution stability of different formulations. Alvarez-Lorenzo et al. 2000 faced this problem in a study of dissolution stability of theophylline tablets with hydroxypropyl cellulose (HPC) of different grades of substitution. They fitted the dissolution data of the tablets with HPC of high substitution to the Higuchi equation before and after the storage, and calculated the corresponding dissolution rate constants. However, the dissolution profile of the tablets with HPC of low substitution cannot be fitted to the Higuchi equation. For this reason, the dissolution rate constants were not useful to compare their formulations.

In the present paper the dissolution parameters Q_{45}, DE^{45}, t_{70}, t_{100} and MDT were used.

These dissolution parameters were calculated from the mathematical equations that fitted the experimental data (model-dependent treatment), instead of straight from the experimental data (independent-model treatment). Both procedures are valid, but the first one has the advantage of masking possible errors in the experimental determination of a given value. The treatment of dependent-model data are usual in pharmacokinetic analysis, as opposed to independent-model treatments, where the experimental errors have more influence on the value of the parameter calculated to characterize the process.

Regarding the dissolution parameters used, the release specifications of dissolution and dissolution stability for immediate-release solid dosage forms are usually the same. Normally they involve a minimum amount of drug to be dissolved at a specified time interval (they are single-point values: Q_t). Many monographs include the requirement that not less than 75% of the label claim must be dissolved at 45 minutes (Q_{45}), using either water or simulated gastric fluid TS as the dissolution medium and following USP method 1 baskets rotated at 100 rpm or USP method 2 paddles set at 50 rpm. However, it would be more informative and rational to follow the total release profile on the samples of the product during storage (Murthy and Ghebre-Sellassie 1993).

In fact, if a revision of the works about dissolution stability is made, it is observed that Q_t is the commonly used parameter. However, in most of these works, a qualitative analysis of the dissolution profiles is included, because of the limitations of this parameter to detect changes in the dissolution process (Rohrs et al. 1999; Bauer et al. 1999). Other authors have also tried to quantify the characteristics of the dissolution profile through the calculation of the drug dissolved in percentage at different times (Q_{15}, Q_{30}, Q_{60}, Q_{90} and Q_{120}) (Al-Zein et al. 1999; Babu and Pandit 1999; Di-Martino et al. 1999; Wang et al. 1993). The problem of this procedure is that the necessity of several parameters to define the same profile makes the comparison among different formulations and/or storage conditions very complex, so finally the authors only use one parameter (usually Q_{45} or Q_{60}) to compare. In order to avoid this problem, other authors prefer to calculate parameters such as DE^t, which characterizes the whole dissolution process (Alvarez-Lorenzo et al. 2000; Mura et al. 1999).

For all mentioned above, the parameters calculated in this work express both the dissolution (Q_{45}, t_{70} and t_{100}), and all the process globally (DE^{45} and MDT). Some of them are time-dependent (Q_{45} and DE^{45}), and others dissolved quantity-dependent (t_{70}, t_{100} and MDT).

All the observed modifications in the dissolution profiles of the tablets involved a drug dissolution rate decrease, except for tablets III after 3 months under low relative humidity (45%), that increased. Obviously, a decrease in the dissolution rate should involve a decrease in the value of the time-dependent dissolution parameters (Q_{45} y DE^{45}). However, the parameter Q_{45} is not always able to detect such modification because it does not represent the whole dissolution process. If the dissolution at initial time is fast ($Q_{45} = 100\%$), an acute decrease of the dissolution rate during storage must be detected by this parameter.

In the case of tablets II and III the dissolution rate diminution was not only to be detected by the Q_{45} parameter after storage at 45% RH. This also happened to Ondari

et al. when they studied the dissolution of sugarcoated chlorpromazine tablets stored under stressed storage conditions (Ondari et al. 1984). A single point determination of the amount of drug in solution at 60 min showed that the product was stable under the storage conditions, whereas the examination of the total release profile would lead to the opposite conclusion.

When the dissolution rate increases, Q_{45} also enhances and when it approaches the value of 100%, it turns out useless to detect changes in the dissolution process.

Where DE^{45} is concerned, this problem does not occur. As it is a parameter that represents the whole dissolution process, it is able to detect any change: a dissolution rate increase causes an increase in the DE^{45} value (i.e. in tablets II after 3 months of storage at 45% RH), and a dissolution rate decrease produces a diminution of the DE^{45} value (i.e. in the rest of the cases).

As for the dissolved quantity-dependent parameters, all of them increase when the dissolution rate decreases and vice versa. They exhibit, in all cases, the capacity of detecting changes in the dissolution characteristics.

2.2. Quantification of the changes in the dissolution characteristics of each formulation due to storage

The first thing observed through both SIR and MF parameters (Figs. 2 and 3) is that the variations in the drug dissolution kinetics were better detected when dissolved quantity-dependent dissolution parameters (t_{70}, t_{100} and MDT) were used. This is explained by the fact that Q_{45} and DE^{45} parameters can only have values within a range 0–100% of the dose. For this reason, the difference between the values before and after storage is quantitatively smaller than in the case of t_{70}, t_{100} and MDT, which can get any value. For example, the MDT value of the tablets III at 75% RH is 66.24 min, in contrast to 6.12 min obtained at initial time (Table 6), giving rise to a MF value of 10.82 (Fig. 3). Whereas, the Q_{45} values of the same tablets are 38.47% and 100% for the same conditions (Table 6), with a MF value of only 2.6 (Fig. 3).

The storage initial ratio (SIR) is an useful parameter to evaluate the influence of one or more factors on the stability of a product, but it is not helpful enough for comparative stability studies among several formulations or among several storage conditions. This is, because the SIR value is higher or smaller than 100 according to the dissolution parameter value which increases or decreases after storage. It is a parameter that normalizes the new value which respect to the initial one, but it does not quantify the modification detected. The Modification Factor (MF), used as a comparative parameter of stability, gets always values equal to or higher than the unit. Equal to the unit, if there are no modifications in the parameter analyzed, and higher than the unit if a modification has taken place, whatever the modification is (whether the dissolution parameter value increases or whether it decreases). Therefore, the greater the detected modification is, the higher the MF value is. This direct relation between the modification detected and the MF value allows to easily determine which formulation is the most stable or which storage condition has the worst influence on stability.

For example, it is needed to compare the stability of tablets I and III to know which formulation is more stable after 3 months of storage at 40 °C – 45% RH, taking into account the MDT values for being the more reliable dissolution parameter. The MDT value of tablets I was greater than the initial one, whereas the MDT value of tablets III was smaller than the initial one. In both cases, a modification in the dissolution kinetics had taken place: for tablets I, the drug dissolution became faster, and, for tablets III, slower. That modification is shown by SIR values of MDT: 174 for tablets I and 59 for tablets III (Fig. 2). However, from these SIR values, it is complex to discern which of the two types of tablets presented the smallest modification of the MDT parameter, or which was the most stable formulation under these storage conditions. From MF values, it is not so complex. The value of MDT increased 1.74 times for tablets I (MF = 1.74), while in tablets III the value of MDT decreased 1.69 times with storage (MF = 1.69) (Fig. 3). Therefore, the values of MF [MDT] of tablets I and III were not significantly different. Then, the modification in the drug dissolution of both tablets I and III presented the same magnitude, although in the opposite sense.

2.3. Dissolution stability evaluation of tablets of amonafide · 2 HCl

For the selection of the most stable formulation, the MF values were used.

According to the MF values (Fig. 3), the environmental humidity is an important instability factor for Amonafide · 2 HCl dissolution from the three types of tablets. This is due to the presence of hygroscopic constituents in the tablets, as Avicel, PVP and amonafide · 2 HCl.

At 45% RH, tablets II and III, with Emcompress as main excipient, are the most stable, especially tablets II, since most of their dissolution parameters are not modified with storage (MF = 1). At this relative humidity, the only constituent that shows a hygroscopic behaviour is PVP, that is in a very low proportion, so its influence on the dissolution parameter is minimum. Amonafide does not show a hygroscopic behaviour: its equilibrium humidity at 45% RH is 0.5%. Tablets II are a little more stable than tablets III, because of Emcompress behaviour and tablet elaboration technique (Ahlneck and Lundgren 1985; Carstensen 1990). Tablets II were elaborated by wet granulation, tablets III by direct compression. At 45% RH and 40 °C, Emcompress loses its crystallization water of the outer layers which is easier when it is not into a granule than when it is. That is, Emcompress loses its water more easily in tablets III than in tablets II, so the modifications are more important in tablets III than in tablets II. Besides, since the tablets III has less water than tablets II, their affinity by the water is higher and, then, their drug dissolution rate is also higher.

At 45% RH, tablets I, with Avicel as main excipient, are unstable. Probably, due to the hygroscopic behaviour of Avicel, which is show from a relative humidity higher than 10%. This instability of tablets I is even more intense when the relative humidity increases: at 75% RH, the value of MDT is more than 10 times the initial one (MF = 10.36). This modification of the dissolution characteristics of tablets containing hygroscopic constituents when stored under high humidity conditions is reported in the literature. Tablets absorb moisture and lose its prestorage characteristics because, to a large extent, the interparticulate bonds formed in the original compact have been removed and replaced by new bonds, resulting in tablets that have a different porosity and pore structure and, hence, different *in vitro* release patterns compared with the original one (Sebhatu 1994). In the case of tablets I, the acute decrease of the dissolution rate detected at 75% RH may also be explained by the sorption of

water, due to the presence of Avicel, amonafide and PVP (they behave as hygroscopic substances at that relative humidity), that decrease the avidity of amonafide for the dissolution fluid.

Although the tablets with Emcompress (II and III) are the most stable at 45% RH, when stored at 75% R.H., they become very unstable and no significant difference between these and the tablets with Avicel exist, in particular if the dissolution parameters that represent all the process like MDT and ED[45] are analyzed. This may be due to the hygroscopicity of the drug at relative humidities over 60%.

It can be concluded that the proposed method to determine the dissolution stability is useful to select the most stable formulation in the development of solid pharmaceutical dosage forms.

In order to determine the dissolution characteristics before and after storage, parameters representative of all the dissolution process should be used. The time-dependent parameters present less sensitivity to detect changes than the dissolved quantity-dependent ones. The parameter Q_{45}, commonly used in the official specifications for the registration and marketing of solid dosage forms, is not useful for dissolution stability studies. A good dissolution parameter is, according to this work, MDT.

To quantify the changes detected in the dissolution characteristics with storage, the calculation of the named modification factor (MF) is especially useful. It allows to compare directly the stability of different formulations and the effect of different storage conditions on the stability of these formulations.

On comparing the dissolution stability of different types of tablets with amonafide stored at relative humidities of 45% and 75%, it can be said that type II is the most stable with Emcompress as the main excipient.

However, due to the hygroscopic behaviour of amonafide at high relative humidities and its influence on the dissolution stability, it is suggested to protect these tablets against the environmental relative humidity by an impermeable container. This statement affects storage conditions of stability studies for the registration application of this medicinal product (tablets with impermeable container). According to the guidelines of the International Conference on Harmonization about stability testing of new drug substances and products, the stability studies of medicines with impermeable container may be done under any controlled relative humidity (2000; 2003).

3. Experimental

3.1. Materials

Active substance: Amonafide dihydrochloride (2 amino-1,8-naphtalimide; CAS 69408-81-7).

Tablet excipients: microcrystalline cellulose (Avicel® PH101), dicalcium phosphate dihydrate (Emcompress®), povidone K30 (Kollidon® 30), and magnesium stearate Eur. Ph. Grade.

Table 7: Composition (in weight%) of the tablets of amonafide · 2 HCl

Constituents	Tablets		
	I	II	III
Amonafide · 2 HCl	62.50	60.03	67.32
Avicel® PH101	31.24	4.96	4.81
Emcompress®	–	30.01	24.04
Providone®	5.46	4.20	3.03
Mg stearate	0.80	0.80	0.80

Main apparatus and devices: dissolution tester (Sotax, Barcelona, Spain), spectrophotometer (DU Beckman, Madrid, Spain), liquid chromatograph (Hewlett-Packard, Madrid, Spain).

3.2. Elaboration of the tablets

Three types of tablets, I, II and III, were manufactured with 251.5 mg of amonafide · 2 HCl. The main excipient was Avicel PH101 for tablets I and Emcompress for types II and III. Their composition (in weight%) is shown in the Table 7. Tablets I and II were manufactured by wet granulation, type III by direct compression. For tablets III, the drug was granulated with a 5% hydroalcoholic solution of Povidone K30 and, then mixed with excipients for direct compression (Torres et al. 1995).

3.3. Storage conditions

The three types of tablets were placed in open containers and stored at 40 ± 2 °C under two different relative humidities: $45 \pm 5\%$ and $75 \pm 5\%$. The tablets were tested after 3 months of storage.

3.4. Dissolution assay.

The dissolution profile of the active substance formulated in the three types of tablets was studied in the recently prepared tablets (initial time) and after the storage under the different conditions. The assay was carried out with 6 tablets.

The USP 26 dissolution test apparatus (with a basket as stirring element) was used at 100 r.p.m. and with 900 ml of HCl 0.1 N as test fluid (from the solubility coefficient of amonafide · 2 HCl at pH 1.2, the drug is completely dissolved in the gastric medium). At pre-established time intervals, a 3 ml aliquot sample was withdrawn from the dissolution vessel (without replacing the extracted volume), filtered through 0.45 μm and analyzed by UV spectrophotometry after suitable dilution with deionized water. Tablet drug content was determined by HPLC (Camacho et al. 1994) to verify that, during storage, the drug substance had not been degraded. In this way, if any modification on the dissolution profile with storage is detected, it is only due to physico-chemical modification of the dosage form.

3.5. Data analysis

3.5.1. Determination of the dissolution characteristics of each formulation

The dissolution profile was defined by the amount of dissolved drug in percentage (Q), versus the dissolution time in minutes (t).

Experimental data (Q versus t) were fitted to different empirical models with the aim of searching the best mathematical fit. Exponential and polynomial mathematical equations were used. The goodness of fit of the experimental data to the proposed mathematical models was evaluated through the χ^2-test.

If the data can be fitted to different models, the best fit was determined by comparing the residuals: it is well known that the smaller the value of weighed sum of squares (WSSQ) is, the better the fit is. It should be taken into account that, within the same mathematical model (exponential or polynomial), normally, the higher the number of equation parameters is, the better the fit is. For this reason, if the same experimental data were fitted to two equations of the same mathematical model, but with m_1 and m_2 parameters ($m_1 < m_2$) and $WSSQ_1 > WSSQ_2$, the significance of the improvement in the WSSQ value might be evaluated by the F-test. When the improvement was not statistically significant, the simplest equation (m_1) was accepted.

From the selected mathematical equations, the following drug dissolution parameters were calculated:

- Time-dependent parameters: the percentage of drug dissolved at 45 min. from the dissolution test beginning (Q_{45}) and the dissolution efficiency at the same time, 45 min. (DE^{45}).
- Dissolved quantity-dependent ones: time in dissolving the 70% and 100% of the dose (t_{70} and t_{100}) and mean dissolution time (MDT).

3.5.2. Quantification of the modifications of the dissolution characteristics of each formulation during the storage

For each type of tablets studied, the values of the dissolution parameters after storage were compared with the values obtained at initial time ($t = 0$) by a t-Student test. If significant differences were detected, they were quantified by means of the calculation of parameters that express the stability as a relative value:

- Storage Initial Ratio (SIR), as a parameter used by other authors (Bos et al. 1991):

$$SIR\,[Y] = (Y_t/Y_0) \cdot 100 \quad (3)$$

Where: Y is the dosage form characteristic to be studied. Y_t is the Y value after storage. Y_0 is the Y value at initial time.

- Modification Factor (MF), as a new parameter proposed in this paper:

$$MF\,[Y] = Y_h/Y_l \quad (4)$$

Where: Y is also the stability characteristic. It can present two values, before and after the storage: Y_h is the highest Y value and Y_l is the lowest one. This parameter is based on the definition of the statistical F-Snedecor parameter used for comparing two variances.

3.5.3. Comparison of the quantified modifications of the different formulations under different storage conditions

The SIR and MF values of the dissolution parameters were compared for the three types of tablets of amonafide · 2 HCl and for the two different storage conditions. This comparison allows:

- To select the amonafide tablets with the highest dissolution stability.
- To determine the influence of the storage relative humidity on the dissolution stability of amonafide tablets.

References

Ahlneck C, Lundgren P (1985) Methods for the evaluation of solid state stability and compatibility between drug and excipient. Acta Pharm 22: 305–314.

Alvarez-Lorenzo C, Gomez-Amoza JL, Martinez-Pacheco R, Souto C, Concheiro A (2000) The stability of theophylline tablets with a hydroxypropylcellulose matrix. Drug Dev Ind Phar 26: 13–20.

Al-Zein H, Riad LE, Abd-elbary A (1999) Effect of packaging and storage on the stability of carbamacepine tablets. Drug Dev Ind Pharm 25: 223–227.

Babu RJ, Pandit JK (1999) Effect of aging on the dissolution stability of glibenclamide/beta-cyclodextrin complex. Drug Dev Ind Pharm 25: 1215–1219.

Banakar UV (1991) Dissolution of dosage forms. In: Banakar UV (ed.) Pharmaceutical Dissolution Testing, New York: Marcel Dekker, p. 260–282.

Bauer JF, Dziki W, Quick JE (1999) Role of an isomorphic desolvate in dissolution failures of an erythromycin tablet formulation. J Pharm Sci 88: 1222–1227.

Bos CE, Bolhuis GK, Lerk CF, De Boer JH, Duineveld, AK Smilde CAA, Doornbos DA (1991) The use of a factorial design to evaluate the physical stability of tablets prepared by direct compression. I. A new approach based on the relative change in tablet parameters. Eur J Pharm Biopharm 37: 204–209.

Camacho MA, Torres AI, Gil ME, Obregón MM, Ruz V (1994) Qualitative and quantitative determination of two new antitumor agents from 1–8 naphtalimides in tablets: Validation of a high performance liquid chromatography method. Arzneim.-Forsch/Drug Res 44: 659–662.

Carstensen JT (1990) Effect of moisture on the stability of solid dosage forms. In: Swarbrick J (ed.) Drug Stability. Principles and Practices, Vol. 43. Drugs and the Pharmaceutical Sciences, New York: Marcel Dekker Inc., p. 191–207.

Di-Martino P, Barthelemy C, Piva F, Joiris E, Palmieri GF, Martelli S (1999) Improved dissolution behavior of fenbufen by spherical crystallization. Drug Dev Ind Pharm 25: 1073–1081.

International Conference on Harmonization (ICH) (Nov. 2000) Stability Testing of new drug substances and products, ICH Harmonized Tripartite Guideline, ICH Q1AR (CPMP/ICH/2736/99).

International Conference on Harmonization (ICH) (Feb. 2003) Stability Testing of new drug substances and products (Revision 2), ICH Harmonized Tripartite Guideline, ICH Q1A(R2).

Malviya VK, Liu PY, O'Toole R, Alberts DS, Surwit E, Rosenoff S, Ward JH, Yu A, O'Sullivan J, Craig JB (1994) Phase II trial of amonafide inpatients with advanced metastatic or recurrent endometrial adenocarcinoma: A Southwest Oncology Group study. American J Clin Oncol 17: 37–40.

Mura P, Faucci MY, Manderioli A, Bramanti G, Parrini P (1999) Thermal behavior and dissolution properties of naproxen from binary and ternary solid dispersions. Drug Dev Ind Pharm 25: 257–264.

Murthy KS, Ghebre-Sellassie I (1993) Current perspectives on the dissolution stability of solid oral dosage forms. J Pharm Sci 82: 113–126.

Ondari CO, Prasad VK, Shah VP, Rhodes CT (1984) Effects of short-term moderate storage stress on the disintegration and dissolution of four types of compressed tablets. Pharm Acta Helv 59: 149–153.

Rohrs BR, Thamann TJ, Gao P, Stelzer DJ, Bergren MS, Chao RS (1999) Tablet dissolution affected by a moisture mediated solid-state interaction between drug and disintegrant. Pharm Res 16: 1850–1850.

Sebhatu T, Elamin AA, Ahlnek C (1994) Effect of moisture sorption on tabletting characteristics of spray dried (15% amorphous) lactose. Pharm Res 11: 1233–1238.

Torres AI, Camacho MA (1991) The behaviour under atmospheric humidity of two benzoisoquinolinediones (Mitonafide and 2ClH Amonafide). Il Farmaco 46 : 1091-1096.

Torres AI, Camacho MA (1992) Studies about the oral bioavailability of mitonafide and 2 HCl amonafide, two new cytotoxic molecules. Farmaco 47: 497–508.

Torres AI, Gil ME, Camacho MA (1995) Design and formulation of tablets of a new antineoplastic drug: Amonafide. Drug Devel Ind Pharm 21: 185–197.

Wang JT, Shiu GK, Ong-Chen T, Viswanathan CT, Skelly JP (1993) Effects of humidity and temperature on "in vitro" dissolution of carbamazepine tablets. J Pharm Sci 83: 1002–1005.